OPTIMIZATION OF PHENYILALANANINE HYDROXILASE STABILIZERS BY A TESTED 'ALCHEMICHAL' FREE-ENERGY APPROACH

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INTRODUCTION

PHENYLKETONURIA
1:10 000 newborn [1,2]
877 known destabilizing mutations
(April, 2015)

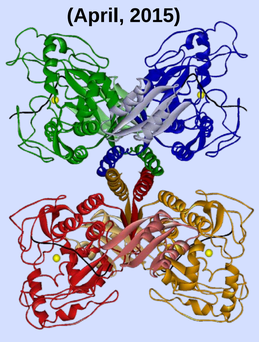


Fig 1. PAH enzyme [3]

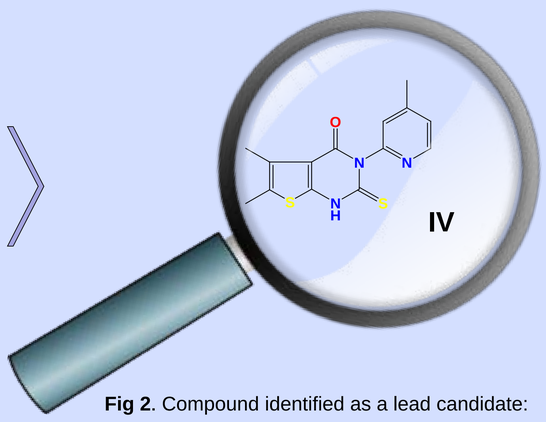


Fig 2. Compound identified as a lead candidate: enhanced the thermal stability, activity and steady-state levels of PAH both *in vitro* and *in vivo* [4].

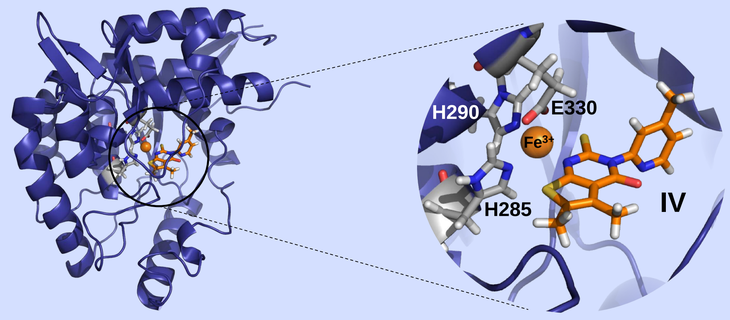


Fig 3. Catalytic domain of PAH complexed with compound **IV** (PDB Code: 4ANP) [5]

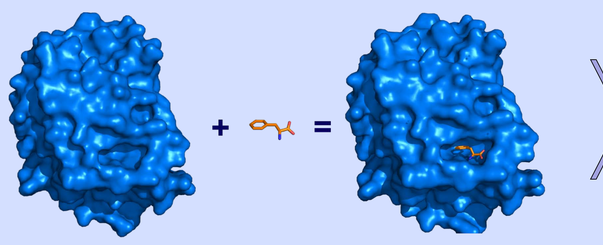
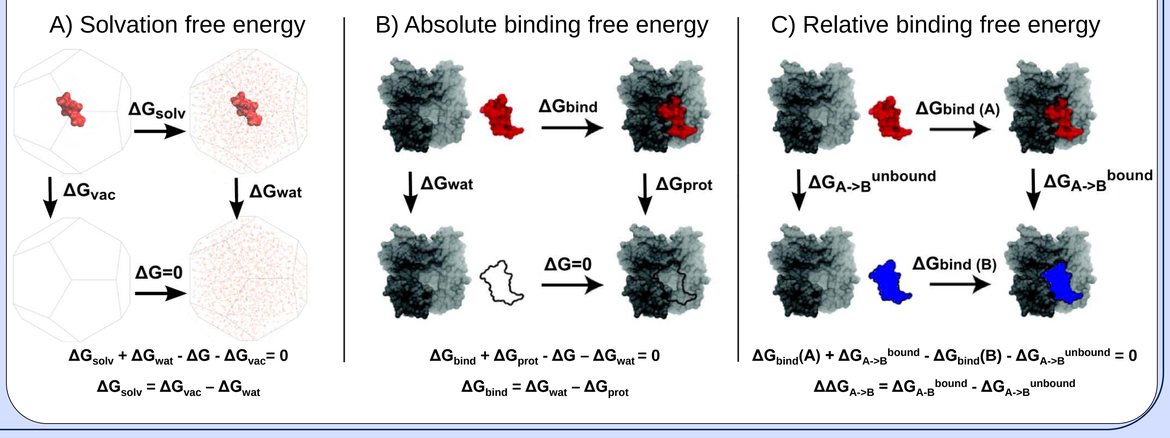


Fig 4. Protein-ligand binding: central event associated with both pharmaceutical activity and modulation of biological function.

