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Evaluation of the Utility of the Rubiatriol Scaffold as Lead in Angiotensin Converting Enzyme (ACE) Modulation for the Management of Hypertension

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Evaluation of the Utility of Rubiatriol Scaffold as Lead in ACE Modulation for the Management of hypertension

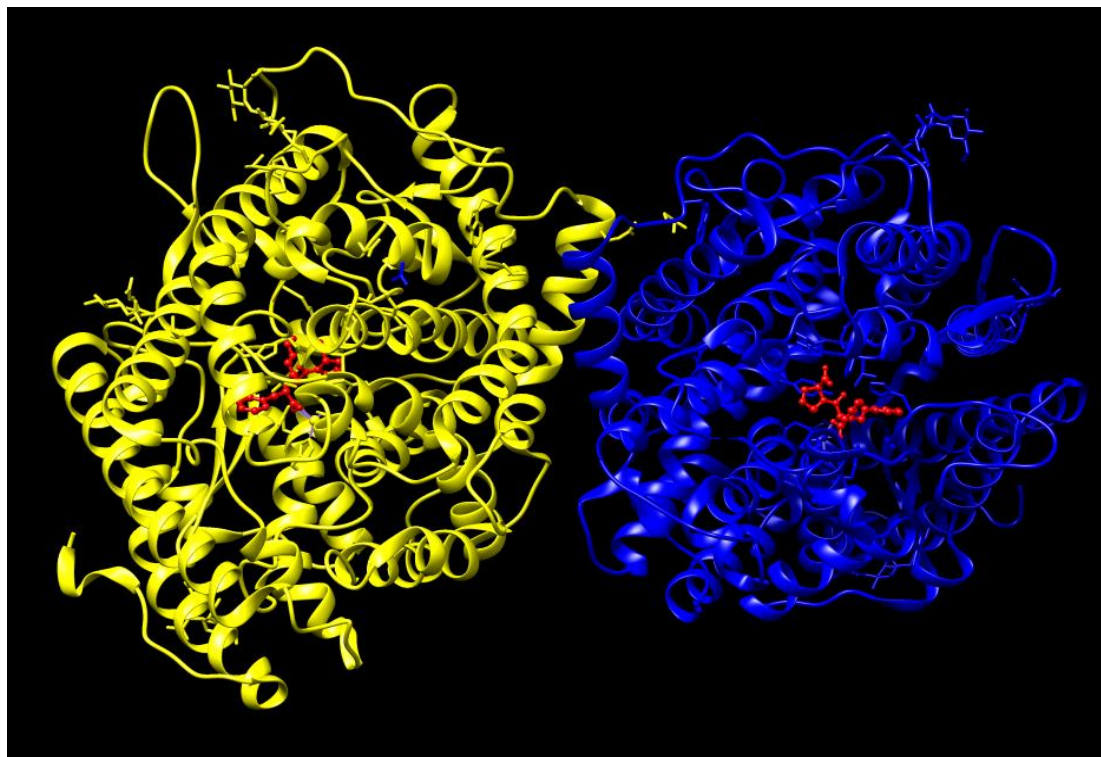


Fig 1 PDB ID 2C6N showing the crystal structure of the N domain of Human Somatic Angiotensin I- converting Enzyme bound to Lisinopril.



Abstract:

Introduction: ACE, a key enzyme in RAS for production of angiotensin II and a mediator for hypertension, is a target for cardiovascular disease management. Arisawa *et al*⁽¹⁾, claim that the naturally occurring triterpene Rubiatriol has ACE-inhibitory activity. This study aimed to, using Rubiatriol as lead molecule, validate this hypothesis using *in silico* techniques and to design novel high affinity structures for the ACE using *de novo* methods.

Methodology: Protein Databank crystallographic deposition 2C6N describing the ACE:Lisinopril complex was selected as a template. Binding affinity of Lisinopril for the ACE was calculated. Structure Based Drug Design methods and Ligand Based Drug Design methods were used to validate the hypothesis made by Arisawa *et al*.

Results: The Lipinski rule compliant molecular cohort was for both methods, segregated into families of similar pharmacophoric structure, and ranked according to binding affinity and physicochemical parameters. The highest ranking molecules were identified for optimisation and *in vitro* validation.

