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Molecular Similarity Methods for Predicting Cross-Reactivity With Therapeutic Drug Monitoring Immunoassays

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Abstract

Immunoassays are used for therapeutic drug monitoring (TDM) yet may suffer from cross-reacting compounds able to bind the assay antibodies in a manner similar to the target molecule. To our knowledge, there has been no investigation using computational tools to predict cross-reactivity with TDM immunoassays. The authors used molecular similarity methods to enable calculation of structural similarity for a wide range of compounds (prescription and over-the-counter medications, illicit drugs, and clinically significant metabolites) to the target molecules of TDM immunoassays. Utilizing different molecular descriptors (MDL public keys, functional class fingerprints, and pharmacophore fingerprints) and the Tanimoto similarity coefficient, the authors compared cross-reactivity data in the package inserts of immunoassays marketed for *in vitro* diagnostic use. Using MDL public keys and the Tanimoto similarity coefficient showed a strong and statistically significant separation between cross-reactive and non-cross-reactive compounds. Thus, two-dimensional shape similarity of cross-reacting molecules and the target molecules of TDM immunoassays provides a fast chemoinformatics methods for *a priori* prediction of potential of cross-reactivity that might be otherwise undetected. These methods could be used to reliably focus cross-reactivity testing on compounds with high similarity to the target molecule and limit testing of compounds with low similarity and ultimately with a very low probability of cross-reacting with the assay *in vitro*.

Keywords

drug monitoring; molecular conformations; molecular models; immunoassay; similarity

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SUPPLEMENTAL DIGITAL CONTENT 1 (Excel spreadsheet). Spreadsheet with multiple tabs containing complete cross-reactivity data sorted and color-coded by classification. Complete details on assays considered in this study are also provided. Similarity data for assays in development also included.

SUPPLEMENTAL DIGITAL CONTENT 2 (pdf file). Contains Supplemental Files S1-S4.

INTRODUCTION

Quantitation of drugs and drug metabolites for therapeutic drug monitoring (TDM) purposes frequently uses immunoassays.¹ This method is limited by interference caused by compounds with structural similarity to the hapten against which the assay antibodies were generated.² Interfering molecules can be metabolites of the parent drug, as well as structurally related molecules such as endogenous compounds, herbal drugs, or natural products. The manufacturers of commercially marketed immunoassays typically test commonly used or co-administered drugs (over-the-counter and prescription), endogenous compounds, and other compounds of interest for possible cross-reactivity.³ For example, for the immunosuppressant sirolimus (rapamycin), structurally related molecules would include sirolimus metabolites and the related drug tacrolimus. While structural similarity can be clearly defined for drugs that share a core structure (e.g., barbiturates) or substructure, similarity is less clear for drugs in a structural class not yet shared by other marketed drugs. Examples of the latter include some of the ‘newer’ anti-convulsant medications such as lamotrigine and zonisamide.

For most TDM assays, the ideal situation is very high specificity of the assay for a single compound and no reactivity with other molecules or even structurally related compounds such as metabolites. This is different from the use of immunoassays in drug of abuse/toxicology screening where an assay may be intended to detect multiple drugs and their metabolites within a class of compounds (e.g., amphetamines, barbiturates, benzodiazepines, opiates).⁴ The package inserts for marketed TDM immunoassays contain information on cross-reactivity testing that is usually generated by either of two main methods. In the first approach, the potential cross-reactive compound is tested together with a therapeutically relevant concentration of the target compound and its signal expressed as an equivalent concentration of the target drug. Alternatively, the potential cross-reactive compound is tested alone in the assay and its signal compared to that of a defined concentration of the target compound. Occasionally package inserts simply state that a compound “cross-reacts” (or a similar description) with the assay without presenting numerical data or they may cite a literature reference pertaining to cross-reactivity. For the purposes of this particular study, the package inserts are considered the ‘official’ data on TDM assay cross-reactivity, with the recognition that the manufacturer or other sources may have additional unpublished data.

