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Shape-Based Reprofiling of FDA-Approved Drugs for the H_1 Histamine Receptor

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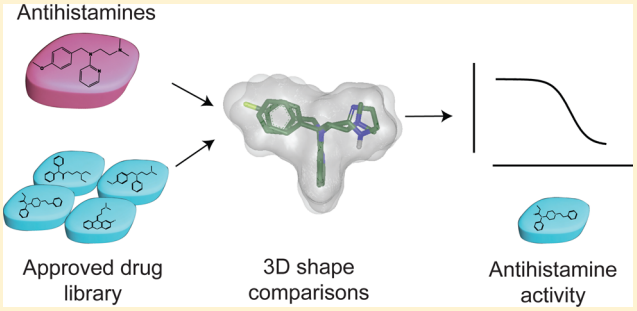
Supporting Information

ABSTRACT: Reprofiling of existing drugs to treat conditions not originally targeted is an attractive means of addressing the problem of a decreasing stream of approved drugs. To determine if 3D shape similarity can be used to rationalize an otherwise serendipitous process, we employed 3D shape-based virtual screening to reprofile existing FDA-approved drugs. The study was conducted in two phases. First, multiple histamine H_1 receptor antagonists were identified to be used as query molecules, and these were compared to a database of approved drugs. Second, the hits were ranked according to 3D similarity and the top drugs evaluated in a cell-based assay. The virtual screening methodology proved highly successful, as 13 of 23 top drugs tested selectively inhibited histamine-induced calcium release with the best being chlorprothixene (IC_{50} 1 nM). Finally, we confirmed that the drugs identified using the cell-based assay were all acting at the receptor level by conducting a radioligand-binding assay using rat membrane.



INTRODUCTION

In light of the increasing costs of drug development and ever-decreasing stream of newly approved drugs, finding novel uses for existing drugs is rapidly gaining popularity. This process, termed reprofiling (also known as redirecting, repositioning, or repurposing),[1] has several advantages over de novo drug development. The expedited bench-to-clinic translation and the potential to address serious illnesses for which treatments have not been developed are unparalleled by any alternative drug discovery approach. Several examples of successful reprofiling exist.[1] Miltefosine, for example, failed phase II clinical trials for tumor reduction but was later discovered to possess anti-infective properties in in vitro and in vivo studies and subsequently passed clinical trials for treating visceral leishmaniasis.[2] Given the vast potential of reprofiling, several approaches are being taken to move reprofiling finds from those of a serendipitous nature to a rational scientific discovery. These include approaches based on transcriptional responses,[3] medicinal chemistry,[4] chemoinformatics,[5] chemical genomics,[6] side effect similarity,[7] molecular topology,[8] and phenotypic screening.[9] Although effective, these approaches require much effort and expertise.

To date, a few computational methods have been described to be of use in reprofiling and to predict adverse drug reactions. These include structure-based approaches such as molecular docking,[10] ligand-based chemoinformatic approaches such as SEA,[5b] bayesian models,[6b] and FEPOPS descriptors.[5b] However, to our knowledge, no study has employed a pure 3D shape-based screening method for reprofiling. As some compounds that do not appear to have commonality in their 2D chemical scaffold will overlap significantly in three-dimensional space and possess similar bioactivity,[11] we determined if 3D shape comparisons can be used as a meaningful method to search for drug activity within the FDA-approved drug database. In order to explore this idea, we compared the shape characteristics of H_1 antihistamines with a database of FDA-approved drugs to identify drugs with previously unknown antihistamine activity. The shape-based screening was not only accurate in picking many of the previously reported drugs that acted on H_1 receptors but also identified drugs including those previously not reported to act on these receptors. These results demonstrate the validity of using a simple measure such as 3D shape similarity in uncovering previously unknown drug activity.

