Seminar iz fizike/astrofizike
Departman za fiziku
6. maj 2016.

Steroidi koji sadrže azot kao potencijalne antitumor supstance

Strukturna analiza i molekularni 'doking'

Prof. dr Olivera Klisurić

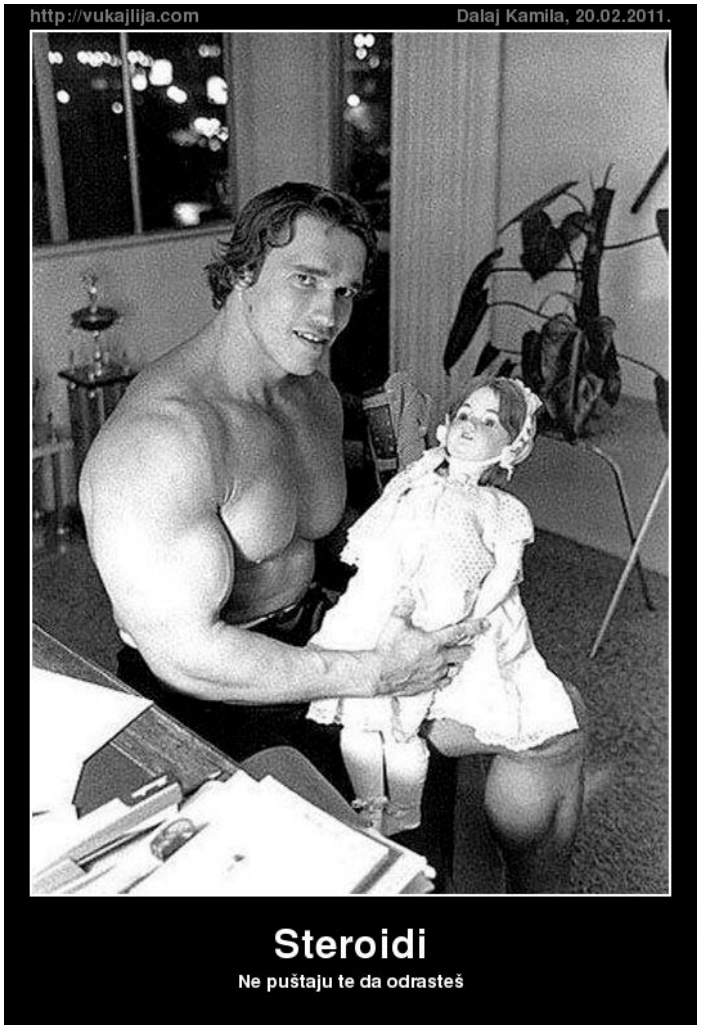
Departman za fiziku

Prirodno-matematički fakultet

Univerzitet u Novom Sadu

olivia@uns.ac.rs

Šta kaže Google...

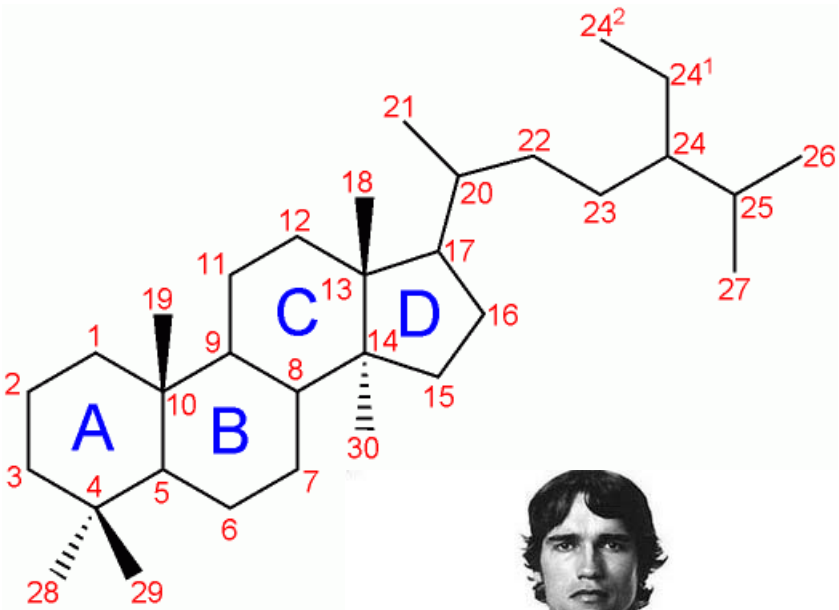


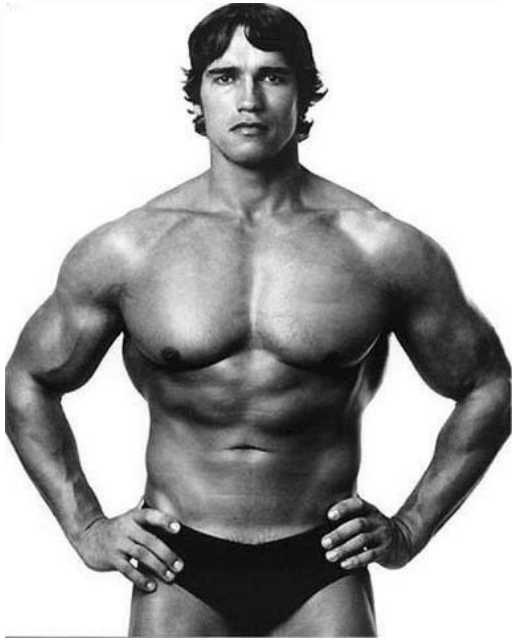
Olivera Klisurić
olivia@uns.ac.rs

Steroidi

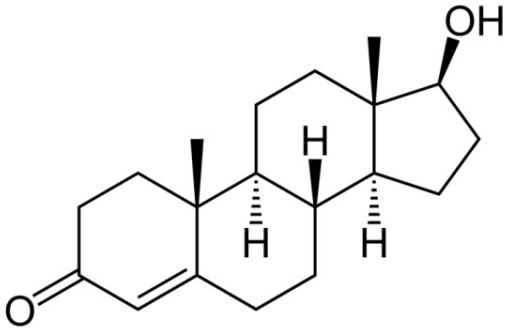
- Steroidi su grupa prirodnih jedinjenja od kojih su najvažnije klase:

1. **polni hormoni**, odgovorni za razvoj i održavanje primarnih i sekundarnih polnih karakteristika,
2. **nadbubrežni kortikoidni hormoni**, važni za metabolizam ugljenih hidrata i održavanje ravnoteže soli i vode,
3. **žučne kiseline** koje omogućuju probavu masti,
4. **steroidni konstituenti membrana ćelija**, itd.

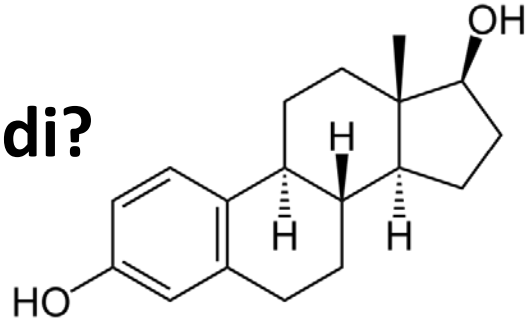




Olivera Klisurić
olivia@uns.ac.rs



Čemu stvarno "služe" steroidi?



Muški polni hormoni - Androgeni

- Stimulacija rasta i razvoja muških sekundarnih polnih karakteristika
- Opšte delovanje na metabolizam jer stimulišu sintezu što predstavlja anaboličko delovanje
- Deluju na:

1. reproduktivno tkivo,
2. mišićno tkivo,
3. centralni nervni sistem,
4. hipofizu,
5. jetru, bubreg,
6. imuni sistem,
7. kožu, kosu, znojne i lojne žlezde i kosti

Ženski polni hormoni - Estrogeni

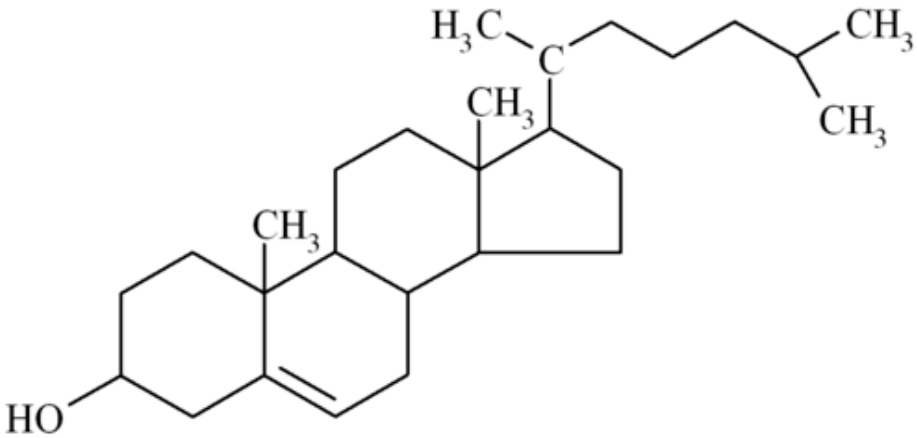
- Razvitak primarnih i sekundarnih ženskih polnih osobina
- Sazrevanje folikula, razvitak mlečnih kanalića
- Utiču na smanjenje koncentracije lipida u krvi
- Odgovorni su i za zadržavanje natrijuma, kalcijuma i fosfora u organima
- Stimulišu razvoj vagine, uterusa, proliferativni rast endometrijuma kao i rast uterinih žlezda
- Utiču na razvoj i građu kostura
- Raspored dlakavosti ženskog tipa

