**In silico Structure-based Drug Screening of Novel Antimycobacterial Pharmacophores by DOCK-GOLD Tandem Screening**

Junichi Taira, Takashi Ito, Hitomi Nakatani, Tomohiro Umei, Hiroki Baba, Shotaro Kawashima, Taira Maruoka, Hideyuki Komatsu, Hiroshi Sakamoto, Shunsuke Aoki

Department of Bioscience and Bioinformatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology, Iizuka 820-8502, Japan

**Abstract**

**Background:** Enzymes responsible for cell wall development in *Mycobacterium tuberculosis* are considered as potential targets of anti-tuberculosis (TB) agents. Mycobacterial cyclopropane mycolic acid synthase 1 (CmaA1) is essential for mycobacterial survival because of its critical role in synthesizing mycolic acids. **Materials and Methods:** We screened compounds that were capable of interacting with the mycobacterial CmaA1 active site using a virtual compound library with an in silico structure-based drug screening (SBDS). Following the selection of such compounds, their antimycobacterial activity was examined. **Results:** With the in silico SBDS, for which we also used DOCK-GOLD programs and screening methods that utilized the structural similarity between the selected active compounds, we identified two compounds with potent inhibitory effects on mycobacterial growth. The antimycobacterial effect of the compounds was comparable to that of isoniazid, which is used as a first-line anti-TB drug. **Conclusion:** The compounds identified through SBDS were expected to be a novel class of anti-TB pharmacophores.

**Keywords:** Cyclopropane mycolic acid synthase 1, DOCK program, GOLD program, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, structure-based drug screening

**Introduction**

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and is a lethal respiratory infection. An increase in the number of TB patients is attributable to the insufficient supply or low quality of anti-TB drugs.[1-4] In addition, the emergence of multidrug-resistant and extensively drug resistant TB has also complicated TB treatment.[1,5] Thus, novel and efficient anti-TB agents with new mechanisms of action are necessary for mitigating the current increase in *M. tuberculosis* infections.

Because the biosynthesis of bacterial cell wall differs from that of mammalian cell wall, enzymes responsible for cell wall development are regarded as potential targets for developing anti-TB agents. The thick and waxy cell wall of *M. tuberculosis* prevents dehydration, protects against varying pH levels, and counters the detrimental effects of free radicals, all of which are essential for pathogenicity, persistence, and drug resistance.[6,7] In pathogenic *M. tuberculosis*, cell wall composition includes a majority of unsaturated α-mycolates that undergo cyclopropanation of the double bonds catalyzed by the family of enzymes called cyclopropane synthases, with S-adenosyl-L-methionine acting as a methyl donor.[8-15] Together with the mycobacterial cyclopropane mycolic acid synthase 1 (CmaA1), these enzymes are responsible for cis-cyclopropanation at the distal position of unsaturated mycolates and are essential drug targets in antimycobacterial drug therapy.

In silico structure-based drug screening (SBDS) is a three-dimensional (3D) protein structural analysis technique.

**Address for correspondence:** Prof. Shunsuke Aoki, Department of Bioscience and Bioinformatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology, Iizuka 820-8502, Japan. E-mail: aokis@bio.kyutech.ac.jp

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Taira J, Ito T, Nakatani H, Umei T, Baba H, Kawashima S, et al. In silico structure-based drug screening of novel antimycobacterial pharmacophores by DOCK-GOLD tandem screening. Int J Mycobacteriol 2017;6:142-8.
that harnesses X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy and is not only considered a breakthrough in computational chemistry but also has accelerated in silico screening of chemical compound libraries. The technique is a faster and less expensive alternative to the traditional high-throughput screening methods. We previously screened various inhibitors of mycobacterial enzymes (e.g., enoyl-acyl carrier protein reductase\textsuperscript{[16-18]} and thymidine monophosphate kinase\textsuperscript{[19]} using in silico SBDS and experimentally confirmed the antimycobacterial activity of the screened compounds. The high-resolution crystal structure of \textit{M. tuberculosis} CmaA1 has been previously published; therefore, in this study, we attempted to identify compounds that were capable of interacting with the mycobacterial CmaA1 active site by combing through a considerably large virtual compound library using \textit{in silico} SBDS with an intent to examine the antimycobacterial activity of the selected compounds.