RESULTS AND DISCUSSION

Our aim was to test if computational shape-based screening could be used to predict the additional effects that a drug might have on targets not originally known, antihistamine activity in this instance. In order to test this hypothesis, we compared the shape characteristics of H_1 antihistamines with a database of 1216 FDA-approved drugs from the U.S. Environmental Protection Agency's DSStox public database.[12] The H_1 receptor was chosen because of a large body of information detailing the actions of various other pharmaceutical agents on this target, which in turn would allow us to validate our results. The FDA database was chosen as it consists of compounds with well-characterized activity in established drug classes, which would allow us to convey more concretely the degree of enrichment the virtual

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screening method confers through the number of classified antihistamines that rank highly.

As a starting point, we evaluated the ability of our protocol for known antihistamines to enrich other known antihistamines from the database. If the method indeed provided enrichment, antihistamines would cluster at the top of the ranking instead of being randomly distributed. Such enrichment implies substantial efficiency gains over random screening. We chose 13 antihistamines from a literature search. As the bioactive conformers of the antihistamines are not known, Omega (version 2.0, OpenEye scientific software)[13] was used to generate up to 100 conformers for both the query antihistamines and the database. An upper limit of 100 was chosen as a previous study reported similar results with databases of either 100 or 1000 conformations.[14] We compared shapes with the program Rapid Overlay of Chemical Structures (ROCS version 2.3, OpenEye scientific software).[15] The output molecules resulting from the ROCS run were ranked by their 3D Tanimoto coefficient, a metric for molecular shape similarity.[16]

To determine if the shape-based protocol alone enriched antihistamine compounds, the top 1% (12 drugs) and 3% (40 drugs) of the database ranked according to shape Tanimoto by the 13 query antihistamines were analyzed for the presence of known antihistamines. Shape Tanimoto scores[11a,17] were used to perform these analyses. Approximately a third of the compounds at the top 1 and 3% were found to be classified antihistamines (Figure 1). When the scoring included the color component of ROCS, an improvement of approximately 10% was observed with regard to enrichment of known antihistamines. Thirty out of a total 1216 drugs in the FDA database are classified as antihistamines acting at the H_1 receptor subtype. This represents 2.4% of the complete database. The enrichment by ROCS represents an enrichment factor of approximately 10-fold when compared to the random distribution of 2.4%. These results demonstrate the nonrandom nature of our method in detecting compounds with similar 3D shape and thus potential similarity in biological activity. In addition to enriching classified antihistamines, the top hits included classes of drugs known to have antihistaminic effects, such as antidepressants, further adding validity to this approach. On average, five of the hits in the top 40 drugs of the database ranked by shape Tanimoto score were antidepressants. As many of the antidepressants were developed by modifying the core structure of histamine, this may not come as a surprise.[18] However, the fact that ROCS identified drugs such as ketanserin (which was initially developed as a selective 5-HT_{2a} antagonist and is structurally unrelated to histamine but was later found to possess antihistaminic activity)[19] demonstrates that shape-based screens can be used to credibly reprofile compounds within the FDA database.

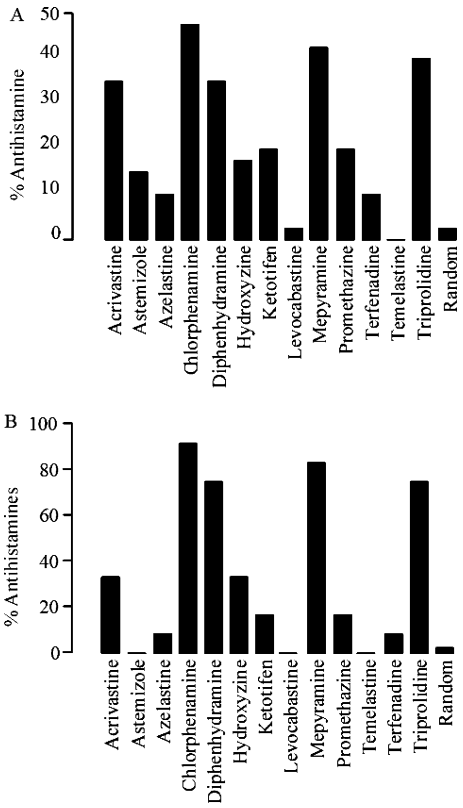


Figure 1. Three-dimensional shape-based screen identifies known antihistamines in the FDA database. Known antihistamine hits in the (A) top 1% and (B) top 3% of the ranked output. The figure illustrates the ability of a query antihistamine to identify other antihistamines from the database and illustrates the variability in shape profile of various antihistamines.

