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Using Molecular Initiating Events to Generate 2D Structure Activity Relationships for Toxicity Screening

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KEYWORDS: Molecular Initiating Event (MIE), Structure Activity Relationship (SAR), Adverse Outcome Pathway (AOP), Human Toxicology, Risk Assessment.

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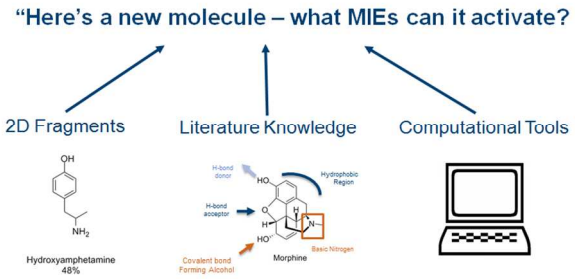

ABSTRACT

Molecular initiating events (MIEs) can be boiled down to chemical interactions. Chemicals that interact must have intrinsic properties that allow them this behavior, be these stereochemical, electronic or otherwise. In an attempt to discover some of these chemical characteristics we have constructed structural alert-style structure activity relationships (SARs) to computationally predict MIEs. This work utilizes chemical informatics approaches, searching the ChEMBL database for molecules that bind to a number of pharmacologically important human toxicology targets, including G-protein coupled receptors (GPCRs), enzymes, ion channels, nuclear receptors, and transporters. By screening these compounds to find common 2D fragments, and combining this approach with a good understanding of the literature, bespoke 2D structural alerts have been written. These SARs form the beginning of a tool for screening novel chemicals to establish the kind of interactions they may be able to make in humans. These SARs have been run through an internal validation to test their quality and the results of this are also discussed. MIEs have proven to be difficult to find and characterize but we believe we have taken a key first step with this work.

INTRODUCTION

The molecular initiating event (MIE) has been defined as a key chemical event in toxicity leading to adverse outcomes through adverse outcome pathways (AOPs).[1,2] With its basis strongly founded in chemistry, the MIE makes a good starting point for the development of *in silico* tools, such as structure activity relationships (SARs). In order for a chemical (or metabolite/breakdown product) to cause an effect *via* an MIE it must conform to specific

chemical characteristics: those which allow it to bind to a receptor, inhibit an enzyme, or modify a protein. An MIE is essentially a chemical interaction. The links between these chemical characteristics and the MIE will undoubtedly be stronger than links to a toxicological endpoint, which is much further down the pathway.

The AOP framework for risk assessment brings together chemical and biological understanding in an attempt to develop predictive methods for human and environmental toxicology.[3,4] AOPs span multiple levels of biological organization, and as such a large amount of knowledge is required to make an AOP risk assessment. This will consist of knowledge of the exposure of an organism to a chemical, followed by an understanding of how that chemical is absorbed, distributed, metabolized and excreted and hence how much of the chemical gets to the active site. Once the chemical reaches the active site how, and to what extent, does it bind to, or interact with, a target? How does this interaction lead to a disturbance of the biosystem within this organism and, finally, how does this disturbance leads to effects at a measurable, level of biological organization? This is indeed a large undertaking. However, the AOP provides a framework that, once these pieces are in place, can provide a genuine alternative to *in vivo* testing.[5–7]

MIEs are already being used as gateways to the development of predictive tools and mechanistic understanding. The MIE has been used in quantitative structure activity relationship (QSAR) development[8] and category formation and read across[9] within *in silico* tools. Molecular modelling is also finding a use for the MIE, in research leading to an increase in mechanistic understanding of biological-chemical interactions.[10] Once the potential for a compound to activate an MIE has been established, this can be linked to *in vitro* studies of downstream biomarkers associated with known AOPs, allowing risk assessment to be carried out.

The development, evaluation and current trends in SAR and QSAR research have been well reviewed.[11,12] Despite advances in these powerful *in silico* techniques they are still treated with some suspicion in the toxicology community. Their role in toxicology risk assessment is generally confined to chemical screening.[2] Anchoring SARs and QSARs at the MIE removes large amounts of biological complexity from the models, providing stronger links between chemical characteristics and the prediction of adverse outcomes. The construction of clear models with sound theoretical backing is of great importance, and these will illustrate the value of the technique.

One such type of SAR in toxicity prediction relies on the use of structural fragments from within molecules to distinguish active compounds from inactive ones. One such approach has been used in the development of SAR "rules" for skin sensitization.[13] Similar approaches are used within computational tools, such as Derek Nexus, a knowledge-based expert system for qualitative toxicity prediction based on chemical structure.[14] The OECD QSAR toolbox (http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm) provides a further example of the use of chemical characteristics being linked to potential mechanism or mode of action.[15] The use of structural alerts can be associated with MIEs in predictive toxicology and tools to make these predictions are already under development.[16,17]

The aim of this work is to build a structural alert-style SAR approach to predict MIEs *in silico*. This study has been conducted with a number of pharmacologically important human targets for systemic toxicity drawn from a paper by Bowes in 2012[18], and several targets of interest from our previous work.[1] In particular the Bowes receptors are highlighted as pharmacologically important off-target interactions, often leading to the failure of new drug candidates in clinical trials.[18] These receptors are also of importance in the development of ingredients for consumer

goods products as they could lead to damaging systemic toxicity outcomes. As important drug safety targets these receptors are also likely to have a good amount of biological *in vitro* data available for analysis and the construction of models, providing a further advantage. In addition to this these targets cover a wide variety of different biomolecules, and a good representation of biological space. As such these receptors provide a good starting point for the development of any *in silico* technique. These targets include G-protein coupled receptors (GPCRs), enzymes, ion channels, nuclear receptors and transporters (Table 1).

These biological targets require MIEs to be described in a different way to the well-characterized skin sensitization alerts based on chemical reactivity,[19] because binders are likely to sit in a sterically restricted receptor binding pockets or enzyme active sites and interact with the biomolecule through hydrogen bonds, or charge-charge and hydrophobic interactions. Fortunately structural and pharmacophore-style 2D fragments coded in SMILES and SMARTS can still describe these alerts well.

2D fragment based approaches to SARs have several advantages. They are computationally simple, allowing the rapid assessment of a large number of chemicals without the use of a large amount of computational resources. 2D SARs are also mechanistically transparent, and can be easily interpretable as to what it is about a molecule that causes it to activate an MIE. Despite this they do have their limitations. For example, this strategy only locates common substructures in the training data when a relatively large common molecular scaffold is available. Some biological targets are promiscuous and only slight changes in the chemistry of binders results in a large change in biological activity, which is sometimes difficult to describe with a chemical fragment. These fragments also do not analyze the chemical characteristics of whole molecules, and so important pieces of information can be missed if they are just outside of the located

fragment. Finally using 2D substructures to model 3D environments is considered an incomplete way to predict biological activity. The shape and size of a molecule may play a bigger role in its ability to fit into, and bind to, a biological target than its chemical features, particularly if the target is a well-defined enzyme active site. This can only be assessed in 3D.

One target may have more than one MIE. From a chemical perspective it is also true that one binding pocket may allow chemicals to bind to it through different interactions, and these may lead to a diversity of alerts. The alerts in this paper are for a specific "mode of binding" (MOB) and this term will be used in this study to avoid confusion. Without additional biological understanding, it is sometimes not clear if two alerts apply to the same MIE, or to different ones.

MATERIALS AND METHODS

Data Set. ChEMBL (https://www.ebi.ac.uk/chembl/, version 19, extracted November 2014) was used, containing more than a million annotated compounds, comprising more than twelve million bioactivities covering more than 10 000 targets, all abstracted from the primary scientific literature.[20] Compounds with a confidence score of 8 or 9 and with reported activities (K_i/K_d/IC50/EC50) better than, or equal to, 10 μM against human protein targets were treated as positives and used for model generation. These cutoffs were chosen to provide chemicals with a pharmacologically-relevant activity at a specific, well-defined, human target. A cut-off of 10 μM will ensure that the compounds have a good degree of biological activity and represents a trade-off between activity and dataset size. A confidence score of 8 represents the assignment of homologous single proteins, and 9 direct single protein interactions.[21] These compounds are binders irrespective of agonistic and antagonistic activity. Non-binders were omitted from the generation of the models because of the small fraction of compounds found in ChEMBL with

reported activities below 10 μM. Only receptors with at least twenty active compounds were used to ensure enough information is available to construct appropriate *in silico* models. The data set for each target was split randomly into 75 % training set and a 25 % test set using a function in Pipeline Pilot,[22] so internal validation could be provided. The data were uniquified to ensure no duplicate data corrupted results. This was performed on the molecular structure of each chemical based on their atomic connectivity, resulting in different tautomers, enantiomers and salts being treated as different data points. The training sets were then combined and uniquified to make an amalgamated training set, and the test sets were combined in an analogous fashion to make an amalgamated test set. These give a representation of ChEMBL chemical space. The amalgamated training set was used to provide fragments present across the whole set. It was not used in the development of models. The amalgamated test set was used in the internal validation. These compounds had unknown activity across each receptor but were used as anticipated negatives to give an idea of chemical space. This is done to overcome bias in the ChEMBL set towards positives, and allow model quality to be better assessed than through the use of sensitivity alone. In total 51 179 activities across 30 349 unique compounds and 45 human targets (Table 1) were extracted for model construction.

Model Construction. The compound's canonical SMILES were used to generate 2D fragment-based structural alerts using custom scripting in Pipeline Pilot.[22] Molecular fragments were identified based on matching atom types and charges between molecules. Partial aliphatic rings were allowed within fragments. The fragment size and frequency within the training set were altered to sample a diversity of potentially active fragments. The outputs were viewed and curated manually to identify key fragments which are associated with positive activity, rather than just being common among medicinal chemicals. Text based literature searches were

conducted using SciFinder to find information on targets that could aid in the construction of models. In some cases, crystal structures were found. In others, well-known molecules with precisely understood binding behavior were found and the structures of these compared to the found fragments. Based on the elucidated fragments, and understanding gained through the literature searches, structural alerts were coded as SMILES strings or SMARTS and models were constructed using a substructure filter.

