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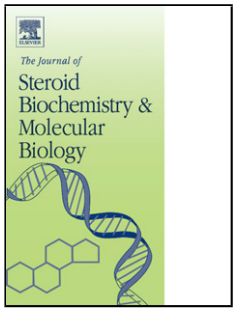

Modified bile acids and androstanes - novel promising inhibitors of human cytochrome P450 17A1

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Modified bile acids and androstanes - novel promising inhibitors of human cytochrome P450 17A1

Running title.

Novel promising ligands of human cytochrome P450 17A1.

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Highlights

- Modified bile acids and androstane derivatives binds with high affinity by human CYP17A1.
- Novel ligands decrease rate of hydroxylation of progesterone – natural CYP17A1 substrate.
- Bile acids derivatives can completely inhibit enzyme activity toward progesterone.
- *In vitro* results and SAR analysis allowed to recognize important pharmacophores.

Abstract.

Cytochromes P450 are key enzymes for steroid hormone biosynthesis in human body. They are considered as targets for the screening of novel high efficient drugs. The results of screening of bile acids and androstane derivatives toward human recombinant steroid 17α-hydroxylase/17,20-lyase (CYP17A1) are presented in this paper. A group of steroids, binding with micromolar or submicromolar affinity (in a range from 9 μM – less than 0.1 μM), was identified. Results presented here showed that these steroidal compounds are able to decrease rate of hydroxylation of essential CYP17A1 substrate – progesterone, while some compounds completely inhibited enzyme activity. Structure-activity relationship (SAR) analysis based on *in vitro* and *in silico* studies showed that high affinity of the enzyme to bile acids derivatives is correlated with side chain hydrophobicity and presence of hydroxyl or keto group at C_3 position. From the other side, bile acid-derived compounds with more polar side chain or substituents at C_7 and C_{12} positions possess higher K_d values. Among androstane-derived steroids couple of Δ^5-steroids with hydroxyl group at C_3 position, as well as 16,17-secosteroids, were found to be high affinity ligands of this enzyme. The data obtained could be useful for the design of novel highly efficient inhibitors of CYP17A1, since the bile acids-derived compounds are for first time recognized as effective CYP17A1 inhibitors.

Abbreviations.

CYP – cytochrome P450; SAR – structure-activity relationship; RMSD – root-mean-square deviation; DMSO – dimethylsulfoxide; CYP17A1 – steroid 17α-hydroxylase/17,20-lyase; IPTG – isopropyl β-D-1-thiogalactopyranoside; PMSF — phenylmethylsulfonyl fluoride; Em-913 – Emulgene-913; DTT — dithiothreitol; CHAPS — 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate; TLC — thin layer chromatography; HRMS — high-resolution mass spectrometry; GAL – galeterone; P4 – progesterone; P5 – pregnenolone; 17OH-P4 – 17α-hydroxy progesterone; 17OH-P5 – 17α-hydroxy pregnenolone; PAINS – pan-assay interference compounds.

Keywords.

Cytochrome P450, androstane compounds, bile acids derivatives, CYP17A1 inhibitors.

1. Introduction

Cytochromes P450 (CYP) present a superfamily of heme-containing enzymes existing in all life kingdoms and participating in many different physiological processes. Human CYP enzymes catalyze biotransformation of xenobiotics (including

drugs), biosynthesis and metabolism of cholesterol, steroid hormones, bile acids, fat-soluble vitamins and polyunsaturated fatty acids [1].

CYP17A1 (steroid 17α-hydroxylase/17,20-lyase or 17,20-desmolase) is an important enzyme, playing crucial role in steroid hormone biosynthesis. This enzyme is expressed in all steroidogenic and few non-steroidogenic tissues [2]. CYP17A1 catalyzes two independent reactions in one active site [3]: hydroxylation at C_{17} position of natural substrates pregnenolone (P5) or progesterone (P4) and breaking of a bond between C_{17} and C_{20} of their 17-hydroxy analogues. The ratio of these two activities is physiologically important and may direct steroid hormone biosynthesis to the production of either corticoid or sex hormones [4] (Figure 1).

Mutations in CYP17A1 gene or overexpression of the protein are associated with different human endocrine diseases: congenital adrenal hyperplasia (due to 17α-hydroxylase deficiency form) [5], isolated 17,20-lyase deficiency [6], polycystic ovary syndrome [7] and Cushing's syndrome [8]. It was also found that changes in the enzyme activity, due to point mutations or other reasons, lead to the castration-resistant prostate cancer, which imposes CYP17A1 as one of the key targets in the treatment of this disease [9]. Having in mind great medical importance of CYP17A1 inhibition, in the present study we tested a panel of modified bile acids and androstane derivatives against human CYP17A1, in order to find novel enzyme ligands – from the one hand, and to determine novel types of biological activity for a group of compounds which suppress cancer cells proliferation. Such biology-driven medicinal chemistry of anticancer drugs is very promising and can make significant progress in the development of drugs for prostate cancer treatment.

2. Materials and methods.

2.1 Protein expression and purification.

Human CYP17A1 was expressed and purified according to known procedure [10] with slight modifications. Protein was truncated by removing 27 amino acids from N-termini and MAKKT sequence was added instead of truncated segment. CYP17A1 was overexpressed in the *E. coli* BL-21 cells. Expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), and 0.4 mM δ-aminolevulinic acid was added as a precursor of heme biosynthesis. After induction cells were cultured at 26 °C during 48 h. Harvested cells were resuspended in Buffer A (50 mM Tris-HCl buffer, pH 7.4, containing 20% glycerol and 0.3 M NaCl), containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF). The cells suspension was frozen under –73 °C.

Cells were disrupted by Emulsiflex C3 homogenizer, followed by addition of Emulgen 913 (Em-913) at final concentration 1%. The lysate was cleared by centrifugation (100000 g) during 1 h. Supernatant was applied to a Ni^{2+}-NTA-agarose column, equilibrated with buffer A. The column was washed with 3 volumes of Buffer

A containing 0.2% Em-913 and then with 20 volumes of Buffer A containing 0.2% Em-913 and 25 mM imidazole with 50 μM P4 (Buffer B). Protein was eluted from the column with buffer A containing 250 mM imidazole and 0.2% Em-913. Eluted fractions of CYP17A1 were applied to a column with hydroxyapatite equilibrated with 10 mM potassium-phosphate buffer, pH 7.4, containing 20% glycerol, 0.2% Em-913, 0.1 mM dithiothreitol (DTT) and 0.3 M NaCl. The column was washed with 10 volumes of 50 mM potassium-phosphate buffer, pH 7.4, containing 20% glycerol, 0.2% Em-913, 0.1 mM DTT and 0.3 M NaCl. The protein was eluted by 0.6 M potassium-phosphate buffer, pH 7.4, containing 20% glycerol, 0.2% Em-913, 0.1 mM DTT and 0.3 M NaCl. Protein preparations were kept under –73 °C.

2.2. Modified steroids used in enzyme binding tests.

Synthesis of tested analytically pure steroidal compounds was performed according to known procedures, while characterization and purity of all substances were tested and documented by thin layer chromatography (TLC), high-resolution mass spectrometry (HRMS), ^{1}H and ^{13}C NMR [11-32]. Purity was also checked by measuring melting points of the synthesized compounds; they were narrow range (1-2 degrees) and in agreement with literature data. Synthetic procedures with short description and raw NMR spectra (^{1}H and ^{13}C) are presented in Supplemental Materials. Stock and working solutions (10^{-2}, 10^{-3}, 10^{-4} M) for titration studies were prepared in dimethylsulfoxide (DMSO).

