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Predicting new molecular targets for known drugs

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Abstract

Whereas drugs are intended to be selective, at least some bind to several physiologic targets, explaining both side effects and efficacy. As many drug-target combinations exist, it would be useful to explore possible interactions computationally. Here, we compared 3,665 FDA-approved and investigational drugs against hundreds of targets, defining each target by its ligands. Chemical similarities between drugs and ligand sets predicted thousands of unanticipated associations. Thirty were tested experimentally, including the antagonism of the β_1 receptor by the transporter inhibitor Prozac, the inhibition of the 5-HT transporter by the ion channel drug Vadilex, and antagonism of the histamine H_4 receptor by the enzyme inhibitor Rescriptor. Overall, 23 new drug-target associations were confirmed, five of which were potent (< 100 nM). The physiological relevance of

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Author contributions: B.K.S., J.J.I., and M.J.K. developed the ideas for SEA. M.J.K. wrote the SEA algorithms, undertook the calculations, and identified the off-targets reported here, typically vetted with J.J.I. and B.K.S., unless otherwise noted below. M.J.K. wrote the Naïve Bayesian classifier algorithms with assistance from J.H. With assistance from B.K.S. and J.J.I., C.L. identified off-targets for Fabahistin, K.L.H.T. identified off-targets for Prozac and Paxil, and D.D.E. identified the off-target for Rescriptor. V.S. and B.L.R. designed empirical tests of the predictions, analyzed and interpreted data, and performed experiments. T.B.T., R.W., R.C.M., A.A., N.H.J., and M.B.K. performed empirical testing of the predictions. S.J.H. and R.A.G. generated materials for the experiments. M.J.K., B.K.S., and B.L.R., the senior author, wrote the manuscript with contributions and review from B.L.R. and V.S. All authors discussed the results and commented on the manuscript.

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one such, the drug DMT on serotonergic receptors, was confirmed in a knock-out mouse. The chemical similarity approach is systematic and comprehensive, and may suggest side-effects and new indications for many drugs.

The creation of target-specific “magic bullets” has been a therapeutic goal since Ehrlich[1] and a pragmatic criterion in drug design for 30 years. Still, several lines of evidence suggest that drugs may have multiple physiologic targets.[2-5] Psychiatric medications, for instance, notoriously act through multiple molecular targets and this “polypharmacology” is likely therapeutically essential.[6] Recent kinase drugs, such as Gleevec and Sutent, though perhaps designed for specificity, modulate multiple targets and these “off-target” activities also may be essential for efficacy.[7,8] Conversely, anti-Parkinsonian drugs such as Permax and Dostinex activate not only dopamine receptors but also 5-HT_{2B} serotonin receptors, thereby causing valvular heart disease and severely restricting their use.[9]

Predicting drug polypharmacology

Drug polypharmacology has inspired efforts to predict and characterize drug-target associations.[10-15] Several groups have used phenotypic and chemical similarities among molecules to identify those with multiple targets,[16,17] and early drug candidates are screened against molecular target panels.[18] To predict new targets for established drugs, Bork and colleagues looked for side-effects shared between two molecules,[19] while Hopkins and colleagues linked targets by drugs that bind to more than one of them.[20] Indeed, using easily accessible associations, one can map 332 targets by the 290 drugs that bind to at least two of them, resulting in a network with 972 connections (Figure 1a). It seemed interesting to calculate a related map that predicts new off-target effects.

Accordingly, we used a statistics-based chemoinformatics approach to predict new off-targets for 878 purchasable FDA-approved small-molecule drugs and 2,787 pharmaceutical compounds. Unlike bioinformatics methods, which might use the sequence or structural similarity among targets, this Similarity Ensemble Approach (SEA)[21] compares targets by the similarity of the ligands that bind to them, expressed as expectation values, adapting the BLAST algorithms[21-23] (other methods such as naïve Bayesian classifiers[23,24] may also be used, see Supplementary Table 1). The approach thus captures ligand-based similarities among what would otherwise be considered disparate proteins. The 3,665 drugs were compared against 65,241 ligands organized into 246 targets drawn from the MDL Drug Data Report (MDDR) database,[25] yielding 901,590 drug-target comparisons.

Most drugs had no significant similarities to most ligand sets. However, 6,928 pairs of drugs and ligand sets were similar, with expectation values (E-values) better than 1×10^{-10}. We analyzed these predictions retrospectively against known associations and prospectively for unreported drug polypharmacology.

