Bioactivity of curcumin on the cytochrome P450 enzymes of the steroidogenic pathway

Patricia Rodríguez Castaño[1,2], Amit V Pandey[1,2,#]

[1]Pediatric Endocrinology, Diabetology and Metabolism, University Children's Hospital Bern, 3010 Bern, Switzerland
[2]Department of Biomedical Research, University of Bern, Bern, Switzerland

*** Correspondence:**
PD Dr. Amit V Pandey
Pediatric Endocrinology, Diabetology & Metabolism,
KIKL C837, University Children's Hospital Bern,
Freiburgstrasse 15, 3010 Bern Switzerland
Tel: +41 31 632 9637
amit@pandeylab.org

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Abstract

Turmeric is a popular ingredient in the cuisine of many Asian countries. Turmeric is known for its use in Chinese and Ayurvedic medicine and comes from the roots of the *Curcuma longa*. Turmeric is rich in curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin has potent anti-inflammatory and anti-carcinogenic activities. Since many anti-cancer drugs target enzymes from the steroidogenic pathway, we tested the bioactivity of curcuminoids on cytochrome P450 CYP17A1, CYP21A2, and CYP19A1 enzyme activities. Curcuminoids were extracted from turmeric with organic solvents. We conducted a cell-based assay for CYP17A1 and CYP21A2 activities using human adrenal cell line NCI-H295R. ^{3}H-pregnenolone was used for CYP17A1 assays, and ^{3}H-17alpha-hydroxyprogesterone was used as a substrate for CYP21A2. Curcuminoids were added at different concentrations and incubated for 24h. Steroids were separated by thin layer chromatography and analyzed by phosphorimager analysis. For CYP19A1 activity, an in vitro assay using endoplasmic reticulum from JEG3 were used with ^{3}H-androstenedione as the substrate. Curcuminoids were incubated for 1h, and the formation of ^{3}H-water from the androstenedione breakdown was measured by scintillation counting. When using 10 µg/ml of curcuminoids, both the 17-hydroxylase as well as 17,20 lyase activities of CYP17A1 were reduced significantly. On the other hand, CYP21A2 activity was only reduced to ~50% control. Furthermore, CYP19A1 activity was reduced to ~20% of control when using 1-100 µg/ml of curcuminoids in a dose-dependent manner. No effect on the activity of 5alpha reductase for the conversion of androstenedione to androstanedione was observed. Molecular docking studies confirmed that curcumin could dock into the active sites of CYP17A1, CYP19A1 as well as CYP21A2. In CYP17A1 and CYP19A1, curcumin docked within 2.5 Å of central heme while in CYP21A2 the distance from heme was 3.4 Å, which is still in the same range or lower than distances of bound steroid substrates. These studies show that curcuminoids may potentially cause inhibition of steroid metabolism, especially at higher dosages. The activities of CYP17A1 and CYP19A1 were inhibited by curcuminoids, which indicate potential anti-carcinogenic effects in case of prostate cancer as well as breast cancer, which can be targeted by inhibition of steroidogenesis. Also, the recent popularity of turmeric powder/curcumin as a dilatory supplement needs further evaluation for the effect of curcuminoids on steroid metabolism. Curcuminoids present in curcumin may affect activities of multiple steroid metabolizing cytochrome P450 enzymes. Computational docking suggests curcumin binds into the active sites of steroid metabolizing P450s and may serve as a model for lead discovery. Molecular structure of curcuminoids could be modified to generate better lead compounds with inhibitory effects on CYP17A1 and CYP19A1 for potential drugs against prostate cancer and breast cancer.

1. Introduction

Turmeric, the well-known yellow spice, and coloring agent is found in the cuisine of numerous Asian countries. Turmeric is also recognized for its use in Chinese and Ayurvedic medicine and has been tested for anti-microbial activity as far back as 1949 [1, 2]. Turmeric is procured from the dried rhizomes of *Curcuma longa.* Curcumin (CI-75300, diferuloylmethane, E100, Natural Yellow 3) is the most abundant of the curcuminoids and enhances wound healing, modulates angiogenesis and the immune system; has anti-inflammatory, anti-oxidant, anti-infective and anti-cancer activity [3-5] (Figure 1). Anti-cancer activity of curcumin was first described by Kuttan et al. in 1985 [6]. Since then, different research groups have been testing curcumin *in vitro* [7] and clinical trials [8]. Curcumin itself has poor solubility in water, and therefore, often shows low bioavailability when consumed directly [9].

Different methods have been developed to increase curcumin bioavailability, and in it has been shown that combining curcumin with piperine, the bioavailability of curcumin increased by 154% in humans. Structurally, curcumin has two aryl moieties connected by a seven-carbon chain. By varying the motifs from the primary structure, synthetic molecules can be created; or a liposomal curcumin preparation can also be prepared [9-11]. Since the discovery of curcumin as a bioactive compound, several biological activities have been described. Curcumin has been shown to modulate molecular signaling pathways, such as the aryl hydrocarbon receptor, the induction of Nrf2 or the inhibition of NF-κB, initiating the activation of inflammatory and immunogenic factors, among others. Curcumin can also inhibit angiogenesis and induce apoptosis on cancerous cells [12-14]. Curcumin is also involved in the increase of insulin in plasma and the decrease of blood glucose level in diabetic patients [15, 16]. Curcumin was also found to exhibit a protective effect against natural and chemical toxicities [17].

Most of the hormonal-dependent cancers, such as breast and prostate cancer, are treated by blocking the synthesis of estrogens and androgens [18, 19], by targeting enzymes from the steroidogenesis pathway. Cytochrome P450 (CYP) 17A1 converts pregnenolone into dehydroepiandrosterone (DHEA), the precursor of both androgens and estrogens (Figure 2). The *CYP17A1* gene (GeneID: 1586, 10q24.32, GRCh38 *chr10:102,830,531-102,837,533,* NCBI: NM_000102.4 , NP_000093.1, OMIM: 609300) regulates sex steroid biosynthesis in humans through 17α-hydroxylase/17,20 lyase activities and is a target of anti-prostate cancer drug abiraterone. Aromatase (CYP19A1) converts androstenedione and testosterone into estrogens and regulates estrogen biosynthesis and is a target for the treatment for breast cancer [20-22]. CYP19A1 protein contains 503 amino acids (NP_000094) and is encoded by the *CYP19A1* gene (GeneID:1588, NCBI: NM_000103, 15q21.2, GRCh38 15:51208056-51338597). The cytochrome P450 21-hydroxylase, coded by *CYP21A2* (GeneID: 1589, NCBI: NM_000500.9, NP_000491.4, 6p21.33, GRCh38 chr6:32,038,265-32,041,670, OMIM: 613815) is needed for biosynthesis of mineralocorticoids and glucocorticoids. In the adrenals, CYP21A2 converts progesterone into 11-deoxycorticosterone and 17-hydroxyprogesterone into 11-deoxycortisol. All these cytochromes P450, the CYP17A1, CYP19A1, and CYP21A2 are membrane-bound proteins and belong to the cytochrome P450 protein superfamily. Cytochromes P450 enzymes are involved in the biosynthesis of drugs, xenobiotics, and steroid hormones [23]. There are different types of cytochrome P450 proteins in humans. The cytochrome P450 proteins located inside the mitochondrion metabolize steroids and sterols in partnership with ferredoxin and ferredoxin reductase and are called type 1 cytochrome P450 [24, 25]. Majority of cytochrome P450 proteins in humans (50 out of 57) are located in the smooth endoplasmic reticulum relying on cytochrome P450 oxidoreductase as their redox partner (type 2 cytochrome P450) and metabolize drugs, xenobiotics as well as endogenous substrates, and many steroid hormones like pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, testosterone, androstenedione [26-33]. Curcumin,

demethoxycurcumin, and bisdemethoxycurcumin are the most abundant components of turmeric; together, these are called curcuminoids (Figure 3). Curcumin has been tested on the five drug-metabolizing enzymes from the cytochrome P450 family, *in vivo* [34] and *in vitro* [35, 36], showing that CYP1A2, CYP2B6, CYP2C9, CYP2D6, and CYP3A4 can be inhibited in a dose-dependent matter.

Here we are reporting that curcumin may inhibit the biosynthesis of steroid hormones. We tested the effect of curcumin at different dosages on the activities of steroid metabolizing enzymes CYP17A1, CYP19A1, and CYP21A2 using adrenal carcinoma cell line NCI-H295R and the endoplasmic reticulum from the placental carcinoma cell line JEG3. Inhibition of CYP17A1 and CYP19A1 activities by curcumin indicates similar molecular entities or modified compounds based on core structures of curcuminoids could be explored as potential treatments for prostate cancer by targeting CYP17A1 and for breast cancer by targeting CYP19A1.

2. Materials and methods

2.1 Materials

Radiolabeled [^{3}H]-pregnenolone [7(N)-^{3}H]-pregnenolone (12.6 Ci/mmol) and [^{3}H]-Androstenedione: Andros-4-ene-3, 17-dione, [1β-^{3}H(N)]-, 250µCi (9.25MBq) were purchased from PerkinElmer (Waltham, MA, USA). [^{3}H]-17α-OH progesterone [1, 2, 6, 7-3H] (60-120 Ci/mmol 2.22-4.44TBq/mmol) from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). Silica gel coated aluminum backed TLC plates were from Macherey-Nagel (Oensingen, Switzerland). The tritium-screens used for the phosphorimaging were purchased from Fujifilm (Dielsdorf, Switzerland). Turmeric extract capsules *(Curcuma longa)* were obtained from the Finest Natural (Item: 943.17, Deerfield, IL, USA). Trilostane was extracted in absolute ethanol

(EtOH) from tablets commercially available as Modrenal® (Bioenvision, NY, USA). Abiraterone was purchased from Selleckchem (Houston, TX, USA). Anastrozole was from AstraZeneca.