Discussion: This study is valuable for validation of the hypothesis of Arisawa *et al*. using *in silico* methods, and for suggesting that the rubiatriol scaffold was a suitable lead for the generation of ACE modulating molecules with a binding affinity superior to that of Lisinopril.



Introduction

Revaluation of Traditional medicines e.g. Chinese medicine is considered as an important resource for the discovery of bioactive molecules with therapeutic effects.²

Drug-like compounds may be discovered using *in silico* methods with enhanced efficacy and effectiveness.³



Rubiatriol

Found in the
dried
root/rhizome
of *Rubia
cordifolia*.

Arisawa *et al*
suggested that
it has ACE-
Inhibitory
activity¹

Naturally
occurring
triterpene

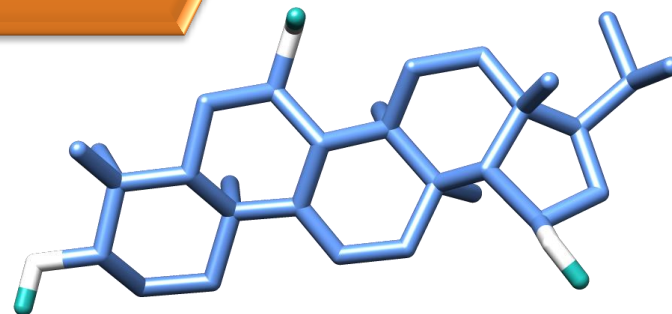
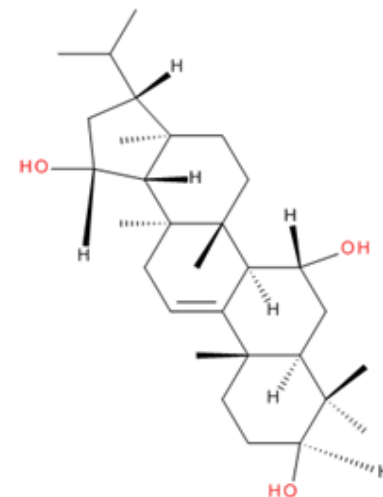


Fig 2. Rubiatriol drawn using Sybyl-X[®] v1.1, rendered using UCSF Chimera



Aims

To validate the work of Arisawa *et al*¹ using *in silico* techniques by utilizing Rubiatriol as a lead molecule.

Design of potential novel structures with similar inhibitory activity at the ACE using *de novo* methods



Methodology

Ligand-based Drug Design

Structure-based Drug Design



Ligand Based Drug design Method


- 
- PDB Crystallographic Deposition 2C6N⁴ was chosen
 - Lisinopril was extracted using Sybyl-X[®] v1.1
 - The extract ie the template ligand was sent to ViCi to obtain *de novo* molecules
 - The molecular cohort was uploaded in Mona[®] in *sdf* format

Fig 2 PDB ID 2C6N showing the crystal structure of the N domain of Human Somatic Angiotensin I-converting Enzyme bound to Lisinopril in yellow.



Ligand Based Drug design Method contd.

- These were segregated according to Lipinski's rules using Mona[®] and saved as a multi-*sdf* file
- Sybyl-X[®] v 1.1 was used to create a protomol of PDB crystallographic deposition 2C6N⁴
- SURFLEX docking was performed by using the multi-*sdf* file and the generated protomol
- The results obtained were saved in a Microsoft[®] Excel spreadsheet for analysis



Structure Based Drug design Method



- PDB Crystallographic Deposition 2C6N⁴ was chosen



- Lisinopril was extracted using Sybyl-X[®] v1.1



- Rubiatriol was docked in the *apo* ACE and conformers were obtained. The optimal conformer was chosen.



- Poseview[®] was used to model the interactions of the optimal conformer occurring at the receptor level



Structure Based Drug design Method contd

- 
- Seeds were generated from the chosen conformer

- 
- Ligbuilder® v1.2 'pocket' was used to identify the ligand binding sites to prepare for 'grow'

- 
- 'Grow' in Ligbuilder® v1.2 was used to generate molecules from the seeds.