2.3. *In vitro* screening of the potential ligands.

Initial screening was performed using high throughput screening (HTS) approach. The 96-well plate was filled with compounds solutions in 50 mM potassium-phosphate buffer (pH 7.4), containing 0.2% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS) and 0.3 M NaCl (final ligand concentration was 80 μM). After adding protein solution (final concentration 1 μM) difference spectrum (350-500 nm) was recorded (protein+ligand vs. protein+DMSO) using SpectraMax i3 spectrophotofluorometer (Molecular Devices, USA). Compounds with typical spectral response, corresponding to binding of substrate (weak field ligand, Type I spectral response; λ_{max}=393 nm, λ_{min}=417 nm) or inhibitor (strong field ligand, Type II spectral response; λ_{min}=413 nm, λ_{max}=432 nm) molecules were detected and picked up for further experiments.

Affinity of the ligands was analyzed using spectrophotometric titration approach in 50 mM potassium-phosphate buffer (pH 7.4), containing 0.2% CHAPS and 0.3 M NaCl with final CYP17A1 concentration 1 μM. Ligand solution (stock solutions with concentrations from 10^{-4} up to 10^{-2} M) was added to the experimental cuvette and equal volume of the solvent (DMSO) to the control cuvette. To determine dissociation constant of enzyme-ligand complex (K_d) equation for the tight binding was used (titration data were approximated using of Levenberg-Marquardt algorithm):

$$A = A_{\max} \cdot \frac{[L]_t + [R]_0 + K_d - \sqrt{([L]_t + [R]_0 + K_d)^2 - 4[R]_0[L]_t}}{2[R]_0}$$

where A – amplitude of the spectral change at [L] ligand concentration; A_{max} – amplitude of the spectral change at saturation concentration of the ligand; $[L]_t$ – concentration of the ligand at saturation; $[R]_0$ – total protein concentration.

2.4. Analysis of protein activity in reconstituted system.

Enzyme activity was reconstituted at 37°C in 25 mM HEPES buffer (pH 7.4), containing 10 mM $MgCl_2$, 0.1% CHAPS, 0.5 μM CYP17A1, 1 μM NADPH-cytochrome P450 reductase (CPR) as a redox partner, 50 μM substrate, 8 mM sodium isocitrate and 1 U/ml isocitrate dehydrogenase. Reaction was started by addition of NADPH (final concentration 0.25 mM). Concentration of P4 and inhibitors in competition test was 50 μM.

Aliquots were taken after 0, 2, 5, 10 and 30 min, and extracted by dichloromethane. Organic phase was evaporated and dissolved in MeOH for HPLC-MS analysis.

Reaction mixture was analyzed by HPLC-MS, using ZORBAX Extend-C18 (2.1 × 50 mm, 1.8 μm) column (Agilent, USA). Mobile phase A consisted of 0.2% formic acid solution in water, while mobile phase B consisted of 0.2% formic acid solution in acetonitrile. The volume of the sample applied to the column was 1.0 μL, and the shape of the elution gradient was the following: 5% phase B for 2 minutes, 5% to 95% phase B for 6 minutes and 95% for 7 minutes. The mobile phase flow rate was 250 μL/min, and column temperature was 40°C. The quadrupole time-of-flight mass analyzer Q-TOF 6550 (Agilent, USA), used for the detection of the products of enzymatic reaction, was equipped with an electrospray ionization source (APESI), with the following parameters: a carrier gas temperature of 400°C, carrier gas flow rate of 12 L/min, capillary voltage of 4 kV, and fragmentor voltage of 90 V. The mass analyzer was operated in range 200–1600 m/z.

2.5. *In silico* screening. Spatial structures of the modified steroids were built from their 2D structures using MolView service. Before docking structures were minimized in UCSF Chimera software (2000 steepest descent steps and 1000 conjugate gradient steps with step size 0.02 Å) [33]. An automated Python-based (v. 3.6) protocol was used for the *in silico* screening of CYP17A1 potential ligands. Docking and further analysis were based on known crystal structures of CYP17A1 with different substrates or inhibitors from Protein Data Bank. Docking was performed using AutoDock Vina software (v.1.1.2) [34] with next parameters: 20 diverse positions of the ligand in the active site of protein; exhaustiveness – 256; energy range – 4 kcal/mol. After docking all hits were ranked according to their root-mean-square deviation (RMSD) towards

similar known ligands from PDB and according to AutoDock Vina scoring function values.

3. Results and Discussion.

Purified human recombinant CYP17A1 possesses a typical P450 absorption spectrum with the heme iron being in a low-spin state, with a Soret region maximum at 418 nm (corresponding α, β-bands located at 567 and 535 nm, respectively) (Figure 2). Protein molecular mass corresponds to the expected value (Figure 2, insets A, B). CYP17A1 binds its substrate P4 and inhibitor econazole (Figure 2, inset C). Carbonyl complex of the protein has characteristic maximum in the absorption spectrum at 450 nm (Figure 2, inset D), indicating that the protein is stable and represents its active form.

Such well characterized human recombinant CYP17A1 was used for the *in vitro* screening of affinity of a series of modified bile acids and androstane derivatives for binding to the active site of this enzyme. Before screening an *in silico* analysis proving that these molecules are not pan-assay interference compounds (PAINS) [35] was provided using functionality of Python3 RDKit library (version 2019.03.3). According to our results, only compound L12 (Table 2) does not satisfy PAINS filter.

Among all compounds tested *in vitro* some synthetic steroids proved themselves as CYP17A1 active site ligands (Table 1), while other did not bind to the enzyme according to spectrophotometric titration results (Table 2).

Spectrophotometric titration experiments showed that human CYP17A1 binds steroids from both classes: bile acids derivatives with long side chain at position 17, as well as D-seco or D-homo androstane derivatives, with similar affinities in some cases. Spectral changes for all compounds correspond to Type I spectral response (‘substrate-like‘ molecules). According to the data presented previously by *Auchus et al.* [36], this enzyme has relatively small active site cavity, so it should be difficult to bind compounds with large differences in structure, in both dimension and substituents. However, bile acid derivatives, having A/B-ring *cis*-conformation, bind to the enzyme with high affinity. This is very important finding because steroidal ligands of CYP17A1, different from androstane-type compounds, has for the first time been indicated. Moreover, according to the literature, among known cytochromes P450 only CYP3A4 is able to bind bile acids in the active site [37]. In other cases bile acids act on the activity of CYP through regulation of the expression of corresponding genes via binding to nuclear receptors [38].

In silico screening of binding affinity of the modified steroids toward CYP17A1 showed that interaction energy for all tested compounds (which was calculated using AutoDock Vina scoring function) is comparable with consequent energy, calculated

for the 'essential' substrates of the enzyme (P4, P5, 17OH-P4, 17OH-P5). However, no correlation between binding energy and the affinity of the ligand was found.

