Supporting Information
Identification of a novel cytochrome P450 17A1 enzyme and its molecular engineering

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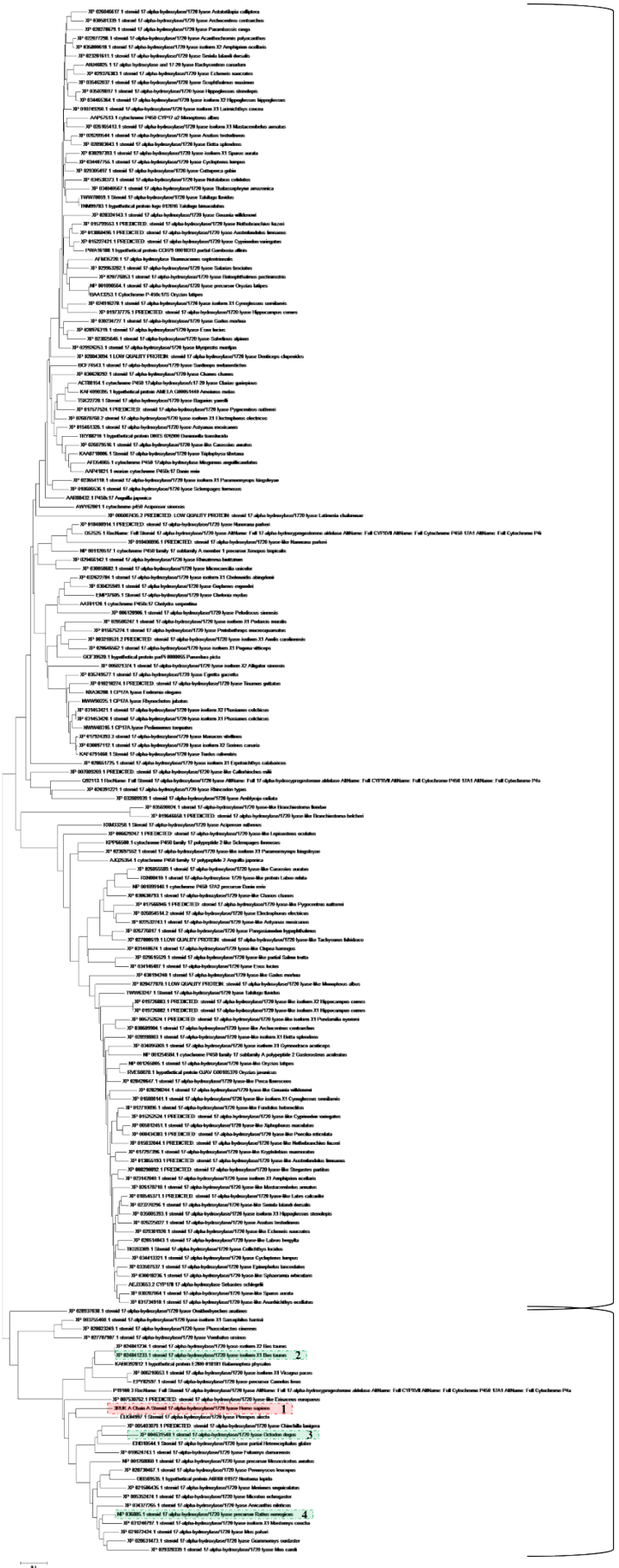
#Kexin Chen and Chao Liu contributed equally to this work



PART B

PART A

Figure S1 Phylogenetic tree of the retrieved sequences of CYP17A1 proteins together with the probe 6CIZ. The tree was constructed with the neighbor-joining method by using the MEGA 6 program. The probe sequence (6CIZ) was boxed in red (No. 1); three selected sequences were boxed in green (No. 2,3,4 in turn).

