Uncovering novel therapeutics for schizophrenia: a multitarget approach using the CANDO platform

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ABSTRACT

Schizophrenia is a complex and debilitating neuropsychiatric disorder characterized by positive, negative, and cognitive symptoms, many remaining insufficiently addressed by current treatments. High rates of treatment resistance and heterogeneous pathophysiology pose significant challenges to traditional single-target drug discovery. To address this, we applied the CANDO platform to identify repurposable drugs for schizophrenia using a multitarget strategy. The platform evaluates how compounds interact with the entire human proteome, generating interaction signatures that capture a compound's effect across all targets. By comparing these signatures, CANDO computes compound similarity scores and enables consensus prediction of novel therapeutics. Benchmarking experiments across all indications and specific to schizophrenia demonstrated that CANDO significantly outperformed controls across multiple evaluation metrics, recovering known drug indication associations with orders of magnitude better accuracy. Using prediction methodology validated through benchmarking, we generated a ranked list of repurposable compounds. Literature review confirmed clinical or biochemical evidence supporting 25 top ranked drug candidates, including phenothiazine and benzamide antipsychotics, tricyclic antidepressants, benzodiazepines, and monoamine oxidase inhibitors. We identified protein targets with the highest likelihood of interaction with these top drugs and assessed prediction quality through overlap analysis with gold standards, showing significantly better concordance with established schizophrenia related biology than controls. Among these protein are corroborated targets such as canonical neurotransmitter receptors, dopamine, serotonin, and adrenergic, as well as monoamine transporters, tyrosine aminotransferase, and lysine specific histone demethylase 1A. Enrichment of calcium-binding proteins among the phenothiazine antipsychotic triethylperazine's top predicted targets highlights a potential role for dysregulated calcium signaling, including calmodulin–CaMKK2 pathways, in schizophrenia pathology and treatment response. Another phenothiazine antipsychotic, triflupromazine, binds the serotonin transporter in addition to its canonical dopamine D2 receptor interaction, warranting further research into its potential effects on depressive symptoms. These findings demonstrate the utility of the CANDO platform in elucidating multitarget pharmacological mechanisms and accelerating the identification of effective repurposable treatments for schizophrenia.

Introduction

Schizophrenia is a complex and debilitating psychiatric disorder characterized by psychotic symptoms such as persistent hallucinations and delusions, negative symptoms including diminished emotional expression, avolition, and social withdrawal, and cognitive deficits such as poor attention, memory, and executive function[1–5]. Individuals with the disorder die on average 28.5 years earlier due to comorbid medical conditions and suicide[2,6]. It has an average lifetime prevalence of approximately 1% of the population[1]. Antipsychotic medications are the cornerstone of schizophrenia treatment, controlling positive symptoms of psychosis by blocking dopamine D_2 receptors in the brain. Treatment resistance remains common, even though second generation antipsychotics (SGAs) are generally more effective than first generation antipsychotics (FGAs) at improving cognitive and negative symptoms[1,5,7,8]. An estimated one third of patients have treatment resistant schizophrenia (TRS), meaning they experience little or no improvement despite adequate trials of multiple antipsychotics[5], and only about one third of patients achieve long term remission of symptoms[6]. Antipsychotics can cause serious adverse effects, including hyperprolactinemia and an elevated risk profile for diabetes mellitus and cardiovascular related mortality. FGAs carry a high risk of extrapyramidal side effects (EPS) such as parkinsonian tremors and tardive dyskinesia while SGAs can present increased risk of weight gain[3]. Antipsychotics interact with multiple neurotransmitter receptors including dopamine, norepinephrine, serotonin, histamine, and muscarinic acetylcholine receptors. The anticholinergic and antihistaminic effects of some antipsychotics may account for deficits in processing speed, verbal memory, and executive functions[9]. Novel therapeutic strategies are needed to address the broad symptom profile of schizophrenia, overcome treatment resistance, and minimize side effects.

Drug discovery is a costly and time consuming endeavor due in part to the need to perform separate biochemical analyses of potentially millions of compounds[10]. As a result, computational strategies, benefiting from advances in computing power and availability of data on ligand properties, binding, and 3D structures of targets, have become indispensable for accelerating

the identification of new drug candidates[10,11]. A key strategy is virtual high throughput screening, using computer simulations to evaluate large libraries of compounds against a biological target and significantly reducing cost by computationally triaging compounds before pharmacological testing[10]. Two main protocols of virtual screening are structure based virtual screening (SBVS) and ligand based virtual screening (LBVS). In SBVS, computational methods dock candidate small molecules into the target's binding site to predict their binding strength and orientation. LBVS leverages known active ligands for a target along with the similarity principle, that molecules with similar structures or properties are likely to exhibit similar biological activities[12]. Recent efforts to improve upon conventional virtual screening protocols include hybrid and multistage screening pipelines where SBVS and LBVS can be merged in sequential or parallel workflows to leverage their respective strengths[13].

The Computational Analysis of Novel Drug Opportunities (CANDO) is our in house platform that enables multitarget and multistage drug repurposing and discovery by ranking compounds for specific indications based on their interaction profiles across the proteome[14,15]. CANDO is based on the principle of polypharmacology, screening drugs across the full ensemble of potential on and off targets simultaneously, rather than focusing on a single target as in traditional SBVS[14,16]. The central hypothesis is that drugs with similar compound–proteome interaction signatures will exhibit similar biological or therapeutic effects[14,17]. This enables the discovery of functionally similar drugs that may be missed by LBVS, as it focuses solely on structural similarity. CANDO's modeling pipeline has been refined and experimentally validated across a range of indications, demonstrating its ability to propose clinically relevant drug–disease connections[15,16,18–22]. In this study, we (1) apply the CANDO platform to identify new potential drug candidates for treating schizophrenia, (2) predict the most likely protein targets of these drugs to elucidate their potential mechanisms of action, and (3) validate these predictions against curated gold standard protein sets.

Methods

Overview of schizophrenia drug repurposing pipeline using the CANDO platform

We started our repurposing pipeline by curating compound, protein, and indication libraries to form the basis for compound proteome interaction scoring. Interaction scores between each compound and protein were generated using an in house scoring protocol that uses algorithmically predicted binding sites and ligand compound fingerprint similarity to produce proteomic interaction signatures. CANDO compares these signatures across all compounds to create ranked similarity lists. We then used these lists in benchmarking to evaluate performance in recapturing known drug–indication associations. We ranked compounds by their frequency of appearance and rank across similarity lists of known drugs to predict potential schizophrenia treatments. Then, we manually curated the top 100 predictions with a literature search to identify 25 high corroboration repurposing candidates based on clinical and biochemical evidence. Control sets were generated by extracting structurally complex compounds from the bottom of similarity rankings and by selecting random entries. Next, we identified predicted protein targets for each compound in all prediction and control sets. To evaluate prediction quality, we created three gold standards and compared our target lists against them using overlap analyses.

Compound and protein library construction

The first step in the CANDO pipeline is compiling a compound library, a proteome library, and an indication library[23]. We used v2.9 of our DrugBank sourced compound list with a filter to include only the 2449 drugs that are approved by at least one regulatory agency, such as the FDA or its international counterparts. We used the Homo sapiens AlphaFold2 library as the proteome, consisting of 20,295 AlphaFold2 structure predicted proteins[23]. We used the Comparative Toxicogenomics Database to link 2,449 approved drugs to 22,771 drug–indication associations, identifying compounds by DrugBank ID and indications by Medline Subject Headings terms[23–25]. Interaction scores between compounds and proteins were computed across the full protein library to generate a compound–proteome interaction matrix. We used this matrix, along with the drug–indication mappings, for benchmarking performance and predicting new therapeutic candidates.

Compound protein interaction scoring

We generated compound compound distances based on proteomic interaction signatures for each molecule in the CANDO library and used them to benchmark the platform and generate predictions. Our in house bioanalytical docking protocol (BANDOCK) computes interaction scores between each compound and every protein in the human proteome by integrating predicted binding site information and chemical similarity[17], with higher scores predicting greater interaction probability, with a maximum of 1.0. Previous publications detail this process[18,21,23,26,27]. BANDOCK was applied to all proteins in the library, generating a vector of interaction scores known as the interaction signature[23]. This process was repeated for each compound to generate the drug proteome interaction matrix. CANDO then calculated cosine distances between compound proteome interaction signatures in an all against all fashion to assess compound similarity. This resulted in ranked similarity lists for each compound that are then used to benchmark known drug–indication associations and to predict novel treatment options[23].

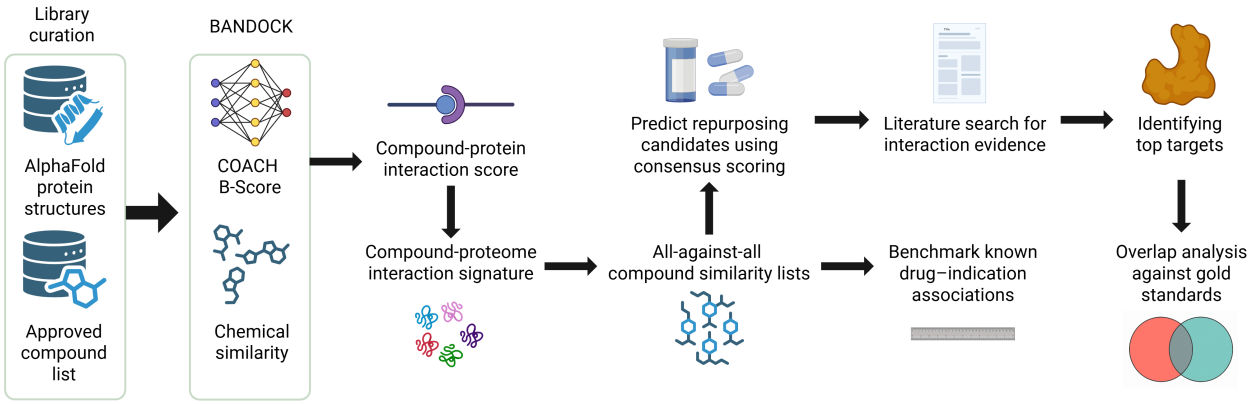

Figure 1. Overview of the CANDO pipeline for generating and corroborating drug repurposing candidates for schizophrenia. We used a protein library of AlphaFold predicted structures and an approved compound list from DrugBank to generate compound protein interaction scores using our in house bioanalytical docking (BANDOCK) protocol. Interaction scores were computed across the full proteome to produce an interaction signature for each compound. CANDO then determined compound compound similarity using cosine distance between interaction signatures in an all against all comparison. We used these similarity rankings to benchmark the platform and to predict repurposing candidates. Top ranked drug predictions were corroborated through literature review, and their most likely predicted protein targets were compared to gold standard protein sets via overlap analysis.

