Addressing the Metabolic Stability of Antituberculars through Machine Learning

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S Supporting Information

ABSTRACT: We present the first prospective application of our mouse liver microsomal (MLM) stability Bayesian model. CD117, an antitubercular thienopyrimidine tool compound that suffers from metabolic instability (MLM $t_{1/2}$ < 1 min), was utilized to assess the predictive power of our new MLM stability model. The S-substituent was removed, a set of commercial reagents was utilized to construct a virtual library of 411 analogues, and our MLM stability model was applied to prioritize 13 analogues for synthesis and biological profiling. In MLM stability assays, all 13 analogues had superior metabolic stability to the parent compound, and six new analogues had acceptable MLM $t_{1/2}$ values greater than or equal to 60 min. It is noteworthy that whole-cell efficacy and lack of relative mammalian cell cytotoxicity could not be predicted simultaneously. These results support the utility of our new MLM stability model in chemical tool and drug discovery optimization efforts.

KEYWORDS: Bayesian, mouse liver microsomal stability, antitubercular, chemical tool optimization, computer-aided analogue design, machine learning

Back to school with machine learning

A naïve Bayesian model for mouse liver microsomal stability

Mycobacterium tuberculosis, the pathogen that causes tuberculosis, is responsible for a global health pandemic. It kills approximately 1.4 million people each year.¹ Multidrug resistant, extensively drug resistant, and even totally drug resistant strains of *M. tuberculosis* continue to increase in frequency and in global distribution. Consequently, new drugs to treat *M. tuberculosis* infections via novel therapeutic approaches represent an urgent public health necessity. To increase the efficiency and accuracy of discovering novel chemical tools that can (a) facilitate studies of the fundamental biology of *M. tuberculosis* and (b) lay the foundation for the discovery and development of new drugs to treat drug-resistant infections, we are creating, honing, and applying novel computational tools and workflows.² Our approaches, and many of the specific machine learning models that we develop, could also be applied to help advance translational research against other diseases. We are (1) developing various types of machine learning models and (2) combining them with each other and with other computational techniques, followed by

(3) prospective predictions and (4) experimental validation, to help overcome each of the hurdles that a compound must pass before it can become either a useful chemical tool for *M. tuberculosis* studies or an antitubercular drug lead.

In the search for small molecule agents that act as chemical tools to probe basic biology as well as to seed novel therapeutic strategies, we must consider as early as possible key molecular properties. In our experience, liver microsomal stability, kinetic solubility, *in vitro* efficacy, and mammalian cell cytotoxicity are among the most important metrics to be profiled.^{3–5} Given our focus on small molecules as antitubercular agents,^{6,7} we are interested in assessing mouse liver microsomal (MLM) stability. Although mouse models fail to recapitulate the entire disease pathology observed for *M. tuberculosis* infection of

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Figure 1. Structure of CD117 with an emphasis on its metabolic liability, and the previous medicinal chemistry optimization to improve metabolic stability that afforded JSF-2070 and JSF-2088.

Scheme 1. Synthesis of CD117 Analogues^a

^aReagents and conditions: (a) NCCH₂CN, S₈, Et₂NH/EtOH; (b) PhC(O)NCS, acetone; (c) NaOH_(aq), EtOH, microwave, (d) RCH₂Br.

humans (e.g., necrotic lesions such as caseous granulomas are not formed in most types of mice),^{8,9} the field has witnessed a significant correlation between acceptable efficacy outcomes in humans and in mice.^{10,11} Thus, we assert that given the goal of demonstrating a small molecule's ability to significantly reduce *M. tuberculosis* infection in mice, we should initially assess the MLM stability of a chemical tool or drug discovery hit.