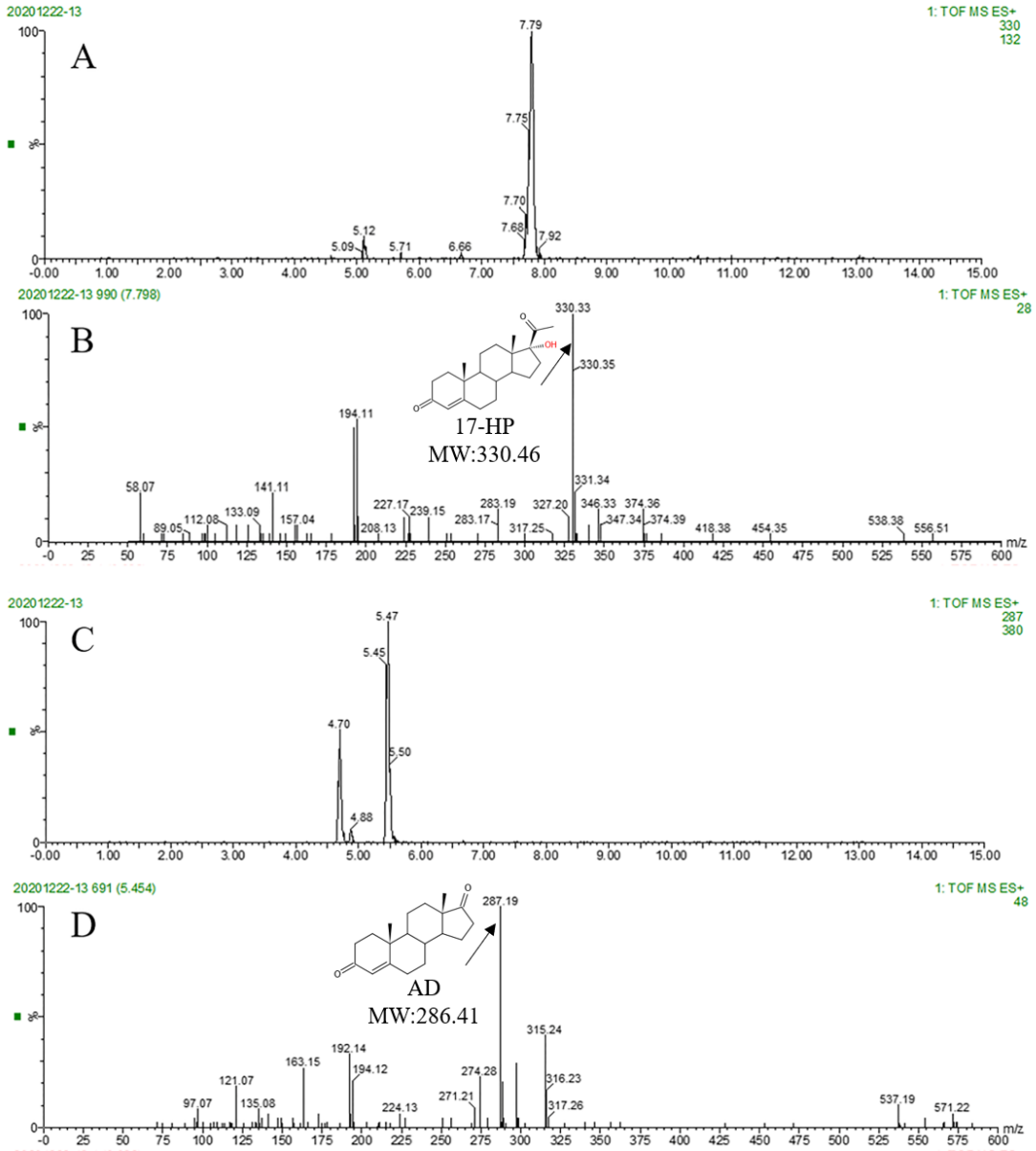


Figure S2 LC-MS analysis of the whole-cell catalytic solution for recombinant *P. pastoris* contained BT_CYP17A1. A, the HPLC profiles of solution for 17-HP detection; B, MS spectrum of solution for17-HP detection. C, the HPLC profiles of solution for AD detection; D, MS spectrum of solution for AD detection.