It was found that novel detected ligands can interact with conserved amino acids A113, F114, N202, IE205, D298, G301, A302, T306, V366, A367, I371 and V483, forming active site surface. Most of them are taking part in stabilization of natural substrates (P4, P5, 17OH-P4, 17OH-P5) of CYP17A1. Especially N202 is important, for which it was found that formation of the hydrogen bond between the C_3 substituent of the steroidal substrate and the amino acid residue is crucial for the first step of activity of CYP17A1 – hydroxylation at C_{17} position [39]. Moreover, 'tightness' of this bond, depending on the stereochemistry of the steroid A/B-rings, increases the possibility of steroid hydroxylation at C_{16} position due to increased mobility of the substrate molecule, as it was shown for the progesterone and pregnenolone: keto group can only serve as hydrogen bond acceptor, but hydroxyl group can serve either hydrogen bond acceptor or hydrogen bond donor [39].

According to our data, localization of the ligands in CYP17A1 active site is quite similar to the position of the known substrates (RMSD is lower than 3 Å) except for compounds **9** and **11**, which have bulky substitution groups at C_{12} and C_6, respectively. In case of compound **11** steroidal plane is turned around the axis C_3-C_{16} so C_6-oximino group of the ligand contacts with the conserved L214 (Figure 3, B). Docking of compound **9** showed that A/B rings moiety of the steroidal core is located farther from the I-helix, in comparison with its location in complex with P5 or P4. This bile acid derivative forms bonds with A105, I205, V236, R239 (Figure 3, A). Relatively high K_d values for these compounds (**9** and **11**), confirm that such localization is not optimal for the ligands of CYP17A1.

Analysis of *in vitro* screening results for bile acids-derived compounds allowed us to determine some crucial structural elements for 'good ligands'.

Compounds with more hydrophobic side chain, including methyl- (compounds **1**, **5**, **6**, **7** and **9**) or butyl- (compound **3**, K_d=0.17±0.04 uM) ester moiety are binding with lower K_d values than those with hydrophilic carboxyl group (compounds **2**, **4**, **8**). According to *in silico* results, compounds with more hydrophobic side chain oriented in such a way that these parts of the molecules occupy hydrophobic pocket formed by V366, A367, I371 and V483 (Figure 3, C). This correlates with the binding mode of a well-known CYP17A1 inhibitor – galeterone (GAL; PDB ID: 3SWZ) – it is known that one of the key points of the binding of this compound is interaction of its benzimidazole moiety with these amino acids [40].

Obtained data also showed that bile acid derivatives with any group (oxo- or ester-derivatives) at C_{12} are binding with lower affinity (possess higher K_d values, as compound **9**), compared to the molecules without any substituent (compounds **7**, which is methyl-ester of lithocholic acid, and **6**), or do not bind at all (as cholic and

deoxycholic acid derivatives **L1**, **L6**, **L4** and **L8**). Analysis of docking results showed that C_{12}-group locates too close to conserved A302 and T306 from I-helix (Figure 3, D). This interaction could decrease stability of the complex 'CYP17A1-steroid molecule'.

Among bile acid-derived compounds with oxo or hydroxyl group at C_3, which bind with high affinity to human CYP17A1 active site heme iron (compounds **3**, **5** and **7**), any correlations between nature of substituent at this position and affinity of the tested molecules could not be found. Our data also showed that bile acid derivatives with acetyl ester or other substitution group at C_3 do not bind to human CYP17A1 (**L1**, **L4**, **L6** and **L9**). According to *in silico* results, it could be due to inappropriate interactions of bulky C_3-group of steroids with A/B *cis*-conformations with residues I198, Y201, L243 and F300, forming pocket over the top of α-helix I and along the underside of α-helices F and G (Figure 4, B). Another reason is failure to form hydrogen bond network with conserved N202 and R239, and other polar residues from α-helices F, G and I, which is necessary for ligand stabilization (Figure 4, A) [40].

Ligands with low K_d values both between bile acid derivatives with planar (Δ^4-steroids) and non-planar (A/B-ring *cis*-junction) steroidal core were found, but in case of A/B-ring *cis* molecules binding affinity was higher (compounds **1**, **3** and **7**). This could be because I-helix contains conserved G301 and A302, whose location permits ligands to adopt right conformation inside the active site (Figure 3, D). Interestingly, for abiraterone and GAL planar α-face of the molecule is one of the key features that make them effective inhibitors of CYP17A1 [40].

There were no ligands found for CYP17A1 by *in vitro* HTS among C-homo lactones derived from cholic acid (Table 2). It was also found that modified bile acids with any group at C_7 position, namely cholic acid derivatives, were not recognized as ligands for the enzyme (no spectral response was detected), except compound **8**, which is binding relatively weak. Nevertheless, in the latter case addition of hydroxyl group dramatically decreases affinity (K_d=108±6 μM for compound **8** comparing to K_d=26.0±0.7 μM for compound **2**). According to docking results this could be due to unfavorable interactions of α-oriented functional group in this position with conserved hydrophobic amino acids arising from I-helix (G301, A302).

In vitro analysis of androstane derivatives binding also allowed us to select compounds that interact with CYP17A1 active site. In general, such androstane-derived steroids were not so good ligands for the protein as bile acid derivatives: only two of the tested compounds (**12** and **13**, Table 1) bind with micromolar or submicromolar affinity. It was found that Δ^5-steroids with hydroxyl group at C_3 (compounds **10**, **12**, **13**) interact with the enzyme more tightly than other androstane derivatives. Addition of long 2-hydroxyethyl ether chain at C_3 position significantly decreases affinity of steroid (K_d =0.33±0.01 μM for compound **13**, towards K_d

=42.8±2.9 μM for compound **14**). According to docking results this could be due to unfavorable interaction of C_3-substituent group of compound **14** with surface of the cavity, formed by residues arising from α-helices F and G (Figure 3, B). Similar situation is for 3,6-dioxime **11**. Results also showed that 16,17-secosteroids (**12**, **13**) are more preferable for binding than D-homo lactones (**10**, **15**, Table 1).

In order to analyze possible products of the enzymatic reaction we performed reconstruction of the enzyme activity, offering modified steroids (Table 1) as substrates. According to the mass spectrometry analysis of the reaction mixture extracts, any hydroxylated derivatives of steroidal compounds were not detected in reconstituted system after 30 min of incubation. From the fact that some of the tested molecules (compounds **1** (K_d=0.72±0.09 μM), **3** (K_d=0.17±0.04 μM), **7** (K_d=0.05±0.03 μM) and **13** (K_d=0.33±0.01 μM)) possess unusually high affinity or moderately high affinity, compared to essential ligands of human CYP17A1 (P5 (K_d=0.86±0.12 μM)), P4 (K_d=1.92±0.09 μM), 17OH-P4 (K_d=2.32±0.35 μM) and 17OH-P5(K_d=0.63±0.05 μM) [10]), they could be considered as competitive inhibitors of the enzyme, that is very useful for the creation of novel drugs with high efficiency.

To check this hypothesis, we analyzed relative activity of CYP17A1 toward progesterone in the presence of these modified steroids. It was found that modified steroids are able to decrease rate of first phase catalytic reaction of CYP17A1, namely P4 hydroxylation (Figure 5). The most prominent compounds among them were compounds **1** and **6** (corresponding K_d values are 0.72±0.09 μM and 5.6±0.4 μM, respectively): they completely blocked hydroxylation of P4. In the latter case (compound **6**) affinity was lower for modified steroid than for progesterone. From the other hand, compounds with K_d values at submicromolar range (compounds **3, 5, 7** and **13**) demonstrated good inhibiting potential.