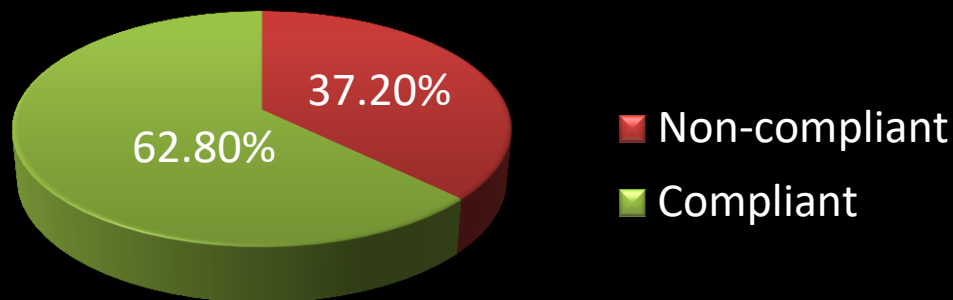
- 
- Molecules were then assessed for Lipinski Rule of 5 compliance



Results and Discussion- Ligand Based Drug design

- From the molecular cohort 62.8% were Lipinski's rule of 5 compliant molecules

Molecules' Compliance to Lipinski's Rules



Results and Discussion-Ligand Based Drug Design contd.

- **628 molecules, which were also Lipinski rule compliant were docked within the protomol**

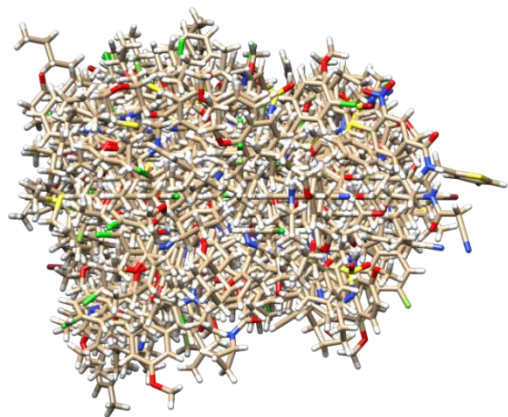
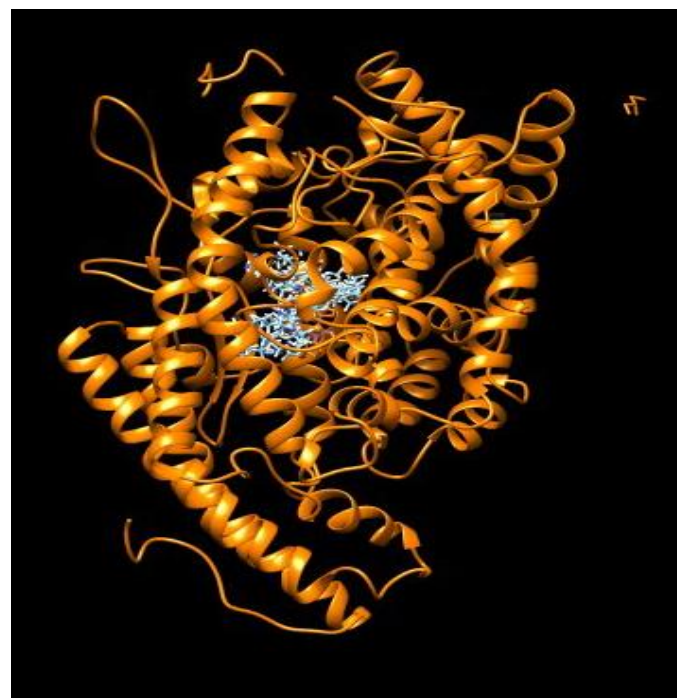


Fig 4 The generated protomol using Sybyl-X and the 628 molecules which were docked, depicted using UCSF Chimera[®].



Results and Discussion Ligand Based Drug Design contd.