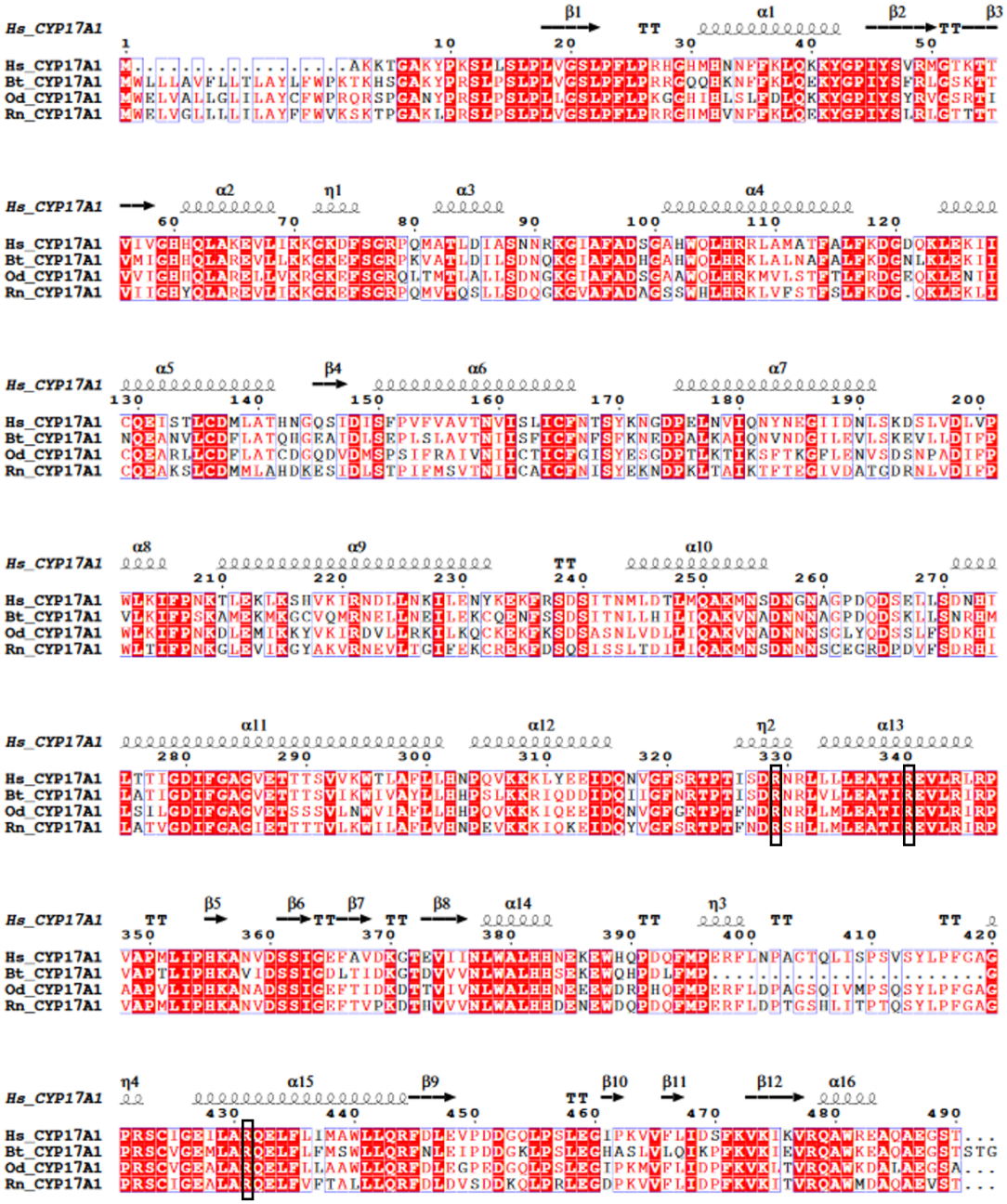


Figure S3 Results of multiple sequence alignment for CYP17A1s, the key amino acid residues were marked in black.