While the package inserts or other manufacturers’ documents collectively contain extensive data on marketed TDM assay cross-reactivity, analysis of interfering compounds has been reported in the literature. Perhaps the most intensively analyzed TDM assay with respect to cross-reactivity is digoxin, with reports of interference by drugs, herbal products, steroid hormones, and as yet incompletely identified factors termed “digoxin-like immunoreactive substances”.⁵⁻⁸ To date there has been no comprehensive computational analysis aimed at predicting cross-reactivity of TDM assays. Our hypothesis is that a given compound is more likely to cross-react with an immunoassay if the compound shares a high level of structural similarity to the target molecule/hapten of the assay. The implication is that a structurally similar molecule can bind to the antibody in a similar manner. If a compound lacking clear structural similarity still causes cross-reactivity, this may be more likely due to interference with the assay at a step other than antibody-drug binding, or because the cross-reacting compound binds to an alternative site on the antibody (e.g., binding of the cross-reactant to another site on the antibody that can cause allosteric interactions influencing binding of the target drug).

We have utilized an *in silico* method known as similarity analysis which determines the similarity between molecules independent of any *in vitro* data.⁹⁻¹⁰ For example, at the level of structure, two molecules may be similar if they possess the same functional group(s), a common substructure, or pharmacophore. Similarity analyses have been used widely in the

pharmaceutical industry for a variety of purposes including: finding molecules with similarity to promising lead compounds, estimating physicochemical and pharmacokinetic parameters, and identifying compounds with a high likelihood of causing false positives in high-throughput activity screens.⁹⁻¹¹⁻¹⁴ Similarity algorithms have the potential advantage of being computationally fast (suitable for interrogating databases of thousands to millions of molecules) and not requiring the structure of the protein being studied.⁹⁻¹⁰

Similarity searching can compare entire molecules or substructures of a molecule at the one-dimensional, two-dimensional (2D), or three-dimensional (3D) levels.¹⁰⁻¹⁵⁻¹⁷ Common 2D similarity approaches use 2D fragment bit strings compared using the Tanimoto coefficient (0 being maximally dissimilar, 1 being maximally similar). 3D similarity methods essentially require the development of a pharmacophore that represents the arrangement of the chemical features and distances between them that are important for biological activity. The 3D pharmacophore method can be used to search a multiconformer 3D database of structures. The potential advantage of the 3D similarity approach is the ability to find structural matches that may not look similar in 2D, but possess the key features (or pharmacophore) for 3D mapping.¹⁸

A potential disadvantage of similarity based methods is not knowing *a priori* which variables are most important for the system being studied. To our knowledge, similarity analysis has not been previously applied to the prediction of cross-reactivity for immunoassays used for TDM. The present study applies similarity analyses to a broad array of commercially marketed TDM immunoassays. Our goal was to develop computational methods that could predict compounds likely to cross-react with immunoassays, ideally identifying cross-reacting compounds (including metabolites and natural products) that would otherwise have been unsuspected.

MATERIALS AND METHODS

CLASSIFICATION OF IMMUNOASSAY CROSS-REACTIVITY DATA

For TDM immunoassays, cross-reactivity data was obtained from manufacturers' package inserts. Information on the manufacturer, analyzer instruments capable of running the assays, assay methodology (including whether monoclonal or polyclonal antibodies are used), reference number, and target compounds are found in Supplemental Digital Content 1. To classify cross-reactivity of compounds for the various assays, compounds were divided into three categories: "Strong Cross-Reactives", "Weak Cross-Reactives", and "Non-Cross-Reactives" (Table 1). For any target compound, meeting the criteria for strong or weak cross-reactivity in any one assay was sufficient for classification in that category. Unpaired t-tests were used to compare average similarity between different classes.

SIMILARITY METHODS

2D Similarity searching uses the 'find similar molecules by fingerprints' protocol in the library analysis module of Discovery Studio 2.0 (Accelrys, San Diego, CA). The MDL public keys and long range functional class fingerprint description 6 keys (referred to as 'FCFP_6')¹⁹ are used separately with the Tanimoto similarity coefficient and an input query molecule representing the target compound of the assay.¹⁰ It should be noted that MDL public keys and FCFP_6 do not recognize differences between racemic mixtures and stereoisomers (e.g., omeprazole and esomeprazole).

Three-point and four-point pharmacophore-based fingerprints were calculated from the 3D conformation using the Molecular Operating Environment (MOE, Chemical Computing Group, Montreal, Canada). Each atom in a molecule is given one of eight atom types computed from three atomic properties ("in π system", "is donor", "is acceptor"). All quadruplets of

atoms are coded as features using the inter-atomic distance, atom types, and chirality of each quadruplet. The Tanimoto coefficient is used as the metric to compare the molecular fingerprints.

