Structure–activity relationships of compounds targeting mycobacterium tuberculosis 1-deoxy-D-xylulose 5-phosphate synthase

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ABSTRACT

We report on a target-based approach to identify possible *Mycobacterium tuberculosis* DXS inhibitors from the structure of a known transketolase inhibitor. A small focused library of analogs was assembled in order to begin elucidating some meaningful structure–activity relationships of 3-(4-chloro-phenyl)-5-benzyl-4H-pyrazolo[1,5-a]pyrimidin-7-one. Ultimately we found that 2-methyl-3- (4-fluorophenyl)-5-(4-methoxy-phenyl)-4H-pyrazolo[1,5-a]pyrimidin-7-one, although still weak, was able to inhibit *M. tuberculosis* DXS with an IC_{50} of 10.6 μM.

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Despite the availability of effective antituberculosis drugs, tuberculosis (TB) is still a major cause of disability and death globally. Improved TB medications need to be developed to shorten the duration of the treatment period, to reduce the amount of drugs required while making costs affordable, and to provide a more effective treatment against persistent TB infection.[1] To develop new treatments for TB, many drug discovery programs target enzymes considered to be essential for *Mycobacterium tuberculosis* survival. It is hoped that this target-based approach will identify specific inhibitors of bacterial enzymes, thus blocking the bacterial growth while showing little if any toxicity toward the host organism. Potential lead compounds against these targets can be identified from high-throughput screening (HTS) campaigns or through ligand- and structure-based design methods.[2]

Within this context, early steps in the isoprenoid biosynthetic pathway are being investigated, as isoprenoids are known to be required for *M. tuberculosis* survival. Examples of these approaches encompass molecules such as decaprenyl phosphate[3] which is involved in the biosynthesis of major cell wall components of *M. tuberculosis* (peptidoglycan,[4] lipoarabinomannan,[5] and arabinomannan[6]), and menaquinone which is the only lipoquinone involved in the mycobacterial electron transport chain.[7] The universal precursors of isoprenoids, isopentenyl diphosphate (IPP), and dimethylallyl diphosphate (DMAPP) are synthesized solely through the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway in *M. tuberculosis*. This biosynthetic pathway does not exist in mammalian biochemistry, and therefore it opens alternative approaches to developing novel antituberculosis drugs.[8–10] In the initial rate-limiting step[11] in the MEP pathway (Scheme 1), 1-deoxy-D-xylulose 5-phosphate (DXP) is formed from the condensation of pyruvate and D-glyceraldehyde-3-phosphate (GAP) catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) in the presence of thiamine pyrophosphate (TPP). The formation of DXP is not a committed step in isoprenoid biosynthesis as this molecule is also a precursor of pyridoxol (vitamin B6)[12] or thiamine (vitamin B1).[13,14]

Himar1-based transposon mutagenesis predicted that *M. tuberculosis* DXS is required for *M. tuberculosis* survival.[15] Although there are no structural data available, *M. tuberculosis* DXS shows 38% amino acid sequence identity with *Escherichia coli* DXS, for which a crystal structure has been reported.[16] These proteins have well-conserved regions predicted to be involved in substrate binding and catalytic activity.[8] The overall structure is similar to the members of the mammalian transketolase (TK) superfamily, including pyruvate dehydrogenase E1 subunit[17] and 2-oxoisovalerate dehydrogenase.[18] These enzymes are all dimers, composed of three different domains, and require TPP as a cofactor.[16]

In this study, a previously established in vitro DXS assay[8] was utilized to study the structure–activity relationships (SARs) of a

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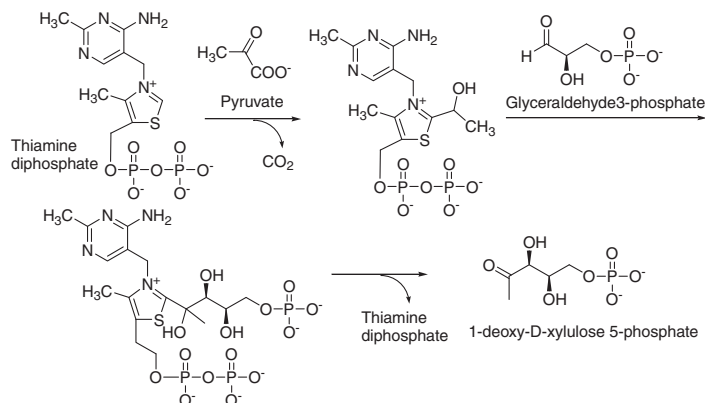
Scheme 1. Mechanism of DXS.

small, focused compound library based on the structure of a known TK inhibitor,[22] 3-(4-chloro-phenyl)-5-benzyl-4H-pyrazolo[1,5-a]pyrimidin-7-one (**1**). Although DXS was previously characterized in several organisms including *E. coli*[23] and *M. tuberculosis*,[8] this is the first study describing the SAR of inhibitors against the *M. tuberculosis* DXS.