Given data shed light on a novel type of biological activity of modified steroids, some of which were recognized as perspective anticancer agents. Among all novel ligands of CYP17A1, D-secoandrostane compound **13** stood out in that, since good anti-proliferative activity of this compound against prostate cancer cells PC-3 line was stated previously (IC_{50} was 6.3μM) [21]. Tight binding to human CYP17A1, one of the key enzymes in biosynthesis of sex hormones and a key target in prostate cancer treatment, followed with high percentage of the enzyme inhibition, allowed us to present these novel ligands of the enzyme as lead compounds for the development of high-efficient therapeutics for the treatment of hormone-responsive diseases, particularly prostate cancer. This is especially emphasized for compounds expressing anti-proliferative effect for prostate cancer cells. From the other side, the results obtained can diminish tested compounds, which can interact with other targets than CYP17A1, from further usage in therapy, or redirect design of novel drugs, as well as their possible pharmacological usage.

Author statement

The authors are very grateful for reviewing our manuscript (SBMB-D-20-00042). We corrected the manuscript according to the comments and suggestions. We send the revised manuscript with corrections visible in 'track changes', while answers are provided for each comment. We hope that corrected manuscript will suitable for publication in Journal of Steroid Biochemistry and Molecular Biology.

Author Contributions.

Conceptualization and design: Y.D. and S. J-Š.; Synthesis and characterization of modified steroids: Lj.G. and M.S.; Protein expression and purification: T.S.; *In vitro* experiments and analysis of data: Y.D., M.Sh. and S. J-Š.; *In silico* experiments and analysis of data: Y.D. and M.Sh.; HPLC analysis of CYP17A1 activity and interpretation of the results: A.Y.; Drafting and revising the article: Y.D., S. J-Š., A.Y. and S.U.

Conflicts of Interest.

No potential conflicts of interest.

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Figure 1. Steroidogenic reaction pathways catalyzed by human CYP17A1.

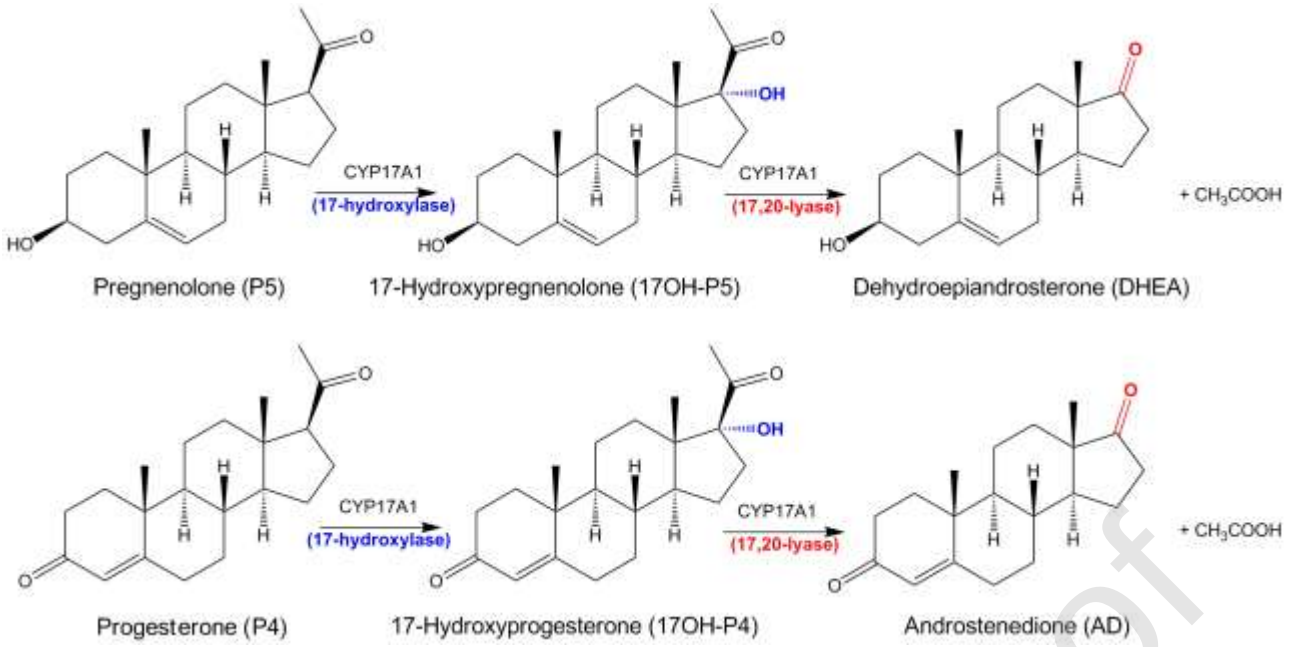


Figure 2. Absorbance spectrum of human recombinant CYP17A1 in ligand-free form. Insets: MALDI-TOF mass-spectrum (A), SDS-PAGE (14% gel, Coomassie Blue G-250 staining) of purified CYP17A1 (ST – protein ladder (Page Ruler^{TM} Plus Prestained Protein Ladder, 10 to 250 kDa (Thermo Fisher Scientific)), 1 – purified protein) (B), difference spectra of CYP17A1 in complex with P4 (substrate – dotted line) and econazole (inhibitor – solid line) (C) and carbonyl complex spectrum (D)