Retrospective drug-target predictions

We first compared the predicted drug-target associations from the MDDR database against reported associations with affinities better than 1 μM in a second database, the World of Molecular Bioactivity (WOMBAT).[26] For instance, the MDDR annotates Azopt (brinzolamide) only as an “antiglaucoma agent,” but WOMBAT reports that it binds carbonic anhydrase II at 3 nM. Correspondingly, when screened internally against all MDDR molecular targets, SEA associated this drug with “Carbonic anhydrase inhibitors” with an E-value of 8.32×10^{-139}. For 184 of the 746 drugs in WOMBAT, the predicted MDDR target agreed with the annotated WOMBAT target with E-values of 1×10^{-10} or better, recapitulating 19% of the

off-targets missing from the MDDR (Supplementary Table 2). Another 257 drug-target predictions were unannotated in either database, and may suggest new polypharmacology.

A second retrospective test predicted targets for the 3,665 drugs uncharacterized in either database but known in the literature. Of the 6,928 drug off-targets predicted, we discarded 430 as highly similar by structure to known target ligands, and another 2,666 as trivial. This left 3,832 predictions, of which we inspected 184 by literature search and by interrogating other databases. Of these, 42 turned out to be known associations (Supplementary Table 3). For instance, when we screened the drug Revanil (lisuride) against the MDDR ligand-target sets, its best E-value was as an α_2 adrenergic antagonist, and when we screened the drug Permax (pergolide) it had an E-value of 8.70×10^{-29} as a 5-HT_{1D} receptor agonist. Consistent with these predictions, Revanil has been reported to bind adrenergic α_2 at 0.055 nM and Permax the 5-HT_{1D} receptor at 13 nM (Supplementary Table 3), although neither activity was reported in the MDDR or WOMBAT databases.

New drug-target predictions

For many of these 184 predictions we found no literature precedent. We therefore tested 30 predictions that were experimentally accessible to us. In radioligand competition binding assays, 23 of these (77%) yielded binding constants (K_i's) less than 15 μM (lower K_i values indicate higher affinity) (Table 1, Table 2, Supplementary Figure 1). Fifteen of these 23 were to aminergic G-protein coupled receptors (GPCRs) (Table 1), and the remainder crossed major receptor classification boundaries (Table 2). For instance, the α_1 antagonist Doralese was predicted and observed to bind to the dopamine D_4 receptor—both α_1 and D_4 are aminergic GPCRs. Conversely, the HIV-1 reverse transcriptase (enzyme) inhibitor Rescriptor was predicted and observed to bind histamine H_4; this prediction crosses major target boundaries. For several predictions, we tested multiple receptor subtypes because the MDDR left these unspecified; e.g., for a predicted "α_1 adrenergic blocker," we tested the drug at α_{1A}, α_{1B}, and α_{1D} subtypes; we count these as a single target. In total, 14 drugs bound 23 previously unknown targets, with 13 having sub-micromolar and five having sub-100 nM affinities (Table 1, Table 2). In cases such as Doralese's, the affinity for the discovered off-target dopamine D_4, to which it binds with a K_i of 18 nM, was better than that for its known therapeutic targets, α_{1A} and α_{1B} adrenergic receptors, for which its K_i values are 611 and 226 nM, respectively (Figure 2a).[27]

How interesting and biologically relevant are these new off-targets? This can be evaluated by the following criteria: when the new targets contribute to the primary activity of the drug, when they may mediate drug side effects, or when they are unrelated by sequence, structure and function to the canonical targets. Whereas not all of the newly predicted off-targets fall into these three categories, several fall into each.

New targets as primary sites of action

The new targets can improve our understanding of drug action. *N*,*N*-dimethyltryptamine (DMT) is an endogenous metabolite and a notorious hallucinogen. Recently the molecule was characterized as a σ_1-receptor regulator at micromolar concentrations, an association implicated in its hallucinogenic properties.[28,29] This surprised us because many drugs, including non-hallucinogens, bind promiscuously to the σ_1 receptor with higher affinity than DMT.[30] Also, DMT's hallucinogenic characteristics are consistent with other hallucinogens thought to act through serotonergic receptors, some of which the molecule is known to bind.[31-33] We therefore screened DMT against the 1,133 WOMBAT targets. SEA predicted it to be similar against multiple serotonergic (5-HT) ligand sets, with expectation values ranging from 9.2×10^{-81} to 7.4×10^{-6}. Upon testing, we find DMT binds 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_6, and 5-HT_7 receptors with affinities from 39 nM to

2.1 μM (Supplementary Table 4, Supplementary Figure 2). Of these, three were previously unknown (Table 1), and all had substantially greater affinities for DMT than that represented by its 14.75 μM K_d for σ_1.[28] To further investigate the role of serotonin receptors in DMT-induced hallucination, we turned to a cell-based assay and an animal model that are predictive of hallucinatory actions.[34] Consistent with SEA prediction, we find that DMT not only is a potent partial agonist at 5-HT_{2A} (Figure 2g) as has been reported,[31] but also that it induces head twitch response in wild type but not 5-HT_{2A} knockout mice (Figure 2h), which is new to this study. The EC_{50} of DMT at 5-HT_{2A} is 100-fold lower (better) than that observed for σ_1.[28] These observations support 5-HT_{2A} as the primary target for DMT's hallucinogenic effects.

