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Chemoinformatic Methods for Predicting Interference in Drug of Abuse/Toxicology Immunoassays

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Abstract

Background—Immunoassays used for routine drug of abuse (DOA) and toxicology screening may be limited by cross-reacting compounds able to bind to the antibodies in a manner similar to the target molecule(s). To date, there has been little systematic investigation using computational tools to predict cross-reactive compounds.

Methods—Commonly used molecular similarity methods enabled 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 DOA/toxicology screening assays. We utilized different molecular descriptors (MDL public keys, functional class fingerprints, and pharmacophore fingerprints) and the Tanimoto similarity coefficient. These data were then compared with cross-reactivity data in the package inserts of immunoassays marketed for *in vitro* diagnostic use. Previously untested compounds that were predicted to have a high probability of cross-reactivity were tested.

Results—Molecular similarity calculated using MDL public keys and the Tanimoto similarity coefficient showed a strong and statistically significant separation between cross-reactive and non-cross-reactive compounds. This was validated experimentally by discovery of additional cross-reactive compounds based on computational predictions.

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Conclusions—The computational methods employed are amenable towards rapid screening of databases of drugs, metabolites, and endogenous molecules, and may be useful for identifying cross-reactive molecules that would be otherwise unsuspected. These methods may also have value in focusing cross-reactivity testing on compounds with high similarity to the target molecule(s) and limiting testing of compounds with low similarity and very low probability of cross-reacting with the assay.

Keywords

toxicology; molecular conformations; molecular models; immunoassay; similarity; substance abuse detection

Introduction

Immunoassays are widely used for detection of drugs or drug metabolites (1). A common application is drug of abuse/toxicology (DOA/Tox) screening performed on urine or other body fluids (2). The use of immunoassays as ‘screening tests’ is distinguished from confirmation methods such as gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry, which can provide definitive identification of drugs and their metabolites. Positive DOA/Tox immunoassay screening results are often designated as “preliminary”, “presumptive”, or “unconfirmed” positives (2). One limitation of immunoassays is interference caused by compounds with structural similarity to the target molecule(s) of the assay (i.e., typically the hapten(s) against which the assay antibodies were generated) (3). Such cross-reactive molecules can be structurally related drugs, drug metabolites, or endogenous compounds. During the development of commercially marketed immunoassays, manufacturers typically test common drugs for cross-reactivity as well as endogenous compounds (4).

DOA/Tox screening assays may have broad specificity towards classes of drugs such as amphetamines, barbiturates, benzodiazepines, cannabinoids, and opiates. Other DOA/Tox screening assays are directed towards detection of a single target drug or metabolite (e.g., buprenorphine, benzoylcegonine). There are advantages to using broad specificity DOA/Tox screening assays. First, for some classes of drugs, the management of an overdose involves the same treatment regardless of which particular drug within the class is involved (e.g., flumazenil as an antidote for benzodiazepine overdose). Second, using a single assay to screen for multiple drugs within a class is less expensive than using separate assays for each individual drug.

While the package inserts or manufacturers' documents collectively contain extensive data on assay cross-reactivity, many of these compounds have been reported post-marketing over the last several decades. Examples of published reports of DOA/Tox assay cross-reactivity include fluoroquinolone antibiotic cross-reactivity with opiates assays (5,6), quetiapine cross-reactivity with tricyclic antidepressant (TCA) assays (7-9), fentanyl cross-reactivity with LSD immunoassays (10,11), sertraline and sertraline metabolite cross-reactivity with benzodiazepine assays (12,13), and venlafaxine and *O*-desmethylvenlafaxine cross-reactivity with PCP assays (14).

To date there has been no comprehensive computational analysis aimed at predicting cross-reactivity of DOA/Tox screening assays. Our hypothesis was 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(s) of the assay.

We utilize an *in silico* method known as similarity analysis which determines the similarity between molecules independent of any *in vitro* data (15-17). Similarity can be assessed at the one-dimensional, two-dimensional (2D), and three-dimensional (3D) levels (17-20). Common 2D similarity approaches use 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 pattern that represents the arrangement of the chemical features and distances between them that are important for biological activity (21).

In this study, we applied similarity analyses to a wide range of marketed immunoassays used for DOA/Tox screening. The overall goal was to develop computational methods that efficiently predict compounds likely to cross-react with immunoassays.

Materials and Methods

Standards and Reagents

Quetiapine fumarate and escitalopram oxalate were obtained from Sequoia Research Products (Pangbourne, United Kingdom). Citalopram hydrobromide was purchased from Molcan (Toronto, Canada). All other drugs were obtained from Sigma.

Classification of Immunoassay Cross-Reactivity Data

Cross-reactivity data was obtained from manufacturers' package inserts or supplemental manufacturers' documents (Data Supplement 1). Often this information is presented as the concentration of the tested compound that produces the same response in the assay as the cutoff concentration of the target compound, or occasionally as percent cross-reactivity. Additional data for cannabinoid (22), opiate (5, 6), and TCA assays (23, 24) were obtained from the peer-reviewed literature.

To classify cross-reactivity of compounds for the various assays, we broadly divided compounds into "Strong True Positives", "Weak True Positives", "Strong False Positives", "Weak False Positives", "True Negatives", and "False Negatives" (Table 1). For any compound, meeting the criteria for strong or weak cross-reactivity in any one assay was sufficient for classification in that category. Within-class compounds were formally defined as drugs and their metabolites (if present) whose detection allows for interpretation that one or more members of the target drug class are present in the sample. For example, for benzodiazepines, within-class compounds include alprazolam, chlordiazepoxide, clonazepam, diazepam, and their metabolites but not the therapeutically similar but non-benzodiazepine drugs eszopiclone and zolpidem.

