Pharmaceutical and Medicinal Chemistry[1], Saarland University, Saarbrücken; Institut für Pharmazeutische und Medizinische Chemie[2], Westfälische Wilhelms-Universität, Münster, Germany; Faculty of Pharmacy[3], Damascus University, Damascus, Syria; Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS)[4], Saarbrücken, Germany

Computational prediction of new CYP17 inhibitors based on pharmacophore modeling, virtual screening and docking approach

S. Haidar [1, 2, 3, *], R. W. Hartmann [1, 4]

Received March 20, 2107, accepted May 26, 2017

** Corresponding author: Dr. Samer Haidar, Institut für Pharmazeutische und Medizinische Chemie, Westfälische Wilhelms-Universität Münster, Pharmacampus, Corrensstr. 48, 48149 Münster, Germany*
shaid_01@uni-muenster.de

Pharmazie 72: 529–536 (2017) *doi: 10.1691/ph.2017.7516*

17α-Hydroxylase/C17-20-lyase (P450 17, CYP 17) is an important enzyme in the androgen biosynthesis and inhibitors of this enzyme can be used for the treatment of prostate cancer. With the aim of developing new inhibitors for the target enzyme, we generated a structure-based pharmacophore model to further explain the binding requirements for human CYP17 inhibitors. Seven common features of steroidal CYP17 inhibitors were determined using MOE software. This pharmacophore model was then used to search the Cambridge Structural Database (CSD) with the aim of developing more potent and selective CYP17 inhibitors by identifying new hits. We were able to identify 36 structures as possible active CYP17 inhibitors. Docking studies for the selected compounds from the database were also performed and the best three compounds were chosen as possible hits.

1. Introduction

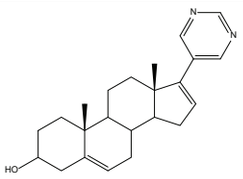
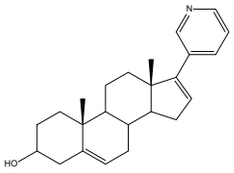
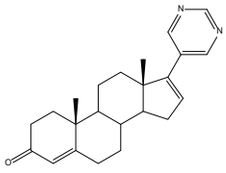
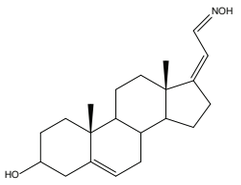
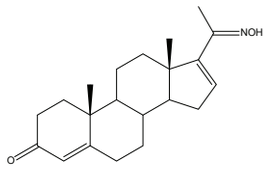
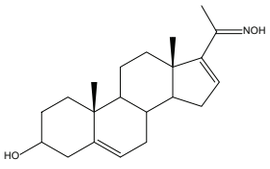
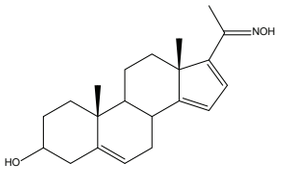
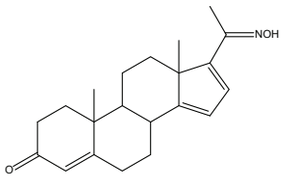
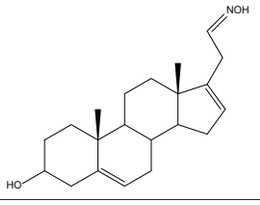
Prostate cancer (PCa) is the most common cause of cancer death in men in Western countries. Statistics indicate that around 80% of PCa are androgen dependent (Jarman et al. 1998), so blocking of androgen production will prevent cancer cells from proliferation (Geller 1993). Castration resistant prostate cancer (CRPC) occurs after the traditional treatment methods, which include surgical or medical castration with gonadotropin-releasing hormone (GnRH) analogs often in combination with antiandrogens. An alternative method of treatment will be total ablation of androgen biosynthesis in the testes as well as in the adrenals. CYP17 (P450-17) is an enzyme consisting of a heme and an apoprotein moiety, and catalyzes two key reactions in steroid hormone biosynthesis in gonadal and adrenal glands. First the substrates progesterone and pregnenolone are hydroxylated in 17α position of the steroid to yield 17α-hydroxyprogesterone and 17α-hydroxypregnenolone, respectively. In the second step, dehydroepiandrosterone (DHEA) and androstenedione are produced, respectively. Inhibiting this enzyme will totally block the androgen biosynthesis from adrenal and testes, since this enzyme is involved in androgen biosyntheses regardless of production location (Hartmann et al. 2002). The antimycotic ketoconazole was the first compound discovered few decades ago as CYP17 inhibitor. Ketoconazole was withdrawn clinically as PCa treatment due to many side effects related to its non-selectivity (Eklund et al. 2006). A large number of steroidal and nonsteroidal inhibitors (Hartmann et al. 1996; Njar et al. 1996; Njar and Brodie 1999; Hartmann et al. 2002; Leroux et al. 2003; Leroux 2005; Baston and Leroux 2007; Moreira et al. 2008; Hille et al. 2009; Owen 2009; Hu et al. 2010; Vasaitis et al. 2011; Salvador et al. 2013) were developed and tested as CYP17 inhibitors, among them the recently approved drug abiraterone acetate (Zytiga®) (de Bono et al. 2011) which is used in the treatment of PCa. Other inhibitors are currently in clinical studies (Hu and Hartmann 2014). Most of the steroidal inhibitors bear functional groups with heteroatoms (N, S, and O) at C16 or C17 of the steroidal backbone. Those functional groups can serve as the sixth ligand with the heme iron of the enzyme.