Benchmarking

We benchmarked CANDO for schizophrenia and across all indications using eight metrics against random controls to validate its ability to generate meaningful predictions. Indication accuracy (IA) measures how effectively the platform ranks drugs approved for the same indication as similar, using a leave one out procedure. For each indication, CANDO identifies the percentage of similarity lists of associated drugs that have at least one other associated drug within a certain cutoff, such as the top 10 or top 25[21,28,29]. The platform calculates average indication accuracy (AIA) by computing the IA for every indication mapped to at least two drugs, the minimum for establishing a similarity list, and averaging the results[29]. New indication accuracy (nIA) measures how well the platform recaptures associated drugs in the consensus list of an indication. It is defined as the percentage of drugs withheld from their indication that appear in the consensus list for that indication within a given cutoff. New average indication accuracy (nAIA) is the mean nIA across every indications with two or more associated drugs[23]. CANDO uses the metric normalized discounted cumulative gain (NDCG), commonly applied in information retrieval to evaluate the quality of ranked lists based on known relevant items, to assess the prioritization of indicated drugs for specific indications and across all indications[23]. The discounted cumulative gain (DCG) metric is calculated based on the principle that associated compounds ranked higher in a compound's similarity list should contribute more to the overall relevance score[28–30]. CANDO computes ideal DCG (IDCG) from a hypothetical perfect ranking where all approved compounds are placed at the top of the similarity list, serving as the reference for computing the normalized score[23,28]. NDCG is calculated by dividing a DCG by the IDCG and takes upon values between 0 and 1, with higher scores indicating better ranking[28]. The platform uses a related metric, new NDCG (nNDCG), to measure prediction quality and is calculated from consensus rankings rather than similarity lists. We compared our results for each benchmark metric to controls generated by inputting 100 randomized compound–protein interaction matrices into CANDO's benchmarking functions, then averaged the resulting scores to model the platform's expected performance under random chance.

Generating putative drug predictions

We used CANDO's canpredict compounds module to generate a list of compounds likely to treat schizophrenia. The module predicts candidates by counting how often a compound appears in the top 10 of similarity lists for drugs already approved for the indication. It prioritizes compounds that appear more frequently and resolves ties by favoring those with higher average ranks across the lists. We selected the top 100 compounds from this ranking for subsequent literature review. As noted earlier, we limited our predictions to approved drugs to ensure acceptable safety profiles.

Literature search to corroborate putative drug candidates

We conducted a literature search using PubMed and Google Scholar to corroborate our drug predictions, using queries that combined "schizophreni*" and each compound name, limited to the title or abstract fields. We marked as highly corroborated drugs with at least 2 two high corroboration evidences, defined as 1) a clinical study that demonstrates the efficacy of the drug as a standalone treatment for schizophrenia 2) a functional assay that demonstrates agonism or antagonism at a relevant receptor, or 3) a binding study that shows the drug has potent binding activity to a relevant receptor. We also marked drugs with 1 high corroboration evidence and at least 1 low corroboration evidence as highly corroborated, where low corroboration is defined as a clinical study that demonstrates the combined efficacy of the drug as an adjunctive treatment along with another drug. We excluded from our high corroboration list drugs with 1 high corroboration evidence and no other evidence, or drugs with no high corroboration evidence.

Generating bottom ranked and random controls

From the similarity list of each schizophrenia associated drug, we selected the lowest ranked compounds with at least 4 heavy atoms, excluding structurally simplistic molecules. The drugs with the most appearances in these bottom lists were selected as the bottom filtered predictions control. We generated the random control by selecting random ranks from the similarity list of risperidone and extracting the compounds from the corresponding rows.

Top protein interactions for putative drug candidates

We applied CANDO's top targets module to generate a list of 100 protein predictions for each of our top corroborated drug predictions. This module ranks proteins for a given compound based on their interaction scores, returning the targets with the strongest predicted associations. We repeated this process to generate control target lists for the bottom filtered and random drug lists.

Generating gold standards and conducting a second literature search

We generated the UniProt gold standard by retrieving protein targets associated with each schizophrenia associated drug in UniProt and compiling the unique results. Next, we created the GeneCards gold standard by searching for schizophrenia associated genes and applying three layers of filters. First, we restricted results to the sections Disorders, Function, Genomics, Pathways, Phenotypes, Proteins, and Publications. By applying this filter, the dataset was enriched for biologically relevant associations and curated to remove entries derived largely from automated text mining or other low evidence sources[31]. Then, we removed genes that do not code for proteins or those with relevance scores of < ten. Finally, we created a PubMed and DrugBank gold standard. We searched PubMed using the UniProt recommended names of our top targets from our top predictions, and the names of every drug associated with that target. We marked pairs with evidence of a direct binding interaction between the drug and protein as high corroboration and included them in the gold standard. Pairs with only evidence of indirect interactions, such as downstream effectors of a direct interaction, were marked as low corroboration and excluded from the gold standard. Following the PubMed review, we conducted a DrugBank search for each top ranked drug to identify proteins involved in its mechanisms of action. We then noted any proteins that overlap with those found in the CANDO top target list for that drug and added them to the gold standard. The results from the PubMed and DrugBank literature search are presented in Figure 4, Table 2, and the discussion section of our paper .

Overlap analyses between top target lists and gold standards

We compared each of the top, bottom, and random prediction target lists against each gold standard (Fig. 4). We computed histogram frequencies as the number of intersections between a target list and a gold standard within each of five rank bins, divided by the total number of intersections for that gold standard. These histograms highlight which rank ranges contribute most to the overlap and help assess prediction quality. We calculated overlap percentage as the cumulative number of intersecting proteins up to a given rank cutoff, divided by the total number of proteins in the gold standard list. We used this metric to compare the top prediction list against the controls to determine differences in their degrees of overlap with each gold standard. The Jaccard coefficient, defined as the size of the intersection divided by the size of the union between the gold standards and prediction sets, was used to quantify and compare their overall similarity.[32,33] The higher the JC, the better our prediction list captures proteins independently corroborated by gold standard libraries.

Exploratory analysis on sources of interaction scores for high ranking targets of phenothiazines:

We observed that canonical targets of thiethylperazine, our fourth ranked prediction, were absent from its top 100 CANDO targets. To better understand its mechanism of action, we ran the CANDO top targets module for thiethylperazine and seven schizophrenia associated compounds whose similarity lists included it, then formed a consensus ranking by ordering proteins by their frequency of appearance across these lists. We repeated this procedure for three comparison cohorts: seven non-phenothiazine antipsychotics from our predictions, eleven non-antipsychotic schizophrenia predictions, and fifteen random

approved DrugBank compounds, and compared functional characteristics across sets. We also modified BANDOCK to output, for each predicted compound–protein interaction, the top ligand from Protein Data Bank (PDB) associated with that score, mapping proteins to binding site predictions and confidence values using the AlphaFold Homo sapiens COACH output. Literature referenced by PDB for each ligand and binding site complex was reviewed to determine the likely mode of action (e.g., inhibition) of predicted interactions, informing our assessment of therapeutic relevance. Predicted targets of phenothiazines that could be traced to a top ligand that was also a phenothiazine, and with literature evidence of being implicated in schizophrenia (e.g. by differential expression) were added to Table 3 and our PubMed/DrugBank gold standard set.

Results

Benchmarking results

Figure 2 presents benchmarking results for schizophrenia (A/B) and across all indications (C/D), showing recapture percentages based on similarity and consensus lists as well as prioritization of indicated drugs. In panel A, schizophrenia IA results show recapture rates ranging from 75% at the top 10 cutoff to 93% at the top 100 cutoff, while nIA increases from 10% to 53%, both markedly better than controls. This indicates CANDO's ability to recover known schizophrenia associated drugs based on interaction signature similarity and thereby reflects its predictive strength, indicating. Panel B reports NDCG values for schizophrenia of 0.220 at the top 10 and 0.151 at the top 50 cutoff, while nNDCG ranges from 0.058 at the top 10 to 0.138 at the top 100. Both ranges are better than controls, underscoring CANDO's ability to prioritize indicated drugs for schizophrenia early in the consensus ranking compared to random chance. Panel C highlights AIA performance between 22% and 44%, exceeding control values by five times at the top 10 cutoff. nAIA ranges from 9% to 26%, compared to control values between 0.7% and 6.8%. These results indicate that CANDO consistently ranks drugs for the same indication as highly similar to one another, and that this effect is broadly distributed across indications. In panel D, NDCG values across all indications span 0.044 to 0.059, while controls remain between 0.005 and 0.014; average nNDCG for all indications ranges from 0.049 to 0.083, better than the control values of 0.003 to 0.014. These findings demonstrate CANDO's robust ability to prioritize therapeutically relevant compounds early in the consensus rankings across a wide range of indications.

Putative drug candidates and compound indication literature corroboration

We used the CANDO platform to generate candidate drugs from our approved drug library. A full list of the top 100 predictions can be found in Supplementary Table S1. Table 1 contains a list of drug candidates that were corroborated through literature search. 25 of the 100 putative drug candidates showed clinical efficacy against schizophrenia, yielding a 25% hit rate. Of these 25, 20 are established or structurally related antipsychotics or antidepressants, lending credibility to the platform. The list also includes drugs from multiple chemical and mechanistic classes, including phenothiazine antipsychotics, benzodiazepines, tricylic antidepressants, benzamide antipsychotics, and N-arylpiperazine, supporting CANDO's polypharmacological insight.

Top targets and compound protein literature corroboration

We identified 29 protein targets from the top target lists of the top 25 drug predictions that were corroborated by the literature. These include canonical neurotransmitter receptors (serotonin, dopamine, adrenergic), monoamine transporters, and calcium binding proteins. Several drugs exhibited notable polypharmacology: amisulpride, with receptor interactions extending beyond its primary targets, and vortioxetine, spanning serotonergic and adrenergic systems. We also observed secondary predictions, or targets not typically emphasized in the primary drug profile. Examples include amisulpride's acetylcholinesterase inhibition that may confer cognitive effects[34], and phenelzine's histone demethylase activity, suggesting an epigenetic role beyond well documented monoamine oxidase inhibition[35]. These findings highlight CANDO's capacity to capture the multitarget activity central to effective schizophrenia treatments, while secondary predictions can motivate new hypotheses for exploring polypharmacology in both schizophrenia and depression.