2.2 Cell lines and culture media

Human placental JEG3 cells were purchased from ATCC (HTB-36™) and cultured in MEM with Earle's Salts (GIBCO), 10% Fetal Bovine Serum, 1% L-Glutamine (200mM GIBCO), 1% penicillin (100 U/ml; GIBCO) and streptomycin (100 µg/ml; GIBCO). Human adrenocortical NCI-H295R (NCI-H295R) was purchased from American Type Culture Collection (ATCC; CRL-2128) and grown in DMEM/Ham's F-12 medium containing L-glutamine and 15mM HEPES medium (GIBCO, Paisley, UK) supplemented with 5% NU-I serum (Becton Dickinson, Franklin Lakes, NJ USA), 0.1% insulin, transferrin, and selenium (100U/ml; GIBCO), 1% penicillin (100 U/ml; GIBCO) and streptomycin (100µg/ml; GIBCO). HEK293 were grown in DMEM GlutaMAXTM supplemented with 10% FBS, 1% antibiotic mix (100x) and 1 mM sodium pyruvate.

2.3 Curcumin extraction procedures

The crude extract was obtained by mixing 20 grams of turmeric in 200ml of ethanol overnight. Then, samples were taken for an initial screening on NCI-H295R cells. The rest was filtered and dried with nitrogen. Pure curcuminoids were obtained by recrystallization as follows: powder was dissolved in hexane and centrifuged at 3000 rpm for 15 minutes, afterward, the supernatant was removed, and the pellet was dissolved in 50% propanol + 50% hexane (warm). After shaking the solution, it was disposed at 4°C overnight. The next day, soluble supernatant was removed, and the curcuminoids were dried completely by using nitrogen. Aliquots were made by mixing 10mg of the powder per 1ml of 100% ethanol. The purity of the extracted curcuminoids was tested by thin layer chromatography [37] (Figure 3).

2.4 Cell viability assay using MTT

Human adrenal NCI-H295R cells were seeded in 96-well culture plates at a density of $0.3x10^6$ cells per well overnight and grown at 37°C under 5% CO_2, and 90% humidity. Then, the media was changed, and different concentrations of curcuminoids were added and incubated for 24h. Later, 20µl of MTT reagent (5mg/ml in PBS) was added into each well and incubated for 4h. At that point, media was removed, and 200µl of DMSO was added, and the plate was incubated 20 minutes in the dark. Finally, absorbances were measured at 570nm.

2.5 CYP17A1 and CYP21A2 assays

Human adrenal NCI-H295R cells were seeded in 6 well tissue culture plates at a density of $1x10^6$ cells per well. The day after, media was changed, and different concentrations of curcuminoids were added and incubated for 24h. Abiraterone (1µM) was used as a control for both assays. Cells were treated with trilostane, an inhibitor of HSD3B2, 90 min before adding radiolabeled substrate in the CYP17A1 assay. To study steroid metabolism, ~100000 cpm/well of [^{3}H]-pregnenolone was added to determine its conversion by CYP17A1. To check CYP21A2 activity, [^{3}H]-17α-OH progesterone (~50000 cpm/well) was added to cells. Media was then collected for steroid extraction and separation by thin layer chromatography on silica gel TLC plates (Macherey-Nagel, Oensingen, Switzerland). Steroids were visualized on a Fuji FLA-7000 PhosphorImager (Fujifilm, Dielsdorf, Switzerland). Conversion from the substrate into the product was measured by MultiGauge software (Fujifilm, Dielsdorf, Switzerland).

2.6 SRD5A2 activity assay

Cloning of SRD5A2 gene into the pcDNA3.1 vectors, transformation into HEK293 cells and assay of SRD5A2 activity has recently been described in an earlier publication [38]. In brief, after the transfection of SRD5A2 into HEK293 cells the activity of SRD5A2 was monitored by

conversion of androstenedione into 5α-dione using tritium labeled androstenedione as a tracer (60000 cpm/well [1ß-3H]-androstadiene). After the incubation with inhibitors, steroids were extracted and separated by TLC and quantitated by phosphor image analysis as described earlier [38].

2.7 JEG3 microsomes preparation

JEG3 cells were collected near confluency and washed in cold PBS. The cell suspension was then centrifuged at 1200 rpm for 5 min to pellet the cells. Afterward, the pellet was suspended in 100 mM Na_3PO_4 containing 150 mM KCl and sonicated to break the cells. Unbroken cells and mitochondria were pelleted by centrifugation at 10000 rpm for 15 min at 4°C. Microsomes containing endoplasmic reticulum were collected by ultracentrifugation at 38000 rpm for 90 min at 4°C and resuspended in 50mM K_3PO_4 containing 20% glycerol [39]. Amount of protein was measured by the BioRad Protein Assay Kit.

2.8 CYP19A1 tritiated water release assay

CYP19A1 activity was measured by calculating the release of tritiated water from the substrates during aromatization. Reactions for the enzyme assays were carried out in a final volume of 200µl, by using 40µg of protein from JEG3 microsomes, containing 100mM NaCl in 100mM potassium phosphate buffer (pH7.4). Different concentrations of curcuminoids were added using ethanol and anastrozole as controls. Mixtures were incubated with 50 nM androstenedione containing ~20000 cpm of [^{3}H]-Androstenedione. Reactions were initiated by adding 1mM of NADPH and incubated at 37°C in constant shaking for 1h. Afterward, 800µl of 5% charcoal with 0.5% dextran was added. Samples were then vortexed and centrifuged for 10 minutes at 13000 rpm. Aliquots (500µl) were taken for counting ^{3}H by mixing them with Rotiszint Universal Cocktail (Carl Roth GmbH, Karlsruhe, Germany). The measured cpm

values were multiplied by 2, as the counted aliquots represented only one half of the total reaction volume [40].

2.9 Docking of curcumin into CYP17A1, CYP19A1, and CYP21A2 protein structures

The published 3D structures of human CYP17A1, CYP19A1, and CYP21A2 were obtained from the PDB database (www.rcsb.org). We made in-silico calculations and structure analysis with the programs YASARA [41] and WHATIF [42]. For docking experiments, X-ray crystal structures of human of CYP17A1 (PDB# 3RUK), CYP19A1 (PDB# 3EQM) and CYP21A2 (PDB # 4Y8W), were used [43-45]. Missing hydrogen atoms into the structures were added with YASARA [41] which was also used for all other calculations. After the cleaning the structures and removing the bound steroid ligands, the system was subjected to 500 ps of explicit solvent MD simulations at 310 K, preceded by 500 steps of steepest descent and simulated annealing minimization with the AMBER14 force field and the TIP3P water model [46, 47]. All MD simulations used similar settings. The resulting minimum energy structures were used for AutoDock VINA [48] to perform docking experiments with curcumin (orthorhombic docking was grid established around the central heme molecule). The final poses of curcumin were selected based on their docking location and scores and resemblance to the co-crystallized ligands in the P450 structures. Structure models were drawn with Pymol (www.pymol.org) and rendered as ray-traced images with POVRAY (www.povray.org). Ligand interactions were analyzed and depicted with LIGPLOT+ (http://www.ebi.ac.uk/thornton-srv/software/LigPlus/)

2.10 Statistical analysis

For the statistical analysis, Microsoft Excel and GraphPad Prism (Graph Pad Software, Inc. San Diego, CA, USA) were used. Data are presented as the mean of 3 independent replicates.

Student t-test was used to calculate the differences between experiments. Significance was set at $p<0.05$.

3. Results

3.1 Toxicity of curcuminoids on NCI-H295R cells

We first performed an initial screening to determine at what concentration the curcuminoids were toxic on NCI-H295R adrenal cancer cell line (Figure 4). We performed serial dilutions of extracted curcuminoids and tested a range of concentrations on NCI-H295R cells to check the maximum concentration that was not toxic for the cells to establish the concentration range for further experiments. At high concentrations, only 25% of the cells survived, while at 25 µg/ml most of the cells were viable. Between 12.5-0.78 µg/ml, there was no cell death, and a standard concentration of 10µg/ml was used in further experiments.

3.2 Bioactivity of curcuminoids

We first did an initial screening of curcuminoids on steroid production by NCI-H295R cells. We compared the effect of curcuminoids on steroid biosynthesis with known inhibition abiraterone using pregnenolone as a substrate. Both the curcuminoids as well as abiraterone blocked the production of 17-hydroxypregnenolone and DHEA, indicating inhibition of CYP17A1 activities (Figure 5A). As a comparison, extracts of plant Saw Palmetto, did not show any effect on steroid production. Finasteride, a known inhibitor of SRD5A2, was used as a positive control and caused a complete block of 5α-reductase activity.

We also tested the effect of curcuminoids in on SRD5A2, which is a critical enzyme in the production of active androgens. However, no effect on SRD5A2 activity was observed by curcuminoids, indicating that cytochrome P450 proteins like CYP17A1, CYP19A1, or CYP21A2 might be better targets for curcuminoids (Figure 5B).

3.3 Bioactivity of curcuminoids on CYP17A1

After seeing the preliminary results showing a block of DHEA production we by curcuminoids that were similar to abiraterone, we tested the effect of curcuminoids on CYP17A1 17-hydroxylase and 17,20 lyase activities (Figure 6A). We observed inhibitory effects of curcuminoids on both activities of CYP17A1 in dose-dependent manner (Figure 6B and 6C). Abiraterone, a known inhibitor for CYP17A1, was used as a control. Overall, inhibition by curcuminoids of CYP17A1 17, 20 lyase activity was stronger compared to the effect on 17α-hydroxylase activity (Figure 6C).

3.4 Bioactivity of curcuminoids on CYP21A2

We used 17-hydroxyprogesterone as a substrate for CYP21A2 activity and monitored the production of 11-deoxycortisol to quantify the effect of curcuminoids. Human adrenal NCI-H295R cells do not use 17-hydroxyprogesterone as a substrate of CYP17A1 and therefore, are a good model for assay of CYP21A2 activities using 17-hydroxyprogesterone. We tested the bioactivity of curcuminoids at 5 µg/ml and 10 µg/ml concentrations for effect on CYP21A2 activity (Figure 7). The activity of CYP21A2 was reduced to 50% of control at 10 µg/ml of curcuminoids, which was less than the effects observed for inhibition of CYP171 and CYP19A1 activities. The anti-prostate-cancer drug abiraterone also inhibited the CYP21A2 activity as we have shown previously [49, 50].

