Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2016 March 09.

Published in final edited form as:

Bioorg Med Chem Lett. 2008 October 1; 18(19): 5320–5323. doi:10.1016/j.bmcl.2008.08.034.

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

Jialin Mao[a,b,†], **Hyungjin Eoh**[c,†], **Rong He**[a], **Yuehong Wang**[b], **Baojie Wan**[b], **Scott G. Franzblau**[b,*], **Dean C. Crick**[c,*], and **Alan P. Kozikowski**[a,*]

[a]Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

[b]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

[c]Department of Microbiology, Immunology and Pathology, Colorado State University, 1682 Campus Delivery, Ft. Collins, CO 80523-1677, USA

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-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one. Ultimately we found that 2-methyl-3-(4-fluorophenyl)-5-(4-meth-oxy-phenyl)-4*H*-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.

Keywords

Drug design; DXS; Enzyme; SAR; Tuberculosis

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]

*Corresponding authors. Tel.: +1 312 996 7577. kozikowa@uic.edu (A.P. Kozikowski).
†These authors contributed equally to this work.

Supplementary data

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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 small, focused compound library based on the structure of a known TK inhibitor,[22] 3-(4-chloro-phenyl)-5-benzyl-4*H*-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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 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.

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 ~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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Hao Xie (Department of Chemistry, Princeton) for his helpful discussions and suggestions for the letter.

References and notes

1. Tomioka H, Sato K, Shimizu T, Sano C. J Infect. 2002; 44:160. [PubMed: 12099742]
2. Selzer PM, Brutsche S, Wiesner P, Schmid P, Mullner H. Int J Med Microbiol. 2000; 290:191. [PubMed: 11045924]
3. Crick DC, Schulbach MC, Zink EE, Macchia M, Barontini S, Besra GS, Brennan PJ. J Bacteriol. 2000; 182:5771. [PubMed: 11004176]
4. Mahapatra S, Yagi T, Belisle JT, Espinosa BJ, Hill PJ, McNeil MR, Brennan PJ, Crick DC. J Bacteriol. 2005; 187:2747. [PubMed: 15805521]
5. Besra GS, Morehouse CB, Rittner CM, Waechter CJ, Brennan PJ. J Biol Chem. 1997; 272:18460. [PubMed: 9218490]
6. Mikusova K, Mikus M, Besra GS, Hancock I, Brennan PJ. J Biol Chem. 1996; 271:7820. [PubMed: 8631826]
7. Putra SR, Disch A, Bravo JM, Rohmer M. FEMS Microbiol Lett. 1998; 164:169. [PubMed: 9675863]
8. Bailey AM, Mahapatra S, Brennan PJ, Crick DC. Glycobiology. 2002; 12:813. [PubMed: 12499403]
9. Dhiman RK, Schaeffer ML, Bailey AM, Testa CA, Scherman H, Crick DC. J Bacteriol. 2005; 187:8395. [PubMed: 16321944]
10. Eoh H, Brown AC, Buetow L, Hunter WN, Parish T, Kaur D, Brennan PJ, Crick DC. J Bacteriol. 2007; 189:8922. [PubMed: 17921290]
11. Kang MJ, Lee YM, Yoon SH, Kim JH, Ock SW, Jung KH, Shin YC, Keasling JD, Kim SW. Biotechnol Bioengin. 2005; 91:636.
12. Hill RE, Himmeldirk K, Kennedy IA, Pauloski RM, Sayer BG, Wolf E, Spenser ID. J Biol Chem. 1996; 271:30426. [PubMed: 8940007]
13. Therisod M, Fischer JC, Estramareix B. Biochem Biophys Res Commun. 1981; 98:374. [PubMed: 6452875]
14. White RH. Biochemistry. 1978; 17:3833. [PubMed: 359046]
15. Sassetti CM, Boyd DH, Rubin E. J Mol Microbiol. 2003; 48:77.
16. Xiang S, Usunow G, Lange G, Busch M, Tong L. J Biol Chem. 2007; 282:2676. [PubMed: 17135236]
17. Nikkola M, Lindqvist Y, Schneider G. J Mol Biol. 1994; 238:387. [PubMed: 8176731]
18. Aevarsson A, Seger K, Turley S, Sokatch JR, Hol WG. Nat Struct Biol. 1999; 6:785. [PubMed: 10426958]
19. Lindqvist Y, Schneider G, Ermler U, Sundstrom M. EMBO J. 1992; 11:2373. [PubMed: 1628611]
20. Gerhardt S, Echt S, Busch M, Freigang J, Auerbach G, Bader G, Martin WF, Bacher A, Huber R, Fischer M. Plant Physiol. 2003; 132:1941. [PubMed: 12913150]
21. Nilsson U, Lindqvist Y, Kluger R, Schneider G. FEBS Lett. 1993; 326:145. [PubMed: 8325361]
22. Du MX, Sim J, Fang L, Yin Z, Koh S, Stratton J, Pons J, Wang JJ, Carte B. J Biomol Screening. 2004; 9:427.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

23. Sprenger GA, Schorken U, Wiegert T, Grolle S, de Graaf AA, Taylor SV, Begley TP, Bringer-Meyer S, Sahm H. Proc Natl Acad Sci USA. 1997; 94:12857. [PubMed: 9371765]
24. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Adv Drug Deliv Rev. 2001; 46:3. [PubMed: 11259830]
25. Falzari K, Zhu Z, Pan D, Liu H, Hongmanee P, Franzblau SG. Antimicrob Agents Chemother. 2005; 49:1447. [PubMed: 15793125]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