Similarly, the new off-targets for Sedalande, a neuroleptic and anxiolytic derived from haloperidol, may illuminate this drug's therapeutic effects. Although used in psychiatric clinical trials as far back as the early 1960s,[35] neither its mechanism of action in the central nervous system (CNS), nor that of the related Dimetholizine, is well understood. In addition to new activities against α_1 adrenergic receptors (1.2 nM – 240 nM, Figure 2b, Table 1), Dimetholizine was found to bind the D_2 and 5-HT_{1A} receptors and Sedalande to bind the 5-HT_{1D} receptor (Table 1, Supplementary Figure 1). This likely contributes to the CNS activity of both drugs, given the association of the former with anxiety and aggression modulation, and the activity of many antipsychotics against the D_2 receptor. We also found analogs of Sedalande that were active against 5-HT_{1D}, often at affinities comparable to or greater than those of Sedalande itself (Supplementary Table 5, Supplementary Figure 3). This supports the possibility of optimizing these drugs for new indications.

An example of a drug now being investigated for a new indication is Fabahistin. Used since the 1950s as a symptomatic antihistamine, Fabahistin is now being investigated for Alzheimer's disease. When screened against 1,133 WOMBAT targets, SEA found an extraordinary similarity to 5-HT_{5A} ligands, with an expectation value of 2.0×10^{-58}. When we measured its binding to the 5-HT_{5A} receptor, Fabahistin had a K^i of 130 nM (Figure 2c, Table 1). This is another example of a drug whose new, “off-target” affinity is much better than that for its canonical H_1 receptor target.[36] Its activity against 5-HT_{5A} and related serotonergic receptors[37] may have implications for Fabahistin's role as an Alzheimer's disease therapeutic.

Off-targets as side-effect mediators

Some of the new off-targets may contribute to a drug's adverse reactions. Motilium is an antiemetic and dopamine $D_{1/2}$ antagonist that achieves peak plasma concentrations of 2.8 μM[38] on intravenous administration. This formulation was withdrawn due to adverse cardiovascular effects, with the US FDA citing cardiac arrest, arrhythmias, and sudden death.[39] While Motilium binds the hERG channel with an IC_{50} of 5 μM,[40] the 71 - 710 nM affinities observed here against α_{1A}, α_{1B}, and α_{1D} may also contribute to these cardiovascular effects (Figure 2d, Table 1, Supplementary Figure 1).

Similarly, the micromolar activity against the β-adrenergic receptors of the widely used selective serotonin reuptake inhibitor (SSRI) antidepressants Prozac and Paxil (Figure 2e, Table 1, Supplementary Figure 1) may explain several of their adverse effects. Abrupt withdrawal of Paxil raises standing heart rate, a symptom of the SSRI discontinuation syndrome.[41] This is counterintuitive, as relieving blockade of serotonin reuptake should reduce synaptic serotonin, inconsistent with the cardiovascular syndrome.[42] β-blockade by these SSRIs may partially explain this effect since β-blockers induce a similar rebound tachycardia upon abrupt withdrawal, due to β receptor up-regulation and sensitization. Despite its higher affinity for β receptors, Prozac has a longer half-life than Paxil, and its withdrawal does not induce SSRI discontinuation syndrome. Also, both SSRIs and many β-blockers can induce sexual dysfunction.[43] Since both serotonergic and adrenergic signaling are involved in sexual

response, the binding of Paxil and Prozac to the β_1-receptor may explain why they induce greater dysfunction than other SSRIs.