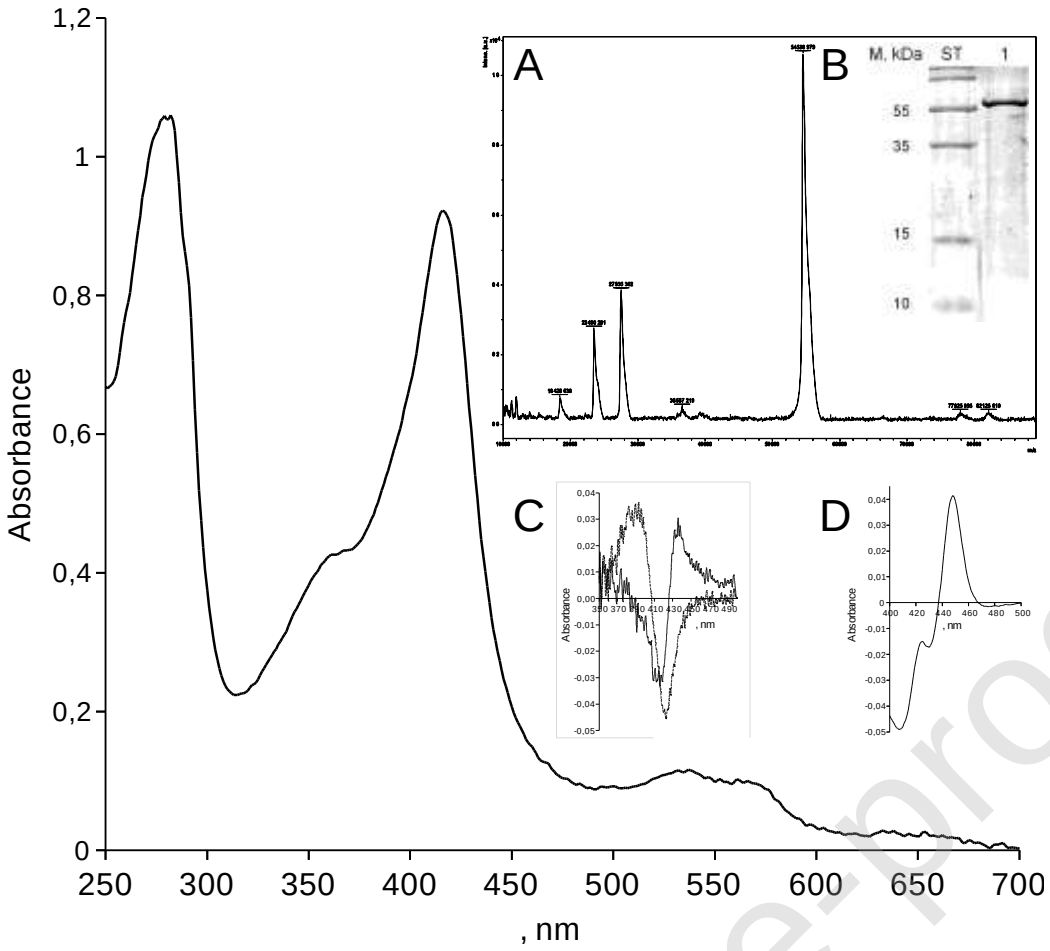


Figure 3. Fragment of human CYP17A1 active site in complex with compound **9** and P5 (A), compound **11** and P5 (B), compound **3** and GAL (C) and compound **1** (D). In the stick and sphere representations, non-carbon atoms are indicated in blue (N) and red (O), Fe atom is indicated as orange sphere. Molecules are colored by dark grey (haem), green (compounds **1**, 3, **9** and **11**), and gold (P5 and GAL). Surface of G301, A302, E305 and T306 is colored by grey.

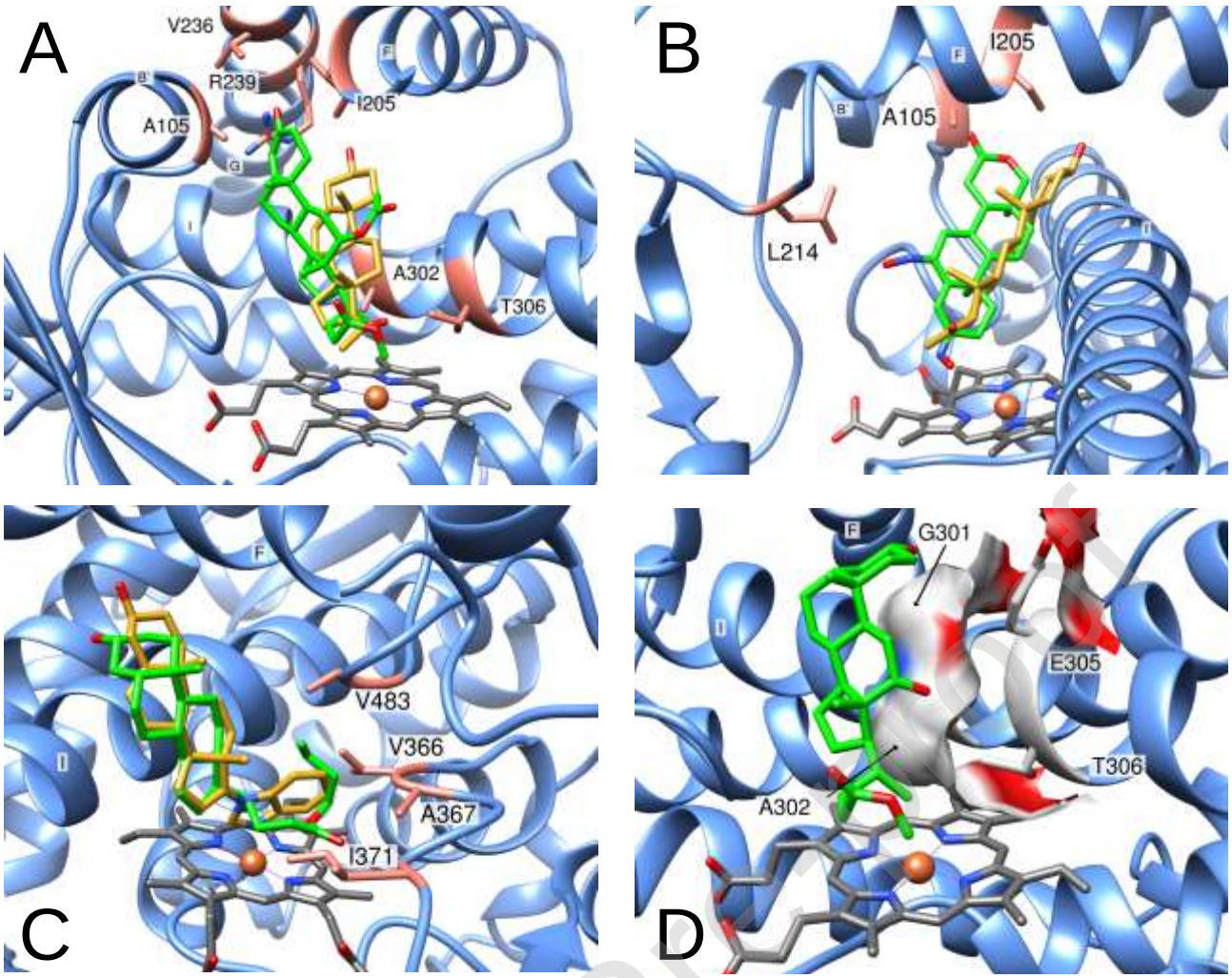


Figure 4. CYP17A1 structure, representing amino acid residues forming active site pocket, in complex with GAL (PDB ID: 3SWZ) (A), compounds **L4** (Table 2) and **14** (B). In the stick and sphere representations, non-carbon atoms are indicated in blue (N) and red (O), Fe atom is indicated as orange sphere. Molecules are colored by dark grey (heme), green (compound **L4**), salmon (compound **14**) and gold (GAL). Hydrogen bonds are indicated as green lines. Surface is colored by cornflower blue.