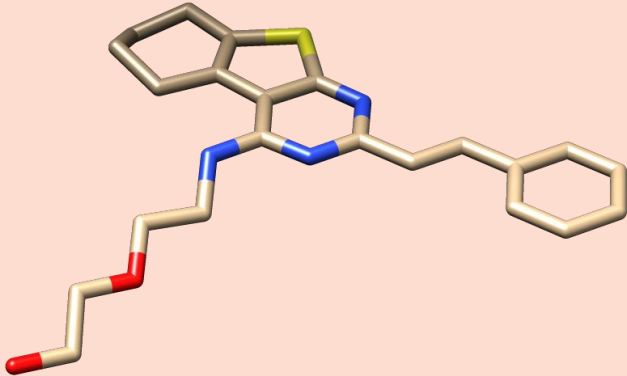
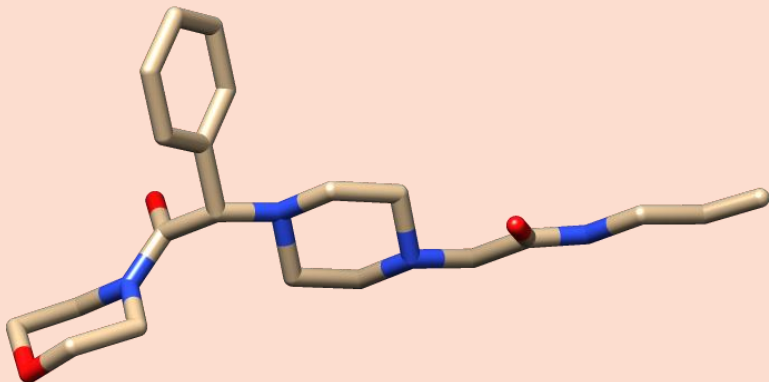
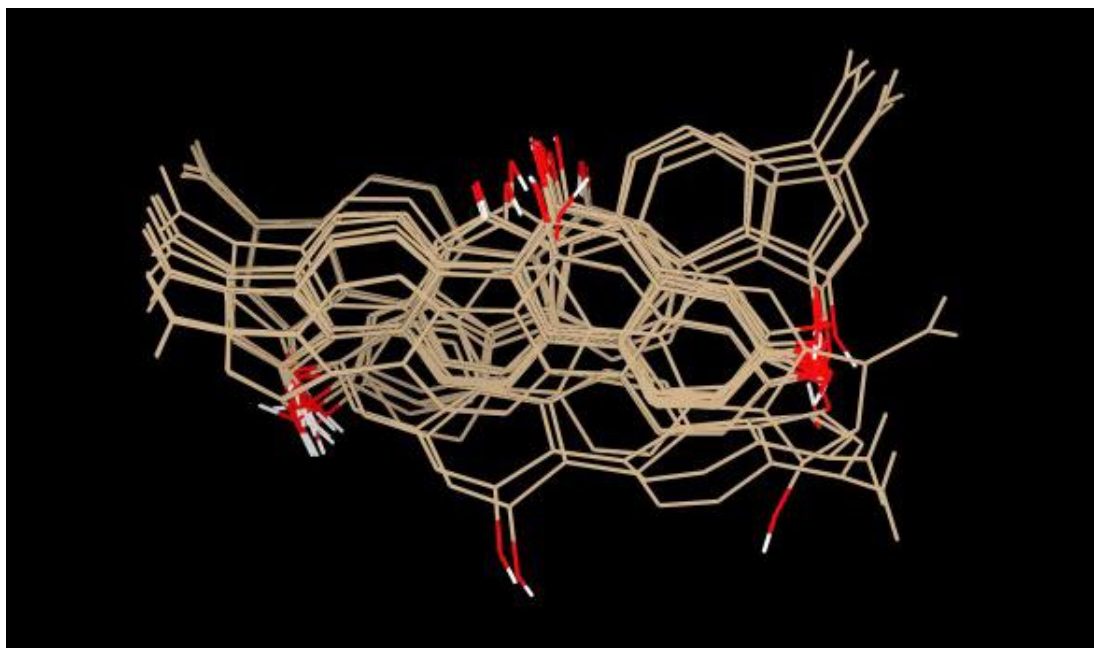
Highest Total Binding Score	Lowest Total Binding Score
0478_enamine_T5692177	0291_ukorysynth_PB196050726
8.27	-17.45
	

Fig 5 The molecules with the highest and lowest total binding scores depicted using UCSF Chimera[®].