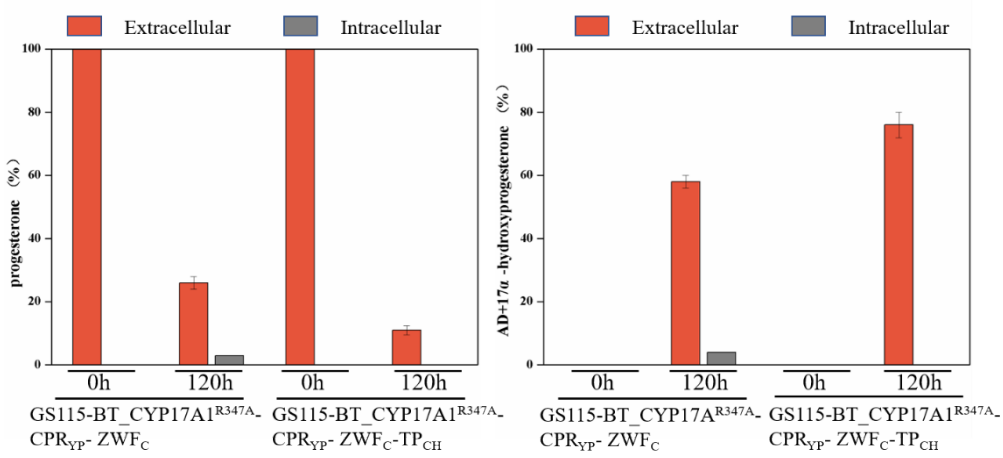


Figure S4 The exogenous TP_{CH} transporter facilitated acetylated cortexolone substrate transportation in *Pichia pastoris*. The extracellular and intracellular progesterone or 17α-hydroxyprogesterone concentrations in strain GS115-BT_CYP17A1^{R347A}-CPR_{YP}-ZWF_C and GS115-BT_CYP17A1^{R347A}-CPR_{YP}-ZWF_C-TP_{CH} were measured after the biotransformation. The description of each strain was listed in Table S2. Cell mass was determined by 30 measuring the optical density at 600 nm (OD600), OD_{600} 1=0.35±0.01 g_{CDW}/L, and the cytoplasmic volume was assumed to be 1.6 μL/mg dry cell weight[1]

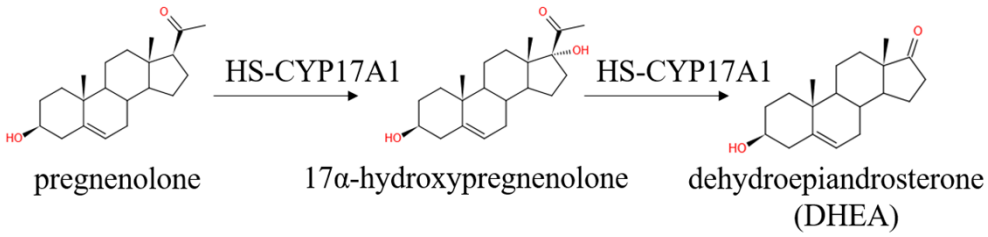


Figure S5 Reaction catalyzed by HS_CYP17A1 enzyme.

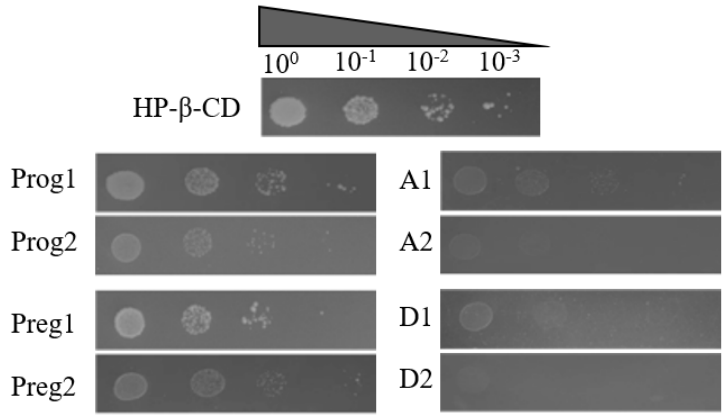


Figure S6 Inhibition of growth of *Pichia pastoris* by steroid. Serial 10-fold dilutions of OD_{600} 1 cell suspensions were spotted on agar
slants containing 0.1mM (1) and 1mM (2) concentrations of steroids. After two days growth was compared to that of control containing only HP-β-CD
Prog: progesterone, Preg: pregnenolone, A: androstenedione, DHEA: dehydroepiandrosterone