Expression and purification of DXS: M. tuberculosis DXS was cloned, expressed, and purified in *E. coli* as a fusion protein bearing a His6-tag. The purified *M. tuberculosis* DXS was estimated to be at least 95% pure by SDS–PAGE analysis on 12% gels. A clear band was observed corresponding to a molecular weight (67.8 kDa) consistent with the expected size. Western blot analysis with anti-His antibody confirmed that the single band corresponds to DXS. Purified DXS (59 pmol) were used for the in vitro enzyme assay to screen the compounds.

Determination of the IC$_{50}$ of active compounds against M. tuberculosis DXS: As has been reported, the catalytic function of *M. tuberculosis* DXS combines the mechanism of the pyruvate decarboxylase and TK.[8] Therefore, the known TK inhibitor **1** (Table 1), inhibiting the TK superfamily with an IC$_{50}$ of 3.9 μM,[22] was expected to inhibit *M. tuberculosis* DXS as well. However, the IC$_{50}$ value of the compound for *M. tuberculosis* DXS (114.1 μM) was much higher than that found for the TK superfamily, suggesting that there may be sufficient variation between the binding pockets of TK and *M. tuberculosis* DXS. Indeed, the domain arrangements of DXS differ from those of TK, resulting in differences in the formation of the active site.[19–21] In DXS, three subunits of one domain form the active site. However, in the TK superfamily, the largest subunit of one domain contacts smaller subunits of the other domains forming the active site.[17] These differences are possibly caused by a longer linker (95 amino acids) between domains in the TK superfamily,[16] whereas DXS contains only 20 amino acid residues in this linker.[16] Although the catalytic mechanism of *M. tuberculosis* DXS appears to be similar to that of TK superfamily, inhibitory specificity may be achieved through these structural differences. Moreover, the *M. tuberculosis* DXS active site can accommodate a relatively broad spectrum of substrates, such as D- or L-glyceraldehyde and D-erythrose 4-phosphate,[8] indicating that there is flexibility in the types of molecules that can be accommodated in the active site. Based on this information together with the difference in IC$_{50}$ values for inhibitor **1** between *M. tuberculosis* DXS and TK, we were encouraged to select some modifications to this molecule in order to gain possibly better selectivity. Based on the compound drug likeness as defined by the guidelines of Lipinski's Rule of Five,[24] we purchased compounds from different commercial vendors in order to build a preliminary body of SAR information (Fig. 1). In order to explore both steric and electronic effects at the active site, various modifications were included: different substitutions were introduced at the 2-position; the
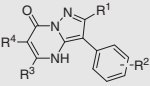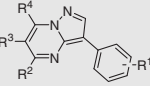
chloro group on the phenyl ring located at the 3-position was replaced by an electron-withdrawing or -donating group; the carbon linker length was varied and electron-withdrawing/donating groups were introduced at different positions of the 5-position benzyl side chain; alkyl or aryl substituents were introduced at the 6 and 7-positions.

Modifications of compound **1** are shown in Table 1. When the benzyl group was replaced with a methoxymethyl group as in compound **2** or a methyl group as in **3**, the inhibitory activity was abolished, indicating that an aromatic ring at the 5-position is required for the activity. When the chloro substituent was removed at the *para*-position of phenyl side chain, and a methylene ethyl ester was added at 6-position, compound **4** also lost its activity against *M. tuberculosis* DXS. In addition, compounds with a trifluoromethyl group (compound **29**) and a phenyl group (compound **30**) at the 7-position increased the IC$_{50}$ on *M. tuberculosis* DXS.