Drug binding across major protein boundaries

Whereas many of the predicted off-targets occur among aminergic GPCRs, a target class for which cross-activity is well-known (see below),[44] four of the drugs bound to targets unrelated by sequence or structure to their canonical targets (Table 2). For instance, the reverse transcriptase (enzyme) inhibitor Rescriptor was predicted and shown to bind to the histamine H_4 receptor, a GPCR. These two targets share no evolutionary history, functional role, or structural similarity whatsoever. Intriguingly, while Rescriptor's K_i for the H_4 receptor is high at 5.3 μM (Table 2, Supplementary Figure 1), this is within its steady-state plasma concentration (C_{min} averages 15 μM) and is consistent with the painful rashes associated with Rescriptor use;[45] likewise, H_4 dysregulation has been associated with atopic dermatitis.[46] Similarly, the vesicular monoamine transporter (VMAT) inhibitor[47] Xenazine binds two different GPCRs at sub-micromolar concentrations (Table 2, Supplementary Figure 1). Despite its use over the last 50 years, Xenazine has not been reported to bind any GPCR. Finally, the selective ion channel inhibitors Vadilex and RO-25-6981 were predicted and found to bind to GPCRs and to transporters to which they were previously unknown to bind (Figure 2f, Table 2, Supplementary Figure 1). Whereas these ion channel drugs have known polypharmacology (Figure 3), a key point is that the new targets for these four drugs are unrelated to their main therapeutic targets except in the similarity of the ligands that modulate their activities.

More broadly, the protein target with highest sequence similarity to any of a drug's known targets is rarely predicted by the SEA approach. Rather, the target predicted by ligand similarity is typically well down in the sequence similarity ranking. Thus for Xenazine, the off-target α_2 adrenergic receptor is 78th most similar to the known target VMAT2 and in fact has no significant similarity at all, with a PSI-BLAST E-value of 125 (Supplementary Table 6), while for Rescriptor, H_4 is the 167th most similar receptor to HIV-1 RT, and even for Prantal, the aminergic δ-opioid receptor is only 26th most similar to its known muscarinic M_3 target.

Certain caveats merit mention. Not all of the new off-targets predicted here would surprise specialists. For instance, Dimetholizine has antihypertensive activity and so its affinity for adrenergic receptors is not wholly unanticipated. Similarly, Kalgut is classified as a "selective β_1 agonist," thought to have little activity on other adrenergic receptors.[48] Whereas the observation that it does bind to the β_3 receptor goes against this classification, structurally this seems easy to credit (Table 1, Supplementary Figure 1). Indeed, ten of the fourteen drugs reported here are active against aminergic GPCRs (Figure 3), and so their cross-activities against other aminergic GPCRs has some precedent.[44] Finally, whereas most of the drugs were active at their predicted off-targets, a third were not; these are examples of the false-positives to which this method is susceptible (Supplementary Table 7). Thus, the anxiolytics Valium and Centrax scored well against Cholecystokinin B ligands, the antipsychotic Emilace was predicted to bind 5-HT_4, the anaesthetic Duocaine the κ-opioid receptor, the antihypertensive Doralese neurokinin receptors, and the narcotic Dromoran and the bradycardic Zatebradine scored well against the D_2 and D_1 receptors. None of these bound their predicted off-targets with affinities better than 10 μM. SEA ignores pharmacophores in its predictions, comparing drugs to ligand sets based on all shared chemical patterns. This is at once a strength, in that it is model-free, and a weakness, in that it may predict activity for drugs that share many features with the ligands of a target, and yet miss a critical chemotype.

Predicting polypharmacology on a large scale

Notwithstanding these caveats, it is the model-free nature of these predictions that allows a comprehensive exploration of drug-target interactions, most of which remain unexplored. We

have focused on a thin slice of pharmacological targets, one dominated by aminergic drugs (Figure 3). Stepping back to view the larger space, 364 additional off-targets for 158 drugs are predicted with E-values better than 1×10^{-50}, while 1,853 new off-targets are predicted with E-values better than 1×10^{-10} (Figure 1b). This compares to the only 972 off-target activities already annotated in the databases (Figure 1a). The Similarity Ensemble Approach and related chemoinformatics methods[16-20] provide tools to explore these associations systematically, both to understand drug effects and explore new opportunities for therapeutic intervention.

Methods Summary

Prediction of off-targets

A collection of 3,665 FDA-approved and investigational drug structures was computationally screened against a panel of over 1,400 protein targets. The drug collection was extracted from the MDL Comprehensive Medicinal Chemistry database. Each target was represented solely by its set of known ligands, which were extracted from three sources of annotated molecules: the MDL Drug Data Report, the World of Molecular Bioactivity (WOMBAT),[26] and the StARlite databases. The 2D structural similarity of each drug to each target's ligand set was quantified as an expectation value (E-value) using the Similarity Ensemble Approach (SEA).[21]

Experimental testing

Predicted “off-targets” with strong SEA E-values were evaluated for novelty against orthogonal databases and the literature. Those off-targets without precedent were subjected to radioligand competition binding assays using standard techniques[49] at the NIMH Psychoactive Drug Screening Program. The role of 5-HT_{2A} agonism in DMT-induced hallucination was examined in cell-based and in knock-out mouse models.[34] Derivatives of Sedalande were identified in the ZINC[50] database by substructure search, and their affinities for 5-HT_{1D} tested using standard techniques.[49]