The typical MLM assay involves determination of the stability of a small molecule in the presence of a microsomal preparation, which contains the enzymes involved in phase I (oxidative) metabolism, except for some MLM assays that contain both phase I and phase II (conjugative) metabolism.¹² The half-life ($t_{1/2}$) and intrinsic clearance (Cl_{int}) are determined. In our programs, we have set the goal for acceptable MLM stability as $t_{1/2} \geq 60$ min and $Cl_{int} \leq 10$ μ L/min/mg protein.¹³ Once a small molecule's MLM stability has been assessed, it may also be helpful to determine what metabolites are formed via mass spectroscopic techniques, followed by synthesizing the dominant metabolites and then assaying them for inhibition of *M. tuberculosis* growth.⁷ We herein characterize the *in vitro* whole-cell growth inhibition of the laboratory wild-type *M. tuberculosis* strain H37Rv with a minimum inhibitory concentration (MIC) equal to the concentration of compound capable of inhibiting 90% of bacterial growth as judged by a microplate Alamar blue assay.¹⁴

We have utilized such early profiling techniques to assess the strengths and shortcomings of a class of antitubercular agents stemming from thienopyrimidine CD117 (Figure 1).¹⁵ CD117 displays promising whole-cell efficacy versus *in vitro* cultured *M. tuberculosis* (MIC = 0.19–0.38 μ g/mL) and selectivity versus Vero cells as a model mammalian cell line (SI = CC_{50} /MIC = 120–240, where CC_{50} = minimum concentration to inhibit the growth of 50% of the Vero cells). However, CD117 suffers from rapid metabolism, in the presence of MLM ($t_{1/2} < 1$ min) or when dosed to BALB/c mice, to generate the corresponding

whole-cell inactive carboxylic acid metabolite (JSF-2000). While this decomposition does not occur within the bacterial growth medium, metabolomics studies confirmed this degradation does take place to a minor extent within CD117-treated *M. tuberculosis* over 18 h. Given that the primary target of CD117 was (and still remains) a subject of intense study, we have been unable to inform our optimization efforts with details as to what protein/s CD117 is/are binding and how its ethyl ester is interacting with the protein/s at an atomic level. This is not an uncommon circumstance with antibacterial chemical biology and drug discovery research and in particular within the tuberculosis field.¹⁶ Thus, we turned to medicinal chemistry heuristics and attempts to mimic structural aspects of the ethyl ester of CD117 with a heterocyclic isostere, which led to isoxazole (JSF-2070) and thiazole (JSF-2088) analogues with MLM $t_{1/2}$ values of 19.0 and 66.6 min, respectively.⁷

While pursuing further evolution of these heterocyclic replacements for the ethyl ester, we sought to design and then apply an orthogonal strategy that would more broadly consider chemical space and not be limited by our medicinal chemistry insights, which are undoubtedly biased by our collective experience. We turned to our recently devised naïve Bayesian (heretofore referred as Bayesian) classifier models for MLM stability.¹³ The models have essentially learned from our curated version of publicly deposited MLM data sets to identify what molecular physiochemical and structural features are associated with sufficient or insufficient MLM stability, as judged by our metric of stable compounds having a $t_{1/2} \geq 60$ min. Using a novel approach for the construction of machine learning models, we discovered that pruning the training set (by discarding moderately stable/moderately unstable models from the training set, such that sufficiently stable molecules were defined by $t_{1/2} \geq 60$ min and insufficiently stable molecules were set as those with a $t_{1/2} < 30$ min, while the intermediate compounds were deleted) led to models that were better able

to predict their respective training set as well as independent external test and validation sets (when compared to the full or unpruned model). In particular, we previously concluded the pruned $t_{1/2}$ MLM Bayesian model constructed with nine descriptors was most useful for filtering a large library of compounds to harvest candidates with MLM stability. Conversely, we observed that the full $t_{1/2}$ MLM Bayesian model constructed using only the FCFP-6 descriptor (Full MLM/FCFP-6 model which had a full training set but a pruned set of descriptors) performed the best when identifying a small number of candidates with MLM stability.¹³ We herein describe the first prospective application of the Full $t_{1/2}$ MLM Bayesian model constructed using only the FCFP-6 descriptor to enhance the MLM stability of CD117.