Fig 5. Discovering of new small-molecule drugs.
Key point: designing tight-binding ligands.

ALCHEMICAL FREE ENERGY CALCULATION (AFEC) [6]

"**Alchemical**" intermediates are defined by λ values. These values determine the way in which charge and Lennard-Jones parameters are varied to attain the "alchemical" changes (coupling or decoupling atoms).



METHODS

A 'bonded' and an 'unbonded' approach have been implemented to model the coordinated metal center, and in the end, the relative binding free energy results are compared with available experimental data. The one showing more accurate results is chosen for subsequent alchemical transformations from compound IV.

MD PACKAGE:
GROMACS 4.6.1
SAMPLING METHOD:
H-REMD
λ VALUES: 16 (x 5 ns)
ESTIMATION METHOD:
MBAR

➢ BONDED MODEL

Interactions between the metal ion and its ligating residues treated via bond, angle, dihedral, electrostatic and van der Waals (VDW) terms (parameters obtained from either QM calculation or experimentally where available).

Metal Center parameterized with:
GAUSSIAN 03; AmberTools (RESP, MCPB) **;** (charges 2+ or 3+ on Fe)

Ligands:
Partial charges: Gaussian 03
Force field: GAFF

The remaining system treated with:
AMBER99SB force field

➢ NON-BONDED MODEL

Interactions between the metal ion and its ligating residues treated only via electrostatic and VDW terms.

The overall protein treated with:
AMBER99SB force field;
(charge 0 on Fe)

Ligands:
Partial charges: Gaussian 03
Force field: GAFF

coulombic-λ = 0.0 0.2 0.4 0.6 0.8 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 **bonded-λ** = 0. 0 0.0 0.0 0.0 0.0 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 **vdw-λ** = 0.0 0.0 0.0 0.0 0.0 0.0 0.08 0.17 0.27 0.38 0.5 0.61 0.72 0.83 0.92 1.0

Open synthesis paths

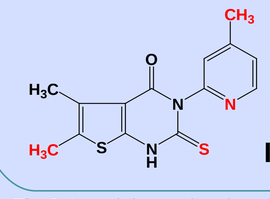
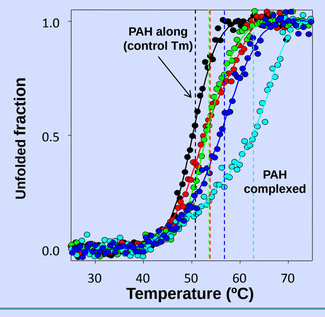


Fig 6. In red those sites by where we are modifying compound **IV.**

Preliminary affinity test
(Increase in Tm,
2,5% DMSO)

Fig 7. Representative thermal denaturation profiles of WT-PAH in the absence (black) or presence of compounds.



Experimental affinities (K_d)
20 µM PAH WT/ 300 µM Cpd
(Tris/NaCl 25°C)

Fig 8. AutoITC200 MicroCal (GE Healthcare).

$$\Delta G = -RT \ln K_a$$

$$\Delta\Delta G_{A\to B} = -RT \ln \frac{K_{a(B)}}{K_{a(A)}}$$

RESULTS

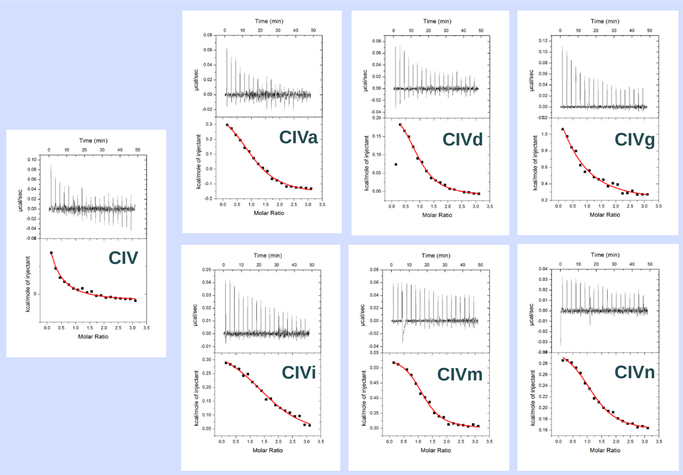


Fig 9. ITC results for CIV and the synthesized derivatives.
Affinity values are shown in **table 1.**

Table 1. Stabilizing effect and affinity data.