**MATERIALS AND METHODS**

**Analysis of the compound library and protein structural data**

The 3D virtual chemical library, comprising 461,383 compounds and supplied by ChemBridge (San Diego, CA, USA), was derived from the Ressource Parisienne en Bioinformatique Structurale (Paris, France) web-based database, as previously described.\textsuperscript{[16]} 2D structures of the compounds were converted to 3D structures using the stereochemistry module of the Molecular Operating Environment (MOE; Chemical Computing Group, Montreal, Canada) application. Protonation states were adjusted at pH 7.0, and multiple conformations of the compounds were generated using the Protonate 3D module and conformation search module in MOE. The 3D structural data of \textit{M. tuberculosis} CmaA1 complexed with S-adenosyl-L-homocysteine and didecylmethylammonium (PDB-id 1KPH; resolution, 2.0 Å)\textsuperscript{[20]} was used for the present \textit{in silico} SBDS. Hydrogen atoms were assigned to the structural data using the DOCK Prep tool. The molecular surface of the structure was calculated using the DMS program, and the docking pocket for drug screening (i.e., active site cavity of the enzyme) was determined using the SPHGEN program.\textsuperscript{[21]}

**\textit{In silico} structure-based drug screening**

The three-step \textit{in silico} SBDS was performed using a combination of two applications—UCSF DOCK (version 6.3)\textsuperscript{[22]} and GOLD,\textsuperscript{[23,24]} as previously described.\textsuperscript{[16-19,25]} In brief, the top 3,000 compounds with a DOCK score of <−55 kcal/mol were selected by screening the library using the DOCK program; this was designated as primary screening. Next, the selected compounds were screened using the GOLD program, which constituted the secondary screening. Ten multiple conformations of the compounds with a high GOLD score were generated using the LowModeMD in MOE and were then rescreened with the GOLD program; this comprised the tertiary screening. Structurally related compounds among the active compounds obtained prior to SBDS were targeted within a chemical library that comprised approximately 1,000,000 compounds (ChemBridge). Ten multiple conformations of compounds with a high Tanimoto coefficient (>0.85 for compound 1; >0.65 for compound 5) were generated, as described earlier, after which the docking simulation was performed using the GOLD program.

**Candidate compounds**

The candidate compounds identified via SBDS were purchased from ChemBridge. The NMR and/or liquid chromatography/mass spectrometry data related to the compounds are available on the manufacturer’s website at http://www.chembridge.com. Hereafter, the series of the screened compounds were designated as follows: 1–10, 1a–1e, and 5a–5e [Tables 1 and 2].

### Table 1: The candidate compounds identified by DOCK-GOLD structure-based drug screening (compounds 1-10)

| Compound numbers | IUPAC names                                                                 | MW     | GOLD scores |
|------------------|-----------------------------------------------------------------------------|--------|-------------|
| 1                | \(N\)-(2,5-diethoxy-4-[[3-(4-nitro-1,3-dioxo-1,3-dihydro-2H-isoinodino-2-yl] propanoyl] amino] phenyl) benzamide | 547    | 89.4        |
| 2                | 2-[[4-amino-5-(7-methoxy-1-benzofuran-2-yl) 4H-1,2,4-triazol-3-yl]thio]-\(N\)-(4-phenyl-1,3-thiazol-2-yl) acetamide | 479    | 89.1        |
| 3                | 2',2''-[2,3-quinaxalinediybis (thio)]bis[N-(tetrahydro-2-furanylmethyl) acetamide] | 477    | 86.5        |
| 4                | 6-oxo-7,8,9,10-tetrahydro-6H-benzo[c] chromen-3-yl-[[(benzyloxyl) carbonyl] amino] butanoate | 436    | 86.2        |
| 5                | 2-[[4-bromo-3-nitro-1H-pyrazol-1-yl] methyl]-2-furoyl]-\(N\)-(4-methyl-2-oxo-2H-chromen-7-yl) hydrazinecarboxamide | 547    | 86.0        |
| 6                | \(N\)-[[5-[[4-bromo-3-chlorophenyl] amino]-2-oxoethyl] thio]-4-methyl-4H-1,2,4-triazol-3-yl] methyl]-3,4-dichlorobenzamide | 564    | 85.8        |
| 7                | \(N\)-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-[[5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-yl] thio] acetamide | 475    | 85.8        |
| 8                | 2-[[1,3-dioxo-1,3-dihydro-2H-isoinodino-2-yl] 1,3-benzothiazol-2-yl] thio]-\(N\)-(2-methoxyphenyl) acetamide | 476    | 85.6        |
| 9                | 2'-[[2-antilino-2-oxoethyl] 4-ethyl-4H-1,2,4-triazol-3-yl] thio]-\(N\)-(4-methyl-3-nitrophenyl) acetamide | 455    | 84.5        |
| 10               | \(N\)-[[4-allyl-5-[[2-[(3-bromophenyl) amino]-2-oxoethyl] thio]-4H-1,2,4-triazol-3-yl] methyl]-4-chlorobenzamide | 521    | 85.4        |