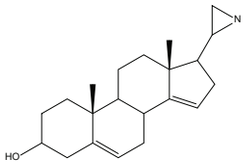
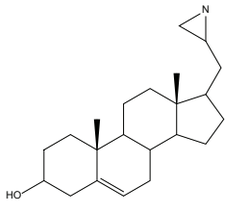
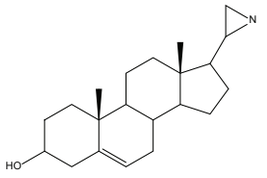
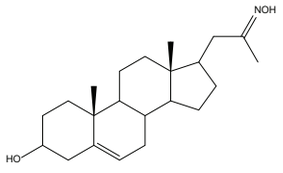
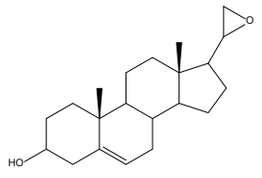
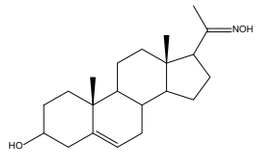
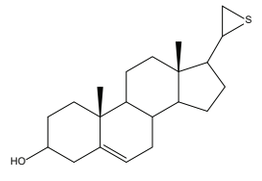
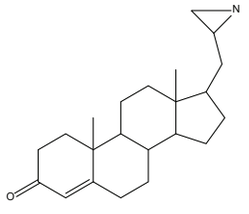
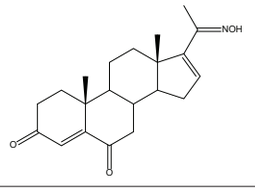
In drug discovery shortening the development cycle of a drug is a very important aspect to reduce the costs, for that reason computer aided drug design has become an important tool in modern drug discovery to accelerate this process. Several modeling methods are commonly used nowadays such as pharmacophore, docking, QSAR and other in silico techniques which play a crucial role in exploring potential drugs to fight against several diseases. For instance, donepezil was discovered by molecular modeling and a QSAR study (Sugimoto et al. 2002). A pharmacophore model is a tool used to describe the 3D arrangement of main features essential for activity in terms of distances, angles, or coordinates. Only few studies were published concerning the development of pharmacophore model based on steroidal CYP17 inhibitors (Miao et al. 2001; Schappach and Höltje 2001; Clement et al. 2003). In this study, our goal was to further understand the binding requirements for the steroidal CYP17 inhibitors and to discover new hits of the target enzyme using the developed pharmacophore model as 3D queries to search in structural databases, which might help in discovering a more active and selective inhibitor for this enzyme. Additionally, docking studies for the selected compounds can help in scoring the compounds fitting best the active site of the enzyme.

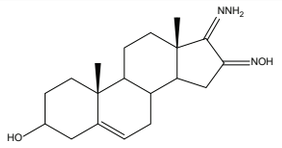
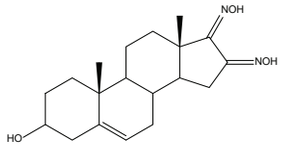
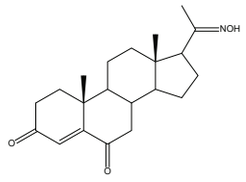
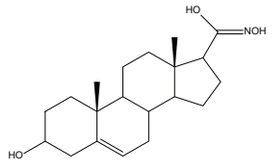
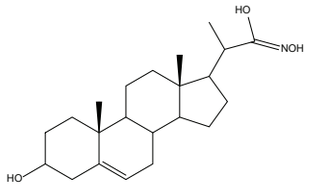
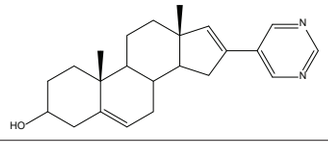
2. Investigations, results and discussion

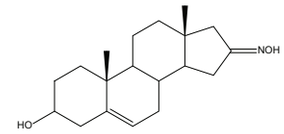
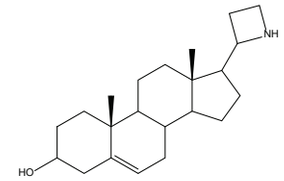
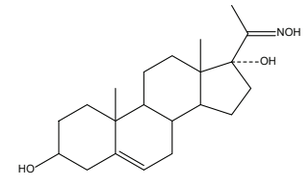
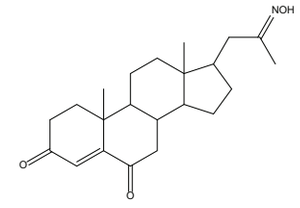
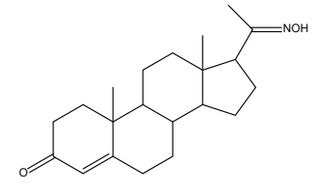
In this work we collected data of different steroidal compounds tested as CYP17 inhibitors in our laboratories. The chemical structures of the 30 tested compounds and their IC_{50}s are shown in Table 1, inhibitors with IC_{50} values of 2.5 μM or less were considered as active compounds, and compounds with IC_{50} values more than 2.5 μM were considered as non-active, so the compounds can be divided into 17 active and 13 non-active. The resultant database contains 146 conformations of 30 compounds, those were divided into training set and test set. The three most active pregnenolone based compounds in the database namely compounds 1, 2, and 4 (Table 1) were aligned to create the pharmacophore model using Molecular Operating Environment softwere (MOE). Those compounds have IC_{50} values of 24, 73, and 77 nM, respectively and are called training set. The MOE`s flexible alignment was

Table 1: Chemical structures of the training and test compounds and their IC_{50}s.

