Interaction of Benzodiazepine Drugs Flunitrazepam and Clonazepam with Aldoketoreductase

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Background

Benzodiazepines:

- Class of drugs that act as central nervous system depressants, among most commonly prescribed drugs in Western hemisphere
- Benzene ring fused to 7-membered diazepine ring, phenyl ring attached to 5-position of diazepine ring
- Clonazepam (CLO; generic: Klonopin)
  - Used to treat seizure and panic disorders, mania
- Flunitrazepam (FLU; generic: Rohypnol or 'roofie')
  - Extremely potent, high abuse potential
  - Often used as a 'date-rape' drug due to sedative and amnesia-inducing properties

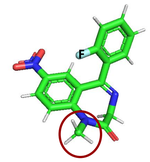


Figure 1. Flunitrazepam

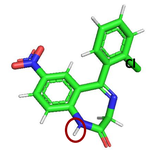


Figure 2. Clonazepam

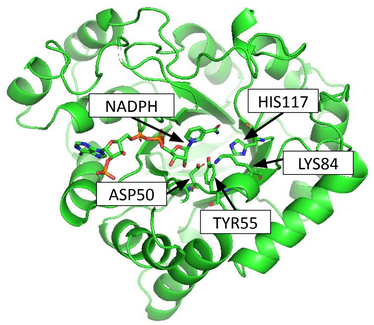


Figure 3. Conserved catalytic tetrad on AKR1C3

Aldoketoreductase (AKR):

- 15 types of AKRs in humans
- AKR1C class: involved in steroid hormone synthesis, recently implicated in drug metabolism
- $(\alpha/\beta)_8$-barrel structure, conserved catalytic center containing cofactor NADPH (Figure 3)
- AKR1C3 has been shown to metabolize flunitrazepam

Objectives:

- Understand why flunitrazepam is metabolized by AKR1C3 and clonazepam is not, despite being very similar molecules.
- Understand why flunitrazepam is not metabolized by the other three members of the AKR1C class (AKR1C1, 2, and 4).

Approach: Molecular modeling, simulations, computer graphics

Methods

Autodock Vina:
Software that explores combinations of rotations, translations and conformations of ligands to generate potential poses in the protein binding site. Scores each pose for best fit against the protein, returns top 9 poses.

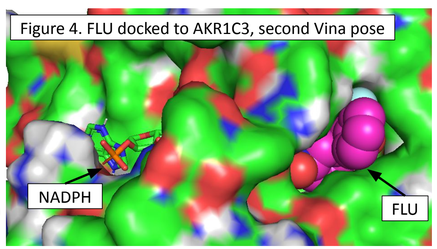


Figure 4. FLU docked to AKR1C3, second Vina pose

Methods (cont.)

Figure 5. Scan for visualization of NAMD output

Nanoscale Molecular Dynamics (NAMD):
Applied software that simulates thermal motion of protein and ligand complex in solution over time. Used to provide mechanistic insight into protein function. Wrote software to analyze docking and molecular dynamics simulations.

Electrostatics:
Calculation of binding free energy using the Poisson-Boltzmann model of electrostatics, provides insight about energetic favorability of binding.

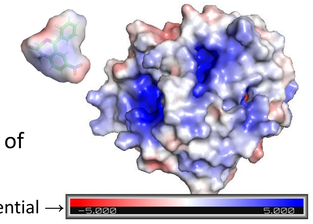
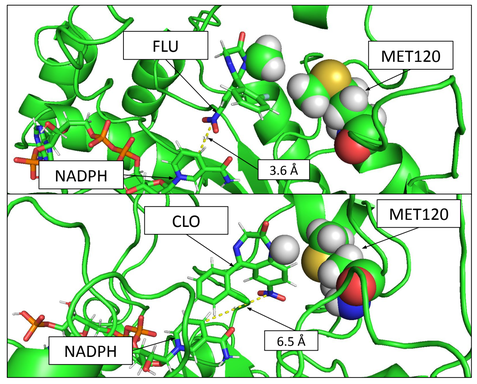


Figure 6. FLU (L) and AKR1C3 surface (R) color-coded by electrostatic potential →