Following the initial validation screen using ROCS the following considerations were used to purchase the top ranking compounds:

1. Possessed a shape Tanimoto score of over 0.80 and appeared more than three times in the 13 runs conducted with the selected antihistamines or appear at least 3 times when combo Tanimoto score was used.
2. Are not known to possess antihistaminic or antidepressant properties (which have broad implications for activity at the H_1 receptor).
3. Are not substructures of the query drug used for the initial shape-based screening.

On the basis of these criteria, we purchased 23 compounds for biological testing. A Tanimoto cutoff of 0.8 was chosen for collation, as we wanted to ensure accurate normalized scores that would reflect the potential to be bioactive while maintaining a reasonable number of compounds to test. The disparity in different antihistamines retaining hits with Tanimoto scores above 0.8 demonstrates that each antihistamine was not equal in its ability to discover potentially bioactive compounds (Figure 2). Therefore, we decided that a compound should be enriched by at least three different antihistamines.

Since the histamine H_1 receptor has a number of ligands,[20] we were able to exploit this rich drug space through group fusion.[21] This approach can be used when structurally diverse compounds known to be active at a certain target are compiled as reference structures. Not every novel drug discovery project seeks to target such a "druggable" receptor. Does this mean we would not have been able to do this if we had only one antihistamine to begin our search with? The answer to this depends entirely on which antihistamine one chooses as a query. The number of antihistamines identified in the top 3% of our database by every other antihistamine is presented in Figure 1A,B. This demonstrates that some antihistamines are more representative of common chemical features expressed in the class than others. In other words, if we had chosen chlorphenamine as our query, we would have ended up with partly similar results to the data presented in the remainder of this article. On the other hand, if we had chosen temelastine as our only query, we would have ended up with a very different hit list from the data presented.

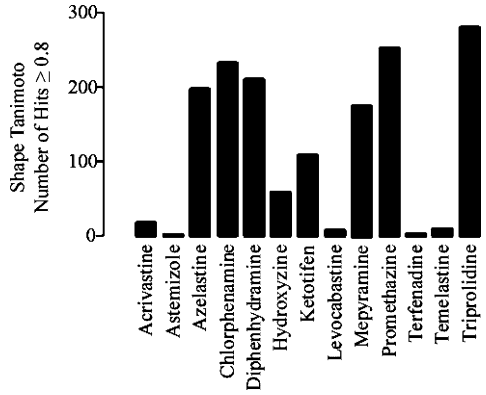


Figure 2. Effect of antihistamine query molecule on retrieval of hits. Plot shows number of known antihistamines retrieved with a shape Tanimoto score over 0.8. The different antihistamines show a varying degree of shape similarity with the molecules in the database; Tripolidine retrived 281 molecules with a shape Tanimoto over 0.8, while astemizole retrieved only itself.

In order to probe for antagonism at the H_1 receptor, we screened the 23 compounds for antagonist activity at 100 μM in HeLa cells, by quantifying their inhibition of the response to histamine at its EC_{50} concentration (2.5 μM). Followed by this, the drugs' selectivity was verified by probing the P2Y receptor by the addition of the EC_{50} concentration of ATP (1 μM). Representative fluorescence results in Figure 4 illustrate various

