Biological evaluation of a library of thiamine-like 1,2,3-triazoles towards the inhibition of DXP synthase

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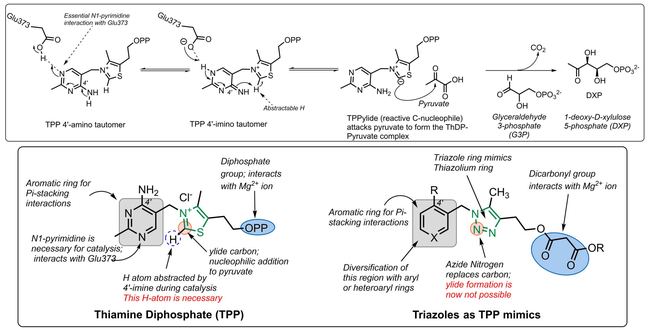
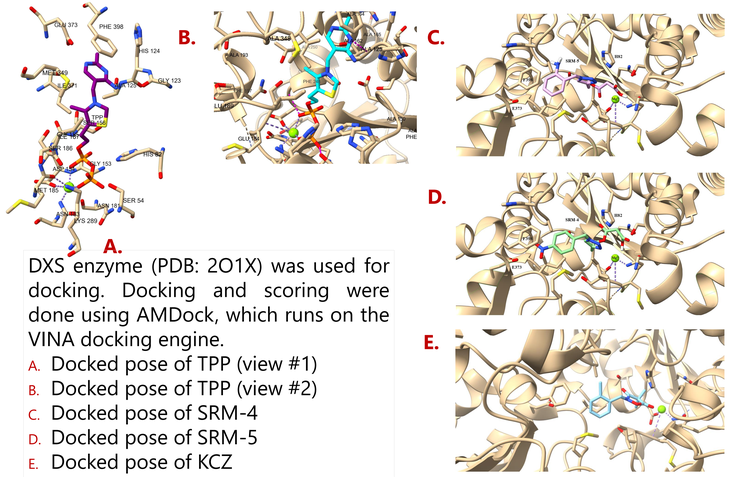
OBJECTIVE

The non-mevalonate pathway (MEP pathway) in bacteria is responsible for synthesizing DMAPP and IPP, critical precursors in the biosynthesis of isoprenoids. DXP synthase (DXS) is a key enzyme in this pathway, and its absence in humans makes it a promising target in the development of potentially newer antibiotics. Thiamine diphosphate (TPP; Vitamin B1) is a cofactor responsible for the C-C bond formation between pyruvate and glyceraldehyde-3-phosphate to yield 1-deoxy-D-xylulose 5-phosphate (DXP). TPP is the template for inhibitor design.

ENZYME CATALYSIS and STRUCTURE-BASED DESIGN





DXS enzyme (PDB: 2O1X) was used for docking. Docking and scoring were done using AMDock, which runs on the VINA docking engine.

A. Docked pose of TPP (view #1)
B. Docked pose of TPP (view #2)
C. Docked pose of SRM-4
D. Docked pose of SRM-5
E. Docked pose of KCZ

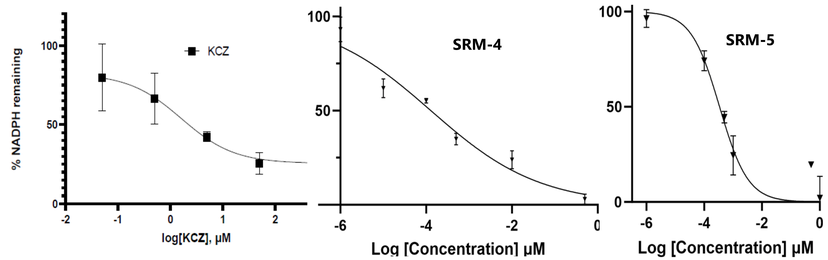
METHODS

The lack of a suitable chromophore in the reaction involving DXS alone led us to discover a DXS-DXR (DXP synthase-DXP reductase) coupled assay. DXR catalyzes the conversion of DXP to MEP, a redox reaction involving NADH. The redox changes (NADH/NAD+) can be measured by UV-Visible spectrophotometry, supporting the use of the coupled assay to indirectly examine the catalytic function of DXS.

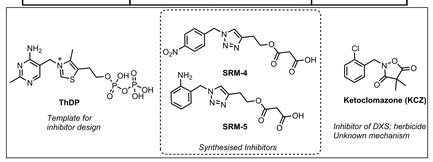
Modified 1,2,3-triazole molecules with superior docking scores were chosen for the DXS-DXR (DXP synthase-DXP reductase; Echelon BioSciences)-coupled assays to evaluate their inhibitory potential. Compounds were tested at 1 mM to 10 nM concentration range. The compounds were dissolved in DMSO (conc <5%), and then added to a 96-well plate. Subsequently, pyruvate (3 mM) and glyceraldehyde-3-phosphate (600 µM) were added, followed by NADPH (225 µM). Incubation at 25°C for 5 minutes with reaction buffer was done, followed by initiating the enzyme reaction by adding DXS (67 nM) and DXR (7 nM) enzymes. The kinetic spectrophotometric readings were then captured at 340 nm over a 15-minute intervals. Ketoclomazone (KCZ) was used as a control. KCZ is an herbicide, and inhibitor of DXP synthase via a yet-unclear mechanism.

RESULTS AND DISCUSSION

- SRM-4, SRM-5, SRM-7, SRM-13 and KCZ had docking (binding) scores of -9.5, -9.7, -9.1, -9.2, and -7.8 kcal/mol, respectively.
- SRM-4, SRM-5, SRM-7, SRM-13 and KCZ exhibited IC50 values of 0.128, 0.314, 27.82, 9.82, and 1.62 µM respectively.



Compound	Docking (kcal/mol)	IC50 (µM)
TPP	-10.5	
SRM-4	-9.2	**0.128**
SRM-5	-9.5	**0.314**
SRM-7	-9.3	27.82
SRM-13	-9.1	9.82
KCZ	-7.8	1.62



- All inhibitors have binding energy lower than TPP (native cofactor; template).
- Among the inhibitors, KCZ (control) has the best ranking *in silico*.
- In the DXS-DXR coupled assay (*in vitro*), SRM-4 and SRM-5 are superior to KCZ.

CONCLUSIONS

We have obtained two inhibitors in the nanomolar range i.e., SRM-4 and SRM-5. This is supported by their binding energy scores. As such, SRM-4 is the most potent inhibitor of DXP synthase.

CHALLENGES and FUTURE DIRECTIONS

- The commercially available enzyme is extremely expensive ($350 for 250 µg), limiting the number of iterations possible in optimization and compound testing
- SRM-4 is a viable lead molecule for further SAR studies.

REFERENCES

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Acknowledgements: AACP New Investigator Award Program; MCPHS University SOP Boston, Pharmaceutical Sciences Department