Name Surname :__Teacher_____ Group Nº ___Teacher_____2025

A. Human Mineralocorticoid Receptor (**MR**-NR3C2) AS4 aldosterone.pdb Task for studies.

ChemScape MDLi RasMol (RasMac); MAGE FireFox application at:
htdocsLocal http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/MineraloCorticoidReceptor/NR-A-G-P-R2AA2.htm

B. RSU Aris Kaksis 2025 molecular tutorials: solution MinerCorticoidAldosteronAnswer.doc.

1) 2AA2.pdb, 2005 The Journal of Biological Chemistry, Departments of Gene Expression and Protein Biochemistry

2) 4FNE.pdb; PLoS Genet. 2012;8(11)

3. Which **steroid** molecule exhibit potent mineralocorticoid receptorMR activation?.....

1. ……..**aldosterone** .....

4. What four activation types induce **MR** weak, antihypertensive, binding selectivity over?

1. **MR** weak ……..**progesterone** 2. antihypertensive **spironolactone**.....

3. binding selectivity..... **cortisol** over 4. **cortisone**.....

5. What four Physiological roles in the **HOMEOSTASIS regulation** in Human body?

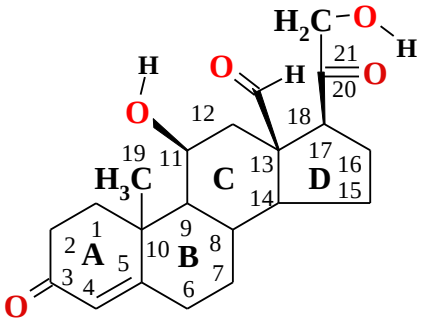
1. ... plays a physiological role in the **HOMEOSTASIS regulation** of **water** and **electrolyte** .....

2. ... primarily sodium **Na^+** and potassium **K^+** levels **regulation** .

3. ... effects on the **colon**.....and **kidney**..... in the **distal nephron**.....

4. largest influence on **vascular blood pressure**.....

6. Put in **Aldosterone** hydrocarbon chains C21 structural formula having two carbonyl **O=C<**, one aldehyde **O=CH–**, and two hydroxyl oxygen atoms **–OH**!



Show carbon –C– cyclic structures **A,B,C,D,** one double bond >C4=C5< and one **–CH_3**!

7. What subgroups super families of the **oxosteroid** nuclear receptors (NR) includes?.....

1. ...Androgen receptor (AR), ...

2. ...Glucocorticoid receptor (GR), ...

3. ...progesterone receptor (PR).....

8. What Nuclear receptor Complexes contains as three major functional **Domains**?.....

1. **N-terminal** domen ..... variable receptor **activations domen** ... DNA binding;

2. **DNA Binding Domain** (**DBD**) is receptor **central** part , and .....

3. **C-terminal** signaling molecule - **Ligand Binding Domain** (**LBD**). .....

9. What helices and beta structure **aldosterone** contacting residues bound in a fully enclosed pocket? ..... helices **H3**,**H4**,**H5**,**H6**, **H7**,**H9**&**H11**,and 4 **β**-coupled **strands** .....

10. What one of three amino acid side chain the hydrogen bond–**O**–**H•••:O=C**< and two amino acids **backbone carbonyls >C=O:** bound to **AS4** in 1AA2Marz.pdb?

Thr945....-**O**-**H**... **O=C.AS4**.22**C**-**O**-**H**...**O=C**<Phe941.... un **AS4.**-**O**-**H**...**O=C**<Cys942.....

11. What type secondary structures dose contains the **LBD** androgen receptor **MR 2AA2.pdb**?

4- β-strands, two β-sheets, 11 Alpha-helices.....

12. How many **alpha helices** has **LBD** polypeptide molecule **2AA2.pdb**?**11 Alpha-helices**.....

13. How many **beta structures**: **sheets** and how many **beta strands** constitute **LBD** molecule **2AA2.pdb**? **4- β-strands, two β-sheets**.....