Overlap analyses

(A) Approximately 47%, 28%, and 33% of top prediction overlaps occur in the top rank bins of the PubMed and DrugBank, UniProt, and GeneCards gold standards, respectively (Figure 3. The apparent outperformance of the random control in the top rank bin is readily explained. Seven of the thirteen targets in this bin interact with the phenothiazine antipsychotic prochlorperazine or the serotonin norepinephrine reuptake inhibitor venlafaxine. If both of these drugs were excluded from our random set, the height of the leftmost random control bar would fall below that of the top predictions bar. The GeneCards gold standard includes the dopamine and serotonin transporters. Venlafaxine targets both, skewing the overlap with the random set that has only six overlaps in total. Similarly, with only four overlaps in the bottom filtered control, the apparent enrichment in the top rank bin is of limited interpretative value. (B) The overlap percentage of targets from top predictions range from approximately 55% to 100% in the PubMed and DrugBank gold standard, 6% to 19% for UniProt, and 7.5% to 18% for GeneCards. The steady rise in overlap with rank cutoff suggests that true targets are spread across the ranked list, supporting

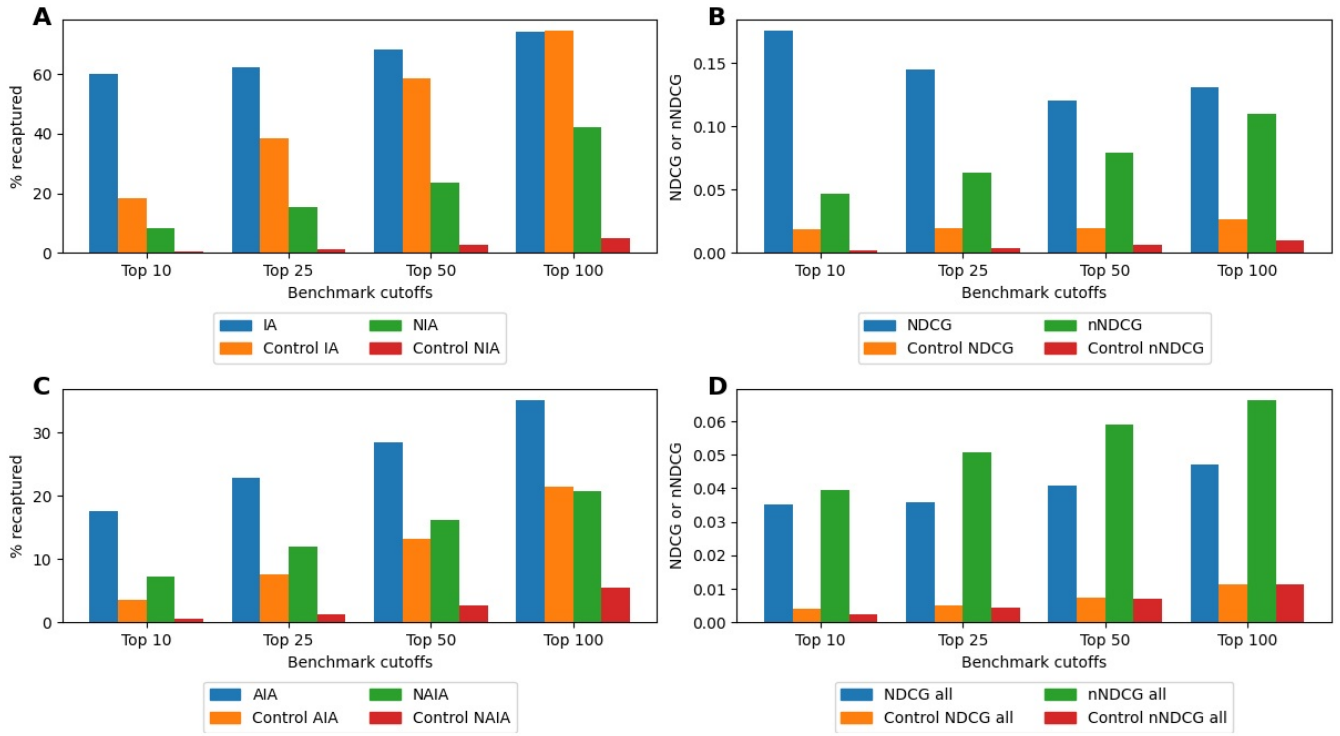

Figure 2. Benchmarking results for schizophrenia and all indications across eight metrics measuring recapture percentage and compound prioritization. (A) IA for schizophrenia outperforms the control by over 3 fold at the top 10 cutoff and remains better until the top 100, where both exceed 90%. nIA maintains over 8 fold improvement across all cutoffs. (B) NDCG for schizophrenia is nearly 5 times better than the control at the top 100 cutoff, with the relative advantage increasing at stricter cutoffs. nNDCG outperforms the control by over 10 times at the top 100 cutoff and nearly 30 times at the top 10 cutoff. (C) AIA across all indications reaches 44% at the top 100 cutoff, outperforming the control significantly, while nAIA hits 26%, a 3.8 fold improvement over control. (D) Mean NDCG and nNDCG across all indications are better than the control by 4.1 fold and 5.8 fold at the top 100 cutoff, respectively, and are even more superior at more stringent cutoffs. These benchmarking results support CANDO's ability to recapture known drug indication associations and prioritize relevant compounds near the top of its consensus lists, validating the predictive utility of the platform.

the use of larger cutoffs for validation. (C) Whereas overlap percentage increased with rank cutoff, reflecting the accumulation of additional true targets, the Jaccard coefficient declined for PubMed and GeneCards as lower ranked predictions were added. This pattern demonstrates that although additional targets are recovered at larger cutoffs, the relative enrichment is diluted because the denominator (union size) grows more quickly than the number of overlaps, implying that higher ranked predictions are of higher quality. Meanwhile, the flat random control lines for PubMed/DrugBank and GeneCards confirm that observed enrichment is not due to chance.

Discussion

We excluded 11 of the 25 corroborated drug predictions that were FDA-approved or approved internationally and selected 5 of the remaining for discussion based on notable corroborated target interactions.

Triflupromazine and trimipramine: The phenothiazine antipsychotic triflupromazine also demonstrates binding activity at the serotonin transporter (SERT)[70,71] (Fig 4). Clinical studies show that triflupromazine significantly improves thinking disorganization, psychiatric ward behavior, and total morbidity scores on the Psychotic Reaction Profile compared with a sedative control[72]. Its potency as a SERT binder suggests potential effects on depressive symptoms, but further research is needed to confirm this, particularly in the context of schizophrenia. Triflupromazine was discontinued from the FDA, but not due to efficacy or side effect reasons[73]. The TCA trimipramine also demonstrates inhibitory activity at SERT[37]. Accordingly, Eikmeier et al. provided evidence that the drug significantly decreased the anxiety / depression factor of the Brief Psychiatric Rating Scale[74]. Chronic administration of a selective serotonin reuptake inhibitor (SSRI) downregulates 5-HT7 receptors[75], already reduced in schizophrenia.

Table 1. Top predicted drug candidates corroborated with PubMed / Google Scholar literature search. CANDO ranks compounds in descending order of consensus score, the number of times they appeared in the top n of similarity lists of drugs associated with schizophrenia. In this case, n = 10. Ties are broken by increasing order of average rank of the candidate in these similarity lists. The low probability values emphasize the statistical strength of these rankings, indicating that the predictions are not due to random chance but instead reflect meaningful proteomic similarities. CANDO not only reproduces known antipsychotic therapies but also uncovers less obvious candidates. These results indicate that the CANDO platform successfully identifies drug candidates for schizophrenia that are corroborated by existing literature and reinforce its predictive validity for drug repurposing.

Drug rank	Consensus score	Average rank	Probability	Drug name
1	8	3.2	4.61E-10	Zuclopenthixol
2	8	5.1	4.61E-10	Butaperazine
3	8	5.6	4.61E-10	Perazine
4	7	5.1	1.48E-08	Thiethylperazine
5	6	3.3	4.09E-07	Lumateperone
7	6	4.7	4.09E-07	Prochlorperazine
10	5	1.6	9.57E-06	Pipotiazine
12	5	4.2	9.57E-06	Promazine
13	5	4.4	9.57E-06	Droperidol
15	5	5.4	9.57E-06	Amoxapine
17	4	2.2	1.84E-04	Clothiapine
18	4	2.5	1.84E-04	Clomipramine
31	3	2.7	2.80E-03	Chlordiazepoxide
33	3	3	2.80E-03	Trimipramine
40	3	5.7	2.80E-03	Vortioxetine
44	3	6	2.80E-03	Pizotifen
48	3	6.7	2.80E-03	Vilazodone
53	2	0	3.18E-02	Sultopride
57	2	1.5	3.18E-02	Amisulpride
63	2	2	3.18E-02	Flunarizine
66	2	3.5	3.18E-02	Halazepam
70	2	4	3.18E-02	Triflupromazine
71	2	4	3.18E-02	Pargyline
77	2	5	3.18E-02	Phenelzine
80	2	5	3.18E-02	Lurasidone

Vortioxetine: Vortioxetine, a multimodal antidepressant with 5-HT1A agonism, 5-HT1B partial agonism, and 5-HT1D antagonism, improves MK-801 induced deficits in object recognition, working memory, and social behavior deficits induced by MK-801 in rats[41,42,76]. The elevated expression of 5-HT1A receptors in the prefrontal cortex and dorsal hippocampus in schizophrenia could be symptomatic of low serotonin (5-HT) in those areas (Fig 4). 5-HT1A receptor agonism counteracts phencyclidine induced social attention deficits and has known antidepressant effects[77,78]. 5-HT1B receptor agonists have anti-aggressive effects in individuals who show moderate as well as high levels of aggression, not specific to schizophrenia[79]. A prior review suggests that activation of 5-HT1A, 5-HT1B, and 5-HT1D mediates antidepressant efficacy[80]; Vortioxetine activates two of these three receptors, making it a strong candidate for treating depressive symptoms.