DATABASES

The main database searched was created using the database of Food and Drug Administration (FDA)-approved drugs derived from the Clinician's Pocket Drug Reference.²⁰⁻²² ('SCUT 2008 database') supplemented with drugs of abuse and drug metabolites ($n = 110$). The drug metabolites added to the database included those for antidepressants, barbiturates, benzodiazepines, cocaine, opiates, and phenothiazines. The total database of 786 compounds will be referred to as the 'Expanded SCUT database' containing compounds of interest to TDM immunoassay cross-reactivity. Some of the package inserts contained cross-reactivity data for compounds not likely of clinical relevance but of importance in demonstrating the specificity of the assay. For example, 3-isobutyl-1-methylxanthine interferes with some theophylline assays (Supplemental Digital Content 1) but is not a naturally occurring xanthine or a known metabolite found in humans. However, 3-isobutyl-1-methylxanthine is an internal standard for some chromatographic assays for theophylline.²³ Data for additional compounds, when present, were considered in the computational analysis for individual assays to gain insight into the relation of molecular similarity and cross-reactivity.

RESULTS

CLASSIFICATION OF CROSS-REACTIVITY DATA

The authors compiled cross-reactivity data for 96 marketed versions of 28 TDM immunoassays (acetaminophen, amikacin, caffeine, carbamazepine, cyclosporine, digoxin, disopyramide, ethosuximide, gentamicin, lamotrigine, lidocaine, methotrexate, mycophenolic acid, *N*-acetylprocainamide, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylate, sirolimus, tacrolimus, theophylline, tobramycin, topiramate, valproic acid, vancomycin, and zonisamide) using information from package inserts. Complete data for each assay (with compounds sorted and color-coded by classification) is in Supplemental Digital Content 1. Drugs that are within the top 200 most prescribed trade-name drugs and top 200 most prescribed generic drugs in the United States in 2007²⁴ are also highlighted.

Classification of all available cross-reactivity data yielded a total of 1111 datapoints – 98 Strong Cross-Reactives, 32 Weak Cross-Reactives, and 981 Non-Cross-Reactives. Two types of molecular descriptors, namely MDL public keys and FCFP₆, were then used to compute 2D similarity for the assay target molecules to compounds in the Expanded SCUT Database (and, where applicable, to any additional compounds reported in the package inserts that were not found in this database). An example of these similarity calculations for carbamazepine and five additional drugs (oxcarbazepine, carbamazepine epoxide, phenobarbital, nortriptyline, and ibuprofen) is shown in Figure 1. All of the five drugs except ibuprofen have been reported to cause greater than 5% interference with at least one marketed carbamazepine assay (Supplemental Digital Content 1). Carbamazepine has the highest similarity to oxcarbazepine (a related anti-convulsant medication) and carbamazepine epoxide (a metabolite of carbamazepine) and weaker similarity to phenobarbital and nortriptyline (another tricyclic molecule). Carbamazepine has minimal 2D similarity to ibuprofen.

SIMILARITY COMPARISONS COMBINING DATA FROM ALL TDM ASSAYS

Plotting similarity coefficients for all TDM datapoints (Figure 2) shows that all values calculated by MDL public keys are generally higher than those calculated using FCFP₆. For the dataset, this leads to the MDL public key data being spread out more evenly between 0 and 1 than the FCFP₆ data. For either method, the average similarity is significantly higher ($p <$

0.001, unpaired t-test) using either MDL public keys or FCFP_6 for Strong Cross-Reactives (MDL: 0.783 ± 0.199 ; FCFP_6: 0.484 ± 0.286) and Weak Cross-Reactives (MDL: 0.692 ± 0.246 ; FCFP_6: 0.406 ± 0.263) compared to Non-Cross-Reactives (MDL: 0.421 ± 0.170 ; FCFP_6: 0.119 ± 0.105) although there is overlap in the similarity coefficient between the three categories of compounds (Figure 2).

Using MDL public keys, nearly 60% of the Strong Cross-Reactives (58.8%) have similarity coefficients of 0.8 or higher relative to the target compound of the assay. For the Non-Cross-Reactives, only 31 of 981 (3.2%) of compounds have similar coefficients 0.8 or higher. Conversely, 48.2% of Non-Cross-Reactives have similarity coefficients of less than 0.4 to the target compounds whereas only 5.2% of Strong Cross-Reactives fit in this category.