3.5 Bioactivity of curcuminoids on CYP19A1

To test the effect of curcuminoids on the aromatase activity of CYP19A1, we used androstenedione as substrate and monitored its metabolism by quantifying the release of tritiated water from radiolabelled androstenedione. A preparation of endoplasmic reticulum

obtained from the placental JEG3 cells was used for the assay of aromatase enzyme activity. A dose-response effect showing the inhibition of aromatase with the increasing the concentration of curcuminoids was seen from 0.78 to 100 µg/ml of curcuminoids (Figure 8). A known inhibitor of CYP19A1, anastrozole was used as a positive control at 100 nM and showed inhibition of CYP19A1 activity as expected.

3.6 Computational docking of curcumin into the human CYP17A1, CYP21A2, and CYP19A1 crystal structures.

After observing the inhibitory effects of curcumin on CYP17A1, CYP19A1, and CYP21A2, we performed computational docking of curcumin into the protein structures of these cytochromes P450 to understand the molecular nature of inhibition. Molecular structure of curcumin resembles steroid substrates of cytochromes P450 studied in this report, and therefore, we wanted to check whether curcumin fits into the active site of these steroid metabolizing enzymes. Curcumin was docked into the crystal structures of human steroid metabolizing cytochrome P450 CYP17A1, CYP19A1, and CYP21A2 using the docking program Autodock VINA (Figure 9). Superimposition of P450 structures with either their substrates or the curcumin docked into the active site revealed similar binding poses (Figure 9). We observed a close binding pattern from the docking of curcumin into the active sites of CYP17A1 and CYP19A1 crystal structures (Heme iron to curcumin distances <2.5 Å) (Figure 9). The binding pose of curcumin to CYP21A2 was also similar to its binding into the CYP17A1 and CYP19A1. A comparison of the CYP17A1 and CYP19A1 crystal structures in complex with curcumin and docked curcumin into the crystal structure of CYP21A2 revealed similar binding conformations but the distance of the curcumin to the central heme iron of CYP21A2 was longer (3.4 Å *versus* 2.4Å) (Figure 9). Binding of curcumin with CYP17A1, CYP19A1 and CYP21A2 shares many similarities with the binding of steroid substrates into the active sites of these cytochrome P450 proteins with many similar active site residues involved in binding. These results indicate that

curcumin may act as an active site inhibitor and occupy the same space in cytochrome P450 active sites as used by their steroid substrates.

4. Discussion

All steroid hormones are produced from cholesterol [28]. The abnormal production of steroid hormones, for example, the hypercortisolemia seen in the Cushing´s syndrome, is a life-threatening condition [51, 52]. The hyperandrogenism is not life-threatening by itself but creates many severe complications during all phases of life [53]. Androgens regulate sexual differentiation in both the female as well as male and disruption of androgen biosynthesis by mutations in steroid metabolizing enzymes, or their redox partners cause metabolic disorders [53-64]. Non-tumoral cases of hyperandrogenism include the most common metabolic disorder in females, the polycystic ovary syndrome, congenital adrenal hyperplasia cuased by to 21-hydroxylase deficiency and Cushing syndrome [65-68]. Overproduction of cortisol and androgens could be therapeutically controlled by inhibitors that block specific steps in steroid biosynthesis.

CYP17A1 catalyzes multiple reactions in the steroid pathway [30, 69-71]. Main activities of CYP17A1 include the 17α-hydroxylase activity needed for the biosynthesis of 17OH-pregnenolone (17OH-PREG) and 17OH-progesterone (17OH-PROG,) the precursors of cortisol. The CYP17A1 provides qualitative regulation of steroid production in humans by its 17,20 lyase activity that produces dehydroepiandrosterone (DHEA), the precursor of sex steroids. The two distinct enzyme activities of the CYP17A1 dictate the nature of steroids synthesized in different cells types [39, 72, 73]. Overproduction of androgens by the activation of CYP17A1-17,20 lyase activity has been linked to polycystic ovary syndrome [72]. CYP17A1 is a metabolic target for chemotherapy of castration-resistant prostate cancer [49, 74-77].

Novel compounds that are safe and non-toxic are needed to target CYP17A1 and CYP19A1 activities to treat metabolic disorders resulting from excess production of androgens or estrogens. Here we have probed the potential of curcuminoids as possible test candidates for synthesizing novel chemicals to target CYP17A1 and CYP21A2. We found a dose-dependent inhibition of both the CYP17A1 and CYP19A1 by curcuminoids. In our current study, curcumin caused a reduction of CYP21A2 activity. A 50% lower CYP21A2 activity was observed at 10 µg/ml concentration of curcumin. Curcuminoids were found not to inhibit 5α-reductase activity. Based on these results, we can conclude that curcumin causes an intricate pattern of changes in steroid metabolites due to its inhibition of CYP19A1 and CYP21A2 activities in addition to inhibition of 17α-hydroxylase as well as 17,20 lyase activity of CYP17A1.

Our studies provide an analysis of the inhibitory effects of curcumin on CYP17A1 and CYP19A1 activities. These results indicate that the steroid production in people with high amounts of Curcuma consumption may be affected not only by the inhibition of CYP17A1 but also by the CYP19A1 and to a smaller extent, CYP21A2 inhibition. Also, use of curcumin as a common over the counter health supplement requires further caution as inhibition of both the CYP17A1 as well as the CYP19A1, may potentially result in complications of steroid metabolism in both males and females. Inhibitors of steroidogenesis act on multiple steps in steroid biosynthesis. Many drugs have been approved for use as steroid biosynthesis inhibitors (Ketoconazole, Metyrapone, Etomidate, Mitotane). All these drugs inhibit the CYP17A1, CYP11A1, CYP11B1, and CYP19A1 [78], and many other candidate molecules are under examination [78-80]. Also, many inhibitors which can block androgen biosynthesis in androgen-dependent prostate cancers are being studied [50, 81-86].

Computational docking showed that curcumin could bind into the active sites of steroid metabolizing P450s and therefore, may act as a competitive inhibitor. The inhibition of CYP17A1 and CYP19A1 by curcuminoids also provides a template for modification to produce effective and safe compounds that can target prostate cancer as well as breast cancer. Lesser inhibition of CYP21A2 activity by curcuminoids compared to inhibition of CYP17A1 (50% inhibition of 17,20 lyase activity at 5 µg/ml compared to no significant effect on CYP21A2 activity at same concentration) was found. This suggests that compounds based on curcumin may be better and safer inhibitors for use in hyperandrogenic states like polycystic ovary syndrome, especially in children and young adults and avoid the toxic effects of abiraterone which severely inhibits CYP21A2 activity [49, 50].

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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References

[1] E. Schraufstatter, H. Bernt, Antibacterial action of curcumin and related compounds, Nature 164(4167) (1949) 456.

[2] H.P. Ammon, M.A. Wahl, Pharmacology of Curcuma longa, Planta Med 57(1) (1991) 1-7.

[3] G.C. Jagetia, B.B. Aggarwal, "Spicing up" of the immune system by curcumin, J Clin Immunol 27(1) (2007) 19-35.

[4] R.K. Maheshwari, A.K. Singh, J. Gaddipati, R.C. Srimal, Multiple biological activities of curcumin: a short review, Life Sci 78(18) (2006) 2081-2087.

[5] S. Parsai, R. Keck, E. Skrzypczak-Jankun, J. Jankun, Analysis of the anticancer activity of curcuminoids, thiotryptophan and 4-phenoxyphenol derivatives, Oncology letters 7(1) (2014) 17-22.

[6] R. Kuttan, P. Bhanumathy, K. Nirmala, M.C. George, Potential anticancer activity of turmeric (Curcuma longa), Cancer Lett 29(2) (1985) 197-202.