Phenothiazine antipsychotics and calcium-binding proteins: Thiethylperazine, our fourth ranked drug prediction with a consensus score of seven and an average rank of 5.1, is a phenothiazine atypical antipsychotic with a known interaction with the dopamine D2 receptor[115]. However, our platform ranks this interaction relatively low, 1,609 out of 20,295 proteins, with an interaction likelihood score of 0.262, substantially lower than expected for a canonical target. We observed this striking result across other antipsychotics in our corroborated predictions, as only one, amisulpride, has a top corroborated interaction with dopamine receptors in Table 2. Similarly, the predicted interaction of thiethylperazine with the serotonin 5-HT2A receptor, another key target of atypical antipsychotics, is ranked 3,532 with a score of 0.193. Some of the interactions ranked as more probable than these known targets may contribute to the broad side effect profiles of antipsychotics. We investigated these unexpected rankings by generating a phenothiazine antipsychotic consensus ranking and found that 44 of the top 50 proteins were calcium binding proteins. For comparison, calcium binding proteins comprise 39 of consensus top 50 targets for non-phenothiazine antipsychotics, 11 for non-antipsychotic schizophrenia predictions, and none in a random set, suggesting a notable enrichment of calcium binding protein interactions among antipsychotic compounds. This observation aligns with existing literature reporting that neuroleptic drugs bind calmodulin, as do tricyclic antidepressants (TCAs), *in vitro*[62,63]. TCAs

Table 2. Literature corroborated top protein targets of putative drug candidates for schizophrenia. Drug rank refers to the compound's position in our consensus based predictions. Target rank is the protein's position in the drug's top target list. Score represents the predicted interaction probability between the drug and protein. Beyond the well-established serotonin and dopamine transporters, we highlight monoamine transporters, adrenergic receptors, calmodulin, monoamine oxidase B, and acetylcholinesterase as being worthy of further investigation. See Supplementary Table S2 for the full set of target predictions for our top 25 compounds.

Drug rank	Drug name	Target rank	Score	UniProt ID	Target name	Interaction Type	Evidence
18	Clomipramine	2	0.78				Maliza et al. 1997[36]
33	Trimipramine	14	0.509	P31645	Sodium dependent serotonin transporter		Gerile et al. 2012[37]
70	Triflu promazine	92	0.516				Tatsumi et al. 1999[38]
18	Clomipramine	3	0.78	P23975	Sodium dependent noradrenaline transporter	Inhibitor	DrugBank[39]
33	Trimipramine	3	0.532				DrugBank[40]
33	Trimipramine	3	0.524	Q01959	Sodium dependent noradrenaline transporter	Inhibitor	DrugBank[40]
40	Vortioxetine	61	0.415	P08908	5-HT1A receptor	Agonist[41]	Sanchez et al. 2015[42]
		46	0.422	P28222	5-HT1B receptor	Partial agonist[41]	Chakroborty et al. 2017[43]
		33	0.435	P28221	5-HT1D receptor	Antagonist[41]	Sanchez et al. 2015[42]
57	Amisulpride	7	0.44	P08908	5-HT1A receptor		Tollens et al. 2018[44]
		84	0.348	P28223	5-HT2A receptor	Antagonist[45]	
		34	0.387	P41595	5-HT2B receptor		Donahue et al. 2024[46]
		27	0.394	P34969	5-HT7 receptor	Antagonist[45]	Meltzer 2012[47]
		1	0.522	P22303	Acetylcholinesterase	Inhibitor	Ouyang et al. 2016[34]
		2	0.519	P14416	Dopamine D2 receptor		Meltzer and Massey 2011[48]
		4	0.473	P35462	Dopamine D3 receptor		Castelli et al. 2001[49]
		5	0.456	P21917	Dopamine D4 receptor		Vasse and Protais 1988[50]
53	Sultopride	27	0.423	P08913	Alpha-2A adrenergic receptor		Chivers et al. 1988[51]
		16	0.443	P18089	Alpha-2B adrenergic receptor		Sahyoun et al. 1982[52]
		37	0.404	P18825	Alpha-2C adrenergic receptor		
57	Amisulpride	15	0.427	P08913	Alpha-2A adrenergic receptor		Chivers et al. 1988[51]
		6	0.446	P18089	Alpha-2B adrenergic receptor		
		19	0.407	P18825	Alpha-2C adrenergic receptor		
40	Vortioxetine	6	0.467	P08588	Beta-1 adrenergic receptor	Ligand	DrugBank[41]
63	Flunarizine	26	0.39	P0DP25	Calmodulin-3	Inhibitor	DrugBank[53]
		27		P0DP24	Calmodulin-2		
		28		P0DP23	Calmodulin-1		
71	Pargyline	5	0.558	P27338	Monoamine oxidase B	Inhibitor	Takao et al. 2019[54]
77	Phenelzine	92	0.56	P17735	Tyrosine aminotransferase		Dyck and Dewar 1986[55]
		93	0.558	O60341	Lysine specific histone demethylase 1A		Tan et al. 2019[56]

are present in our set of non-antipsychotic schizophrenia predictions. Recent evidence has suggested that trifluoperazine binds to Calmodulin-2 *in vivo* in the brain, but further studies are needed to substantiate this interaction[116]. The top ligand driving high scoring (0.65–0.71) predicted interactions in our phenothiazines set is a calmodulin–trifluoperazine complex[117,118]. This complex has been experimentally shown to be inhibitory: trifluoperazine competes with target peptides for calmodulin binding and induces conformational changes that block calmodulin's interaction with those targets[60,61]. Importantly, calmodulin activates Ca^{2+}/calmodulin dependent protein kinase kinase 2 (CaMKK2), and reduced CaMKK2 activity has been associated with schizophrenia in human studies[119]. As a result, inhibition of calmodulin may have a negative effect on disease prognosis. Calcium homeostasis is tightly regulated in the brain and plays a critical role in intracellular signaling cascades, including those downstream of 5-HT2A receptor activation that converge on intracellular calcium release. As the brain's primary calcium sensor, calmodulin mediates calcium dependent processes, and its dysregulation may disrupt protein interactions and signaling cascades essential for cerebellar circuit formation and function. Such disruptions could contribute to the connectivity deficits observed in schizophrenia, supporting calmodulin's potential as a therapeutic target[57]. The calmodulin trifluoperazine complex also drives our predictions of phenothiazine interactions with several calmodulin like proteins and myosin light chains and polypeptides. The relevance of these targets to schizophrenia remains to be elucidated, and future research may determine whether their inhibition contributes to therapeutic efficacy or to adverse effects. Several S100 family proteins also appear

Table 3. Literature corroborated top protein targets of thiethylperazine and seven schizophrenia associated drugs that include it in their similarity lists. We retained proteins that 1) appear on a consensus top targets list across the eight compounds and 2) have literature evidence of involvement in schizophrenia (e.g., differential expression). All eight drugs are phenothiazine antipsychotics. Proteins are ranked by the number of appearances ("apps") across the eight lists; average rank is the mean position of a protein on the lists where it appears, and average score is the mean predicted interaction probability across those same lists. All but the bottom three proteins are calcium binding. Notably, calmodulin mediates many calcium dependent processes in the brain, and its dysregulation could contribute to connectivity deficits observed in schizophrenia, supporting its candidacy as a therapeutic target[57]. S100B has likewise been proposed as a treatment target, as elevated concentrations may be toxic whereas deletion or inhibition yields beneficial effects[58,59]. This convergence on calcium-binding proteins provides novel support for investigating them as central mediators of both therapeutic effects and adverse outcomes of phenothiazine antipsychotics.

Apps	Average rank	Average score	UniProt ID	Target name	Interaction Type	Evidence
8	19.5	0.711	P0DP23	Calmodulin-1	Inhibitor[60,61]	Lejoyeux and Maziere 1991[62] Roufogalis et al. 1983[63]
8	18.5	0.711	P0DP24	Calmodulin-2		
8	17.5	0.711	P0DP24	Calmodulin-3		
7	20.4	0.695	P04271	Protein S100-B	Inhibitor	Donato et al. 1984[64]
7	24	0.689	P26447	Protein S100-A4		Malashkevich et al. 2010[65]
6	84.2	0.5387	Q96C19	EF-hand domain containing protein D2		Gao et al. 2020[66]
3	80	0.589	Q8IVG9	Humanin		Murakami et al. 2017[67]
2	36.5	0.611	P11511	Aromatase		da Silva et al. 2023[68]
2	45	0.592	Q00535	Cyclin dependent kinase 5		Barrera-Conde et al. 2023[69]

among the top predicted targets for phenothiazines. These predictions are driven by the structural complex of S100A4 bound to a phenothiazine, and prior work has shown that chlorpromazine inhibits S100 proteins *in vitro*[64,65,120]. Previous studies indicate that S100B is a potential therapeutic target for various neural disorders, including schizophrenia, as its overexpression or administration induces disease worsening, while its deletion or inhibition produces beneficial effects[59]. Similarly, Sorci et al. report that the effects of S100B are context dependent. At nanomolar concentrations and low cellular expression of the receptor for advanced glycation end products (RAGE), S100B may have trophic effects, while at micromolar concentrations and / or in the presence of high RAGE density, it may become toxic, contributing to inflammation and cell death[58]. The protein has been shown to be upregulated in the frontal cortex and downregulated in the deep white matter of the anterior cingulate gyrus and the corpus callosum of schizophrenia patients[59]. Future research should evaluate whether inhibition of S100B can produce therapeutic benefits in schizophrenia, particularly in patient subgroups characterized by elevated S100B levels and / or high RAGE expression, and experimentally verify whether phenothiazines bind S100B *in vivo*.

MAO-B: High platelet levels of monoamine oxidase B (MAO-B) activity has been found to be associated with severe agitation in subjects with schizophrenia[125]. Pargyline, a MAO-B inhibitor, warrants investigation as a treatment for agitation. As an adjunctive treatment, pargyline is effective in alleviating depression, anergy, aberrant affect, mood, and self-confidence[126,127].