Strong Cross-Reactive compounds with MDL public key similarity of 0.5 or less were found for only 5 TDM assays (carbamazepine, gentamicin, salicylates, tobramycin, and topiramate). For each of these 5 assays, Strong Cross-Reactives that had MDL public key similarity of 0.5 or less were reported only for a single marketed assay and not by multiple manufacturers. The topiramate assay (currently marketed versions use a single methodology) was somewhat unusual in having three compounds which qualified as Strong Cross-Reactives but had low similarity to topiramate (tiagabine, MDL: 0.297, FCFP_6: 0.036; phenytoin, MDL: 0.256, FCFP_6: 0.068; ibuprofen, MDL: 0.180, FCFP_6, 0.068).

SIMILARITY COMPARISONS WITHIN INDIVIDUAL TDM IMMUNOASSAYS

Figure 3 shows data using MDL public keys for four TDM immunoassays (cyclosporine, lamotrigine, theophylline, and valproic acid). For these four assays, all reported cross-reactive compounds are either metabolites of the target drug or closely related derivatives. All of these cross-reactive compounds have similarity coefficients relative to the target molecules that are greater than the highest similarity coefficient for a Non-Cross-Reactive compound that is not a metabolite or derivative of these four drugs (see Figure 3C,D). The highest similarity coefficient for a Non-Cross-Reactive compound that is not a metabolite or derivative of these four drugs is for cyclosporine (oxytocin, MDL public keys similarity coefficient = 0.680).

Figure 4 shows data using MDL public keys for carbamazepine, digoxin, phenobarbital, and quinidine. For these four assays, there is less clear separation between the similarity coefficients of cross-reactive compounds and Non-Cross-Reactives. The data for carbamazepine assays (Figure 4A) illustrates this best with substantial overlap in the similarity coefficients between Strong Cross-Reactives and Non-Cross-Reactives. This may relate to the similarity between the tricyclic structure of carbamazepine and other tricyclic molecules such as oxcarbazepine (a related anti-convulsant), tricyclic antidepressants (e.g., imipramine, nortriptyline), and phenothiazines (e.g., anti-psychotic medications such as thioridazine). Plots using MDL public keys for all other TDM immunoassays not shown in Figures 3 or 4 are found in Supplemental Figure S1 (part of Supplemental Digital Content 2). Plots using FCFP_6 are found in Supplemental Figure S2 (also part of Supplemental Digital Content 2).

Another way to examine the data is to analyze how the similarity for the cross-reactive compounds relates to Non-Cross-Reactives. Using MDL public keys data, the average similarity for the Non-Cross-Reactives (indicated as a dashed line in the Non-Cross-Reactives columns of all plots in Figures 3 and 4 and Supplemental Figures S1 and S2) is lower than the similarity for nearly all cross-reactive compounds. The only exceptions are the assays for carbamazepine (nortriptyline and probenecid in two marketed assays using the same methodology), gentamicin (carbenicillin in one marketed assay), quinidine (isoproterenol and hydrochlorothiazide as weak cross-reactives in one marketed assay), tobramycin (tetracycline in one marketed assay), topiramate (ibuprofen, tiagabine, and valproic acid), and valproic acid (phenobarbital as weak cross-reactive in one marketed assay). Using FCFP_6 descriptors, the

similarity for Non-Cross-Reactives overlapped more with cross-reactive compounds, with 10 of 28 assays (amikacin, carbamazepine, ethosuximide, gentamicin, quinidine, salicylates, theophylline, tobramycin, topiramate, valproic acid) having cross-reactive compounds that had similarity coefficients lower than the average similarity for all Non-Cross-Reactives. For eight of these assays, there were also Strong Cross-Reactives having FCFP_6 coefficient lower than the average similarity for Non-Cross-Reactives.

3D similarity classification approaches were also explored using three- or four-point pharmacophore fingerprints. However, even by varying cutoff settings, the three- or four-point pharmacophore algorithms were too restrictive and missed many cross-reactive compounds (e.g., the four-point pharmacophore method assigned zero similarity to some Strong Cross-reactives; see Supplemental Data Figure S3 found in Supplemental Digital Content 2).

FRAMEWORK FOR IDENTIFYING CROSS-REACTIVE COMPOUNDS

As shown in Supplemental Digital Content 1, for most TDM immunoassays, extensive cross-reactivity testing has already been performed. However, as is evident from the peer-reviewed literature, some cross-reactive compounds are also still only recognized post-marketing. Supplemental Figure S4 (found in Supplemental Digital Content 2) plots the similarity coefficients for drugs and drug metabolites in the Expanded SCUT database for which experimental cross-reactivity data has not yet been reported. These plots showed that particularly for carbamazepine, gentamicin, lamotrigine, and tobramycin assays, there are untested compounds with similarity coefficient equal or greater than the similarity coefficient for known cross-reactive compounds. Such high similarity “untested” compounds could form the basis for future studies aimed at identification of previously undetected cross-reactive compounds across marketed immunoassay platforms.