412 [7] P. Anand, C. Sundaram, S. Jhurani, A.B. Kunnumakkara, B.B. Aggarwal, Curcumin and cancer:
413 an "old-age" disease with an "age-old" solution, Cancer letters 267(1) (2008) 133-164.
414 [8] S.C. Gupta, S. Patchva, B.B. Aggarwal, Therapeutic roles of curcumin: lessons learned from
415 clinical trials, The AAPS journal 15(1) (2013) 195-218.
416 [9] P. Anand, A.B. Kunnumakkara, R.A. Newman, B.B. Aggarwal, Bioavailability of curcumin:
417 problems and promises, Molecular pharmaceutics 4(6) (2007) 807-818.
418 [10] P. Anand, S.G. Thomas, A.B. Kunnumakkara, C. Sundaram, K.B. Harikumar, B. Sung, S.T.
419 Tharakan, K. Misra, I.K. Priyadarsini, K.N. Rajasekharan, B.B. Aggarwal, Biological activities of
420 curcumin and its analogues (Congeners) made by man and Mother Nature, Biochemical
421 pharmacology 76(11) (2008) 1590-1611.
422 [11] C.M. Mach, J.H. Chen, S.A. Mosley, R. Kurzrock, J.A. Smith, Evaluation of liposomal curcumin
423 cytochrome p450 metabolism, Anticancer research 30(3) (2010) 811-814.
424 [12] S.C. Gupta, S. Patchva, W. Koh, B.B. Aggarwal, Discovery of curcumin, a component of golden
425 spice, and its miraculous biological activities, Clin Exp Pharmacol Physiol 39(3) (2012) 283-299.
426 [13] G. Kumar, S. Mittal, K. Sak, H.S. Tuli, Molecular mechanisms underlying chemopreventive
427 potential of curcumin: Current challenges and future perspectives, Life Sciences 148 (2016) 313-328.
428 [14] G.C. Jagetia, B.B. Aggarwal, "Spicing up" of the immune system by curcumin, Journal of
429 Clinical Immunology 27(1) (2007) 19-35.
430 [15] K. Karlowicz-Bodalska, S. Han, J. Freier, M. Smolenski, A. Bodalska, Curcuma Longa as
431 Medicinal Herb in the Treatment of Diabetic Complications, Acta Pol Pharm 74(2) (2017) 605-610.
432 [16] T. Khaliq, M. Sarfraz, M.A. Ashraf, Recent Progress for the Utilization of Curcuma longa, Piper
433 nigrum and Phoenix dactylifera Seeds against Type 2 Diabetes, West Indian Med J 64(5) (2015) 527-
434 532.
435 [17] A. Hosseini, H. Hosseinzadeh, Antidotal or protective effects of Curcuma longa (turmeric) and
436 its active ingredient, curcumin, against natural and chemical toxicities: A review, Biomedicine &
437 pharmacotherapy = Biomedecine & pharmacotherapie 99 (2018) 411-421.
438 [18] M.A. Pufall, Glucocorticoids and Cancer, Advances in experimental medicine and biology 872
439 (2015) 315-333.
440 [19] S.P. Valentine, M.J. Le Nedelec, A.R. Menzies, M.J. Scandlyn, M.G. Goodin, R.J. Rosengren,
441 Curcumin modulates drug metabolizing enzymes in the female Swiss Webster mouse, Life Sci 78(20)
442 (2006) 2391-2398.
443 [20] M. Shozu, K. Akasofu, T. Harada, Y. Kubota, A new cause of female pseudohermaphroditism:
444 placental aromatase deficiency, J Clin Endocrinol Metab 72(3) (1991) 560-566.
445 [21] N. Harada, H. Ogawa, M. Shozu, K. Yamada, K. Suhara, E. Nishida, Y. Takagi, Biochemical and
446 molecular genetic analyses on placental aromatase (P-450AROM) deficiency, J Biol Chem 267(7)
447 (1992) 4781-4785.
448 [22] E.R. Simpson, Aromatase cytochrome P450, the enzyme responsible for estrogen
449 biosynthesis, Endocr. Rev. 15 (1994) 342-355.
450 [23] U.M. Zanger, M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene
451 expression, enzyme activities, and impact of genetic variation, Pharmacol Ther 138(1) (2013) 103-
452 141.
453 [24] A. Zalewski, N.S. Ma, B. Legeza, N. Renthal, C.E. Flück, A.V. Pandey, Vitamin D-Dependent
454 Rickets Type 1 Caused by Mutations in CYP27B1 Affecting Protein Interactions With Adrenodoxin, J
455 Clin Endocrinol Metab 101(9) (2016) 3409-3418.
456 [25] T. Omura, Mitochondrial P450s, Chem Biol Interact 163(1-2) (2006) 86-93.
457 [26] T. Omura, Contribution of cytochrome P450 to the diversification of eukaryotic organisms,
458 Biotechnol Appl Biochem 60(1) (2013) 4-8.
459 [27] B.C. Chung, K.J. Matteson, W.L. Miller, Structure of a bovine gene for P-450c21 (steroid 21-
460 hydroxylase) defines a novel cytochrome P-450 gene family, Proc Natl Acad Sci U S A 83(12) (1986)
461 4243-4247.
462 [28] B.C. Chung, K.J. Matteson, R. Voutilainen, T.K. Mohandas, W.L. Miller, Human cholesterol
463 side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and
464 expression in the placenta, Proc Natl Acad Sci U S A 83(23) (1986) 8962-8966.

465 [29] K.J. Matteson, B.C. Chung, M.S. Urdea, W.L. Miller, Study of cholesterol side-chain cleavage
466 (20,22 desmolase) deficiency causing congenital lipoid adrenal hyperplasia using bovine-sequence
467 P450scc oligodeoxyribonucleotide probes, Endocrinology 118(4) (1986) 1296-1305.
468 [30] B.C. Chung, J. Picado-Leonard, M. Haniu, M. Bienkowski, P.F. Hall, J.E. Shively, W.L. Miller,
469 Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17,20 lyase): cloning of human adrenal and testis
470 cDNAs indicates the same gene is expressed in both tissues, Proc Natl Acad Sci U S A 84(2) (1987)
471 407-411.
472 [31] Y. Morel, J. Picado-Leonard, D.A. Wu, C.Y. Chang, T.K. Mohandas, B.C. Chung, W.L. Miller,
473 Assignment of the functional gene for human adrenodoxin to chromosome 11q13----qter and of
474 adrenodoxin pseudogenes to chromosome 20cen----q13.1, Am J Hum Genet 43(1) (1988) 52-59.
475 [32] A.V. Pandey, C.E. Flück, NADPH P450 oxidoreductase: structure, function, and pathology of
476 diseases, Pharmacol Ther 138(2) (2013) 229-254.
477 [33] C.E. Flück, A.V. Pandey, Steroidogenesis of the testis -- new genes and pathways, Ann
478 Endocrinol (Paris) 75(2) (2014) 40-47.
479 [34] S.B. Kim, S.S. Cho, H.J. Cho, I.S. Yoon, Modulation of Hepatic Cytochrome P450 Enzymes by
480 Curcumin and its Pharmacokinetic Consequences in Sprague-dawley Rats, Pharmacognosy magazine
481 11(Suppl 4) (2015) S580-584.
482 [35] T. Sasaki, Y. Sato, T. Kumagai, K. Yoshinari, K. Nagata, Effect of health foods on cytochrome
483 P450-mediated drug metabolism, Journal of pharmaceutical health care and sciences 3 (2017) 14.
484 [36] R. Appiah-Opong, J.N. Commandeur, B. van Vugt-Lussenburg, N.P. Vermeulen, Inhibition of
485 human recombinant cytochrome P450s by curcumin and curcumin decomposition products,
486 Toxicology 235(1-2) (2007) 83-91.
487 [37] P. Phattanawasin, U. Sotanaphun, L. Sriphong, Validated TLC-Image Analysis Method for
488 Simultaneous Quantification of Curcuminoids in Curcuma longa, Chromatographia 69(3) (2009) 397-
489 400.
490 [38] E. Katharopoulos, K. Sauter, A.V. Pandey, C.E. Fluck, In silico and functional studies reveal
491 novel loss-of-function variants of SRD5A2, but no variants explaining excess 5alpha-reductase
492 activity, J Steroid Biochem Mol Biol 190 (2019) 263-272.
493 [39] A.V. Pandey, W.L. Miller, Regulation of 17,20 lyase activity by cytochrome b5 and by serine
494 phosphorylation of P450c17, J Biol Chem 280(14) (2005) 13265-13271.
495 [40] E.D. Lephart, E.R. Simpson, [45] Assay of aromatase activity, Methods in Enzymology,
496 Academic Press, 1991. pp. 477-483.
497 [41] E. Krieger, T. Darden, S.B. Nabuurs, A. Finkelstein, G. Vriend, Making optimal use of empirical
498 energy functions: force-field parameterization in crystal space, Proteins 57(4) (2004) 678-683.
499 [42] G. Vriend, WHAT IF: a molecular modeling and drug design program, J Mol Graph 8(1) (1990)
500 52-56, 29.
501 [43] N.M. DeVore, E.E. Scott, Structures of cytochrome P450 17A1 with prostate cancer drugs
502 abiraterone and TOK-001, Nature 482(7383) (2012) 116-119.
503 [44] D. Ghosh, J. Griswold, M. Erman, W. Pangborn, Structural basis for androgen specificity and
504 oestrogen synthesis in human aromatase, Nature 457(7226) (2009) 219-223.
505 [45] P.S. Pallan, C. Wang, L. Lei, F.K. Yoshimoto, R.J. Auchus, M.R. Waterman, F.P. Guengerich, M.
506 Egli, Human Cytochrome P450 21A2, the Major Steroid 21-Hydroxylase: STRUCTURE OF THE
507 ENZYME·PROGESTERONE SUBSTRATE COMPLEX AND RATE-LIMITING C–H BOND CLEAVAGE, Journal
508 of Biological Chemistry 290(21) (2015) 13128-13143.
509 [46] Y. Duan, C. Wu, S. Chowdhury, M.C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T.
510 Lee, J. Caldwell, J. Wang, P. Kollman, A point-charge force field for molecular mechanics simulations
511 of proteins based on condensed-phase quantum mechanical calculations, Journal of Computational
512 Chemistry 24(16) (2003) 1999-2012.
513 [47] W.L. Jorgensen, J. Tirado-Rives, Potential energy functions for atomic-level simulations of
514 water and organic and biomolecular systems, Proc Natl Acad Sci U S A 102(19) (2005) 6665-6670.
515 [48] O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new
516 scoring function, efficient optimization, and multithreading, Journal of Computational Chemistry
517 31(2) (2010) 455-461.