Nr.	Chemical structure	IC_{50} (nM)
1		24
2		73
3		38
4		77
5		100
6		170
7		200
8		200
9		270

Nr.	Chemical structure	IC_{50} (nM)
10		290
11		400
12		540
13		1180
14		1250
15		1650
16		1800
17		1800
18		<2500

Nr.	Chemical structure	IC_{50} (nM)
19		<2500
20		<2500
21		<2500
22		<2500
23		<2500
24		<2500
25		<2500

Nr.	Chemical structure	IC_{50} (nM)
26		<2500
27		<2500
28		<2500
29		<2500
30		<2500

used for the alignment of the training set and all confirmations of the molecules were considered for the alignment. Due to the identical pregnenolone backbones of the training set, the three compounds were very well aligned (Fig. 1). A multi-conformer database which is called test set was obtained by the conformational search for each of the 27 compounds using MOE. The developed query was created depending on the alignment of three

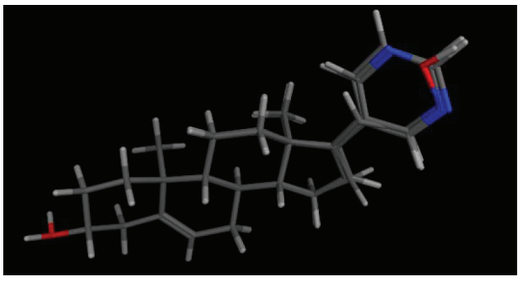
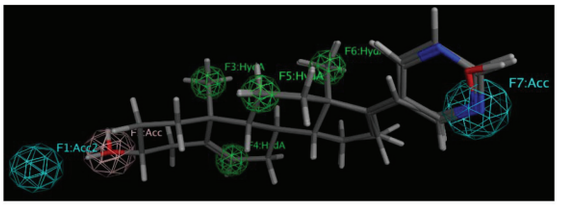


Fig. 1: Alignment of the training set CYP17 steroidal inhibitors compounds **1**, **2**, and **4**.

most potent compounds (training set) as presented in Fig. 1. Only seven features were necessary for the development of our model, namely one hydrogen bond acceptor (F7) on the heterocycle, four hydrophobes (F3-F6) on rings B and C, and one hydrogen bond donor acceptor which was changed to only acceptor (F2) on ring A, as well as one hydrogen bond accepter projection (F1). The radius of F1 and F2 were set at 1 ºA, F3-F6 were set at 0.7 ºA and F7 was set at 1 ºA (Fig. 2). In order to evaluate the pharmacophore model, its ability to identify only the medium and highly active compounds (compounds 4-17) was challenged. The test set was scanned using the pharmacophore search function of MOE and applying the default options.

The developed model was able to select 12 out of the 14 active compounds (86%); compounds 16, and 17 were not selected.

(A)



(B)

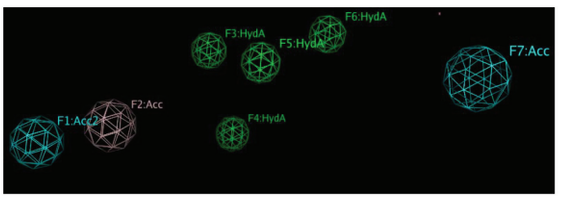
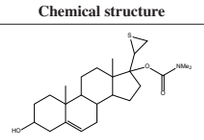
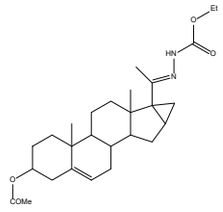
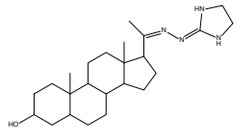
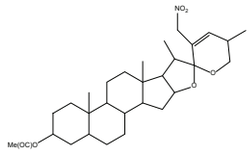
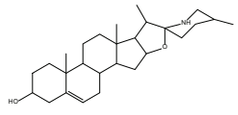
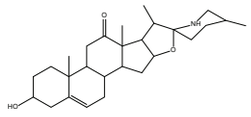
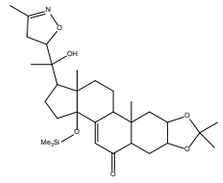
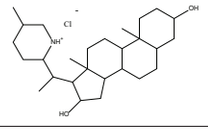
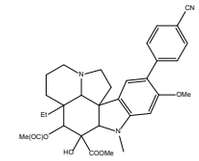
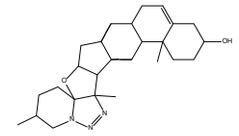
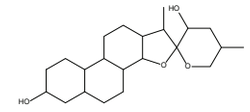
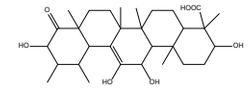
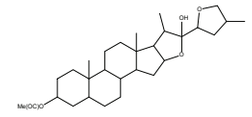
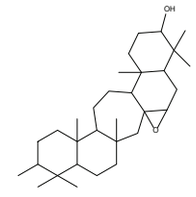
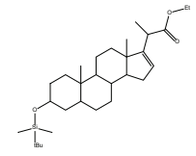
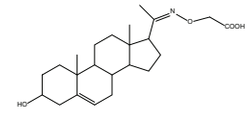
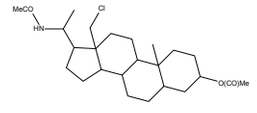
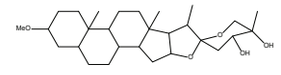
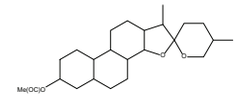
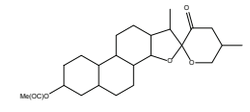
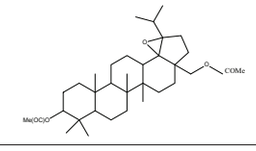