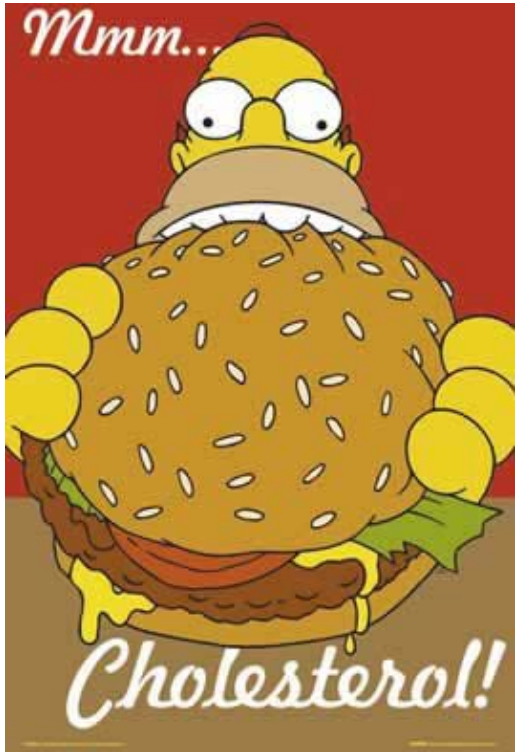
Olivera Klisurić
olivia@uns.ac.rs

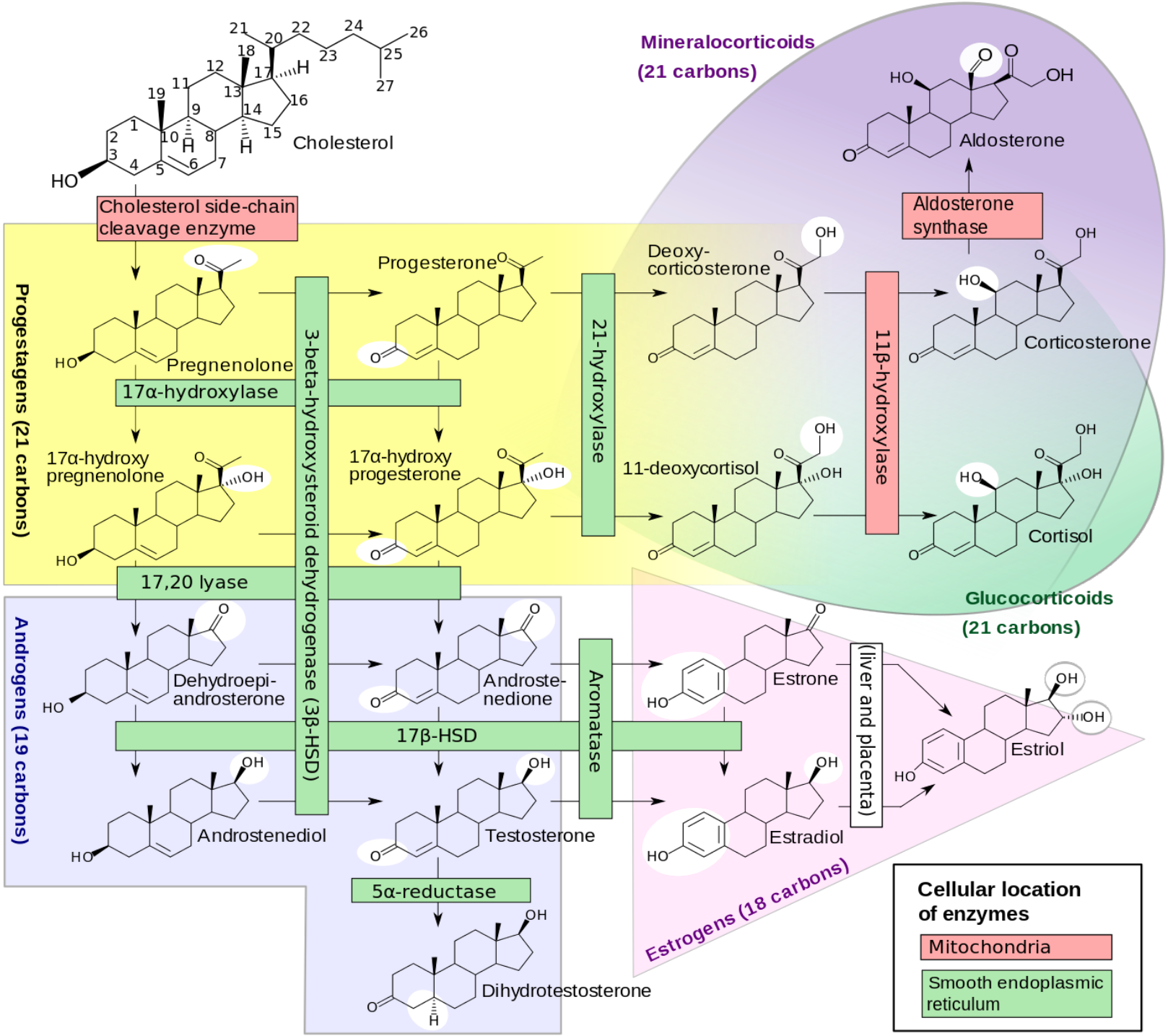
Proizvodnja steroidnih hormona

- Steroidni hormoni nastaju u odgovarajućim tkivima od molekula **holesterola**
- Holesterol se uglavnom smatra "lošim" molekulom?
- Holesterol je istovremeno nužan sastojak svih naših ćelija, bez kojeg ćelije ne bi mogle preživeti, pa ni proizvoditi

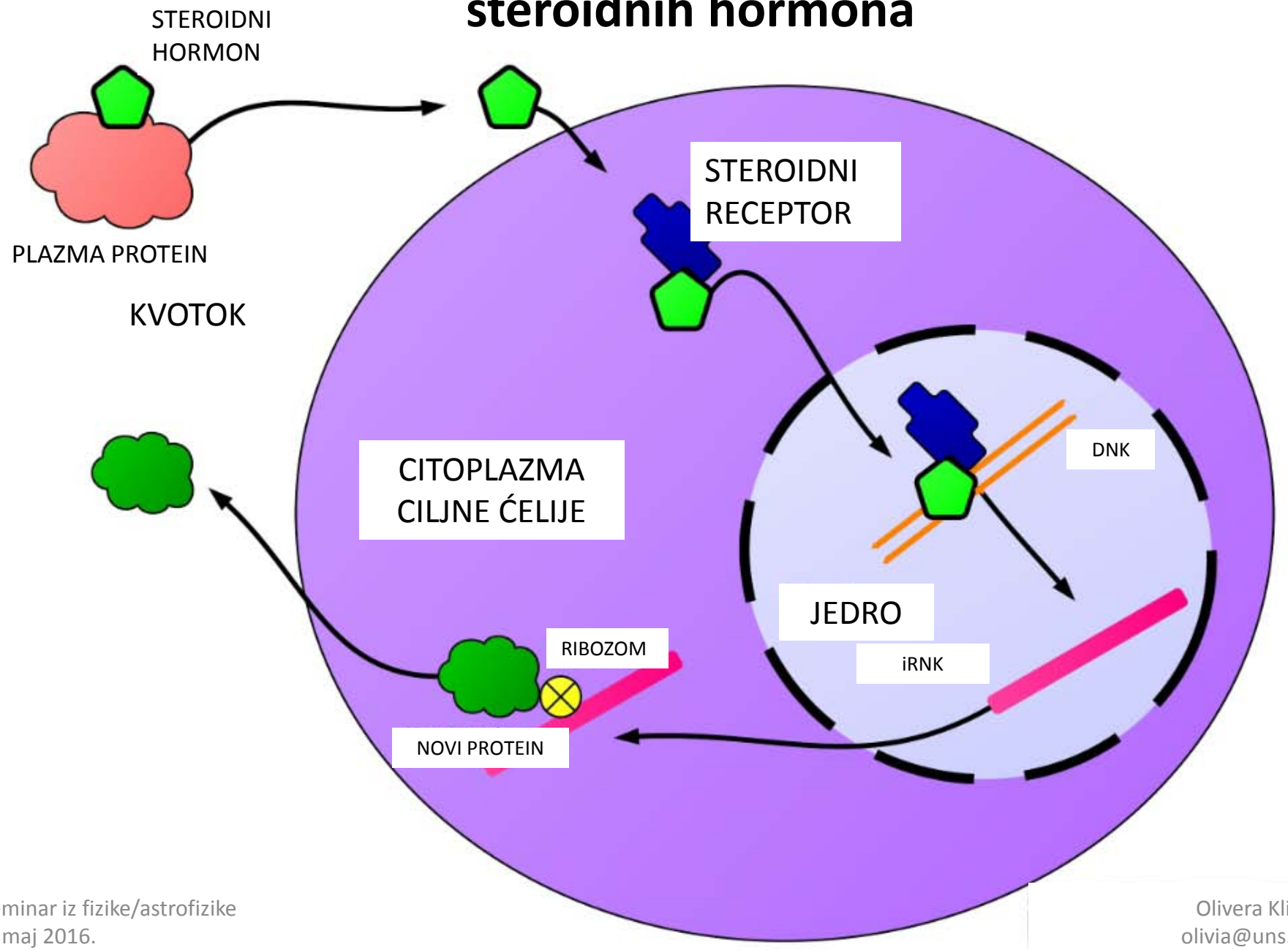
1. steroidni hormoni,
2. kortikoidni hormoni,
3. žučne kiseline i
4. vitamin D






Cholesterol
Cholesterol side-chain cleavage enzyme
Progestagens (21 carbons)
Pregnenolone
17α-hydroxylase
17α-hydroxy pregnenolone
17α-hydroxy progesterone
Progesterone
3-beta-hydroxysteroid dehydrogenase (3β-HSD)
17,20 lyase
Androgens (19 carbons)
Dehydroepi-androsterone
Androste-nedione
17β-HSD
Androstenediol
Testosterone
5α-reductase
Dihydrotestosterone
21-hydroxylase
Aromatase
Estrogens (18 carbons)
Estrone
Estradiol
(liver and placenta)
Estriol
Mineralocorticoids (21 carbons)
Aldosterone
Aldosterone synthase
Deoxy-corticosterone
11β-hydroxylase
Corticosterone
11-deoxycortisol
Cortisol
Glucocorticoids (21 carbons)
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

Mehanizam delovanja steroidnih hormona



Olivera Klisurić
olivia@uns.ac.rs

...kad se stvari otmu kontroli

- **Androgeni**: testosteron ili DHT uzrokuju kontrolisanu deobu ćelija prostate
- Ako je koncentracija androgena u krvi visoka deoba ćelija može postati nekontrolisana
- To dovodi do benigne hiperplazije prostate i kancera prostate
- Karcinom prostate je danas jedan od najčešće dijagnostifikovanih karcinoma kod muškaraca

- **Antiandrogeni** se koriste u terapiji različitih tzv. androgen-zavisnih oboljenja

1. hirsutizam
2. akne
3. seboreja
4. benigna hiperplazija prostate (BHP)
5. karcinom prostate

Olivera Klisurić
olivia@uns.ac.rs

Inhibitori enzima koji učestvuju u steroidogenezi
Antiandrogeni

- ***Inhibitori 3β-hidroksisteroid dehidrogenaze /Δ^5, Δ^4 – izomeraze***

Osnovna funkcija prekidanje konverzije na primer pregnenolona u progesteron ili DHEA u androstendion

- ***Inhibitori 17α-hidroksilaze /$C_{17,20}$-liaze***

Uzrokuju prekidanje konverzije progesterona u 17α-hidroksiprogesteron i androstendion, kao i pregnenolona u 17-hidroksipregnenolon i dehidroepiandrosteron

- ***Inhibitori 5α-reduktaze***

Prekomerna produkcija 5α-dihidrotestosterona je osnovni uzrok mnogih androgen-zavisnih poremećaja. Logičan tretman u slučaju ovih bolesti je inhibicija delovanja androgena pomoću inhibitora 5α-reduktaze (5αR).