[49] J. Malikova, S. Brixius-Anderko, S.S. Udhane, S. Parween, B. Dick, R. Bernhardt, A.V. Pandey, CYP17A1 inhibitor abiraterone, an anti-prostate cancer drug, also inhibits the 21-hydroxylase activity of CYP21A2, J Steroid Biochem Mol Biol 174 (2017) 192-200.
[50] S.S. Udhane, B. Dick, Q. Hu, R.W. Hartmann, A.V. Pandey, Specificity of anti-prostate cancer CYP17A1 inhibitors on androgen biosynthesis, Biochem Biophys Res Commun 477(4) (2016) 1005-1010.
[51] J. Lindholm, S. Juul, J.O. Jorgensen, J. Astrup, P. Bjerre, U. Feldt-Rasmussen, C. Hagen, J. Jorgensen, M. Kosteljanetz, L. Kristensen, P. Laurberg, K. Schmidt, J. Weeke, Incidence and late prognosis of cushing's syndrome: a population-based study, J Clin Endocrinol Metab 86(1) (2001) 117-123.
[52] O.M. Dekkers, E. Horváth-Puhó, J.O.L. Jørgensen, S.C. Cannegieter, V. Ehrenstein, J.P. Vandenbroucke, A.M. Pereira, H.T. Sørensen, Multisystem Morbidity and Mortality in Cushing's Syndrome: A Cohort Study, The Journal of Clinical Endocrinology & Metabolism 98(6) (2013) 2277-2284.
[53] G. Öcal, M. Berberoğlu, Z. Sıklar, Z. Aycan, B. Hacıhamdioglu, Ş.S. Erdeve, E. Çamtosun, P. Kocaay, H.I. Ruhi, B.G. Kılıç, A. Tukun, Clinical Review of 95 Patients with 46,XX Disorders of Sex Development Based on the New Chicago Classification, Journal of Pediatric and Adolescent Gynecology 28(1) (2015) 6-11.
[54] C.E. Flück, M. Meyer-Boni, A.V. Pandey, P. Kempna, W.L. Miller, E.J. Schoenle, A. Biason-Lauber, Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation, Am J Hum Genet 89(2) (2011) 201-218.
[55] N. Bouchoucha, D. Samara-Boustani, A.V. Pandey, H. Bony-Trifunovic, G. Hofer, Y. Aigrain, M. Polak, C.E. Flück, Characterization of a novel CYP19A1 (aromatase) R192H mutation causing virilization of a 46,XX newborn, undervirilization of the 46,XY brother, but no virilization of the mother during pregnancies, Mol Cell Endocrinol 390(1-2) (2014) 8-17.
[56] N. Camats, A.V. Pandey, M. Fernandez-Cancio, J.M. Fernandez, A.M. Ortega, S. Udhane, P. Andaluz, L. Audi, C.E. Flück, STAR splicing mutations cause the severe phenotype of lipoid congenital adrenal hyperplasia: insights from a novel splice mutation and review of reported cases, Clin Endocrinol (Oxf) 80(2) (2014) 191-199.
[57] C.E. Flück, A.V. Pandey, Testicular Steroidogenesis, in: M. Simoni, I.T. Huhtaniemi (Eds.), Endocrinology of the Testis and Male Reproduction, Springer International Publishing, Cham, 2017. pp. 343-371.
[58] C.E. Flück, T. Tajima, A.V. Pandey, W. Arlt, K. Okuhara, C.F. Verge, E.W. Jabs, B.B. Mendonca, K. Fujieda, W.L. Miller, Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome, Nat Genet 36(3) (2004) 228-230.
[59] N. Huang, A.V. Pandey, V. Agrawal, W. Reardon, P.D. Lapunzina, D. Mowat, E.W. Jabs, G. Van Vliet, J. Sack, C.E. Flück, W.L. Miller, Diversity and function of mutations in p450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis, Am J Hum Genet 76(5) (2005) 729-749.
[60] M. Janner, A.V. Pandey, P.E. Mullis, C.E. Flück, Clinical and biochemical description of a novel CYP21A2 gene mutation 962_963insA using a new 3D model for the P450c21 protein, Eur J Endocrinol 155(1) (2006) 143-151.
[61] I. Martinez de LaPiscina, C. de Mingo, S. Riedl, A. Rodriguez, A.V. Pandey, M. Fernandez-Cancio, N. Camats, A. Sinclair, L. Castano, L. Audi, C.E. Fluck, GATA4 Variants in Individuals With a 46,XY Disorder of Sex Development (DSD) May or May Not Be Associated With Cardiac Defects Depending on Second Hits in Other DSD Genes, Front Endocrinol (Lausanne) 9 (2018) 142.
[62] W.L. Miller, N. Huang, C.E. Flück, A.V. Pandey, P450 oxidoreductase deficiency, Lancet 364(9446) (2004) 1663.
[63] A.V. Pandey, P. Kempna, G. Hofer, P.E. Mullis, C.E. Flück, Modulation of human CYP19A1 activity by mutant NADPH P450 oxidoreductase, Mol Endocrinol 21(10) (2007) 2579-2595.
[64] S. Parween, F. Roucher-Boulez, C.E. Flück, A. Lienhardt-Roussie, D. Mallet, Y. Morel, A.V. Pandey, P450 Oxidoreductase Deficiency: Loss of Activity Caused by Protein Instability From a Novel L374H Mutation, J Clin Endocrinol Metab 101(12) (2016) 4789-4798.

[65] E. Carmina, F. Rosato, A. Jannì, M. Rizzo, R.A. Longo, Relative Prevalence of Different Androgen Excess Disorders in 950 Women Referred because of Clinical Hyperandrogenism, The Journal of Clinical Endocrinology & Metabolism 91(1) (2006) 2-6.
[66] A. Kumar, K.S. Woods, A.A. Bartolucci, R. Azziz, Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS), Clinical Endocrinology 62(6) (2005) 644-649.
[67] R. Azziz, L.A. Sanchez, E.S. Knochenhauer, C. Moran, J. Lazenby, K.C. Stephens, K. Taylor, L.R. Boots, Androgen Excess in Women: Experience with Over 1000 Consecutive Patients, The Journal of Clinical Endocrinology & Metabolism 89(2) (2004) 453-462.
[68] N. Krone, W. Arlt, Genetics of congenital adrenal hyperplasia, Best Practice & Research Clinical Endocrinology & Metabolism 23(2) (2009) 181-192.
[69] M.X. Zuber, E.R. Simpson, M.R. Waterman, Expression of bovine 17 alpha-hydroxylase cytochrome P-450 cDNA in nonsteroidogenic (COS 1) cells, Science 234(4781) (1986) 1258-1261.
[70] S. Nakajin, M. Shinoda, M. Haniu, J.E. Shively, P.F. Hall, C21 steroid side chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17 alpha-hydroxylase/C17,20-lyase cytochrome P-450, J Biol Chem 259(6) (1984) 3971-3976.
[71] M. Fernandez-Cancio, N. Camats, C.E. Fluck, A. Zalewski, B. Dick, B.M. Frey, R. Monne, N. Toran, L. Audi, A.V. Pandey, Mechanism of the Dual Activities of Human CYP17A1 and Binding to Anti-Prostate Cancer Drug Abiraterone Revealed by a Novel V366M Mutation Causing 17,20 Lyase Deficiency, Pharmaceuticals 11(2) (2018).
[72] W.L. Miller, R.J. Auchus, The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders, Endocr Rev 32(1) (2011) 81-151.
[73] A.V. Pandey, S.H. Mellon, W.L. Miller, Protein phosphatase 2A and phosphoprotein SET regulate androgen production by P450c17, J Biol Chem 278(5) (2003) 2837-2844.
[74] T.S. Vasaitis, R.D. Bruno, V.C. Njar, CYP17 inhibitors for prostate cancer therapy, J Steroid Biochem Mol Biol 125(1-2) (2011) 23-31.
[75] G. Attard, A.H. Reid, R.J. Auchus, B.A. Hughes, A.M. Cassidy, E. Thompson, N.B. Oommen, E. Folkerd, M. Dowsett, W. Arlt, J.S. de Bono, Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer, J Clin Endocrinol Metab 97(2) (2012) 507-516.
[76] J.S. de Bono , C.J. Logothetis , A. Molina , K. Fizazi , S. North , L. Chu , K.N. Chi , R.J. Jones , O.B.J. Goodman , F. Saad , J.N. Staffurth , P. Mainwaring , S. Harland , T.W. Flaig , T.E. Hutson , T. Cheng , H. Patterson , J.D. Hainsworth , C.J. Ryan , C.N. Sternberg , S.L. Ellard , A. Fléchon , M. Saleh , M. Scholz , E. Efstathiou , A. Zivi , D. Bianchini , Y. Loriot , N. Chieffo , T. Kheoh , C.M. Haqq , H.I. Scher Abiraterone and Increased Survival in Metastatic Prostate Cancer, New England Journal of Medicine 364(21) (2011) 1995-2005.
[77] G. Attard, A.H. Reid, T.A. Yap, F. Raynaud, M. Dowsett, S. Settatree, M. Barrett, C. Parker, V. Martins, E. Folkerd, J. Clark, C.S. Cooper, S.B. Kaye, D. Dearnaley, G. Lee, J.S. de Bono, Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 26(28) (2008) 4563-4571.
[78] M. Fleseriu, F. Castinetti, Updates on the role of adrenal steroidogenesis inhibitors in Cushing's syndrome: a focus on novel therapies, Pituitary 19(6) (2016) 643-653.
[79] X. Bertagna, R. Pivonello, M. Fleseriu, Y. Zhang, P. Robinson, A. Taylor, C.E. Watson, M. Maldonado, A.H. Hamrahian, M. Boscaro, B.M.K. Biller, LCI699, a Potent 11β-hydroxylase Inhibitor, Normalizes Urinary Cortisol in Patients With Cushing's Disease: Results From a Multicenter, Proof-of-Concept Study, The Journal of Clinical Endocrinology & Metabolism 99(4) (2014) 1375-1383.
[80] M. Fleseriu, R. Pivonello, J. Young, A.H. Hamrahian, M.E. Molitch, C. Shimizu, T. Tanaka, A. Shimatsu, T. White, A. Hilliard, C. Tian, N. Sauter, B.M.K. Biller, X. Bertagna, Osilodrostat, a potent oral 11β-hydroxylase inhibitor: 22-week, prospective, Phase II study in Cushing's disease, Pituitary 19 (2016) 138-148.
[81] B. Montgomery, M.A. Eisenberger, M.B. Rettig, F. Chu, R. Pili, J.J. Stephenson, N.J. Vogelzang, A.J. Koletsky, L.T. Nordquist, W.J. Edenfield, K. Mamlouk, K.J. Ferrante, M.-E. Taplin, Androgen

Receptor Modulation Optimized for Response (ARMOR) Phase I and II Studies: Galeterone for the Treatment of Castration-Resistant Prostate Cancer, Clinical Cancer Research 22(6) (2016) 1356-1363.
[82] K. Fizazi, R. Jones, S. Oudard, E. Efstathiou, F. Saad, R.d. Wit, J.D. Bono, F.M. Cruz, G. Fountzilas, A. Ulys, F. Carcano, N. Agarwal, D. Agus, J. Bellmunt, D.P. Petrylak, S.-Y. Lee, I.J. Webb, B. Tejura, N. Borgstein, R. Dreicer, Phase III, Randomized, Double-Blind, Multicenter Trial Comparing Orteronel (TAK-700) Plus Prednisone With Placebo Plus Prednisone in Patients With Metastatic Castration-Resistant Prostate Cancer That Has Progressed During or After Docetaxel-Based Therapy: ELM-PC 5, Journal of Clinical Oncology 33(7) (2015) 723-731.
[83] T. Kaku, T. Hitaka, A. Ojida, N. Matsunaga, M. Adachi, T. Tanaka, T. Hara, M. Yamaoka, M. Kusaka, T. Okuda, S. Asahi, S. Furuya, A. Tasaka, Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer, Bioorganic & medicinal chemistry 19(21) (2011) 6383-6399.
[84] I. Schuster, R. Bernhardt, Inhibition of cytochromes p450: existing and new promising therapeutic targets, Drug Metab Rev 39(2-3) (2007) 481-499.
[85] T. Hakki, R. Bernhardt, CYP17- and CYP11B-dependent steroid hydroxylases as drug development targets, Pharmacol Ther 111(1) (2006) 27-52.
[86] S.S. Udhane, S. Parween, N. Kagawa, A.V. Pandey, Altered CYP19A1 and CYP3A4 Activities Due to Mutations A115V, T142A, Q153R and P284L in the Human P450 Oxidoreductase, Frontiers in Pharmacology 8 (2017).