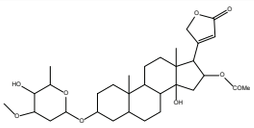
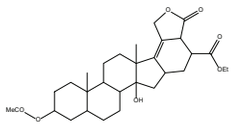
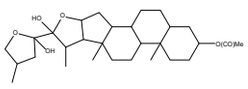
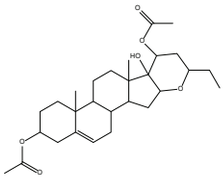
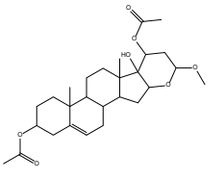
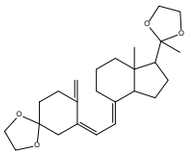
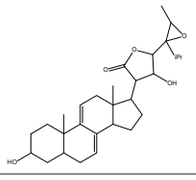
Fig. 2: (A) Alignment the pharmacophore model features and the training set. (B) The pharmacophore model features of steroidal CYP17 inhibitors. Four hydrophobes (Hyd), and two hydrogen bond acceptors (Acc), as well as one hydrogen bond accepter projection (Acc2).

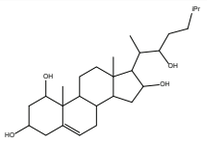
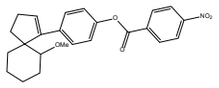
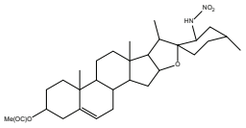
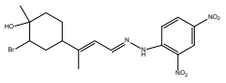
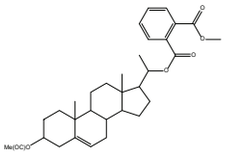
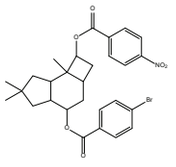
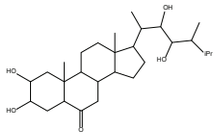
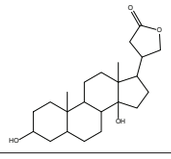
Only two out of 13 non-active compounds were also selected as false positive (15%), namely compounds 21 and 28. Clement et al. (2003) were able to develop a pharmacophore model of azole steroidal based CYP17 inhibitors. Their result is somehow similar to our new pharmacophore model, where they have three hydrophobe features and two hydrogen bond acceptors.

In order to use this pharmacophore model in finding potential CYP17 inhibitors, the features of the developed pharmacophore were used to search the (CSD) Cambridge structural database using the ConQuest software from CCDC (Cambridge Crystallographic Data Centre). The CSD contains around one million compounds from published literature, beside tens of journals that deposit their crystal structure data exclusively in the CSD, additionally the CSD contains unique directly deposited data ((http://www.ccdc.cam.ac.uk). The results of the search are shown in Table 2, where 36 compounds were selected and can be considered as hits compounds.

Table 2: Chemical structures of the (hit) compounds from 3D search of (CSD) Cambridge structural database using the developed pharmacophore features of the steroidal CYP17 inhibitors.

Code	Chemical structure	S score
AJURUT		-11.5708
GIHCIJ		-12.1021
KATXIO		-12.1665
SOLFEF		-13.4737
TAFXON		-11.4714
VIJMUX		-12.6482
BAFMOM		-12.3122
ISFLOR01		-12.2677
POQJOV		-10.5914
UWANIQ		-11.8521
SOLFAB		-10.7855
FUZSUP		-13.8825
EGUFOB		-11.4286
EXURIY		-12.2145
GIHLUE		-11.6724
KATXAG		-12.7535
KUTXIH		-10.3093
LAKJAJ		-10.5443
IWFEZ02		-12.1213
ZEWXUW		-13.5461
AXELUP		-11.7851

Code	Chemical structure	S score
BAVJEN		-13.8730
BEYBEM		-13.2133
DAZKEV		-12.2573
DENYIE		-12.5612
DENYOK		-11.9845
EDPRGT		-13.4903
KOPPOU		-12.8904

Code	Chemical structure	S score
LAHFOR		-12.6449
OLBOB		-11.3816
POQSOE		-12.5421
RIJPEF		-11.5010
WICFAP		-11.875
VIKXUI		-12.3681
QULWUP		-11.7835
JIDNUF		-12.851

In order to further filter the resultant structures, a docking study was performed using MOE software. MOE was able to define 816 confirmations using the conformational search implemented in the software for the 36 compounds from the database. The conformation structures were then docked into the crystal structure of CYP17 from PDB ID (3RUK, resolution 2.6 Å) (http://www.rcsb.org/pdb/explore/explore.do?structureId=3RUK). With the attempt to classify the selected compounds, all the conformations were scored according to S score and the three best conformations with minimum S score were selected. Docking software uses an energy based scoring method to obtain the most favorable ligand conformation and orientation which is important for binding the active site of the target enzyme. Compound (FUZSUP) was selected as top conformation followed by compound (BAVJEN), and then compound (ZEWXUW), Table 2. Beside the minimum S score, top conformation for every compound was visually checked to control the interaction between each compound and the amino acid residue in the active site, such as the hydrogen binding and *pi-pi* interactions. The 2D interactions between the active site of the enzyme and the ligands for the three top ranked compounds are shown in Fig. 3, while Fig. 4 presents the docking complexes of the ligands with the binding site of the enzyme.

