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Drug–nutrient interactions: discovering prescription drug inhibitors of the thiamine transporter ThTR-2 (SLC19A3)

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ABSTRACT

Background: Transporter-mediated drug–nutrient interactions have the potential to cause serious adverse events. However, unlike drug–drug interactions, these drug–nutrient interactions receive little attention during drug development. The clinical importance of drug–nutrient interactions was highlighted when a phase III clinical trial was terminated due to severe adverse events resulting from potent inhibition of thiamine transporter 2 (ThTR-2; SLC19A3).

Objective: In this study, we tested the hypothesis that therapeutic drugs inhibit the intestinal thiamine transporter ThTR-2, which may lead to thiamine deficiency.

Methods: For this exploration, we took a multifaceted approach, starting with a high-throughput in vitro primary screen to identify inhibitors, building in silico models to characterize inhibitors, and leveraging real-world data from electronic health records to begin to understand the clinical relevance of these inhibitors.

Results: Our high-throughput screen of 1360 compounds, including many clinically used drugs, identified 146 potential inhibitors at 200 μM . Inhibition kinetics were determined for 28 drugs with half-maximal inhibitory concentration (IC_{50}) values ranging from 1.03 μM to >1 mM. Several oral drugs, including metformin, were predicted to have intestinal concentrations that may result in ThTR-2–mediated drug–nutrient interactions. Complementary analysis using electronic health records suggested that thiamine laboratory values are reduced in individuals receiving prescription drugs found to significantly inhibit ThTR-2, particularly in vulnerable populations (e.g., individuals with alcoholism).

Conclusions: Our comprehensive analysis of prescription drugs suggests that several marketed drugs inhibit ThTR-2, which may contribute to thiamine deficiency, especially in at-risk populations. *Am J Clin Nutr* 2020;111:110–121.

Keywords: drug–nutrient interactions, vitamin B1, transporters, vitamin deficiency, clinical data, high-throughput screen, electronic health records, machine learning

Introduction

In 2012, a phase III clinical trial involving the development of the Janus kinase 2 (JAK2) inhibitor fedratinib was terminated when several patients developed Wernicke’s encephalopathy (WE) (1). WE is a serious, life-threatening neurologic condition which occurs as a result of vitamin B1 (thiamine) deficiency (2, 3). Following termination of the fedratinib trial, subsequent studies indicated that fedratinib potently inhibits the primary intestinal absorptive transporter for thiamine, thiamine transporter 2 (ThTR-2; SLC19A3).

This project was part of the “Drug Vitamin Interactions Mediated by the Thiamine Transporter, SLC19A3” project and supported by R01 DK108722. EAEG is supported by NIH/NIGMS T32 GM007546. MS is supported by K01LM012381.

Supplemental Tables 1–7 and Supplemental Figures 1–6 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>. BV and EAEG contributed equally to this work.

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Abbreviations used: auROC, area under receiver operating characteristic curve; DDI, drug–drug interaction; DNI, drug–nutrient interaction; EHR, electronic health record; IC_{50} , half maximal inhibitory concentration; ICD, International Classification of Diseases; prIC_{50} , predicted half-maximal inhibitory concentration; QSAR, quantitative structure activity relationship; RF, random forest; ROC, receiver operating characteristic; SDF, structure data file; SLogP, octanol–water partition coefficient; TPP, thiamine pyrophosphate; ThTR-2, thiamine transporter 2; UCSF, University of California, San Francisco; WE, Wernicke’s encephalopathy.

Received June 3, 2019. Accepted for publication September 11, 2019.

First published online November 25, 2019; doi: <https://doi.org/10.1093/ajcn/nqz255>.

Thiamine is a water-soluble vitamin that is obtained from exogenous sources and primarily from the diet (4, 5). The vitamin is absorbed in the small intestine via facilitated transport and is rapidly converted via thiamine kinases into thiamine monophosphate, thiamine pyrophosphate (TPP), and thiamine triphosphate (6). TPP accounts for approximately 80% of total thiamine stores in the human body and is the active form of the vitamin, acting as a coenzyme for various enzyme complexes (6).

Membrane transporters are known sites for drug–drug interactions (DDIs), especially in the small intestine, kidney, and liver (7–9). Transporter-mediated DDIs occur when one drug induces or inhibits a transporter, which results in a change in the influx or efflux of another drug and potentially leads to drug toxicities and adverse events (9, 10). As a result, DDIs are thoroughly investigated throughout drug development and impact drug dosing and labeling (8).

In sharp contrast, transporter-mediated drug–nutrient interactions (DNIs) had been largely ignored during drug development until the fedratinib trial. It was suggested that the toxicity observed in this clinical trial was a result of a DNI in a population vulnerable for thiamine deficiency, patients with myelofibrosis (11–15). This incident raised awareness in the drug development and regulatory communities about the potential for transporter-mediated DNIs. Although thiamine deficiency has been primarily associated with alcoholism, malnutrition, and various disease states such as infection with HIV, the catastrophic event brought to light a new mechanism for thiamine deficiency, drug-induced deficiency (3, 5, 16–18).

Using a multifaceted approach to determine the impact and clinical relevance of drug-induced thiamine deficiency beyond the fedratinib trial, we were able to do the following: 1) identify 146 inhibitors of ThTR-2, some of which were predicted to cause a DNI based on current FDA DDI guidelines, by conducting a high-throughput screen of 1360 FDA-approved compounds, 2) elucidate key descriptors of ThTR-2 inhibition by building a quantitative structure activity relationship (QSAR) model with machine learning methodology which could serve as a tool for drug discovery programs to evaluate the potential for ThTR-2 inhibition for novel compounds, and 3) identify thiamine deficiency in both a general patient population and in patients who have been diagnosed with malnutrition, alcoholism, or HIV and are taking 1 or more of the clinically relevant inhibitors identified in our prescription drug library screen using real-world data from electronic health records (EHRs).

Methods

Chemicals and reagents

Radiolabeled thiamine was purchased from American Radio-labeled Chemical Incorporations. The specific activity of the tritium-labeled thiamine hydrochloride was 20 Ci/mmol. Unlabeled chemicals were purchased from Sigma Aldrich. Fedratinib was purchased from Med Chem Express. Cell culture supplies were purchased from the cell culture facility at the University of California, San Francisco (UCSF), and Life Tech. The ThTR-2 stable HEK 293 cell line used was created by the Giacomini laboratory and described in Liang et al. (13). Cell lines were obtained from -80°C storage at the the UCSF Cell Culture Facility for the purposes of this study. The compound library, the

US Drug Collection, was purchased from Microsource Discovery Systems. The starting concentration of each drug was 10 mM in 100% DMSO.