Introduction of an electron-withdrawing trifluoromethyl group at the 2-position reduced the IC$_{50}$ on *M. tuberculosis* DXS compared with the 2-H series (compound **8** vs compound **1**, Table 1). Addition of a *para*-methoxy group on the 3-phenyl group did not increase the inhibitory activity (compound **9** vs compound **7**). In contrast, introduction of a *para*-chloro substituent on this phenyl ring decreased the IC$_{50}$ value (compound **8** vs compound **6**). Furthermore, when we compared the impact of different halogens, a *para*-chloro substituent on the phenyl ring showed better inhibitory activity than bromo (compound **12** vs compound **11**). Additionally, compound **7** served as a better inhibitor than compound **6**, which indicated that a phenyl substituent is better than a benzyl group at the 5-position. Compound **10** exhibited greater inhibitory activity than that of compound **11**, suggesting that there is some space in the binding cavity located in the area surrounding the 5-position of the main core in the active site. However, this is apparently not the case for the 6-position of the main scaffold (cf. compound **13** with compound **12**).

The 2-methyl series (compounds **14–27**, Table 1) showed less inhibitory activity than the 2-trifluoromethyl series, but the activity was still greater than the 2-H series (compound **17** vs compound **8**, compound **17** vs compound **1**). A phenyl ring bearing an electron-withdrawing group is preferred at the 3-position as compound **17** served as a better inhibitor than compound **14**. Halogen size and/or electronic effects played a role at the 3-phenyl side chain as the smaller fluoro and chloro substituents (compound **19** and compound **18** vs compound **20**) showed better inhibition than the bromo substitution. The notion that a phenyl substituent is better than a benzyl group at the 5-position is supported by the comparison of compounds **15** and **14**. A *para*-methoxy group at the 5-position increased the inhibitory activity (cf. compound **16** vs compound **15**). As mentioned earlier, the replacement of the benzyl group with a

Table 1
Inhibition activity of different scaffold compounds against *M. tuberculosis* DXS

Compound[a]	R[1]	R[2]	R[3]	R[4]	DXS IC$_{50}$ (μM)
1	H	4-Cl	PhCH$_2$	H	114.1 ± 0.6
2	H	4-Cl	CH$_3$OCH$_2$	H	>200
3	H	4-Cl	CH$_3$	H	>200
4	H	H	CH$_3$	CH$_2$COOEt	>200
5	H	H	ClCH$_2$	H	>200
6	CF$_3$	H	PhCH$_2$	H	71.1 ± 5.1
7	CF$_3$	H	Ph	H	23.0 ± 4.1
8	CF$_3$	4-Cl	PhCH$_2$	H	14.0 ± 1.1
9	CF$_3$	4-CH$_3$O	Ph	H	20.2 ± 0.6
10	CF$_3$	4-Br	CH$_3$OCOCH$_2$	H	73.0 ± 6.4
11	CF$_3$	4-Br	CH$_3$	H	>200
12	CF$_3$	4-Cl	CH$_3$	H	98.4 ± 13.8
13	CF$_3$	4-Cl	CH$_3$	PhCH$_2$	>200
14	CH$_3$	4-CH$_3$O	Ph	H	>200
15	CH$_3$	4-CH$_3$O	Ph	H	90.7 ± 4.6
16	CH$_3$	4-CH$_3$O	4-CH$_3$OC$_6$H$_4$	H	42.4 ± 3.8
17	CH$_3$	4-Cl	Ph	H	34.2 ± 5.8
18	CH$_3$	4-Cl	4-CH$_3$OC$_6$H$_4$	H	10.9 ± 1.5
19	CH$_3$	4-F	4-CH$_3$OC$_6$H$_4$	H	10.6 ± 3.1
20	CH$_3$	4-Br	2-CH$_3$OC$_6$H$_4$	H	53.5 ± 0.5
21	CH$_3$	H	Ph	H	30.6 ± 1.1
22	CH$_3$	4-Cl	(1-Phenyl-1*H*-tetrazol-5-ylsulfanyl)-methylene-	H	55.1 ± 4.4
23	CH$_3$	4-Cl	4-ClC$_6$H$_4$SCH$_2$	H	41.1 ± 0.4
24	CH$_3$	H	4-ClC$_6$H$_4$SCH$_2$	H	63.7 ± 1.0
25	CH$_3$	4-Cl	CH$_3$OCH$_2$	H	>200
26	CH$_3$	H	Naphthalene-2-yl	H	19.5 ± 2.9
27	CH$_3$	H	Ph	CH$_3$CH$_2$CH$_2$	71.9 ± 10.1
28	CH$_3$CH$_2$	4-Cl	4-CH$_3$OC$_6$H$_4$	H	19.7 ± 3.4