Similarity Searching in Discovery Studio 2.0

Similarity searching used the 'find similar molecules by fingerprints' protocol in 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') (25) were used separately with the Tanimoto similarity coefficient and an input query molecule (17). It should be noted that these similarity algorithms do not recognize differences between stereoisomers and racemic mixtures (e.g., citalopram and escitalopram).

Pharmacophore Fingerprints

Three-point and four-point pharmacophore-based fingerprints were calculated from the 3D conformation using the Molecular Operating Environment (Chemical Computing Group, Montreal, Canada). Each atom in a molecule was given one of eight atom types computed

from three atomic properties (“in π system”, “is donor”, “is acceptor”). All quadruplets of atoms were coded as features using the inter-atomic distance, atom types, and chirality.

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 (26-28) ('SCUT 2008 database'). The database was supplemented with drugs of abuse and drug metabolites ($n = 110$) important in clinical toxicology testing. The total database of 786 compounds was referred to as the 'Expanded SCUT database'.

Cross-Reactivity Testing

Tests for cross-reactivity determined the lowest concentration of a compound added to drug-free urine that caused a reaction result that equaled or exceeded the cutoff concentration for the target compound of the assay (4). Cross-reactivity testing was performed following manufacturers' instructions using two different assay systems: (1) Emit® II plus assays (amphetamines, barbiturate, benzodiazepine, cannabinoids, cocaine metabolite, methadone, opiate, phencyclidine, propoxyphene) and Emit® tox™ serum (tricyclic antidepressants) run on Siemens (Dade-Behring) Viva-E analyzers; and (2) Biosite Triage® Tox screen.

Results

Classification of Cross-Reactivity Data

We compiled cross-reactivity data for 84 marketed versions of 18 DOA/Tox screening assays (amphetamines, barbiturates, benzodiazepines, benzoylecgonine, buprenorphine, cannabinoids, cotinine, 6-AM, LSD, MDMA, methadone, EDDP, methaqualone, opiates, oxycodone, phencyclidine, propoxyphene, and TCAs) using information from package inserts, supplemental manufacturers' data, and five peer-reviewed articles (Data Supplement 1) (5, 6, 22-24). Top prescribed medications in the United States in 2007 (29) are also highlighted. Classification of all available cross-reactivity data yielded a total of 1961 datapoints – 162 strong true positives, 20 weak true positives, 20 strong false positives, 69 weak false positives, 1681 true negatives, and 9 false negatives. We then used two algorithms, MDL public keys and FCFP_6, to compute 2D similarity for the most common target compounds for the 18 immunoassay target compounds (*d*-amphetamine, secobarbital, diazepam, buprenorphine, 9-carboxy-11-nor- Δ_9 -tetrahydrocannabinol, carisoprodol, benzoylecgonine, cotinine, EDDP, 6-acetylmorphine, LSD, methadone, methaqualone, morphine, oxycodone, phencyclidine, propoxyphene, and desipramine) to compounds in the Expanded SCUT Database and, where applicable, to any additional compounds reported in the package inserts.

Fig. 1 shows the similarity of desipramine (target compound of several TCA assays) to five compounds. Desipramine has the highest similarity (in descending order) to clomipramine (another TCA), chlorpromazine (a phenothiazine antipsychotic), and quetiapine (another tricyclic). Desipramine has low similarity to secobarbital and essentially no 2D similarity to ibuprofen.

The data analysis is complicated for some DOA/Tox screening assays in that the same target compound is not always used for all marketed versions (Data Supplement 1). This issue applies to amphetamine, benzodiazepine, and TCA assays. We chose *d*-amphetamine, diazepam, and desipramine as the target compound for amphetamine, benzodiazepine, and TCA screening assays, respectively, for the total data comparisons but also provide data using alternative target compounds (e.g., nordiazepam for benzodiazepines) in Data Supplement 2.

Similarity Comparisons Combining Data from All Assays

Plotting the similarity coefficients for all datapoints (Fig. 2) shows MDL public keys are generally higher than those calculated using FCFP₆. However, for either method, the mean similarity (mean±SD) is significantly higher ($p < 0.001$, unpaired t-test) using either MDL public keys or FCFP₆ for Strong True Positives (MDL: average similarity 0.773 ± 0.154 ; FCFP: 0.517 ± 0.254), Weak True Positives (MDL: 0.763 ± 0.145 ; FCFP: 0.507 ± 0.242), Strong False Positives (MDL: 0.574 ± 0.106 ; FCFP: 0.217 ± 0.100), or Weak False Positives (MDL: 0.478 ± 0.151 ; FCFP: 0.199 ± 0.124) compared to True Negatives (MDL: 0.362 ± 0.138 ; FCFP: 0.133 ± 0.071). Interestingly, the mean similarity for False Negatives (MDL: 0.720 ± 0.113 ; FCFP: 0.328 ± 0.142) was very close to that for True Positive using MDL public keys but in between Strong and Weak True Positives using FCFP₆ (Fig. 2).