Radiometric cell uptake screen

The ThTR-2 cell line [stably transfected HEK293 cells (13)], was maintained in DMEM supplemented with penicillin (100 U/mL), streptomycin (100 mg/mL), puromycin (5 $\mu\text{g/mL}$), and 10% FBS. Penicillin, streptomycin, and puromycin were included in the growth media and removed prior to the radiometric study. All 3 compounds were tested in our screen and did not induce more than a 10% inhibition of thiamine uptake compared to control. Cells were seeded at 70,000 cells per well and cultured on poly-D-lysine-coated 96-well plates for 24 h to reach 90% confluence. Uptake assay solutions were prepared at 2 times their final assay concentrations. The inhibitor compound solution and the substrate (thiamine) solution were combined at a 1:1 ratio to allow for throughput assay design. The inhibitor compound solution was prepared by transferring 20 μL of the US Drug Collection into 180 μL of HBSS, pH 7.4, in a 96-well plate, producing a stock solution of 1 mM. On the day of the assay, the compound stock solutions were used to make compound assay solutions at a concentration of 400 μM . The thiamine solutions were prepared at a concentration of 500 nM thiamine hydrochloride in HBSS, with 450 nM unlabeled thiamine and 50 nM tritium labeled thiamine. The plates were designed to test 80 compounds in columns 2–11. The negative control, DMSO, and the positive control, fedratinib, were assayed in alternating wells in columns 1 and 12. The results from the negative control on each plate were used to determine hits for that plate. Each compound was tested at 200 μM against a thiamine concentration of 250 nM, except for 2 of the 17 plates, for which the compounds were screened at 500 μM . The exact concentrations of substrate, inhibitor, and the positive control can be found in **Supplemental Table 1**. To initiate the assay, the cells were washed once in 80 μL of warm HBSS, and then incubated in 80 μL of the assay buffer at 37°C for 5 min. Following the incubation, the cells were washed twice with 80 μL ice-cold HBSS buffer. MicroScint-20 (Perkin Elmer) was added to the 96-well plates and sealed with an adhesive plastic cover. Following radiometric uptake assays, the plates were placed on a shaker overnight. The plates were read in a MicroBeta2 (Perkin Elmer) using the dual counting mode.

Radiometric cell uptake dose–response curves

The ThTR-2 cell line was cultured in the same manner as for the screen. Compounds selected for potency validation were assessed in dose–response curves with concentrations ranging from 2.5 nM to 200 μM at a substrate concentration of 250 nM thiamine. The compound and substrate buffers were made at 2 times their assay concentration and then combined at a 1:1 ratio to reach the final assay concentrations as in the primary screen. The compound buffer was made by serial dilutions in HBSS. The substrate buffer was made with 450 nM unlabeled thiamine and 50 nM tritium-labeled thiamine, which was subsequently diluted to reach final thiamine concentrations of 225 nM unlabeled thiamine and 25 nM tritium-labeled thiamine. Each plate contained control wells with no inhibitor which were used

for normalization in data analysis. To initiate the assay, the cells were washed once in 80 μL warm HBSS, and then incubated in 80 μL of the assay buffer at 37°C for 5 min. Following the incubation, the cells were washed twice with 80 μL ice-cold HBSS buffer. MicroScint-20 (Perkin Elmer) was added to the 96-well plates and sealed with an adhesive plastic cover. Following radiometric uptake assays, the plates were placed on a shaker overnight. The plates were read in a MicroBeta2 (Perkin Elmer) using the dual counting mode.

Data analysis for radiometric cell uptake assays

Hit calling for the primary screen was conducted and Z-prime scores were calculated within each plate using the positive and negative controls (19). The mean \pm SD of the vehicle negative control for each plate was determined and all compounds were normalized to this control. A hit threshold was set as 3 SDs below the average of the negative control respective to each plate. Compounds were classified as hits if they were below this cutoff. All the hits and nonhits from each plate were compiled together and the normalized values were plotted (with respect to plate) using ggplot. Data from half-maximal inhibitory concentration (IC_{50}) curves were exported to Excel for normalization and entered into GraphPad Prism 8.0 for graphing and nonlinear fitting.

Clinical relevance ratios

One-point thiamine inhibition values for each compound from the primary screen were used in combination to determine the predicted IC_{50} (prIC_{50}) (20) with the equation $V = V_0 / \{1 + [(I)/\text{prIC}_{50}]\}$, where V and V_0 are the activity with and without inhibitor, respectively, and I is the inhibitor concentration of 200 μM . Dosing information for each of the primary screen hit compounds was reviewed from the clinical databases IBM Micromedex and Lexicomp, and a maximal reported single dose was documented. The maximal intestinal concentration for each drug was determined by dividing the maximal dose by 250 mL (21). A ratio was calculated using the prIC_{50} and the maximal intestinal concentration.

Data preparation for the QSAR model

Compound names ($n = 1360$) were used as queries to the PubChem database (22) to retrieve compound identification numbers and structure data files (SDFs). Four compounds were removed due to failure to compute SDF files. SDF files were processed with PaDEL software (23) to compute 770 molecular descriptors for each screened compound. Molecular descriptors were filtered to remove descriptors with missing values ($n = 595$) and descriptors with zero variance ($n = 60$). To identify highly correlated features (correlation coefficient >0.95), pairwise descriptor correlations were computed and, from each highly correlated pair, 1 randomly chosen feature was removed ($n = 41$), leaving 74 molecular descriptors for 1356 compounds. Feature selection was implemented using the *caret* R package (24). Correlation-based feature selection was performed, in which a greedy heuristic is employed to pick a subset of features that are independent of each other but are correlated with a class label

(25). In vitro screening data were used to label 144 compounds as hits (label = 1) and 1212 as nonhits (label = 0).

Analysis of physicochemical properties for QSAR model

MayaChemTools package (26) was used to compute 8 physicochemical descriptors, namely, molecular weight, molecular volume, number of rotatable bonds, number of heavy atoms, number of hydrogen bond donors and acceptors, octanol–water partition coefficient (SLogP), and total polar surface area. Distributions of physicochemical properties for hit and nonhit compounds were analyzed in the R statistical package. Pairwise Student's t test was performed, using `t.test` in R, for the 8 physicochemical properties to identify those that differed significantly. Results were plotted using the `boxplot` function in R.

QSAR model development with machine learning

Four machine learning algorithms from the *caret* package in R (k-nearest neighbors, partial least squares regression discriminant analysis, support vector machine, and random forest), were employed to build binary classifiers. A double loop cross-validation (20) was used to assess the predictive power of each algorithm. First, the train function in the *caret* R package was used to fit predictive models for the 4 algorithms for 75% of the original data (training data set; $n = 1017$). Parameter tuning was done with 10-fold cross-validation as follows. The training set was divided into 10 subsets with each subset comprising the same ratio of hits ($\sim 90\%$) and nonhits ($\sim 10\%$) as the original data set. Model parameters were optimized by fitting classifiers to 9 out of 10 subsets and assessing them with the 1 out of 10 subsets. Next, the performance of each tuned model was assessed on 25% of the data (holdout set; $n = 340$) using the area under the receiver operating characteristic curve (auROC) (27) as the performance measure. Double-loop cross-validation was repeated 10 times for both full and reduced feature sets. First, classifiers were built with 74 molecular descriptors (full feature set). Second, feature reduction was performed with the `cfs` filtering algorithm in the *FSelector* R package for each of the training data sets. We identified a subset of 18 features that overlapped between different training sets to create a reduced feature set. Finally, cross-validation of models trained with these 18 features was performed. ROC curves were plotted with the *ROCR* package in R (28).

