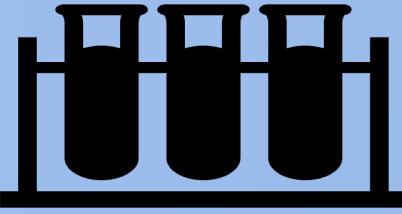


# Pipeline to identify stable conformers of APIs

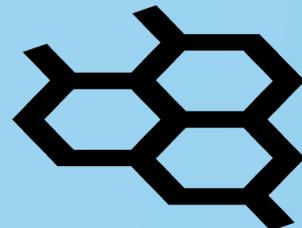
A case study with Rotigotine,  
Diflorasone diacetate &  
Ritonavir

# Context: Why low energy conformers of SMOLs are of interest?



## Property modelling

- The physical properties of a molecule, such as melting point and solubility, are often dependent on the stability of its conformers.



## Drug discovery

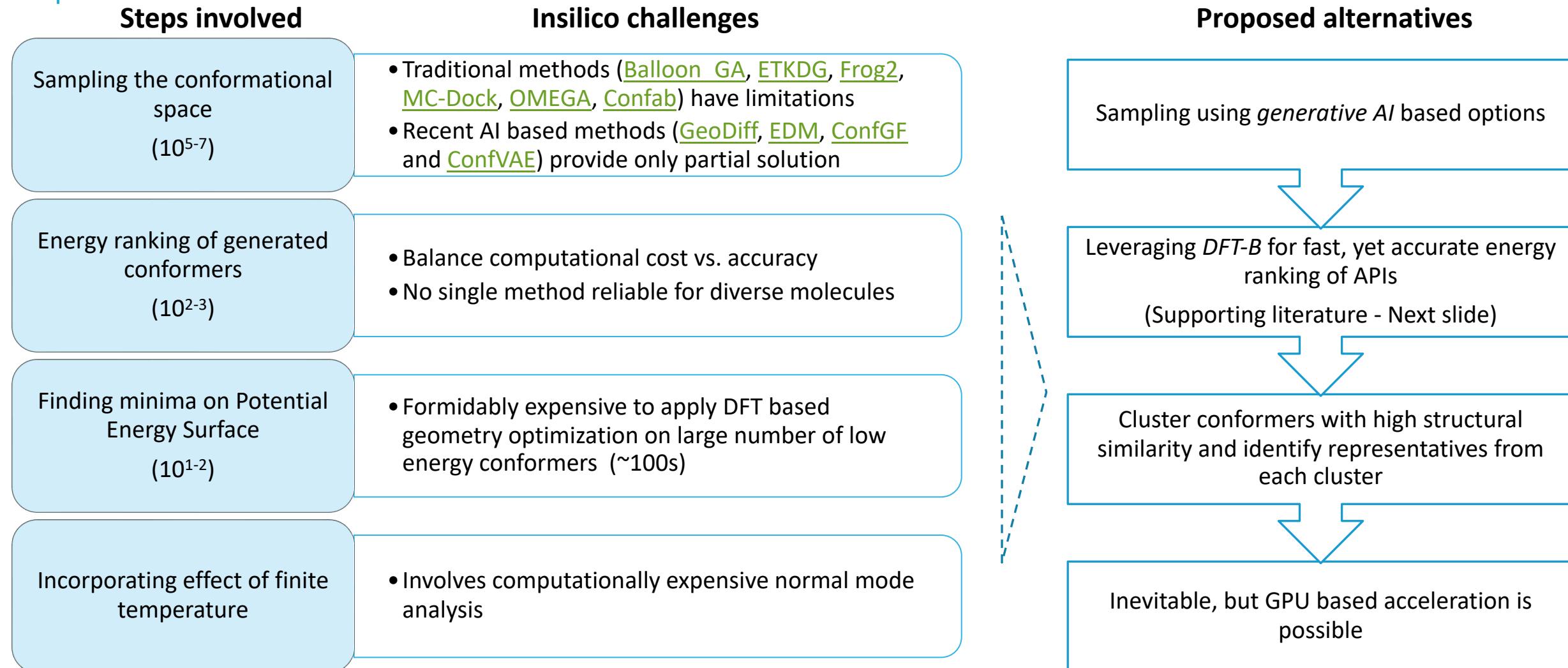
- 3D structure of SMOLs influence their interaction with target biomolecules such as protein, RNA, etc.
- Knowing which molecular conformations are most likely to interact with a specific receptor helps in designing drugs with improved efficiency and reduced side-effects e.g., unwanted off-target binding



## ADMET profiling

- Conformers with lower energy are more likely to be present in solution, and thus are more likely to participate in chemical reactions.
- Solid state properties such as tabletability also depend on the ensemble of stable conformers

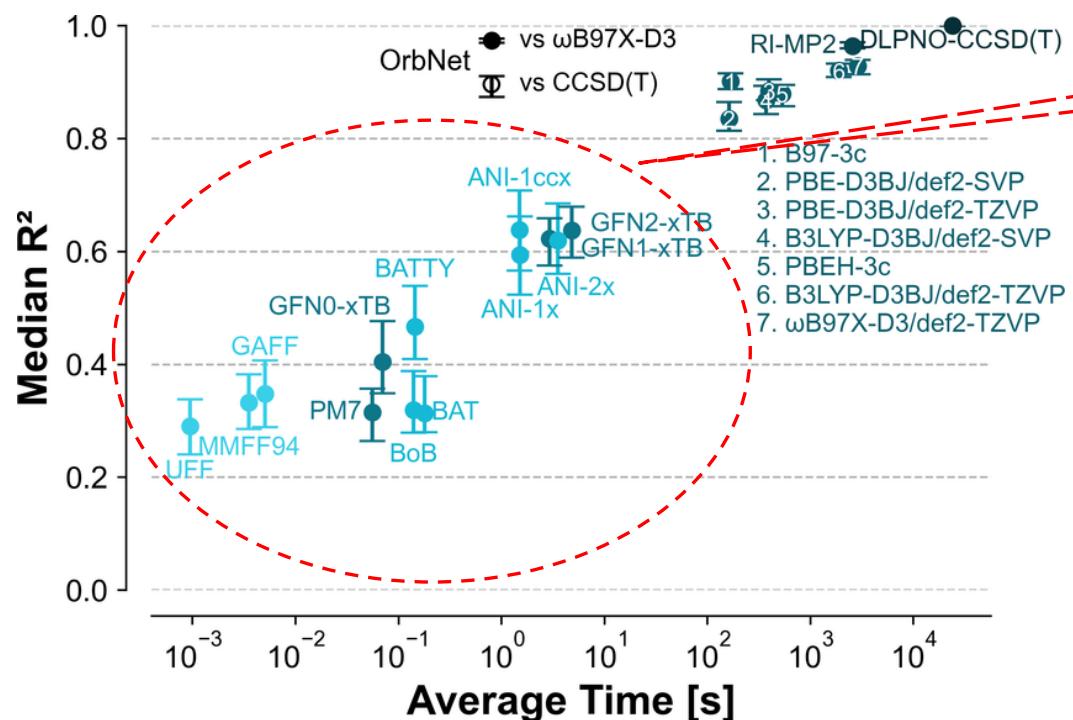
# Conformation search: Limitations of conventional methods