Using MDL public keys, 45.6% of the strongly cross-reactive compounds (Strong True Positives and Strong False Positives) have similarity coefficients of 0.8 or higher relative to the target compound of the assay. For the True Negatives, only 24 of 1681 (1.4%) of compounds have similarity coefficients of 0.8 or higher to the target compounds. Thus, a cutoff of 0.8 would have a positive predictive value of 77.6% in distinguishing compounds capable of strong cross-reactivity from the True Negatives. Conversely, 65.9% of True Negatives have similarity coefficients of less than 0.4 to the target compounds whereas only 3 of 1681 (1.6%) of strongly cross-reactive compounds fit in this category. The three examples of strongly cross-reactive compounds with similarity coefficients of 0.4 or less were all from amphetamine assays (MDMA; 3,4-methylenedioxy- α -ethyl-*N*-methylphenethylamine, also known as MDDP; and 3,4-methylenedioxy-*N*-ethylamphetamine, also known as MDEA). Adopting a lower cutoff of 0.4 would have a negative predictive value of 99.8% in distinguishing compounds capable of strong cross-reactivity from the True Negatives.

Strongly cross-reactive compounds with MDL public keys similarity of 0.5 or less to the target compound were found for only 5 DOA/Tox screening assays (amphetamines, benzodiazepines, methadone, PCP, and TCAs). These compounds represented only 14 of 182 (7.6%) of the total strongly cross-reactive compounds. Ten of the 14 examples came from the benzodiazepines assays (5 compounds) and TCAs assays (5 compounds). In contrast, 1473 of 1681 (87.6%) of True Negatives had similarity coefficients of 0.5 or less.

Similarity Comparisons Within Individual Doa/Tox Screening Assays

Fig. 3 shows data using MDL public keys for four broad-specificity assays (amphetamines, barbiturates, benzodiazepines, and TCAs). These data show the challenge in identifying weakly cross-reactive compounds for broadly specific assays, in that the similarity for weakly cross-reactive compounds can overlap with that for True Negatives. Fig. 4 shows data using MDL public keys for cannabinoid, benzoylecgonine, opiate, and PCP assays. For these four assays, all cross-reactive compounds (strong or weak) have similarity coefficients to the respective targets compounds higher than the average similarity of all True Negatives. For the opiate assays (Fig. 4C), several fluoroquinolone antibiotics, identified in the literature as cross-reacting with some opiate screening assays (5,6), have similarity coefficients that overlap with those of the True Negatives but that are higher than the majority of compounds classified as True Negatives. In addition, fentanyl has relatively high similarity (MDL: 0.621, FCFP: 0.152) to LSD, consistent with studies indicating cross-reactivity of fentanyl with LSD immunoassays (10,11). Plots for DOA/Tox screening assays not shown in Figs. 3 or 4 are found in Supplemental Data Figs. 1 and 2. These plots show the same general trends for DOA/Tox screening assays shown in Figs. 3 and 4.

Another way to examine the data is to analyze how the 2D structural similarity for the cross-reactive compounds relates to that for True Negatives. Using MDL public keys data, the mean similarity for the True Negatives (indicated as a dashed line in Figs. 3 and 4 and Supplemental Data Fig. 1) is lower than the similarity for nearly all cross-reactive compounds. The only exceptions are the assays for benzodiazepines (ketoprofen, lovastatin, modafanil are weak false positives in one marketed assay each), propoxyphene (methaqualone is a weak false positive in one marketed assay), and TCAs (six compounds are weak false positives in a single marketed assay). The similarity for True Negatives overlapped more with cross-reactive compounds when using FCFP_6, with four assays (benzodiazepine, opiate, propoxyphene, and TCA) having cross-reactive compounds that had similarity coefficients lower than the average similarity for all True Negatives. Consequently, using MDL public keys or FCFP_6, Strong True Positives with similarity lower than the mean similarity coefficient for True Negatives are rare.

The 2D similarity results suggest that the targets of DOA/Tox screening immunoassays can be categorized on a continuum based on whether they have low to high similarity to other compounds that can be encountered clinically. At one extreme would be benzoylecgonine, which has high similarity to cocaine and other cocaine metabolites, but low predicted similarity to other clinically encountered drugs (Fig. 4B). This may explain why marketed benzoylecgonine screening immunoassays have very few documented cross-reacting substances. At the other extreme would be the TCA desipramine. There is substantial overlap between the similarity of desipramine to other TCAs and to other three-ringed molecules such as cyclobenzaprine, phenothiazines, or quetiapine (Fig. 3D), suggesting that cross-reactivity is likely to be a problem with TCA screening immunoassays no matter which TCA is chosen as the target.

We also explored 3D similarity classification approaches using three- or four-point pharmacophore fingerprints. However, we found that even by varying cutoff settings, these algorithms were too restrictive and missed many cross-reactive compounds, including some Strong True Positives (e.g., the four-point pharmacophore method assigned zero similarity to some Strong True Positives; Supplemental Data Fig. 3 found in Data Supplement 2).

Cross-Reactivity Testing Guided by Similarity Predictions

As evidenced in published reports, some cross-reactive compounds are recognized post-marketing. We used similarity analyses to predict additional cross-reactive compounds for ten assays: amphetamine, barbiturate, benzodiazepine, benzoylecgonine, cannabinoid, methadone, opiate, PCP, propoxyphene, and TCA (Supplemental Data Fig. 4 in Data Supplement 2). We then tested 46 such compounds on two different platforms (Biosite Triage, Syva EMIT assays on Siemens Viva-E analyzers), identifying additional cross-reactive compounds for amphetamines, barbiturates, opiates, PCP, and TCA assays (Table 2). Eight of the cross-reactivities we identified have not, to our knowledge, yet been reported in the published literature or package inserts to cross-react with any marketed version of a particular DOA/Tox immunoassay (footnote 4, Table 2). These eight new cross-reactivities were: atropine (Biosite Triage opiates assay), citalopram (Syva EMIT TCA assay), dextromethorphan (Syva EMIT opiates and propoxyphene assays), escitalopram (Syva EMIT TCA assay), mirtazapine (Syva EMIT TCA assay), oxcarbazepine (Biosite Triage barbiturates assay), and selegiline (Syva EMIT amphetamines assay). Cetirizine was previously identified as cross-reacting with fluorescence polarization immunoassays (30). Quetiapine was previously identified as cross-reacting with some marketed TCA screening immunoassays (7-9).