- ***Inhibitori aromataze***

Inhibitori aromataze su jedinjenja koja inhibiraju aromatazu i imaju potencijalnu primenu u tretmanu bolesti kao što su: karcinom dojke, karcinom endometrijuma, hiperplazija prostate i kancer prostate

Olivera Klisurić
olivia@uns.ac.rs

Steroidi koji sadže azot

- Steroidi koji sadže azot su već poznata jedinjenja koja se koriste u lečenju širokog spektra bolesti:

1. kancer dojke[1]
2. kancer prostate[3]
3. leukemija[2]
4. osteoporoza[4]

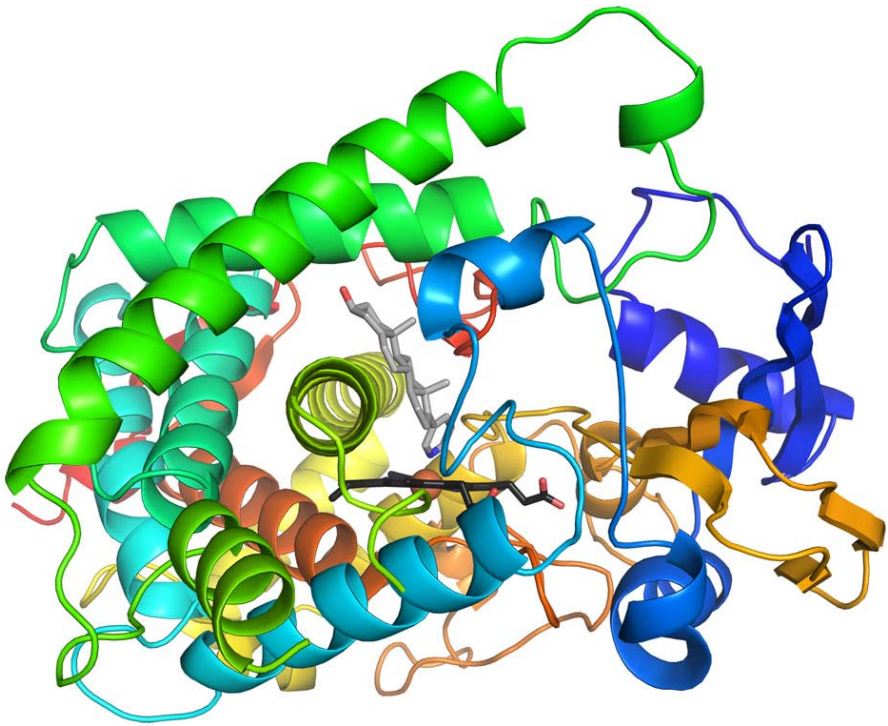
1. Yadav, M. R. Sabale, P. M. Giridhar, R. Zimmer, C. Hartmann, R. W**. Steroids 2012**, 77, 850.
2. Mitchell, H. J. Dankulich, W. P. Hartman, G. D. Prueksaritanont, T. Schmidt, A. Vogel, R. L. Bai, C. McElwee-Witmer, S. Zhang, H. Z. Chen, F. Leu, C. T. Kimmel, D. B. Ray, W. J. Nantermet, P. Gentile, M. A. Duggan, M. E. Meissner**, R. S. J. Med. Chem. 2009**, 52, 4578.
3. Salvador, J. A. Pinto, R. M. Silvestre, S. M**. J. Steroid Biochem. Mol. Biol. 2013**.
4. Minorics, R. Szekeres, T. Krupitza, G. Saiko, P. Giessrigl, B. Wolfling, J. Frank, E. Zupko, I. **Steroids 2011**, 76, 156.
5. Roy, J. DeRoy, P. Poirier, D. J. Comb. Chem. 2007, 9, 347.
6. Latham, K. A. Zamora, A. Drought, H. Subramanian, S. Matejuk, A. Offner, H. Rosloniec, E. F. **J. Immunol. 2003**, 171, 5820.

CYP17A1

- Citohrom P450 enzim CYP17[1] vezan za membranu endoplazmatičnog retikuluma

- Multifunkcionalni protein sa 17α-hidroksilaza i $C_{17,20}$-liaza aktivnošću

- Obe funkcije smeštene na jednoj aktivnoj strani proteina

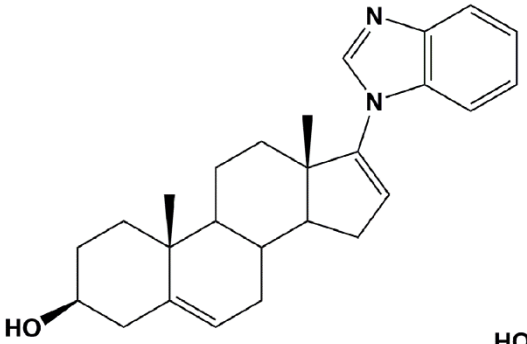


1. N. M. DeVore, E. E. Scott, Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. **Nature 2012** Jan 22;482(7383):116-9

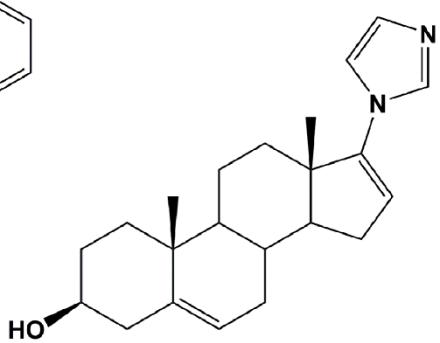
Inhibitori 17α-hidroksilaze/17,20-liaze

- Snižavaju koncentraciju androgena u krvi tako što inhibiraju 17α-hidroksilazu/$C_{17,20}$-liazu (CYP17)

1. *Abiraterone*
2. VN/124-1 (*Galeterone*)
3. VN/85-1



VN/124-1
galeterone



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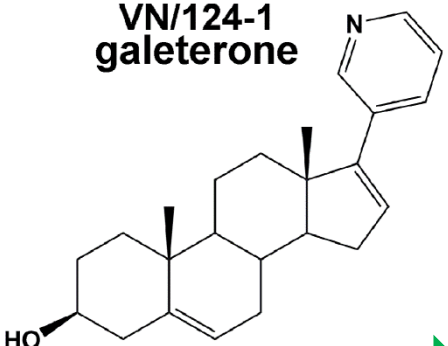


abiraterone

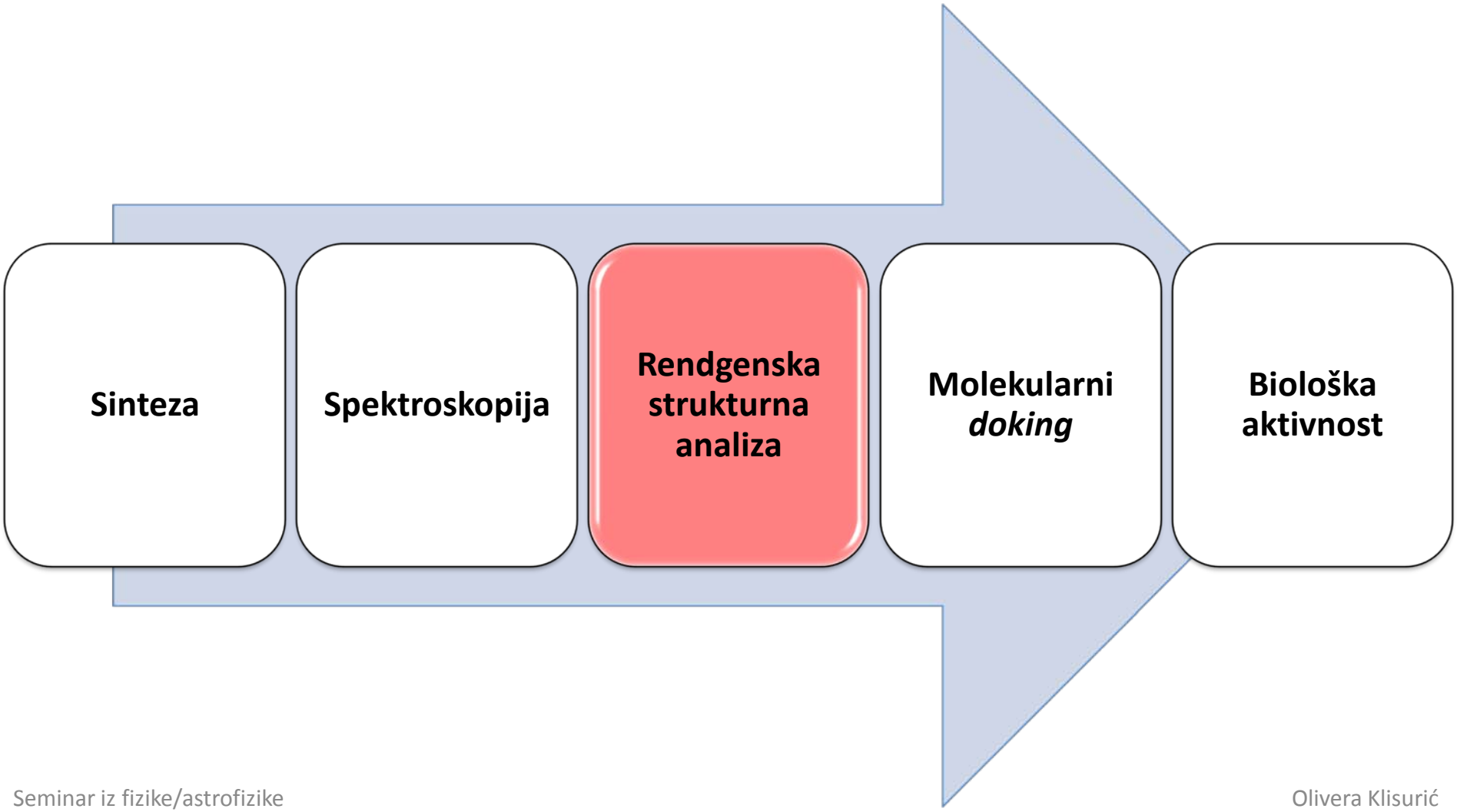
Olivera Klisurić
olivia@uns.ac.rs

Naši radovi...