Internal Validation. Test sets were used to calculate sensitivity (SE) for each structural alert.

$$SE = \frac{TP}{TP + FN}$$

In order to provide a negative test set for each human receptor target the test set for the target of interest was subtracted from the amalgamated test set. As such every negative test set is not the same. This is to give an idea of chemical space, but the chemicals are untested against the target of interest and are treated as negatives only for the purpose of this analysis. This is done to provide analysis and get a confidence score for models. As such these results must be treated carefully, but should provide more guidance greater than sensitivity-only calculations. Specificity (SP) is calculated from these results.

$$SP = \frac{TN}{TN + FP}$$

Overall quality (Q) is calculated based on the total number of correct predictions.

$$Q = \frac{TP + TN}{TP + TN + FP + FN}$$

To overcome the issue of having a larger negative test set than positive the Matthews correlation coefficient (MCC) was used;[23]

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}$$

RESULTS AND DISCUSSION

Amalgamated Set

In the search for common 2D fragments in the ChEMBL dataset it is inevitable that some fragments will be found that are simply common among medicinal chemicals within ChEMBL. In order to take this into account, and to prevent models being built using fragments that are simply common across the entire dataset of all receptors, a bespoke model building process is used. The fragments are subjected to human analysis, and appropriate fragments selected, rather than simply using a computer to select all fragments. In addition to this, a dataset was constructed containing all compounds across all test sets analyzed in this study. This training set was uniquified, and then run through the same protocols used to generate 2D fragments for each receptor. This generated a number of fragments that are simply common among medicinal chemicals of this type, and so should not be considered indicative of any particular receptor-ligand interaction, unless exceptionally high percentages of the training set include them. These are shown in Figure 1.

As may have been expected, some fragments, including benzene rings, aliphatic amines, and short carbon chains are commonly present.

Structural Alerts

Across the 45 human targets analyzed in this work, 126 structural alerts have been developed, and these are shown in Table 2. These structural alerts include those found using maximal common substructure searches using Pipeline Pilot, those found in typical ligands for the target, those identified in previously accepted pharmacophores, those identified in existing crystal structures, and combinations of these. Each alert is labelled in Table 2 to indicate its origin and pharmacophores, crystal structures and typical binders found in the literature are referenced. The SMILES and SMARTS that were coded for these structural alerts are provided in supporting information. Table 2 also provides the fraction of positives identified by each alert in the training set.

Results for the internal validation of structural alerts are shown in Table 3. A total of 126 alerts have been produced across 45 human targets. In large part this has been successful, with 77 models across 39 targets producing MCC values of greater than 0.2 (representing 60 % correct predictions for a balanced data set), 28 models across 21 targets giving MCC values greater than 0.4 (70 % correct predictions for a balanced data set), and seven models across seven receptors scoring MCC values of greater than 0.6 (80 % correct predictions for a balanced data set) in internal validation. These results must be treated with a note of caution, however, as the remainder of the ChEMBL training set was used as a negative test set, meaning these compounds have not been confirmed as true negatives. Despite this, these results show that the structural alerts that have been developed do not tend to over-fire, and as such the results obtained by running a novel chemical through these structural alerts should be of benefit to a toxicologist wanting to be able to perform a pragmatic risk assessment when combined with other data, or identify targets of interest for further investigation.

This study has produced an average of just under three structural alerts (2.93) per target. It is notable that a number of targets have only one structural alert covering a large proportion of their active compounds (Dihydrofolate reductase, GAR transformylase, tyrosine-protein kinase, the glutamate (NMDA) receptor, the HERG channel, and the voltage gated K channel subunit Kv7.1). This suggests that these biological targets are quite specific in the compounds that they accept and hence there is less structural variability in their binders. Only two targets have more than five fragments, the adrenergic 2a receptor (6) and monoamine oxidase (7), indicating that these are more promiscuous receptors with more structurally varied binders.

Combined Models

2D structural alerts, such as those developed in this work, can be used in toxicity screening, as a hazard identification tool, or as a tool in risk assessment to support a decision on chemical safety. In each of these cases the structural alerts will be used in different ways. In hazard identification tools need to be calibrated to provide maximum safety; that is to minimize the number of false negatives at the expense of the number of false positives predicted. In risk assessment tools will be adjusted to provide maximum accuracy; to provide the best possible predictivity as measured by a metric such as MCC.

To provide results on the structural alerts developed in this work for hazard identification, the structural alerts for each target were combined into a model requiring a molecule to contain any of the structural alerts to be predicted a positive. These models were tested against the positive test set for each target to provide a sensitivity value in each case. These results are shown in Table 4. This provides perspective on the proportion of binders likely to be predicted by these models, in a hazard assessment exercise. 45 models were tested and their sensitivity values

calculated. 29 models scored sensitivity values of 50% or more, 13 scored 75% or more and five scored 90% or more. These results provide a promising basis for the construction of a toxicity screening tool for hazard identification based on these structural alerts predicting MIEs. 2D structural alert methods such as this have an advantage in hazard assessment as they are computationally quick and inexpensive, and often hazard assessment requires the processing of large numbers of chemicals to screen out potentially toxic molecules at an early stage of compound development.

Risk assessment for individual chemicals is very different to hazard identification, and as such requires a different approach. Small numbers of chemicals are assessed on a case-by-case basis to ensure their safety. This requires expert input and pragmatic decision making by an expert scientist, and as such a batch test of these structural alerts is not an appropriate way to assess their value. 2D structural alerts such as these may find use in this field, and if they do it is important that the expert takes into account the results from our study to make an informed decision as to whether a chemical is likely to bind to a given target or not. The statistical results for sensitivity, specificity, overall quality and MCC values provided in Table 2 give guidance as to which of the alerts are most predictive and which are likely to over-fire. Alerts with a high sensitivity and MCC are very predictive and should be most useful in risk assessment. Alerts with a low specificity are hitting a number of compounds that are not considered binders in ChEMBL, and as such will produce a large number of false positives in risk assessment.

Further research will be required to expand our work to other key MIEs and improve it to the point where it can quantitatively predict the amount of a toxicant required at a target to exhibit a toxicological response. In addition to this a number of other tools will be required to accurately predict the amount of a toxicant that is able to reach the site of the MIE, and the biological

response that will result from the activation of the MIE, in order to represent a complete risk assessment.

Biological Relevance

The AOP Wiki represents the best current repository for AOP information, and as such a search was performed to identify AOPs which could result from the binding of a molecule to the targets examined in this work.[24] The majority of the AOPs that may be associated with this work are currently under development (totaling 19 pathways) and the associated targets are listed below:

- Acetylcholinesterase
- Androgen Receptor
- Cyclooxygenase (5 pathways)
- Ether-a-go-go voltage gated potassium channel
- Glucocorticoid Receptor (2 pathways)
- Glutamate Receptor
- Histamine H2 Receptor
- Serotonin Transporter (2 pathways)
- Sodium Channel (3 pathways)

This shows much promise for the future of AOP-based toxicology studies, as the AOP Wiki is currently in its infancy and will continue to grow over time, providing further scope for the identification of potentially toxic pathways using work such as this.

One pathway from the AOP wiki is well developed for agonism of the androgen receptor leading to reproductive dysfunction in adult female fish. While this pathway is not based on human toxicology studies, with time the AOP Wiki will be able to provide such detailed pathways for human toxicity pathways. In addition to this there are a number of MIEs, KEs and AOPs that transcend species and as such this research may be relevant to human toxicology. In essence agonism at the androgen receptor leads to a decrease in the concentrations of the hormones gonadotropin, testosterone, estradiol and vitellogenin. A decrease in vitellogenin uptake leads to impaired development of oocytes, decreased spawning rates and a population decline. A graphic of this AOP is shown in Figure 2.

Further information on clinical pathways that may be affected by the agonism or antagonism of the targets studied in this work is presented in the Bowes 2012 article.[18]

CONCLUSIONS

As toxicology moves away from animal based approaches and towards in silico and in vitro methods, further understanding and new tools are required. The MIE is the first KE in the AOP, the boundary between chemistry and biology where a chemical makes its first key interaction with the body. By understanding the chemical properties of existing receptor-binding chemicals we aim to be able to predict the MIEs of new molecules computationally. The focus of this work

was to utilize open source data to develop 2D structural alert-based SAR models for the prediction of MIEs associated with pharmacologically important human targets.

We have produced a number of 2D SAR models in an attempt to describe the characteristic fragments that allow molecules to cause effects *via* MIEs. A total of 126 alerts across 45 human targets have been developed in this work. A number of these alerts performed well, with an emphasis on being able to combine alerts into models for individual targets which will have high levels of specificity and therefore will not over-fire. When the 45 combined models were tested, 29 models scored sensitivity values of 50% or more, 13 scored 75% or more and five scored 90% or more, providing overall a good level of coverage. To prevent the models over-firing the whole ChEMBL data set was analyzed to provide fragments that are common in chemicals in this set, and as such should not be used as structural alerts. This information was used, along with existing pharmacophores, crystal structures and an understanding of typical binders found in the literature, to manually curate structural alerts provided by a maximal common substructure searches and develop structural alerts. This has resulted in a number of structural alerts with high specificity values: 95 of 126 alerts scored 95% or greater specificity values.

Understanding of the chemical characteristics that govern receptor MIEs will be a key step in the development of AOP based tools for toxicity risk assessment. 2D fragment based approaches are not the only way to attempt to answer this complex problem, and a number of approaches will need to be combined to provide a quantitative risk assessment. In this first step we have begun to explore this area, with sights set on the development of *in silico* screening tools. We believe that these fragment alerts can provide useful information in compound development, regarding the potential toxic effects of lead chemical compounds, and provide a basis for exciting new developments in the understanding of receptor MIEs, and how they can be used in toxicology.