"Symptom treaters": Not all predicted compounds in our prediction set target the underlying mechanisms of delusions, hallucinations, and disorganized speech—the three positive symptoms required for a schizophrenia diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition[128]. In most patients, these symptoms are attributed to hyperactive dopaminergic signaling in the associative striatum. Antipsychotics mitigate this dysfunction by antagonizing downstream D2 receptors, thereby reducing the severity of positive symptoms[129]. However, a subset of our predicted compounds target negative or cognitive symptoms, and these may not be part of the primary diagnostic criteria. This pattern reflects the diversity of compounds associated with schizophrenia in our library. These compounds may possess polypharmacological profiles affecting multiple neurotransmitter systems. For example, the "symptom treating" compound pizotifen, an alleviator of depressive symptoms in patients with schizophrenia, appeared in the similarity lists of several associated antipsychotic drugs that were not symptom treaters themselves. These antipsychotics shared broad compound proteome interaction signature overlaps with pizotifen, particularly involving the 5-HT receptor family, adrenergic receptors, and muscarinic acetylcholine receptors[130–133]. Halazepam, a drug that has been reported to reduce anxiety and tension in schizophrenia patients, was included in the similarity lists of associated compounds also used to treat anxiety[134,135], rather than psychosis. These compounds exhibited interaction similarities with halazepam at GABA(A) receptors, the GABA(A) benzodiazepine binding site, and the translocator protein[136–140]. This limitation may, in another view, represent an advantage of the study, potentially facilitating the

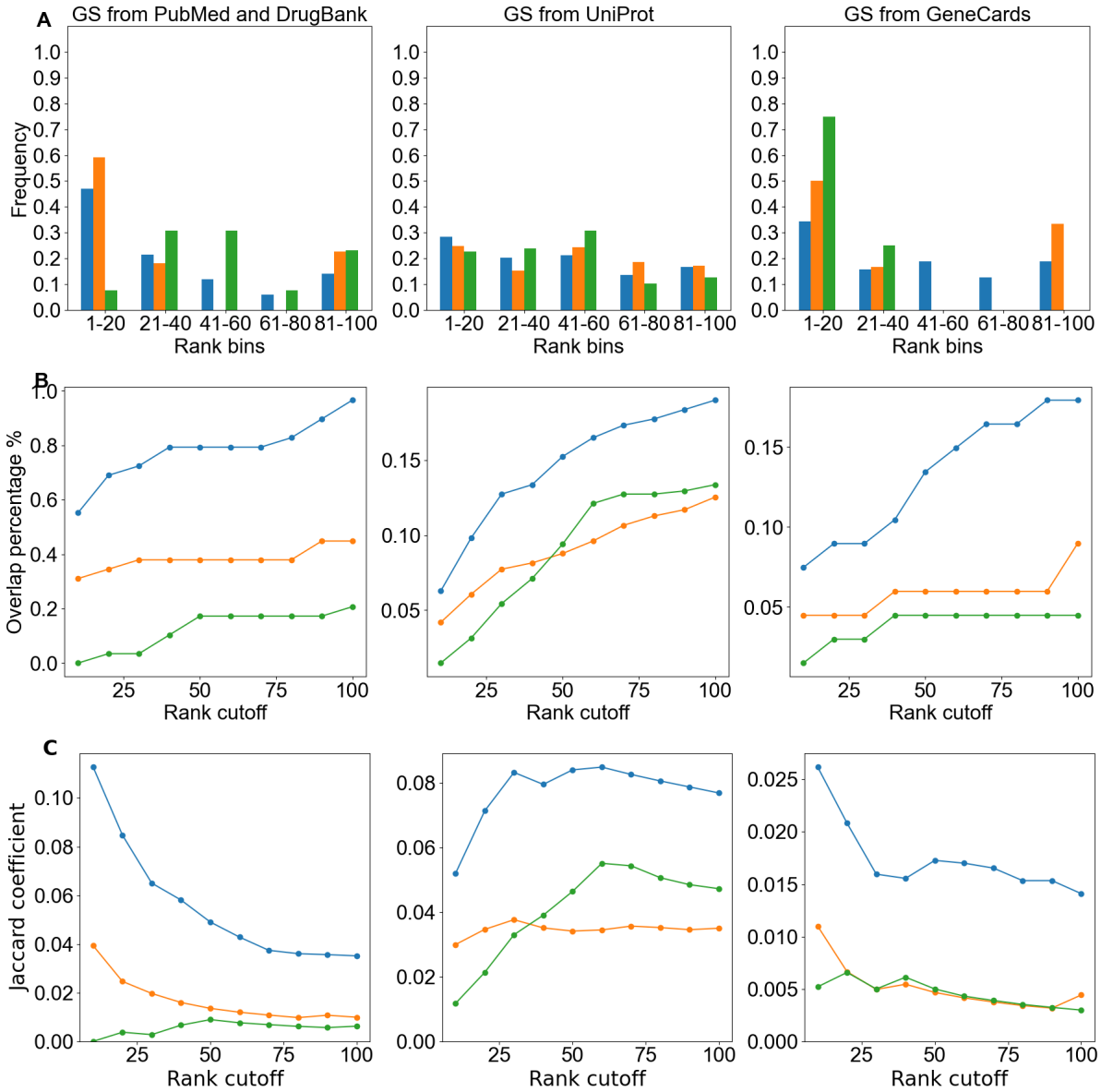

Figure 3. Overlap analyses between top targets of top predictions and controls, and gold standard sets (A) For all three gold standards, overlaps with top predictions are most enriched in the top rank bin (1–20), indicating that overlaps are not randomly distributed and that higher ranked predictions are more likely to be meaningful. (B) A greater percentage of targets from our top 25 predictions overlap with gold standards than the bottom and random controls across all rank cutoffs. The top 100 overlap % for top predictions is nearly 20% for both UniProt and GeneCards, while that of the controls stayed below 12.5% and about 9%, respectively. These results indicate that on the whole, our top predictions are more enriched for biologically meaningful targets, supporting the validity of the compound rankings generated by CANDO. (C) The JC demonstrates better performance across all rank values for top predictions compared to controls across all three GSs, reinforcing the biological relevance and consistency of CANDO's target rankings. The signal for top predictions is concentrated at the top ranks for PubMed/DrugBank and GeneCards, whereas progression into the lower ranks introduces increasing noise. In contrast to random or bottom-ranked sets, the clear enrichment of top targets in top predictions confirms that CANDO's rankings prioritize biologically relevant associations.

serendipitous discovery of therapeutics that address unmet needs or reveal novel pathways to treating certain symptoms. Future research could refine the schizophrenia indication in CANDO by subdividing it into symptom or domain specific subindications and clustering drugs accordingly. These clusters could be annotated using the NIMH Research Domain Criteria (RDoC)

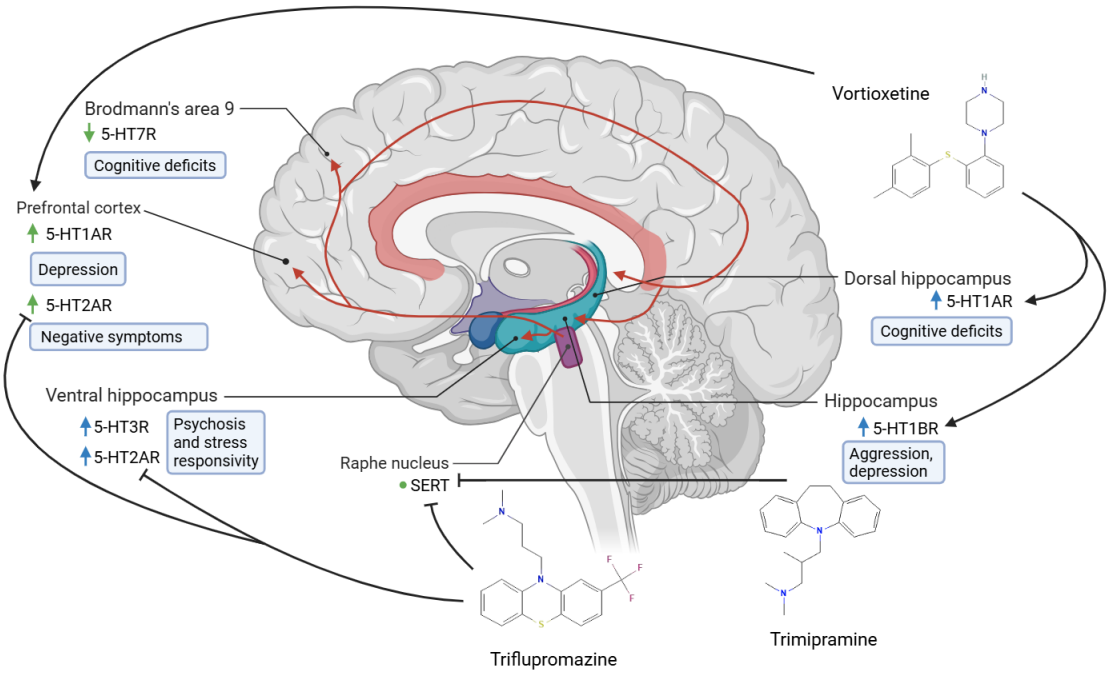

Figure 4. Serotonergic pathway abnormalities implicated in schizophrenia Green arrow: 2+ evidences corroborate expression change. Blue arrow: single evidence. 5-HT1A receptor upregulation in schizophrenia in the prefrontal cortex[81] as well as the dorsal hippocampus is indicative of low [5-HT]; in the latter region, serotonergic hypofunction is linked to cognitive deficits[82]. de Almeida et al found that A 5-HT1B receptor can have anti-aggressive effects[83], and it has been shown that 5-HT1B is more expressed in the hippocampus in schizophrenia[84]. Taken together, we formulate the hypothesis that 5-HT1B overexpression is symptomatic of low 5-HT, and that decreased 5-HT1B signaling causes the symptom of aggression. Prior research has shown that 5-HT1A, 5-HT1B, and 5-HT1D agonism mediates antidepressant efficacy[80]. The active conformation of 5-HT2A receptor is upregulated in antipsychotic-free schizophrenia patients in the prefrontal cortex[85,86]. The receptor is also hyperactive in the ventral hippocampus, where it is associated with psychosis and stress responsitivity, respectively. Triflupromazine, an atypical antipsychotic, antagonizes the 5-HT2A receptor and reduces negative symptoms[87]. SERT is expressed in the raphe nucleus[88,89]. Not shown in the figure: SERT blockers have activity at the axon terminals of serotonergic projections. Chronic treatment of SSRI leads to a decrease in 5-HT2A binding potential in young subjects[90]. Increased [5-HT] in the cortex as a result of SERT inhibitor administration may ameliorate 5-HT7 signaling deficits[91,92], but more research is needed to confirm this.

framework, as it categorizes symptoms along dimensions such as cognition, social processes, and arousal. This stratified approach would allow CANDO to generate more precise and clinically relevant drug predictions by capturing the heterogeneity of schizophrenia. It may also enhance benchmarking performance and increase the interpretability of overlap analyses by aligning predicted targets with distinct neurobiological domains.

Exclusion of investigational compounds: We used the library of approved drugs to prioritize compounds with established safety profiles for compound prediction. However, this choice excluded investigational compounds with potentially high therapeutic value. Future studies could expand the search space by incorporating both approved and investigational compounds, as well as virtual libraries of computationally designed molecules. These in silico compounds can be computationally screened, with the most promising candidates selected for synthesis and experimental validation[12].

Approved abroad and discontinued compounds: Our drug indication mapping excludes drugs not approved for schizophrenia in the U.S. but is associated with the indication in other regions such as Canada and Europe. Notable among these is our best ranking prediction, zuclopenthixol[141]. Also excluded are drugs that were once approved for schizophrenia but later discontinued for reasons unrelated to efficacy or side effects, including butaperazine and perazine[142–144]. Although these compounds could have been classified as schizophrenia associated in our drug indication mapping, it is validating that CANDO recovered them in strong positions within our predictions.