Similarity-Based Predictions for Assays in Development

There are at least four other drugs with potential abuse liability for which immunoassays are in development: carisoprodol, fentanyl, ketamine, and meperidine. We calculated similarity of these four drugs to the compounds in the Expanded SCUT database (Data Supplement 1). Carisoprodol showed high similarity to its active metabolite meprobamate (MDL=0.949; FCFP=0.724) but low similarity to other compounds. The next closest similarities for carisoprodol are to barbiturates (MDL~0.500-0.575; FCFP 0.2 or less). For fentanyl, the compounds with the closest similarity by MDL public keys are bupivacaine (0.712), imatinib (0.684), meperidine (0.673), buspirone (0.667), and meclizine (0.646). Ketamine has generally low similarity to other compounds but is moderately similar to several antidepressants or their metabolites (sertraline, bupropion, desmethylcitalopram).

For meperidine, a number of common drugs are close in similarity: loperamide (MDL: 0.726), fentanyl (0.696), linezolid (0.696), buspirone (0.667), ketoconazole (0.667), meclizine (0.648), risperidone (0.639), and aripiprazole (0.632). Cross-reactivity testing for a meperidine immunoassay could focus on these drugs.

Discussion

Cross-reactivity by structurally related compounds remains a challenge in the design and clinical use of DOA/Tox screening immunoassays (3). In this study we applied similarity analysis as a new tool to classify compounds that are likely to cross-react with common DOA/Tox screening tests. Using our predictions, we performed cross-reactivity testing and identified eight assay cross-reactivities not previously reported. Of the three molecular descriptors evaluated, MDL public keys were shown as the most useful for this purpose. The similarity coefficients generated by the MDL analysis are well distributed with clear separation (on average) between cross-reactive compounds and those that do not cross-react. FCFP₆ and pharmacophore fingerprints are best suited towards identifying very close structural analogs but not compounds with lower degrees of similarity. There are other molecular fingerprints that could be evaluated in the future (31).

There are several screening strategies that could be employed using the techniques we report in this study. One approach 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 between 0.4 and 0.8, additional selection criteria could be used such as pharmacokinetics and frequency of overdoses.

A limitation of the similarity approaches is that these cannot account for the complex 3D molecular interactions inherent in antibody-antigen binding. To our knowledge, a 3D structure of an antibody used in a DOA/Tox screening assay and its antigen target has not been reported, although there has been structural determination of antibodies being evaluated as novel antidotes to DOA overdose (e.g., PCP (32) and cocaine (33,34)), in which the antibody interacts with all portions of the target molecule. For DOA/Tox screening assays where similar antibody-drug interactions apply, whole molecule similarity measures (as used in this study) seem appropriate for prediction. However, a crystal structure of morphine bound to a monoclonal antibody showed the antibody interacting with the more hydrophobic portion of morphine, while the hydrophilic half was mostly exposed to solvent (35). The crystal structure of digoxin with a Fab fragment revealed the carbohydrate portions of the drug unbound by antibody and exposed to solvent (36). For target compounds like these, similarity searching using substructures may be worth evaluating.

An additional limitation of the similarity methods used in this study is that these do not account for the concentration-dependence of cross-reactivity. For instance, a substance with poor cross-reactivity may be problematic if present in serum/plasma or urine at much greater concentrations than the analyte. This is likely to especially be an issue for drugs used in low doses, resulting in low concentrations in body fluids. The synthetic opioid fentanyl would be an example of such a drug. For a drug such as fentanyl, a wider range of similarities may need to be considered in testing potentially cross-reactive substances.

Lastly, biological specimens may contain several cross-reacting substances. The situation can be quite complex for classes of drugs such as benzodiazepines that have multiple metabolites. Total cross-reactivity to a DOA/Tox immunoassay can be derived from multiple compounds, each cross-reacting to varying degrees. Future studies can be directed at predicting such total cross-reactivity by extensions of the similarity methods used in this study.

False positive DOA/Tox screening results present a challenge for clinical chemists and clinicians, as these assays are used by emergency departments, substance abuse treatment programs, transplant programs, and pain clinics, as well as other settings. Clinicians may use the results for a variety of decisions including antidote administration, prescribing of narcotic medications, and whether to proceed with or delay elective surgeries. Clinical chemists may be consulted as to the likely cause of an unexpectedly positive screening assay. The extensive similarity calculations we performed (summarized in the Data Supplement 1) highlight drugs or metabolites that have high similarity to the assay target antigen(s) but whose cross-reactivity has not been reported. These data can aid clinical chemists in determining what drugs or metabolites may contribute to assay positivity unexplained by known assay cross-reactivities, clinical history, or confirmatory testing. The similarity tools also provide a rational framework for manufacturers and regulators to focus cross-reactivity testing on drugs or metabolites most likely to cross-react.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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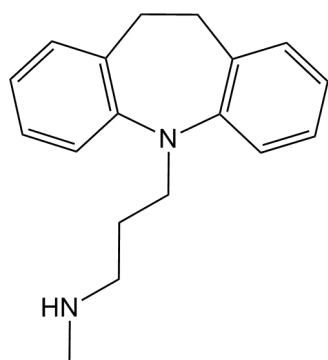
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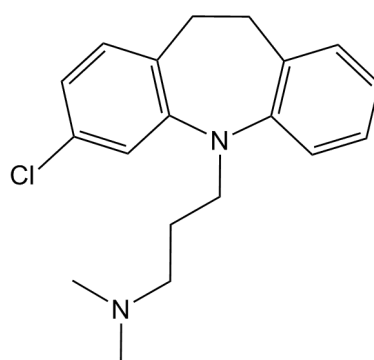
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Nonstandard abbreviations