ASSOCIATED CONTENT

Supporting Information.

SMARTS/SMILES for the structural alerts developed in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Data Statement

According to the University of Cambridge data management policy, all the data used in this paper is available either in the paper or in the SI. A copy of the data is also available in the University of Cambridge repository at: https://www.repository.cam.ac.uk/

ABBREVIATIONS

AA2aR, adenosine A2a receptor; AC, acetylcholinesterase; ADR, adrenergic receptor; AOP, adverse outcome pathway; AR, androgen receptor; CCKAR, cholecystokinin A receptor; COX, cyclooxygenase; CR, cannabinoid receptor; DHFR, dihydrofolate reductase; DR, dopamine receptor; DT, dopamine transporter; E2, estradiol; ER ET-A, endothelin receptor ET-A; GART, GAR transformylase; GPCR, G-protein coupled receptor; GR, glucocorticoid receptor; GRSZ1, glutamate receptor subunit zeta 1; GtH, gonadotropin hormone; HDAC 1, histone deacetylase 1; HERG; human ether-a-go-go related gene; HR, histamine receptor; LCK, tyrosin protein kinase; MAO A, monoamine oxidase A; MAR, muscarinic acetylcholine receptor; MCC, Matthews correlation coefficient; MIE, molecular initiating event; MOB, mode of binding; NMDA, N-methyl-D-aspartate; NT, norepinephrine transporter; OR, opioid receptor; PDE, phosphoidesterase; Q, overall quality; (Q)SAR, (quantitative)structure activity relationship; SCV-A, sodium channel V subunit alpha; SE, sensitivity; SP, specificity; SR, serotonin receptor; ST, serotonin transporter; T, testosterone; TS, thymidylate synthase; VGKC Kv7.1, voltage gated K channel subunit Kv7.1; VTG, vitellogenin; VV1a, vasopressin V1a.

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FIGURES

Figure 1A

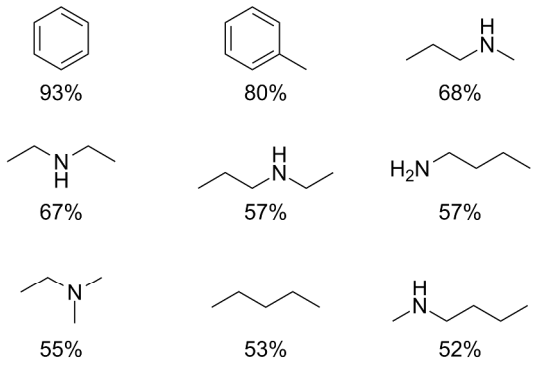


Figure 1B

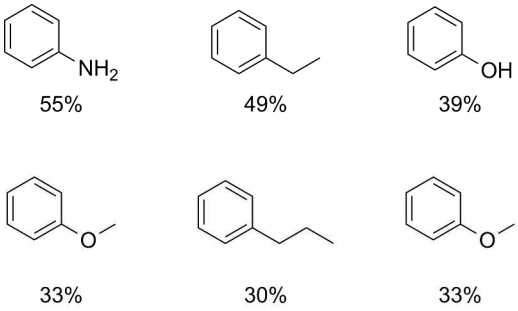


Figure 1C

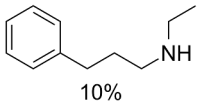


Figure 1. 2D fragments common within the entire dataset for this study. Part 1A shows fragments larger than four chemical bonds present within 50 % or greater of the data set. Part 1B shows fragments larger than seven chemical bonds present within 30 % or greater of the data set. Part 1C shows fragments larger than 12 chemical bonds present within 10 % or greater of the data set.

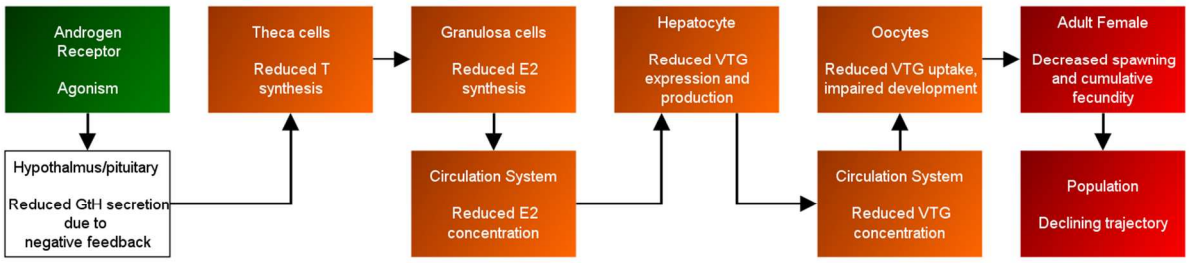


Figure 2. AOP for androgen receptor agonism leading to reproductive dysfunction. The MIE is shown in green, KEs in orange and adverse outcomes in red as per the AOP Wiki template. Reduced GtH secretion in the hypothalamus/pituitary is shown in white as there is uncertainty as to the specific mechanism through which androgen receptor agonism elicits a negative feedback response at this key event. E2: estradiol, GtH: gonadotropin hormone, T: testosterone, VTG: vitellogenin.

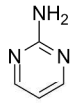
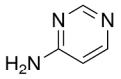
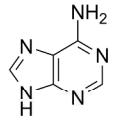
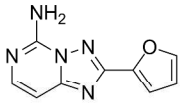
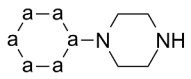
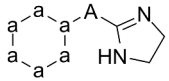
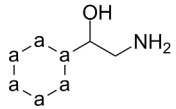
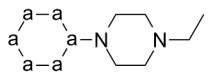
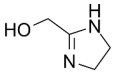
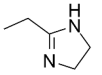
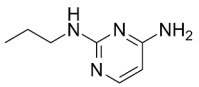
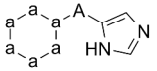
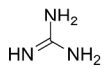
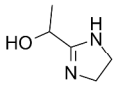
TABLES

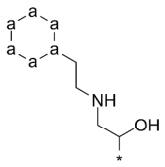
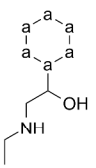
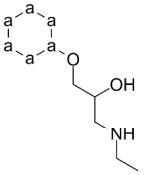
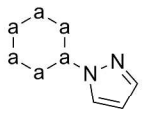
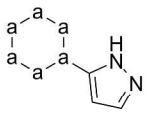
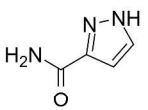
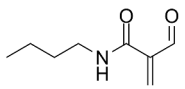
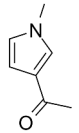
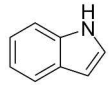
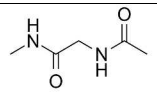
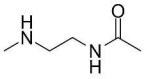
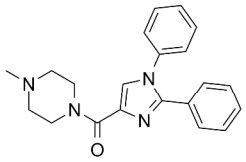
Target **G-Protein Coupled Receptors**	Binders	Binders Training Set	Binders Test Set (positive)	Test Set (negative)
Adenosine A2a Receptor	2960	2150	810	10820
Alpha-1a Adrenergic Receptor	705	510	195	11435
Alpha-2a Adrenergic Receptor	380	269	111	11519
Beta-1 Adrenergic Receptor	694	505	189	11441
Beta-2 Adrenergic Receptor	770	540	230	11400
Cannabinoid CB1 Receptor	3738	2688	1050	10580
Cannabinoid CB2 Receptor	3405	2435	970	10660
Cholecystokinin Receptor A	255	177	78	11552
Dopamine D1 Receptor	453	322	131	11499
Dopamine D2 Receptor	2589	1831	758	10872
Endothelin Receptor A	100	72	28	11602
Histamine H1 Receptor	672	499	173	11457
Histamine H2 Receptor	191	141	50	11580
Muscarinic Acetylcholine Receptor M1	887	639	248	11382
Muscarinic Acetylcholine Receptor M2	620	456	164	11466
Muscarinic Acetylcholine Receptor M3	1067	772	295	11335
Delta Opioid Receptor	2550	1657	893	10737
Kappa Opioid Receptor	2347	1721	626	11004
Mu Opioid Receptor	2793	2056	737	10893
Serotonin 1A Receptor	1777	1273	504	11126
Serotonin 1B Receptor	396	282	114	11516
Serotonin 2A Receptor	1612	1162	450	11180
Serotonin 2B Receptor	750	555	195	11435
Vasopressin V1A Receptor	651	471	180	11450

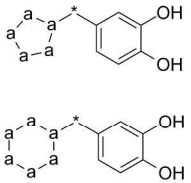
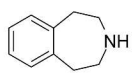
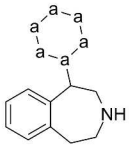
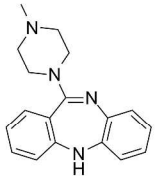
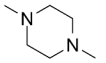
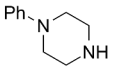
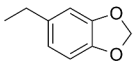
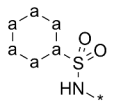
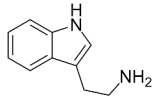
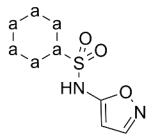
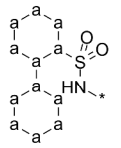
Enzymes				
Acetylcholinesterase	1355	989	366	11264
Cyclooxygenase 1	379	274	105	11525
Cyclooxygenase 2	964	686	278	11352
Dihydrofolate Reductase	404	294	110	11520
GAR Transformylase	36	23	13	11617
Histone Deacetylase 1	1202	882	320	11310
Monoamine Oxidase A	533	382	151	11479
Phosphodiesterase 3A	132	94	38	11592
Phosphodiesterase 4D	385	267	118	11512
Thymidylate Synthase	239	178	61	11569
Tyrosine-Protein Kinase	568	414	154	11476
Ion Channels				
Glutamate (NMDA) Receptor	25	18	7	11623
Potassium Voltage Gated Channel KQT-like Member 1	295	215	80	11550
Serotonin 3A Receptor	316	230	86	11544
Sodium Channel V Subunit Alpha	153	111	42	11588
Voltage Gated K Channel Subunit Kv7.1	28	21	7	11623
Nuclear Receptors				
Androgen Receptor	1598	1186	412	11218
Glucocorticoid Receptor	2201	1622	579	11051