SIMILARITY-BASED PREDICTIONS FOR ASSAYS IN DEVELOPMENT

To our knowledge, there are at least six other TDM immunoassays in various stages of development for commercial marketing: digitoxin, gabapentin, levetiracetam, oxcarbazepine metabolite (10-hydroxycarbazepine), pregabalin, and tramadol. We have therefore calculated the similarity of these molecules to the compounds in the Expanded SCUT database; the results are in Supplemental Digital Content 1. As expected, digitoxin has high similarity to the related cardiac glycoside digoxin (MDL=0.980, FCFP_6=0.812) but also moderate similarity to ciclesonide (0.818, 0.172), budesonide (0.786, 0.167), cortisone (0.727, 0.156), and mycophenolic acid (0.703, 0.067), as well as several widely used statin drugs (e.g., simvastatin, 0.691, 0.168).

The highest similarities to gabapentin are to ϵ -aminocaproic acid (MDL=0.655, FCFP_6=0.379), baclofen (0.629, 0.262), pregabalin (0.559, 0.344; a therapeutically related medication), fexofenadine (0.553, 0.136), methylphenidate (0.537, 0.135), norpropoxyphene (0.512, 0.105), and γ -hydroxybutyrate (0.512, 0.423). Pregabalin's highest similarities are similar to those for gabapentin: ϵ -aminocaproic acid (MDL=0.576, FCFP_6=0.268), baclofen (0.576, 0.268), and γ -hydroxybutyrate (0.500, 0.440).

Interestingly, for the anti-convulsant drug levetiracetam, the closest similarity matches are to the angiotensin-converting enzyme inhibitor drugs such as enalapril (MDL=0.745, FCFP_6=0.197), captopril (0.712, 0.286), and lisinopril (0.709, 0.188). In terms of other anti-convulsant medications, only ethosuximide had MDL public keys similarity to levetiracetam greater than 0.5. Levetiracetam is therefore much more similar to drugs in other therapeutic classes besides the anti-convulsants.

For 10-hydroxycarbazepine (active metabolite of oxcarbazepine), the highest similarities are to oxcarbazepine (parent drug; MDL=0.837, FCFP_6=0.412), carbamazepine (0.805, 0.625), and carbamazepine epoxide (0.783, 0.447). All other compounds had MDL public keys similarity to 10-hydroxycarbazepine less than 0.6. Some widely used drugs with similarity coefficients to 10-hydroxycarbazepine between 0.5 and 0.6 include multiple benzodiazepines (e.g., lorazepam, oxazepam), nevirapine, and candesartan. Lastly, tramadol has high similarity to its main metabolite M1 (MDL=0.955, FCFP_6=0.574) but also to the anti-depressant venlafaxine (0.909, 0.344). Lesser degrees of 2D similarity to tramadol are to verapamil, propoxyphene, and fexofenadine.

DISCUSSION

Structurally related compounds present a challenge in the design and clinical use of immunoassays for drugs and drug metabolites due to their cross-reactivity.² We have therefore applied the widely used similarity analysis as a novel approach to efficiently classify compounds likely to cross-react with immunoassays used for TDM. MDL public keys were more useful than FCFP_6 and pharmacophore fingerprints as molecular descriptors for the similarity analysis. The similarity coefficients generated with the MDL analysis are well-distributed with a clear separation (on average) between cross-reactive compounds and those that do not cross-react. FCFP_6 and pharmacophore fingerprints are best suited toward identifying very close structural analogs; however, with these methods, compounds with lower degrees of similarity that may have weak cross-reactivity are not easily identified. There are many other molecular fingerprints that could be evaluated in future using the current datasets.

We have shown that using MDL public keys, cross-reactive compounds with similarity coefficients of 0.4 or less to the target compound were rare whereas many Non-Cross-Reactives fit in this category. Conversely, few True Negatives had similarity coefficients of 0.8 or above, whereas nearly 60% of the Strong Cross-Reactives fit in this category. Consequently, one screening strategy could be to test all clinically relevant compounds with similarity coefficients of 0.8 or higher to the target compound and avoid any testing of those with a coefficient of 0.4 or less. For compounds with similarity coefficients that are between 0.4 to 0.8, additional selection criteria could be selected such as frequency of co-administration with the target drug, pharmacokinetics, and therapeutic uses.