Our approach began with the obvious decision that the metabolically labile ethyl ester moiety had to be replaced. Leveraging our published route to CD117 and its analogues (Scheme 1), we chose to consider a library of 411 alkyl bromides and aldehydes (which could be converted into alkyl bromides via straightforward reduction and bromination) available from Sigma-Aldrich. A virtual library was created and then scored via three different workflows (Figure 2;

Figure 2. Computational workflows focused on enhancing the MLM stability of CD117.

Supporting Information for an Excel file with all 411 candidates scored with all models). The majority of the candidates occupy different property space from those CD117 analogues previously synthesized (**Supporting Information** Figure S1). In workflow (i), the library was scored with the Full MLM/FCFP-6 model, and the top 20 scoring molecules were noted.

Next, we sought to predict molecules with sufficient MLM stability as well as acceptable antitubercular whole-cell efficacy and relative Vero cell cytotoxicity. The intersection was probed for virtual library members predicted to be MLM stable through the Full MLM/FCFP-6 model and active (acceptable MIC versus *M. tuberculosis*)/noncytotoxic (acceptable CC₅₀ versus Vero cell cells as judged through the SI value) with our published TAACF-CB2 dual-event Bayesian model (defining an active such that MIC ≤ 10 μg/mL and SI ≥ 10), which was trained with a 1783 molecule library subset as reported by the Southern Research Institute.¹⁷ However, none of the 411 candidates was predicted to be MLM stable, whole-cell active, and sufficiently noncytotoxic. To refine the dual-event model, we pruned its training set (defining an active such that MIC ≤ 10 μg/mL and SI ≥ 15 instead of SI ≥ 10) to generate the CB2v2_SI15 dual-event Bayesian (further model details are in the **Supporting Information**). Alteration of the training set

active definition allowed the model to consider different areas of chemical space as being more favorable to metabolic stability. We also explored the use of both FCFP12 and FCFP6 descriptors to investigate whether these new CB2v2_SI15 models would (a) enhance the accuracy of ranking the 88 previously synthesized CD117 analogues, as an external test of the accuracy of these new models (see Table S2 in the **Supporting Information**) given our specific focus on thienopyrimidines in this optimization, and (b) produce new consensus workflows that allowed some analogues in the new virtual library to pass through these filters (Figure 2, workflow (ii)). Both of these goals were achieved with the new FCFP12-based CB2v2_SI15 model. This new model was found to exhibit acceptable (ROC ≥ 0.7)¹³ internal and external statistics of 0.73 and 0.76, respectively (Tables S1 and S2 in the **Supporting Information**). In workflow (ii), the consensus scoring of the virtual library of analogues using this revised model and the Full MLM/FCFP-6 model afforded ten candidates predicted to meet the criteria of MLM stable, whole-cell active, and relatively noncytotoxic.

In addition, the concept of a chemical series specific Bayesian model for whole-cell efficacy was explored. We utilized the Collaborative Drug Discovery software (www.collaboratedrug.com) to create a single-event model, using 88 previously synthesized CD117 analogues⁷ and the activity definition of MIC ≤ 10 μg/mL (further model details are in the **Supporting Information**). With acceptable internal statistics (see Figure S5 in the **Supporting Information**), the CDD_CD117 model was used in workflow (iii) to score the virtual library. The intersection of those compounds predicted to be active with the CDD_CD117 model and stable with the Full MLM/FCFP-6 model consisted of four molecules.