Compound	ΔTm (ºC)	K_a(mol/L)	K_d(µmol/L)
CIV	5	6.5×10^4	15.4
CIVa	5.3	8.2×10^4	12.2
CIVd	11	2.7×10^5	3.7
CIVg	7.3	5.6×10^4	17.9
CIVi	14.1	1.6×10^5	6.3
CIVm	12.7	2.8×10^5	3.6
CIVn	17.2	2.3×10^5	4.3

Table 2. AFEC results: comparison s with free energy changes obtained from the experimental affinity data.

'Alchemical' transformation	ΔΔG exp (kJ/mol)	ΔΔG calc (kJ/mol)					
		Bonded (Fe^{2+})	Diff (exp - calc)	Bonded (Fe^{3+})	Diff (exp - calc)	Unbounded	Diff (exp - calc)
CIV -> CIVa	-0.58	-3.01 ± 0.04	2.43	-2.14 ± 0.04	1.56	1.46 ± 0.05	-2.04
CIV -> CIVd	-3.53	-5.18 ± 0.11	1.65	-4.41 ± 0.13	0.88	2.51 ± 0.08	-6.04
CIV -> CIVg	0.37	1.37 ± 0.03	-1	1.39 ± 0.03	-1.02	4.04 ± 0.02	-3.67
CIV -> CIVi	-2.23	-6.11 ± 0.17	3.88	-5.47 ± 0.23	3.24	-1.36 ± 0.19	-0.87
CIV -> CIVm	-3.62	-8.06 ± 0.14	4.44	-5.70 ± 0.27	2.08	0.83 ± 0.23	-4.45
CIV -> CIVn	-3.13	-10.45 ± 0.24	7.32	-6.16 ± 0.16	3.03	-0.59 ± 0.21	-2.54
Ave (ABS(Diff))			**3.45**		**1.97**		**3.27**

Correct sign , in general, compared with ΔΔG exp, but values are too overestimated.

Incorrect sign in most of the cases or underestimated values.

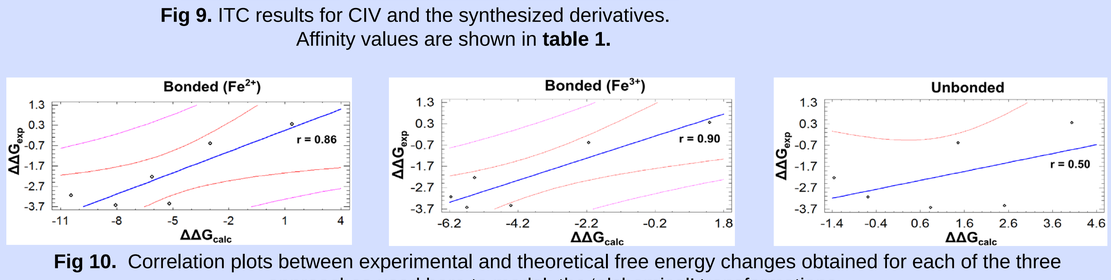


Fig 10. Correlation plots between experimental and theoretical free energy changes obtained for each of the three approaches used here to model the 'alchemical' transformations .

CONCLUSIONS

The possibility of starting, in parallel, a synthesis project to obtain and test new derivatives of the compound **IV**, allowed us to acquire additional experimental affinity data, and therefore to validate our AFEC implementation. In this sense, out of the methodologies followed to model our system, the 'bonded' one in which charge on the iron ion was set to 3+, resulted better correlated (r=0.9) with experimental data, so we decided to chose it to continuing refining our method (by fixing the lambda values: step in which we are focused now). Subsequently, the results of this work will allow us to design new promising candidates with improved binding affinity and with a more potent stabilizing effect on PAH enzyme.

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