An important limitation of the similarity approaches is their inability to account for the complex 3D molecular interactions that are inherent in antibody-antigen binding. We are not aware of a published 3D structure of an antibody used in a TDM immunoassay, although there are structures of antibodies bound to drugs that may be of some relevance. For example, the X-ray crystallographic structure of digoxin with a Fab fragment revealed the carbohydrate portions of the drug to be solvent exposed and not bound by antibody.²⁶ There are also X-ray crystal structures of drugs bound to antibodies that are currently being evaluated as possible novel antidotes to drug overdose and toxicity. These include a crystal structure of morphine bound to a monoclonal antibody in which the antibody interacted with the more hydrophobic portion of the drug, while the hydrophilic half of morphine was predominantly solvent exposed.²⁷ Other drug-antibody structures show the antibody interacting with all portions of the drug molecule, e.g., antibodies complexed with cocaine^{28, 29} (additionally supported by molecular modeling studies³⁰) or phencyclidine.³¹ For those TDM immunoassays where antibody-drug interactions similar to cocaine and phencyclidine apply, whole molecule similarity measures (as applied in the current study) seem appropriate for prediction. For target compounds like morphine and digoxin where the drug molecule is large enough such that the assay antibody may interact only with a portion of the drug structure, similarity searching using molecule substructures may therefore be worth evaluating further.

It would be of interest to see if one-dimensional³² or other descriptors or methods³³⁻³⁵ can improve upon the data obtained with MDL public keys and the Tanimoto coefficient. TDM assays which have highly similar (>0.8 similarity coefficient by MDL public keys) but non-cross-reactive compounds would suggest high specificity of the antibody and those assays in this category may be of particular interest to further examine crystallographically to learn about the hapten-antigen interactions. For example, antibodies that completely interact with all of the hapten may be more specific and less liable to cross-reactivity by structurally different compounds than those antibodies that allow part of the hapten to be solvent exposed.

Overall, 2D molecular similarity assessment represents a novel approach to predict interference in TDM immunoassays by effectively setting a statistically significant difference in average similarity for cross-reactive and non-cross-reactive compounds. In turn, there is a high probability of correct predictions of cross-reactivity using similarity alone, with low levels of false positive and negative predictions. The approach as applied with the currently marketed TDM assays may be of value for prediction of cross-reactivity for assays for future novel targets, and in particular for identification of cross-reactive drugs that are outside the therapeutic class of the assay target drug. Several TDM immunoassays in development are for drugs (e.g., levetiracetam, pregabalin, tramadol) that have relatively unique chemical structures compared to other drugs on the market. Computational methods such as similarity analysis may be valuable in identifying cross-reactive compounds that might otherwise not be suspected. Our results also demonstrate that strongly cross-reactive compounds for TDM immunoassays tend to have high similarity (greater than 0.8 using MDL public keys). In some cases, metabolites of the parent drugs are the only compounds with similarities. Reference standards of drug metabolites may be difficult to obtain and thus not be tested. Similarity analysis can help identify which metabolites are most likely to cross-react and thus worthwhile to synthesize and test for cross-reactivity. Overall, computational methods could be used to better identify cross-reactive compounds so that clinicians have improved knowledge of factors that may influence TDM assay results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Moyer, TP.; Burritt, MF.; Butz, J. Therapeutic drugs and their management. In: Burtis, CA.; Ashwood, ER.; Bruns, DE., editors. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. Elsevier Saunders; St. Louis, MO: 2006. p. 1237-1285.
2. Kricka LJ. Interferences in immunoassays - still a threat. Clin Chem 2000;46:1037-1038. [PubMed: 10926879]
3. Powers, DM.; Boyd, JC.; Glick, MR. Interference testing in clinical chemistry (EP7-A). NCCLS; Villanova, PA: 1986.
4. Hammett-Stabler CA, Pesce AJ, Cannon DJ. Urine drug screening in the medical setting. Clin Chim Acta 2002;315:125-135. [PubMed: 11728415]
5. Dasgupta A, Reyes MA. Effect of Brazilian, Indian, Siberian, Asian, and North American ginseng on serum digoxin measurement by immunoassays and binding of digoxin-like immunoreactive