Finally, visual inspection of the total candidates from the three workflows was conducted to remove molecules with reactive functionality (e.g., an electrophile such as an aldehyde, epoxide, or alkyl halide),⁸ a molecular weight in excess of 600 g/mol, or an AlogP > 6.0.¹⁹ The resulting 13 candidates for synthesis and biological testing (Table 1 excluding CD117 and INH controls) comprised six from the Full MLM/FCFP-6 model, six from this model in consensus with the CB2v2_SI15 dual event Bayesian, and one in consensus with the CDD_CD117 model. Although these candidates were selected using the Full MLM/FCFP-6 model, they were all also predicted to be stable with the pruned $t_{1/2}$ MLM Bayesian model constructed using all nine descriptors. While these 13 candidates cluster more closely to the previously synthesized CD117 analogues than the initial set of 411 (**Supporting Information** Figures S1 and S2), their respective sulfide pendant moieties contain different chemotypes than those previously explored through a medicinal chemistry heuristic approach.⁷ As evident from inspection of the candidate structures, nitro aromatics were not triaged due to their prevalence in anti-infectives.²⁰ We do note that the potential exists for other liabilities to have been introduced into the candidates as the models were only trained to learn about MLM $t_{1/2}$ or {MLM $t_{1/2}$, MIC, and SI}.

The synthetic efforts recalled our previously published methodology,⁷ based on the two-step/one-pot cyclization–alkylation of the key benzoyl thiourea intermediate **1** (Scheme 1). Compared to our previous route, we were pleased to find that microwave heating significantly decreased the time for the base-promoted cyclization to the proposed thiolate intermediate. The electrophiles chosen to react with this intermediate to

Table 1. Biological Profiling of Computationally Evolved Analogues of CD117

Entry	Structure	MLM Stability ^d	Clearance ^e	MIC ^f	CC ₅₀ ^g
CD117		<1	>1390	0.19 – 0.38	45
JSF-2450 ^a		120	5.80	12.5 – 25	>50
JSF-2509 ^a		95.0	7.30	25 – 50	50
JSF-2486 ^a		87.7	7.90	>200	12.5
JSF-2489 ^b		64.2	10.8	>200	>50
JSF-2790 ^b		60.8	11.4	10	100
JSF-2812 ^b		60.3	11.5	25	6.2
JSF-2449 ^a		58.2	11.9	200	50
JSF-2452 ^a		47.5	14.6	10	>200
JSF-2433 ^a		34.3	20.1	>200	>50
JSF-2771 ^b		31.8	21.8	>200	12.5
JSF-2432 ^c		21.7	31.9	100 – 200	1.6
JSF-2772 ^b		19.7	35.1	200	3.1
JSF-2430 ^b		17.0	40.8	>200	25
INH		N.D.	N.D.	0.039	N.D.

^aWorkflow (i), shown in Figure 2. ^bWorkflow (ii), shown in Figure 2. ^cWorkflow (iii), shown in Figure 2. ^dMLM stability from assay as represented by $t_{1/2}$ (min). ^eMLM Cl_{int} as represented by $\mu\text{L}/\text{min}/\text{mg}$ protein. ^fMIC against *Mtb* H37Rv in $\mu\text{g}/\text{mL}$. ^gVero Cell toxicity as represented by CC_{50} in $\mu\text{g}/\text{mL}$.

form the resulting sulfide were commercially available or prepared by straightforward functional group interconversions (reduction followed by bromination) from the commercially available aldehyde. The resulting CD117 analogues were characterized by NMR spectroscopy (^1H and ^{13}C), HPLC, and HRMS (Supporting Information).

Each of the synthesized candidates was assayed for MLM stability, as judged primarily by $t_{1/2}$ given the training criterion of the Bayesian models. All 13 analogues displayed larger MLM $t_{1/2}$ values than the parent compound CD117 (Table 1). Six of the 13 analogues displayed MLM $t_{1/2}$ values above our stability criterion of 60 min, which represents a hit rate of 46% (6/13). One analogue displayed a $t_{1/2}$ of 58.2 min, which is very close to our intended goal and could also be viewed as exhibiting sufficient MLM stability. Only three of the 13 analogues (23%) had an MLM $t_{1/2}$ less than 30 min. Interestingly, the MLM stability model fared similarly, with or without the inclusion of predictions from the dual-event model for whole-cell efficacy

and lack of relative Vero cell cytotoxicity. Failing to predict a metabolically stable compound with an extremely limited test set ($N = 1$), workflow (iii) and the potential to leverage a chemical series Bayesian model will need to be investigated with a significantly larger virtual library of analogues. While the MLM models were not trained with Cl_{int} data, one may also employ a stability criterion of MLM $Cl_{int} \leq 10 \mu\text{L}/\text{min}/\text{mg}$ protein. Through this lens, three of the 13 candidates (23%) may be judged suitably stable, with another five analogues (38%) nearly meeting this criterion with $10 < Cl_{int} < 15$.