Substructure similarity search

The ChemBioServer web service (29) was used to search for compounds containing fragments similar to the 2,4-diaminopyrimidine fragment. To that end, the structure of 2,4-diaminopyrimidine in SDF format was queried against the 1356 SDF files using an online interface with default settings.

EHR data and analysis

We used the UCSF Research Data Browser to search for patients who had a reported numeric thiamine pyrophosphate laboratory (measured in whole blood by the UCSF Health Clinical Laboratories) test value <1200 nM, which gave us a total of 1433 patients and 2016 laboratory values. Thiamine laboratory

values reported as <7 nM were assigned the value of 0 nM. Only patients with 1 thiamine laboratory value were included in the analysis, which reduced our sample size to 1133 individuals.

Patients were divided into 2 groups depending on their medication use. Patients prescribed 1 or more of the orally dosed drugs that were identified as potentially clinically relevant hits in the primary screen and analysis (metformin, verapamil, amitriptyline, sertraline, amoxapine, pencillamine, quinidine, quinapril, and/or hydroxychloroquine) were grouped into the “on” drug group, which resulted in 236 patients. In addition, sulfamethoxazole-trimethoprim was an inclusion criterion for the on group only in the HIV analysis since this drug is not chronically taken among the general population but can be chronically used in HIV patients as a prophylaxis for pneumonia. Fedratinib was not included in any of the analyses since it was not an approved therapeutic agent. The 897 remaining patients (i.e., individuals who were never prescribed any of the clinical hits mentioned above) were grouped into the “off” drug group. Patients in the on group were further filtered based on their laboratory collection date relative to their first medication order start date. Thiamine laboratory values measured before the on group patient’s first medication order start date or within 30 d after their first medication order start date were excluded. A minimum of 30 d between medication start date and the thiamine laboratory value measurement was chosen since it can take a few weeks for thiamine stores to deplete. In total, 155 patients met this criterion and were in the on group.

For patient population–specific analyses, patients were further assigned to subgroups based on a diagnosis of malnutrition, alcoholism, or HIV. Malnutrition diagnosis was defined using the International Classification of Diseases (ICD) as ICD10 level 1 “endocrine, nutritional and metabolic diseases” (E00–E89) and ICD10 level 2 “malnutrition” (E40–E46). Alcoholism diagnosis was defined as ICD10 level 3 “alcohol related disorders,” “alcoholic liver disease,” “evidence of alcohol involvement determined by blood alcohol level,” and “toxic effect of alcohol.” HIV diagnosis was defined as ICD10 level 1 “certain infectious and parasitic diseases (A00–B99)” and ICD10 level 2 “human immunodeficiency virus [HIV] disease.” There were 221, 121, and 19 patients with 1 reported thiamine laboratory value and a malnutrition, alcoholism, and HIV diagnosis, respectively, without any regard to medication use. Patients which met the inclusion criteria for 2 or more diagnoses were included in both patient population-specific analyses.

We further subdivided the patient groups in each respective patient population based on their laboratory collection date relative to their first diagnosis date. Laboratory values taken any time before diagnosis were considered for patients diagnosed with malnutrition. For patients with alcoholism, laboratory values taken within 1 year of diagnosis were used. Lastly, for patients diagnosed with HIV, laboratory values taken 1 year prior to or any time after diagnosis were considered. This resulted in 45 patients with malnutrition, 76 patients with alcoholism, and 16 patients with HIV before filtering for medication use/prescriptions was performed. The total number of patients in the on and off groups after filtering based on medication order start date and prescriptions is listed in the Results section. All analyses were performed relative to only the date of initial diagnosis, laboratory value collection dates, and medication start dates; no other covariates were included in the analysis.

Welch’s 2-sample *t*-test was performed to evaluate if there was a significant difference in laboratory values when comparing both groups and ggplot was used to plot the data using R (version 3.4.0).

Results

Overview

The overview of our study design is presented in **Figure 1**. Our first goal was to perform a cell-based high-throughput screen to identify prescription drugs and other bioactive compounds that inhibit ThTR-2. Using the data from the primary screen, we validated a subset of the most potent compounds as well as investigated several drugs to assess their potential to cause clinically relevant DNIs at ThTR-2 (**Figure 1B**). Additionally, we leveraged computational methods to identify features specific to hits and nonhits from our screen. To complement our screen and model, we investigated and compared thiamine laboratory values in patient populations that are prone to thiamine deficiency. Specifically, we compared thiamine pyrophosphate plasma concentrations from patients taking 1 or more of the clinically relevant inhibitors that had been identified in our high-throughput screen with concentrations from patients who were not on any of those inhibitors. Our goal here was to explore the hypothesis that these inhibitors may exacerbate thiamine deficiency in vulnerable populations.

In vitro radiometric thiamine inhibition screen identified 146 potential thiamine inhibitors

A radiometric screen to identify marketed drugs that inhibit ThTR-2-mediated thiamine uptake was performed using tritium-labeled thiamine hydrochloride. Prior to the screen, the optimal conditions for thiamine uptake in cells overexpressing ThTR-2 were determined (i.e., duration of uptake, concentration of thiamine, and plating density of cells, see the Methods section). Most compounds were screened once in a 96-well plate using a 5-min uptake assay. To determine the inhibitor concentration to use for the screen, the single maximum doses for commonly prescribed drugs were collected and used to predict intestinal concentrations (**Supplemental Figure 1**). This informal *in silico* review guided the selection of the final drug concentration of prescription drugs and other compounds in the library (200 μ M) used in the primary screen.

The drug library used for the primary screen comprised 1360 diverse compounds with various mechanisms of action and from many therapeutic classes (**Figure 2**). Inhibitors, or hits, were defined by a decrease in thiamine uptake greater than 3 SDs from the thiamine only (no inhibitor) control (**Supplemental Figure 2**). Slight enrichment of hits was seen in certain therapeutic classes, including drugs used in the treatment of gastrointestinal and central nervous system disorders (**Figure 3**). Out of the 1360 compounds screened, 146 were determined to be ThTR-2 inhibitors (*z*-prime: 0.44–0.81) (**Supplemental Table 1**) (19).

