Development of MTHFR modulating compounds for treatment of MTHFR deficiency

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Introduction

Methylenetetrahydrofolate reductase (MTHFR) deficiency

- Caused by single nucleotide polymorphisms
- Mild reduced enzyme activity
- Appear in late childhood and adulthood
- Associated with multifactorial diseases

- Autosomal recessive
- Residual enzyme activity <20%
- Inborn error (ca 200 patients known today)
- Neuropsychiatric diseases, possibly fatal

AIM: To design MTHFR activity modulating compounds based on the structures of SAM (inhibition) and SAH (dis-inhibition)

References

**Approach**

Study of the structure-function relationship of SAM inhibition and SAH dis-inhibition to optimise binding and fine tune level of inhibition

**Binding studies**

Differential scanning fluorimetry S-SKI-72 to stabilise hsMTHFR$_{RD}$ stronger than SAM and SAH

<table>
<thead>
<tr>
<th>Compound</th>
<th>MeltT (°C)</th>
<th>R squared</th>
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<tbody>
<tr>
<td>DMSO</td>
<td>47.95</td>
<td>0.9933</td>
</tr>
<tr>
<td>TAM-4-61</td>
<td>50.45</td>
<td>0.9922</td>
</tr>
<tr>
<td>TAM-4-59</td>
<td>50.45</td>
<td>0.9912</td>
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<tr>
<td>Sinfungin</td>
<td>50.85</td>
<td>0.9984</td>
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<tr>
<td>WZ-16</td>
<td>51.75</td>
<td>0.9855</td>
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<tr>
<td>SAM</td>
<td>52.85</td>
<td>0.9912</td>
</tr>
<tr>
<td>SAH</td>
<td>56.15</td>
<td>0.9934</td>
</tr>
<tr>
<td>[S]-SKI-72</td>
<td>58.05</td>
<td>0.9933</td>
</tr>
</tbody>
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Surface plasmon resonance show S-SKI-72 bind with stronger affinity to hsMTHFR$_{RD}$ than SAM

**Published results**

Activity studies

In vitro inhibition assay using hsMTHFR and derivatives of SAM show structures affecting inhibition potency and efficacy

1) R-stereochemistry of amino-group increase inhibitory efficacy

2) Removal of methyl-group does not guarantee dis-inhibition

3) Carboxyl-group substitution affects both potency and efficacy

Conclusion

- Structure of S-SKI-72 has been found to bind and stabilised hsMTHFR_{RD} stronger than SAM, and resulting in partial inhibition of hsMTHFR.
- Structural modification to S-SKI-72 have been identified increasing potency and effect the level of inhibition