Results and Discussion Structure Based Drug Design

- 21 conformers were obtained after docking Rubiatriol
- Conformer 12 was chosen for further modelling highlighted in yellow



Results and Discussion Structure Based Drug Design contd.

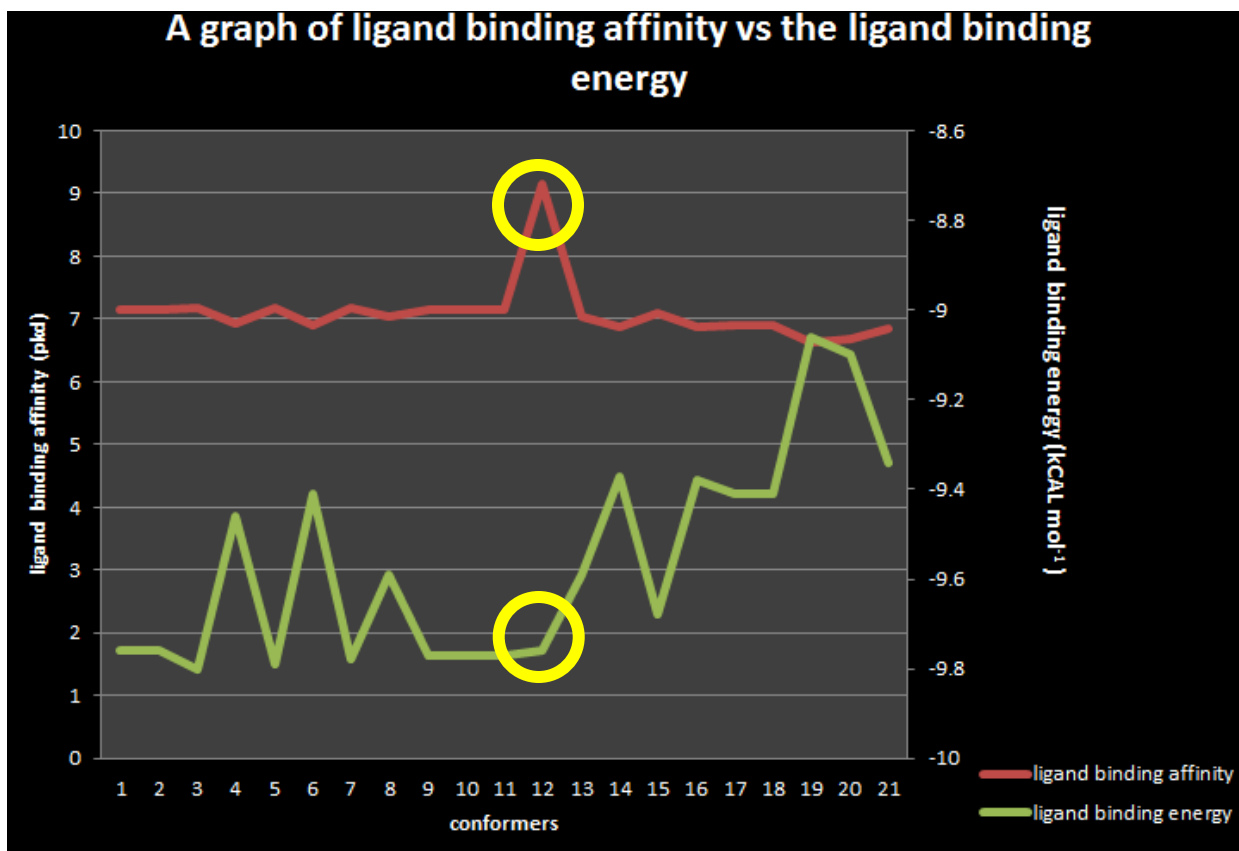


Fig 7. A graph depicting the ligand binding affinity vs the ligand binding energy using Microsoft® Excel 2010. Optimal conformer chosen highlighted values in yellow

LBA (pKd)	LBE (Kcal mol ⁻¹)
9.16	-9.76



Results and Discussion Structure Based Drug Design contd.