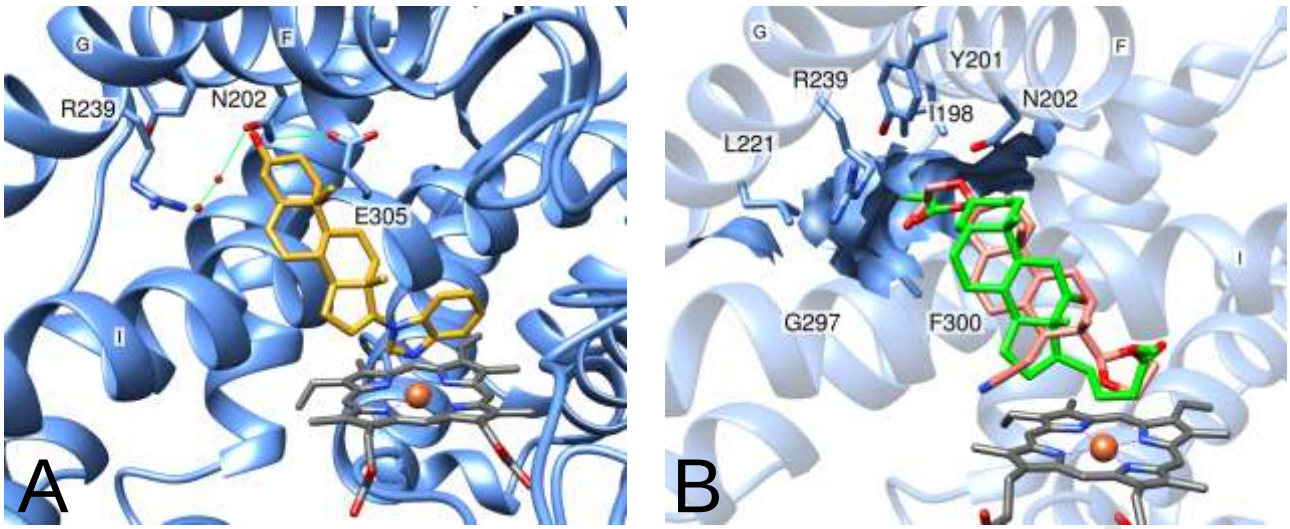
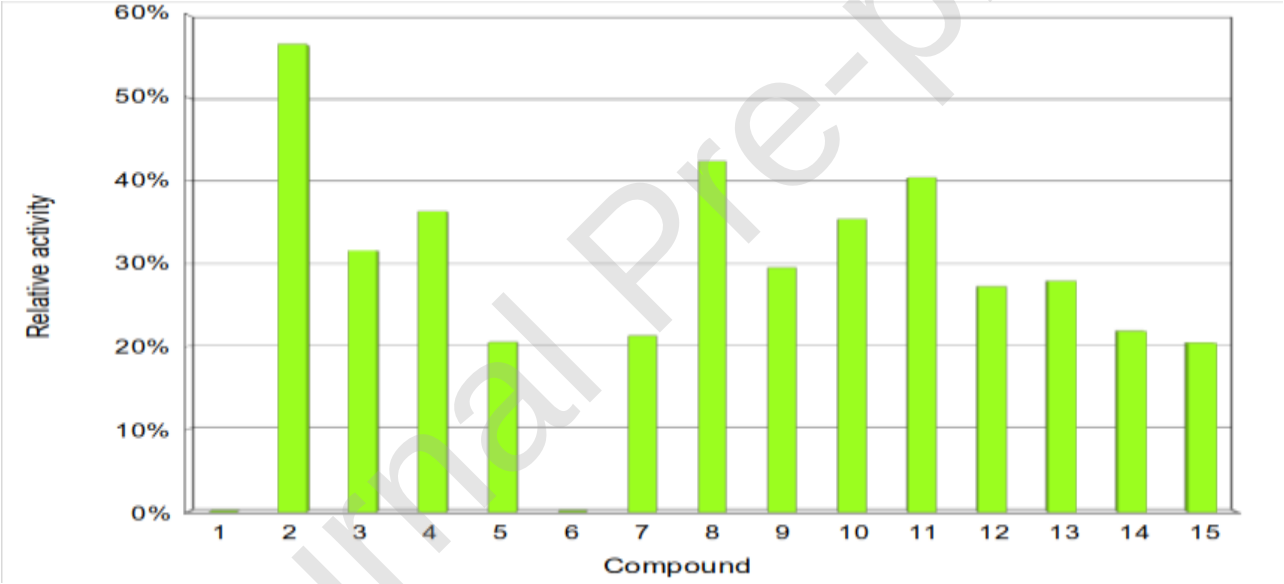
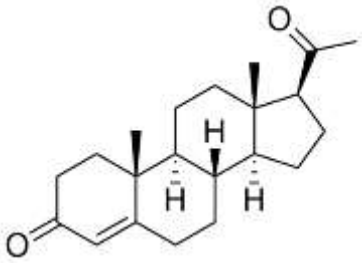
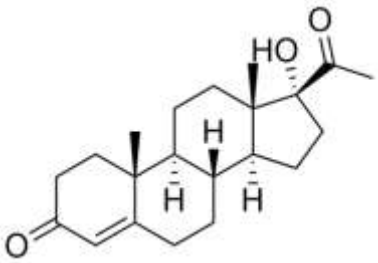
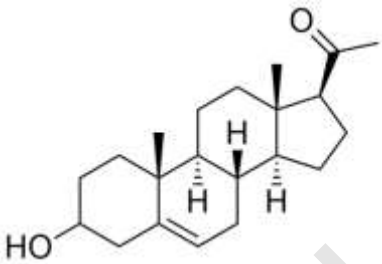
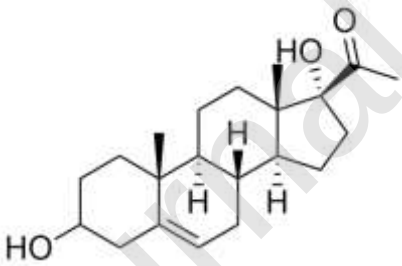
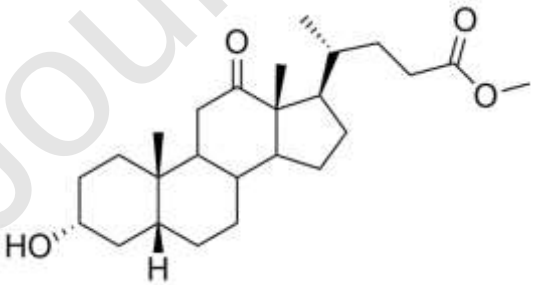


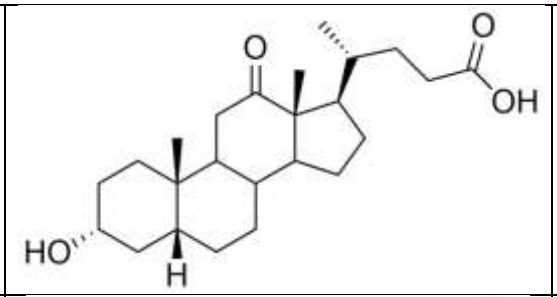
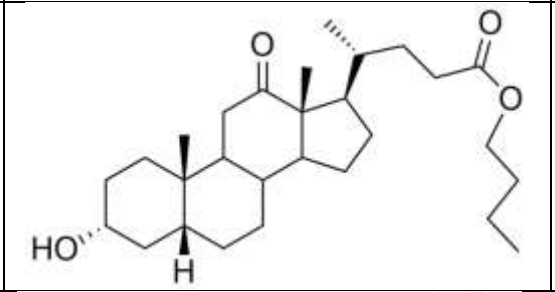
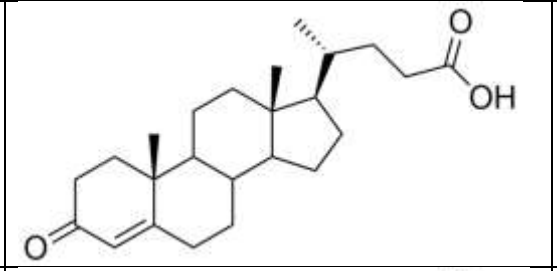
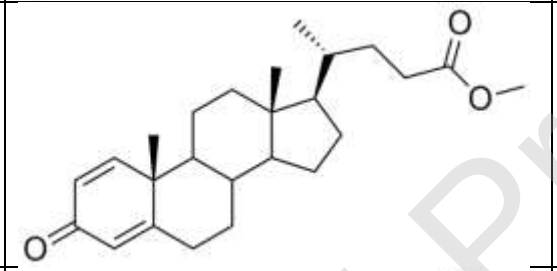
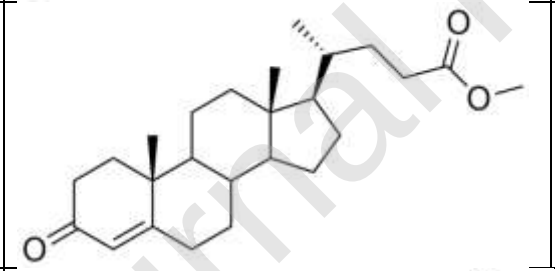
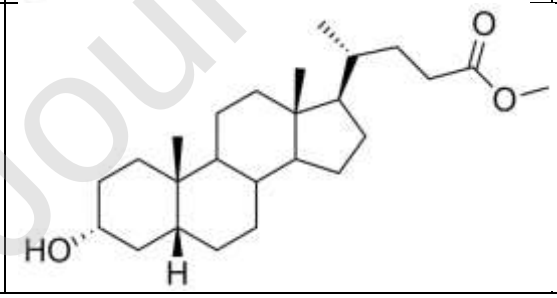
Figure 5. Relative conversion rate of P4 in the presence of novel human CYP17A1 inhibitors. Conversion rate of P4 in the absence of modified steroids is taken as 100%. Tick labels of X axis correspond the labels of compounds in Table 1.