Compound FUZSUP has a hydrogen bonding with arginine D96, arginine D440, and Isoleucine D299, as it is shown in Fig. 3 (a), while compound BAVJEN has interaction with arginine D239 via hydrogen bond, and indirect binding with the same amino acid residue via a water molecule, Fig. 3 (b). Compound ZEWXUW, has interaction via only one hydrogen binding with threonine D306, Fig. 3 (c). Compound FUZSUP: 3,11,12,21-tetrahydroxy-22-oxo-,(3β,4β,11α,21β)-12-en-23-oic acid, was discovered recently as one of three new triterpenoids camellisins, which were found in the roots of Camellia sinensis (Lei et al. 2010). While compound BAVJEN: 16-(acetyloxy)-3-[(2,6-dideoxy-3-*O*-methyl-L-*arabino*-hexopyranosyl)oxy]-14-hydroxy-,(3β,5β,16β)-cardanolide, was published as part of study concerning the discovery of cardenolide glycosides from larvae of the sphingid moth *Daphnis nerii,* (Abe et al. 1996) and later its cytotoxicity was determined against several cell lines (Jung et al. 2015). On the other hand compound ZEWXUW was discovered a few years ago by Jastrzebska et al. (2009) among other compounds through the reactions of steroidal 23-oxo and 23, 24-epoxysapogenins with Lewis acids. It is important to mention that the compounds in the training set namely compounds 1, 2 and 4 were also docked in the active site of the enzyme for comparison, and their S scores were -11.6676, -10.3540, and -10.9992 respectively. Table 3 compares the training set and the best three selected compounds from the database in term of the S score and some other basic and important physicochemical parameters such as TPSA (topological polar surface aria), *C*log *P,* number of hydrogen bond donor and acceptor and molecular weight. It is clear that the three selected compounds

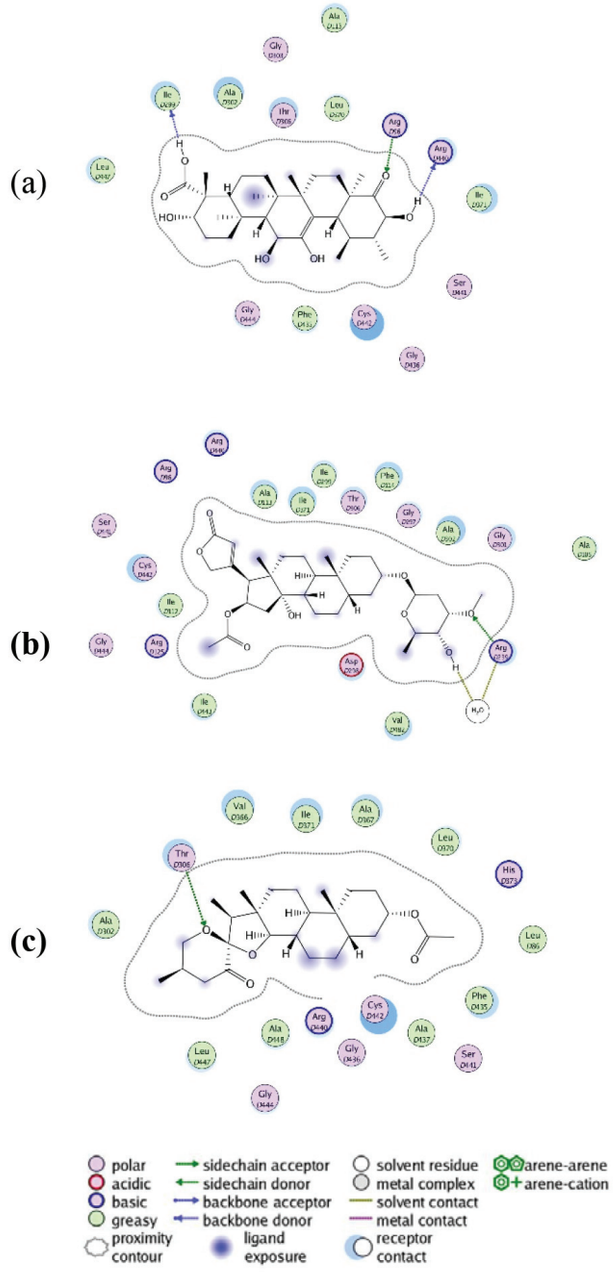


Fig. 3: 2-D interactions between the selected compounds and the receptor. (a): FUZSUP, (b): BAVJEN, (c): ZEWXUW.