13a. N-terminus amino acid is Leu727..... and C-terminus amino acid is Arg983.....

How meny amino acids have MR polypeptide 984.... (see 2nd page) and **2AA2.pdb** molecule 983-727+1=256+1=257....?

2AA2, **Aldosterone**
2AA7, Deoxycorticosterone
2AA5, **Progesterone**
2AA6, **Progesterone, mutant S810L**
2AAX, **Cortisone , mutant**
2AB2, **Spironolactone, mutant**
3VHU,**deoxycorticosterone**,**2011J.Med.Chem.** 54: **8616-8631**

13b. N-terminus amino acid is Ser600.....and **C-terminus** amino acid is Arg671.....! How many amino acids have MR polypeptide 984.... (see 2nd page) and **4TNT.pdb** 671-600+1=72.....

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>2AA2;4TNT|P08235|MCR_HUMAN receptor OS=Homo sapiens 984 AA
1                                                          60
METKGYHSLPEGLDMERRWGQVSQAVERSSLGPTERTDENNYMEIVNVSCVSGAIPNNST
61                                                         120
QGSSKEKQELLPCLQQDNNRPGILTSDIKTELESKELSATVAESMGLYMDSVRDADYSYE
121                                                        180 DNS
QQNQQGSMSPAKIYQNVEQLVKFYKGNGHRPSTLSCVNTPLRSFMSDSGSSVNGGVMRAV
181                                                        2400 DNS
VKSPIMCHEKSPSVCSPLNMTSSVCSPAGINSVSSTTASFGSFPVHSPITQGTPLTCSPN
241                                                        300
VENRGSRSHSPAHASNVGSPLSSPLSSMKSSISSPPSHCSVKSPVSSPNNVTLRSSVSSP
301                                                        360
ANINNSRCSVSSPSNTNNRSTLSSPAASTVGSICSPVNNAFSYTASGTSAGSSTLRDVVP
361                                                        420
SPDTQEKGAQEVPFPKTEEVESAISNGVTGQLNIVQYIKPEPDGAFSSSCLGGNSKINSD
421                                                        480
SSFSVPIKQESTKHSCSGTSFKGNPTVNPFPFMDGSYFSFMDDKDYYSLSGILGPPVPGF
481                                                        540
DGNCEGSGFPVGIKQEPDDGSYYPEASIPSSAIVGVNSGGQSFHYRIGAQGTISLSRSAR
541                                                        600
DQSFQHLSSFPPVNTLVESWKSHGDLSSRRSDGYPVLEYIPENVSSSTLRSVSTGSSRPS
601                                                        660
KICLVCGDEASGCHYGVVTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACRL   4TNT.PDB
661                                                        720
QKCLQAGMNLGARKSKKLGKLKGIHEEQPQQQQPPPPPPPPQSPEEGTTYIAPAKEPSVN     DNA
721                                                        780
TALVPQLSTISRALTPSPVMVLENIEPEIVYAGYDSSKPDTAENLLSTLNRLAGKQMIQV
781                                                        840
VKWAKVLPGFKNLPLEDQITLIQYSWMCLSSFALSWRSYKHTNSQFLYFAPDLVFNEEKM  2AA2.PDB
841                                                        900 Aldosterone
HQSAMYELCQGMHQISLQFVRLQLTFEEYTIMKVLLLLSTIPKDGLKSQAAFEEMRTNYI
901                                                        960
KELRKMVTKCPNNSGQSWQRFYQLTKLLDSMHDLVSDLLEFCFYTFRESHALKVEFPAML
961      970       980 984                                 1020
VEIISDQLPKVESGNAKPLYFHRK
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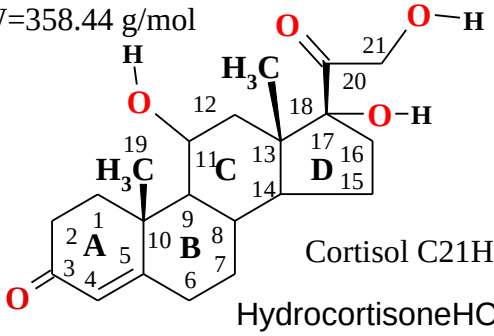
14. What difference has **Hydrocortisone (HCY)** relative to Aldosterone?
instead aldehyde C13 **O=CH**– is methyl group –**CH_3** and C17 instead C-H is C-**O**H.....

