Name Surname :_____ Group Nº _____2023

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 2023 molecular tutorials: solution

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. .....

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

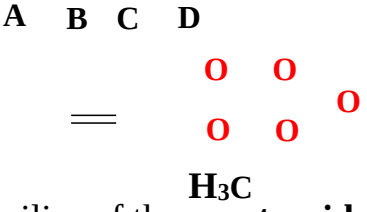
1. **MR** weak .....2. antihyprtensive .....

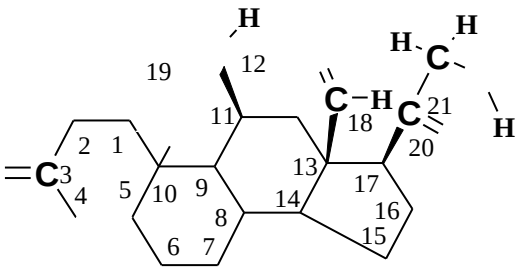
3. binding selectivity .....over 4. .....

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

1. physiological role in the **HOMEOSTASIS** of ..... and .....**regulation**
2. primarily physiologic .....and .....levels **regulation**
3. on in the ..... and.....in **distal** .....
4. largest influence on **vascular** .....

6. Put in **Aldosterone** hydrocarbon chain ring symbols: stabilizing double bond from C4 to C5 >**C=C**< five oxygen atoms and methyl group at C10





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

1. ..... receptor (.....
2. ..... receptor (.....
3. ..... receptor (.....

8. Nuclear receptor Complexes functional **Domains** short cut names are :

1. **N-terminal** domen ..... variable receptor **activations domen** ... DNA binding;
2. receptor **central** part is a **DNA Binding Domain** .....and
3. a **C-terminal** signaling molecule - **Ligand Binding Domain** . .....

9. What helices and beta structure **aldosterone** contacting residues bound in a fully enclosed pocket? Helices H...,H...,H...,H..., H...,H... & H.....,and ...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?

Thr....-**O**-**H**... **O=C**.**AS4**.22**C**-**O**-**H**...**O=C**<Phe.... un **AS4**.-**O**-**H**...**O=C**<Cys.....

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

..... **β-strands,** .....**β-sheets**, ...**Alpha-helices**

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

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

13a. N-terminus amino acid is Leu..... and C-terminus amino acid is Arg.....

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

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 Ser.....and **C-terminus** amino acid is Arg.....! How many amino acids have MR polypeptide .... (see 2nd page) and **4TNT.pdb** 671-600+1=.....