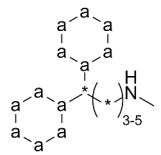
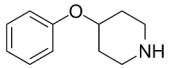
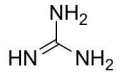
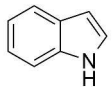
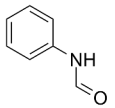
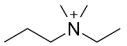
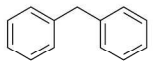
Transporters				
Dopamine Transporter	1908	1414	494	11136
Norepinephrine Transporter	2616	1923	693	10937
Serotonin Transporter	3480	2578	902	10728

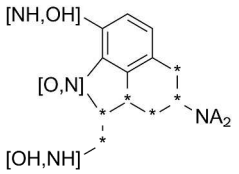
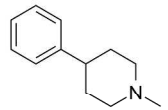
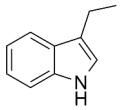
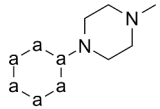
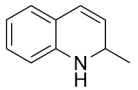
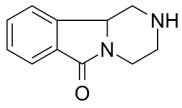
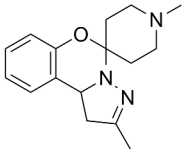
Table 1. Pharmacological targets analyzed in this work. Data was extracted from ChEMBL version 19. The total test set in each case was 11 630 compounds.

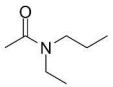
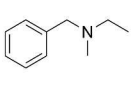
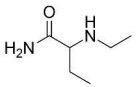
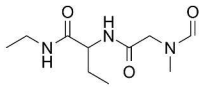
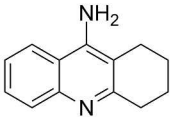
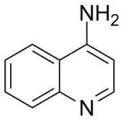
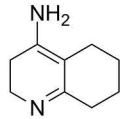
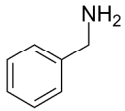
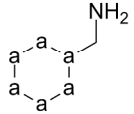
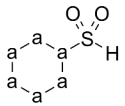
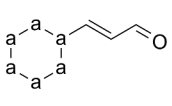
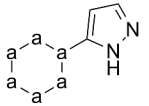
Receptor	MIE	Origin	Training Set Hits	Alerts
G-Protein Coupled Receptors				
Adenosine A2a Receptor				
	Alert AA2aR 1 - 2-Pyrimidine	S	842/2150	 Alert AA2aR 1 2-Aminopyrimidine
	Alert AA2aR 2 - 4-Pyrimidine	S,L[25,26]	647/2150	 Alert AA2aR 2 4-Aminopyrimidine
	Alert AA2aR 3 - Adenine	S,L[25,26]	349/2150	 Alert AA2aR 3 Adenine
	Alert AA2aR 4 - AA2aR Frag 1	S,L[27]	364/2150	 Alert AA2aR 4 AA2aR Frag 1
Adrenergic Receptors				
Alpha	Alert A-ADR 1 - Phenylpiperazine-like	S,L	207/758	 Alert A-ADR 1 Phenylpiperazine-like
	Alert A-ADR 2 - Tolazoline-like	S,L[28–32]	7/758	 Alert A-ADR 2 Tolazoline-like
Alpha-1a	Alert A-1aADR 1 - Phenylethanolamine-like	L[29,33–36]	10/510	 Alert A-1aADR 1 Phenylethanolamine-like
	Alert A-1aADR 2 - 1-Ethyl-4-phenylpiperazine-like	S	158/510	 Alert A-1aADR 2 1-Ethyl-4-phenylpiperazine-like
Alpha-2a	Alert A-2aADR 1 - CID 5145436	S	55/269	 Alert A-2aADR 1 CID 5145436 55/269
	Alert A-2aADR 2 - CID 13001	S	52/269	 Alert A-2aADR 2 CID 13001 52/269
	Alert A-2aADR 3 - A2aADR Frag 2	S	37/269	 Alert A-2aADR 3 A2aADR Frag 2 37/269
	Alert A-2aADR 4 - 4-Benzyl-1H-imidazole-like	L[37]	3/269	 Alert A-2aADR 4 4-Benzyl-1H-imidazole-like 3/269
	Alert A-2aADR 5- Guanidine	L[31,38]	9/269	 Alert A-2aADR 5 Guanidine 9/269
	Alert A-2aADR 6 - 2-(1-Hydroxyl ethyl)-2-imidazole	S	50/269	 Alert A-2aADR 6 2-(1-Hydroxyl ethyl)-2-imidazole 50/269

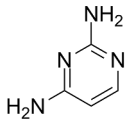
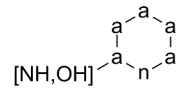
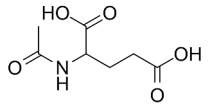
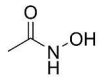
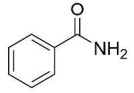
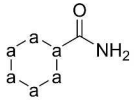
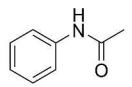
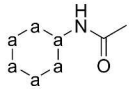
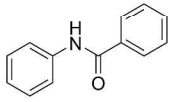
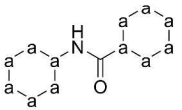
Receptor	MIE	Origin	Training Set Hits	Alerts
Beta	Alert B-ADR 1 - 1-(Phenethylamino)propan-2-ol-like	S	383/774	 Alert B-ADR 1 1-(Phenethylamino) Propan-2-ol-like
	Alert B-ADR 2 - 2-(Ethylamino)-1-phenylethanol-like	S	509/774	 Alert B-ADR 2 2-(Ethylamino)-1-Phenylethanol-like
	Alert B-ADR 3 - 1-(Ethylamino)-3-phenoxyl-2-propanol-like	S	194/774	 Alert B-ADR 3 1-(Ethylamino)-3-Phenoxy-2-propanol-like
Cannabinoid Receptor				
CB1	Alert CCB1R 1 - 2-Phenylpyrazole-like	S	391/2688	 Alert CCB1R 1 2-Phenylpyrazole-like
	Alert CCB1R 2 - 5-Phenyl-1H-pyrazole-like	S	286/2688	 Alert CCB1R 2 5-Phenyl-1H-pyrazole-like
	Alert CCB1R 3 - Pyrazole-3-carboxamide	S	321/2688	 Alert CCB1R 3 Pyrazole-3-carboxamide
CB2	Alert CCB2R 1 - CCB2R Frag 1	S	298/2435	 Alert CCB2R 1 CCB2R Frag 1
	Alert CCB2R 2 - 1-(1-Ethyl-1H-pyrrol-3-yl)ethanone	S	217/2435	 Alert CCB2R 2 1-(1-Ethyl-1H-pyrrol-3-yl)ethanone
CB1 & CB2	Alert CR 1 - Indole	S	489/3362	 Alert CR 1 Indole
Cholecystokinin A Receptor				
	Alert CCKAR 1 - 2-Acetamido-N-methylacetamide	S,L[39–41]	72/177	 Alert CCKAR 1 2-Acetamido-N-methylacetamide
	Alert CCKAR 2 - N-[2-(methylamino)ethyl]acetamide	S,L[39–41]	159/177	 Alert CCKAR 2 N-[2-(Methylamino)ethyl]acetamide
	Alert CCKAR 3 - CID 9957635	S	63/177	 Alert CCKAR 3 CID 9957635

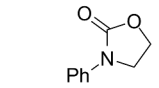
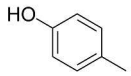
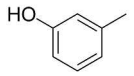
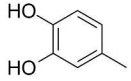
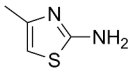
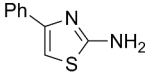
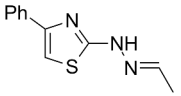
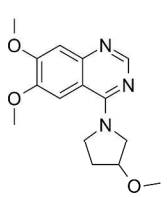
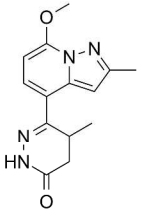
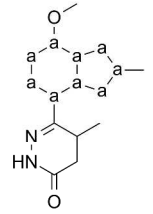
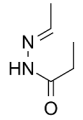
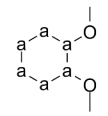
Receptor	MIE	Origin	Training Set Hits	Alerts
Dopamine Receptors				
D1	Alert DD1R 1 - Dihydrexine-like	L[42]	21/322	 Alert DD1R 1 Dihydrexine-like
	Alert DD1R 2 - Benzazepine-like	L[42]	20/322	 Alert DD1R 2 Benzazepine-like
	Alert DD1R 3 - Benzazepine-like with aromatic	L[42]	19/322	 Alert DD1R 3 Benzazepine-like with aromatic
	Alert DD1R 4 - CID 15288	S	84/322	 Alert DD1R 4 CID 15288
D2	Alert DD2R 1 - Piperazine	S	1006/1831	 Alert DD2R 1 Piperazine
	Alert DD2R 2 - 1,4 Dimethylpiperazine	S	1000/1831	 Alert DD2R 2 1,4 Dimethylpiperazine
	Alert DD2R 3 - Phenylpiperazine	S	727/1831	 Alert DD2R 3 Phenylpiperazine
Endothelin Receptor A				
	Alert ER ET-A 1 - 5-Ethyl-1,3-benzodioxole	S,L[43]	23/72	 Alert ER ET-A 1 5-Ethyl-1,3-benzodioxole
	Alert ER ET-A 2 - N-Methylbenzenesulfonamide-like	S	53/72	 Alert ER ET-A 2 N-Methylbenzenesulfonamide-like
	Alert ER ET-A 3 - Tryptamine	S	25/72	 Alert ER ET-A 3 Tryptamine
	Alert ER ET-A 4 - ER ET-A Frag 1-like	S	30/72	 Alert ER ET-A 4 ER ET-A Frag 1-like
	Alert ER ET-A 5 - N-Methyl-2-biphenylsulfonamide-like	S	25/72	 Alert ER ET-A 5 N-Methyl-2-biphenylsulfonamide