Potency studies of top inhibition hits validate primary screen

As the primary screen was conducted with single point determinations, the top 11 compounds, based on percentage

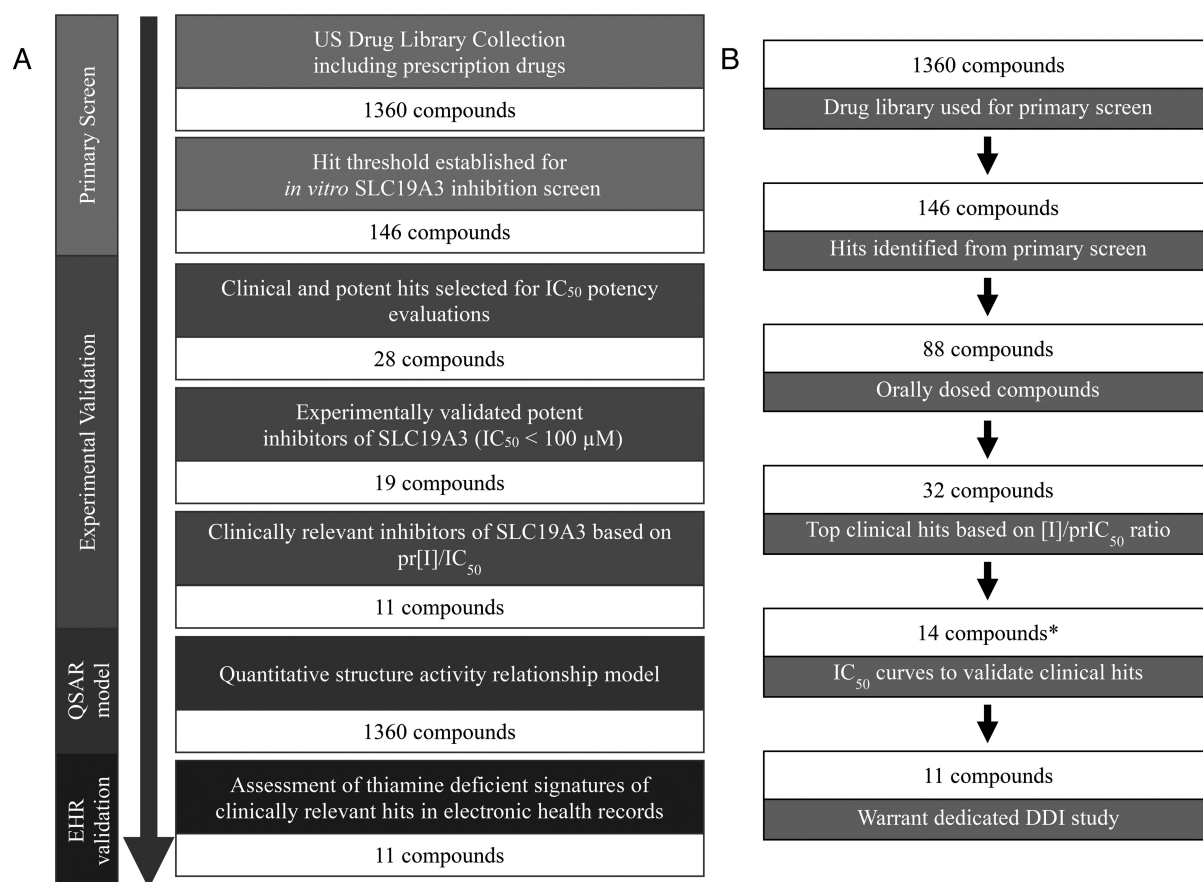


FIGURE 1 Workflow of experimental and computational methods for primary screen and identification of clinically relevant hits. Schematic of workflow used to identify, validate, and determine the potential clinical relevance of ThTR-2 inhibitors (A). Shown on the left are the 4 major stages to identify marketed drug inhibitors of ThTR-2. These major stages are accompanied by substages of each effort (on the right) with the selection criteria in the colored box and the number of compounds selected for progression to the next stage below each colored box. Detailed methods used to select compounds at each stage can be found in the Methods section. Detailed workflow used to identify and validate clinically relevant hits (B). Parallel steps were applied to the hits identified from the primary screen to determine the clinical relevance and potential to cause a drug–nutrient interaction. The number of compounds entering each step is noted above and the criteria that filtered that collection of compounds is summarized in the box below it. * 12 compounds were validated using literature derived IC₅₀ values and 2 compounds (metformin hydrochloride and trimethoprim) were validated using literature derived IC₅₀ values (12, 13). IC₅₀, concentration which causes 50% inhibition of uptake; pr[I], predicted inhibitor concentration in the small intestine following maximum single dose; ThTR-2, thiamine transporter 2.

inhibition of ³H-thiamine uptake, were selected to test in 8-point dose response curves to validate the accuracy of the primary screen. Ten of the 11 compounds were validated as hit compounds (**Supplemental Table 2**). Citric acid was determined to be a false positive. In general, the 10 validated hits have IC₅₀ values, the half-maximal inhibitory concentration, <100 μM (**Figure 4A** and **Supplemental Figure 3**). Additionally, randomly selected compounds that were identified as noninhibitor compounds were also tested and confirmed to not inhibit the transporter.

Selected clinically relevant screen hits maintain predicted potency

As screening of the top hit compounds validated the primary screen, a clinical relevance ratio, calculated using the predicted intestinal concentration following a single dose divided by the IC₅₀, was employed to identify which of the 146 hits from the primary screen would be clinically relevant. These compounds were chosen based on their clinical relevance ratios (≥10), availability in oral dosage forms, chronic dosing schedules, which

could result in prolonged inhibition of intestinal ThTR-2, and use in patient populations at-risk for thiamine deficiency. Of the 146 hits, 88 were orally administered drugs. Out of the 88 orally administered compounds, 10 compounds were selected for further validation by utilizing the additional selection criteria, noted above (**Supplemental Table 3**). Of the 10 compounds, 5 were identified as potent inhibitors of ThTR-2 with low nanomoles per liter IC₅₀ (**Figure 4B** and **Supplemental Figure 4**). Five inhibitors with predicted clinical relevance, and 3 of the other selected inhibitors, despite higher IC₅₀ values, were estimated to reach intestinal concentrations 10 times greater than their IC₅₀ values, suggesting the potential to cause a DNI at ThTR-2 (**Table 1** and **Figure 1B**).

Computational characterization of properties of hit compounds

We used several methods to characterize and differentiate properties of hit and nonhit compounds identified by high-throughput screening.

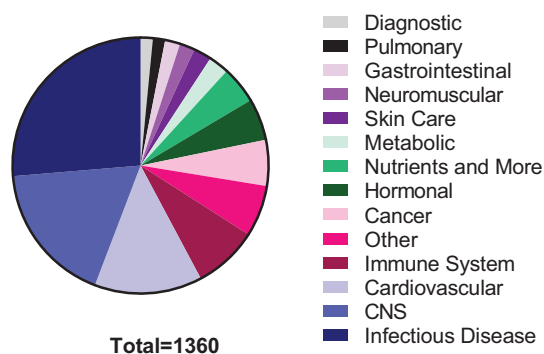


FIGURE 2 Classification of drug library by therapeutic class. The US drug collection library used for the high-throughput screen comprised a diverse set of compounds which ranged across various pharmacological classes. CNS, central nervous system.

First, we computed and analyzed 8 physicochemical properties of hits and nonhits, such as molecular weight, molecular volume, number of heavy atoms, number of rotatable bonds, number of hydrogen bond donors and acceptors, SLogP, and topological polar surface area (Table 2 and Supplemental Figure 5). No statistical differences were observed in molecular weight, volume, number of heavy atoms, or number of rotatable bonds. The values for the number of hydrogen bond donors and acceptors and total polar surface area were significantly lower among hit compared to nonhit compounds ($P < 0.0001$). Moreover, hit compounds were more lipophilic as evidenced by

significantly higher SLogP values ($P < 0.0001$) compared to those of nonhits.