# DFT-B, a fair compromise between cost & accuracy for energy ranking

Computational cost vs. accuracy of methods

Hutchison conformer benchmark set ([GitHub](#)).



Faster methods provide poor estimate of the total energy of the molecule.

- However, good spearman rank correlation coefficient ( $>0.7$ ) is reported for DFT-B=> Can be used for ranking



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Assessing conformer energies using electronic structure and machine learning methods

Dakota Folmsbee, Geoffrey Hutchison

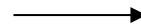
First published: 09 July 2020 | <https://doi.org/10.1002/qua.26381> | Citations: 27

Source: OrbNet Denali @ [J. Chem. Phys. 155, 204103 \(2021\)](#)

# Proposed pipeline: Stable conformer search

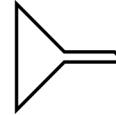
## A. Conformer generation and screening

- Torsional diffusion<sup>1</sup>: Leveraging Gen AI
- Screen out conformers with chemically infeasible geometries
- 3-5 hrs for 100K conformers<sup>2</sup>



## B. Preliminary energy screening

- DFT-B: Good compromise between computational cost vs. accuracy (Prev. slide)
- 6 hours for 100K conformers<sup>3</sup>
- Select N lowest energy conformers (N=1K in this study)



## C. Clustering of low energy conformers

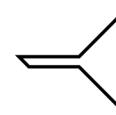
- Based on pairwise RMSD
- Optimal #clusters decided based on elbow plot
- $\sim 1 \text{ hr}^3$



Expected total time  $\sim 2\text{-}3$  days

## E. Incorporate temperature effects

- Hessian calculation at experimental temperature
- $\sim 1 \text{ day}^4$



## D. Geometry optimization of the cluster centroids

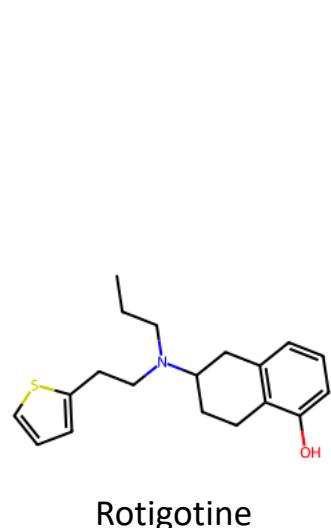
- Using DFT with appropriate functional and basis set
- $\sim 1 \text{ day}^4$
- Select conformers with energy  $< \Delta E_{\text{cutoff}}$
- $\Delta E_{\text{cutoff}} = 20 \text{ kJ mol}^{-1}$  for this study
- $\Delta E_{\text{cutoff}}$  based on the energy distribution of the geometry optimized cluster centroids

- Operates on the space of torsion angles, and leaves the other degrees of freedom fixed
- Trained on MIT's GEOM-DRUGS dataset (304K drug-like molecules with energies)

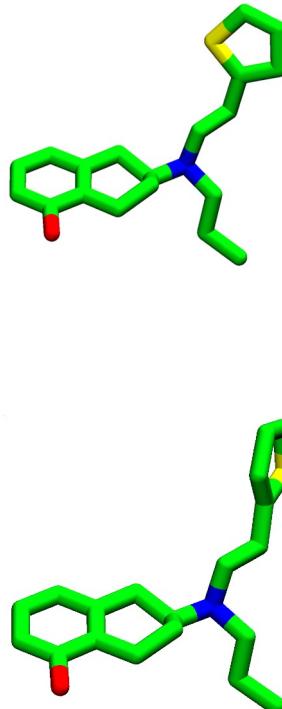
1. <https://arxiv.org/abs/2206.01729>
2. Performed on GPU with 11 GB memory
3. Performed on Mac M1 with 8 GB memory
4. Performed on GPU with 24 GB memory

# Case study: APIs showing conformational polymorphism leading to different physicochemical properties

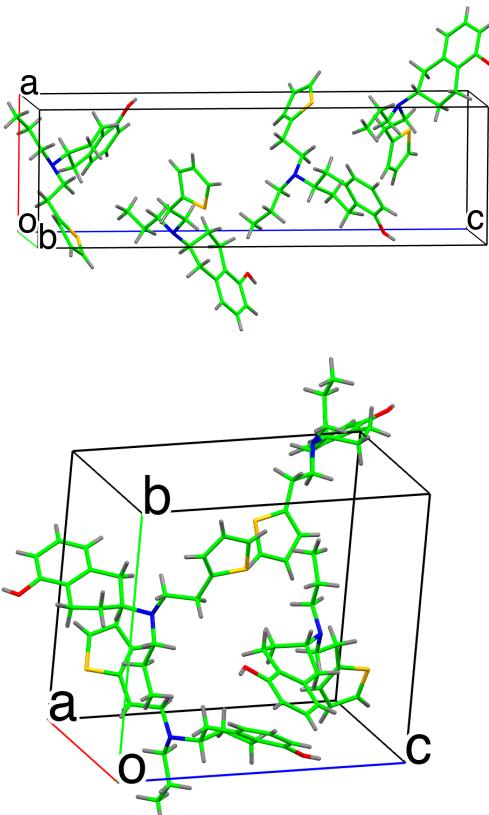
Molecule



Conformations



Crystal structure



APIs showing conformational polymorphism

	API	MW (g/mol)	#rot. bonds
1	Tolbutamide	270.35	5
2	Chlorpropamide	276.74	4
3	<b>Rotigotine</b>	315.5	6
4	N-(4'-methoxyphenyl)-3-bromothiobenzamide	322.22	3
5	Acitretin	326.4	6
6	Furosemide	330.74	5
7	Axitinib	386.5	5
8	Aripiprazole	448.4	7
9	Diflorasone diacetate	494.5	6
10	Ritonavir	720.9	18