The candidates were also assessed for their antitubercular whole-cell efficacy and Vero cell cytotoxicity. The most potent antitubercular compounds were JSF-2452 and 2790, exhibiting a MIC value of $10 \mu\text{g}/\text{mL}$. The Vero cell cytotoxicities of these two compounds were $>200 \mu\text{g}/\text{mL}$ ($SI > 20$) and $100 \mu\text{g}/\text{mL}$ ($SI = 10$), respectively. The consensus approach in workflow (ii) delivered one analogue (JSF-2790) with sufficient MLM $t_{1/2}$ and MIC, although its SI value of 10 fell short of the target value of 15. Thus, at this juncture, workflow (ii) does not add significant value over workflow (i). Workflow (i) lacked inclusion of a dual-event model but did produce one compound (JSF-2452) with acceptable MLM $t_{1/2}$, whole-cell activity, and relative Vero cell cytotoxicity.

To further demonstrate the predictive power of our Bayesian approach, we synthesized the lowest scoring five CD117 candidates according to the Full MLM/FCFP-6 model. The model correctly predicted the metabolic instability of 4/5 compounds (see Table S3 in the Supporting Information), demonstrating its ability to triage analogues unlikely to exhibit a sufficient $t_{1/2}$. Using the random number generator functionality within LibreOffice Calc (www.libreoffice.org), five candidate analogues (not previously assessed) were chosen randomly from a set of 124 candidate analogues (reduced from the list of 411 compounds via the previously mentioned molecular weight and AlogP filters), prepared, and assayed for MLM $t_{1/2}$ (see Table S4 in the Supporting Information). All five analogs in this random set were unstable, and the Bayesian model's prediction was correct for only 1/5 candidates. This is consistent with the proposed workflow and our previous studies showing that the Full MLM/FCFP-6 model performed best when utilized to select the top- or bottom-scoring candidates.¹³ Given the fundamentals of Bayesian modeling, one has the greatest confidence in the highest-scoring entities as their probability of stability scales with their Bayesian score. Inversely, the lowest scoring candidates have the least likelihood of being stable.

The novel workflows presented herein represent a complementary approach to the utilization of medicinal chemistry heuristics when faced with the challenge of enhancing the metabolic stability of a chemical tool and/or drug discovery entity. We have demonstrated the utility of a Bayesian model, cognizant of the physiochemical and structural features related to metabolic stability in the presence of mouse liver microsomes. Regardless of the inclusion of a dual-event Bayesian model to account for whole-cell activity and lack of relative Vero cell cytotoxicity, our chosen MLM Bayesian model correctly selected for novel CD117 analogues with acceptable MLM stability 50% of the time, while triaging 80% of the unstable (and lowest scoring) candidates.

This outcome represents a significant extension from our previous work,¹³ which made available to the scientific community machine learning models for MLM stability, while pharmaceutical industry models have been published but not

disclosed due to intellectual property concerns.²¹ We are unaware of other published reports where such a model has been used alone or in combination with efficacy and cytotoxicity models in prospective predictions to guide compound optimization. This machine learning approach is complementary to MetaSite,²² which computationally predicts the metabolic hot spots of small molecules due to phase I metabolism.