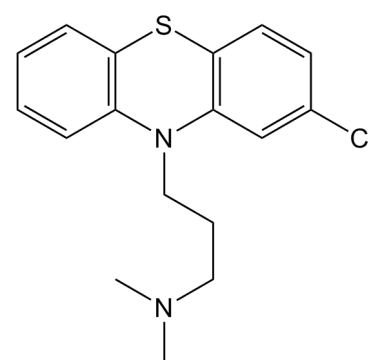
DOA	drug of abuse
Tox	toxicology
6-AM	6-acetylmorphine
LSD	lysergic acid diethylamide
MDMA	3,4-methylenedioxymethamphetamine (Ecstasy)
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
PCP	phencyclidine
TCA	tricyclic antidepressant
2D	two-dimensional
3D	three-dimensional
FCFP_6	long range functional class fingerprint description 6 keys
FDA	Food and Drug Administration
MDBD	3,4-methylenedioxy- α -ethyl- <i>N</i> -methylphenethylamine
MDEA	3,4-methylenedioxy- <i>N</i> -ethylamphetamine



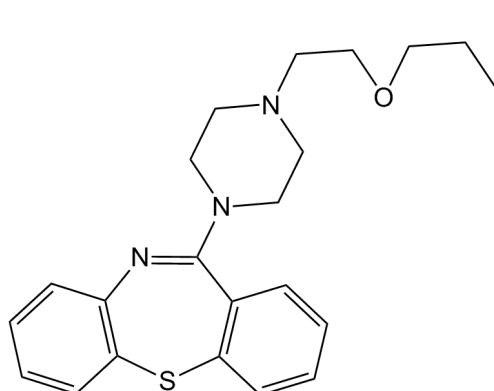
Desipramine
(target compound)



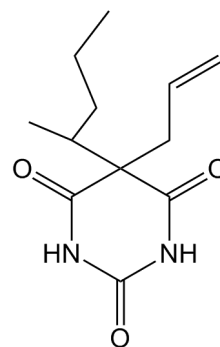
Clomipramine
MDL 0.750, FCFP 0.456



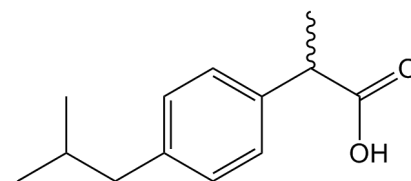
Chlorpromazine
MDL 0.630, FCFP 0.194



Quetiapine
MDL 0.485, FCFP 0.177



Secobarbital
MDL 0.286, FCFP 0.083



Ibuprofen
MDL 0.146, FCFP 0.127

Fig. 1. Illustration of similarity measures

Using desipramine (target compound of some TCA screening assays) as the target compound, 2D similarity was calculated using MDL public keys and FCFP₆ to five different compounds, three of which (clomipramine, chlorpromazine, quetiapine) are three-ringed molecules and two of which (secobarbital, ibuprofen) have a single ring in their structures. Of the five test compounds, clomipramine (a TCA like desipramine) has the highest similarity to desipramine, while ibuprofen has the lowest similarity.