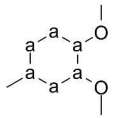
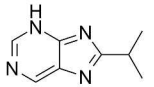
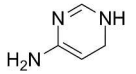
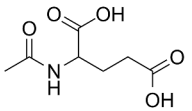
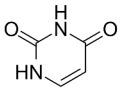
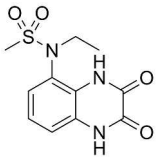
Receptor	MIE	Origin	Training Set Hits	Alerts
Histamine Receptors				
H1	Alert HH1R 1 - Doxepine-like	S,X[44]	58/444	 Alert HH1R 1 Doxepine-like
	Alert HH1R 2 - 4-Phenoxypiperidine	S	66/444	 Alert HH1R 2 4-Phenoxypiperidine
H2	Alert HH2R 1 - Imidazole	S	36/126	 Alert HH2R 1 Imidazole
	Alert HH2R 2 - Guanidine	S	46/126	 Alert HH2R 2 Guanidine
	Alert HH2R 4 - Indole	S	36/126	 Alert HH2R 4 Indole
Muscarinic Acetylcholine Receptors				
M1 and M2	Alert MAR 1 - Formanilide	S,L[45,46]	238/844	 Alert MAR 1 (M1 and M2) Formanilide
M2 and M3	Alert MAR 2 - N-Ethyl-N,N-dimethylpropanaminum	S,L[47,48]	316/915	 Alert MAR 2 (M2 and M3) N-Ethyl-N,N-dimethylpropanaminum
				 Alert MAR 3 (M3) Tetramethylamonium
M3	Alert MAR 3 - Tetramethylamonium	S,L[47,48]	316/772	 OR  OR  Cationic Nitrogen — Diphenylmethane — Reuterin
All	Alert MAR 4 - MAR Pharmacophore	X[49]	393/1236	Alert MAR 4 (M1, M2 or M3) MAR Pharmacophore

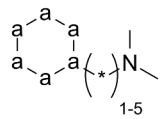
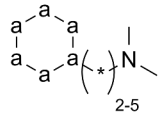
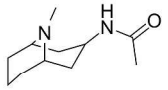
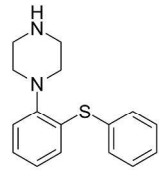
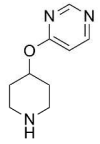
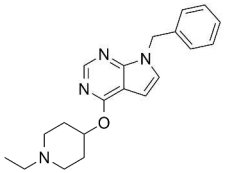
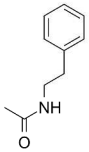
Receptor	MIE	Origin	Training Set Hits	Alerts
Opioid Receptors				
	Alert OR 1 - Morphine-like (4 or more)	X,P[50]	164/1385	 Alert OR 1 Morphine-like
	Alert OR 1 - Morphine-like (3 or more)	X,P[50]	459/1385	
	Alert OR 2 - 1-Methyl-4-phenylpiperidine	S	668/1385	 Alert OR 2 1-Methyl-4-phenylpiperidine
Serotonin Receptors				
All	Alert SR 1 - 3-Ethyl Indole	S	299/2633	 Alert SR 1 3-Ethyl Indole
1A, 1B, 2A	Alert SR 1a1b2a 1 - 1-Methyl-4-Phenylpiperazine-like	S	908/2377	 Alert SR 1a1b2a 1 1-Methyl-4-phenylpiperazine
1A	Alert SR 1a 1 - SR 1a Frag 2	S	65/1208	 Alert SR 1a 1 SR 1a Frag 2
1B	Alert SR 1b 1 - 2-Methyl-1,2-dihydroquinoline	S	116/282	 Alert SR 1b 1 2-methyl-1,2-dihydroquinoline
2B	Alert SR 2b 1 - CID 15206310	S	41/555	 Alert SR 2b 1 CID 15206310
	Alert SR 2b 2 - SR 2b Frag 2	S	31/555	 Alert SR 2b 2 SR 2b Frag 2

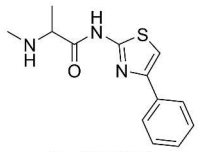
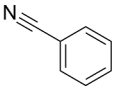
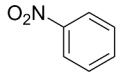
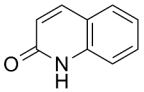
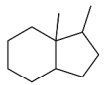
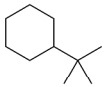
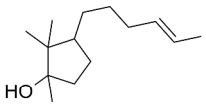
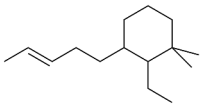
Receptor	MIE	Origin	Training Set Hits	Alerts
Vasopressin V1a				
	Alert VV1a 1 - VV1a Frag 1	S,L[51–53]	224/471	 Alert VV1a 1 VV1a Frag 1
	Alert VV1a 2 - n-benzyl-n-ethylmethylamine	S	243/471	 Alert VV1a 2 n-Benzyl-n-ethylmethylamine
	Alert VV1a 3 - VV1a Frag 3	S,L[51–53]	187/471	 Alert VV1a 3 VV1a Frag 3
	Alert VV1a 4 - VV1a Frag 4	S,L[51–53]	128/471	 Alert VV1a 4 VV1a Frag 4
Enzymes				
Acetylcholinesterase				
	Alert AC 1a - Tacrine	S,L[54]	184/989	 Alert AC 1a Tacrine
	Alert AC 1b - 4-Quinolinamine	S,L[54]	192/989	 Alert AC 1b 4-Quinolinamine
	Alert AC 1c - AC Frag 1	S,L[54]	245/989	 Alert AC 1c AC Frag 1
	Alert AC 1d - Dimethyl benzylamine	S,L[54]	395/989	 Alert AC 1d Dimethyl benzylamine
	Alert AC 1e - Dimethyl benzylamine-like SMARTS	S,L[54]	401/989	 Alert AC 1e Dimethyl benzylamine-like SMARTS
Cyclooxygenases				
	Alert COX 1 - Sulfonated aromatic rings	S	392/816	 Alert COX 1 Sulfonated aromatic rings
	Alert COX 2 - Cinnamaldehyde-like	S	183/816	 Alert COX 2 Cinnamaldehyde-like
	Alert COX 3 - 5-Phenyl-1H-pyrazole-like	S	70/816	 Alert COX 3 5-Phenyl-1H-pyrazole-like

Receptor	MIE	Origin	Training Set Hits	Alerts
Dihydrofolate Reductase				
	Alert DHFR 1 (SMILES) - Diaminopyrimidine	S,L[55],X[56]	145/237	 Alert DHFR 1 (SMILES) Diaminopyrimidine
	Alert DHFR 1 (SMARTS) - Diaminopyrimidine-like	S,L[55],X[56]	158/237	 Alert DHFR 1 (SMARTS) Diaminopyrimidine-like
GAR Transformylase				
	Alert GART 1 - N-Acetyl-DL-glutamic acid	S	23/23	 Alert GART 1 N-Acetyl-DL-glutamic acid
Histone Deacetylase 1				
	Alert HDAC 1 1 - Hydroxamic acids	S,L[57,58]	416/882	 Alert HDAC 1 Acetohydroxamic acid
	Alert HDAC 1 2a - Benzamide	S,L[57,58]	271/882	 Alert HDAC 2a Benzamide
	Alert HDAC 1 2b - Benzamide-like SMARTS	S,L[57,58]	316/882	 Alert HDAC 2b Benzamide-like
	Alert HDAC 1 2c - Acetanilide	S,L[57,58]	355/882	 Alert HDAC 2c Acetanilide
	Alert HDAC 1 2d - Acetanilide-like SMARTS	S,L[57,58]	372/882	 Alert HDAC 2d Acetanilide-like
	Alert HDAC 1 2e - Benzanilide	S,L[57,58]	165/882	 Alert HDAC 2e Benzanilide
	Alert HDAC 1 2f - Benzanilide-like SMARTS	S,L[57,58]	198/882	 Alert HDAC 2f Benzanilide-like

Receptor	MIE	Origin	Training Set Hits	Alerts
Monoamine Oxidase				
	Alert MAO A 1 - 3-Phenyl-2-oxazolidone	L[59,60]	12/382	 Alert MAO A 1 3-Phenyl-2-oxazolidone
	Alert MAO A 2a - 4-Methylphenol	S	99/382	 Alert MAO A 2a 4-Methylphenol
	Alert MAO A 2b - 3-Methylphenol	S	80/382	 Alert MAO A 2b 3-Methylphenol
	Alert MAO A 2c - Methyl-3,4-diphenol	S	10/382	 Alert MAO A 2c Methyl-3,4-diphenol
	Alert MAO A 3a - Aminomethiazole	S	58/382	 Alert MAO A 3a Aminomethiazole
	Alert MAO A 3b - 4-Phenyl-1,3-thiazole-2-amine	S	57/382	 Alert MAO A 3b 4-Phenyl-1,3-thiazole-2-amine
	Alert MAO A 3c - CID 7958070	S	54/382	 Alert MAO A 3c CID 7958070
Phosphodiesterases				
3A	Alert PDE 3A 1 - PDE 3A Frag 2	S,X[61]	18/94	 Alert PDE 3A 1 PDE 3A Frag 2
	Alert PDE 3A 2a - PDE Frag 3	S,X[61]	11/94	 Alert PDE 3A 2a PDE 3A Frag 3
	Alert PDE 3A 2b - PDE Frag 3-like SMARTS	S,X[61]	15/94	 Alert PDE 3A 2b PDE 3A Frag-like SMARTS
	Alert PDE 3A 3 - PDE 3A Frag 4	S	34/94	 Alert PDE 3A 3 PDE 3A Frag 4
	Alert PDE 3A 4 - Veratrol-like	S,X[61]	36/94	 Alert PDE 3A 4 Veratrol-like