Drug-target networks and out-group analysis

Comprehensive networks of known drug-target associations (by WOMBAT) and predicted off-targets (by SEA) were constructed. Additionally, SEA off-target predictions were compared to those derived from naïve Bayesian classifiers and from PSI-BLAST[21-23] comparisons of a drug's known protein target(s) against the panel of potential protein targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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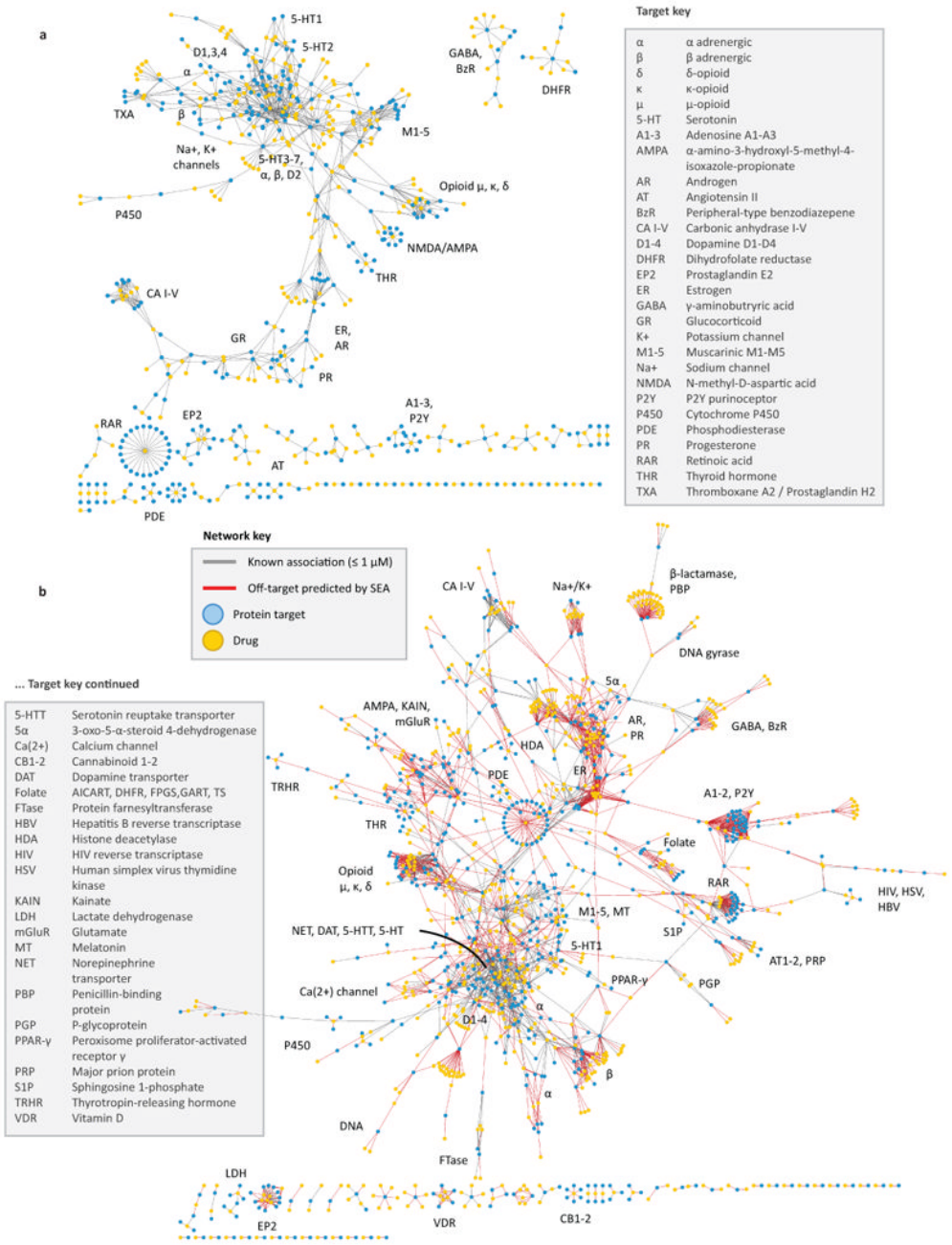


Figure 1. Drug-target networks, before and after predicting off-targets

(A) Known drug-target network. Each drug (gold) is linked to its known protein targets (cyan) by a gray edge. Each edge denotes a K^i of 1 μM or better for that drug to its target. (B) Predicted drug-target network. Drugs and proteins are linked as per the known drug-target network in (A), but with the addition of red edges representing SEA off-target predictions with E-values $\leq 10^{-10}$.