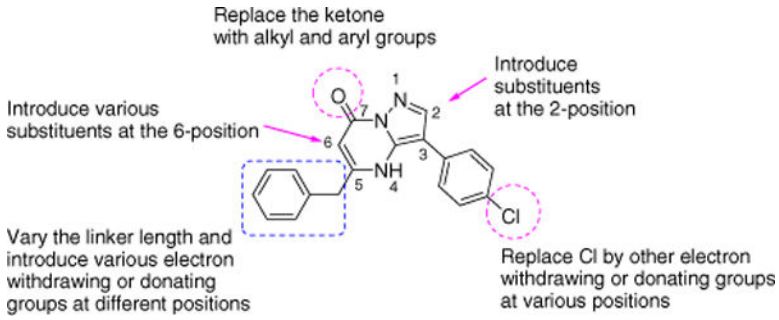


Figure 1.
Modifications of reported TK inhibitor.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

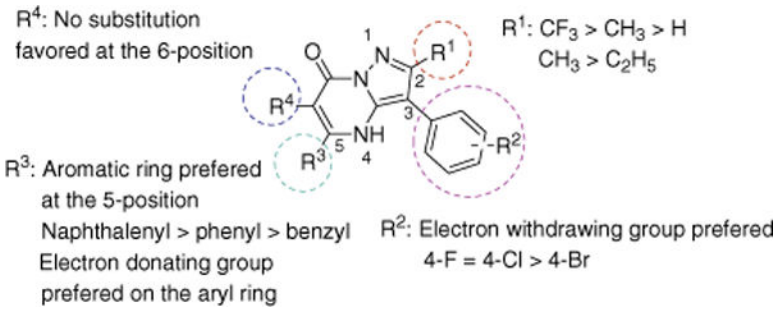
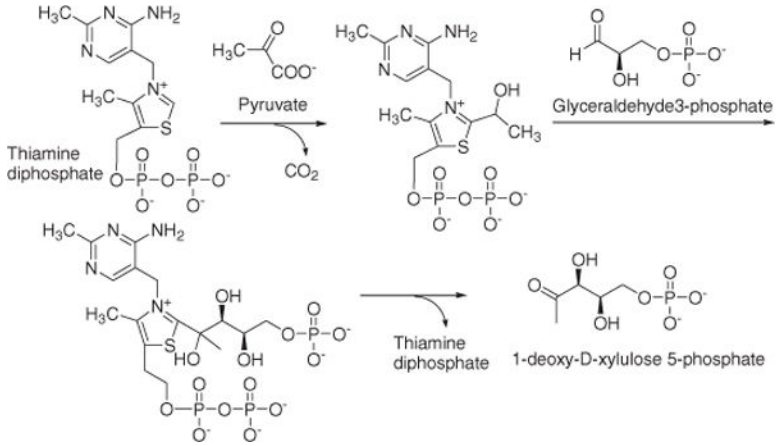


Figure 2.
SAR summary of 3-phenyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



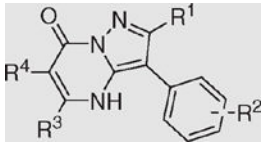
Scheme 1.
Mechanism of DXS.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

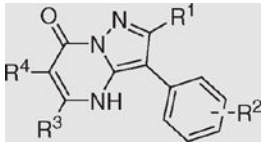
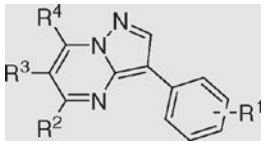
Bioorg Med Chem Lett. Author manuscript; available in PMC 2016 March 09.

Table 1

Inhibition activity of different scaffold compounds against M. tuberculosis DXS

Compound[a]					DXS IC_{50} (μM)
	R^1	R^2	R^3	R^4	
1	H	4-Cl	$PhCH_2$	H	114.1 ±0.6
2	H	4-Cl	CH_3OCH_2	H	>200
3	H	4-Cl	CH_3	H	>200
4	H	H	CH_3	CH_2COOEt	>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_3O	Ph	H	20.2 ± 0.6
10	CF_3	4-Br	CH_3OCOCH_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_3O	Ph	H	>200
15	CH_3	4-CH_3O	Ph	H	90.7 ± 4.6
16	CH_3	4-CH_3O	4-$CH_3OC_6H_4$	H	42.4 ± 3.8
17	CH_3	4-Cl	Ph	H	34.2 ± 5.8
18	CH_3	4-Cl	4-$CH_3OC_6H_4$	H	10.9 ±1.5
19	CH_3	4-F	4-$CH_3OC_6H_4$	H	10.6 ± 3.1
20	CH_3	4-Br	2-$CH_3OC_6H_4$	H	53.5 ± 0.5
21	CH_3	H	Ph	H	30.6 ± 1.1
22	CH_3	4-Cl	(1-Phenyl-1H-tetrazol-5-ylsulfanyl)-methylene-	H	55.1 ± 4.4

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Compound[a]					DXS IC_{50} (μM)
	R^1	**R^2**	**R^3**	**R^4**	
23	CH_3	4-Cl	4-$ClC_6H_4SCH_2$	H	41.1 ± 0.4
24	CH_3	H	4-$ClC_6H_4SCH_2$	H	63.7 ± 1.0
25	CH_3	4-Cl	CH_3OCH_2	H	>200
26	CH_3	H	Naphthalene-2-yl	H	19.5 ± 2.9
27	CH_3	H	Ph	$CH_3CH_2CH_2$	71.9 ± 10.1
28	CH_3CH_2	4-Cl	4-$CH_3OC_6H_4$	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**.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

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