Receptor	MIE	Origin	Training Set Hits	Alerts
4D	Alert PDE 4D 1 - Homoveratrol-like	S,X[61]	83/267	 Alert PDE 4D 1 Homoveratrol-like
	Alert PDE 4D 2 - 8-Isopropyl-1H-purine Frag	S	24/267	 Alert PDE 4D 2 8-Isopropyl-1H-purine Frag
	Alert PDE 4D 3 - PDE 4D Frag 3	S	74/267	 Alert PDE 4D 3 PDE 4D Frag 3
Thymidylate Synthase				
	Alert TS 1 - N-Acetyl-DL-glutamic acid	S,L[62]	21/145	 Alert TS 1 N-Acetyl-DL-glutamic acid
	Alert TS 2 - Pyrimidine	L[62]	8/145	 Alert TS 2 Pyrimidine
	Alert TS 3 - Uracil	L[62]	2/145	 Alert TS 3 Uracil
Tyrosine Protein Kinase				
	Alert LCK 1 - Pyrimidine-like	S,L[63]	212/383	 Alert LCK 1 Pyrimidine
Ion Channels				
Glutamate (NMDA) Receptor				
	Alerts GRSZ1 1 - GRSZ1 Frag 1	S	18/18	 Alert GRSZ1 1 GRSZ1 Frag 1

Receptor	MIE	Origin	Training Set Hits	Alerts
Potassium Voltage Gated Channel Subunit H Member 2				
	Alert HERG 1a - Flex Aromatic Amine 1-5	S,P[64–67]	167/215	 Alert HERG 1a Flex Aromatic Amine 1-5
	Alert HERG 1b - Flex Aromatic Amine 2-5	S,P[64–67]	147/215	 Alert HERG 1b Flex Aromatic Amine 2-5
Serotonin 3A Receptor				
	Alert S3AR 1 - S3AR Frag 1	S,L[68]	47/229	 Alert S3AR 1 5HT 3a Frag 1
	Alert S3AR 2 - 1-[2-(Phenylsulfanyl)phenyl]piperazine	S,L[69]	27/229	 Alert S3AR 2 1-[2-(Phenylsulfanyl)phenyl]piperazine
Sodium Channel V Subunit alpha				
	Alert SCV-A 1a - Pyrimidine-like aromatic	S	49/111	 Alert SCV-A 1a Pyrimidine-like aromatic
	Alert SCV-A 1b - Pyrimidine-like wildcard	S	49/111	 Alert SCV-A 1b Pyrimidine-like wildcard
	Alert SCV-A 2 - 4-(4-Piperidineyloxy)pyrimidine	S	30/111	 Alert SCV-A 2 4-(4-Piperidineyloxy)pyrimidine
	Alert SCV-A 3 - SCV Frag 1	S	28/111	 Alert SCV-A 3 SCV Frag 1
	Alert SCV-A 4 - N-(2-Phenylethyl)acetamide	S	25/111	 Alert SCV-A 4 N-(2-Phenylethyl)acetamide

Receptor	MIE	Origin	Training Set Hits	Alerts
Voltage Gated K Channel Sub Unit Kv7.1				
	Alert VGKC Kv7.1 1 - VGKV Kv7.1 Frag 1	S	21/21	 Alert VGKC 1 VGKC Frag 1
Nuclear Receptors				
Androgen Receptor				
	Alert AR 1a - Benzonitrile	S,L[70]	294/764	 Alert AR 1a Benzonitrile
	Alert AR 1b - Nitrobenzene	S,L[70–72]	73/764	 Alert AR 1b Nitrobenzene
	Alert AR 1c - Quinolone	S,L[70]	128/764	 Alert AR 1c Quinolone
Glucocorticoid Receptor				
	Alert GR 1a - GR Frag 1	S,L[73–75]	228/1232	 Alert GR 1a GR Frag 1
	Alert GR 1b - tert-Butylcyclohexane	S,L[73–75]	166/1232	 Alert GR 1b tert-Butylcyclohexane
	Alert GR 1c - GR Frag 4	S,L[73–75]	201/1232	 Alert GR 1c GR Frag 4
	Alert GR 1d - GR Frag 5	S,L[73–75]	198/1232	 Alert GR 1d GR Frag 5

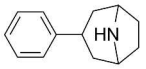
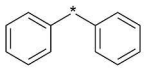
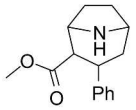
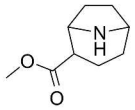
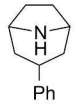
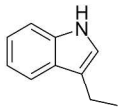
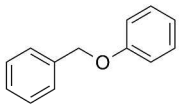
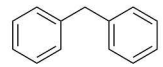
Receptor	MIE	Origin	Training Set Hits	Alerts
Transporters				
Dopamine Transporter				
	Alert DT 1 - 3-Phenyl-8-azabicyclo[3.2.1]octane	S,L[76,77]	147/1159	 Alert DT 1 3-Phenyl-8-azabicyclo[3.2.1]octance
	Alert DT 2 - Diphenylmethane-like	S,L[78]	268/1159	 Alert DT 2 Diphenylmethane-like
Norepinephrine Transporter				
	Alert NT 1a - Cocaine-like 1	S,L[79]	60/1573	 Alert NT 1a Cocaine-like 1
	Alert NT 1b - Cocaine-like 2	S,L[79]	61/1573	 Alert NT 1b Cocaine-like 2
	Alert NT 1c - Cocaine-like 3	S,L[79]	106/1573	 Alert NT 1c Cocaine-like 3
	Alert NT 1d - Cocaine-like 4	S,L[79]	117/1573	 Alert NT 1d Cocaine-like 4
	Alert NT 2 - Amphetamines-like	S,L[79]	1164/1573	 Alert NT 2 Amphetamines-like
Serotonin Transporter				
	Alert ST 1 - 3-Ethyl-indole	S,L[80]	175/2166	 Alert ST 1 3-Ethyl-indole
	Alert ST 2 - DMEA	S,L[80]	1438/2166	 Alert ST 2 DMEA
	Alert ST 3 - Benzyloxybenzene	S,L[80]	172/2166	 Alert ST 3 Benzyloxybenzene
	Alert ST 4 - Diphenylmethane	S,L[80]	192/2166	 Alert ST 4 Diphenylmethane

Table 2. Structural alerts developed for each receptor with the fraction of hits within each training set shown. Alerts are labelled based on their origin, S for fragments identified by maximal common substructure search using Pipeline Pilot, L for fragments found in typical ligands found in the literature, P for fragments based on an existing pharmacophore and X for fragments derived from an existing crystal structure. Alerts are shown pictorially as they have been coded in SMARTS or SMILES, with aromatic heavy atoms depicted as (a), aliphatic heavy atoms depicted as (A), and wildcard atoms (any heavy atom) depicted as (*).

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
G-Protein Coupled Receptors									
Adenosine A2a Receptor									
	Alert AA2aR 1 - 2-Aminopyrimidine	319	491	10591	229	39.4	97.9	93.8	0.448
	Alert AA2aR 2 - 4-Aminopyrimidine	251	559	10441	379	31.0	96.5	91.9	0.309
	Alert AA2aR 1 OR 2	505	305	10343	477	62.3	95.6	93.3	0.530
	Alerts AA2aR 1 AND 2	65	745	10689	131	8.0	98.8	92.5	0.135
	Alert AA2aR 3 - Adenine	137	673	10771	49	16.9	99.5	93.8	0.334
	Alert AA2aR 4 - AA2aR Frag 1	155	655	10820	0	19.1	100.0	94.4	0.425
Adrenergic Receptors									
Alpha-1a/-2a	Alert A-ADR 1 - Phenylpiperazine-like	69	234	10318	1009	22.8	91.1	89.3	0.076
	Alert A-ADR 2 - Tolazoline-like	6	297	11326	1	2.0	100.0	97.4	0.128
Alpha-1a	Alert A-1aADR 1 - Phenylethanolamine-like	6	189	11136	299	3.1	97.4	95.8	0.004
	Alert A-1aADR 2 - 1-Ethyl-4-phenylpiperazine-like	54	141	10705	730	27.7	93.6	92.5	0.109
Alpha-2a	Alert A-2aADR 1 - CID 5145436	24	86	11513	7	21.8	99.9	99.2	0.408
	Alert A-2aADR 2 - CID 13001	22	88	11507	13	20.0	99.9	99.1	0.351
	Alert A-2aADR 1 OR 2	24	86	11505	15	21.8	99.9	99.1	0.363
	Alert A-2aADR 3 - A2aADR Frag 2	6	104	11484	36	5.5	99.7	98.8	0.083
	Alert A-2aADR 4 - 4-Benzyl-1H-imidazole-like	3	107	11515	5	2.7	100.0	99.0	0.099
	Alert A-2aADR 5- Guanidine	10	100	11342	178	9.1	98.5	97.6	0.058
	Alert A-2aADR 6 - 2-(1-Hydroxyl ethyl)-2-immidazole	22	88	11515	5	20.0	100.0	99.2	0.401