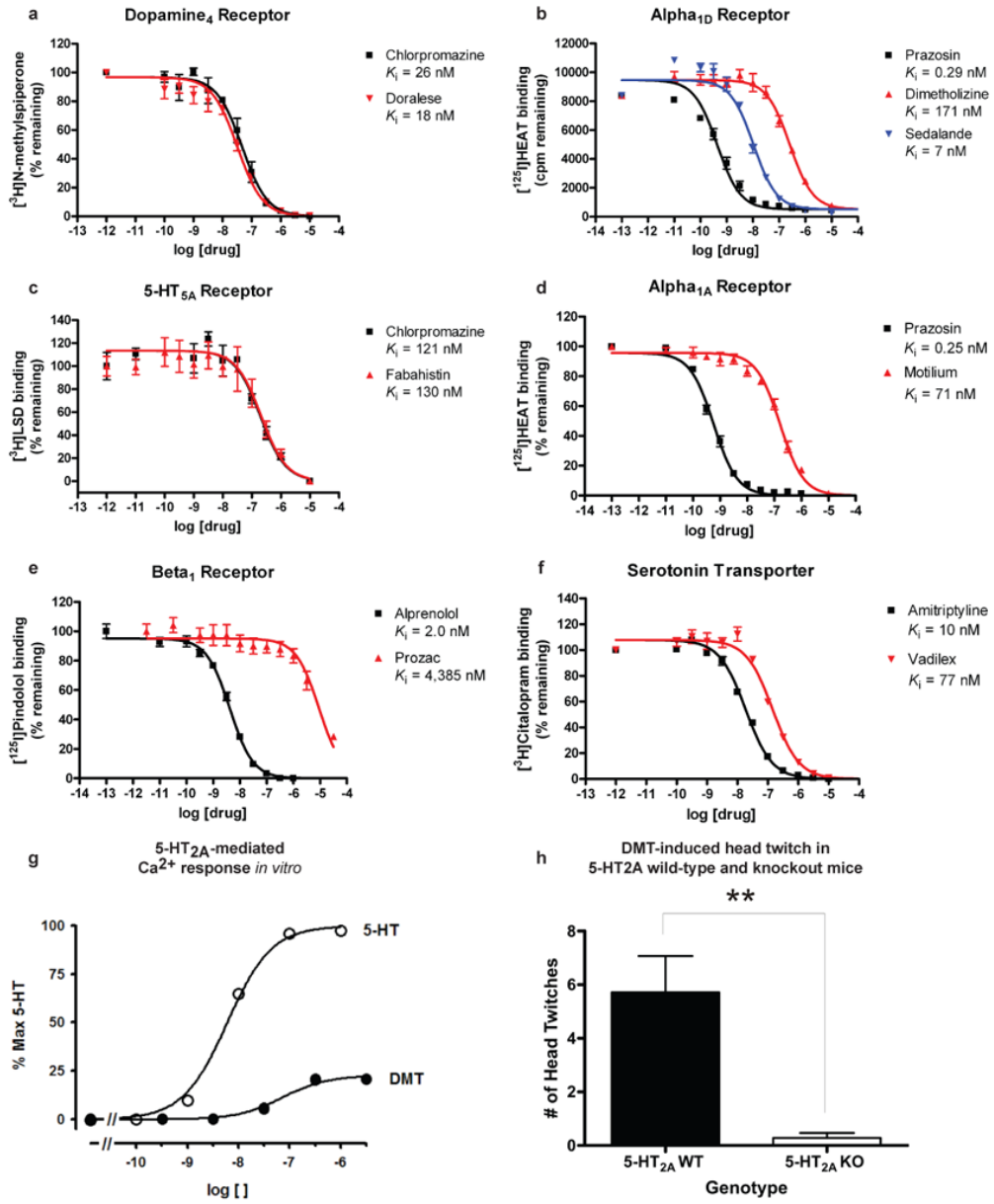


Figure 2. Testing new off-target activities

(A-F) Radioligand competition binding assays: (A) Doralese at D_4, (B) Sedalande and Dimetholizine at α_{1D}, (C) Fabahistin at 5-HT_{5A}, (D) Motilium at α_{1A}, (E) Prozac at β_1, and (F) Vadilex at the serotonin transporter. (G-H) Investigating 5-HT_{2A} as the target of DMT-induced hallucination: (G) 5-HT_{2A}-mediated Ca^{2+} response was measured after treating HEK 293 cells stably expressing the human 5-HT_{2A} receptor with DMT or 5-HT. DMT's EC_{50} was found to be 118±29 nM (vs. 5-HT's 6.6±0.4 nM baseline, n = 3), with an E_{max} of 23±0.4% (n = 3), confirming that DMT is a potent partial agonist at 5-HT_{2A} receptors. (H) DMT elicited head twitch behavior only in 5-HT_{2A} wild-type mice, confirming that it is a hallucinogenic 5-HT_{2A} agonist. **, $p < .01$.