Table S1 Strains and plasmids used in this study

Strains and plasmids	Description	Source
Strains		
E. coli JM109	endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F‘ traD36 proAB+ lacIq lacZΔM15]hsdR17(rK-mK+)	Beijing ComWin Biotech Co., Ltd.
P. pastoris GS115	Wild type	Invitrogen
GS115-CK1	*P. pastoris* GS115 electro-transformed with pPIC3.5K empty vector as control	[2]
GS115-CK2	*P. pastoris* GS115 electro-transformed with pPICZB empty vector as control	[2]
GS115-CK3	*P. pastoris* GS115 electro-transformed with pPIC3.5K and pPICZB empty vector as control	[2]
GS115-MA_CYP17A2	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*ma_cyp17a2*, expressing the *ma_cyp17a2* gene alone	[3]
GS115-MA_CYP17A2-CPR_{RAT}-ZWF_K	*P. pastoris* GS115-MA_CYP17A2 electro-transformed with binary-pPICZB-cpr_{rat}-zwf_k , co-expressing the *ma_cyp17a2*, cpr_{rat} and zwf_k genes	[3]
GS115-HS_CYP17A1	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*hs_cyp17a1*, expressing the *hs_cyp17a2* gene alone	[2]
GS115-HS_CYP17A1-CPR_{YP}-ZWF_C	*P. pastoris* GS115-HS_CYP17A1 electro-transformed with binary-pPICZB- cpr_{yp}-zwf_c, co-expressing the *hs_cyp17a1*, cpr_{yp} and zwf_c genes	[2]
GS115-BT_CYP17A1	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*, expressing the *bt_cyp17a2* gene alone	
GS115-OD_CYP17A1	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*od_cyp17a1*, expressing the *od_cyp17a2* gene alone	
GS115-RT_CYP17A1	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*rt_cyp17a1*, expressing the *rt_cyp17a2* gene alone	
GS115-BT_CYP17A1^{R347A}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1* ^{R347A}, expressing the *hs_cyp17a2* ^{R347A} gene alone	
GS115-BT_CYP17A1^{R347A}-TP_{AC}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ac}, expressing the *bt_cyp17a1*^{R347A} and tp_{ac} genes	This study
GS115-BT_CYP17A1^{R347A}-TP_{CH}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ch}, expressing the *bt_cyp17a1*^{R347A} and tp_{ch} genes	This study
GS115-BT_CYP17A1^{R347A}-TP_{CL}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{cl}, expressing the *bt_cyp17a1*^{R347A} and tp_{cl} genes	This study
GS115-BT_CYP17A1^{R347A}-TP_{EV}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ev}, expressing the *bt_cyp17a1*^{R347A} and tp_{ev} genes	This study
GS115-BT_CYP17A1^{R347A}-TP_{NF}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{nf}, expressing the *bt_cyp17a1*^{R347A} and tp_{nf} genes	This study
GS115-BT_CYP17A1^{R347A}-TP_{SC}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{sc}, expressing the *bt_cyp17a1*^{R347A} and tp_{sc} genes	This study
GS115-BT_CYP17A1^{R347A}-CPR_{YP}-ZWF_C-TP_{CH}	GS115-BT_CYP17A1^{R347A}-TP_{CH} electro-transformed with binary-pPICZB- cpr_{yp}-zwf_c, co-expressing the *bt_cyp17a1*^{R347A}, tp_{ch}, cpr_{yp} and zwf_c genes	This study
GS115-HS_CYP17A1-TP_{CH}	*P. pastoris* GS115 electro-transformed with pPIC3.5K-	This study