Second, we developed machine learning classifiers to differentiate between hit and nonhit compounds from the high-throughput screen. The performance of 4 machine learning algorithms was estimated by means of double-loop cross-validation, consisting of internal 10-fold cross-validation and external validation using 10 test sets consisting of 25% of samples (see Methods section). We evaluated 4 machine learning algorithms, namely k-nearest neighbors, partial least squares regression discriminant analysis, support vector machines, and random forest (RF). For each algorithm, we trained each classifier with 770 and 18 molecular descriptors, respectively. The 18 descriptors were identified via a recursive feature selection method (Supplemental Table 4). These descriptors represent a common subset of features selected from different training sets during the cross-validation. The receiver operating characteristic (ROC) curves for external validation experiments of 4 algorithms are shown in Supplemental Figure 6A for 18 features and in Supplemental Figure 6B for 770 features. The RF classifier outperformed the other 3 models as assessed by the average auROC for the 10 external validation tests. Performance of all models was considered better than random. Notably, the auROC of RF classifier with 770 features was 0.71 ± 0.03 and with 18 features 0.74 ± 0.05 , respectively. The performances of the other models, though slightly worse than that of the RF model (Supplemental Figure 6A), improved with use of the 18 descriptors over the full 770 features (Supplemental Figure

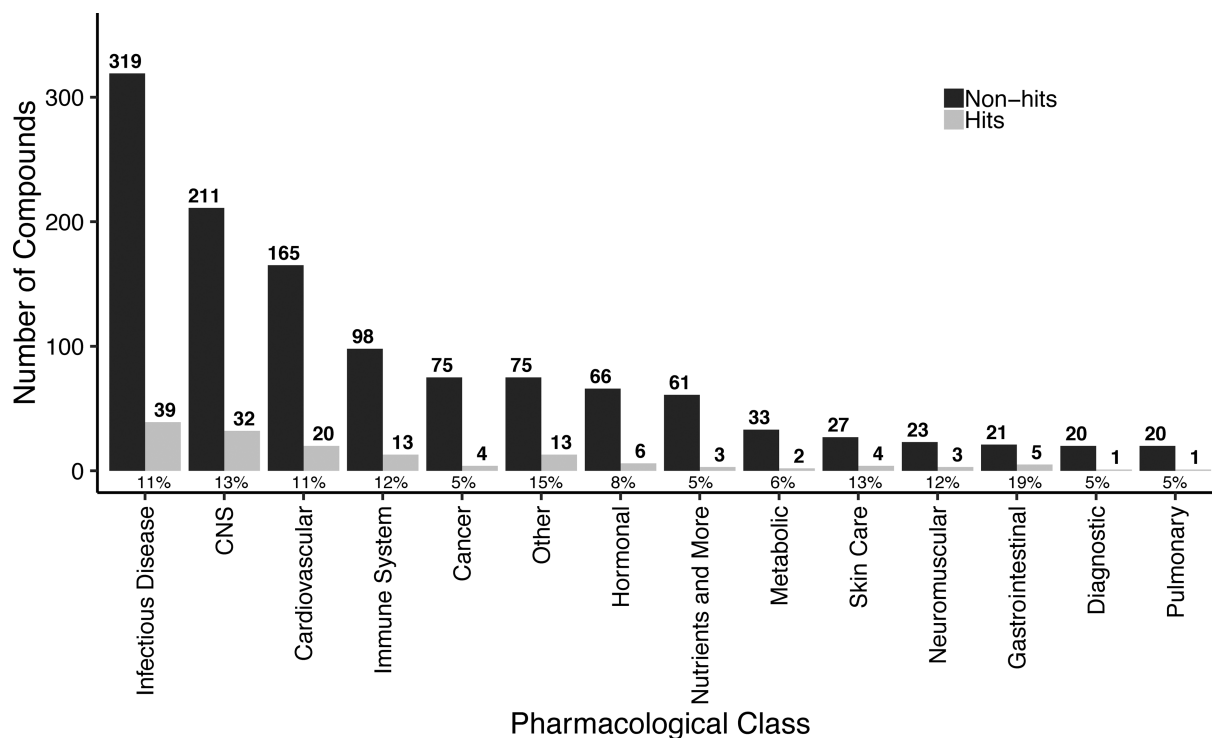


FIGURE 3 Total number of hits and nonhits in each therapeutic class. Although the diversity of the hits trended similarly to the diversity of the prescription drug library, there was enrichment of certain pharmacological classes such as drugs used in the treatment of gastrointestinal and CNS disorders. Numbers above bars represent raw count of hits and non-hits in each therapeutic class respectively. Percent represents enrichment of hits in a therapeutic class (i.e., hits which fall in a class/total number of compounds in that same class). CNS, central nervous system.