- components of ginseng with Fab fragment of antidigoxin antibody (Digibind). *Am J Clin Pathol* 2005;124:229–236. [PubMed: 16040294]
6. McMillin GA, Owen WE, Lambert TL, et al. Comparable effects of DIGIBIND and DigiFab in thirteen digoxin immunoassays. *Clin Chem* 2002;48:1580–1584. [PubMed: 12194938]
 7. Steimer W, Müller C, Eber B. Digoxin assays: frequent, substantial, and potentially dangerous interference by spironolactone, canrenone, and other steroids. *Clin Chem* 2002;48:507–516. [PubMed: 11861441]
 8. Wahed A, Dasgupta A. Positive and negative interference of Chinese medicine dan shen in serum digoxin measurement. Elimination of interference by monitoring free digoxin concentration. *Am J Clin Pathol* 2001;116:403–408. [PubMed: 11554169]
 9. Bender A, Glen RC. Molecular similarity: a key technique in molecular informatics. *Org Biomol Chem* 2004;2:3204–3218. [PubMed: 15534697]
 10. Willett P. Similarity-based approaches to virtual screening. *Biochem Soc Trans* 2003;31:603–606. [PubMed: 12773164]
 11. Ekins S, Mestres J, Testa B. *In silico* pharmacology for drug discovery: applications to targets and beyond. *Br J Pharmacol* 2007;152:21–37. [PubMed: 17549046]
 12. Feng BY, Simeonov A, Jadhav A, et al. A high-throughput screen for aggregation-based inhibition in a large compound library. *J Med Chem* 2007;50:2385–2390. [PubMed: 17447748]
 13. Glen RC, Bender A, Arnby CH, et al. Circular fingerprints: flexible molecular descriptors with applications from physical chemistry to ADME. *IDrugs* 2006;9:199–204. [PubMed: 16523386]
 14. Reddy AS, Pati SP, Kumar PP, et al. Virtual screening in drug discovery - a computational perspective. *Curr Protein Pept Sci* 2007;8:329–351. [PubMed: 17696867]
 15. Gillet VJ, Willett P, Bradshaw J. Identification of biological activity using substructural analysis and genetic algorithms. *J Chem Inf Comput Sci* 1998;38:165–179. [PubMed: 9538517]
 16. Hert J, Willett P, Wilton DJ, et al. Comparison of fingerprint-based methods for virtual screening using multiple bioactive reference structures. *J Chem Inf Comput Sci* 2004;44:1177–1185. [PubMed: 15154787]
 17. Hert J, Willett P, Wilton DJ, et al. Comparison of topological descriptors for similarity-based virtual screening using multiple bioactive reference structures. *Org Biomol Chem* 2004;2:3256–3266. [PubMed: 15534703]
 18. Zhang Q, Muegge I. Scaffold hopping through virtual screening using 2D and 3D similarity descriptors: ranking, voting, and consensus scoring. *J Med Chem* 2006;49:1536–1548. [PubMed: 16509572]
 19. Paolini GV, Shapland RH, van Hoorn WP, et al. Global mapping of pharmacological space. *Nat Biotechnol* 2006;24:805–815. [PubMed: 16841068]
 20. Chang C, Bahadduri PM, Polli JE, et al. Rapid identification of novel P-glycoprotein ligands. *Drug Metab Dispos* 2006;34:1976–1984. [PubMed: 16997908]
 21. Ekins S, Johnston JS, Bahadduri P, et al. In vitro and pharmacophore-based discovery of novel hPEPT1 inhibitors. *Pharm Res* 2005;22:512–517. [PubMed: 15846457]
 22. Gomella, LG.; Haist, S.; Adams, AG., et al. Clinician's pocket drug reference 2008. McGraw-Hill Companies; New York: 2008.
 23. Bailey DG, Davis HL, Johnson GE. Improved theophylline serum analysis by an appropriate internal standard for gas chromatography. *J Chromatogr* 1976;121:263–268. [PubMed: 932145]
 24. Red Book. Thomson Healthcare; Montvale, NJ: 2008.
 25. Todeschini, R.; Consonni, V. Handbook of molecular descriptors. Wiley-VCH; Weinheim: 2000.
 26. Jeffrey PD, Strong RK, Sieker LC, et al. 26-10 Fab-digoxin complex: affinity and specificity due to surface complementarity. *Proc Natl Acad Sci U S A* 1993;90:10310–10314. [PubMed: 8234291]
 27. Pozharski E, Wilson MA, Hewagama A, et al. Anchoring a cationic ligand: the structure of the Fab fragment of the anti-morphine antibody 9B1 and its complex with morphine. *J Mol Biol* 2004;337:691–697. [PubMed: 15019787]
 28. Larsen NA, Zhou B, Heine A, et al. Crystal structure of a cocaine-binding antibody. *J Mol Biol* 2001;311:9–15. [PubMed: 11469854]