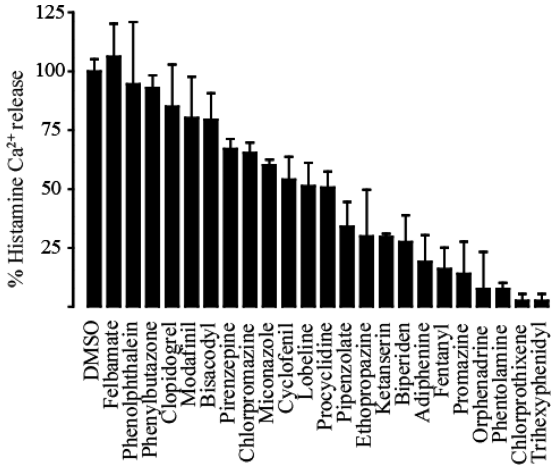


Figure 3. Biological screening of the top ranking virtual hits. Results confirm effective inhibition by known antihistamines and identify novel antihistamines. HeLa cells grown in 96-well plates were loaded with fura-2AMm and the corresponding Ca^{2+} response to histamine (2.5 μM) was measured in the presence and absence of the 23 top ranking hit compounds (100 μM). Data are mean ± SEM (n = 6−9).

scenarios possible during the experiments. As can be seen from Figure 3, in total, 16 drugs significantly reduced histamine-induced intracellular calcium release (one-tailed t test, $P < 0.05$). Of these, only biperiden significantly reduced intracellular calcium release in response to ATP (Figure 5) and was therefore not included in further studies. Thirteen drugs that reduced the basal histamine response in excess of 50% were pursued further to determine the mode of inhibition. This demonstrates the selectivity of the VS hits for the histamine H_1 receptor, and these results stand in stark contrast to those obtained for the P2Y receptor.

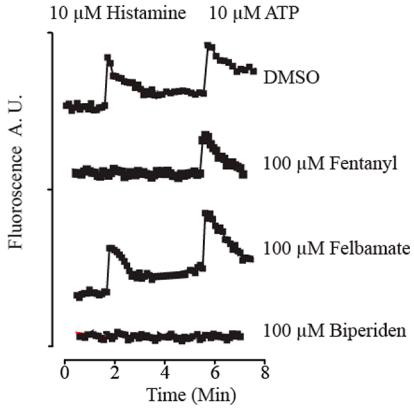


Figure 4. Representative traces of the biological responses to the virtual hits. HeLa cells grown in 96-well plates were loaded with fura-2AM, and the corresponding Ca^{2+} response to histamine and ATP was measured in the presence and absence of indicated compounds (100 μM dose). Felbamate is an example of a high-scoring drug that failed to block histamine and did not interrupt ATP-induced Ca^{2+} release. Fentanyl, by contrast, effectively blocks histamine-induced Ca^{2+} release without blocking ATP in a manner validating the virtual screening process. Biperiden inhibits both receptor-based responses.

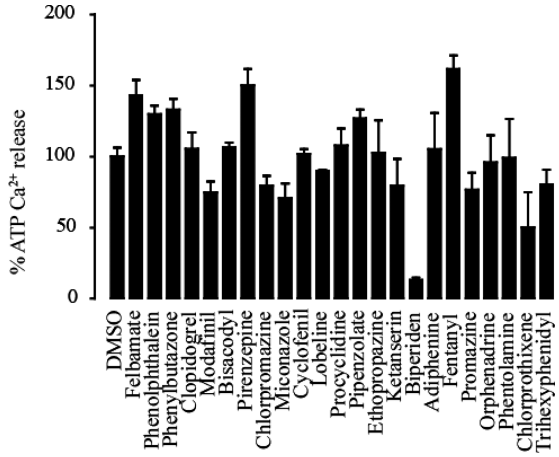
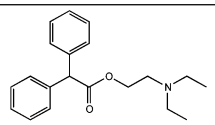
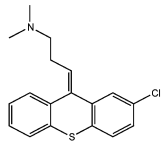
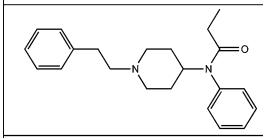
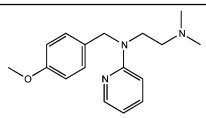
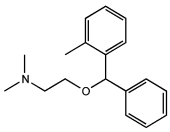
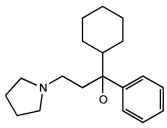
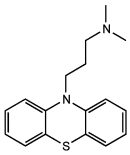
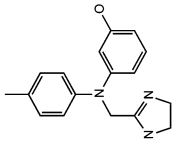
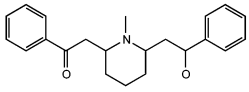


Figure 5. Antihistamines identified by virtual screening do not block ATP-induced signaling. HeLa cells grown in 96-well plates were loaded with fura-2AM, and the corresponding Ca^{2+} response to ATP (10 μM) was measured in the presence and absence of the 23 top ranking hit compounds. Data are mean ± SEM (n = 6−9).