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Beta	Alert B-ADR 1 - 1-(Phenethylamino)propan-2-ol-like	168	203	11154	105	45.3	99.1	97.4	0.515
	Alert B-ADR 2 - 2-(Ethylamino)-1-phenylethanol-like	242	129	11207	52	65.2	99.5	98.4	0.725
	Alert B-ADR 3 - 1-(Ethylamino)-3-phenoxyl-2-propanol-like	102	269	11178	81	27.5	99.3	97.0	0.378
	Alert B-ADR 1 OR 2 OR 3	350	21	11025	234	94.3	97.9	97.8	0.742
Cannabinoid Receptor									
CB1	Alert CCB1R 1 - 2-Phenylpyrazole-like	170	880	10296	284	16.2	97.3	90.0	0.200
	Alert CCB1R 2 - 5-Phenyl-1H-pyrazole-like	117	933	10445	135	11.1	98.7	90.8	0.194
	Alert CCB1R 3 - Pyrazole-3-carboxamide	148	902	10533	47	14.1	99.6	91.8	0.305
	Alert CCB1R 1 OR 2	178	872	10210	370	17.0	96.5	89.3	0.182
	Alerts CCB1R 1 AND 2	109	941	10531	49	10.4	99.5	91.5	0.246
	Alerts CCB1R 1, 2 AND 3	86	964	10576	4	8.2	100.0	91.7	0.267
CB2	Alert CCB2R 1 - CCB2R Frag 1	111	859	10612	48	11.4	99.5	92.2	0.262
	Alert CCB2R 2 - 1-(1-Ethyl-1H-pyrrol-3-yl)ethanone	96	874	10621	39	9.9	99.6	92.1	0.246
CB1 & CB2	Alert CR 1 - Indole	207	1594	9043	786	11.5	92.0	79.5	0.045
Cholecystokinin A Receptor									
	Alert CCKAR 1 - 2-Acetamido-N-methylacetamide	35	43	11209	343	44.9	97.0	96.7	0.193
	Alert CCKAR 2 - N-[2-(methylamino)ethyl]acetamide	76	2	10595	957	97.4	91.7	91.8	0.256
	Alert CCKAR 3 - CID 9957635	30	48	11552	0	38.5	100.0	99.6	0.619

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Dopamine Receptor									
D1	Alert DD1R 1 - Dihydrexine-like	10	121	11447	52	7.6	99.5	98.5	0.104
	Alert DD1R 2 - Benzazepine-like	9	122	11454	45	6.9	99.6	98.6	0.101
	Alert DD1R 3 - Benzazepine-like with aromatic	9	122	11499	0	6.9	100.0	99.0	0.261
	Alert DD1R 4 - CID 15288	32	99	11472	27	24.4	99.8	98.9	0.359
D2	Alert DD2R 1 - Piperazine	417	341	9678	1194	55.0	89.0	86.8	0.315
	Alert DD2R 2 - 1,4 Dimethylpiperazine	416	342	9928	944	54.9	91.3	88.9	0.355
	Alert DD2R 3 - Phenylpiperazine	321	437	10285	587	42.3	94.6	91.2	0.340
Endothelin Receptor A									
	Alert ER ET-A 1 - 5-Ethyl-1,3-benzodioxole	3	25	11573	29	10.7	99.8	99.5	0.098
	Alert ER ET-A 2 - N-Methylbenzenesulfonamide-like	18	10	11107	495	64.3	95.7	95.7	0.143
	Alert ER ET-A 3 - Tryptamine	8	20	11340	262	28.6	97.7	97.6	0.086
	Alert ER ET-A 4 - ER ET-A Frag 1-like	14	14	11602	0	50.0	100.0	99.9	0.707
	Alert ER ET-A 5 - N-Methyl-2-biphenylsulfonamide-like	13	15	11585	17	46.4	99.9	99.7	0.447
	Alert ER ET-A 4 OR 5	14	14	11585	17	50.0	99.9	99.7	0.474
Histamine Receptors									
H1	Alert HH1R 1 - Doxepine-like	24	134	11106	366	15.2	96.8	95.7	0.077
	Alert HH1R 2 - 4-Phenoxypiperidine	21	137	11412	60	13.3	99.5	98.3	0.178
H2	Alert HH2R 1 - Imidazole	15	33	10635	947	31.3	91.8	91.6	0.054
	Alert HH2R 2 - Guanidine	14	34	11408	174	29.2	98.5	98.2	0.141
	Alert HH2R 4 - Indole	20	28	10609	973	41.7	91.6	91.4	0.076
	Alert HH2R 1, 2 OR 4	38	10	9558	2024	79.2	82.5	82.5	0.104

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Muscarinic Acetylcholine Receptors									
M1 and M2	Alert MAR 1 - Formanilide	102	270	9782	1476	27.4	86.9	85.0	0.074
M2 and M3	Alert MAR 2 - N-Ethyl-N,N-dimethylpropanaminum	129	282	11175	44	31.4	99.6	97.2	0.473
M3	Alert MAR 3 - Tetramethylamonium	118	177	11245	90	40.0	99.2	97.7	0.465
All	Alert MAR 4 - MAR Pharmacophore	198	394	10541	497	33.4	95.5	92.3	0.268
Opioid Receptors									
	Alert OR 1 - Morphine-like (4 or more)	164	1221	10238	7	11.8	99.9	89.4	0.317
	Alert OR 1 - Morphine-like (3 or more)	459	926	9867	378	33.1	96.3	88.8	0.369
	Alert OR 2 - 1-Methyl-4-phenylpiperidine	668	717	9957	288	48.2	97.2	91.4	0.536
Serotonin Receptors									
All	Alert SR 1 - 3-Ethyl Indole	125	1041	9946	518	10.7	95.0	86.6	0.076
1A, 1B, 2A	Alert SR 1a1b2a 1 - 1-Methyl-4-Phenylpiperazine-like	397	621	10011	601	39.0	94.3	89.5	0.336
1A	Alert SR 1a 1 - SR 1a Frag 2	33	471	11118	8	6.5	99.9	95.9	0.222
1B	Alert SR 1b 1 - 2-Methyl-1,2-dihydroquinoline	46	68	11323	193	40.4	98.3	97.8	0.269
2B	Alert SR 2b 1 - CID 15206310	15	180	11425	10	7.7	99.9	98.4	0.211
	Alert SR 2b 2 - SR 2b Frag 2	8	187	11435	0	4.1	100.0	98.4	0.201

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Vasopressin V1a									
	Alert VV1a 1 - VV1a Frag 1	70	110	10811	639	38.9	94.4	93.6	0.172
	Alert VV1a 2 - n-benzyl-n-ethylmethylamine	90	90	9928	1522	50.0	86.7	86.1	0.131
	Alert VV1a 3 - VV1a Frag 3	66	114	11144	306	36.7	97.3	96.4	0.238
	Alert VV1a 4 - VV1a Frag 4	41	139	11372	78	22.8	99.3	98.1	0.271
Enzymes									
Acetylcholinesterase									
	Alert AC 1a - Tacrine	74	292	11261	3	20.2	100.0	97.5	0.435
	Alert AC 1b - 4-Quinolinamine	77	289	11233	31	21.0	99.7	97.2	0.378
	Alert AC 1c - AC Frag 1	97	269	11261	3	26.5	100.0	97.7	0.501
	Alert AC 1d - Dimethyl benzylamine	127	239	9596	1668	34.7	85.2	83.6	0.096
	Alert AC 1e - Dimethyl benzylamine-like SMARTS	142	224	9710	1554	38.8	86.2	84.7	0.124
Cyclooxygenases									
	Alert COX 1 - Sulfonated aromatic rings	187	168	10567	708	52.7	93.7	92.5	0.299
	Alert COX 2 - Cinnamaldehyde-like	58	297	10912	363	16.3	96.8	94.3	0.121
	Alert COX 3 - 5-Phenyl-1H-pyrazole-like	34	321	11057	218	9.6	98.1	95.4	0.090
	Alerts COX 1 OR 2	226	129	10218	1057	63.7	90.6	89.8	0.298
Dihydrofolate Reductase									
	Alert DHFR 1 (SMILES) - Diaminopyrimidine	63	37	11397	133	63.0	98.8	98.5	0.444
	Alert DHFR 1 (SMARTS) - Diaminopyrimidine-like	65	35	10535	995	65.0	91.4	91.1	0.181

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
GAR Transformylase									
	Alert GART 1 - N-Acetyl-DL-glutamic acid	10	3	11592	25	76.9	99.8	99.8	0.468
Histone Deacetylase 1									
	Alert HDAC 1 1 - Hydroxamic acids	147	173	11296	14	45.9	99.9	98.4	0.641
	Alert HDAC 1 2a - Benzamide	83	237	10066	1244	25.9	89.0	87.3	0.077
	Alert HDAC 1 2b - Benzamide-like SMARTS	100	220	9846	1464	31.3	87.1	85.5	0.088
	Alert HDAC 1 2c - Acetanilide	127	193	10380	930	39.7	91.8	90.3	0.179
	Alert HDAC 1 2d - Acetanilide-like SMARTS	135	185	9654	1656	42.2	85.4	84.2	0.125
	Alert HDAC 1 2e - Benanilide	61	259	11173	137	19.1	98.8	96.6	0.226
	Alert HDAC 1 2f - Benanilide-like SMARTS	73	247	11135	175	22.8	98.5	96.4	0.241
Monoamine Oxidase									
	Alert MAO A 1 - 3-Phenyl-2-oxazolidone	6	145	11477	2	4.0	100.0	98.7	0.171
	Alert MAO A 2a - 4-Methylphenol	37	114	9431	2048	24.5	82.2	81.4	0.020
	Alert MAO A 2b - 3-Methylphenol	35	116	9698	1781	23.2	84.5	83.7	0.024
	Alert MAO A 2c - Methyl-3,4-diphenol	3	148	10901	578	2.0	95.0	93.8	-0.016
	Alert MAO A 3a - Aminomethaziole	26	125	11415	64	17.2	99.4	98.4	0.215
	Alert MAO A 3b - 4-Phenyl-1,3-thiazole-2-amine	24	127	11452	27	15.9	99.8	98.7	0.268
	Alert MAO A 3c - CID 7958070	24	127	11479	0	15.9	100.0	98.9	0.396
Phosphodiesterases									
3A	Alert PDE 3A 1 - PDE 3A Frag 2	9	29	11592	0	23.7	100.0	99.8	0.486
	Alert PDE 3A 2a - PDE Frag 3	6	32	11592	0	15.8	100.0	99.7	0.397
	Alert PDE 3A 2b - PDE Frag 3-like SMARTS	7	31	11592	0	18.4	100.0	99.7	0.429
	Alert PDE 3A 3 - PDE 3A Frag 4	14	24	11574	18	36.8	99.8	99.6	0.400
	Alert PDE 3A 4 - Veratrol-like	13	25	11139	453	34.2	96.1	95.9	0.088