MW: Molecular weight
Table 2: The structurally-related compounds of 1 and 5 (1a-1e, 5a-5e)

| Compound numbers | IUPAC names                                                                 | MW  | GOLD scores |
|------------------|------------------------------------------------------------------------------|-----|-------------|
| 1a               | 2-{[(4-[(2-methoxyphenyl) carbonyl]-3-nitrophenyl) amino]ethyl}-1,3-dioxo-5-isooindolinecarboxylic acid | 504 | 86.7        |
| 1b               | 4-{[(3-(1,3-dioxo-1,3-dihydro-2H-isooindol-2-yl) propyl] amino}-N-(4-methoxyphenyl)-3-nitrobenzamide | 475 | 78.5        |
| 1c               | Benzyl 4-{[(3-(4-nitro-1,3-dioxo-1,3-dihydro-2H-isooindol-2-yl) propoxy] oxy} benzoate | 474 | 78.1        |
| 1d               | 2-{3-[(4-(methoxyphenyl) amino] carbonyl}1-2-nitrophenyl) amino}propyl]-1,3-dioxo-5-isooindolinecarboxylic acid | 519 | 77.9        |
| 1e               | N-[4-[[benzyl]oxy] phenyl]-3-(4-nitro-1,3-dioxo-1,3-dihydro-2H-isooindol-2-yl) Propanamide | 445 | 76.3        |
| 5a               | N-[3-(N-[5-{4-bromo-3-nitro-1H-pyrazol(-1-yl) methyl]-2-furoyl} ethanehydrazonyloyl]phenyl]-2-(4-methoxyphenyl) acetamide | 570 | 84.5        |
| 5b               | Methyl 2-{[(2-{5-{4-bromo-3-nitro-1H-pyrazol-1-yl} methyl]-2-furoyl} hydrazino] carbonothioyl} amino]-5,6-dihydro-4H-cyclopenta[b] thiophene-3-carboxylate | 569 | 84.4        |
| 5c               | 2-{{[2-{5-[4-bromo-3-nitro-1H-pyrazol-1-yl] methyl]-2-furoyl}]-N-{2-(3,4-dimethoxyphenyl) ethyl} hydrazinecarbothioamide | 553 | 84.3        |
| 5d               | N-[2-{{[2-oxo-2Hchromen-3-yl] carbonyl] amino} ethyl}]-3-{[2-(trifluoromethyl)-1H-benzimidazol-1-yl] methyl}-1,2-oxadiazole-5-carboxamide | 557 | 82.9        |
| 5e               | 4-{4-[4-chloro-3-nitro-1H-pyrazol-1-yl] methyl] benzoyl} amino)-1-ethyl-N-(2-furylmethyl)-1H-pyrazole-3-carboxamide | 471 | 81.9        |

MW: Molecular weight

Antimicrobial assay

*Mycobacterium smegmatis* (IAM 12065 strain; RIKEN BioResource Center, Saitama, Japan) was cultured at 37°C for 24 h in 3.7% brain–heart infusion broth (Sigma, St. Louis, MO, USA). Then, the culture was diluted 8-fold with a broth containing the candidate compounds in a 96-well flat bottom clear plates (CORNING, Corning, NY, USA); the quantity of the cultures was 200 µL per well. Isoniazid (LKT Laboratories, St. Paul, MN, USA) and 0.3% dimethyl sulfoxide were used as the positive and negative controls, respectively. After incubation of the plate at 37°C for 24 h, the cell culture was used for the growth inhibition assay. The inhibition of bacterial growth was determined using OD₅₉₅ with the Bio-Rad Model 680 Microplate Reader (Bio-Rad, Hercules, CA, USA).

Results

Structure-based drug screening of *Mycobacterium tuberculosis* cyclopropane mycolic acid synthase 1 inhibitor

In this study, *in silico* SBDS of the 461,383-compound library was performed to identify CmaA1-targeted novel antibiotics for *M. tuberculosis*. The van der Waals molecular surface of the drug-binding cavity in *M. tuberculosis* CmaA1 (1KPH) is shown in Figure 1. The three-step screening strategy using the DOCK and GOLD applications has been previously described. The primary DOCK screening identified the top 3,000 ranked chemical compounds with a DOCK score of \(-55 \text{ kcal/mol}\). The subsequent GOLD screenings (secondary screening: Single conformation; tertiary screening: Multiple conformations) led to the identification of 10 compounds (1–10) with high GOLD scores. The IUPAC names, molecular weights, and GOLD scores of the candidate compounds are summarized in Table 1, and their chemical structures are represented in Figure S1.