Figure Legends

Figure 1: Activity of curcumin on different biological processes. Curcumin is a common ingredient in the cuisine of many Asian countries and has been used in traditional medicine for centuries. Several medicinal properties have been linked to curcumin that ranges from antiproliferative activity in cancers, an antioxidant activity as well as anti-inflammatory, anti-bacterial, antifungal activities.

Figure 2: Curcuminoids, chemical structure, and separation. A thin layer chromatography separation is shown indicating the different curcuminoids present in turmeric extracts. A major component of turmeric is curcumin, desmethoxycurcumin, and bis-desmethoxycurcumin.

Figure 3: Synthesis of steroid hormones from cholesterol in humans. After entering the mitochondrion, cholesterol is converted into pregnenolone, which is used as a substrate by CYP17A1 in the endoplasmic reticulum to produce sex steroids. The CYP19A1 converts androgens into estrogens. Abbreviations: Preg=pregnenolone, Prog=progesterone, DOC=deoxycorticosterone, 11DOC=11-deoxycortisol, DHEA=dehydroepiandrosterone, HSD3B2=3β-hydroxysteroid dehydrogenase type 2, HSD17B1/2=17β-hydroxysteroid dehydrogenase type 1/2, SRD5A2=5α-reductase type 2.

Figure 4: Cell Viability Assays. Cell toxicity and viability of human adrenal NCI-H295R cells were determined using a range of curcuminoids between 100-0.78 µg/ml over 24 h. Cells were treated with varying dosages of curcumin, and cell viability was determined by MTT assay. No significant effect on viability of cells was observed at 25 µg/ml or lower doses of curcumin. In subsequent experiments, the time of incubations was much shorter (few hours), and the concentration of curcumin was 10 µg/ml or lower in most experiments.

Figure 5. Effect of curcumin on steroid production. **A.** Curcumin inhibited 17OH-pregnenolone and DHEA production in human adrenal NCI-H295R cells. A block of DHEA production indicates that curcumin was causing inhibition of both the 17α-hydroxylase as well as 17,20 lyase activities of CYP17A1. **B.** Effect of curcumin on SRD5A2 activity. Curcumin did not cause inhibition of 5α-reductase activity. A known inhibitor of 5α-reductase, finasteride, caused complete blockage of activity.

Figure 6: Inhibitory effects of curcuminoids on CYP17A1 activity. Abiraterone, a known inhibitor for CYP17A1, was used as a control. Each experiment was done in triplicate. **A.** Inhibition of CYP17A1 at a single curcumin concentration of 10μg/ml. *$p<0.05$, **$p<0.05$. **B.** Differential inhibition of curcuminoids on CYP17A1 17α-hydroxylase activity and **C.** Differential inhibition of curcuminoids on CYP17A1 17, 20 lyase activity. ***$p<0.05$. The CYP17A1 converts pregnenolone into 17α-hydroxypregnenolone by its 17α-hydroxylase activity, and 17α-hydroxypregnenolone is converted into DHEA by the 17,20 lyase activity of CYP17A1.

Figure 7: Effect of curcuminoids on CYP21A2 activity. Bioactivity of curcuminoids was measured at two concentrations on CYP21A2. For assay of CYP21A2 activity, 17α-hydroxyprogesterone was used as substrate and reaction was monitored by measuring the production of 11-deoxycortisol by TLC and phosphor image analysis. The activity of CYP21A2 was reduced to 50% of control at 10 μg/ml. Data shown here are from the mean and standard deviation of three independent experiments.

Figure 8: Effect of curcuminoids on CYP19A1 activity. A dose-response curve is showing the inhibition of aromatase while increasing the concentration of curcuminoids in the enzyme

preparation of endoplasmic reticulum obtained from placental JEG3 cells. For assay of aromatase activity of CYP19A1, tritium-labeled androstenedione was used as a substrate, and product formation was monitored by quantifying the amount of tritiated water released by CYP19A1 using scintillation counting. A known inhibitor of CYP19A1, anastrozole, was used as control at a dose of 100 nM.

Figure 9: Docking of curcumin into the protein structures of CYP17A1, CYP19A1, and CYP21A2. Published crystal structures of CYP17A1, CYP19A1, and CYP21A2 were used for docking of curcumin by software AUTODOCK-VINA as described in materials and methods. Bound steroid ligands were removed before docking of curcumin into the active site of P450s. The poses in which curcumin docks are similar to steroid substrates of all three P450s, with closer fitting in case of CYP17A1 and CYP19A1 as compared to CYP21A2 (distance from heme 2.5 Å for CYP17A1/CYP19A1 versus 3.4 Å for CYP21A2).

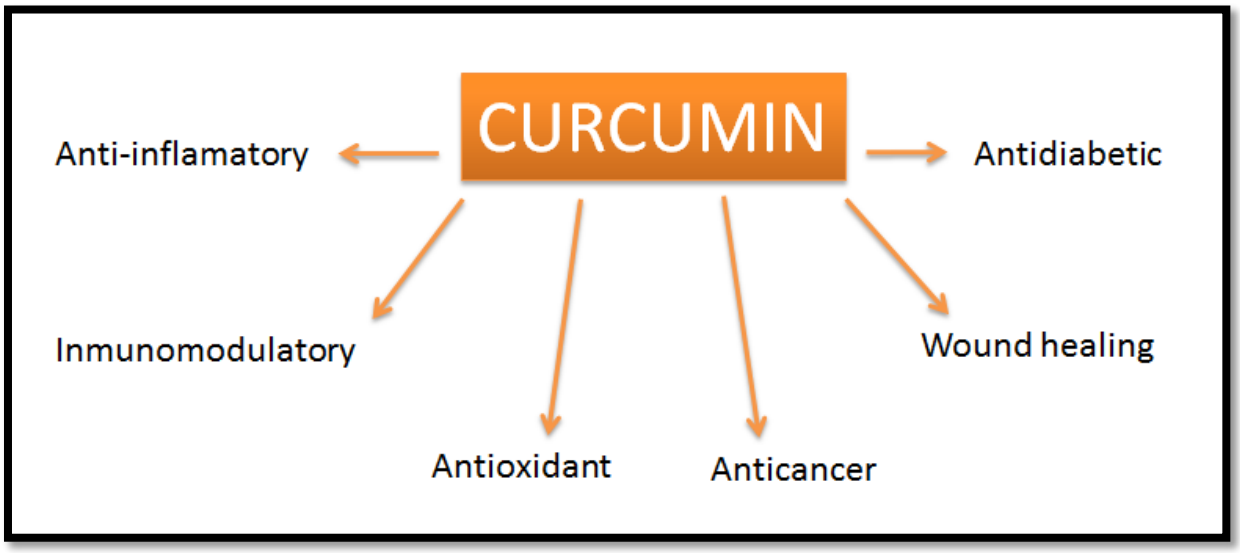

CURCUMIN
Anti-inflamatory
Antidiabetic
Inmunomodulatory
Wound healing
Antioxidant
Anticancer