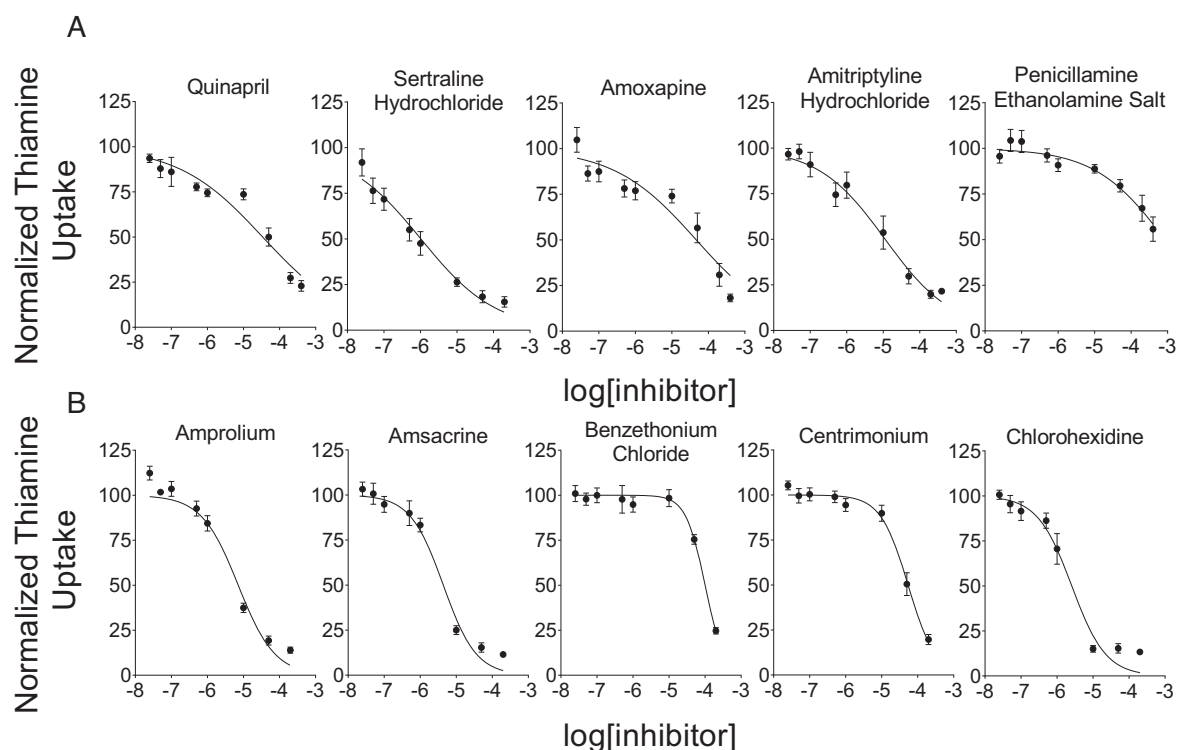


FIGURE 4 IC₅₀ curves of a subset of (A) potent and (B) clinically relevant hits. IC₅₀ values were determined for selected drugs in HEK293 cells stably overexpressing ThTR-2. Eight-point curves were conducted on 96-well plates with inhibitor concentrations ranging from 200 μM to 2.5 nM and 250 nM thiamine. Data points and error bars are presented as means ± SEMs, respectively. Each curve is representative of 3 experiments and each concentration was tested in duplicate within each experiment. IC₅₀ values range from 2.56 to 100 μM in panel A and 1.03 to 4.04 mM in panel B and are listed in detail in Supplemental Tables 2 and 3. IC₅₀, concentration which causes 50% inhibition of uptake; ThTR-2, thiamine transporter 2.

6B). Analysis of the 18 selected features revealed that the lipophilicity, descriptors of topological and chemical diversity, and descriptors of hydrogen bond counts were important for the accuracy of differentiating between hit and nonhit compounds.

More specifically, the number of nitrogen atoms and number of CrippenLogP descriptors were ranked as the 2 most important descriptors for the RF classifier (Supplemental Table 4). Four discrepancies in hit calling between screen and the RF QSAR

TABLE 1 Prescription drug inhibitors predicted to be clinically relevant based on experimental and computational methods¹

Drug name	Max single dose, mg	IC ₅₀ , μM	[Predicted Intestinal]/IC ₅₀	DDI study recommended ²
Trimethoprim ³	320	5.6 ± 0.59	793	Yes
Fedratinib	500	7.50 ± 0.883	600	Yes
Hydroxychloroquine sulfate	400	17.0 ± 5.24	217	Yes
Sertraline hydrochloride	200	1.03 ± 0.255	201	Yes
Amitriptyline hydrochloride	100	11.3 ± 2.85	116	Yes
Metformin hydrochloride ³	2500	680 ± 372	88.8	Yes
Amoxapine	300	46.6 ± 14.7	81.4	Yes
Penicillamine ethanolamine salt	2000	857 ± 372	62.6	Yes
Verapamil	480	141 ± 46	27.7	Yes
Quinidine gluconate	648	181 ± 52.8	27.5	Yes
Quinapril	80	34.0 ± 7.36	19.8	Yes
Didanosine	400	4040 ± 5740	8.29	No
Posaconazole	400	1896 ± 1767	1.80	No
Telmisartan	160	Not convergent	—	No

¹Based on the workflow detailed in Figure 1B, 14 compounds were predicted to be clinically relevant hits and selected for further validation. Hit inhibitors were defined as clinically relevant if the ratio of the compound's predicted intestinal concentration (following maximum single dose given at one time) divided by its IC₅₀ was > 10. DDI, drug–drug interaction; IC₅₀, half maximal inhibitory concentration; [Predicted Intestinal], predicted intestinal concentration.

²A subset of these compounds would be recommended for a DDI trial based on current FDA guidelines.

³Trimethoprim and metformin hydrochloride IC₅₀ values were obtained from published literature (12, 13).

TABLE 2 Physicochemical descriptors of hit and nonhit compounds identified in a screen of ThTR-2¹

Descriptor	Hits	Nonhits	<i>P</i> value
Molecular weight (Da)	330 ± 116	343 ± 194	2.24 × 10 ⁻¹
Molecular volume (Å ³)	304 ± 105	309 ± 180	6.14 × 10 ⁻¹
Heavy atoms (<i>n</i>)	23.0 ± 8.02	23.5 ± 13.3	4.51 × 10 ⁻¹
Rotatable bonds (<i>n</i>)	5.07 ± 4.35	5.05 ± 4.50	9.60 × 10 ⁻¹
Hydrogen bond donors (<i>n</i>)	1.06 ± 1.13	2.04 ± 2.42	1.04 × 10 ⁻¹⁵
Hydrogen bond acceptors (<i>n</i>)	4.32 ± 2.70	5.96 ± 4.60	1.19 × 10 ⁻⁹
SLogP	4.02 ± 1.90	2.90 ± 2.44	6.48 × 10 ⁻¹⁰
Total polar surface area (Å ²)	56.1 ± 42.2	88.5 ± 74.3	7.57 × 10 ⁻¹⁴

¹Mean ± SD values for 8 physicochemical descriptors are shown for 144 hits and 1213 nonhits. *P* < 0.005 for differences in distributions of physicochemical properties of hits compared with those of nonhits, estimated with the Student's pairwise *t*-test. SLogP, log of the octanol–water partition coefficient, including implicit hydrogens; ThTR-2, thiamine transporter 2.

model were observed (**Supplemental Tables 5 and 6**). To analyze the tradeoff between the precision and recall of the RF classifier with 18 features, we computed the average F1 measure, which was 0.58. The average precision and recall were 0.7 and 0.5, respectively. The lower recall indicates that the model is less successful at filtering out false negatives than false positives.

EHRs validate identified clinically relevant inhibitors

To investigate the clinical relevance of the inhibitors identified, we mined EHRs from UCSF and identified drug-induced decreases in thiamine laboratory values associated with the use of the drugs identified in our screen in both the general population and distinct patient populations. Specifically, we compared thiamine laboratory values in patients prescribed 1 or more of the 9 (10 for HIV patients) clinically relevant inhibitors identified in the primary screen (**Table 1**) with thiamine laboratory values in patients who were never prescribed any of the respective inhibitors. Based on the inclusion and exclusion criteria described in the methods, we were able to classify patients as “on” drug (i.e., on 1 or more of the clinically relevant inhibitors) or “off” drug.

In the general population, we observed a significant difference in thiamine laboratory values between the 2 groups (*P* = 0.02; *n* = 154 on drug; *n* = 878 off drug). If we add thiamine medication orders as an exclusion criterion, we still observed a significant difference (*P* = 0.003) in thiamine laboratory values between the on drug (*n* = 36) and off drug (*n* = 215) groups, demonstrating the robustness and sensitivity of our analysis. Population demographics, such as age and sex, as well as number of medication orders based on unique pharmaceutical class (i.e., each pharmaceutical class is counted only once per patient irrespective of number of prescriptions), were comparable between both groups (**Supplemental Table 7**).