Chosen for the case study

CHEMICAL  
REVIEWS

Review

pubs.acs.org/CR

## Conformational Polymorphism

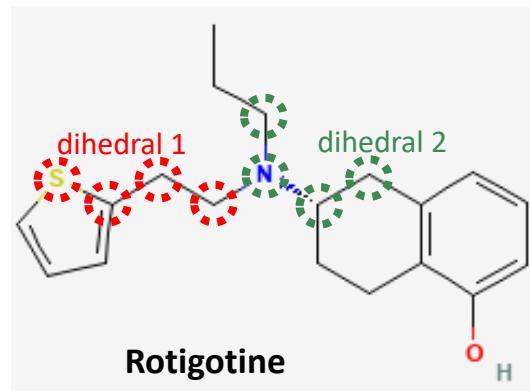
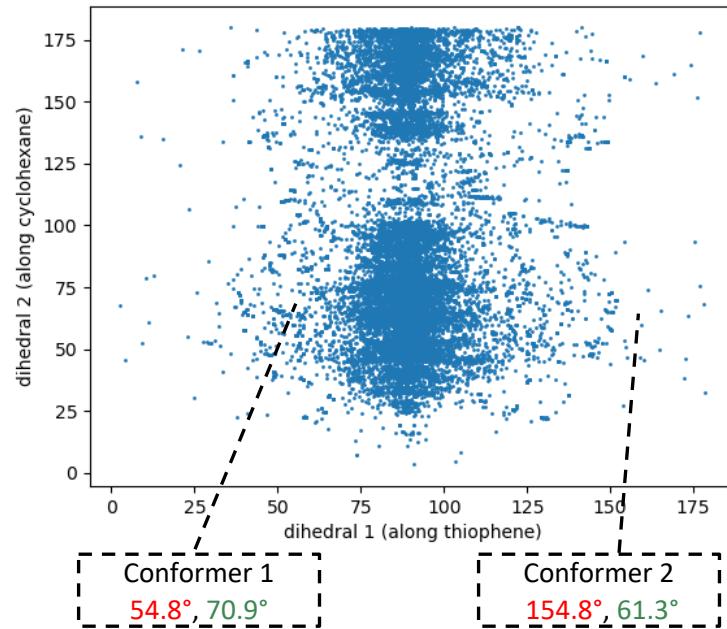
Aurora J. Cruz-Cabeza\*† and Joel Bernstein‡§

\*Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

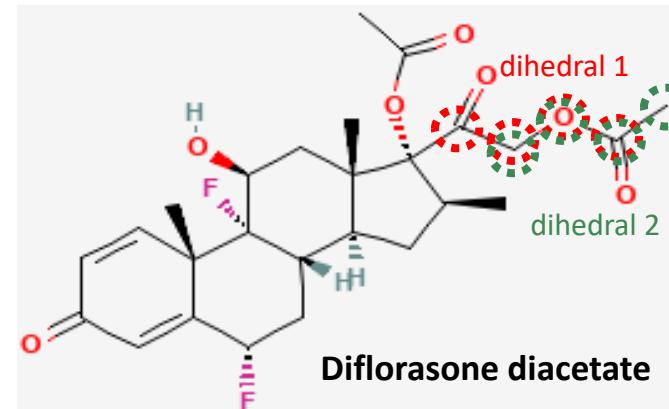
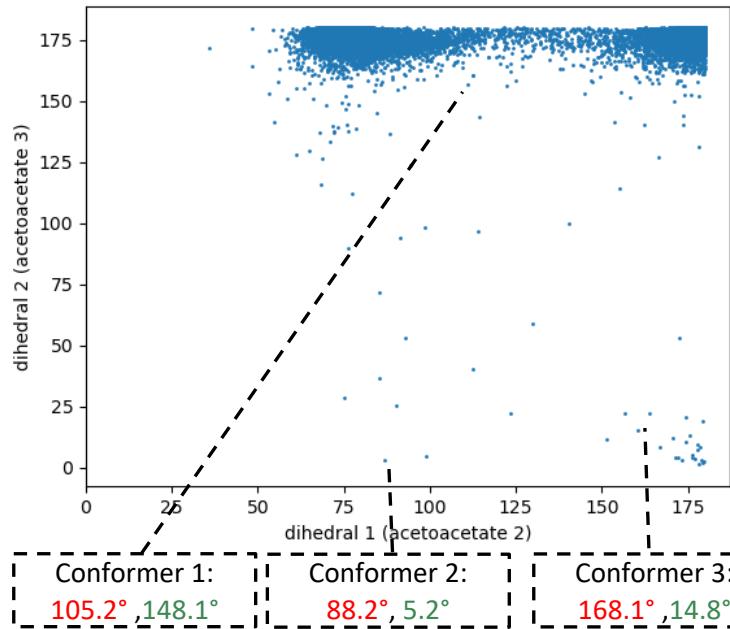
†Faculty of Natural Sciences, New York University Abu Dhabi, P.O. Box 129188, Abu Dhabi, United Arab Emirates

‡Department of Chemistry, Ben-Gurion University of the Negev, P.O. Box 653, Beer Sheva, Israel 84120