Since our hypothesis was that 3D shape screens can be used as a tool to find approved drugs with a similar mechanism of action as existing antihistamines, it was important to verify that all of these inhibitors block histamine responses in a dose-dependent and reversible fashion. In order to verify this, a full concentration−response curve was performed in the presence of varying concentrations of the compound and 2.5 μM histamine. The concentration−response curves for the eight drugs (Table 1) that displayed dose-dependent inhibition are shown in Figure 6. Of the 13 compounds selected from the initial screen, seven were excluded as they showed less that 50% inhibition at the concentrations tested. Next, to determine if these compounds acted at the same site as the known antihistamines, the ability of these drugs to compete with [^{3}H]mepyramine for binding to the histamine H_1 receptor through the established rat brain membrane preparation[22] protocol was investigated. This approach validated our previous

Table 1. Details of the Active Compounds from the Virtual Screening

Structure	Drug	Original target (therapeutic indication)	Log binding IC_{50} (M)[a]	Log Ca^{2+} release IC_{50} (M)[b]
	Adiphenine	nACh antagonist (Antispasmodic)	-4.77	-3.95
	Chlorprothixene	5-HT_2 antagonist (Antipsychotic)	-8.97	-6.06
	Fentanyl	μ-opioid agonist (Narcotic analgesic)	-4.7	-3.83
	Mepyramine	Antihistamine	-8.24	-7.33
	Orphenadrine	mACh antagonist (Antispasmodic)	-6.79	-4.01
	Procyclidine	mACh antagonist (Parkinsonism)	-5.17	-4.75
	Promazine	Unknown-multiple (Antipsychotic)	-5.9	-5.61
	Phentolamine	α-Adrenergic antagonist (Acute hypertension)	-4.9	-3.5
	Lobeline	Unknown-multiple (Smoking cessation)	-4.95	-4.35

[a]Half maximal inhibitory concentrations of drugs for the histamine receptor in rat brain membrane [^{3}H]mepyramine binding assay. [b]Half maximal inhibitory concentrations of drugs for the histamine receptor in the HeLa cell Ca^{2+} release assay.

finding obtained via the Ca^{2+} release assay, in that all drugs competed fully at the [^{3}H]mepyramine binding site (Figure 6) with the most potent being chlorprothixene with a binding IC_{50} of 1 nM and lobeline, which to our knowledge has never been predicted or experimentally demonstrated to act on histamine receptor (Table 1). Finally, in order to evaluate if the compound identified by shape similarity could have been identified using a simpler 2D substructure searching, a substructure search was performed using inbuilt substructure search function in Canvas 1.2. It was observed that only Orphenadrine was identified as a hit, furthering the validity of using our shape-based screening protocol.

With enormous effort being directed toward crystallizing the human proteome, it is conceivable that several crystal structures of membrane proteins or otherwise would become available in the future.[23] If cocrystals of the protein with query ligands used for the shape-based virtual screening become available, such as the newly discovered H_1 receptor,[24] we would hypothesize that incorporating this information to postprocess results from ROCS might provide one with better lead candidate selection. We believe this improvement would mainly be provided by (1) avoiding molecules that sterically clash with the protein and (2) eliminating molecules that have significantly different pose in comparison to the cocrystallized query. Such a dual-layered approach has previously been attempted and resulted in identification of an entirely novel class of active molecules.[25] It should be noted, however, that non-availability of structural data should not be seen as a handicap; especially where inhibitors for a