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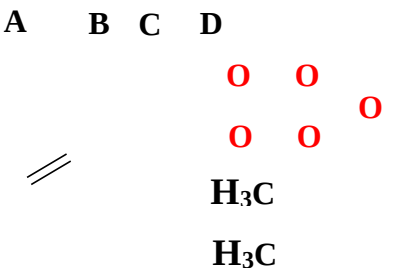
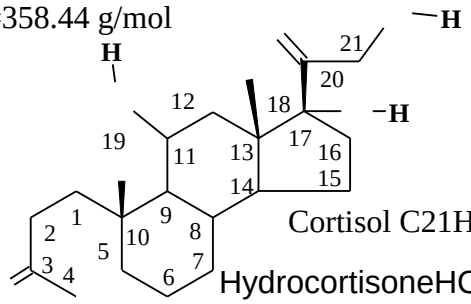
>2AA2;4TNT|P08235|MCR_HUMAN receptor OS=Homo sapiens 984 AA
1                                                          60
METKGYHSLPEGLDMERRWGQVSQAVERSSLGPTERTDENNYMEIVNVSCVSGAIPNNST
61                                                         120
QGSSKEKQELLPCLQQDNNRPGILTSDIKTELESKELSATVAESMGLYMDSVRDADYSYE
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
```

14. What difference has **Hydrocortisone (HCY)** relative to Aldosterone?
instead aldehyde C13 **O=CH**– is methyl group ..... and C17 instead C-H is C-.....

15. Put in **Hydrocortisone** (**HCY**) hydrocarbon chains ring symbols:
stabilizing double bond from C4 to C5 >**C=C**<
five oxygen atoms and
methyl group at C10



MW=358.44 g/mol



Cortisol C21H26O5

HydrocortisoneHCY.tgf

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

HOH.....,**HOH**.....

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

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

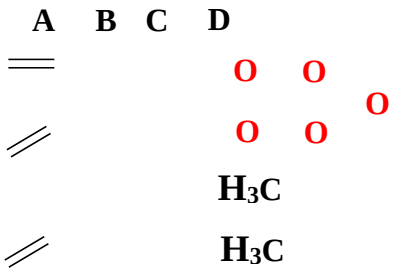
19. What fifteen amino acids make **active** of **MR** by hydrophilic & hydrophobic pocket for **aldosterone** complementary connection of **receptor** with **AF-2 Helix** and **loop H3**?

Ser.....,Asn.....,Ala.....,Gln.....,Phe.....,Ser.....,Arg.....,

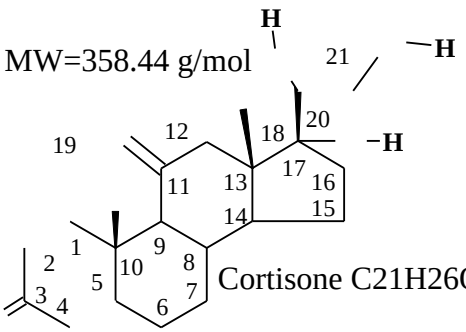
Met.....,Leu.....,Met.....,Phe.....,Cys.....,Thr.....,Phe.....,Glu.....

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

21. Put in **Cortisone** hydrocarbon chains ring symbols, stabilizing double bond from C1=C2,C4=C5, C20=C21 >**C=C**<
five oxygen atoms and
methyl groups at C10, C13 !



MW=358.44 g/mol



Cortisone C21H26O5

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

..... disulfide bond Cys.....- **S** - **S** -Cys.....

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

22. Put in **Progesterone STR**

hydrocarbon chains

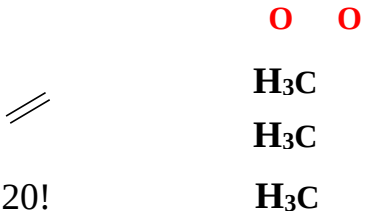
ring symbols: **A B C D**

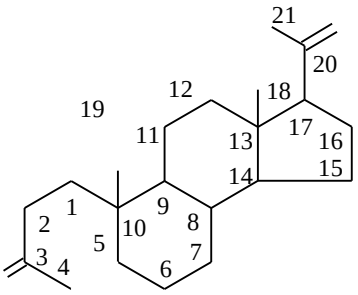
stabilizing double bond

from C4 to C5 >**C=C**<

two oxygen atoms and

methyl groups at C10, C13, C20!





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

24. Put in **Desoxycorticosterone DOC** hydrocarbon chains

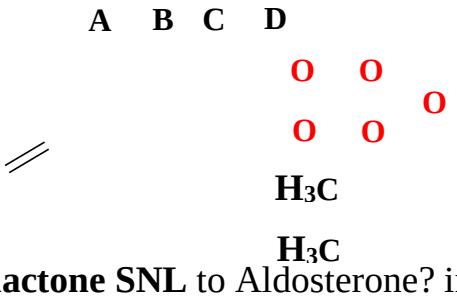
ring symbols: **A B C D**

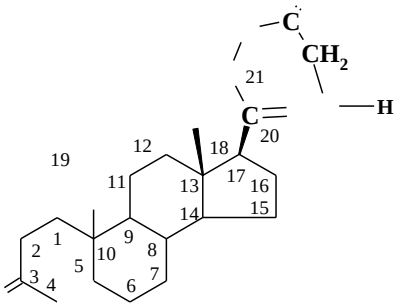
stabilizing double bond

from C4 to C5 >**C=C**<

five oxygen atoms and

methyl groups at C10, C13!





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

26. Put in **Spironolactone SNL**

hydrocarbon chains

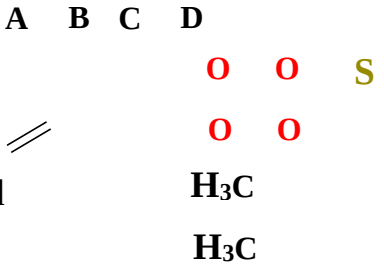
ring symbols: **A B C D**

stabilizing double bond

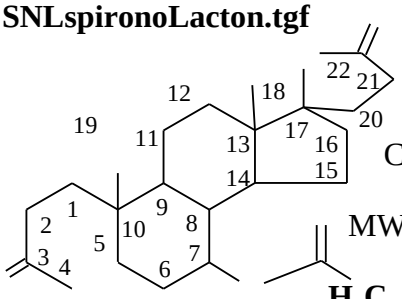
from C4 to C5 >**C=C**<

four oxygen atoms, sulfur and

methyl groups at C10, C13!



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 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 and thus behaves as a passive **MR antagonist**.