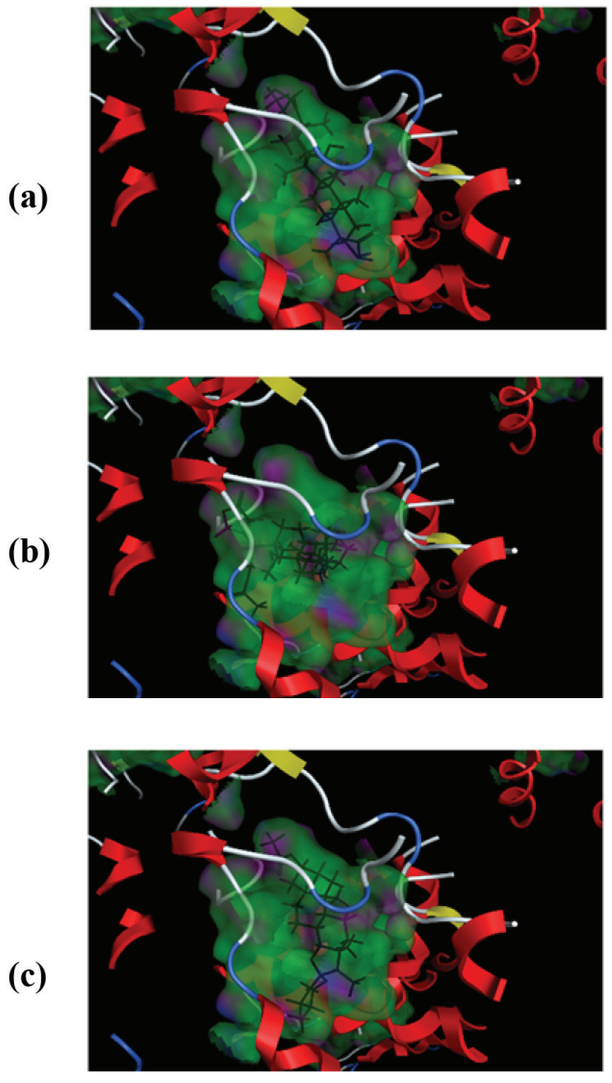


Fig. 4: 3-D docking complexes of the three selected compounds from the database with the binding site of CYP17. (a): FUZSUP, (b): BAVJEN, (c): ZEWXUW.

Table 3: Calculated physicochemical properties and docking result of the training set and the best selected compounds from the database

Compound	MW	TPSA (A2)	*C*Log *P*	H-bond Donor	H-bond Acceptor	Docking S score
1	350.506	46.010	4.794	1	3	-11.6676
2	349.518	33.120	5.399	1	2	-10.3540
4	329.484	52.820	4.697	2	3	-10.9992
FUZSUP	518.691	135.290	4.096	5	7	-13.8825
BAVJEN	576.727	120.750	3.681	2	7	-13.8730
ZEWXUW	432.601	61.830	4.908	0	4	-13.5461

MW: molecular weight, *C*log*P*: calculated partition coefficient (octanol: water), TPSA: topological polar surface.

have comparable properties to the training set, keeping in mind that compound 2 namely abiraterone acetate (Zytiga®) is a drug in the market used for the treatment of prostate cancer.

In this study we participate in finding hypothetically the necessary ligand binding requirement for steroidal CYP17 inhibitors. In our pharmacophore model the F7 feature, which presents the hydrogen bond acceptor is important for the interaction with the heme moiety in CYP17. The three selected compounds from the database can be considered as hit compounds and can be evaluated *in vitro* in order to develop lead compounds. This model can be also used to identify new inhibitors for this enzyme by searching other databases such as ZINC, TosLab, Hitfinder, NCI and Enamine. Unfortunately none of the selected compounds was available to us for *in vitro* testing. However, the target of this study was to develop a pharmacophore model as well as primary in silico filter of the database. Even though no *in vitro* activity was determined in this work, the selected compounds from the database can be considered as a starting point for the development of new CYP17 inhibitors in future studies.

3. Experimental

3.1. Chemical compounds

All compounds used in the current study were prepared in our laboratories (Saarland university) and the synthesis procedures together with the analytical data as well as

the biological activities have been published previously (Haidar et al. 2001a; Hartmann et al. 2000; Haidar et al. 2001b; Haidar and Hartmann 2002).

3.2. *In vitro assay of CYP17*

The inhibitory activity toward CYP 17 human enzyme was determined using testicular microsomes. The RP-HPLC with UV detection was employed for product determination, progesterone was used as substrate as described earlier (Sergejew and Hartmann 1994; Wachall et al. 1999).

3.3. *Computational method*

To perform this study the software package Molecular Operating Environment (MOE, Chemical Computing Group, Montreal, Canada) was applied (Molecular Operating Environment (MOE)). Using Intel Core, Duo based, 3.40 GHz processor. All chemical structures were built using the builder function implemented in the software and energy was minimized in the MMFF94x force field.

3.4. *Generation of pharmacophore model*

A database of 30 known steroidal inhibitors was created in order to develop a reliable pharmacophore model to identify new CYP17 inhibitors. The structures of the used steroidal inhibitors as well as their inhibitory activity were published earlier (Haidar et al. 2001a; Hartmann et al. 2000; Haidar, Klein et al. 2001b; Haidar and Hartmann 2002). The pharmacophore model was developed by aligning compounds 1, 2, 4 with IC_{50} values of 24, 73, and 77 nM, respectively (Table 1), which is called training set. This alignment was obtained using MOE`s flexible alignment and we considered all confirmations of the molecules for the alignment (Molecular Operating Environment (MOE)). A conformational search was performed for each of the 27 compounds using the MMFF94x force field within MOE to generate a multi-conformer database which is called test set, and the default settings were used for performing the search. The consensus query implemented in the software was used to create all necessary features of the pharmacophore model, tolerance was set at 1 and threshold was set at 90%. In order to optimize this multi features model, each individual pharmacophore feature was removed and the test set was scanned using the remaining features.