Antimycobacterial growth assessment of the selected compounds via structure-based drug screening

Antimycobacterial activity of the 10 candidate compounds was measured to evaluate their inhibitory effect on mycobacterial growth. Nonpathogenic *M. smegmatis* (biosafety level 1) was used as a model *Mycobacterium* considering its similarity of primary structures with those of *M. tuberculosis*; the similarity of *M. smegmatis* CmaA1 to *M. tuberculosis* CmaA1 is sufficient (80% similar). Figure 2 shows the inhibitory effects of the candidate compounds on the growth of *M. smegmatis*. Compounds 1 and 5 exhibited strong inhibition,
and compounds 6 and 10 showed moderate inhibition. Dose dependence of the inhibitory effect of compounds 1 and 5 was further examined [Figure 3]. IC$_{50}$ values, concentration at which 50% of $M$. $smegmatis$ growth is inhibited, of compounds 1 and 5 were 5.1 and 31 µM, respectively.

**In silico screening of the structurally related compounds 1 and 5**

Among the candidate compounds, 1 and 5 showed remarkable inhibitory effect on $M$. $smegmatis$ growth. To further study the association between their structure and activity, structurally related compounds, that is, 1a–1e for compound 1 and 5a–5e for compound 5, were identified within the chemical library, as previously described.[17,18,25] The IUPAC names, molecular weights, GOLD scores, and chemical structures of the compounds are summarized in Table 2 and Figure 4. As shown in Figure 5, compounds 1c and 1e exhibited a significant inhibitory effect on $M$. $smegmatis$ growth, whereas the inhibitory effect of compound 5-related composites was undetectable. The IC$_{50}$ values of the compounds 1c and 1e were 45.6 µM, 5.6 µM, respectively [Figure 5].

**Discussion**

The *in silico* SBDS approach has been used to identify novel chemicals from virtual compound libraries with widely used docking simulation tools. The DOCK-GOLD tandem screening employed in this study resulted in the identification of two compounds with antimycobacterial activity (compounds 1 and 5), and the following GOLD screening based on the

Figure 2: Antimicrobial activity of the candidate compounds selected via structure-based drug screening (compounds 1–10) on the growth of *Mycobacterium smegmatis*. Inhibition of bacterial growth was monitored at OD$_{595}$ after 0 and 24 h of treatment with the candidate compounds. Concentration of the tested compounds was set at 100 µM. All experiments were performed in quadruplicate, and the values obtained in the absence (−) and presence (+) of the candidate compounds were compared using the Bonferroni’s test for significance: n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001.
structural similarity of compounds 1 and 5 identified two more active compounds (compounds 1c and 1e). Among the four compounds, the IC$_{50}$ value of 1 (5.1 µM) and 1e (5.6 µM) was comparable to that of isoniazid (5.4 µM). The in silico screening used in this study identified two promising compounds among the experimentally validated 20 compounds (hit rate, 10%), despite the hit rate of random high-throughput screening approaches generally being 1%–3%. Therefore, a combination of the DOCK-GOLD tandem in silico SBDS and successive screening for structural similarity may help in cost and labor savings in general drug screening.

The protein–ligand interaction fingerprint and the ligand interaction in MOE predicted the putative interaction of compounds 1, 5, 1c, and 1e with Phe$_{200}$, Arg$_{204}$, and Leu$_{205}$ of CmaA1, which involves a binding loop (residues 199–207) for α-mycolate acyl group.$^{[26]}$ According to our preliminary experiments, the aforementioned active compounds (1, 5, 1c, and 1e) did not inhibit the activity of the representative Gram-negative bacteria, *Escherichia coli* BL21 strain [Figure S2]; we should also mention that CmaA1 has not been identified in *E. coli*.

Elucidation of further antibacterial mechanisms of the candidate compounds such as target–enzyme specificity, biophysical binding properties, including co-crystal structure, and effect on enzyme kinetics and inhibiting mechanism remain to be studied in the future. Nonetheless, irrespective of the evident experimental insufficiency, we believe that an accumulation of knowledge on pharmacophores related to mycobacterial growth inhibition would be valuable for the development of novel anti-TB drugs. The structural features of compounds 1, 1c, and 1e, including the nitric groups on 1,3-dioxo-isoinold and the central amide bond, constitute structural requirements for exerting their potent antimycobacterial activity. In addition, the 4-methyl-2-oxo-chromen group of compound 5 is structurally
unique among the tested compounds, suggesting that the group is important in the compound’s antimycobacterial action.