29. Pozharski E, Moulin A, Hewagama A, et al. Diversity in hapten recognition: structural study of an anti-cocaine M82G2. *J Mol Biol* 2005;349:570–582. [PubMed: 15885702]
30. Paula S, Tabet MR, Farr CD, et al. Three-dimensional quantitative structure-activity relationship modeling of cocaine binding by a novel human monoclonal antibody. *J Med Chem* 2004;47:133–142. [PubMed: 14695827]
31. Lim K, Owens SM, Arnold L, et al. Crystal structure of monoclonal 6B5 Fab complexed with phencyclidine. *J Biol Chem* 1998;273:28576–28582. [PubMed: 9786848]
32. Wang N, DeLisle RK, Diller DJ. Fast small molecule similarity searching with multiple alignment profiles of molecules represented in one-dimension. *J Med Chem* 2005;48:6980–6990. [PubMed: 16250656]
33. Jennings A, Tennant M. Selection of molecules based on shape and electrostatic similarity: proof of concept of “electroforms”. *J Chem Inform Model* 2007;47:1829–1838.
34. Kortagere S, Chekmarev D, Welsh WJ, et al. New predictive models for blood-brain barrier permeability of drug-like molecules. *Pharm Res* 2008;25:1836–1845. [PubMed: 18415049]
35. Meek PJ, Liu Z, Wang CY, et al. Shape Signatures: speeding up computer aided drug discovery. *Drug Discov Today* 2006;11:895–904. [PubMed: 16997139]

FIGURE 1.

Illustration of similarity measures. Using carbamazepine as the target compound, 2D similarity was calculated using MDL public keys and FCFP₆ to five different compounds. Of the five test compounds, oxcarbazepine (a structurally similar anti-convulsant) has the highest similarity to carbamazepine, while ibuprofen has the lowest similarity.

FIGURE 2.

Plot of similarity of all data for TDM assays. As described in “Materials and Methods”, cross-reactivity data for each TDM assay were used to classify compounds in one of three categories: “Strong Cross-Reactives”, “Weak Cross-Reactives”, and “Non-Cross-Reactives”. (A) Plot of data for all compounds using MDL public keys similarity and the Tanimoto coefficient. (B) Plot of data for all compounds using FCFP₆ and the Tanimoto coefficient.

FIGURE 3.

Similarity of drugs and drug metabolites relative to the target compounds for four TDM assays. As described in the legend to Fig. 2, cross-reactivity data for four TDM assays were sorted into three categories. The similarity (using MDL public keys and the Tanimoto coefficient) of each tested compound to the target compound of each assay was plotted. (A) Cyclosporine assays. (B) Lamotrigine assays. (C) Theophylline assays. (D) Valproic acid assays.

FIGURE 4.

Similarity of drugs and drug metabolites relative to the target compounds for four additional TDM assays. As described in the legend to Fig. 2, cross-reactivity data for four TDM assays were sorted into three categories. The similarity (using MDL public keys and the Tanimoto coefficient) of each tested compound to the target compound of each assay was plotted. (A) Carbamazepine assays. (B) Digoxin assays. (C) Phenobarbital assays. (D) Quinidine assays.

Table 1

Criteria for classifying cross-reactivity of compounds in TDM immunoassays.

Classification	Definition
TDM assays	
Target compound	For a given TDM assay, the drug or drug metabolite used as the antigenic target
Strong Cross-Reactives	Compound causing 5% or greater positive interference in measurement of clinically relevant concentrations of the target compound or described as a “cross-reacting compound” (or similar language such as “cross-reacts” or “strongly cross-reacts”) in the package insert of at least one marketed assay.
Weak Cross-Reactives	Compound causing greater than 1% but less than 5% positive interference in the measurement of clinically relevant concentrations of the target compound as described in the package insert of at least one marketed assay.
Non-Cross-Reactives	Compound causing less than 1% interference in measurement of the target compound in all marketed assays considered for a particular target compound.