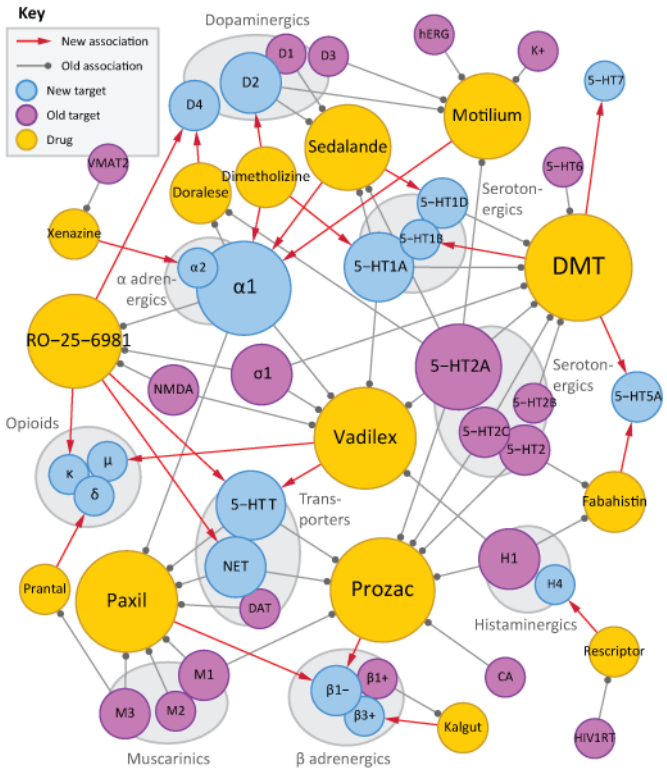
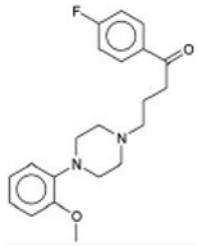
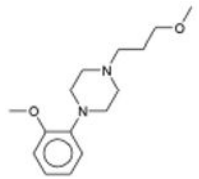
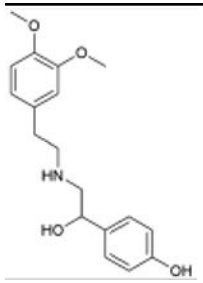
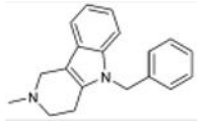
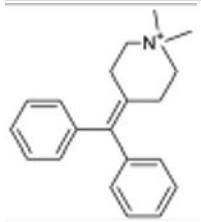


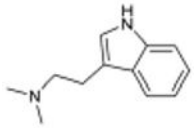
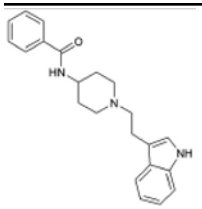
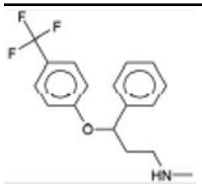
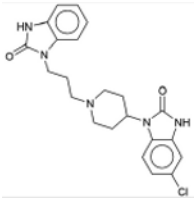
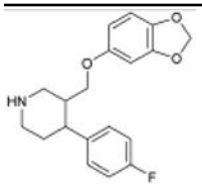
Figure 3. Discovered off-targets network

Bipartite network where drugs (gold) are linked by gray edges to their known targets (violet) and by red arrows to their discovered off-targets (cyan). Gray edges denote binding at 1 μM or better, where these affinities are known. Node sizes increase with number of incident edges. Target abbreviations: 5-HT*x*, serotonin receptor type *x*; 5-HTT, serotonin transporter; β1+, β_1 adrenergic agonist; β1-, β_1 adrenergic antagonist; β3+, β_3 adrenergic agonist; σ1, σ_1-receptor; CA, carbonic anhydrase; DAT, dopamine transporter; HIV1RT, HIV-1 reverse transcriptase; hERG, human Ether-a-go-go Related Gene channel; K+, Potassium channel; NET, norepinephrine transporter; NMDA, *N*-methyl-*D*-aspartate receptor; VMAT2, vesicular monoamine transporter 2.