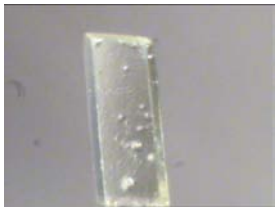
- **17(E)-pikoliniliden** derivati androstana
- **17α-pikolil** derivati androstana
- **D-homo steroidni tetrazoli**
- **A-modifikovani** (17α-pikolil i 17(E)-pikoliniliden) derivati androstana

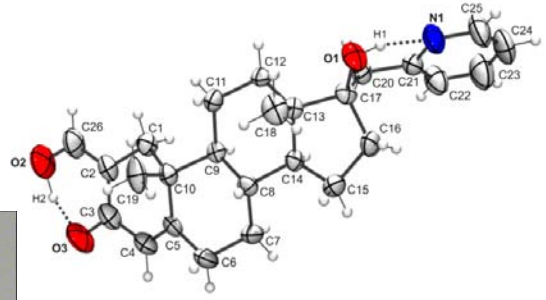
Olivera Klisurić
olivia@uns.ac.rs

Istraživanje



Olivera Klisurić
olivia@uns.ac.rs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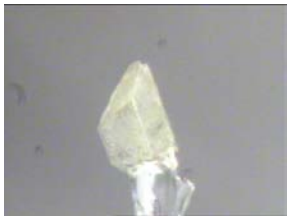
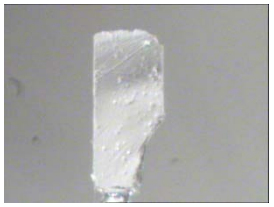
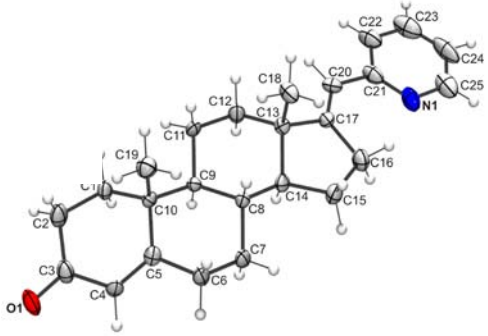
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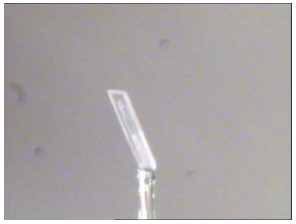

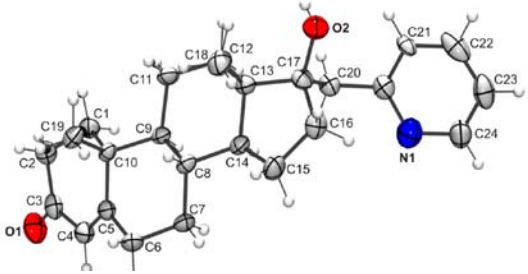
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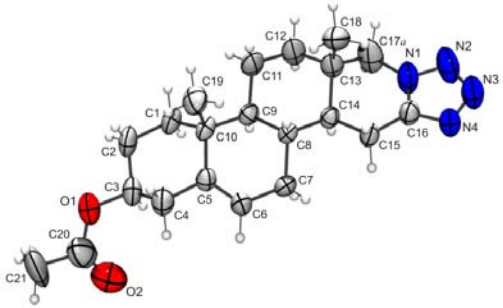
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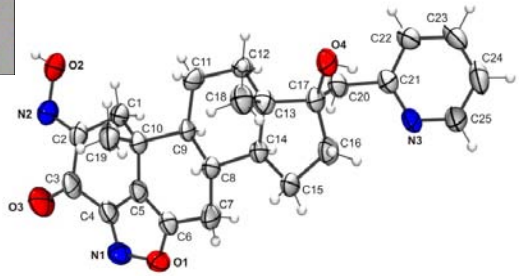
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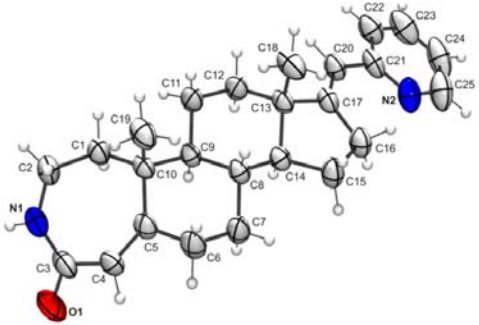
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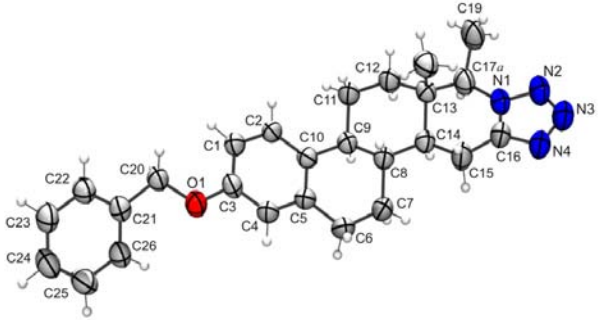
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C26

Naši radovi...

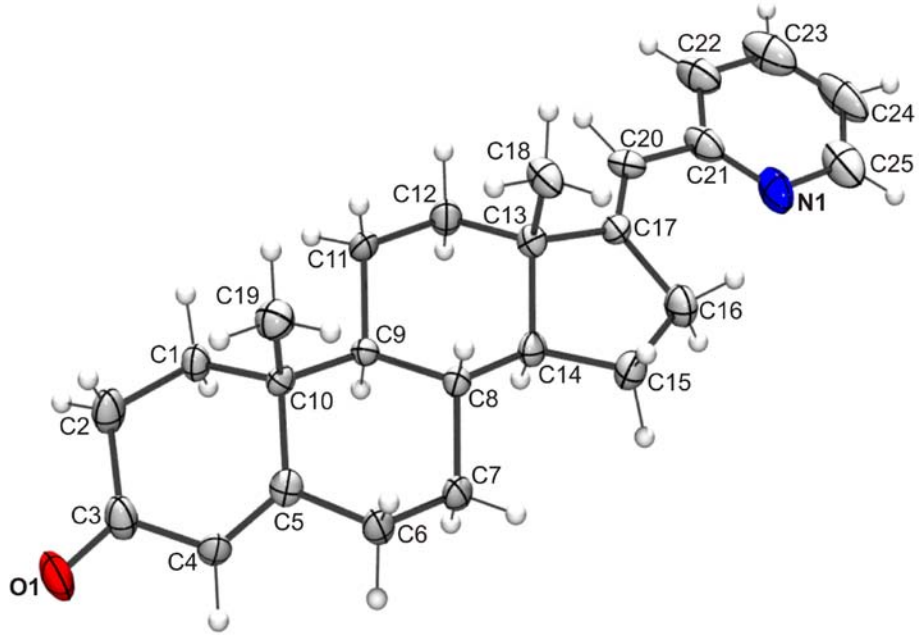
- **17(E)-pikoliniliden** derivati androstana

- **17α-pikolil** derivati androstana

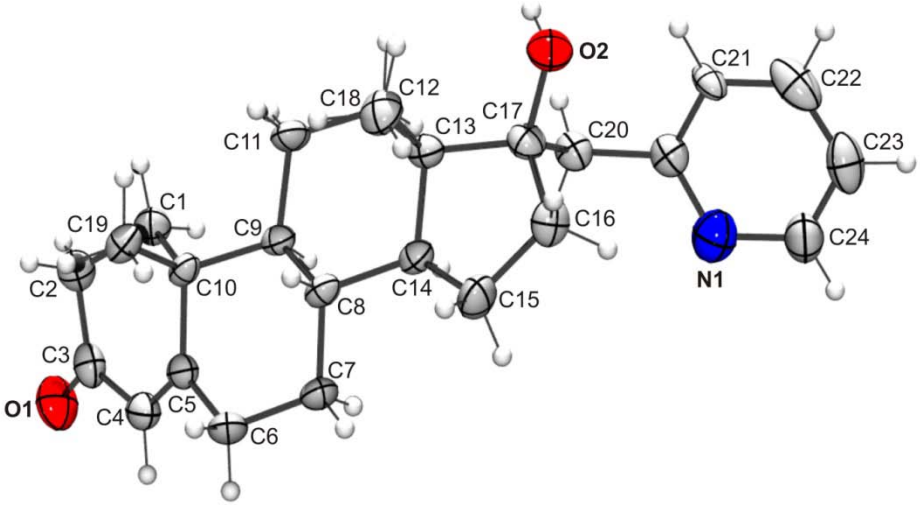
Olivera Klisurić
olivia@uns.ac.rs

17(E)-pikoliniliden i **17α-pikolil** derivati androstana

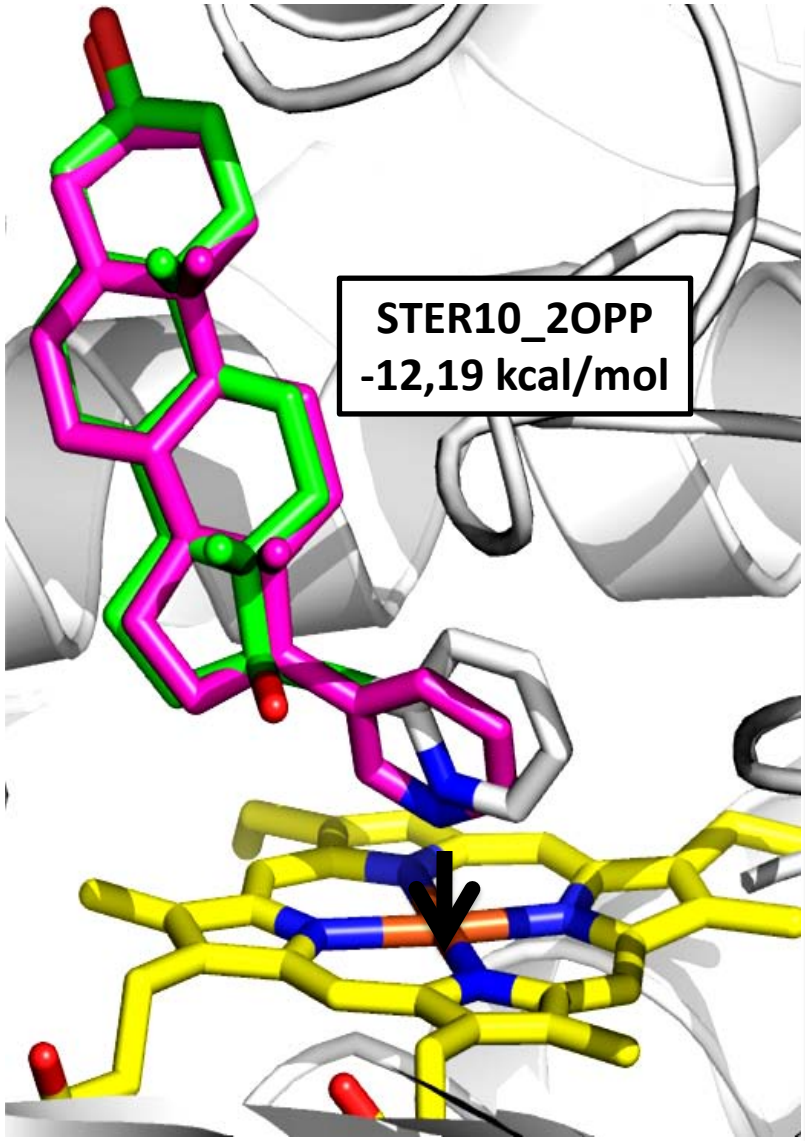
STER10_2OPP	**$C_{25}H_{31}ON$**
Dimenzije kristala (mm)	0,40 × 0,25 × 0,18
Kristalografski sistem	Ortorombični
Prostorna grupa	$P2_12_12_1$
Parametri elementarne ćelije	a = 7,274 (4) Å b = 11,857 (5) Å c = 23,920 (7) Å
$R(F^2>2\sigma(F^2))$	5,6%
S	0,83