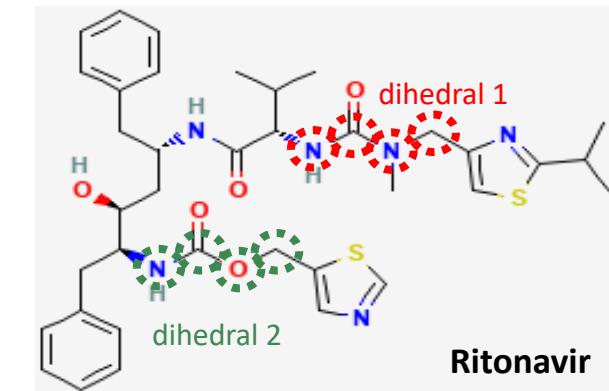
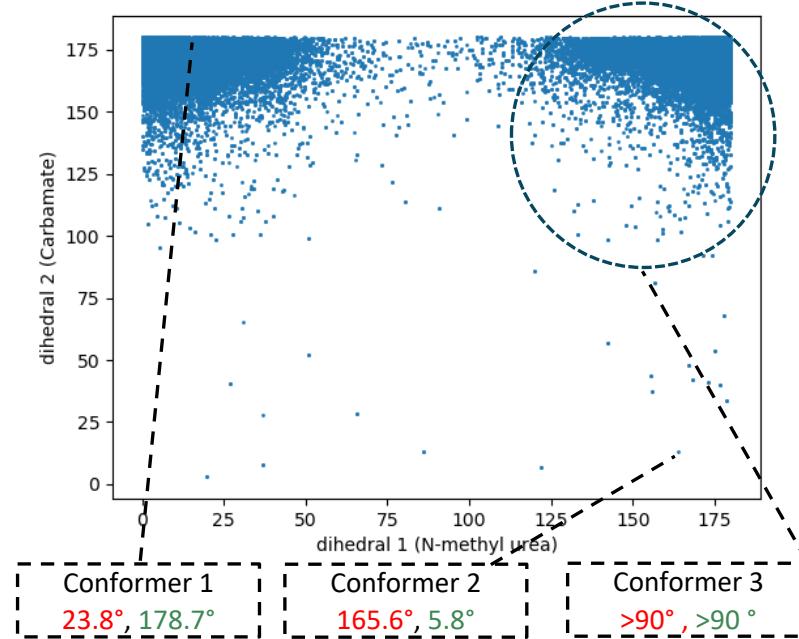
# Generative AI helps sample (100K) the conformation space well, including conformers in known crystal structures



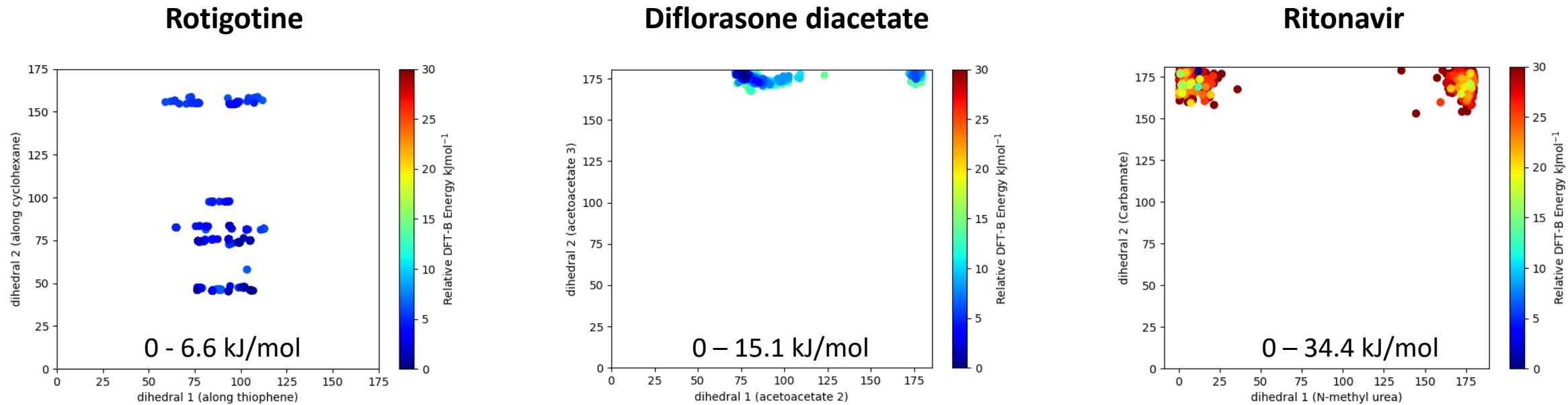
Conformers found in  
known crystal structures



Confidential and Proprietary. Copyright © 2017-24 Aganitha



# Preliminary energy screening: Top-N conformers to be chosen based on the molecule complexity (N=1000 for this study)

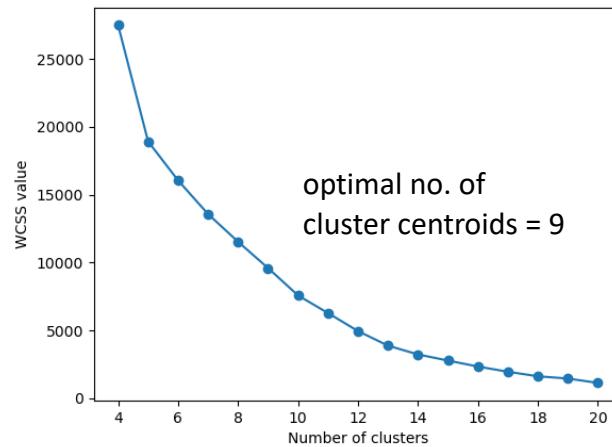


$\Delta E$  1000<sup>th</sup> conformer: DFT-B energy (kJ/mol) of the 1000<sup>th</sup> conformer relative to that of the most stable conformer