	hs_cyp17a1^{R347A}-tp_{ch}, expressing the *hs_cyp17a1*^{R347A} and tp_{ch} genes	
GS115-HS_CYP17A1-CPR_{YP}-ZWF_C -TP_{CH}	*P. pastoris* GS115-HS_CYP17A1-TP_{CH} electro-transformed with binary-pPICZB- cpr_{yp}-zwf_c, co-expressing the *hs_cyp17a1*, cpr_{yp}, zwf_c and tp_{ch} genes	This study
Plasmid		
pPIC3.5K	9.0 kb, Amp^R	Invitrogen
pPICZB	3.3 kb, Zeo^R	Invitrogen
pPIC3.5K-*ma_cyp17a2*	pPIC3.5K carrying a CYP17A2 gene from *Mastacembelus armatus*	[3]
pPIC3.5K-*hscyp17a1*	pPIC3.5K carrying a CYP17A1 gene from *Homo sapiens*	[2]
pPICZB-cpr_{rat}-zwf_k	pPICZB carrying a CPR gene from *Rattus norvegicus* and a *zwf* gene from *Kluyveromyces lactis*	[3]
pPICZB-cpr_{yp}-zwf_c	pPICZB carrying a CPR gene from *Yorkshire pig* and a *zwf* gene from *Candida tropicalis*	[2]
pPIC3.5K-tp_{ac}	pPIC3.5K carrying a transporter gene from *Aspergillus clavatus*	This study
pPIC3.5K-tp_{ch}	pPIC3.5K carrying a transporter gene from *Cochliobolus heterostrophus*	This study
pPIC3.5K-tp_{cl}	pPIC3.5K carrying a transporter gene from *Cochliobolus lunatus*	This study
pPIC3.5K-tp_{ev}	pPIC3.5K carrying a transporter gene from *Emericella variicolor*	This study
pPIC3.5K-tp_{nf}	pPIC3.5K carrying a transporter gene from *Neosartorya fumigate*	This study
pPIC3.5K- tp_{sc}	pPIC3.5K carrying a transporter gene from *Saccharomyces cerevisiae*	This study
pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ac}	pPIC3.5K carrying a CYP17A1 gene from *Bovine taurus* and a transporter gene from *Aspergillus clavatus*	This study
pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ch}	pPIC3.5K carrying a CYP17A1 gene from *Bovine taurus* and a transporter gene from *Cochliobolus heterostrophus*	This study
pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{cl}	pPIC3.5K carrying a CYP17A2 gene from *Bovine taurus* and a transporter gene from *Cochliobolus lunatus*	This study
pPIC3.5K-*bt_cyp17a1*^{R347A}-tp_{ev}	pPIC3.5K carrying a CYP17A2 gene from *Bovine taurus* and a transporter gene from *Emericella variicolor*	This study
pPIC3.5K-*bt_cyp17a1*^{R347}-tp_{nf}	pPIC3.5K carrying a CYP17A2 gene from *Bovine taurus* and a transporter gene from *Neosartorya fumigate*	This study
pPIC3.5K-*bt_cyp17a1*^{R347}-tp_{sc}	pPIC3.5K carrying a CYP17A2 gene from *Bovine taurus* and a transporter gene from *Saccharomyces cerevisiae*	This study
pPIC3.5K-*hscyp17a1*-tp_{ch}	pPIC3.5K carrying a CYP17A1 gene from *Homo sapiens* and a transporter gene from *Cochliobolus*	This study

