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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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*. Naturally occurring triterpene

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

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

To validate the work of Arisawa et al\textsuperscript{1} using \textit{in silico} techniques by utilizing Rubiatriol as a lead molecule.

Design of potential novel structures with similar inhibitory activity at the ACE using \textit{de novo} methods
Methodology

- Ligand-based Drug Design
- Structure-based Drug Design
Ligand Based Drug design Method

- PDB Crystallographic Deposition 2C6N\(^4\) was chosen
- Lisinopril was extracted using Sybyl-X\(^\text{®}\) v1.1
- The extract ie the template ligand was sent to ViCi to obtain *de novo* molecules
- The molecular cohort was uploaded in Mona\(^\text{®}\) 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 2C6N\(^4\) 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

- 62.80% Compliant
- 37.20% Non-compliant
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.

<table>
<thead>
<tr>
<th>Highest Total Binding Score</th>
<th>Lowest Total Binding Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0478_enamine_T5692177</td>
<td>0291_ukorysynth_PB196050726</td>
</tr>
<tr>
<td><strong>8.27</strong></td>
<td><strong>-17.45</strong></td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>LBA (pKd)</th>
<th>LBE (Kcal mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.16</td>
<td>-9.76</td>
</tr>
</tbody>
</table>
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.

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

<table>
<thead>
<tr>
<th></th>
<th>Protomol</th>
<th>Key-Site file</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (Å³)</td>
<td>1035</td>
<td>633.4</td>
</tr>
<tr>
<td>Area(Å²)</td>
<td>700.4</td>
<td>570.2</td>
</tr>
</tbody>
</table>
Conclusion

The study was valuable for validation of the hypothesis of Arisawa et al.

The Rubiatriol scaffold was a suitable lead for the generation of ACE modulating molecules.

Both methods generated a cohort of Lipinski Rule of 5 compliant molecules.

The highest ranking molecules will be proposed for *in vitro* validation studies.

The entire molecular cohort is considered as suitable for inclusion in a database for high throughput screening.
References