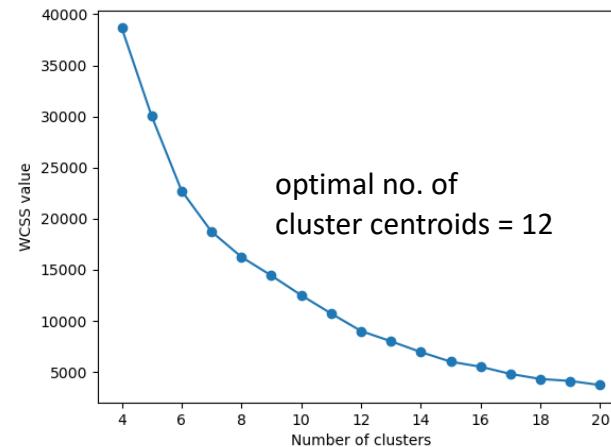
In this case,  $\Delta E$  (in kJ/mol) 1000<sup>th</sup> conformer of Ritonavir (34.4) > Diflorasone diacetate (15.1) > Rotigotine (6.6)

# Clustering of low energy conformers to identify geometrically diverse low energy conformers

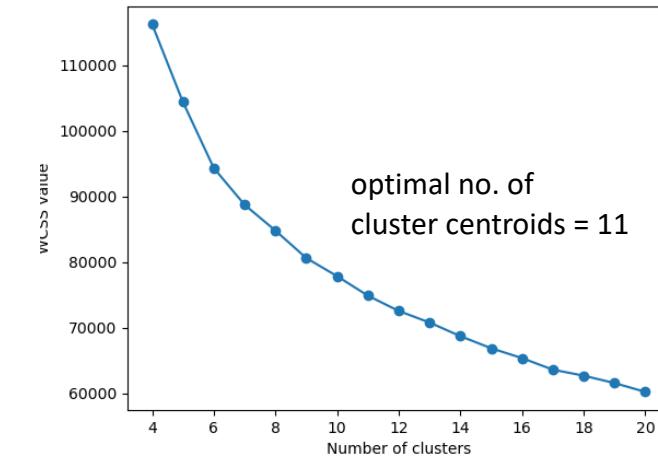
**Rotigotine**



**Diflorasone diacetate**



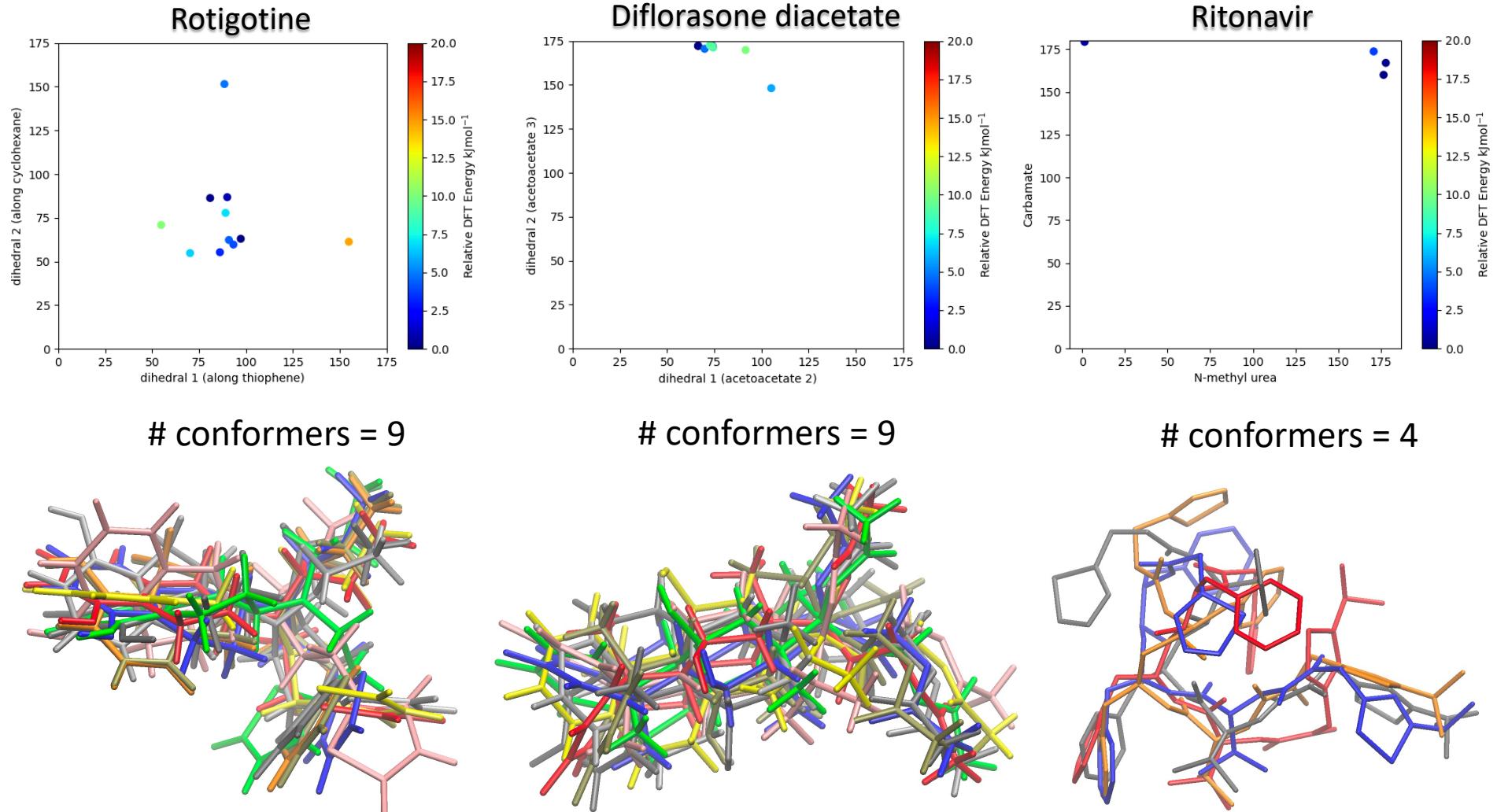
**Ritonavir**



- K-Means clustering of the Top-1000 lowest energy conformers based on pairwise RMSD.
- RMSD is calculated by aligning two sets of atomic coordinates using the [Kabsch algorithm](#)
- WCSS = Within Cluster Sum of Squares of RMSD w. r. t. centroid
- Optimal number of cluster centroids decided based on the rate of change of slope w. r. t. increase in number of clusters.