Further work is required to understand how *in vitro* efficacy, Vero cell cytotoxicity, and MLM stability can be predicted simultaneously, at the risk of certain features intrinsic to each model not being shared (i.e., one structural feature or physiochemical property may correlate strongly with MLM stability and yet be inconsistent or inversely correlated with whole-cell activity and/or Vero cell cytotoxicity). In fact, medicinal chemistry optimizations involve even more criteria beyond those considered in this report,²³ and ultimately, we will search for a subset of those that can be successfully predicted simultaneously. The evolved CD117 analogues disclosed here are now the subjects of downstream pharmacokinetic studies to enable consideration for *in vivo* assays of antitubercular efficacy. Coupled with insights from our ongoing mechanistic studies of these thienopyrimidines, we aim for near-term *in vivo* validation of their novel mechanism of action.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.7b00299.

Details with regard to the computational workflows and inherent Bayesian models, syntheses and characterization data for the reported compounds, and protocols for the biological assays (PDF)

Summary information for the candidate analog virtual library (XLSX)

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Author Contributions

[†]These authors contributed equally. T.P.S., S.G.L., and J.S.P. synthesized the compounds. A.L.P. constructed and validated the Bayesian models and utilized them to score the virtual library of candidates. C.V. conducted the *M. tuberculosis* growth inhibition assays. R.R. and E.S. carried out the Vero cell cytotoxicity assays. J.S.F., S.E., W.R.J., and N.C. directed the research. J.S.F. conceived of and originated the project. J.S.F., A.L.P., T.P.S., and S.E. wrote the manuscript. All authors contributed to editing the manuscript. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MLM, mouse liver microsomal; NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry

■ REFERENCES

- (1) *Global Tuberculosis Report*; WHO: Geneva, 2016.
- (2) Ekins, S.; Freundlich, J. S.; Choi, I.; Sarker, M.; Talcott, C. Computational databases, pathway and cheminformatics tools for tuberculosis drug discovery. *Trends Microbiol.* **2011**, *19*, 65–74.
- (3) Workman, P.; Collins, I. Probing the probes: fitness factors for small molecule tools. *Chem. Biol.* **2010**, *17*, 561–77.
- (4) Lakshminarayana, S. B.; Huat, T. B.; Ho, P. C.; Manjunatha, U. H.; Dartois, V.; Dick, T.; Rao, S. P. Comprehensive physicochemical, pharmacokinetic and activity profiling of anti-TB agents. *J. Antimicrob. Chemother.* **2015**, *70*, 857–67.
- (5) Ekins, S.; Freundlich, J. S.; Hobrath, J. V.; Lucile White, E.; Reynolds, R. C. Combining computational methods for hit to lead optimization in Mycobacterium tuberculosis drug discovery. *Pharm. Res.* **2014**, *31*, 414–35.
- (6) Ekins, S.; Perryman, A. L.; Clark, A. M.; Reynolds, R. C.; Freundlich, J. S. Machine Learning Model Analysis and Data Visualization with Small Molecules Tested in a Mouse Model of Mycobacterium tuberculosis Infection (2014–2015). *J. Chem. Inf. Model.* **2016**, *56*, 1332–43.
- (7) Li, S.-G.; Vilcheze, C.; Chakraborty, S.; Wang, X.; Kim, H.; Anisetti, M.; Ekins, S.; Rhee, K. Y.; Jacobs, W. R., Jr.; Freundlich, J. S. Evolution of a thienopyrimidine antitubercular relying on medicinal chemistry and metabolomics insights. *Tetrahedron Lett.* **2015**, *56*, 3246–3250.
- (8) Kramnik, I.; Beamer, G. Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. *Semin. Immunopathol.* **2016**, *38*, 221–37.
- (9) Harper, J.; Skerry, C.; Davis, S. L.; Tasneen, R.; Weir, M.; Kramnik, I.; Bishai, W. R.; Pomper, M. G.; Nuermberger, E. L.; Jain, S. K. Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. *J. Infect. Dis.* **2012**, *205*, 595–602.
- (10) Lanoix, J. P.; Lenaerts, A. J.; Nuermberger, E. L. Heterogeneous disease progression and treatment response in a C3HeB/FeJ mouse model of tuberculosis. *Dis. Models & Mech.* **2015**, *8*, 603–10.
- (11) Irwin, S. M.; Driver, E.; Lyon, E.; Schrupp, C.; Ryan, G.; Gonzalez-Juarrero, M.; Basaraba, R. J.; Nuermberger, E. L.; Lenaerts, A. J. Presence of multiple lesion types with vastly different microenvironments in C3HeB/FeJ mice following aerosol infection with Mycobacterium tuberculosis. *Dis. Models & Mech.* **2015**, *8*, 591–602.
- (12) Di, L.; Kerns, E. H.; Ma, X. J.; Huang, Y.; Carter, G. T. Applications of high throughput microsomal stability assay in drug discovery. *Comb. Chem. High Throughput Screening* **2008**, *11*, 469–76.