STER11_3OPP	**$C_{25}H_{31}NO$**
Dimenzije kristala (mm)	0,58 × 0,36 × 0,22
Kristalografski sistem	Ortorombični
Prostorna grupa	$P2_12_12_1$
Parametri elementarne ćelije	a =7,021 (4) Å b = 8,967 (4) Å c =33,740 (7) Å
$R(F^2>2\sigma(F^2))$	4,7%
S	1,03



17(E)-pikoliniliden i **17α-pikolil** derivati androstana



Abirateron (ciklama)
STER10_2OPP (zelena)

STER10_2OPP **STER11_3OPP**

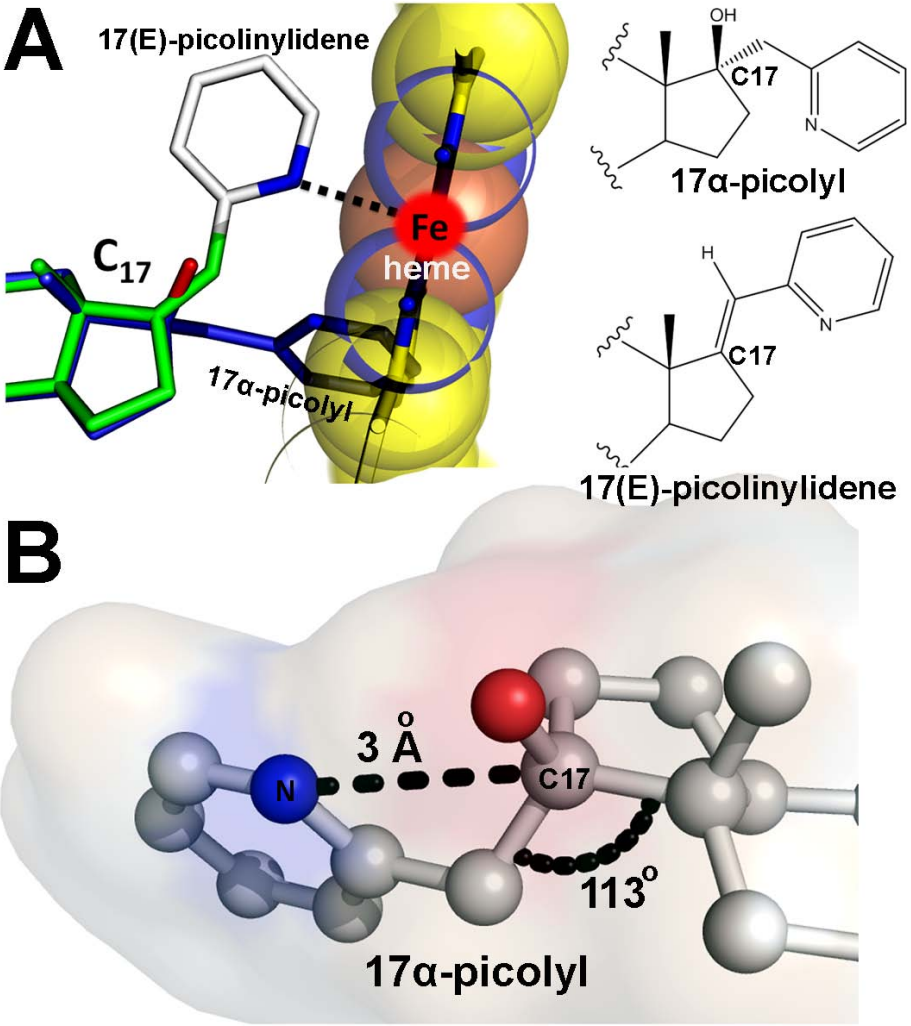
NS15 **NS13, NS14**

VEZIVANJE ZA ENZIM		**ENERGIJA VEZIVANJA**
STER10_2OPP 12,7 Å	**VEZUJE SE** ZA CYP17	-12.19 kcal/mol
STER11_3OPP 13,91 Å	**NE VEZUJE** SE ZA CYP17	-

17(E)-pikoliniliden i **17α-pikolil** derivati androstana

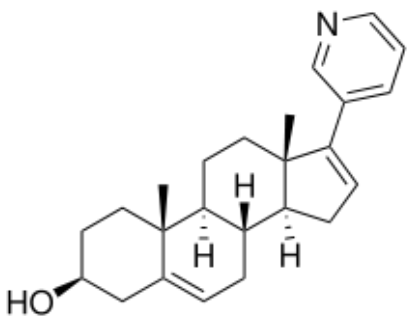
- Derivati androstana sa 17α-pikolil grupom u kontaktu sa CYP17A1

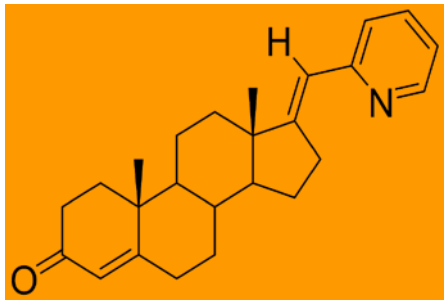
1. Veliki sterni sudar i Van der Valsovo preklapanje 17α-pikolil grupe i *HEM* grupe u CYP17A1
2. Nema vezivanja za *HEM* (slika A)
3. Tetraedarska sp^3 geometrija na C17 atomu u 17α-pikoli (slika B) u odnosu na planarnu sp^2 geometriju pronađenu na C17 u 17-pikoliniliden funkcionalnoj grupi

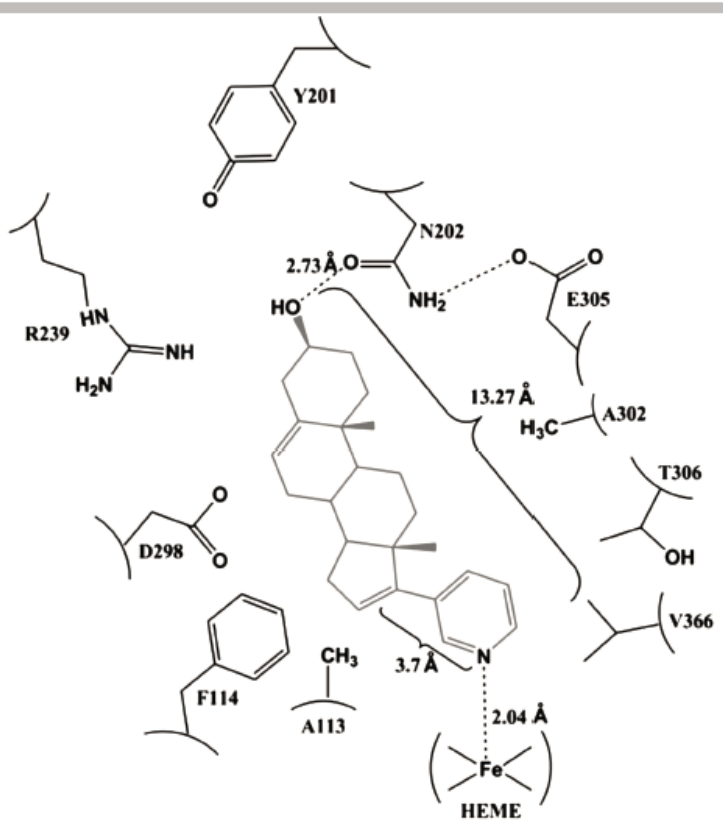


J. J. Ajduković, E. A. Đurendić, E. T. Petri, O. R. Klisurić, A. S. Ćelić, M. N. Sakač, D. S. Jakimov, K. M. Penov Gaši, **Bioorganic & Medicinal Chemistry** 21 (**2013**) 7257–7266

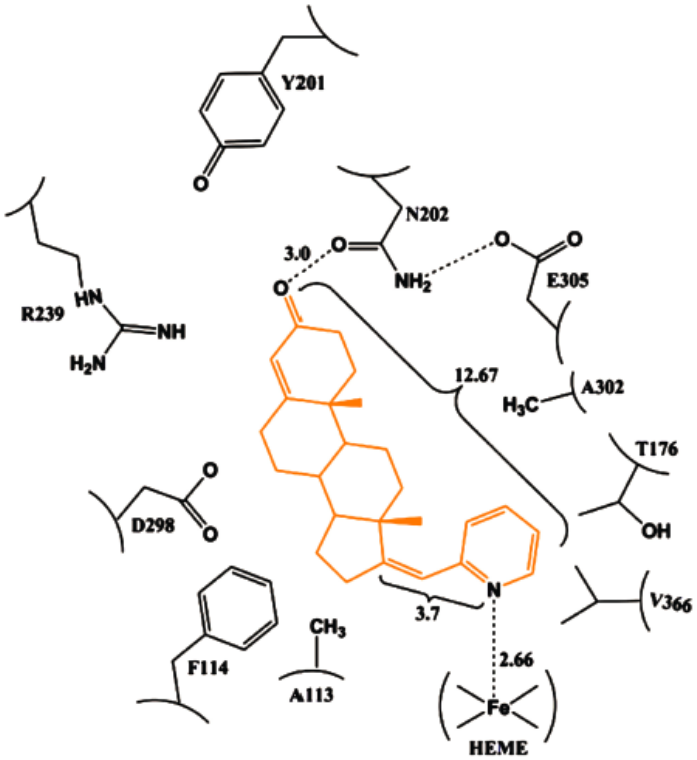
...za dalja istraživanja







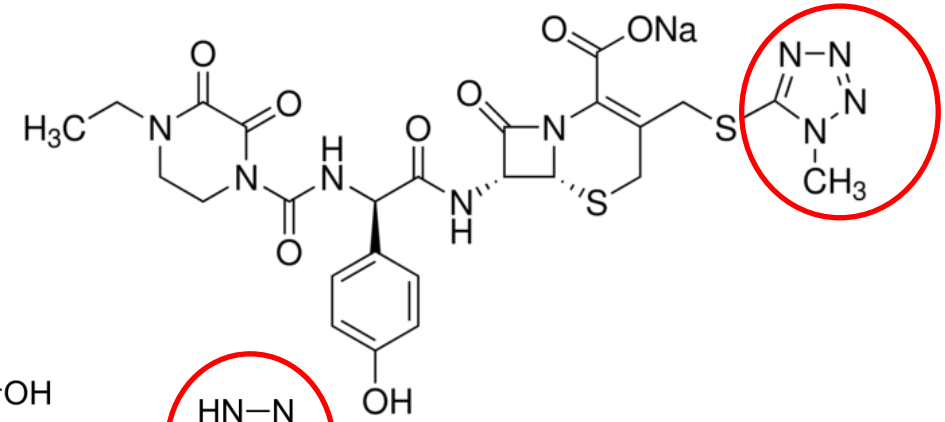
ABIRATERONE
IC_{50} (PC3) 9.32 μM



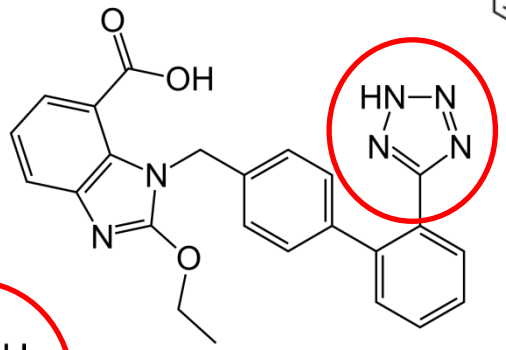
STER10 2OPP
IC_{50} (PC3) 12.9 μM

Tetrazolska grupa_D-homo steroidni tetrazoli

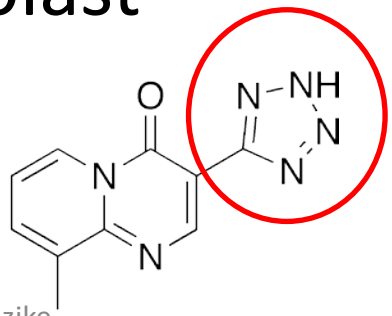
- Tetrazolska grupa je pronađena u nekoliko poznatih klinički odobrenih lekova
- Cefoperazon