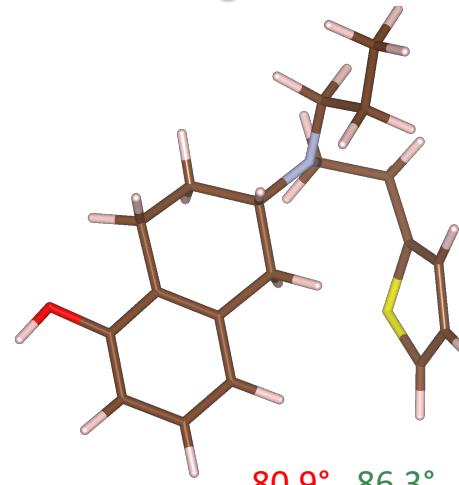
# DFT optimization of cluster centroids reveal unique local minima

- Unique low energy conformers identified with  $\Delta E < 20 \text{ kJ mol}^{-1}$  for this study
- Represent the local minima on the PES at  $T = 0 \text{ K}$
- DFT calculations were carried out at B3LYP/6-31G level of theory



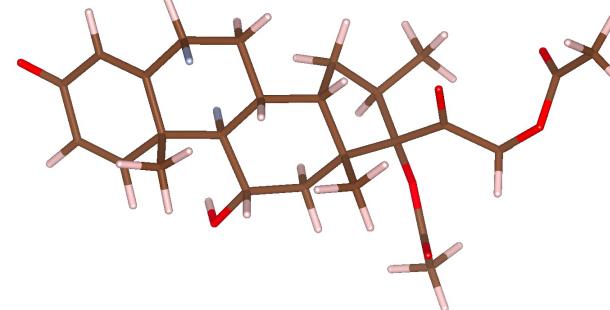
# Temperature correction on the local minima to identify the most stable conformer (in vacuum) at room temperature<sup>1</sup>

Rotigotine



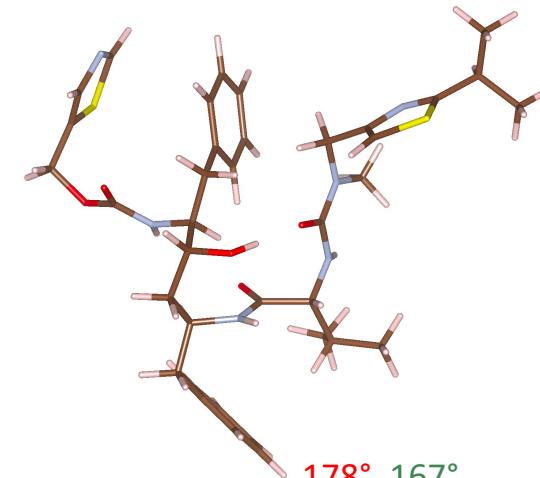
80.9° , 86.3°

Diflorasone diacetate



74.0° , 172.1°

Ritonavir



178° , 167°

## Comparison with the in vacuum optimized geometries of the conformers found in known crystal structures

Expt. known conformer	$\Delta E_{298}$ (expt.) in kJ/mol <sup>2</sup>	#local minima with $\Delta E_{298} <$ $\Delta E_{298}$ (expt.)
1	-6.9	0/9
2	+5.6	4/9

Expt. known conformer	$\Delta E_{298}$ (expt.) in kJ/mol <sup>2</sup>	#local minima with $\Delta E_{298} <$ $\Delta E_{298}$ (expt.)
1	+1.2	6/9
2	+66.4	9/9
3	+93.0	9/9

Expt. known conformer	$\Delta E_{298}$ (expt.) in kJ/mol <sup>2</sup>	#local minima with $\Delta E_{298} <$ $\Delta E_{298}$ (expt.)
1	+26.0	4/4
2	+60.5	4/4
3	NA <sup>3</sup>	-

1.  $\Delta E_{298}$  = Relative enthalpy from Hessian calculations at T = 298 K. Some of the conformers have one imaginary frequencies that is  $> 100 \text{ cm}^{-1}$ .

2.  $\Delta E_{298}$ (expt.) = Thermally corrected enthalpy<sub>Most stable conformer identified Insilico</sub> - Thermally corrected enthalpy<sub>DFT optimized geometry (vacuum) of the conformers in known crystal structures</sub>

3. Crystal structure corresponding to conformer 3 is not available

# Pipeline predicted stable conformers

APIs that display conformational polymorphism	{SC} = Set of <i>in silico</i> identified stable conformers	Conformers from known polymorphs present in {SC} †	Stability of the conformers in {SC} w.r.t. gas phase optimized geometry of conformers found in known polymorphs †
<b>Rotigotine</b> (MW: 315 g mol <sup>-1</sup> , #rot. bond: 6)	9	1/2	<ul style="list-style-type: none"><li>• 0/9 are more stable than *conformer 1</li><li>• 4/9 are more stable than *conformer 2</li></ul>
<b>Diflorasone diacetate</b> (MW: 494 g mol <sup>-1</sup> , #rot. bond: 6)	9	1/3	<ul style="list-style-type: none"><li>• 6/9 are more stable than *conformer 1</li><li>• 9/9 are more stable than the *conformer 2 &amp; 3</li></ul>
<b>Ritonavir</b> (MW: 720 g mol <sup>-1</sup> , #rot. bond: 18)	4	2/3	<ul style="list-style-type: none"><li>• All the insilico identified conformers are more stable than the known conformers</li></ul>