**Conclusion**

In the present study, we achieved to identify two compounds (compounds 1 and 1e) with potent inhibitory effects on mycobacterial growth. These compounds identified via present SBDS were expected to be a novel class of antituberculosis pharmacophores and the SBDS techniques are expected as powerful tool for antimycobacterial drug screening.

**Financial support and sponsorship**

This work was supported in part by a grant from Takeda Science Foundation to J.T. and a Grants-in-Aid for Young Scientists (B) (16K21226) and a Grant-in-Aid for Scientific Research (C) (26460145) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Dye C, Williams BG. The population dynamics and control of tuberculosis. Science 2010;328:856-61.

Figure 5: Antimicrobial activity and dose dependence of compounds 1c (upper panel) and 1e (lower panel) on the growth of *Mycobacterium smegmatis*. Left panel: Inhibition of mycobacterial growth monitored at OD$_{595}$ after 0 h and 24 h of treatment with the compounds. Concentration of the tested compounds was set at 100 µM. All experiments were performed in quadruplicate, and the values obtained in the absence (−) and presence (+) of the candidate compounds were compared using the Bonferroni’s test for significance: n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001. Right panel: IC$_{50}$ values were estimated with nonlinear curve-fitting analysis with GraphPad Prism.
tuberculosis cmaA2 gene encodes a mycolic acid trans-cyclopropane synthetase. J Biol Chem 2001;276:2228-33.
14. Glickman MS. The mmaA2 gene of Mycobacterium tuberculosis encodes the distal cyclopropane synthase of the alpha-mycolic acid. J Biol Chem 2003;278:7844-9.
15. Boissier F, Bardou F, Guillet V, Uttenweiler-Joseph S, Daffé M, Quémard A, et al. Further insight into S-adenosylmethionine-dependent methyltransferases: Structural characterization of Hma, an enzyme essential for the biosynthesis of oxygenated mycolic acids in Mycobacterium tuberculosis. J Biol Chem 2006;281:4434-45.
16. Izumizono Y, Arevalo S, Koseki Y, Kuroki M, Aoki S. Identification of novel potential antibiotics for tuberculosis by in silico structure-based drug screening. Eur J Med Chem 2011;46:1849-56.
17. Kinjo T, Koseki Y, Kobayashi M, Yamada A, Morita K, Yamaguchi K, et al. Identification of compounds with potential antibacterial activity against Mycobacterium through structure-based drug screening. J Chem Inf Model 2013;53:1200-12.
18. Kanetaka H, Koseki Y, Taira J, Umei T, Komatsu H, Sakamoto H, et al. Discovery of InhA inhibitors with anti-mycobacterial activity through a matched molecular pair approach. Eur J Med Chem 2015;94:378-85.
19. Koseki Y, Kinjo T, Kobayashi M, Aoki S. Identification of novel antimycobacterial chemical agents through the in silico multi-conformational structure-based drug screening of a large-scale chemical library. Eur J Med Chem 2013;60:333-9.
20. Huang CC, Smith CV, Glickman MS, Jacobs WR Jr., Sacchettini JC. Crystal structures of mycolic acid cyclopropane synthases from Mycobacterium tuberculosis. J Biol Chem 2002;277:11559-69.
21. Kuntz ID, Blaney JM, Oatley SJ, Langridge R, Ferrin TE. A geometric approach to macromolecule-ligand interactions. J Mol Biol 1982;161:269-88.
22. Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC, Thomas V, et al. DOCK 6: Combining techniques to model RNA-small molecule complexes. RNA 2009;15:1219-30.
23. Jones G, Willett P, Glen RC. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. J Mol Biol 1995;245:43-53.
24. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. J Mol Biol 1997;267:727-48.
25. Kobayashi M, Kinjo T, Koseki Y, Bourne CR, Barrow WW, Aoki S. Identification of novel potential antibiotics against Staphylococcus using structure-based drug screening targeting dihydrofolate reductase. J Chem Inf Model 2014;54:1242-53.
26. Choudhury C, Deva Priyakumar U, Sastry GN. Molecular dynamics investigation of the active site dynamics of mycobacterial cyclopropane synthase during various stages of the cyclopropanation process. J Struct Biol 2014;187:38-48.
Figure S1: The chemical structures of compounds 1–10.

Figure S2: Growth of *Escherichia coli* BL21 strain in the presence of active compounds (1, 5, 1c, and 1e). Ampicillin (100 mg/mL) and dimethyl sulfoxide (0.3%) were used as positive and negative control, respectively.