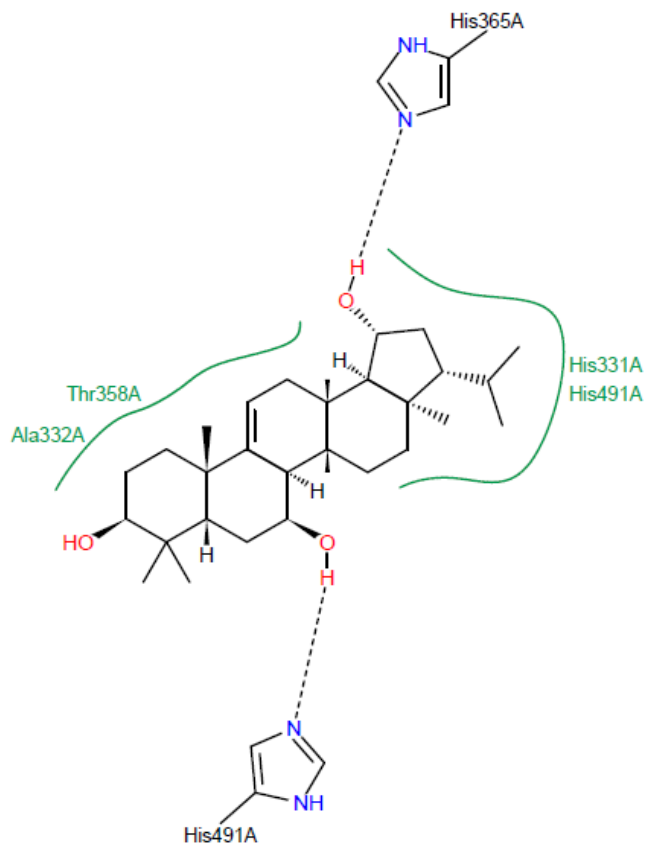


Fig 8. A 2D representation of the interactions between Rubiatriol Conformer 12 and the ACE. Rendered using Poseview[®].

It highlights the most important atomic interactions between rubiatriol and the amino acids lining the ligand binding pocket of the ACE that was generated in Poseview and guided the seed structure design process.



Results and Discussion Structure Based Drug design contd.

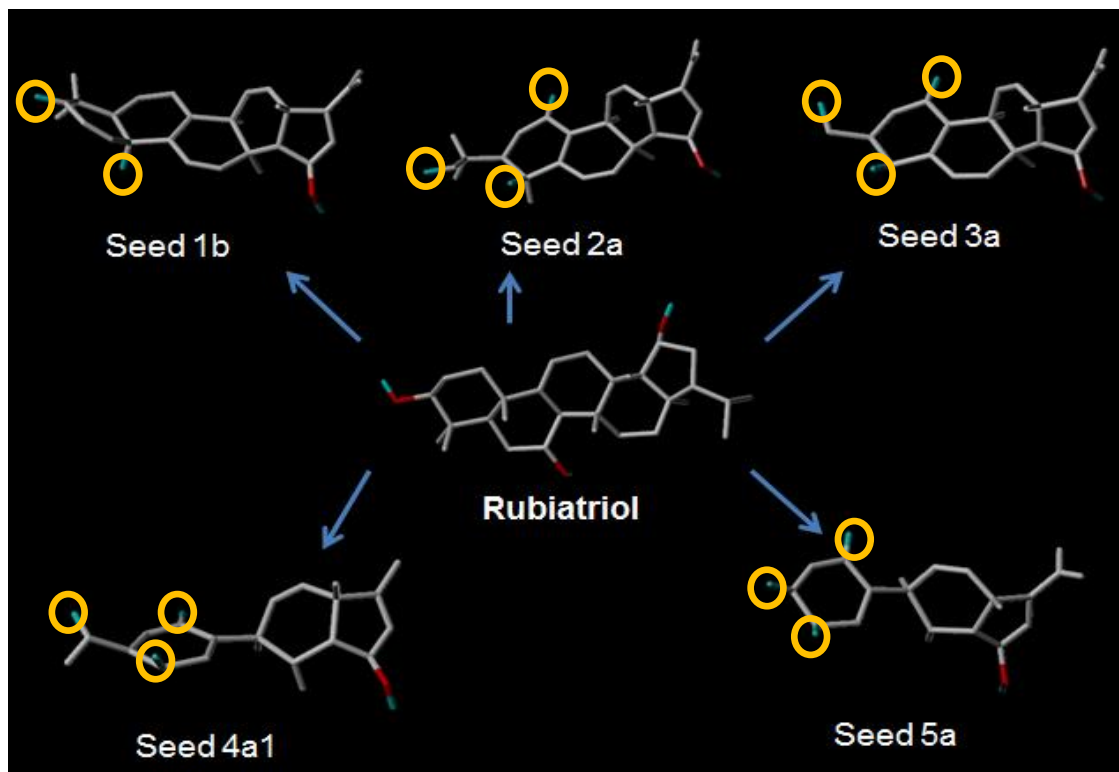
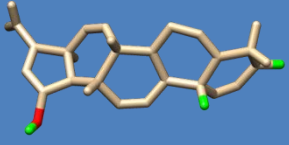
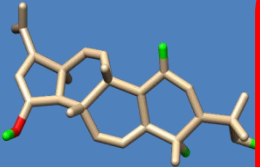
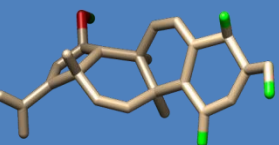
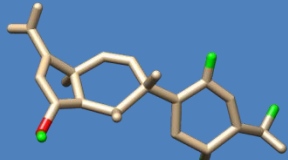
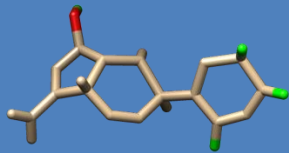