- Kandesartan



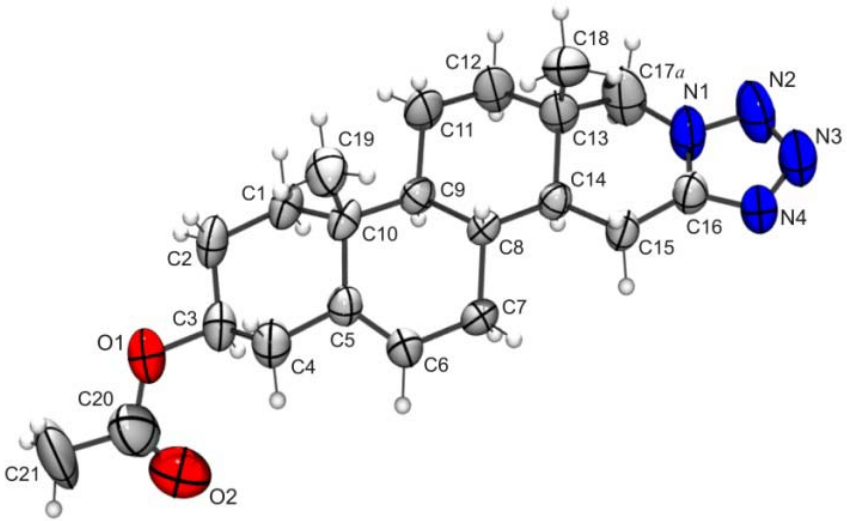
- Pemirolast



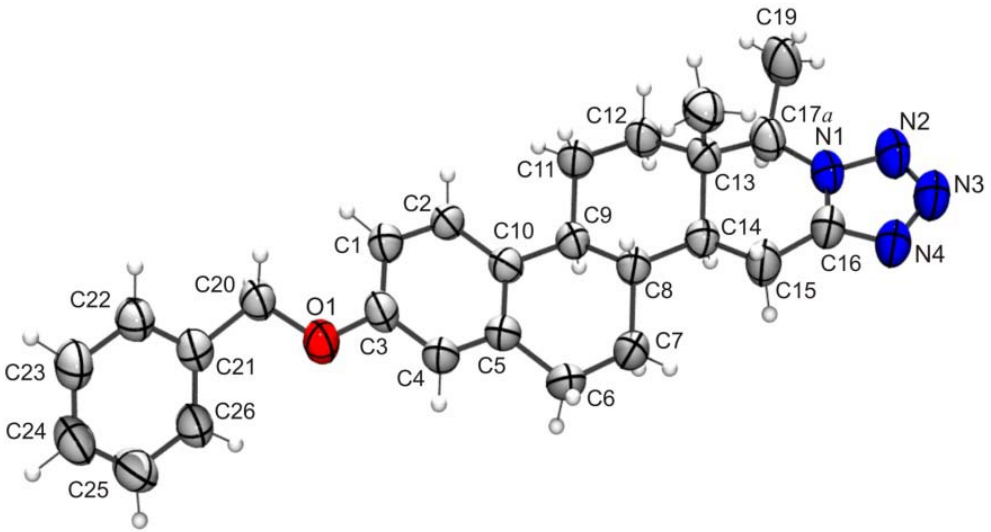
Olivera Klisurić
olivia@uns.ac.rs

D-homo steroidni tetrazoli

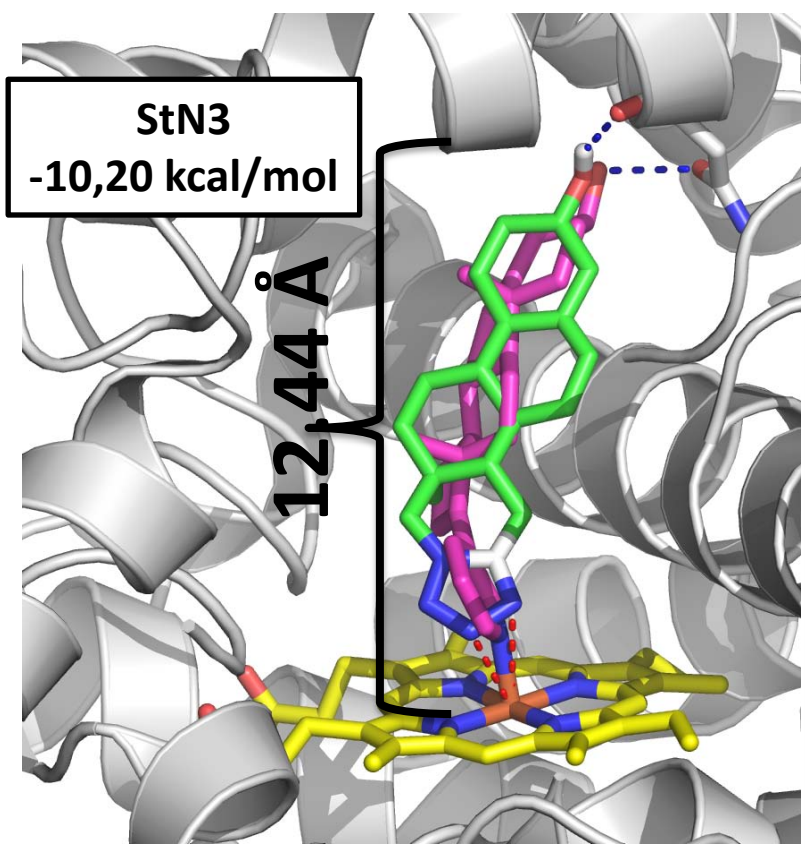
StN3	$C_{21}H_{30}N_4O_2$
Dimenzije kristala (mm)	0,18 × 0,17 × 0,05
Kristalografski sistem	Ortorombični
Prostorna grupa	$P2_12_12_1$
Parametri elementarne ćelije	a = 8,099(3) Å b = 10,195(5) Å c = 24,458(4) Å
$R(F^2>2\sigma(F^2))$	5,5%
S	0,89



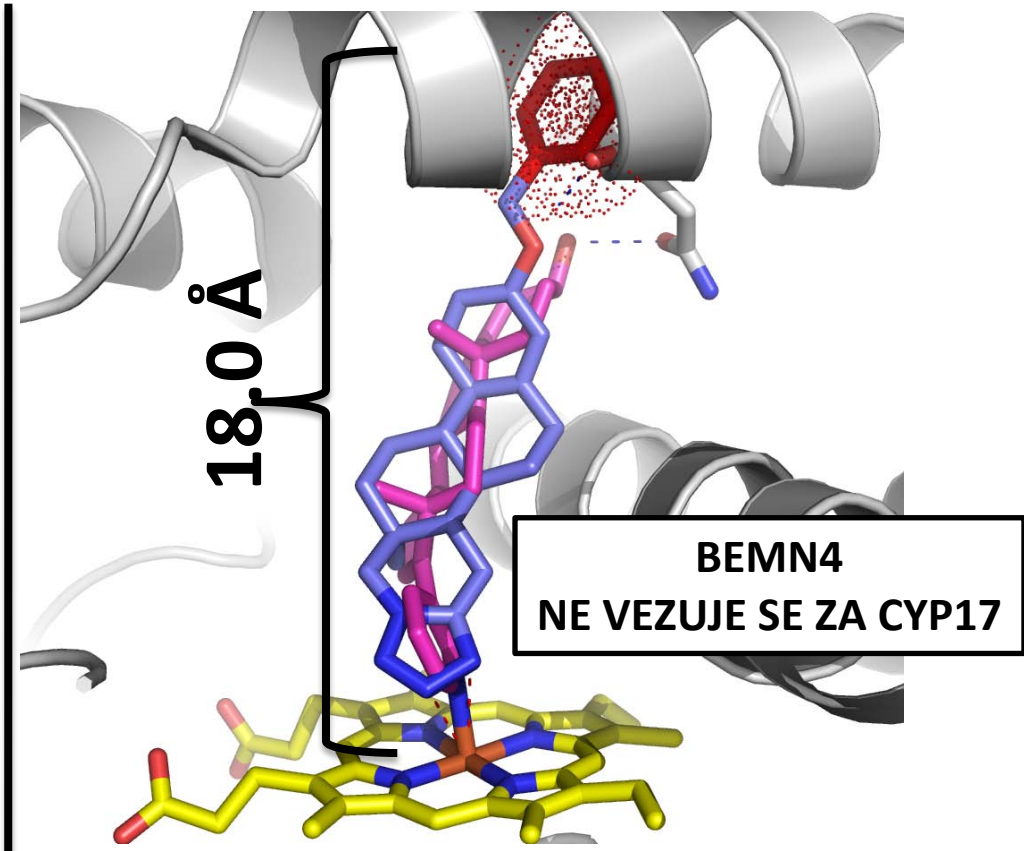
BEMN4	$C_{26}H_{30}N_4O$
Dimenzije kristala (mm)	0,28 × 0,18 × 0,05
Kristalografski sistem	Monoklinični
Prostorna grupa	$P2_1$
Parametri elementarne ćelije	a = 9,039(5) Å b = 12,320(5) Å c = 9,996(5) Å β= 93,034(5)°
$R(F^2>2\sigma(F^2))$	3,8%
S	1,07