Table S2 Primers used in this study

Primer	Sequence (5`-3`)
The following primers were used for amplification and constructing the plasmid of pPIC3.5K-tp_{ch}	
TP_{CH}-F	CATCATCACCATCACCATATGGGATTAACTTTATTAC
TP_{CH} -R	TTAGAATCTAGCAAGACCTTACGTCCTGGAACGTATAGT
3.5K- TP_{CH}-F	ACTATACGTTCCAGGACGTAAGGTCTTGCTAGATTCTAA
3.5K- TP_{CH}-R	GTAATAAAGTTAATCCCATATGGTGATGGTGATGATG
The following primers were used for elimination of the *Pme* I restriction site	
d*Pme* I-pPIC3.5K-F	AAACTGACAGTTGAAACGCTGTCTTGGAACCT
d*Pme* I-Ppic3.5K-R	CCAAGACAGCGTTTCAACTGTCAGTTTTGGG
The following primers were used for mutation	
R347A-F	CAACCATCTCAGACGCAAACCGTCTAGTACTGC
R347A-R	GCAGTACTAGACGGTTTGCGTCTGAGATGGTTG
Primers were used for amplification and constructing the plasmid of pPIC3.5K-*bt_cyp17a1-tp* and pPIC3.5K- *hcyp17a1-tp*	
P-TP-T-F	CTATATGCGTTGATGCAATTTCTATGCGATCTAACATCCAAAGAC GAAAGG
P-TP-T-R	CAGTGCTCCGAGAACGGGTGCTCTCACTTAATCTTCTGTACTCTGA AG
3.5K-CYP F	CCTTTCGTCTTTGGATGTTAGATCGCATAGAAATTGCATCAACGCA TATAG
3.5K-CYP R	CTTCAGAGTACAGAAGATTAAGTGAGAGCACCCGTTCTCGGAGCA CTG

Table S3 Sequence identity percentages of four selected sequences of CYP17s. The identity percentages between two sequences were calculated using the on-line software SIAS (Sequence Identity and Similarity, http://imed.med.ucm.es/Tools/sias.html)

HS_CYP17A1	100%			
BT_CYP17A1	66.38%	100%		
OD_CYP17A1	66.59%	63.37%	100%	
RT_CYP17A1	67.64%	63.37%	67.06%	100%
	HS_CYP17A1	BT_CYP17A1	OD_CYP17A1	RT_CYP17A1

Table S4 Sequence identity percentages of six selected sequences of transporters. The identity percentages between two sequences were calculated using the on-line software SIAS (Sequence Identity and Similarity, http://imed.med.ucm.es/Tools/sias.html)

TP_{AC}	100%					
TP_{CH}	82.15%	100%				
TP_{CL}	78.83%	75.06%	100%			
TP_{EV}	84.85%	80.58%	76.64%	100%		
TP_{MF}	70.95%	68.33%	71.81%	68.56%	100%	
TP_{SC}	57.53%	55.70%	56.38%	57.18%	57.18%	100%
	TP_{AC}	TP_{CH}	TP_{CL}	TP_{EV}	TP_{MF}	TP_{SC}

>TP_{AC} from *Aspergillus clavatus* (NCBI Reference Sequence:XP_001276075.1,4341bp)

CACGACACATTCCTGGGGGGGTGAATGCGACCCAGTATGCCAGCCACATGCGAGACGTGA
TCATGGCCATGTTTGGTATTAGCCATACGAAGAACACGATTGTAGGAAACGACTTCATTCG
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CTGGCGACACCAGGTCAGCTCCCAGGGTTCTGGATCTTCATGTATCGCGTTTCGCCGTTTA
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GGAATTTTGGGCTCATGTGGGTATTTATTGTTTTTAATATCTTTGCAGCTTGTTTGTTGTAC
TGGTGGGTCCGTGTCCCAAGAGTCAAGAAGCCAGTCGCTAAGACCGAGTGA

>TP_{CH} from *Cochliobolus heterostrophus* (NCBI Reference Sequence: XM_014229532,4482bp)

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ACCCATACGGACGACAATCAAGCACATCCTCTTAGGACACTGGGCGTCTCCTTTTCTAACC
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>TP_{CL} from *Cochliobolus lunatus* (NCBI Reference Sequence:, bp)

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>*TPEV from Emericella variicolor (*NCBI Reference Sequence: A0A1V1GB10.1,4465bp)

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>TP_{MF} from *Neosartorya fumigate* (NCBI Reference Sequence: XM_747710,4494bp)

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>TP_{SC} from *Saccharomyces cerevisiae* (NCBI Reference Sequence: DQ332357.1,4536bp)

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