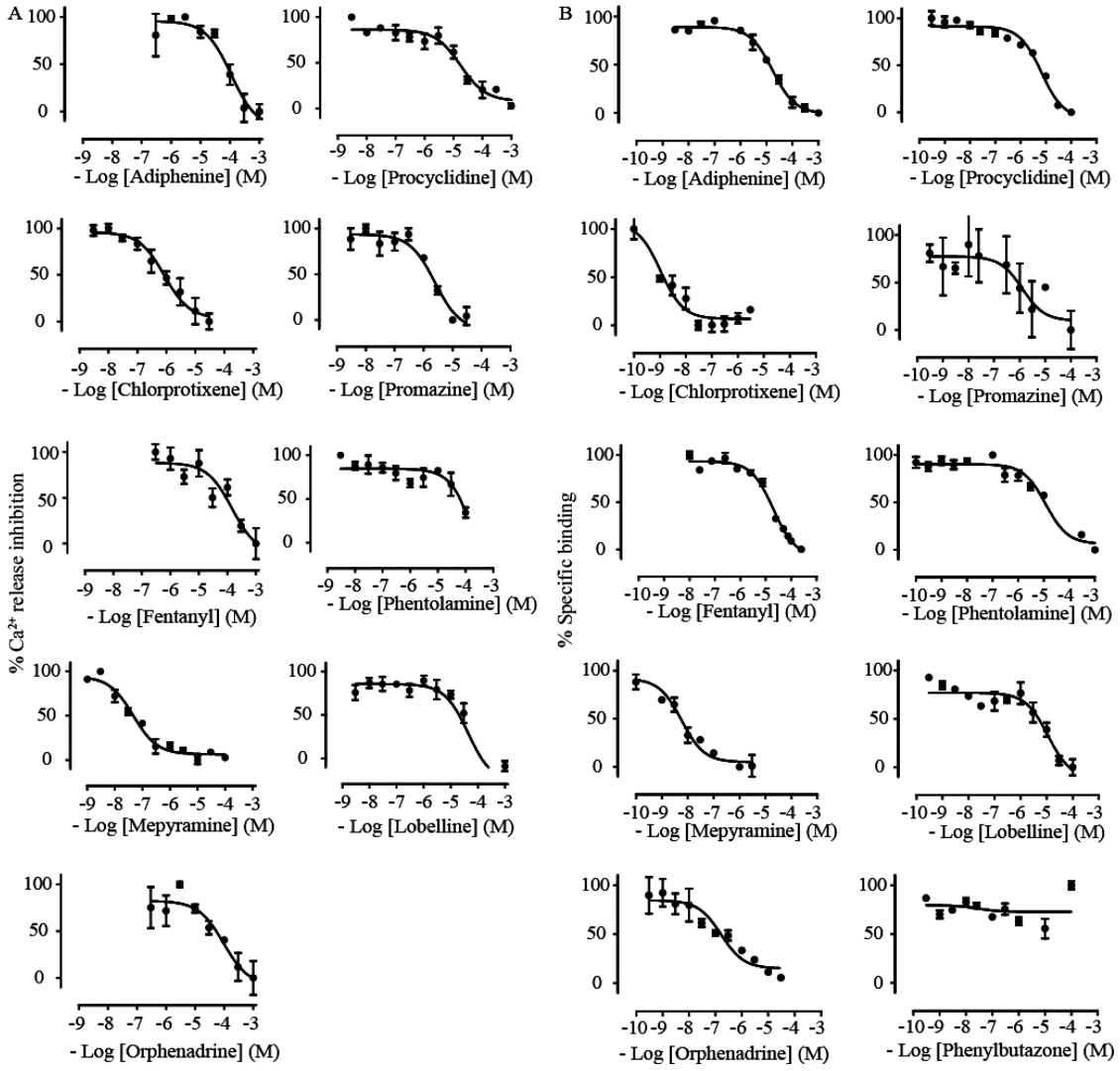


Figure 6. Validation that the hits act competitively at the histamine H_1 receptor. (A) Concentration response curves demonstrating the effect of indicated compounds on histamine-mediated Ca^{2+} release. Data are mean ± SEM (n = 3−4). (B) Concentration response curves demonstrating the effect of indicated compounds on [^{3}H]histamine binding. Data are mean ± SEM (n = 3−6).