Results and Discussion

- FLU moves rapidly closer to binding site as shown in Figure 9 movie (initial Vina pose is 5.3 Å away from NADPH)
- FLU is within hydrogen bonding distance of active site on average, remains within 4 Å of NADPH 95% of the time (Table 1)
- CLO is on average 1.7 Å further away from NADPH than FLU, further than required for catalytic interaction
  - Only within 4 Å 6.8% of the time
- FLU has more favorable binding free energy than CLO
- Molecular dynamics supports FLU being a substrate of AKR1C3, CLO not being a substrate

Figure 7. Snapshot of binding site in molecular dynamics trajectories for FLU (upper) and CLO (lower)



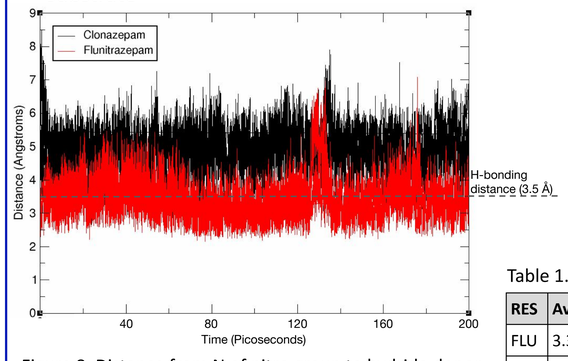


Figure 8. Distance from N of nitro group to hydride donor of NADPH for Flunitrazepam and Clonazepam

Figure 9. Scan for visualization of FLU binding progression

Table 1. Proximity to NADPH for each ligand

RES	Avg dst from N to NADPH	% time within 4 Å
FLU	3.3 Å	95.0
CLO	5.0 Å	6.8

Results and Discussion (cont.)

RESID	FREQ
CLO	
TRP86	93.7%
SER118	79.6%
MET120	65.5%
FLU	
MET120	79.5%
TYR319	55.2%

← Table 2. Residues within 4 Å of CLO nitrogen proton and FLU methyl group in more than 50% of the molecular dynamics trajectories.

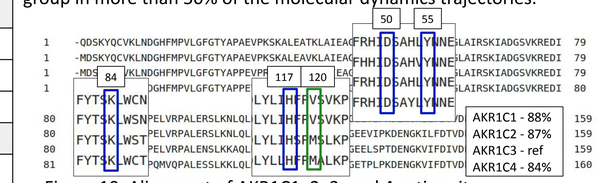
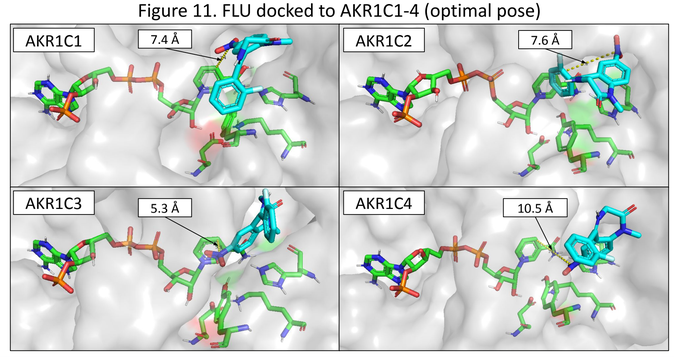


Figure 10. Alignment of AKR1C1, 2, 3, and 4 active site sequences; percent sequence identities of AKR1C1, 2, and 4 with AKR1C3.

- Analysis of residues near FLU methyl group and CLO nitrogen proton in molecular dynamics data suggests that MET120 plays key role in FLU binding to AKR1C3. CLO has less interaction with MET120 (Table 2)
  - Residue 120 is a methionine only in AKR1C3 and 4 (Figure 10)
- Vina docking of FLU to AKR1C1, 2, and 4 does not yield poses with nitro group pointed towards NADPH inside binding site (Figure 11)
- This analysis supports the experimental finding that FLU is only metabolized by AKR1C3

Figure 11. FLU docked to AKR1C1-4 (optimal pose)



Key Takeaways

- FLU nitro group remains within hydrogen bonding distance of the NADPH hydride donor throughout most of molecular dynamics simulation
- Hydrophobic interaction between FLU methyl group and MET120 plays key role in positioning drug close to catalytic center

Acknowledgements

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