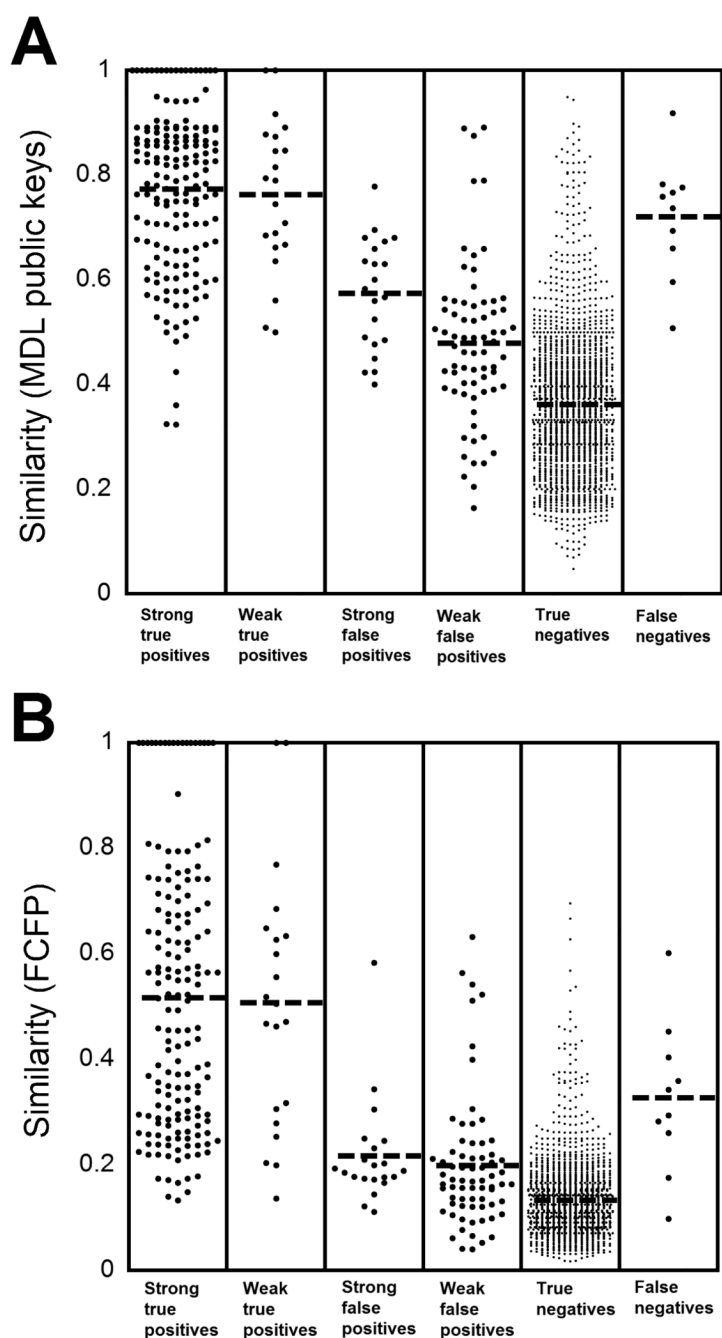


Fig. 2. Plot of similarity of all data for DOA/Tox screening assays

As described in “Materials and Methods”, cross-reactivity data for each DOA/Tox assay were used to classify compounds in one of six categories: “Strong True Positives”, “Weak True Positives”, “Strong True Positives”, “Weak True Positives”, “True Negatives”, and “False Negatives”. (A) Plot of data for all compounds using MDL public keys similarity and the Tanimoto coefficient. See text of “Results” for standard deviation values. (B) Plot of data for all compounds using FCFP₆ and the Tanimoto coefficient. See text of “Results” for standard deviation values.

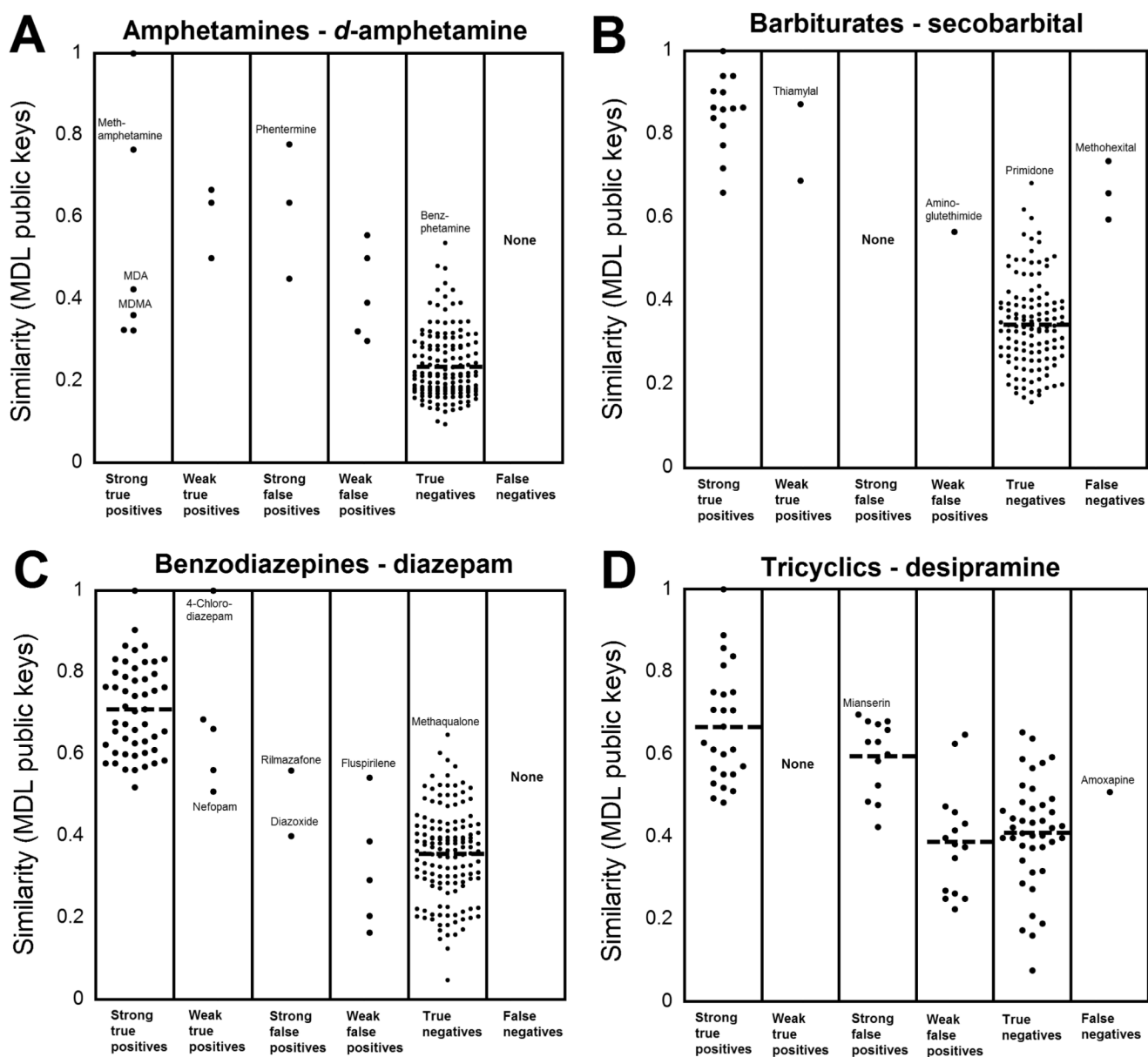


Fig. 3. Similarity of drugs and drug metabolites relative to the target compounds for four broadly specific DOA/Tox assays