D-homo steroidni tetrazoli – Vezivanje za CYP17A1



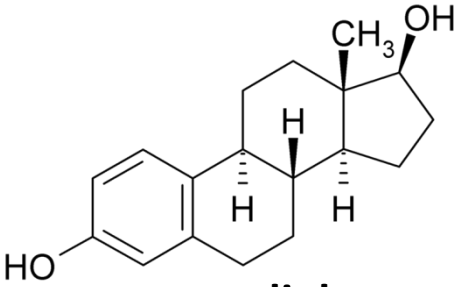
StN3



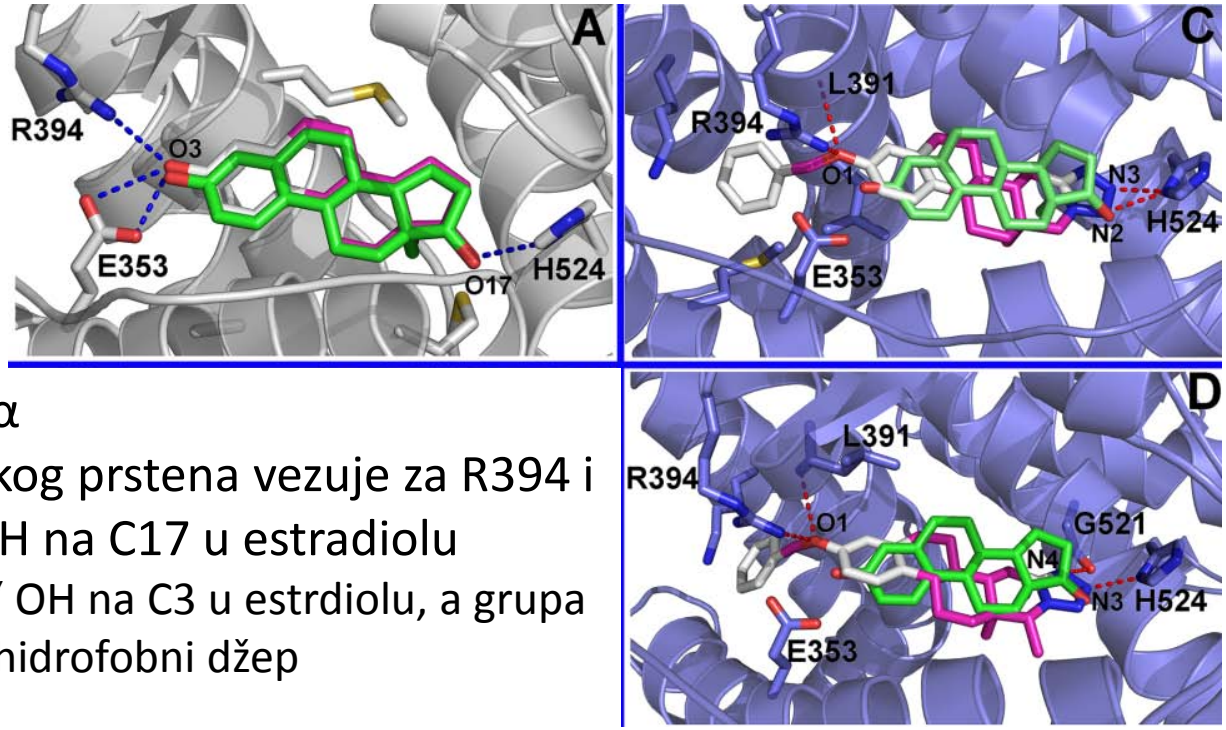
BEMN4

Gaši, K. P. Oklješa, A. Petri, E. Ćelić, A. Đurendić, E. Klisurić, O. Csanadi, J. Batta, G. Nikolić, A. Jakimov, D. Sakač, M.
MedChemCommun. 2013, 4, 317–323

D-homo steroidni tetrazoli – Vezivanje za ERα-LBD



estradiol



A. Vezivanje estradiola za ERα
B. BEMN4 se preko tetrazolskog prstena vezuje za R394 i H524 slično kako to radi OH na C17 u estradiolu
C. O1 na BEMN4 takođe ‘glumi ‘ OH na C3 u estrdiolu, a grupa na O1 u BEMN4 se smešta u hidrofobni džep

	IC50 (μM)							Energija vezivanja
	MCF-7 ER+	**MDA-MB-231**	**PC-3**	**HeLa**	**K562**	**HT-29**	**MRC-5**	**Autodock (kcal/mol)**
StN3	56,23	>100	78,96	>100	45,22	>100	>100	- 8,04
BEMN4	**4,58**	>100	>100	>100	>100	18,02	>100	- 11,01
Doxorubicin	0,75	0,12	95,61	1,17	0,36	0,32	0,12	

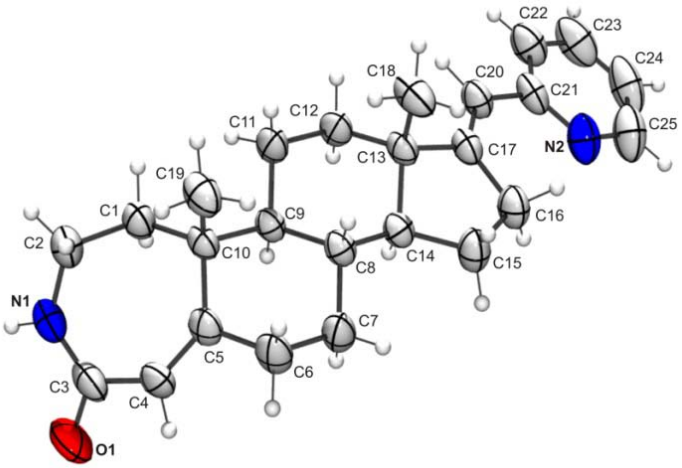
Naši radovi...

- **A-modifikovani** (17α-pikolil i 17(E)-pikoliniliden) derivati androstana

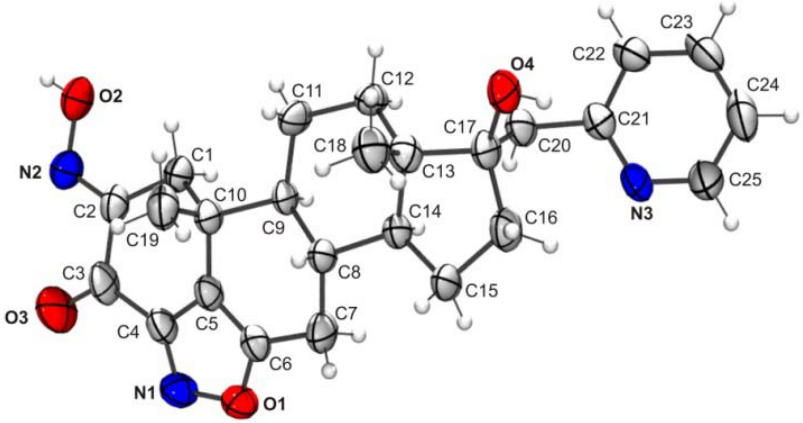
Olivera Klisurić
olivia@uns.ac.rs

A-modifikovani (17α-pikolil i 17(E)-pikoliniliden) derivati androstana

3LAKTAM	**$C_{25}H_{32}N_2O$**
Dimenzije kristala (mm)	0,42 × 0,28 × 0,17
Kristalografski sistem	Ortorombični
Prostorna grupa	$P2_12_12_1$
Parametri elementarne ćelije	a = 7,3301 (3) Å b = 8,5393 (4) Å c = 33,1817 (17) Å
$R(F^2>2\sigma(F^2))$	3,9%
S	1,05

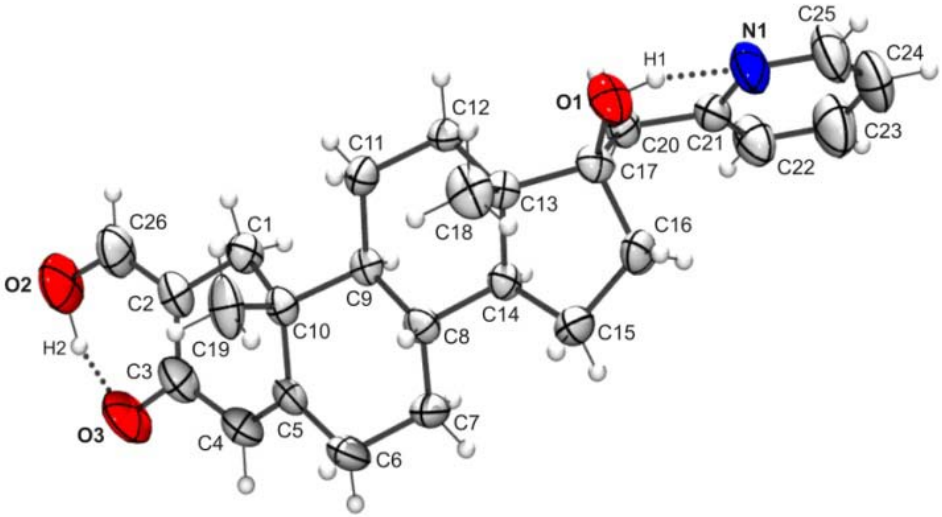


2OPPOKSIM	**$C_{25}H_{29}N_3O_4$**
Dimenzije kristala (mm)	0,49 × 0,31 × 0,22
Kristalografski sistem	Ortorombični
Prostorna grupa	$P2_12_12_1$
Parametri elementarne ćelije	a = 12,2836 (5) Å b = 13,2366 (6) Å c = 16,5473 (10) Å
$R(F^2>2\sigma(F^2))$	7,9%
S	1,12



A-modifikovani (17α-pikolil i 17(E)-pikoliniliden) derivati androstana

2OPPCHOH	**$C_{26}H_{33}NO_3$**
Dimenzije kristala (mm)	0,44 × 0,26 × 0,18
Kristalografski sistem	Monoklinični
Prostorna grupa	$P2_1$
Parametri elementarne ćelije	a = 11,161 (2) Å b = 6,2498 (13) Å c = 16,253 (3) Å β= 91,564(19)°
$R(F^2>2\sigma(F^2))$	5,8%
S	1,05



D–H…A	D–H (Å)	H…A (Å)	D…A (Å)	D–H…A (°)
O1-H1…N1	0,82(3)	2,01(4)	2,762(5)	151(4)
O2-H2…O3	1,07(5)	1,49(5)	2,496(6)	155(5)