Other limitations: In generating the protein target lists for each compound, we used the top 100 highest ranking proteins without applying a score cutoff, potentially including weak or excluding strong interactions. Our interaction scoring protocol

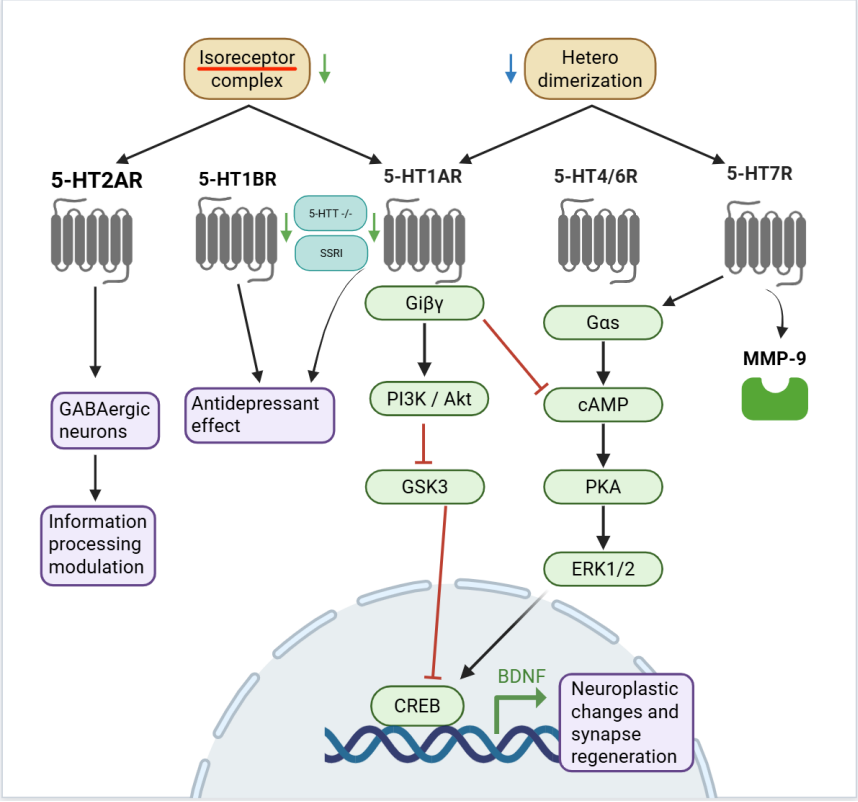

Figure 5. Key protein protein interactions and downstream pathways of 5-HT receptors involved in schizophrenia pathology and treatment. Green arrow: 2+ evidences corroborate expression change. Blue arrow: single evidence. 5-HT1A and 5-HT7 receptors form heterodimers, decreasing 5-HT1A receptor mediated activation of G(i) protein[93,94]. 5-HT1A and 5-HT2A form isoreceptor complexes, where agonism of 5-HT2A receptor reduces affinity of a 5-HT1A agonist for its binding site[95,96]. In 5-HTT-/- mice, 5-HT1A receptors in the dorsal raphe nucleus show reduced binding-site density/protein levels and decreased mRNA expression, while 5-HT1B receptors in the substantia nigra show decreased binding-site density and diminished G-protein coupling[97–99]. Chronic treatment with SSRI reduces 5-HT1A stimulated G protein activation and desensitizes terminal 5-HT1B autoreceptors[100–102]. Trimipramine and triflupromazine block serotonin transporter, increasing serotonin concentration in the synaptic cleft. Effects of elevation of 5-HT include activation of 5-HT1A receptor, 5-HT4 and 5-HT6 receptors, and 5-HT7 receptors[75,103,104]. Upon activation of 5-HT1A receptor, inhibitory G protein beta gamma complex (Gi$\beta\gamma$) activates the phosphoinositide 3-kinase / protein kinase B (PI3K/Akt) pathway, leading to the inhibition of glycogen synthase kinase 3 (GSK3). G3K3 inhibits cyclic AMP (cAMP) response element binding protein (CREB), a transcription factor that binds to and controls the expression of brain derived neurotrophic factor (BDNF), leading to long term neuroplastic changes and synapse regeneration[105–109]. Activation of 5-HT4 and 5-HT6 receptors stimulates the associated Gαs subunit. This, in turn, activates the cAMP/protein kinase A (PKA) pathway, leading to the activation of extracellular signal regulated kinases 1 and 2 (ERK1/2). ERK1/2 then activates CREB, promoting the expression of (BDNF)[106,110–112]. Both activation and blockade of the 5-HT7 receptor have been shown to have beneficial effects[113]. Activation of 5-HT7, coupled to Gαs, also leads to downstream BDNF activation[106,112]. However, activation of the 5-HT7R/MMP-9 signaling pathway has also been shown to induce depressive like behavior, while pharmacological *blockade* of 5-HT7R produces antidepressant effects[114]. Vortioxetine activates 5-HT1AR, as well as 5-HT1BR, both having been shown to mediate antidepressant efficacy[41,42,80]. These findings highlight CANDO's utility in identifying key targets and mechanistic pathways that align with current models of schizophrenia neurobiology. The predicted compounds have the potential to address not only positive symptoms but also the negative and cognitive domains. The context dependent therapeutic effects of both 5-HT7R activation and blockade underscore the complex pharmacodynamics of schizophrenia and reflect CANDO's strength in capturing such multifaceted interactions. Moreover, the implicated targets suggest opportunities for modulating neural circuits disrupted in schizophrenia, offering a systems level approach to treatment.

considers only protein structure and not the physiological or biochemical context where a protein operates *in vivo*; as a result, actual relative binding affinities may differ from our predicted rankings. Future research could incorporate additional data

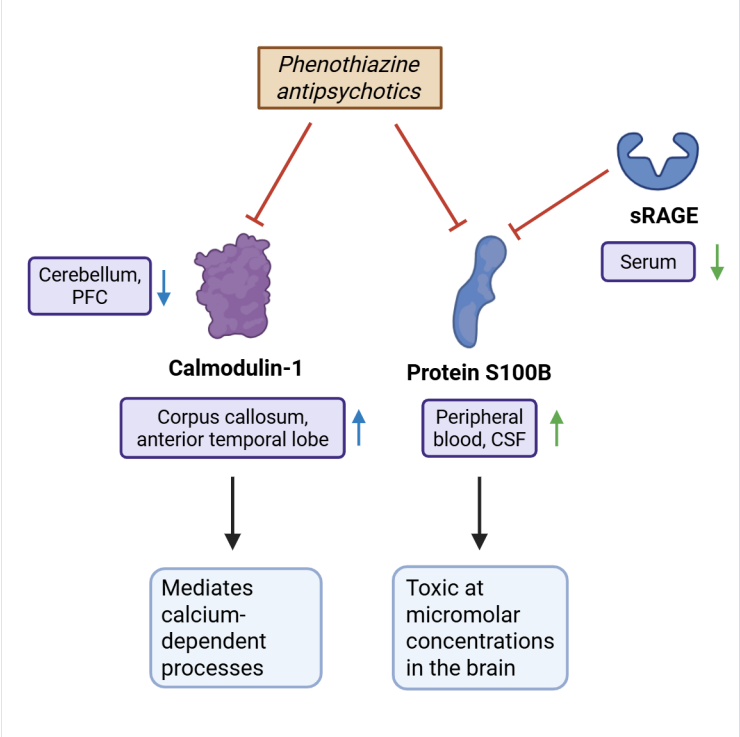

Figure 6. Corroborated phenothiazine antipsychotic interactions with potential therapeutic targets. Green arrow: 2+ evidences corroborate expression change. Blue arrow: single evidence. We investigated the effect pathways of two key interactions involving Calmodulin-1, the fourth ranked consensus target prediction in our phenothiazine set (Supplementary Materials S3) and Protein S100B, twelfth ranked. Dysregulation of calmodulin disrupts signaling cascades and could contribute to connectivity deficits in schizophrenia[57]. Calmodulin is decreased in the cerebellum and a loss of its active form is found in the prefrontal cortex[57,121]. It is also upregulated in the corpus callosum and anterior temporal lobe in schizophrenia, but it is unknown whether this is a cause or consequence of the disorder[57]. S100B has trophic effects at nanomolar concentrations and low cellular expression of RAGE, but may become toxic at micromolar concentrations and / or high RAGE expression[58]. Increased levels of it are found in the cerebrospinal fluid and serum in schizophrenia patients[122], while sRAGE, which sequesters and neutralizes S100B and reduces RAGE signaling, is found in reduced levels in serum[123,124]. CANDO's prediction of these corroborated targets supports the platform's validity and encourages further investigation of additional interactions. Created in BioRender. Hu, Y. (2025) https://BioRender.com/4eued7m

that reflects the *in vivo* state of the protein, such as tissue specific expression levels or protein conformational dynamics under physiological conditions.

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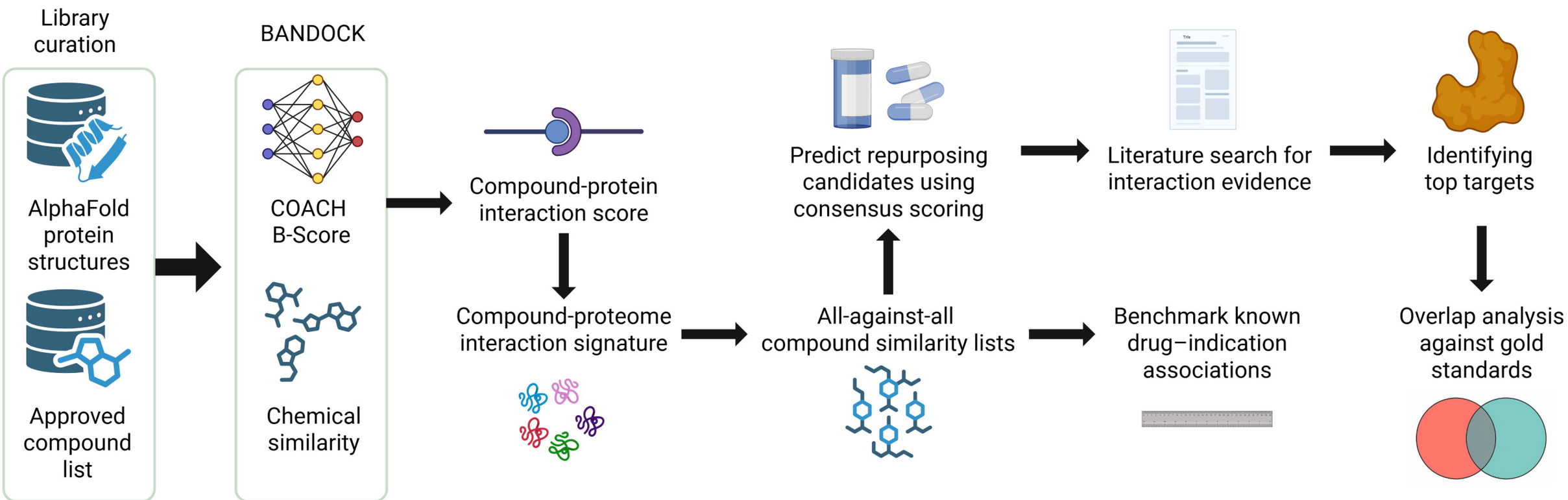
Author contributions statement

Y.H. performed the experiments, carried out the data analysis. Y.H., Z.F., and R.S. jointly contributed to the experimental design and interpretation. Y.H. drafted the initial manuscript, and all authors reviewed, edited, and approved the final version. R.S. supervised the project."