Three distinct patient populations: malnourished, alcoholic, and HIV patients, which have been associated with thiamine deficiency (3, 5, 16–18), were used to further investigate and elucidate drug-induced decreases in thiamine laboratory values. In all 3 patient populations, patients in the on drug group had lower thiamine pyrophosphate blood concentrations than those

in the off drug group (**Figure 5**). In malnourished patients, when comparing thiamine laboratory values from samples taken any time before diagnosis, we observed lower concentrations of thiamine pyrophosphate for individuals on drug (*n* = 8) than individuals off drug (*n* = 30) at a statistically significant level (*P* = 0.015) (**Figure 5A**). In patients diagnosed with alcoholism, there were significantly lower concentrations (*P* = 0.000002) in the on than the off drug groups when we compared thiamine laboratory values taken within 1 y of diagnosis (*n* = 2 on drug; *n* = 68 off drug) (**Figure 5B**). Lastly, although we did not observe a significant difference between the 2 groups in HIV patients when including thiamine laboratory values taken within 1 y before diagnosis or any time after diagnosis (*P* = 0.20; *n* = 9 on drug; *n* = 4 off drug), we still observed lower thiamine laboratory values in on patients compared to off patients, which was consistent with our other patient populations (**Figure 5C**). If we combine all 3 patient populations with the same inclusion and exclusion criteria used in the individual analyses, we observe a statistically significant difference, as previously observed (*P* = 0.0004; *n* = 18 on drug; *n* = 96 off drug) (**Figure 5D**).

Finally, if we added thiamine medication orders (i.e., prescribed thiamine or vitamin B1 supplements and/or given thiamine intravenously) as an exclusion criterion, we still observed a significant difference between the 2 groups (on drug and off drug) in the alcoholic patient population (*P* = 0.00008 for laboratory values within 1 y of diagnosis, *n* = 2 on drug, *n* = 24 off drug) and we still observed a downward trend in malnourished and HIV patients; that is, thiamine laboratory values were lower in malnourished and HIV patients on a ThTR-2 inhibitor than in those not on a ThTR-2 inhibitor (*P* = 0.056 and *P* = 0.27 respectively).

Discussion

DDI studies are a routine and necessary component of clinical drug development. In contrast, DNI studies are rarely performed. The clinical trial of fedratinib, with the development of WE in a handful of patients, underscored the importance of DNIs and the effect of drugs on transporter-mediated nutrient absorption in clinical drug development (1, 30, 31). The current study explored the idea that DNIs mediated by intestinal ThTR-2 occur with clinically used drugs and that such interactions may contribute to thiamine deficiency, especially in vulnerable populations.

This study resulted in 4 major findings, and highlighted the potential for commonly prescribed drugs to contribute to thiamine deficiency. First, we identified many prescription drug inhibitors of ThTR-2-mediated thiamine uptake, representing a surprising fraction (approximately 10%) of the prescription drug library that was screened. Second, 4 key molecular descriptors were identified that can aid in distinguishing ThTR-2 inhibitors from noninhibitors, including increased hydrophobicity, lower polar surface area, and reduced ability to form hydrogen bonding as acceptors or donors. Third, many of the prescription drug ThTR-2 inhibitors are predicted to inhibit intestinal ThTR-2-mediated thiamine absorption at clinically relevant intestinal concentrations. Finally, a thiamine deficient signature was observed in patients diagnosed with HIV, malnutrition, and alcoholism taking 1 or more of the drugs predicted to inhibit intestinal ThTR-2-mediated thiamine uptake. The

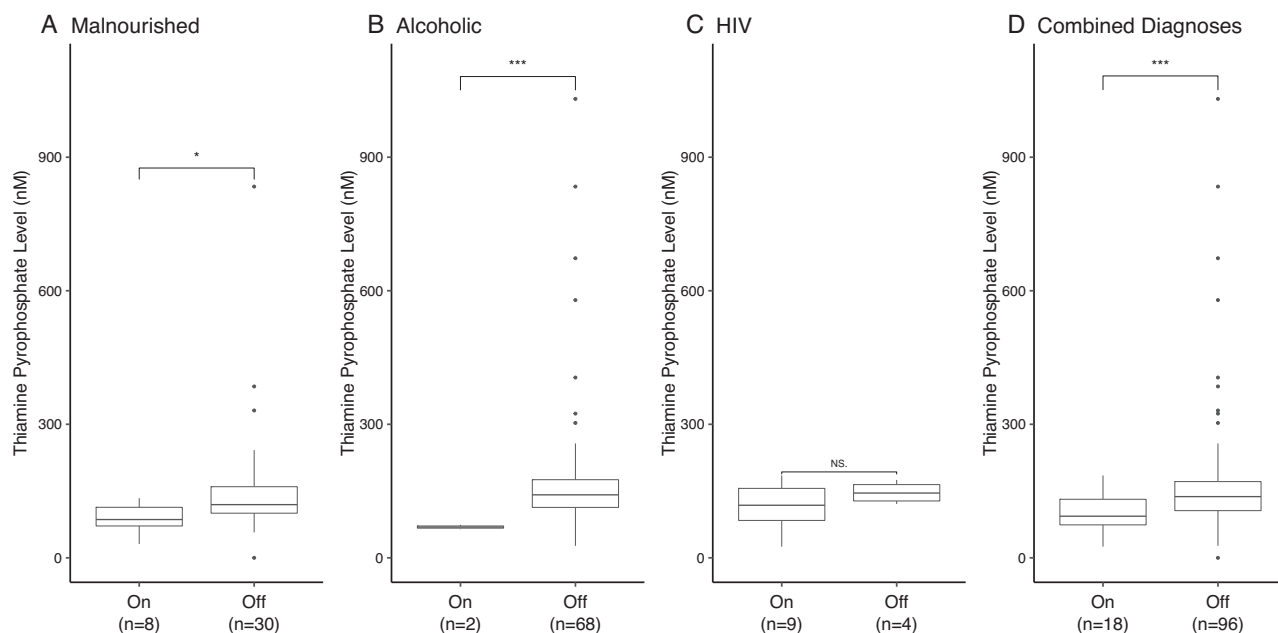


FIGURE 5 Thiamine laboratory values in patients on 1 or more clinically relevant inhibitors compared with concentrations in patients in vulnerable populations but not on a clinically relevant inhibitor. Boxplots of thiamine laboratory values in malnourished, $P = 0.015$ (A); alcoholic, $P = 0.000002$ (B); and HIV, $P = 0.20$ (C) patients, as well as all 3 patient populations combined, $P = 0.0004$ (D), comparing laboratory values of individuals on 1 or more of the clinically relevant inhibitors identified in the primary screen with patients who were not. Inhibitors are noted in Table 1 (based on the DDI recommended column). * $P \leq 0.05$, *** $P \leq 0.001$ following a Welch's 2-sample *t*-test. Sulfamethoxazole-trimethoprim was an inclusion criterion for the on group only in the HIV analysis since this drug is not chronically taken among the general population but can be chronically used in HIV patients as a prophylaxis for pneumonia. DDI, drug–drug interaction.

results suggest the potential of these drugs to cause DNIs and contribute to thiamine deficiency and WE in susceptible patient populations.