Fig 9. The seeds which were generated from conformer 12. Rendered using Sybyl-X[®] v1.1. The encircled moieties show the changes that were made to the chosen conformer. These were mainly Hspc growing sites which allow user directed molecular growth within the pharmacophoric space of the receptor.



Results and Discussion Structure Based Drug design contd.

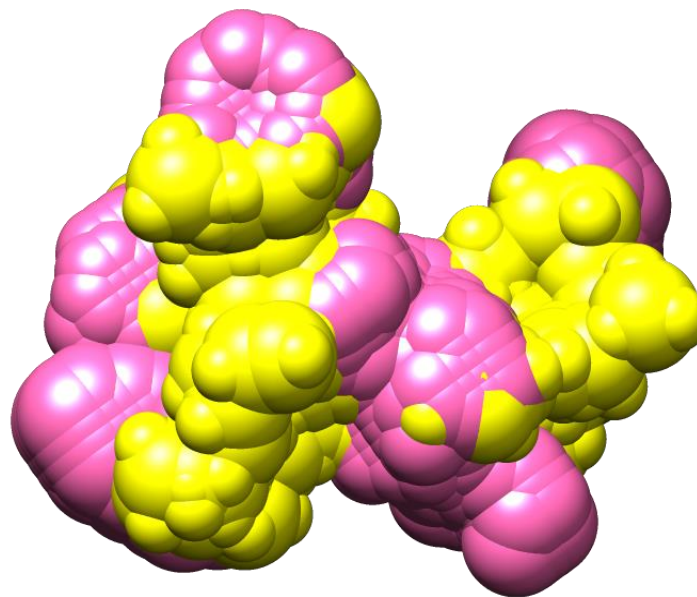
					
Seed Name	Seed_1b	Seed_2a	Seed_3a	Seed_4a1	Seed_5a
Families	17	14	12	4	7
Lipinski Rule Compliant Molecules	1	2	7	1	2
Mwt Range	455-599	428-599	394-600	467-537	412-589
Log P Range	4.27-6	4.08-5.99	4.04-5.99	4.48-5.96	4.55-6
H-Bond Donor Range	3-7	3-7	2-6	2-4	2-6



Results and Discussion Both Methods

Fig 10. Superimposition of the Key site volume in Yellow and the Protomol in pink depicted using Chimera.