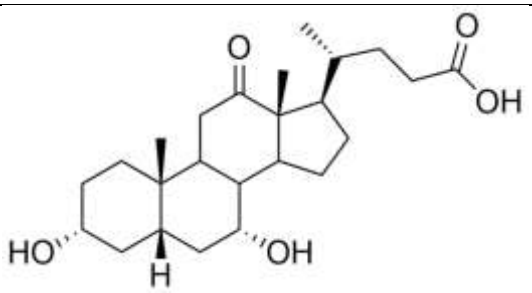
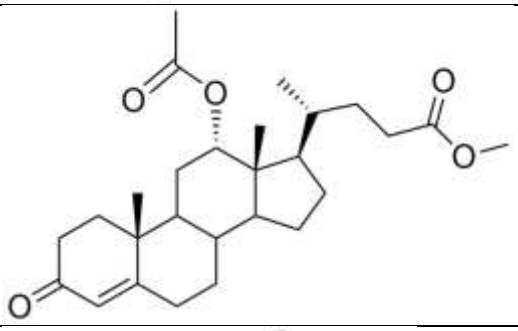
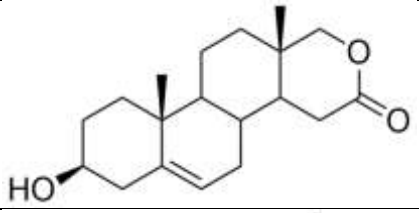
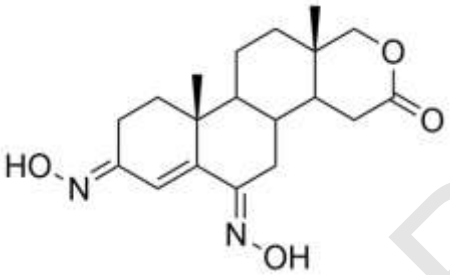
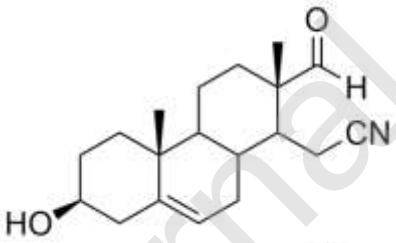
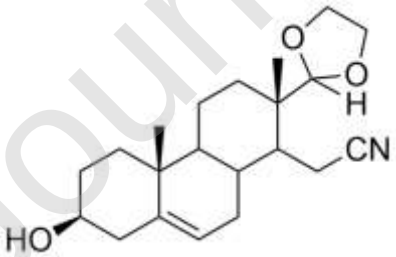
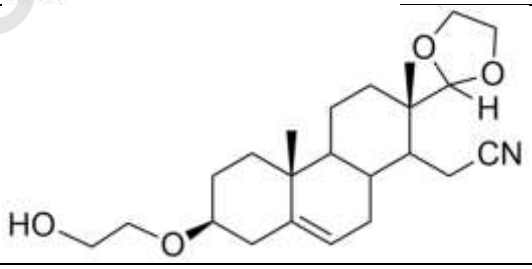


Tables

Table 1. Novel steroidal ligands of human recombinant CYP17A1, found during *in vitro* screening, and their binding parameters.

Compound	Structure	Titration limits, μM	Spectral parameters	K_d, μM
P4 [10]		0.1-50	Type I (max 390 nm, min 420 nm) ΔA = 0.050	1.92±0.09
17OH-P4 [10]		0.1-50	Type I (max 390 nm, min 420 nm) ΔA = 0.020	2.32±0.35
P5 [10]		0.1-50	Type I (max 390 nm, min 420 nm) ΔA = 0.070	0.86±0.12
17OH-P5 [10]		0.1-50	Type I (max 390 nm, min 420 nm) ΔA = 0.050	0.63±0.05
1[11]		0.1-49	Type I (max 387 nm, min 423 nm) ΔA = 0.046	0.72±0.09

2 [12]		0.1-97	Type I (max 387 nm, min 424 nm) ΔA = 0.034	26.0±0.7
3 [13]		0.1-10	Type I (max 387 nm, min 421 nm) ΔA = 0.053	0.17±0.04
4 [14]		0.1-59	Type I (max 388 nm, min 418 nm) ΔA = 0.017	13.2±0.4
5 [15]		0.1-20	Type I (max 388 nm, min 424 nm) ΔA = 0.044	0.46±0.04
6 [14]		0.1-10	Type I (max 386 nm, min 419 nm) ΔA = 0.038	5.6±0.4
7 [16]		0.1-20	Type I (max 388 nm, min 425 nm) ΔA = 0.053	0.05±0.03

8 [12]		0.1-97	Type I (max 385 nm, min 419 nm) ΔA = 0.022	108±6
9 [17]		0.1-89	Type I (max 389 nm, min 417 nm) ΔA = 0.010	9.59±1.01
10 [18]		0.1-97	Type I (max 388 nm, min 424 nm) ΔA = 0.030	18.5±0.4
11 [19]		0.1-108	Type I (max 389 nm, min 423 nm) ΔA = 0.010	97.3±25.1
12 [20]		0.1-97	Type I (max 383 nm, min 419 nm) ΔA = 0.040	6.4±0.5
13 [21]		0.1-20	Type I (max 387 nm, min 419 nm) ΔA = 0.075	0.33±0.01
14 [21]		0.1-59	Type I (max 389 nm, min 427 nm) ΔA = 0.019	42.8±2.9