Additional information

The authors disclose that they formed multiple startup ventures engaged in the commercialization of outputs derived from the CANDO platform. The funders had no involvement in the study's conception or design, the acquisition, analysis, or interpretation of data, the preparation of the manuscript, or the decision to submit it for publication.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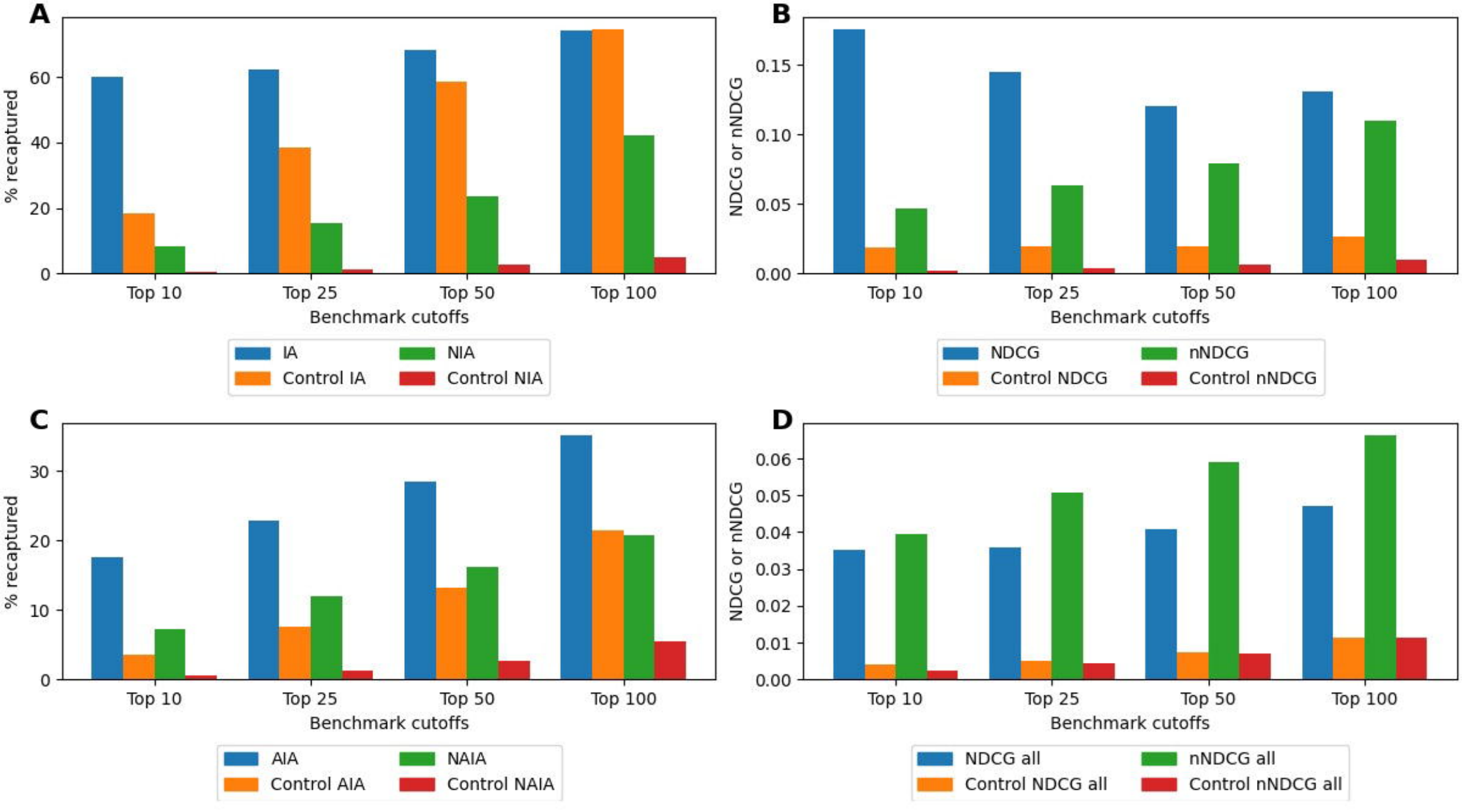
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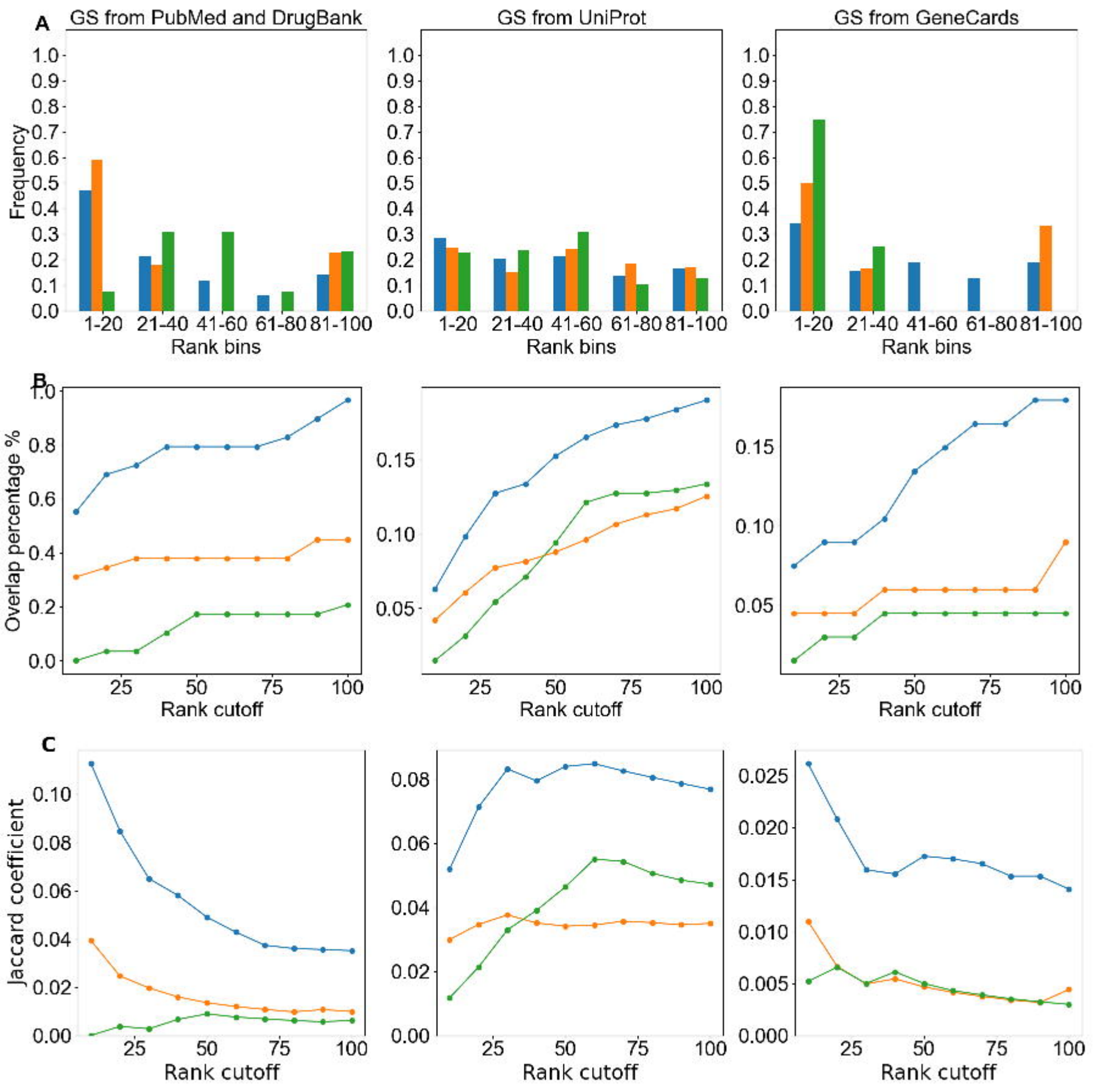
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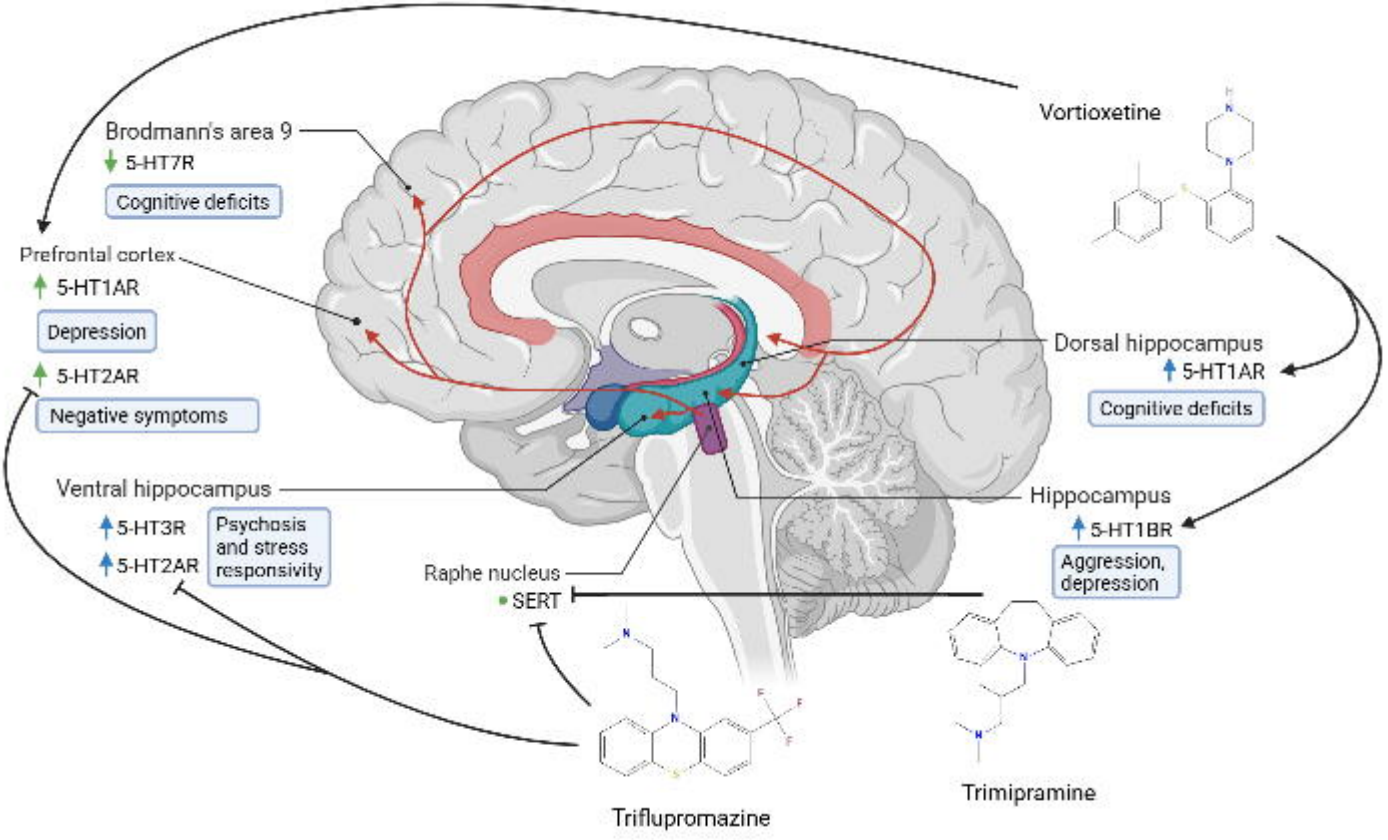
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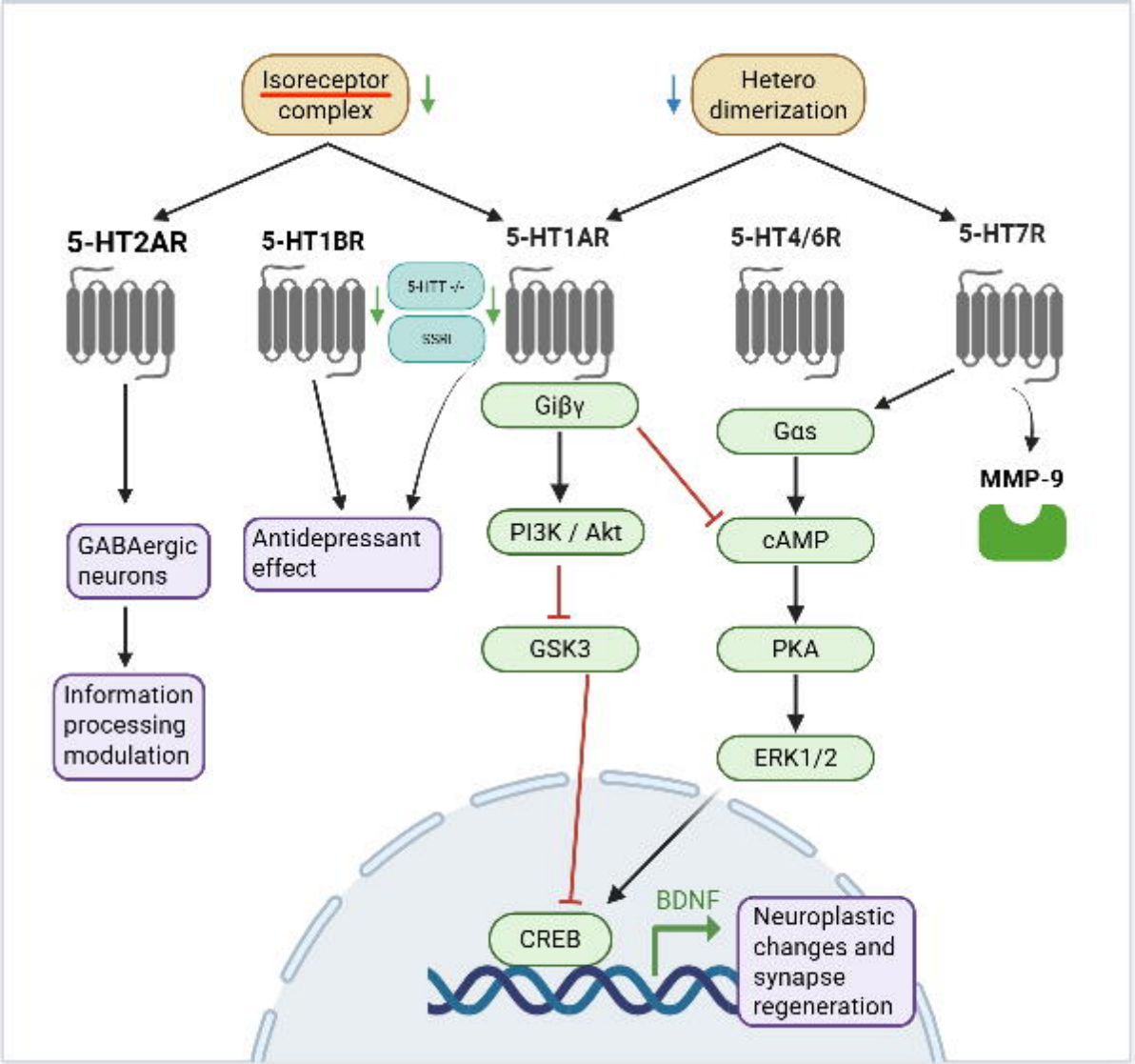