As described in the legend to Fig. 2, cross-reactivity data for four DOA/Tox assays were sorted into six categories. The similarity (using MDL public keys and the Tanimoto coefficient) of each tested compound to the target compound of the DOA/Tox assay was plotted. (A) Amphetamine assays (using *d*-amphetamine as the target). (B) Barbiturate assays (using secobarbital as the target compound). (C) Benzodiazepine assays (using diazepam as the target compound). (D) TCA assays (using desipramine as the target compound).

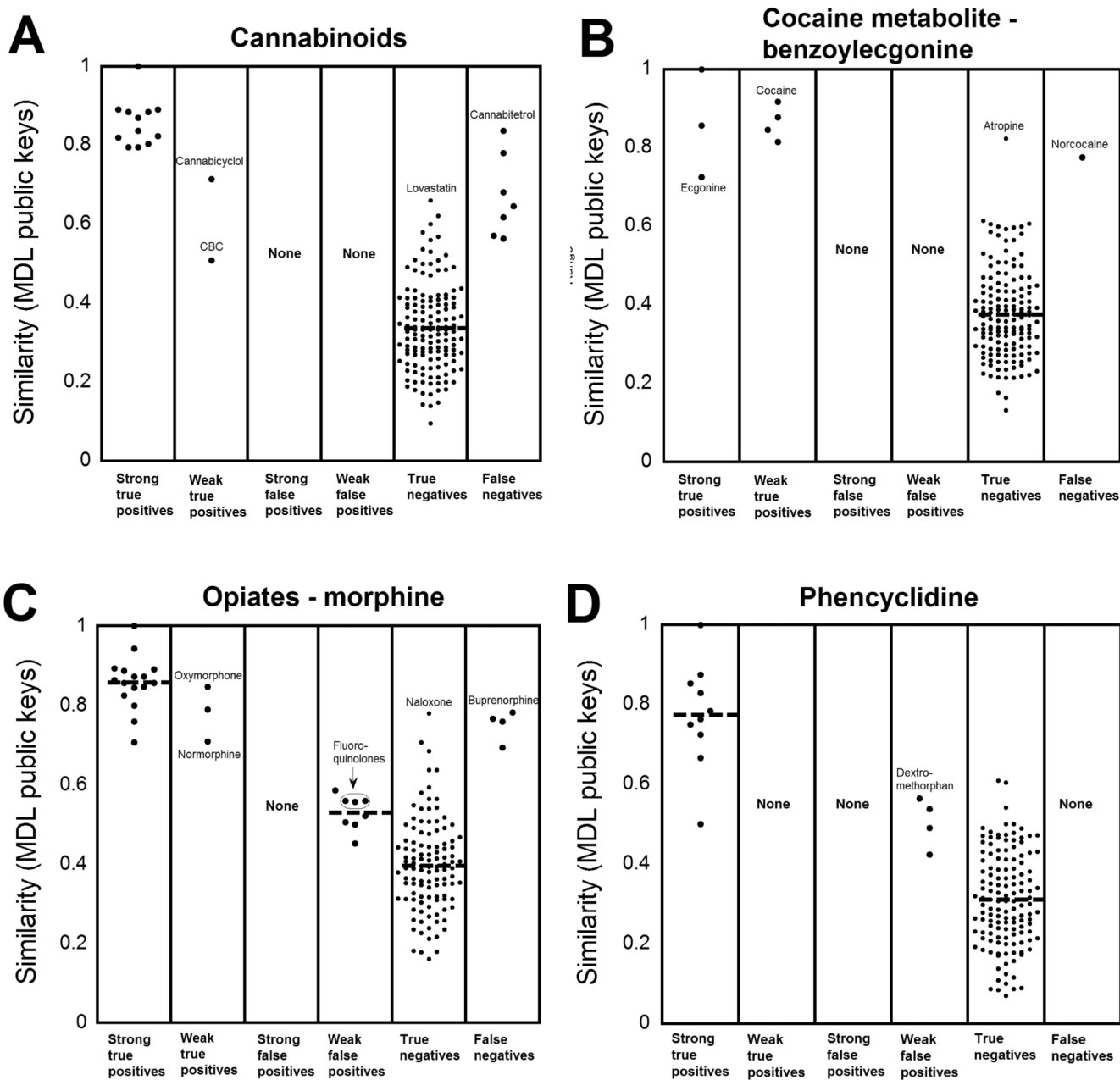


Fig. 4. Similarity of drugs and drug metabolites relative to the target compounds for four DOA/Tox assays

As described in the legend for Fig. 2, cross-reactivity data for four DOA/Tox assays were sorted into six categories. The similarity (using MDL public keys and the Tanimoto coefficient) of each tested compound to the target compound of the DOA/Tox assay of the DOA/Tox assay was plotted. (A) Cannabinoid assays (using 9-carboxy-11-nor- Δ_9 -tetrahydrocannabinol as the target compound). (B) Cocaine metabolite (benzoylecgonine) assays. (C) Opiate assays (using morphine as the target compound). (D) Phencyclidine assays.