3.5. *Database search*

The Cambridge structural database CSD (http://www.ccdc.cam.ac.uk) was applied to search for hits that fit to the features of the developed pharmacophore. The Cambridge structure database contains over 850,000 entries; this accurate 3D structure database is considered as an important resource to scientists around the world. The developed pharmacophore features were implemented in ConQuest supplementary software provided by Cambridge Crystallographic Data Centre (CCDC) and a search was performed to obtain the hit compounds. Each compound from CSD has a unique six letters "Refcode" (entry ID), beside two digits identifying additional structures (http://www.ccdc.cam.ac.uk).

3.6. *Receptor refinement*

Three dimensional structure of the CYP17 complexed with abiraterone was obtained from the Protein Data Bank (PDB) using PDB ID: 3RUK (http://www.rcsb.org/pdb/explore/explore.do?structureId=3RUK). The protein structure was prepared by adding hydrogen atoms, then water molecules were removed from the structure and 3D protonation was performed to change the state into ionization level. Additionally, energy minimization was performed using defaults parameters in MOE by applying the AMBER99 force field.

3.7. *Database generation*

All compounds which were selected from the CSD database were rebuilt using the builder option implemented in the software (Molecular Operating Environment (MOE)). The Energy of the compounds was minimized using the following parameters gradient: 0.05, Force Field: MMFF94X, all other parameters were set to default. A database having all the compounds and their conformations was created and saved in mdb format and was employed for the docking study.

3.8. *Molecular docking*

The 36 selected compounds from the database were docked into the active site of P450-17 enzyme (3RUK) (http://www.rcsb.org/pdb/explore/explore.do?structureId=3RUK) using MOE-Dock implemented in the software. The docking parameters were set as Rescoring 1: London dG, Placement: triangle matcher, Retain 30, Refinement Force field, and Rescoring 2: London dG. The docking function of MOE can give the correct conformation of the ligand to obtain minimum energy structure. In order to rank the best fitting compound in the active site the S score function was used and the top conformation for each compound was selected based on the S score. Also visual inspection was carried out by using the "ligand interaction" function implemented in MOE. The best compounds were selected as promising hits based on their significant interactions with the amino acid residues of binding pocket of CYP17.

Acknowledgments: Many thanks for DAAD (Deutscher Akademischer Austauschdienst) for the fellowship for the first author, also thanks to Dr. Matthias Negri for his support and important discussion.

Conflicts of interest: None declared.

References

Abe F, Yamauchi T, Minato K (1996) Presence of cardenolides and ursolic acid from oleander leaves in larvae and frass of Daphnis nerii. Phytochemistry 42: 45-49.

Baston E, Leroux FR (2007) Inhibitors of steroidal cytochrome p450 enzymes as targets for drug development. Recent Pat Anticancer Drug Discov 2: 31-58.

Clement OO, Freeman CM, Hartmann RW, Handratta VD, Vasaitis TS, Brodie AM, Njar VC (2003) Three dimensional pharmacophore modeling of human CYP17 inhibitors. Potential agents for prostate cancer therapy. J Med Chem 46: 2345-2351.

de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OB Jr, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Fléchon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI; COU-AA-301 Investigators (2011) Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 364: 1995-2005.

Eklund J, Kozloff M, Vlamakis J, Starr A, Mariott M, Gallot L, Jovanovic B, Schilder L, Robin E, Pins M, Bergan RC (2006) Phase II study of mitoxantrone and ketoconazole for hormone-refractory prostate cancer. Cancer 106: 2459-2465.

Geller J (1993) Basis for hormonal management of advanced prostate cancer. Cancer 71(3 Suppl): 1039-1045.

Haidar S, Ehmer PB, Hartmann RW (2001a) Novel steroidal pyrimidyl inhibitors of P450 17 (17 alpha-hydroxylase/C17-20-lyase). Arch Pharm 334: 373-374.

Haidar S, Klein CD, Hartmann RW (2001b) Synthesis and evaluation of steroidal hydroxamic acids as inhibitors of P450 17 (17 alpha-hydroxylase/C17-20-lyase). Arch Pharm 334: 138-140.

Haidar S, Hartmann RW (2002) C16 and C17 substituted derivatives of pregnenolone and progesterone as inhibitors of 17alpha-hydroxylase-C17, 20-lyase: synthesis and biological evaluation. Arch Pharm 335: 526-534.

Hartmann RW, Ehmer PB, Haidar S, Hector M, Jose J, Klein CD, Seidel SB, Sergejew TF, Wachall BG, Wächter GA, Zhuang Y (2002) Inhibition of CYP 17, a new strategy for the treatment of prostate cancer. Arch Pharm 335: 119-128.

Hartmann RW, Frotscher M, Ledergerber D, Wächter GA, Grün GL, Sergejew TF (1996) Synthesis and evaluation of azole-substituted tetrahydronaphthalenes as inhibitors of P450 arom, P450 17, and P450 TxA2. Arch Pharm 329: 251-261.