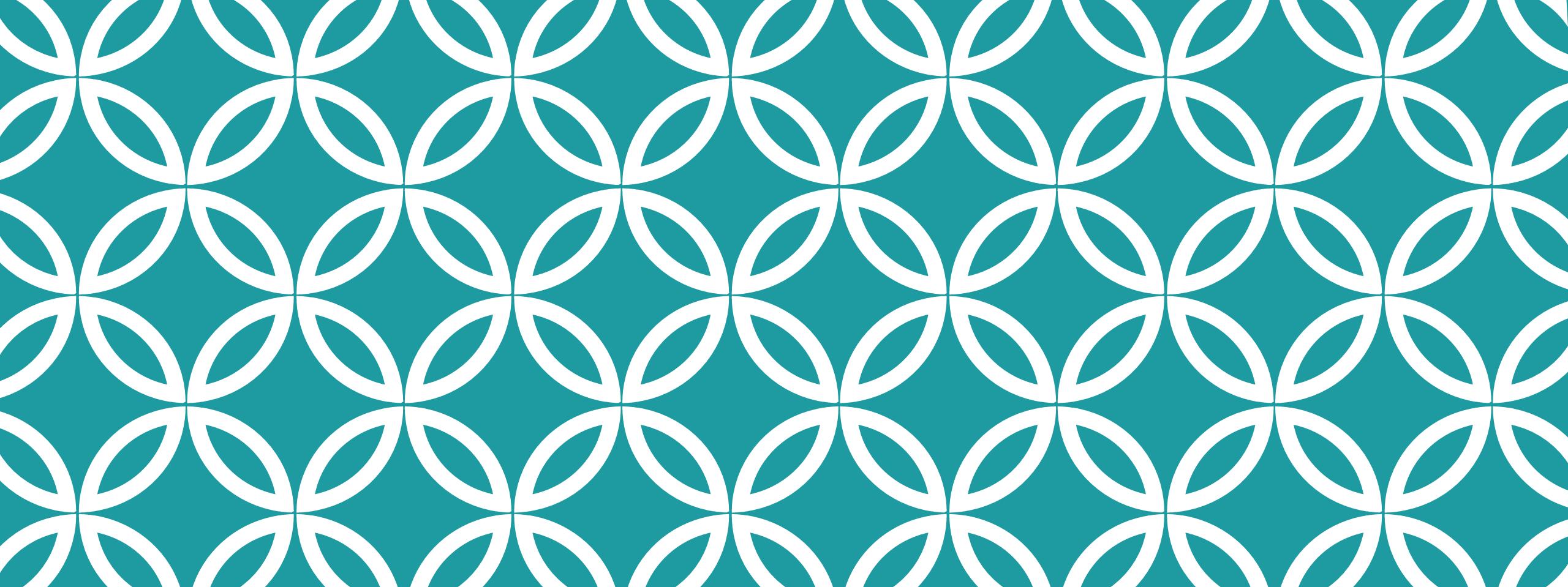
† For the purpose of PoC,

(a) Only  $10^5$  conformers were generated. Ideally,  $10^{6-7}$  conformers are to be generated

(b) Only top 1% lowest energy conformers were considered for clustering. Ideally, top 10% should be considered

(c) DFT calculations performed using a relatively small basis set (6-31G) to reduce the computational overhead. Ideally, larger basis sets augmented with polarization & diffuse functions for drug-like molecules are to be used

\* Conformer 'x' is the gas phase optimized geometry of each conformer found in known crystal structures.