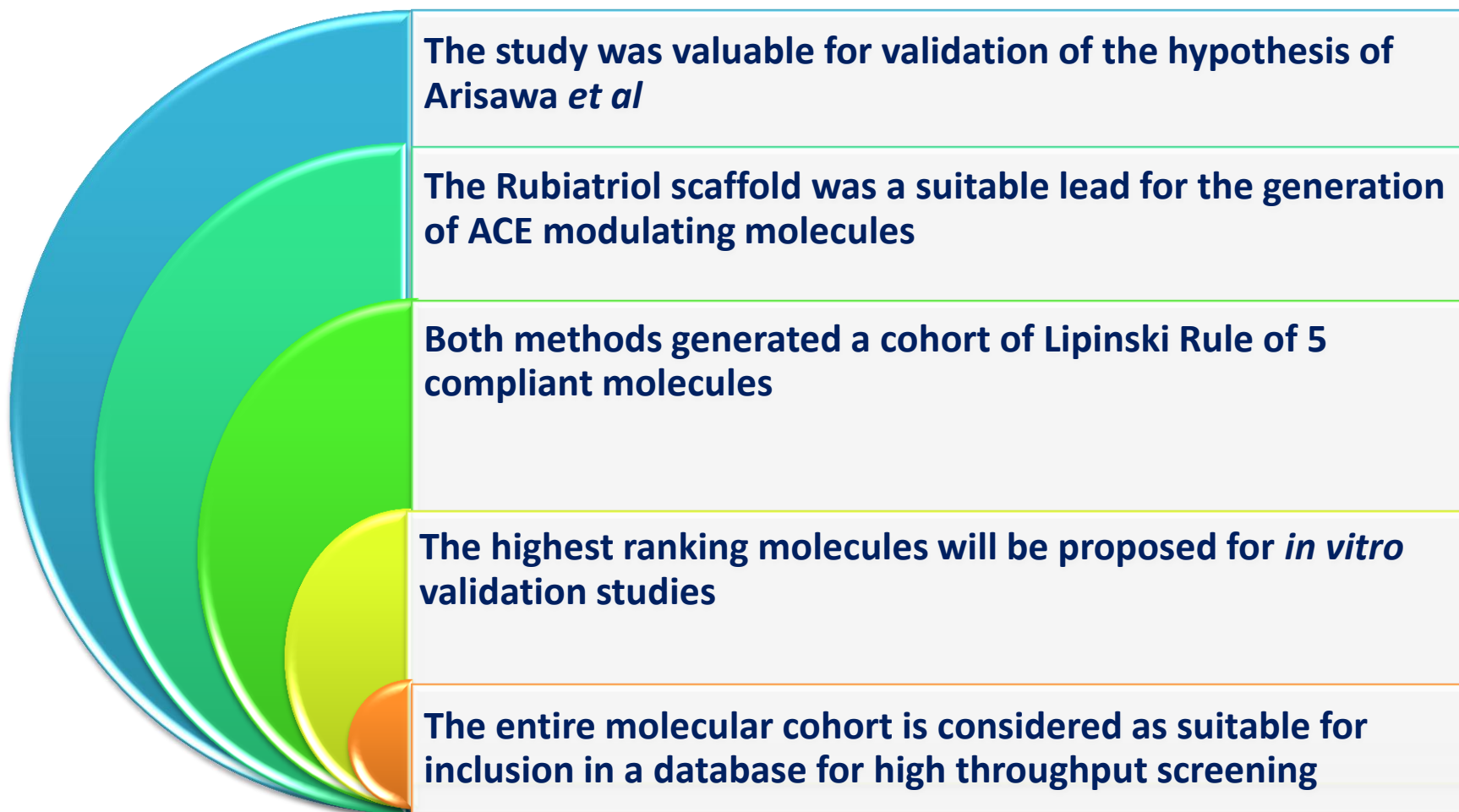
The protomol has a larger area and volume as depicted and calculated.



	Protomol	Key-Site file
Volume (Å ³)	1035	633.4
Area(Å ²)	700.4	570.2



Conclusion



References

1. Arisawa M, Ueno H, Nimura M, Hayashi T, Morita N. Rubiatriol, a new triterpenoid from the Chinese drug, 'qián cáo gēn', *Rubia cordifolia*. Journal of natural products. 1986; 49, 6, 1114-1116.
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