Table 1

Prediction and testing of new aminergic GPCR targets for drugs

Drug / Pharmacological Action	E-valuePredicted Target	K_i (nM)‡	
 Sedalande Neuroleptic	8.3×10^{-136} α_1 Adrenergic Blocker †	α_{1A} α_{1B} α_{1D}	1.2 14 7.0
	1.7×10^{-14} 5-HT_{1D} Antagonist		140
 Dimetholizine Antihistamine; Antihypertensive	1.6×10^{-129} α_1 Adrenergic Blocker †	α_{1A} α_{1B} α_{1D}	70 240 170
	2.7×10^{-113} 5-HT_{1A} Antagonist		110
	7.4×10^{-56} Dopamine D_2 Antagonist		180
 Kalgut Cardiotonic	3.1×10^{-79} β_3 Adrenergic Agonist		2.1×10^{3}
 Fabahistin Antihistamine	5.7×10^{-57} 5-HT_{5A} Antagonist		130
 Prantal Anticholinergic; Antispasmodic	5.5×10^{-32} δ Opioid Agonist		1.4×10^{4}

Drug / Pharmacological Action	E-value	Predicted Target		K_i (nM)‡
 *N*,*N*-dimethyltryptamine Serotonergic Hallucinogen	3.1×10^{-21}	5-HT$_{1B}$ Agonist		130
	1.2×10^{-13}	5-HT$_{2A}$ Agonist §		130
	1.1×10^{-7}	5-HT$_{5A}$ Antagonist		2.1×10^{3}
	5.0×10^{-6}	5-HT$_{7}$ Modulator		210
 Doralese Adrenergic α_1 Blocker; Antihypertensive; Antimigraine	2.8×10^{-27}	Dopamine D$_4$ Antagonist		18
 Prozac 5-HT Reuptake Inhibitor; Antidepressant	3.9×10^{-15}	β Adrenergic Blocker †	β_1	4.4×10^{3}
 Motilium Antiemetic; Peristaltic Stimulant	4.8×10^{-11}	α_1 Adrenergic Blocker †	α_{1A} α_{1B} α_{1D}	71 530 710
 Paxil 5-HT Reuptake Inhibitor; Antidepressive Agent	1.3×10^{-7}	β Adrenergic Blocker †	β_1	1.0×10^{4}

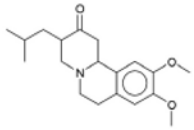
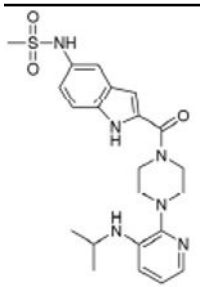
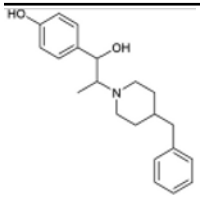
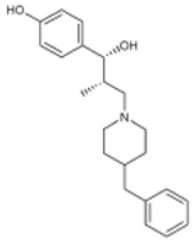
† For the targets marked, the reference dataset did not specify the receptor subtype, requiring a separate assay for each one. For instance, the MDDR contains an "α1 adrenergic Blocker" set, for which it was necessary to test the α_{1A}, α_{1B}, and α_{1D} subtypes.

‡ K_i values are accurate ± 20% at two significant figures.

§ 5-HT2A is a known target of DMT, but is shown here with its retrospective SEA E-value for comparison purposes.

Table 2

Prediction and testing of new cross-boundary targets for drugs

Drug / Canonical Target		E-value	Predicted Target		K_i (nM)[‡]
	Xenazine VMAT2 (transporter)	1.4×10^{-61}	α_2 Adrenergic receptor [†] (GPCR)	α_{2A}	960
				α_{2C}	1.3×10^{3}
	Rescriptor HIV-1 RT (enzyme)	1.05×10^{-30}	Histamine H_4 receptor (GPCR)		5.3×10^{3}
	Vadilex NMDAR (ion channel)	5.14×10^{-13}	μ Opioid receptor (GPCR)		1.4×10^{3}
		1.98×10^{-4}	5-HTT; Serotonin transporter (transporter)		77
	RO-25-6981 NMDAR (ion channel)	1.53×10^{-8}	5-HTT; Serotonin transporter (transporter)		1.4×10^{3}
		1.94×10^{-6}	Dopamine D_4 receptor (GPCR)		120
		3.61×10^{-6}	NET; Norepinephrine transporter (transporter)		1.3×10^{3}
		9.08×10^{-5}	κ Opioid receptor (GPCR)		3.1×10^{3}

[†] The MDDR database did not specify the α_2 adrenergic receptor subtype, requiring a separate assay for each one (α_{2A}, α_{2C}).

[‡] K_i values are accurate ± 20% at two significant figures.