- (13) Perryman, A. L.; Stratton, T. P.; Ekins, S.; Freundlich, J. S. Predicting Mouse Liver Microsomal Stability with "Pruned" Machine Learning Models and Public Data. *Pharm. Res.* **2016**, *33*, 433–49.
- (14) Palomino, J. C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **2002**, *46*, 2720–2.
- (15) Vilcheze, C.; Baughn, A. D.; Tufariello, J.; Leung, L. W.; Kuo, M.; Basler, C. F.; Alland, D.; Sacchettini, J. C.; Freundlich, J. S.; Jacobs, W. R., Jr. Novel inhibitors of InhA efficiently kill *Mycobacterium tuberculosis* under aerobic and anaerobic conditions. *Antimicrob. Agents Chemother.* **2011**, *55*, 3889–98.
- (16) Cooper, C. B. Development of *Mycobacterium tuberculosis* whole cell screening hits as potential antituberculosis agents. *J. Med. Chem.* **2013**, *56*, 7755–60.
- (17) Ekins, S.; Reynolds, R. C.; Kim, H.; Koo, M.-S.; Ekonomidis, M.; Talaue, M.; Paget, S. D.; Woolhiser, L. K.; Lenaerts, A.; Bunin, B. A.; Connell, N.; Freundlich, J. S. Bayesian models leveraging bioactivity and cytotoxicity information for drug discovery. *Chem. Biol.* **2013**, *20*, 370–378.
- (18) Walters, W. P.; Stahl, M. T.; Murcko, M. A. Virtual screening - an overview. *Drug Discovery Today* **1998**, *3*, 160–178.
- (19) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. Prediction of Hydrophobic (Lipophilic) Properties of Small Organic Molecules Using Fragmental Methods: An Analysis of ALOGP and CLOGP Methods. *J. Phys. Chem. A* **1998**, *102*, 3762–3772.
- (20) Thompson, A. M.; O'Connor, P. D.; Marshall, A. J.; Yardley, V.; Maes, L.; Gupta, S.; Launay, D.; Braillard, S.; Chatelain, E.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Cooper, C. B.; Denny, W. A. 7-Substituted 2-Nitro-5,6-dihydroimidazo[2,1-b][1,3]oxazines: Novel Antitubercular Agents Lead to a New Preclinical Candidate for Visceral Leishmaniasis. *J. Med. Chem.* **2017**, *60*, 4212–4233.
- (21) Hu, Y.; Unwalla, R.; Denny, R. A.; Bikker, J.; Di, L.; Humblet, C. Development of QSAR models for microsomal stability: identification of good and bad structural features for rat, human and mouse microsomal stability. *J. Comput.-Aided Mol. Des.* **2010**, *24*, 23–35.
- (22) Cruciani, G.; Carosati, E.; De Boeck, B.; Ethirajulu, K.; Mackie, C.; Howe, T.; Vianello, R. MetaSite: understanding metabolism in human cytochromes from the perspective of the chemist. *J. Med. Chem.* **2005**, *48*, 6970–9.
- (23) Wermuth, C. G.; Aldous, D.; Raboisson, P.; Rognan, D., Eds. *The Practice of Medicinal Chemistry*; Elsevier: Amsterdam, 2015.