15. Put in **Hydrocortisone** (**HCY**) hydrocarbon chains C21 structural formula having two carbonyl **O=C**< and three hydroxyl oxygen atoms–**OH**!
Show carbon –C– cyclic structures **A,B,C,D** double bond >**C=C**< and two methyl groups –**CH_3**!

MW=358.44 g/mol



Cortisol C21H26O5

HydrocortisoneHCY.tgf

16. What two water molecules with hydrogen bonds stabilize **Aldosterone** binding in **LBD**?

..... **HOH**26,**HOH**5 .....

17. What three amino acids bind with hydrogen bonds hydroxyl group –**O**–**H** of **aldosterone**?

.....Phe941,Cys942,Thr945 .....

18. What four amino acids bind with hydrogen bonds carbonyl group **O=C**< of **aldosterone**?

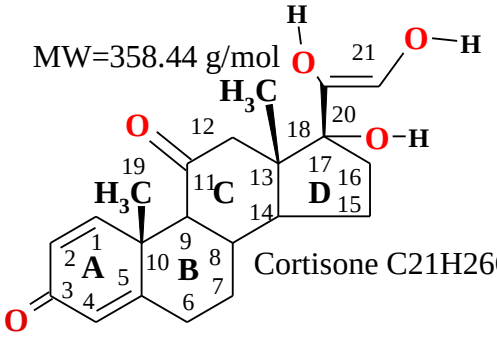
.....Arg817,Ser810,Phe829,Gln776 .....

19. What fifteen amino acids make **active** of **MR** by hydrophilic & hydrophobic pocket for **aldosterone** complementary connection of **receptor** with **AF-2 Helix** and **loop H3**?
Ser767.....,Asn770.....,Ala773.....,Gln776.....,Phe829.....,Ser810.....,Arg817.....,
Met852.....,Leu938.....,Met807.....,Phe941.....,Cys942.....,Thr945.....,Phe956.....,Glu955.....

20. What difference has **Cortisone** relative to Aldosterone? instead **O=CH**– methyl group –**CH_3**....., double bonds C1=C2.....,C20=C21..... and C11 C**=O**..... .

21. Put in **Cortisone** hydrocarbon chains C21 structural formula two carbonyl **O=C**< and three hydroxyl oxygen atoms –**OH**! at C4=C5, C1=C2 and C20=C21 and two methyl groups –**CH_3**!

MW=358.44 g/mol



Cortisone C21H26O5

Show carbon –C– cyclic structures **A,B,C,D,** double bond >**C=C**<

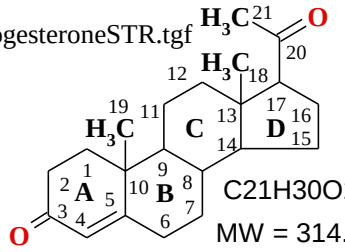
20. What two amino acids make disulfide bond in **LBD** protein unit structure 1E3G.pdb?

..... disulfide bond Cys669.....- **S** - **S** -Cys 844 .....

21. What difference has **Progesterone** relative to Aldosterone? instead **O=CH**– is methyl group –**CH$_3$**...., at C20 carbonyl group >**C=O**.... no hydroxyls –**OH** at C11...,C17...,C21... .