protein exist, previous studies directly comparing ROCS to commercially available docking suites have demonstrated that the shape-based method performs at least as well as the docking studies.[26] We believe the primary attraction in the shape-based approach lies in the fact that hits obtained by any other means could be compared to FDA-approved drugs in the absence of either protein data or complex chemical pharmacophore representations and thereby provide researchers a significantly valuable additional resource for reprofiling.

■ CONCLUSION

In summary, we tested the hypothesis that 3D molecular shape could be used to find drugs that were not classified antihistamine but could act on the histamine receptor. We have shown that shape can predict antihistaminic activity among a range of unrelated drugs. Our results not only demonstrate the effectiveness of our protocol in identifying compounds with antihistaminic activity, but we also confirm the direct effect of various drugs previously speculated to act on histamine receptors[27] by demonstrating their effect in various biological preparations.

It is notable that other efforts to identify novel antihistamines have shown results consistent with our study. For example, Duart et al.[8] used a mathematical model specifically developed for competitive histamine antagonists (molecular topology) to predict antagonists of histamine H_1 receptor in a database of 20 000 compounds. The top 70 molecules were investigated for reported antihistaminic activity in a literature search, and this list features chlorprothixene and promazine, both of which were identified and biologically verified in our study. In the literature, chlorprothixene was shown to suppress histamine-induced bronchospasms in guinea pig, antagonising the histamine H_1 receptor,[28] and promazine was shown to block histamine-induced ileum contraction.[29] Fentanyl was also predicted to have antihistaminic activity, of which to date there has been no reported experimental observation. Lobeline, which we identify as a histamine antagonist, to our knowledge has never been predicted or experimentally verified to possess antihistaminic activity. For the first time, we are now able to show this at both the computational as well as biological level.

In this paper, we have presented the application of prospective computational science, validated by experimental biology, to provide useful insight into approaches for ligand identification or

in drug repositioning. This method can easily be translated to other targets and has an added advantage in being incredibly fast, simple, and economically viable. Therefore, our approach opens up a range of possibilities in reprofiling drugs for other targets.

EXPERIMENTAL SECTION

Virtual Screening. We performed virtual screening on a computer running a single Intel Core 2 Duo processor central processing unit running Windows Vista Ultimate operating system with software from OpenEye (OpenEye Scientific Software), Schrodinger (Schrodinger LLC), ChemAxon (http://www.chemaxon.com), and a database (FDAMDD version 3b) containing 1216 FDA-approved compounds downloaded from the U.S. Environmental Protection Agency's DSSTox public database (http://www.epa.gov/ncct/dsstox/DataFiles.html).[12] From a literature search, 13 antihistamines were selected as queries for conducting the 3D shape comparisons (mepyramine, chlorphenamine, triprolidine, temelastine, diphenhydramine, promethazine, astemizole, hydroxyzine, terfenadine, acrivastine, levocabastine, ketotifen, and azelastine). The antihistamines were drawn and energy-minimized with the Merck Molecular Force Field 94 using the ChemBioOffice Ultra 11.0 (CambridgeSoft Corporation, Cambridge, MA, USA). OMEGA[13] was used to generate 100 conformations for each of the query compounds and the compounds in the FDA database to enable shape comparisons. Rapid Overlay of Chemical Screens (ROCS 2.3) was used for three-dimensional shape comparisons, and all resulting hits were ranked with shape Tanimoto. In order to evaluate the 2D substructure similarity, the inbuilt function in Schrodinger Canvas 1.2[30] was used. ChemAxon Instant JChem, version 5, 2008, was used for structure database management.

