

Copper-catalyzed cyclization reactions in pursuit of molecules that inhibit DXP synthase

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OBJECTIVE

DXP synthase is a bacterial enzyme of the non-mevalonate pathway and is being explored as target for the development of potential antibiotics. The objective is to synthesize a small library of TPP (enzyme cofactor)-resembling triazole compounds using a diverse set of aromatic or heteroaromatic cores alongside a terminal alkyne using copper catalysts. Biological results will also be presented.

METHODS

Standard synthetic chemistry methods are employed. A curated set of aryl or heteroaryl cores (that mimic the enzyme cofactor) were selected based on modeling results. These cores bear either a primary alcohol or alkyl halide, which are converted to their corresponding azides. The isolated azides are cyclized in the presence of a specific copper catalyst to yield the corresponding aryl/heteroaryl triazole, which resembles the central ring of the enzyme cofactor, TPP. Reaction outcomes varied depending upon the catalyst, solvent, and temperature used. The synthesis of 15 compounds (8 selected azides, 7 triazoles) will be presented. These compounds will be evaluated in biological assays.

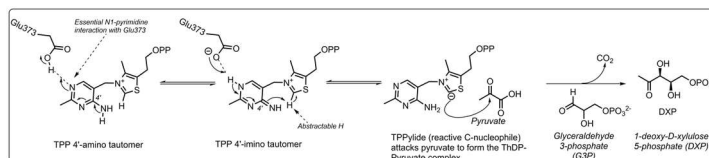
RESULTS

An array of suitable aryl/heteroaryl triazoles were generated from precursor azides using CuAAC chemistry. Product yields vary depending upon the type of solvent, mol % of catalyst (CuI or CuSO₄), reaction times (3 to 18 hours) and temperature (25-35 °C). The triazoles were evaluated in cell-based assays towards lead identification as bacterial DXP synthase inhibitors.

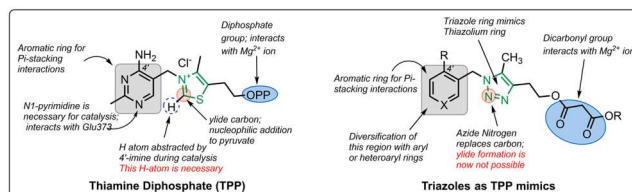
CONCLUSIONS

Copper-based triazole generating reactions are well-studied. In this study, we demonstrate the utility of this procedure towards the synthesis of suitable enzyme inhibitors. Assay results will guide the future direction of this project.

CATALYTIC MECHANISM



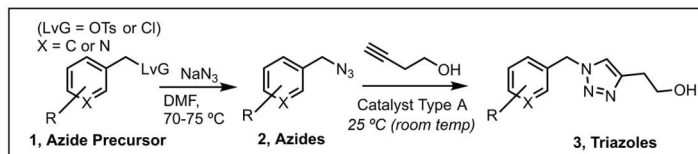
DESIGN STRATEGY



- The C2-position of the cofactor needs an abstractable H to generate the ylide to enable the ligation of pyruvate with glyceraldehyde 3-phosphate.
- The creation of the triazole replacing the thiazole serves to:
 - (a) block the formation of the ylide due to the replacement of C (thiazole) by N (triazole)
 - (b) increase hydrophobic interactions at the floor of the active site by virtue of the 1,2,3-triazole
- Anchoring of the molecule with the Mg²⁺ ion is achieved by replacing the pyrophosphate with dicarbonyl group.

CHEMISTRY

The creation of 1,2,3-triazoles was accomplished using known reactions, commonly termed "click" reactions. These reactions involve a Cu-catalyst, and a suitably paired azide and alkyne counterparts.

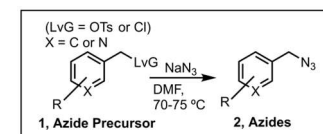


RESULTS AND DISCUSSION

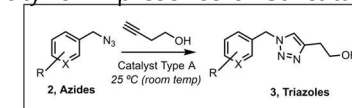
- We tested various Cu-catalyst and solvent combinations that gave us the best yields of final product. Type A appears to be the most ideal.

Type	Copper Catalyst	Reaction Solvent
A	CuSO ₄ ·5H ₂ O (0.01 eq)	Water: t-butanol (1:2)
B	CuI ₂ (0.2 eq)	Water: DMF (1:4)
C	Cu(OAc) ₂ (0.2 eq)	Water: t-butanol (1:2)

- A series of suitable azides (2), involving the 6-membered ring region of the TPP mimic was then synthesized using sodium azide in DMF.



- The synthesis of our desired triazoles (3) was accomplished using the relevant azides (2) and 1-butynol in presence of Cu-catalyst (type A)



- Reactions and Yields of each set given below

#	1 (Precursor)		2 (Azide)		3 (Triazole)	
	X	R	Time (hr)	Yield (%)	Time (hr)	Yield (%)
1	C	4-H	3.5	85	12	50
2	C	4-F	4.5	85	12	50
3	C	4-CF ₃	4.5	85	12	45
4	C	4-NO ₂	4.5	84	12	50
5	C	4-CN	2.5	80	12	50
6	C	2-NH ₂	3	80	12	72
7	N	4-NH ₂	16	48	12	21
8	-	Uracil	8	59	24	42

FUTURE GOALS

- Docking studies to guide analog development
- Explore Ru-based click reactions with internal alkynes.
- Conduct isolated enzyme assays to determine compound activity