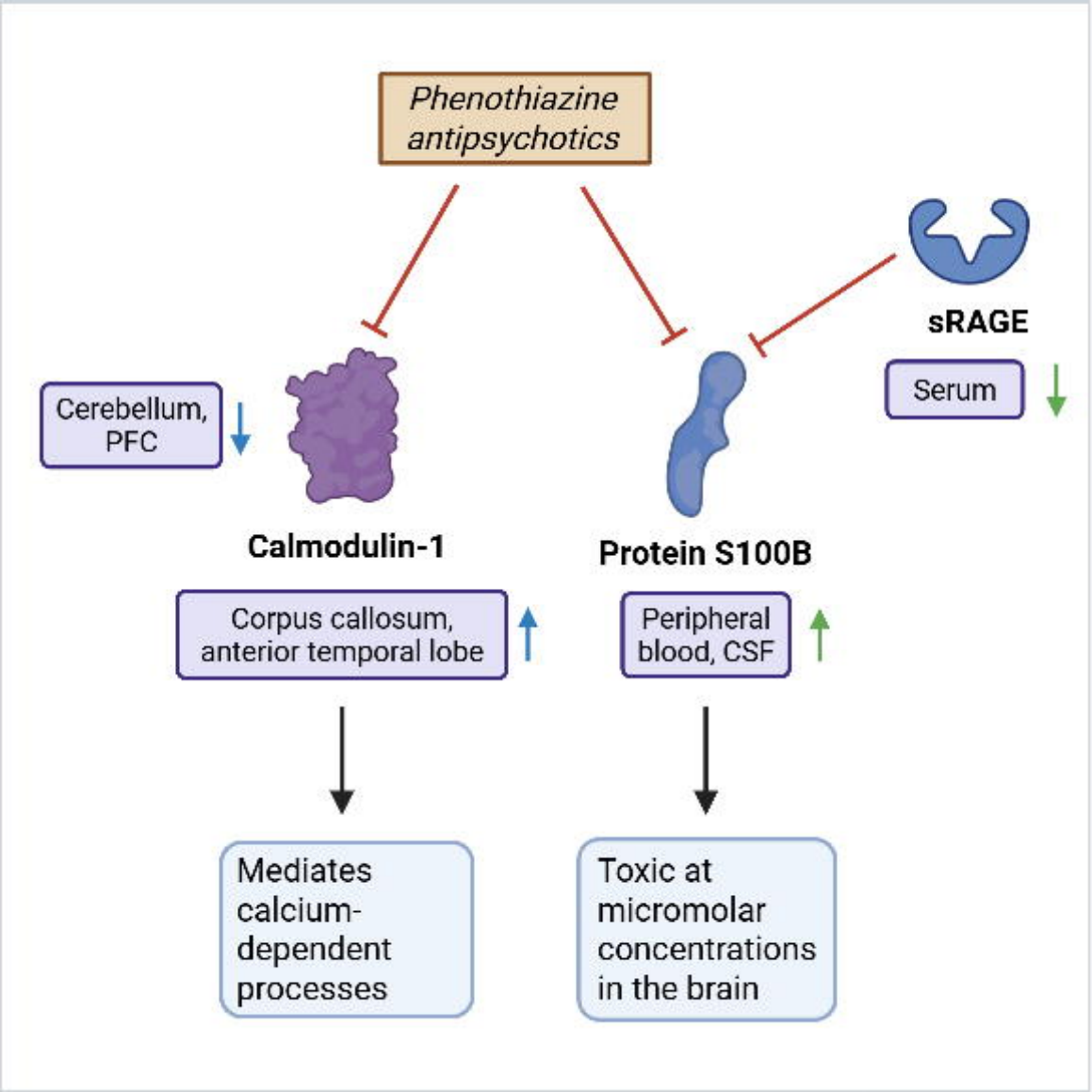
Cognitive deficits
Ventral hippocampus
5-HT3R
5-HT2AR
Psychosis and stress responsivity
Hippocampus
5-HT1BR
Aggression, depression
Raphe nucleus
SERT
Trifluproma zine
Trimipramine


Isoreceptor complex
Hetero dimerization
5-HT2AR
5-HT1BR
5-HT1AR
5-HT4/6R
5-HT7R
5-HTT -/-
SSRI
Giβγ
Gαs
MMP-9
GABAergic neurons
Antidepressant effect
PI3K / Akt
cAMP
GSK3
PKA
ERK1/2
Information processing modulation
BDNF
CREB
Neuroplastic changes and synapse regeneration


Phenothiazine antipsychotics
sRAGE
Serum
Cerebellum, PFC
Calmodulin-1
Protein S100B
Corpus callosum, anterior temporal lobe
Peripheral blood, CSF
Mediates calcium-dependent processes
Toxic at micromolar concentrations in the brain