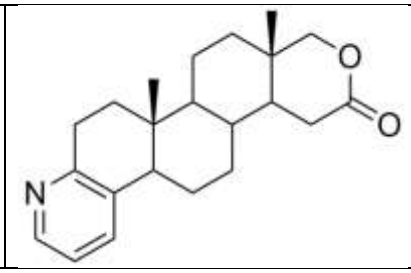
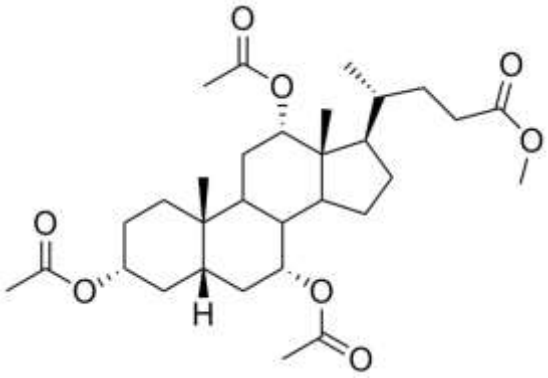
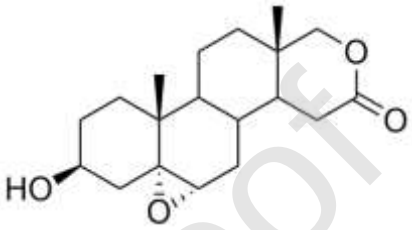
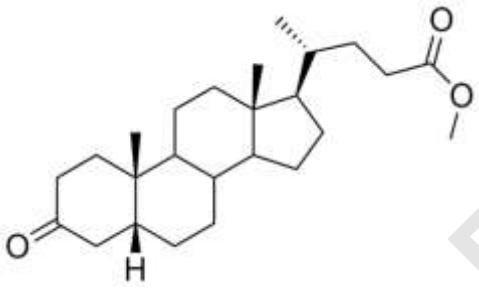
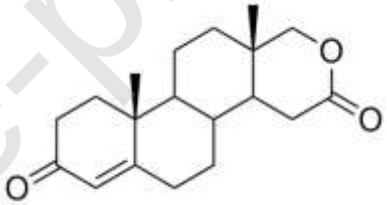
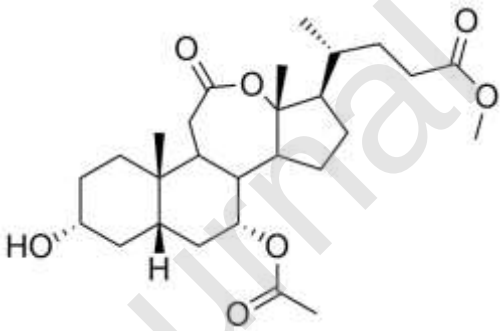
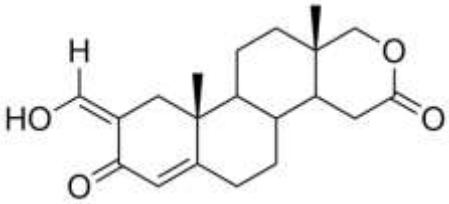
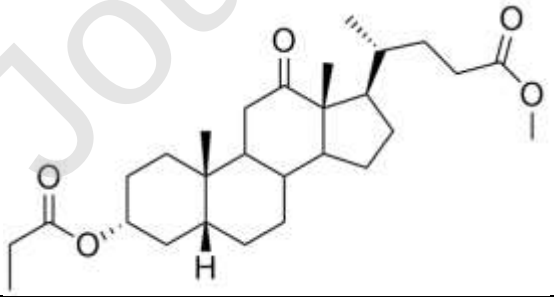
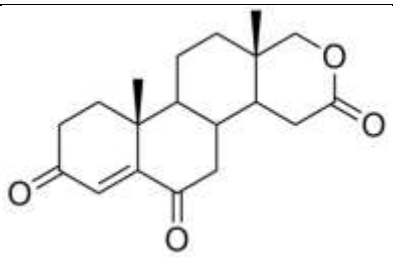
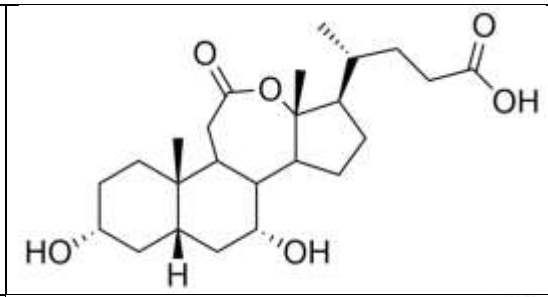
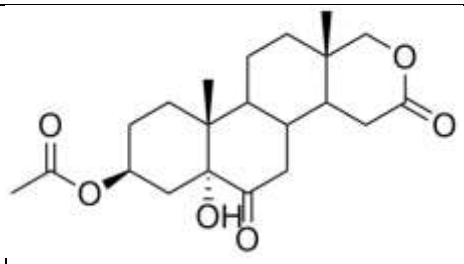
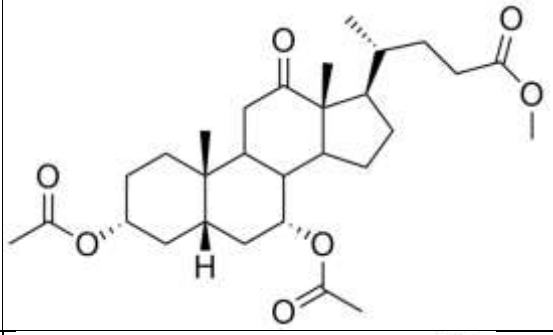
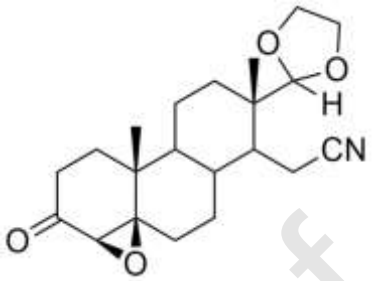
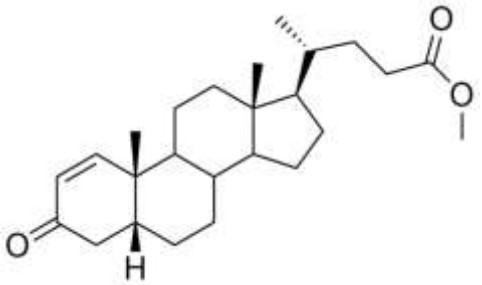
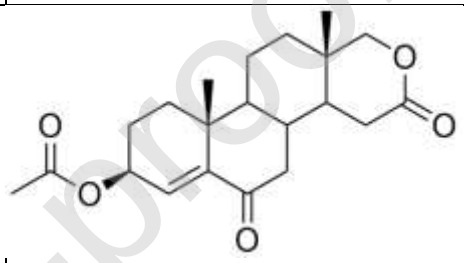
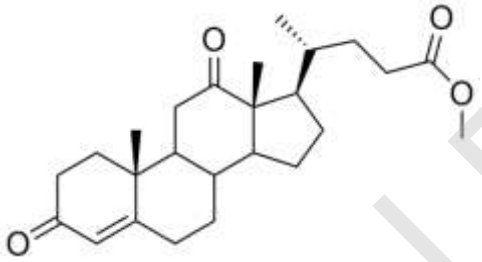
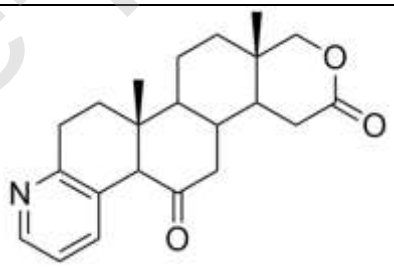
15 [22]		0.1-97	Type I (max 389 nm, min 423 nm) ΔA = 0.044	11.2±0.6

Table 2. Steroids that showed weak (low) or no binding to human recombinant CYP17A1.

Comp.	Structure	Comp.	Structure
L1 [23]		**L10** [29]	
L2 [24]		**L11** [18]	
L3 [13]		**L12** [30]	
L4 [13]		**L13** [31]	

L5 [25]		**L14** [30]	
L6 [26]		**L15** [21]	
L7 [17]		**L16** [32]	
L8 [27]		**L17** [22]	
L9 [28]