Hit Ranking and Selection. Hits with a shape Tanimoto ≥0.80 were compiled for further evaluation. Antihistamines and antidepressants (which have broad implications for activity at the H1-receptor) were eliminated from the ranking. Drugs that appeared in the output files of at least three antihistamines were incorporated into a separate database. The shape Tanimoto values in each file were then summed and normalized according to the number of antihistamine ROCS files in which the drugs appeared. For the shape screen, all drugs with normalized shape Tanimoto values ≥0.85 across the spectrum of antihistamines were considered for purchase. For the color screens, the FDA drugs were scored against each antihistamine based upon a combined Tanimoto score consisting of shape Tanimoto and color Tanimoto score representing chemical similarity, which consists of a numerical sum of the shape Tanimoto and scaled color Tanimoto scores. Drugs appearing at least three times in the top 300 hits of the antihistamine files were normalized by dividing the summation of the combined Tanimoto scores by the number of times it appeared in the top 300 hits. The top 40 drugs were sorted and ranked. Drugs classified as antihistaminic (to include antipruritics that contain histamine-blocking compounds) and antidepressants (long known to exert antihistaminic actions) were excluded. Five drugs (orphenadrine, ketanserin, ethopropazine, promazine, and chlorpromazine) with known antihistaminic activity but not formerly classified as antihistamines were ordered for reprofiling validation in the assays. Once ranking was complete, 23 compounds in total were ordered based upon their previously reported or unreported activity, overlap between the shape and color algorithm, and availability. The drugs were dissolved in Ca^{2+}-free Hank's balanced salt solution (HBSS) or 10% DMSO.

Biological Testing in HeLa Cells. Compounds were tested for their ability to antagonize the Ca^{2+} release induced by histamine in HeLa cells grown in Dulbecco's modified Eagle medium containing 10% fetal bovine serum at their EC_{50} concentration, 2.5 μM (3−4 replicates). Ca^{2+} measurements were performed on confluent cells growing in 96-well plates by incubating with 5 μM fura-2-acetoxymethylester for 45 min at room temperature. The fluorescence measurements were performed using a Novostar plate reader (BMG LABTECH Ltd.). Following the initial screens for histamine antagonism, the compounds were further subjected to a secondary screen to probe their ability to block ATP-induced Ca^{2+} release at their EC_{50} concentration (10 μM). Compounds that passed the initial screens were subjected to a complete dose response analysis. For those drugs demonstrating antagonism of histamine-induced calcium release, half maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism. To characterize the nature of antagonism, the concentration of the drugs was maintained at their EC_{50} concentration reported from the previous step, and the concentration of histamine was varied.

Radioligand Displacement Binding Assay. For confirmation of drug activity at the level of the H_1 receptor, radioligand binding assays with [^{3}H]mepyramine were performed (3−6 replicates). Adult male Sprague−Dawley rat brains were collected by sacrificing the animals with cervical dislocation following unconsciousness induced with a rising concentration of CO_2. The brains were immediately obtained and processed according to the method established by Hill and colleagues.[22] Two milliliters of brain membrane preparation in Na−K phosphate buffer was added to a range of concentrations of hit compounds to give a final concentration of 0.5 mg protein/aliquot. After incubation for 10 min, [^{3}H]mepyramine was added to give a final concentration of 2 nM. Following 2 h of sample incubation, radioactivity was measured by scintillation counting.

Materials. All salts, physiological buffers, and drugs were purchased from Sigma Aldrich (St. Louis, MO, USA), unless otherwise specified. Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum were purchased from Invitrogen (Grand Island, USA). The GF/B filter was purchased from Brandel (Gaithersburg, USA).

ASSOCIATED CONTENT

Supporting Information

Additional virtual screening information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

†These authors contributed equally to the work.

Author Contributions

S.R.V. and G.C.C. conceived the project. S.R.V. and J.B.M. performed the virtual screening. S.R.V., J.B.M., and Y.S. performed the Ca^{2+} release assays. S.R.V. and J.B.M. performed the [^{3}H]mepyramine binding assay. S.R.V. wrote the manuscript with input from J.B.M. and G.C.C. All authors reviewed and commented on the edits.

Notes

The authors declare no competing financial interest.

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