Figure 1

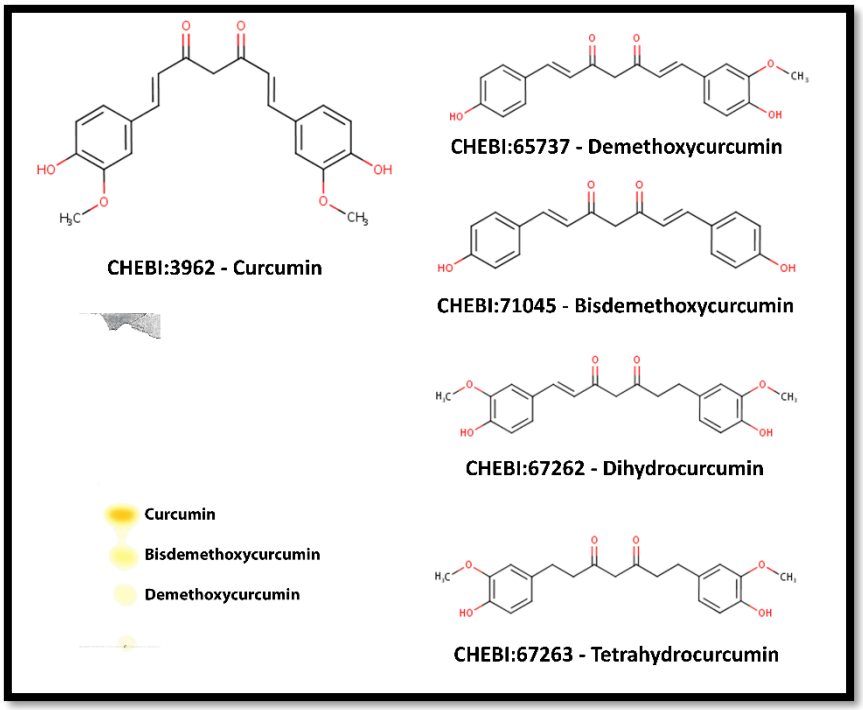
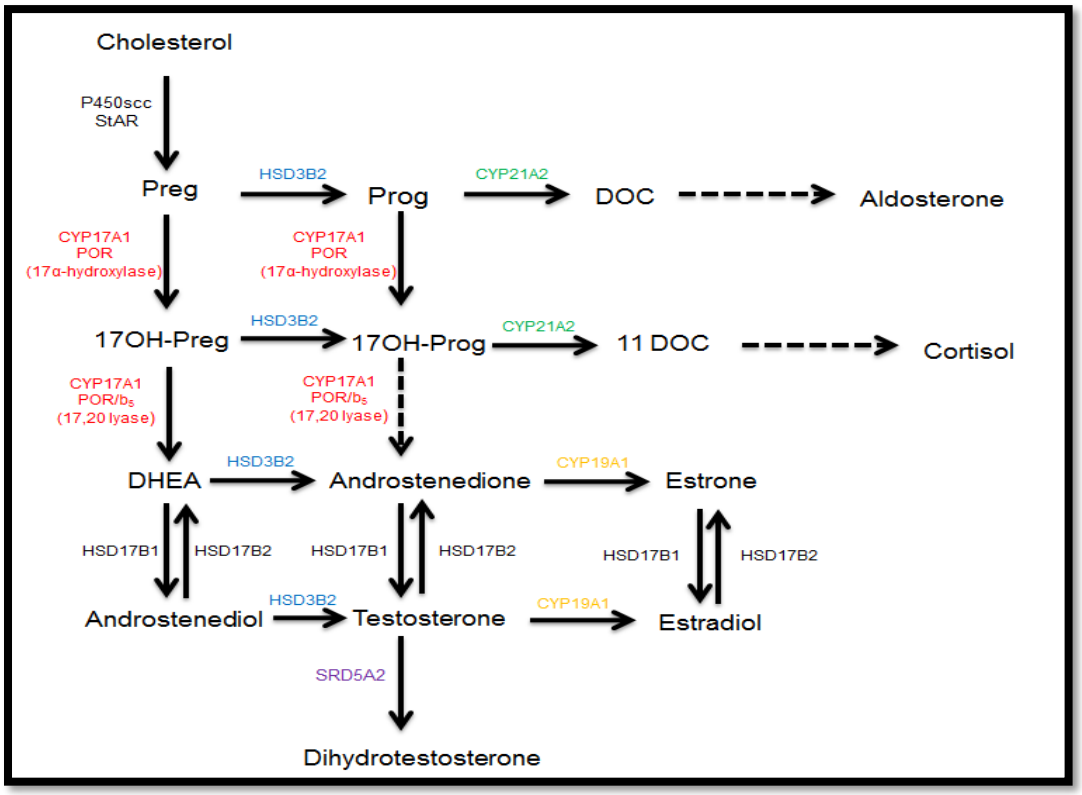

CHEBI:3962 - Curcumin
CHEBI:65737 - Demethoxycurcumin
CHEBI:71045 - Bisdemethoxycurcumin
CHEBI:67262 - Dihydrocurcumin
CHEBI:67263 - Tetrahydrocurcumin
Curcumin
Bisdemethoxycurcumin
Demethoxycurcumin

Figure 2


Cholesterol
P450scc
StAR
Preg
HSD3B2
Prog
CYP21A2
DOC
Aldosterone
CYP17A1
POR
(17α-hydroxylase)
CYP17A1
POR
(17α-hydroxylase)
17OH-Preg
HSD3B2
17OH-Prog
CYP21A2
11 DOC
Cortisol
CYP17A1
POR/b5
(17,20 lyase)
CYP17A1
POR/b5
(17,20 lyase)
DHEA
HSD3B2
Androstenedione
CYP19A1
Estrone
HSD17B1
HSD17B2
HSD17B1
HSD17B2
HSD17B1
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Androstenediol
HSD3B2
Testosterone
CYP19A1
Estradiol
SRD5A2
Dihydrotestosterone

Figure 3

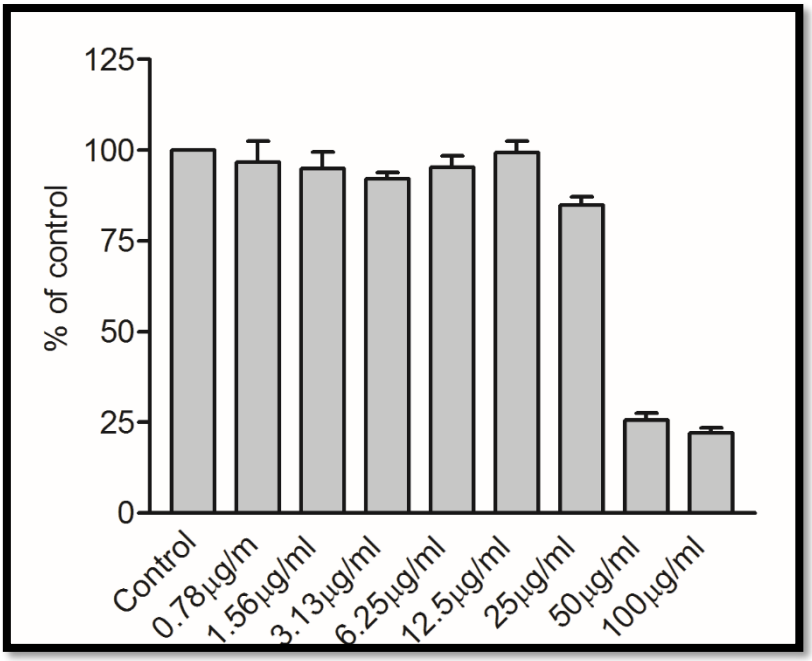
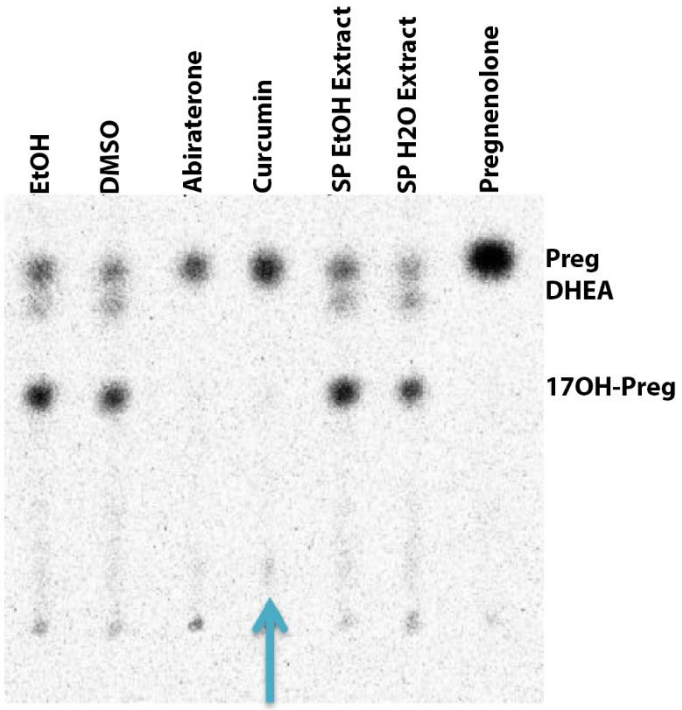

125
100
75
50
25
0
% of control
Control
0.78µg/m
1.56µg/ml
3.13µg/ml
6.25µg/ml
12.5µg/ml
25µg/ml
50µg/ml
100µg/ml

Figure 4

A


EtOH
DMSO
Abiraterone
Curcumin
SP EtOH Extract
SP H2O Extract
Pregnenolone
Preg
DHEA
17OH-Preg