Thiamine deficiency has been repeatedly associated with a cascade of events linked to cognitive decline, many of which are commonly observed in Alzheimer disease and Parkinson disease, where thiamine has been suggested as a potential therapeutic modality (32–36). Thiamine deficiency is a treatable condition when recognized, as seen in global populations where malnutrition is a major concern (37–40). Additionally, the dire consequences of thiamine deficiency have repeatedly been observed in children of developing countries where thiamine deficiency disorders, often triggered by infectious diseases, are a major cause of infant mortality (41–46). Though severe outcomes of thiamine deficiency can lead to a clear diagnosis, mild to moderate thiamine deficiency symptoms are frequently overlooked or misdiagnosed (3, 18, 47–51). Furthermore, even in conditions known to predispose patients to thiamine deficiency, the variability in the presentation of the disorder suggests that unknown confounding factors may also contribute to the deficiency syndrome. Unfortunately, recent studies echo the complexity of recognizing thiamine deficiency and suggest that thiamine deficiency even in developed countries is underdiagnosed and undertreated (52–54). Unrecognized thiamine deficiency is a real problem as the neurologic sequelae may increase the burden on healthcare systems and reduce the general health of world populations.

This study represents 1 of the first studies to evaluate the broad potential of approved drugs to contribute to nutrient deficiency

syndromes, and to our knowledge, is 1 of the first high-throughput in vitro screens for ThTR-2. Hit compounds from our primary screen did not show enrichment for any 1 therapeutic class, suggesting that drug–thiamine interactions may occur across drug classes. The broad inhibitor specificity identified in this study is consistent with recent studies from our laboratory, which have indicated that human ThTR-2 may be more promiscuous in terms of its substrate selectivity than rodent orthologs, which narrowly transport thiamine (13).

Previous studies have indicated that ThTR-2 inhibitors share a common structural feature, a 2,4-diaminopyrimidine, specifically within the Janus kinase inhibitor class (11, 12). To assess if ThTR-2 inhibition could be caused by compounds without this structure, we utilized a diverse compound library. Preliminary computational analysis of our screen results revealed that inhibitors were significantly smaller, less polar, and more hydrophobic. Prediction of a compound's inhibitory potential based on these broad molecular descriptors alone would be difficult given the large molecular and structural diversity of our library and would likely result in many false positives. Therefore, we attempted to use machine learning and the identified descriptors to build models that may more reliably predict compounds that may inhibit the transporter. The models built with the RF algorithm performed better than random, as determined by the cross-validated auROC of about 0.7. Our RF model differs from published computational models that predict drug–food interactions and/or food bioactivities in that it aims to identify properties of molecules that inhibit ThTR-2 and may lead to thiamine deficiency (55).

The aim of this study was not only to assess the extent to which ThTR-2 may be inhibited by marketed prescription drugs but to also determine their potential to contribute to thiamine deficiency clinically. Current FDA DDI guidelines provide a ratio, previously described in the Methods sections, for which >10 indicates a dedicated healthy volunteer DDI study may be warranted (21). Eleven of the 14 selected orally prescribed drugs were predicted to reach this benchmark, by estimating the ratio of predicted intestinal concentration following maximum single dose to experimental IC_{50} , suggesting that they may result in clinically relevant inhibition of ThTR-2. Metformin was among the drugs deemed clinically relevant and mirrored previous suggestions about its ability to cause DNI at ThTR-2 (13). Additionally, some of the clinically relevant drugs identified in our study are used chronically in patients who may already be at risk for thiamine deficiency (56–64).

By examining the EHRs for patients at risk for thiamine deficiency, we were able to use real world data to support the idea that prescription drugs may contribute to thiamine deficiency, and indeed may be major risk factors for WE or beriberi in vulnerable populations. Additionally, inhibition of ThTR-2 may contribute to adverse events associated with some of the therapeutics. For example, a common adverse event associated with metformin use is lactic acidosis (65), a potentially fatal adverse event also associated with thiamine deficiency. If, as our results suggest, metformin inhibits intestinal ThTR-2–mediated thiamine transport, the resulting low concentrations of thiamine and TPP could contribute to metformin-induced lactic acidosis (18, 50, 66–68).

The limitations of this study include the high concentrations used in the initial screen, which were much higher than concentrations used in typical drug discovery screens (69–71). A high screening concentration was used to reflect the concentrations predicted to be achieved in the intestine after therapeutic doses of drugs. Another limitation of the high-throughput screen was that each compound was evaluated in a single well. This approach was taken due to the fact that no fluorescent probe was available and may have resulted in false negatives. Another limitation was the performance of the machine learning classifier, which was constrained due to the large structural diversity of the compound library, limited number of compounds (<2000), and lack of novel molecular structures currently in development. Screening a larger library with many molecules having particular structural backbones may help refine the structure–activity relationships as well as improve the prediction capability of the model. Furthermore, although we used FDA guidance as a benchmark to select compounds which have the potential to cause clinically relevant DNIs, this guidance is meant for drugs and not nutrients and for efflux transporters (and not influx transporters) that are targets for DDIs. Since inhibition of efflux transporters requires inhibitors to access the intracellular compartment, which is not required for inhibition of an influx transporter, the guidelines may have been overly stringent for our study. In addition, our EHR analysis was limited by small sample sizes. Finally, the use of publicly available data rather than a designated clinical trial allowed us to show an association between these drugs and low thiamine laboratory values but prevented us from differentiating between the role of disease and role of the drug, assessing the effect of nutritional status on thiamine laboratory values, and determining whether

these drugs inhibit intestinal ThTR-2–mediated thiamine uptake. Additional samples or designated clinical studies powered to detect drug-related differences as well as assess nutritional status are needed to make broader conclusions.

Overall, our comprehensive study was able to identify 146 inhibitors of ThTR-2, most of which were not previously known. These compounds aided in elucidating structural and chemical features of ThTR-2 inhibitors and, though further screens are needed, provided a preliminary *in silico* model for identifying compounds that inhibit ThTR-2. Compounds that may cause clinically relevant drug–nutrient interactions were predicted, and real-world data from the EHR in vulnerable patient populations were consistent with our predictions. Future work includes investigating the effects of ThTR-2 genetic variants on the plasma concentrations of both thiamine as well as prescription drugs, and conducting prospective DNI studies of prescription drugs and thiamine. This study has led to the largest available dataset of ThTR-2 inhibitors and underscores the potential importance of drug–nutrient interactions at ThTR-2 as well as other vitamin transporters.

We acknowledge Xiaomin Liang, Sook Wah Yee, Huan-Chieh Chien, John Newman, Theresa Pedersen, and Andrew Greenberg for helpful discussions and comments on the manuscript.

The authors' contributions were as follows—BV, EAEG, KMG: conceived of the study and the designed research; BV, EAEG, NK, FB: conducted research and carried out experiments; BV, EAEG, NK, FB: performed data analysis; all authors: contributed to writing and editing the manuscript; KMG: had primary responsibility for final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

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