	R[1]	R[2]	R[3]	R[4]	
29	4-Cl	4-ClPh	H	CF$_3$	>200
30	3-Br	Ph	H	Ph	>200

a Compounds in 2-H series: **1**–**5**, **29**, and **30**; compounds in 2-CF$_3$ series: **6**–**13**; compounds in 2-CH$_3$ series: **14**–**27**.
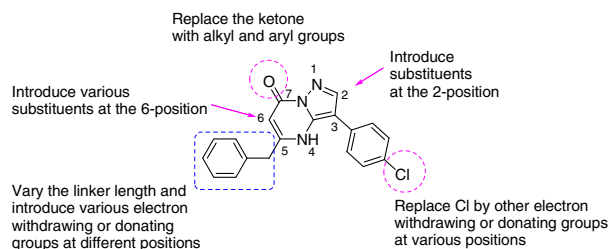

Figure 1. Modifications of reported TK inhibitor.

non-aromatic substituent (compound **25**) at the 5-position resulted in a loss in activity. However, compounds containing aromatic rings with various linkers (compounds **22** and **23**) still maintained some inhibitory activity. The 5-naphthalenyl-substituted compound **26** showed slightly greater inhibitory activity than the 5-benzyl analog **21**, indicating that a conjugated aromatic ring system was favored at this position. As indicated earlier, introduction of an appendage at the 6-position led to a loss of activity (**27** vs **21**).

A 2-ethyl substituent showed somewhat less inhibition than the corresponding 2-methyl (compound **28** vs compound **18**), therefore, we did not investigate this series further.

Table 2
Antituberculosis activity and cytotoxicity of selected inhibitors

Compound	MABA MIC (μM)	Vero cell IC$_{50}$ (μM)
16	61.8	80.9
17	14.2	39.5
18	7.6	16.0
19	7.7	35.6

Anti-TB activity and toxicity of compounds: The best two inhibitors (the inhibition curve of compound **18** is shown in Fig. S1) were tested for inhibition of growth of *M. tuberculosis* (Table 2). Both compound **18** and compound **19** demonstrated reasonably good inhibition of bacterial growth. However, they also showed some toxicity against Vero cells,[25] with a selectivity index of 2.1 and 4.6, for compounds 18 and **19**, respectively. The MICs of compounds **16** and **17** showed some correlation with their IC$_{50}$s, but their cytotoxicity indicates that these compounds may have off-target activity against mammalian TK or other unknown targets in the Vero cells.

In this study, we have successfully utilized a previously established in vitro DXS assay to screen for new inhibitors. Several potential inhibitors of *M. tuberculosis* DXS, with IC$_{50}$ values of

R[4]: No substitution favored at the 6-position

R[1]: $CF_3 > CH_3 > H$
$CH_3 > C_2H_5$

R[3]: Aromatic ring prefered at the 5-position
Naphthalenyl > phenyl > benzyl
Electron donating group prefered on the aryl ring

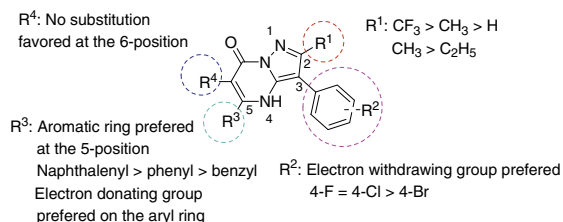R[2]: Electron withdrawing group prefered
4-F = 4-Cl > 4-Br

Figure 2. SAR summary of 3-phenyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one.

~10 μM, have been identified, and preliminary SAR data have been generated. As it is apparent from Figure 2, a trifluoromethyl group is the preferred substituent at the 2-position of the 3-phenyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one scaffold; ethyl, methyl, and hydrogen result in increasingly poorer activity. An electron-donating group *para*-methoxy attached to the phenyl ring is favored, and the 5-naphthenyl analog is even more potent. One of the best inhibitors also exhibited a reasonably good MIC (7.7 μM) against *M. tuberculosis* H37Rv with a 4.6-fold selectivity index. Active compounds did not inhibit DXS from other organisms or other enzymes from *M. tuberculosis* (data not shown). Therefore, solution of the X-ray structures of *M. tuberculosis* DXS in complex with these inhibitors could provide valuable insights into the subtleties of the *M. tuberculosis* DXS binding site that may allow the design of a new generation of inhibitors specific for *M. tuberculosis* DXS.

Acknowledgment

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.034.

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