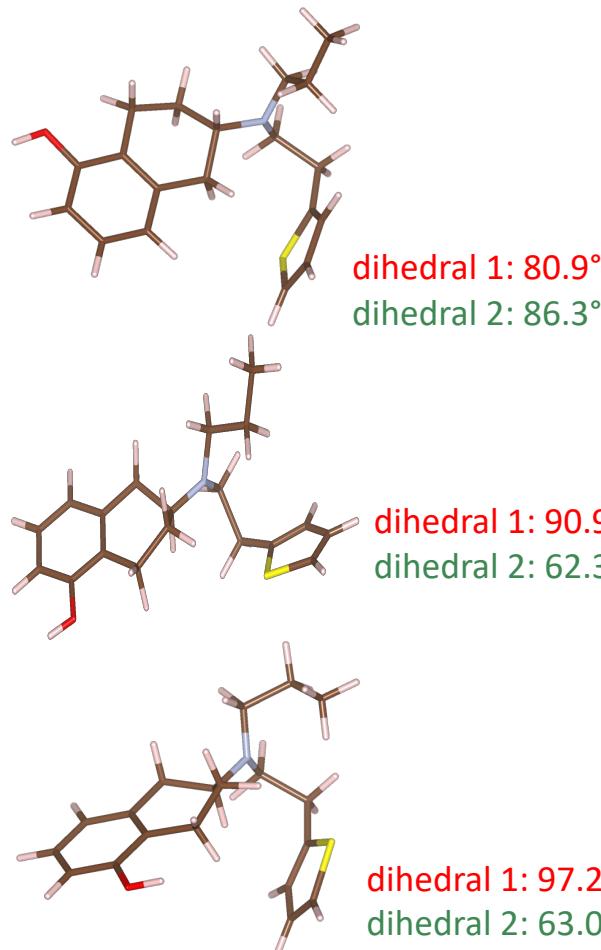


# Appendix

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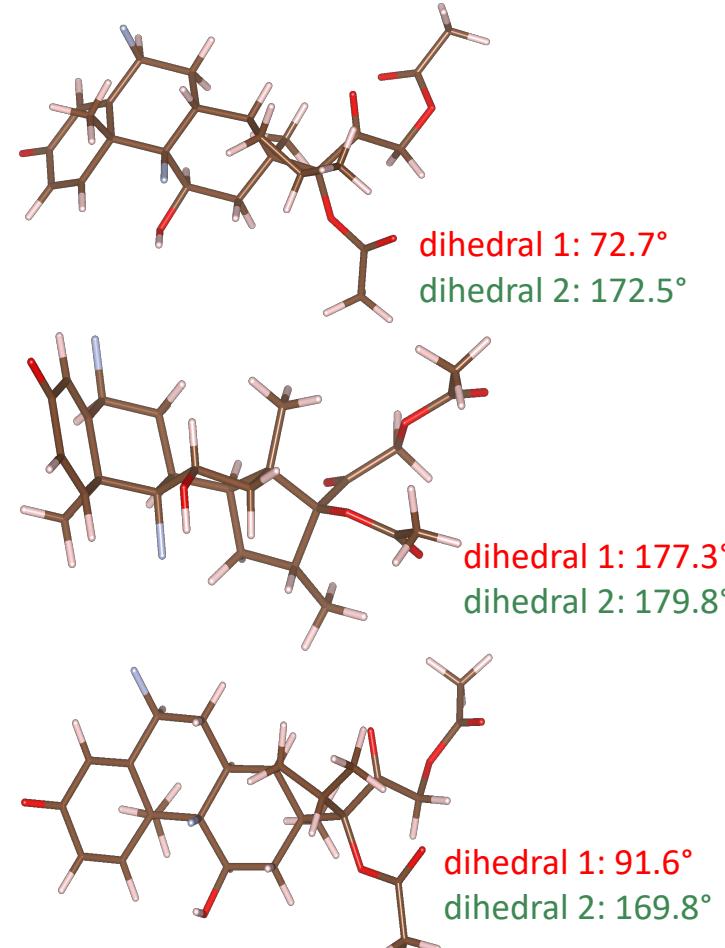
# Sample structures of insilico identified stable conformers

**Rotigotine** (MW: 315.5 g mol<sup>-1</sup>)

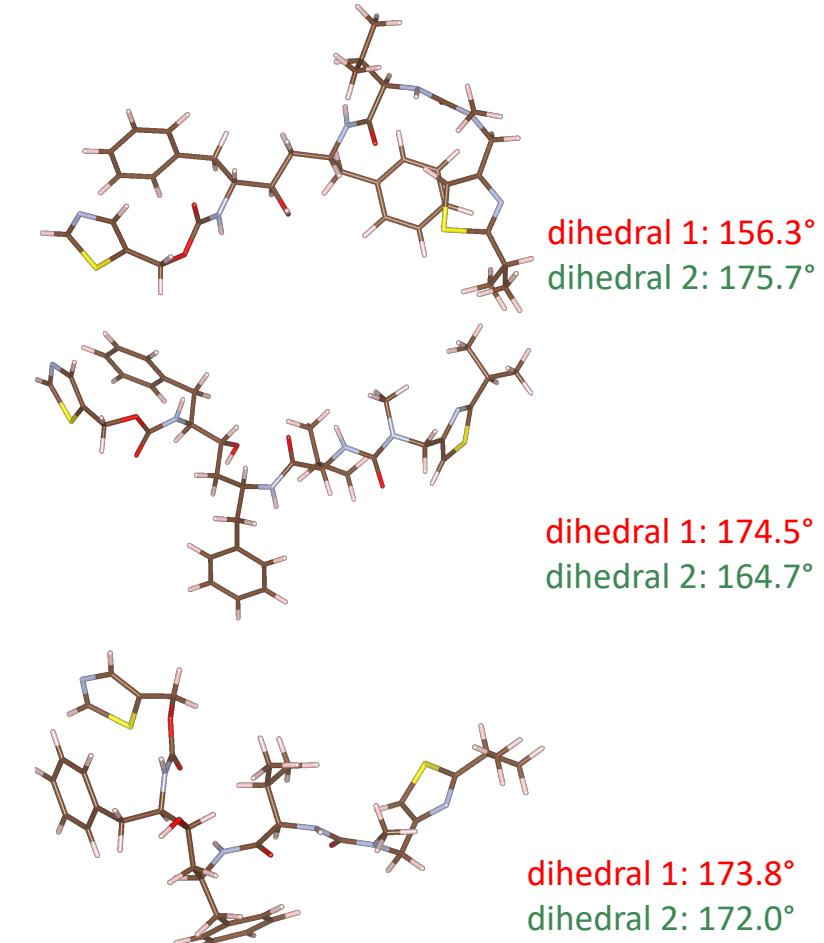


14

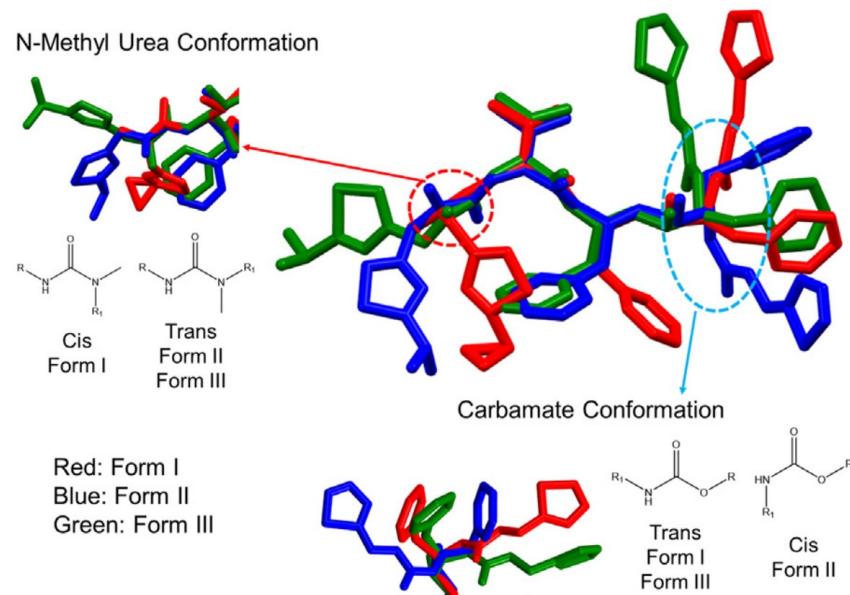
**Diflorasone diacetate**  
(MW: 494.5 g mol<sup>-1</sup>)



**Ritonavir** (MW: 720.9 g mol<sup>-1</sup>)



Increasing the number of generated conformers leads to better sampling of the conformational space



Number of <i>Ritonavir</i> conformers generated	Number of <i>conformer 2 like</i> structures observed
10K	3
20K	9
30K	13
100K	45

Molecular conformations in Ritonavir polymorphs

Ref: [Zhang et al. Journal of Pharmaceutical Sciences \(2023\)](#)