B

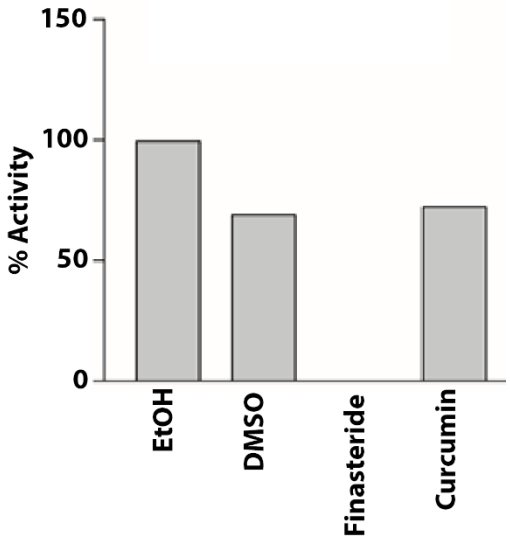

150
100
50
0
% Activity
EtOH
DMSO
Finasteride
Curcumin

Figure 5

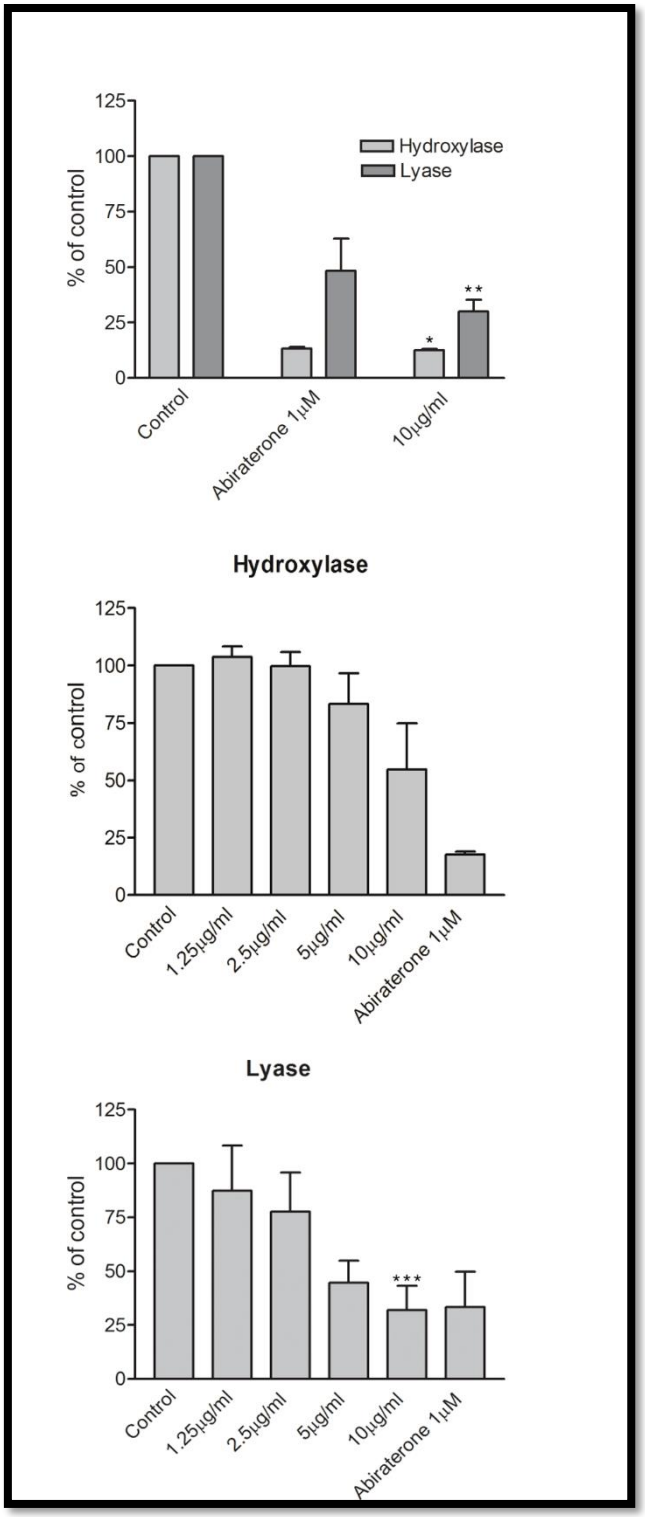

125
100
75
50
25
0
% of control
Hydroxylase
Lyase
*
**
Control
Abiraterone 1μM
10μg/ml
Hydroxylase
125
100
75
50
25
0
% of control
Control
1.25μg/ml
2.5μg/ml
5μg/ml
10μg/ml
Abiraterone 1μM
Lyase
125
100
75
50
25
0
% of control
***
Control
1.25μg/ml
2.5μg/ml
5μg/ml
10μg/ml
Abiraterone 1μM

Figure 6

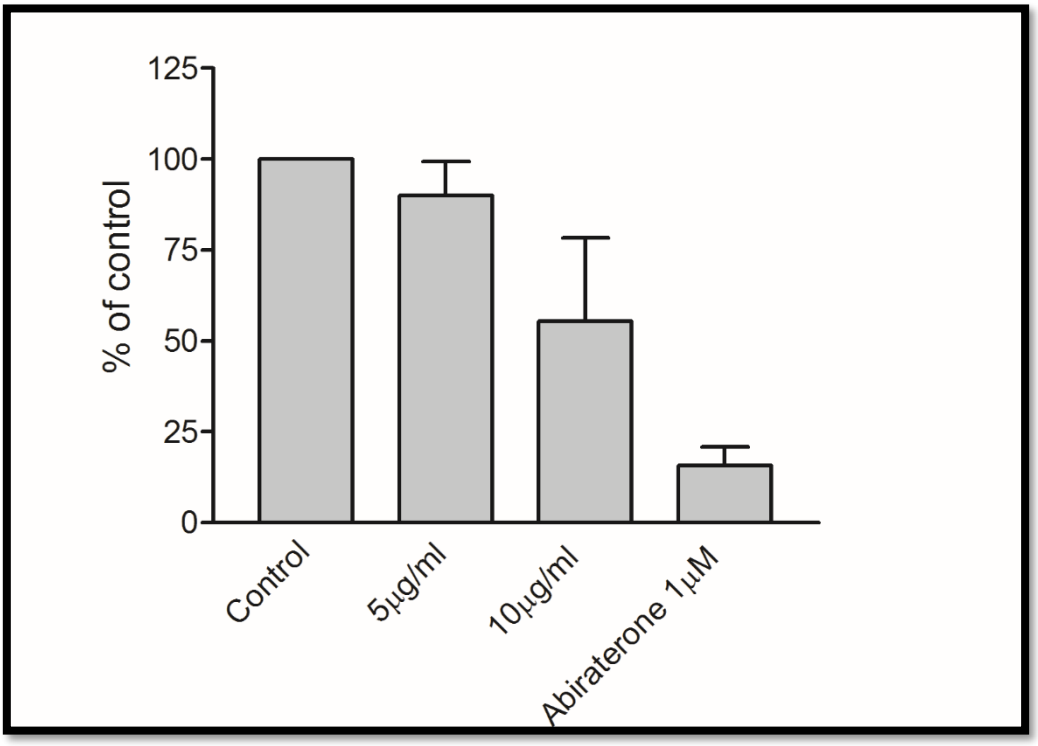

125
100
75
50
25
0
% of control
Control
5µg/ml
10µg/ml
Abiraterone 1µM

Figure 7

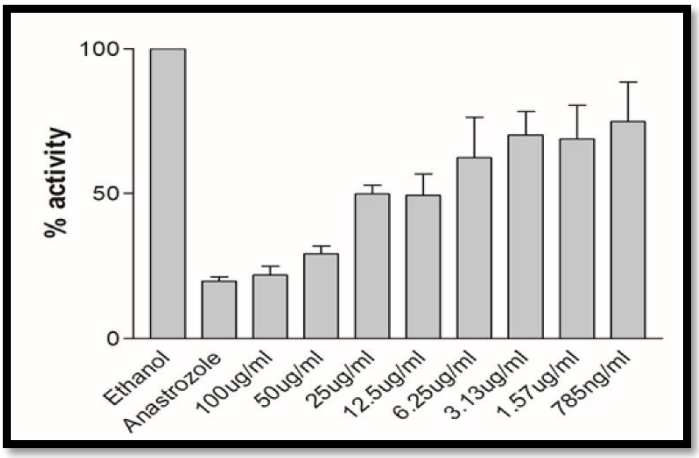

100
50
0
% activity
Ethanol
Anastrozole
100ug/ml
50ug/ml
25ug/ml
12.5ug/ml
6.25ug/ml
3.13ug/ml
1.57ug/ml
785ng/ml

Figure 8

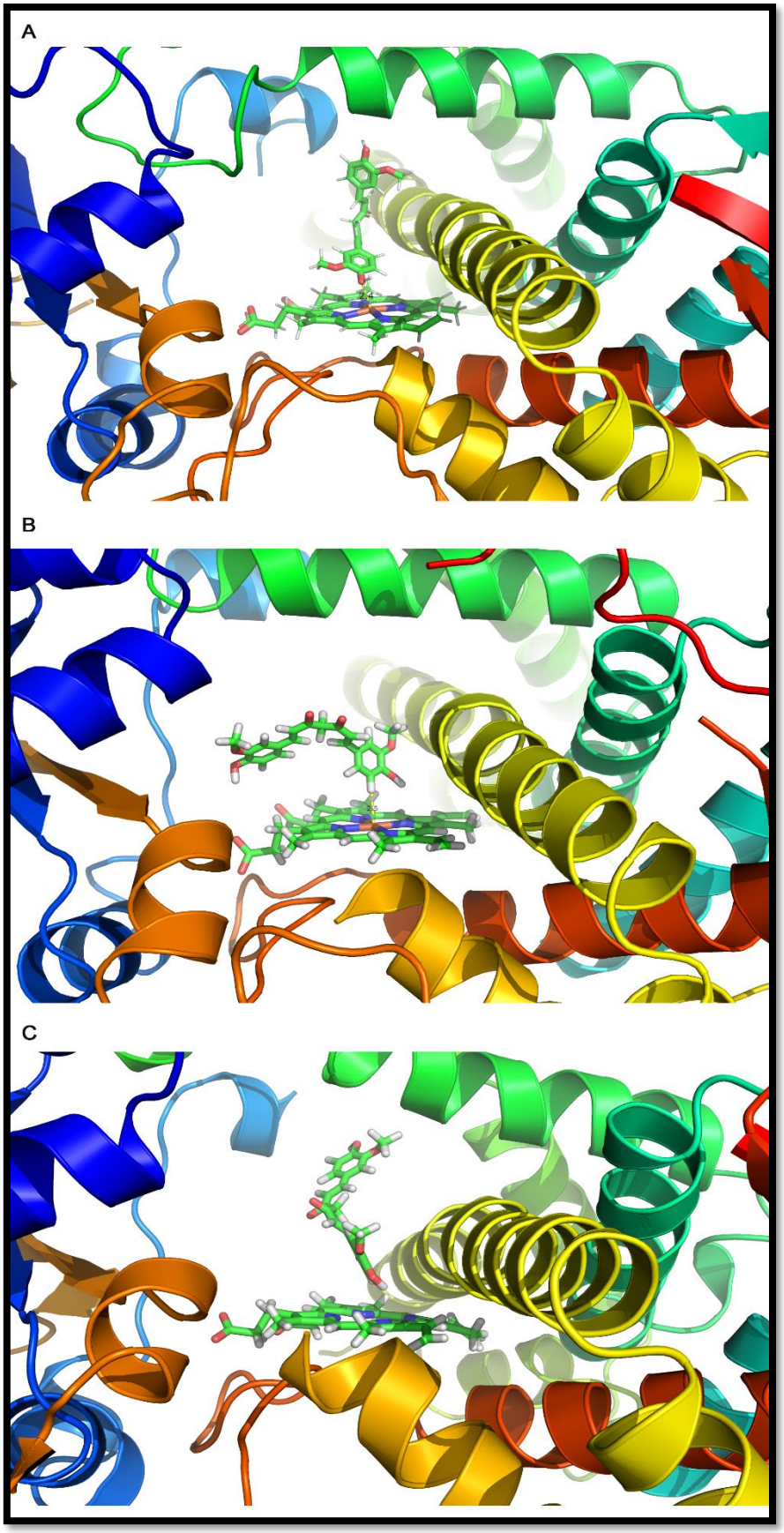


Figure 9