Table 1

Criteria for classifying cross-reactivity of compounds.

Classification	Definition
DOA/Tox assays	
Target compound	For a given DOA/Tox assay, the drug or drug metabolite used as the antigenic (hapten) target (e.g., morphine for opiates assay)
Strong true positives	Within-class compounds causing cross-reactivity equal to the positive cutoff of the assay at concentrations of less than 10,000 ng/mL
Weak true positives	Within-class compounds causing cross-reactivity equal to the positive cutoff of the assay at concentrations of 10,000 to 100,000 ng/mL
Strong false positives	Out-of-class compounds causing cross-reactivity equal to the positive cutoff of the assay at concentrations of less than 10,000 ng/mL
Weak false positives	Out-of-class compounds causing cross-reactivity equal to the positive cutoff of the assay at concentrations of 10,000 to 100,000 ng/mL
True negatives	Out-of-class compounds causing no cross-reactivity or cross-reactivity equal to the positive cutoff of the assay only at concentrations of greater than 100,000 ng/mL
False negatives	Within-class compounds causing no cross-reactivity or cross-reactivity equal to the positive cutoff of the assay only at concentrations of greater than 100,000 ng/mL

Table 2

Results of cross-reactivity testing.

Compound	Closest similarity to target compounds (MDL, FCFP ₆) ^I	Cross-reactivity positives ^I
Acetaminophen	AMPH 0.310, 0.132	None
Atropine	OPI 0.581, 0.159	OPI 500,000 ng/mL ^{2,4}
Azithromycin	PROP 0.532, 0.121	None
Bupirone	BENZO 0.522, 0.088	None
Caffeine	BENZO 0.524, 0.082	None
Carbamazepine	TCA 0.460, 0.306	None
Carisoprodol	BARB 0.508, 0.184	None
Cetirizine	TCA 0.429, 0.120	TCA 250,000 ng/mL ³
Chlorambucil	TCA 0.442, 0.177	None
Chloroquine	TCA 0.686, 0.060	None
Chlorzoxazone	BARB 0.508, 0.184	None
Citalopram	PROP 0.534, 0.107	TCA 500,000 ng/mL ^{3,4}
Citalopram metabolite (<i>N</i> -desmethyl)	TCA 0.517, 0.176	None
Clotrimazole	BENZO 0.473, 0.171	None
Clozapine	BENZO 0.650, 0.174	None
Dexamethasone	CANN 0.542, 0.098	None
Dextromethorphan	PCP 0.565, 0.157	OPI 250,000 ng/mL ^{3,4} PCP 500,000 ng/mL ² 25,000 ng/mL ³ PROP 500,000 ng/mL ^{3,4}
Digoxin	CANN 0.621, 0.071	None
Escitalopram	PROP 0.534, 0.107	TCA 200,000 ng/mL ^{3,4}
5-Fluorouracil	BARB 0.625, 0.220	None
Fluoxetine	TCA 0.434, 0.288	None
Gemfibrozil	CANN 0.604, 0.188	None
Ipratropium	OPI 0.529, 0.105	None
Maprotiline	TCA 0.659, 0.250	TCA 10,000 ng/mL ³
Meprobamate	BARB 0.500, 0.190	None
Mexiletine	AMPH 0.500, 0.286	AMPH 20,000 ng/mL ³
Mirtazapine	TCA 0.653, 0.115	TCA 500,000 ng/mL ^{3,4}
Nifedipine	BARB 0.333, 0.090	None
Norclozapine	BENZO 0.581, 0.179 PCP 0.519, 0.091	None
Oxcarbazepine	BARB 0.375, 0.067	BARB 500,000 ng/mL ^{2,4}
Phenytoin	BARB 0.542, 0.190	None
Propylthiouracil	BARB 0.593, 0.312	None

Compound	Closest similarity to target compounds (MDL, FCFP_6) ¹	Cross-reactivity positives ¹
Quetiapine	TCA 0.485, 0.177	TCA 100,000 ng/mL ³
Rapamycin	BARB 0.534, 0.073	None
Reserpine	OPI 0.597, 0.108	None
Rifampicin	OPI 0.587, 0.132	None
Selegiline	AMPH 0.407, 0.333 MTD 0.556, 0.177	AMPH 40,000 ng/mL ^{3,4}
Sibutramine	MTD 0.610, 0.231	None
Simvastatin	CANN 0.604, 0.188	None
Succinylcholine	PROP 0.549, 0.123	None
Tamoxifen	MTD 0.578, 0.233 PROP 0.617, 0.235	None
Terbinafine	MTD 0.579, 0.156	None
Thioridazine	PCP 0.490, 0.171	TCA 100,000 ng/mL ³
Venlafaxine	PROP 0.696, 0.179	None
Vinblastine	OPI 0.640, 0.133	None
Warfarin	CANN 0.639, 0.198	None

¹ Abbreviations and target compounds: AMPH, amphetamines (*d*-amphetamine); BARB, barbiturates (secobarbital); BENZO, benzodiazepines (diazepam); CANN, cannabinoids (9-carboxy-11-nor- Δ 9-tetrahydrocannabinol); MTD, methadone; OPI, opiates (morphine); PCP, phencyclidine; PROP, propoxyphene; TCA, tricyclic antidepressants (desipramine)

² Biosite Triage

³ Syva Emit

⁴ Previously unreported cross-reactive drug for a DOA/Tox screening immunoassay