22. Put in **Progesterone STR** hydrocarbon chains C21 structural formula two carbonyl **O=C**< and no one hydroxyl oxygen atoms –**OH**!

progesteroneSTR.tgf



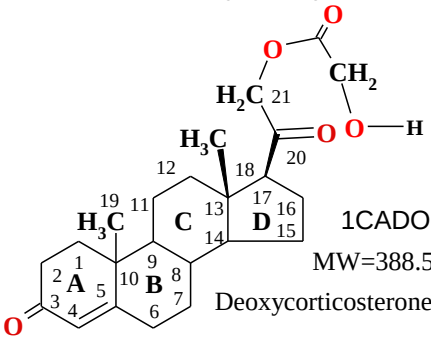
C21H30O2

MW = 314.47 g/mol

Show carbon –C– cyclic structures **A,B,C,D,** double bond **>C=C<** at C4=C5 and two methyl groups –**CH$_3$**!

23. Show difference **Desoxycorticosterone** to Aldosterone? instead **O=CH**– methyl –**CH$_3$**... at C20 carbonyl >**C=O**... at C21 hydroxyl acetate ester... no hydroxyl –**OH** at C11...,C17... .

24. Put in **Desoxycorticosterone DOC** hydrocarbon chains C23 structural formula two carbonyl **O=C**< and no hydroxyl oxygen –**OH** C11,C17. Show carbon –C– cyclic structures **A,B,C,D,** double bond **>C=C<** at C4=C5 and two methyl groups –**CH$_3$**!



1CADOC.tgf

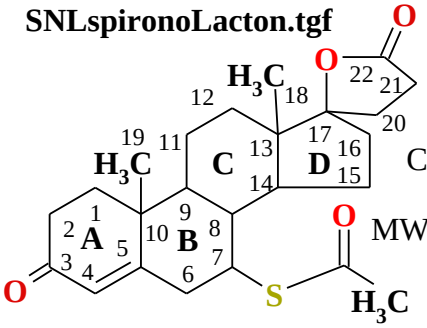
MW=388.51 g/mol

Deoxycorticosterone C23H32O5

25. Show difference **Spironolactone SNL** to Aldosterone? instead C13 aldehyde **O=CH**– methyl group –**CH$_3$**...., carboxylate at C22 >**COO-**..... and at C7 sulfur **S**... .

26. Put in **Spironolactone SNL** hydrocarbon chains C22 structural formula two carbonyl **O=C**< and no one hydroxyl oxygen –**OH**! Show carbon –C– cyclic structures **A,B,C,D,** double bond **>C=C<** at C4=C5 and two methyl groups –**CH$_3$**!

SNLspironoLacton.tgf



C24H32O4S

MW=416.58 g/mol

Antagonism of **MR** and **MR**—**Spironolactone** is an **antihypertensive** that has been used clinically for several decades. The crystal structures of **MR** C808S/S810L with **spironolactone** and **progesterone** provide insights into the requirements needed for **receptor activation** and also provide insights into the molecular basis of **MR** modulation. **MR antagonism** by **progesterone** and **spironolactone** is a "**passive**" **antagonism**. These ligands bind and prevent **MR** from adopting the active conformation by failing to mediate **hydrogen bonding** to **Asn770** and **Thr945**. The result is that both **helix 3** and the **AF-2 helix** are not arranged in the proper position to allow efficient binding of **co activators** Activation Domain (**AD**) of transcription.

CONCLUSION We have shown that maximum **MR activation** occurs only when there is simultaneous stabilization of the l**oop** preceding the **AF-2 helix** and a strong interaction of the ligand (**aldosterone**) with **helix 10**. Stabilization of the **loop** preceding the **AF-2** requires **hydrogen bonds** between **Asn770** and **Ser767** on **helix 3** and **Glu955** present on this **loop**. Ligands that promote this **hydrogen bond** network and interact with **helix 10** via **hydrogen bonds** or **hydrophobic** interaction with **Thr945** induce a stabilization of **helix 3** and a movement of the **AF-2**, enabling **co activator** recruitment and ultimately gene transcription. This Series of **ligand**-mediated activation steps ensures that **ligands** such as **progesterone** and **cortisone** fail to activate **MR** even though these **ligands** will be in excess over **aldosterone** in many tissues. Likewise, **spironolactone** also fails to activate **MR** because of an inability to create the **hydrogen bonding** network as hydrogen bonding **MR antagonist**.