Olivera Klisurić
olivia@uns.ac.rs

BIOLOŠKA AKTIVNOST

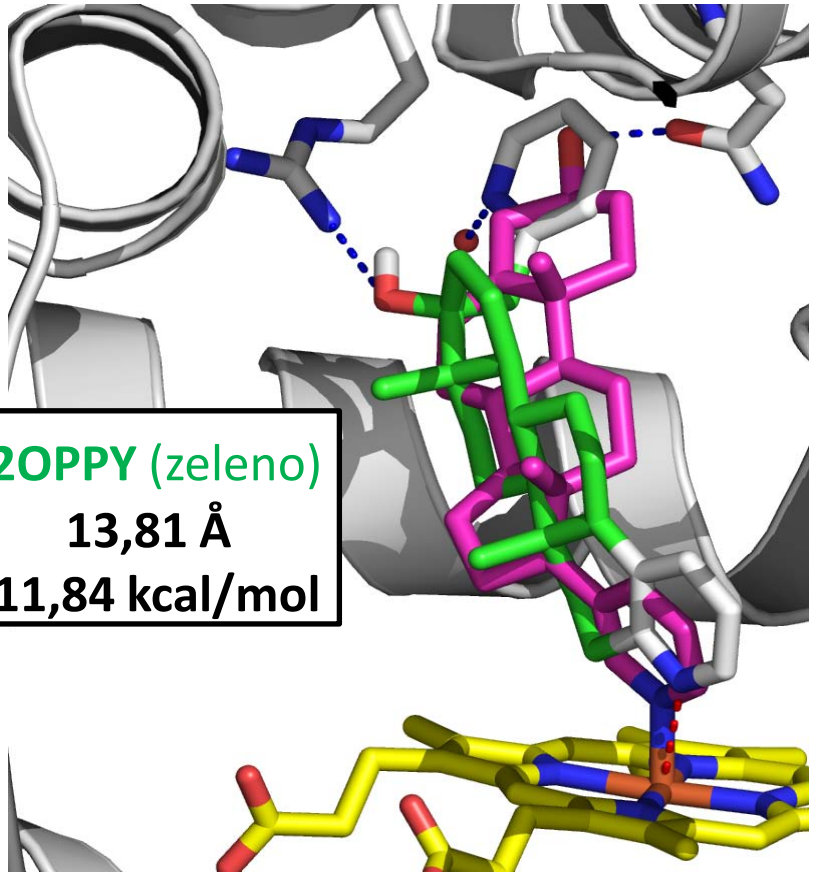
A-modifikovani (17α-pikolil i 17(E)-pikoliniliden) derivati androstana

	IC50 (μM)						
	MCF-7	**MDA-MB-231**	**PC-3**	**HeLa**	**HT-29**	**A549**	**MRC-5**
3LAKTAM	**12.7**	32.2	60.1	>100	**19.8**	>100	>100
2OPPCHOH	**19.0**	34.4	>100	>100	>100	>100	>100
2OPPOKSIM	50.4	**25.3**	**6.6**	>100	>100	>100	>100

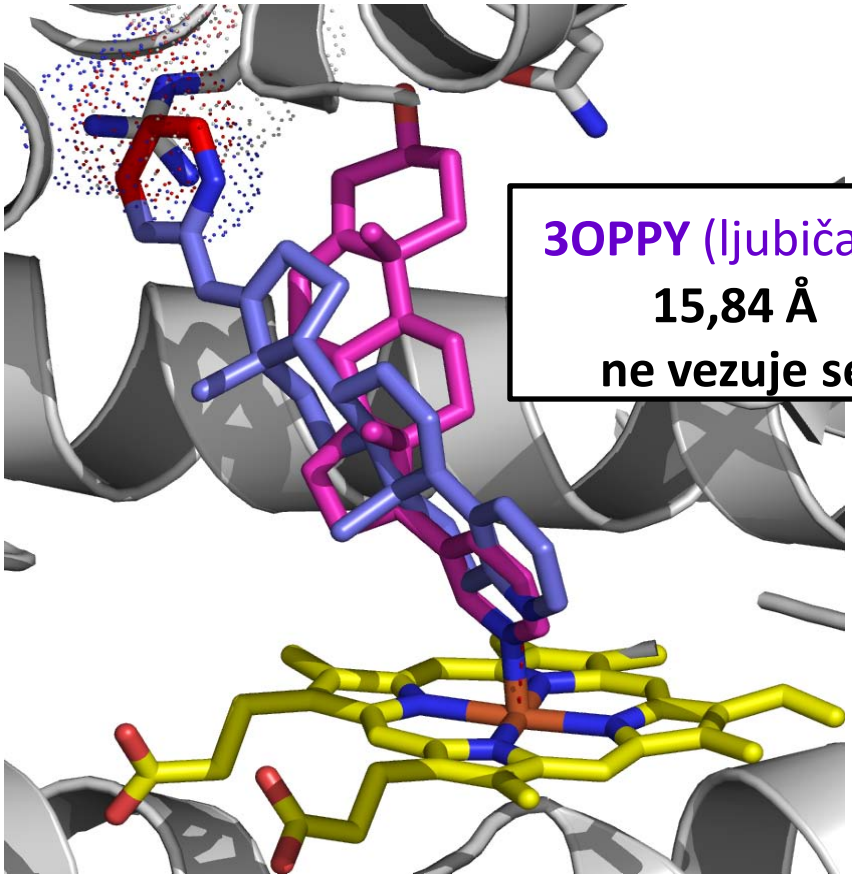
1. Atom azota N1 u piridinskom prstenu jedinjenja **2OPPCHOH** je već involviran u vodoničnu vezu sa O1 atomom isto kao i atom kiseonika O3 sa OH hidroksilnom grupom
2. Kako direktna koordinacija sa enzimom ide preko N atoma iz piridinskog prstena (sa Fe iz HEM) i preko O3 atoma, a oba atoma su već u intramolekularnim vodoničnim vezema, to je verovatno i razlog smanjene biološke aktivnosti

Ajduković, J. Gaši, K. P. Jakimov, D. Klisurić, O. Šanta, S. J. Sakač, M. Aleksić, L. Đurendić, E.
Bioorganic & Medicinal Chemistry 2015, 23, 1557–1568

A-modifikovani (17α-pikolil i 17(E)-pikoliniliden) derivati androstana



2OPPY

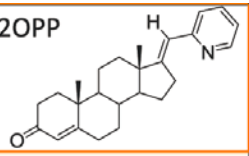
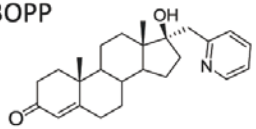
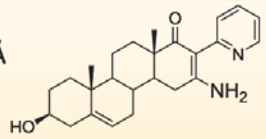
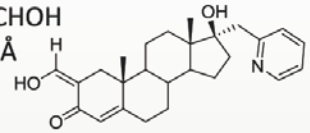
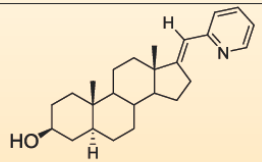
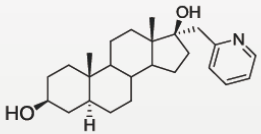
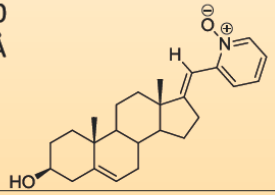
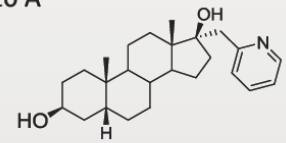
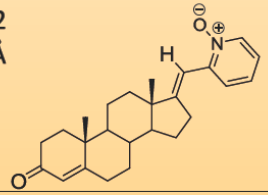
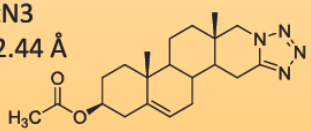
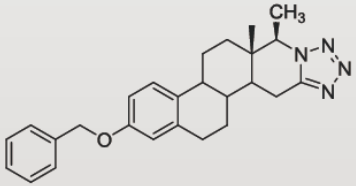
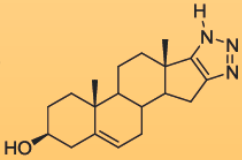


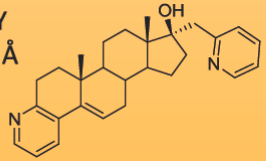
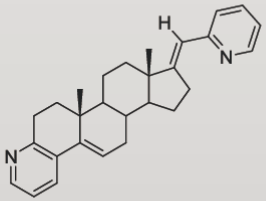
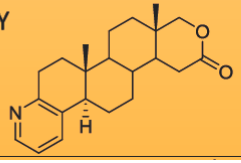
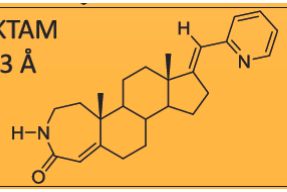
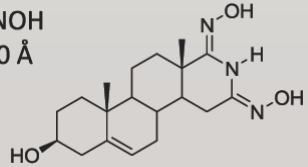
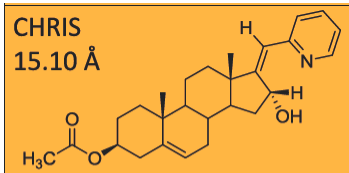
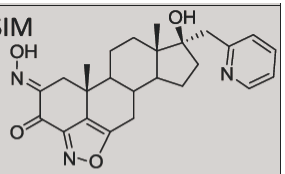
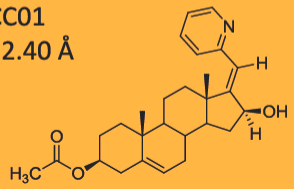
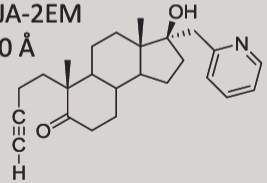
3OPPY

Fe←

Olivera Klisurić
olivia@uns.ac.rs

Naši radovi - Steroidi koji sadže azot

BINDING TO CYP17	ENERGY [kcal/mol]	NO BINDING TO CYP17
STER10_2OPP 12.7 Å 	-12.19	STER11_3OPP 13.91 Å 
DHAPY 15.55 Å 	-10.63	2OPPCHOH 13.21 Å 
NS15 12.7 Å 	-10.53	NS13 14.40 Å 
DJN10 12.7 Å 	-11.87	NS14 13.10 Å 
DJN12 12.7 Å 	-11.04	
StN3 12.44 Å 	-10.20	BEMN4 18.01 Å 
SCVIII 12.10 Å 	-10.02	

BINDING TO CYP17	ENERGY [kcal/mol]	NO BINDING TO CYP17
2OPPY 13.81 Å 	-11.84	3OPPY 15.84 Å 
L-OPP-PY 11.49 Å 	-10.21	
3LAKTAM 13.53 Å 	-10.53 -11.54	SACNOH 12.50 Å 
CHRIS 15.10 Å 	-10.21	2OPPOKSIM 12.51 Å 
CC01 12.40 Å 	-10.44	EDJ-JA-2EM 12.10 Å 

Olivera Klisurić
olivia@uns.ac.rs

Zaključak

1. Atom azota u okviru piridinskog prstena povezanog sa *D* ili *A* prstenom steroida povećava antitumornu aktivnost tog steroida

2. Dužina steroidnog molekula je presudna kod vezivanja za enzim

3. Planarnost steroidnog molekula je važna kod vezivanja atoma azota za HEM

4. 17(E)-pikoliniliden derivati androstana predstavljaju dobre kadidate za dalja istraživanja vezano za citotoksičnost ovih jedinjenja kod kancera prostate

5. Kristalna struktura liganda (steroida) i njihovih targetnih proteina je neophodan uslov za *in silico* predikcije interakcija protein-ligand kao i za dizajn lekova

Hvala na pažnji!
Hvala kolegama!