Hartmann RW, Hector M, Haidar S, Ehmer PB, Reichert W, Jose J (2000) Synthesis and evaluation of novel steroidal oxime inhibitors of P450 17 (17 alpha-hydroxylase/C17-20-lyase) and 5 alpha-reductase types 1 and 2. J Med Chem 43: 4266-4277.

Hille UE, Hu Q, Vock C, Negri M, Bartels M, Müller-Vieira U, Lauterbach T, Hartmann RW (2009) Novel CYP17 inhibitors: synthesis, biological evaluation, structure-activity relationships and modelling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls. Eur J Med Chem 44(7): 2765-2775.

http://www.rcsb.org/pdb/explore/explore.do?structureId=3RUK.

Hu Q, Hartmann RW (2014) The renaissance of CYP17 inhibitors for the treatment of prostate cancer. In: Neidle S (ed) Cancer Drug Design and Discovery (Second Edition). San Diego, Academic Press, pp 319-356.

Hu Q, Yin L, Jagusch C, Hille UE, Hartmann RW (2010) Isopropylidene substitution increases activity and selectivity of biphenylmethylene 4-pyridine type CYP17 inhibitors. J Med Chem 53: 5049-5053.

Jarman M, Smith HJ, Nicholls PJ, Simons C (1998) Inhibitors of enzymes of androgen biosynthesis: cytochrome P450(17) alpha and 5 alpha-steroid reductase. Nat Prod Rep 15: 495-512.

Jastrzebska I, Siergiejczyk L, Tomkiel AM, Urba□czyk-Lipkowska Z, Wójcik D, Morzycki JW (2009) On reactions of steroidal 23-oxo and 23,24-epoxysapogenins with Lewis acids. Steroids 74: 675-683.

Jung JW, Baek NI, Hwang-Bo J, Lee SS, Park JH, Seo KH, Kwon JH, Oh EJ, Lee DY, Chung IS, Bang MH (2015) Two new cytototoxic cardenolides from the whole plants of Adonis multiflora Nishikawa Koki Ito. Molecules 20: 20823-20831.

Lei C, Hu Z, Pu JX, Wang YY, Xiao WL, Gong NB, Li Y, Lu Y, Zheng QT, Sun HD (2010) Camellisins A-C, three new triterpenoids from the roots of Camellia sinensis. Chem Pharm Bull 58: 939-943.

Leroux F (2005) Inhibition of p450 17 as a new strategy for the treatment of prostate cancer. Curr Med Chem 12: 1623-1629.

Leroux F, Hutschenreuter TU, Charrière C, Scopelliti R, Hartmann RW. (2003) N-(4-Biphenylmethyl)imidazoles as potential therapeutics for the treatment of prostate cancer: metabolic robustness due to fluorine substitution? Helv Chim Acta 86: 2671-2686.

Miao J, Ling YZ, Zhu N, Lei XP (2001) [Three dimensional quantitative structure activity relationship of P450(17) alpha inhibitors of 17-substituted steroids.] Yao Xue Xue Bao 36: 507-510.

Molecular Operating Environment (MOE), C. C. G. I., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2010.

Moreira VM, Salvador JA, Vasaitis TS, Njar VC (2008) CYP17 inhibitors for prostate cancer treatment--an update. Curr Med Chem 15: 868-899.

Njar VC, Brodie AM (1999) Inhibitors of 17alpha-hydroxylase/17,20-lyase (CYP17): potential agents for the treatment of prostate cancer. Curr Pharm Des 5: 163-180.

Njar VC, Hector M, Hartmann RW (1996) 20-Amino and 20,21-aziridinyl pregnene steroids: development of potent inhibitors of 17 alpha-hydroxylase/C17,20-lyase (P450 17). Bioorg Med Chem 4: 1447-1453.

Owen CP (2009) 17alpha-hydroxylase/17,20-lyase (p450(17alpha)) inhibitors in the treatment of prostate cancer: a review. Anticancer Agents Med Chem 9: 613-626.

Salvador JAR, Pinto RMA, Silvestre SM (2013) Steroidal 5α-reductase and 17α-hydroxylase/17,20-lyase (CYP17) inhibitors useful in the treatment of prostatic diseases. J Steroid Biochem Mol Biol 137: 199-222.

Schappach A, Höltje HD (2001) Investigations on inhibitors of human 17 alpha-hydroxylase-17,20-lyase and their interactions with the enzyme. Molecular modelling of 17 alpha-hydroxylase-17,20-lyase, Part II. Pharmazie 56: 835-842.

Sergejew T, Hartmann RW (1994) Pyridyl substituted benzocycloalkenes: new inhibitors of 17 alpha-hydroxylase/17,20-lyase (P450 17 alpha). J Enzyme Inhib 8: 113-122.

Sugimoto H, Ogura H, Arai Y, Limura Y, Yamanishi Y (2002) Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. Jpn J Pharmacol 89: 7-20.

Vasaitis TS, Bruno RD, Njar VC (2011) CYP17 inhibitors for prostate cancer therapy. J Steroid Biochem Mol Biol 125: 23-31.

Wachall BG, Hector M, Zhuang Y, Hartmann RW (1999) Imidazole substituted biphenyls: a new class of highly potent and in vivo active inhibitors of P450 17 as potential therapeutics for treatment of prostate cancer. Bioorg Med Chem 7: 1913-1924.