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
4D	Alert PDE 4D 1 - Homoveratrol-like	47	71	11311	201	39.8	98.3	97.7	0.264
	Alert PDE 4D 2 - 8-Isopropyl-1H-purine Frag	19	99	11512	0	16.1	100.0	99.1	0.400
	Alert PDE 4D 3 - PDE 4D Frag 3	50	68	10932	580	42.4	95.0	94.4	0.165
Thymidylate Synthase									
	Alert TS 1 - N-Acetyl-DL-glutamic acid	13	41	11554	22	24.1	99.8	99.5	0.296
	Alert TS 2 - Pyrimidine	2	52	11423	153	3.7	98.7	98.2	0.014
	Alert TS 1 AND 2	1	53	11576	0	1.9	100.0	99.5	0.136
	Alert TS 3 - Uracil	3	51	11537	39	5.6	99.7	99.2	0.059
Tyrosine Protein Kinase									
	Alert LCK 1 - Pyrimidine-like	78	71	10494	987	52.3	91.4	90.9	0.171
Ion Channels									
Glutamate (NMDA) Receptor									
	Alerts GRSZ1 1 - GRSZ1 Frag 1	7	0	11623	0	100.0	100.0	100.0	1.000
Potassium Voltage Gated Channel Subunit H Member 2									
	Alert HERG 1a - Flex Aromatic Amine 1-5	60	20	5493	6057	75.0	47.6	47.7	0.037
	Alert HERG 1b - Flex Aromatic Amine 2-5	53	27	6046	5504	66.3	52.3	52.4	0.031
Serotonin 3A Receptor									
	Alert S3AR 1 - S3AR Frag 1	12	74	11544	0	14.0	100.0	99.4	0.372
	Alert S3AR 2 - 1-[2-(Phenylsulfanyl)phenyl]piperazine	7	79	11533	11	8.1	99.9	99.2	0.175
	Alert S3AR 1 or 2	19	67	11533	11	22.1	99.9	99.3	0.372

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Sodium Channel V Subunit alpha									
	Alert SCA-A 1a - Pyrimidine-like aromatic	24	18	10466	1122	57.1	90.3	90.2	0.096
	Alert SCA-A 1b - Pyrimidine-like wildcard	24	18	10466	1122	57.1	90.3	90.2	0.096
	Alert SCA-A 2 - 4-(4-Piperidineyloxy)pyrimidine	9	33	11588	0	21.4	100.0	99.7	0.462
	Alert SCA-A 3 - SCV Frag 1	8	34	11588	0	19.0	100.0	99.7	0.436
	Alert SCA-A 4 - N-(2-Phenylethyl)acetamide	11	31	11133	455	26.2	96.1	95.8	0.068
Voltage Gated K Channel Subunit Kv7.1									
	Alert VGKC Kv7.1 1 - VGKV Kv7.1 Frag 1	7	0	11618	5	100.0	100.0	100.0	0.764
Nuclear Receptors									
Androgen Receptor									
	Alert AR 1a - Benzonitrile	132	205	11075	218	39.2	98.1	96.4	0.366
	Alert AR 1b - Nitrobenzene	39	298	11191	102	11.6	99.1	96.6	0.164
	Alert AR 1c - Quinolone	59	278	11247	46	17.5	99.6	97.2	0.303
	Alert AR 1a, 1b OR 1c	230	107	10927	366	68.2	96.8	95.9	0.495
Glucocorticoid Receptor									
	Alert GR 1a - GR Frag 1	74	378	11159	19	16.4	99.8	96.6	0.352
	Alert GR 1b - tert-Butylcyclohexane	55	397	11145	33	12.2	99.7	96.3	0.265
	Alert GR 1c - GR Frag 4	68	384	11168	10	15.0	99.9	96.6	0.354
	Alert GR 1d - GR Frag 5	68	384	11023	155	15.0	98.6	95.4	0.192
	Alerts GR 1a OR 1c OR 1d	76	376	11005	173	16.8	98.5	95.3	0.204

Receptor	MIE	TP	FN	TN	FP	SE	SP	Q	MCC
Transporters									
Dopamine Transporter									
	Alert DT 1 - 3-Phenyl-8-azabicyclo[3.2.1]octane	63	372	11136	59	14.5	99.5	96.3	0.260
	Alert DT 2 - Diphenylmethane-like	101	334	9902	1293	23.2	88.5	86.0	0.068
Norepinephrine Transporter									
	Alert NT 1a - Cocaine-like 1	15	623	10961	31	2.4	99.7	94.4	0.075
	Alert NT 1b - Cocaine-like 2	16	622	10960	32	2.5	99.7	94.4	0.079
	Alert NT 1c - Cocaine-like 3	33	605	10903	89	5.2	99.2	94.0	0.098
	Alert NT 1d - Cocaine-like 4	37	601	10789	203	5.8	98.2	93.1	0.063
	Alert NT 2 - Amphetamines-like	486	152	4158	6834	76.2	37.8	39.9	0.066
Serotonin Transporter									
	Alert ST 1 - 3-Ethyl-indole	78	749	10238	565	9.4	94.8	88.7	0.047
	Alert ST 2 - DMEA	68	759	4256	6547	8.2	39.4	37.2	-0.272
	Alert ST 3 - Benzyloxybenzene	81	746	10635	168	9.8	98.4	92.1	0.146
	Alert ST 4 - Diphenylmethane	227	600	10258	545	27.4	95.0	90.2	0.231

Table 3. Results for test sets of each receptor with the remaining test sets acting as negatives. TP=True Positive, FN=False Negative, TN=True Negative, FP=False Positive, SE=Sensitivity, SP=Specificity, Q=Overall Quality, MCC=Matthews Correlation Coefficient.

Target		TP	FN	SE
GPCRs				
Adenosine A2a Receptor	Alert 1, 2 3 OR 4	505	305	62.35
Alpha-1a Adrenergic Receptor	Alert 1, 2 OR 3	57	138	29.23
Alpha-2a Adrenergic Receptor	Alert 1, 2, 3, 4, 5 OR 6	43	67	39.09
Beta-1 Adrenergic Receptor	Alert 1, 2 OR 3 (beta alerts)	182	7	96.30
Beta-2 Adrenergic Receptor	Alert 1, 2 OR 3 (beta alerts)	215	15	93.48
Cannabinoid CB1 Receptor	Alert 1, 2 OR 3	179	871	17.05
Cannabinoid CB2 Receptor	Alert 1 OR 2	207	763	21.34
Cholecystokinin Receptor A	Alert 1, 2 OR 3	76	2	97.44
Dopamine D1 Receptor	Alert 1, 2, 3 OR 4	45	86	34.35
Dopamine D2 Receptor	Alert 1, 2 OR 3	417	341	55.01
Endothelin Receptor A	Alert 1, 2, 3, 4 OR 5	25	3	89.29
Histamine H1 Receptor	Alert 1 OR 2	45	113	28.48
Histamine H2 Receptor	Alert 1, 2 OR 4	38	10	79.17
Muscarinic Acetylcholine Receptor M1	Alert 1 OR 4	109	139	43.95
Muscarinic Acetylcholine Receptor M2	Alert 1, 2 OR 4	74	90	45.12
Muscarinic Acetylcholine Receptor M3	Alert 2, 3 OR 4	128	167	43.39
Delta Opioid Receptor	Alert 1 (3 or more) OR 2	268	241	52.65
Kappa Opioid Receptor	Alert 1 (3 or more) OR 2	286	271	51.35
Mu Opioid Receptor	Alert 1 (3 or more) OR 2	353	306	53.57
Serotonin 1A Receptor	Alert SR1, SR1a1b2a1 OR SR1a1	313	191	62.10
Serotonin 1B Receptor	Alert SR1, SR1a1b2a1 OR SR1b1	98	16	85.96
Serotonin 2A Receptor	Alert SR1 OR SR1a1b2a1	140	310	31.11
Serotonin 2B Receptor	Alert SR1, SR2b1 OR SR2b2	37	158	18.97
Vasopressin V1A Receptor	Alert 1, 2, 3 OR 4	133	47	73.89

Target		TP	FN	SE
Enzymes				
Acetylcholinesterase	Alert 1a, 1b, 1c, 1d, OR 1e	244	122	66.67
Cyclooxygenase 1	Alert 1, 2 OR 3	45	60	42.86
Cyclooxygenase 2	Alert 1, 2 OR 3	197	81	70.86
Dihydrofolate Reductase	Alert 1 (SMARTS)	65	35	65.00
GAR Transformylase	Alert 1	10	3	76.92
Histone Deacetylase 1	Alert 1, 2b, 2d OR 2f	244	76	76.25
Monoamine Oxidase A	Alert 1, 2a, 2b, 2c, 3a, 3b OR 3c	77	74	50.99
Phosphodiesterase 3A	Alert 1, 2b, 3 OR 4	27	11	71.05
Phosphodiesterase 4D	Alert 1, 2 OR 3	78	40	66.10
Thymidylate Synthase	Alert 1, 2 OR 3	17	37	31.48
Tyrosine-Protein Kinase	Alert 1	78	71	52.35
Ion Channels				
Glutamate (NMDA) Receptor	Alert 1	7	0	100.00
Potassium Voltage Gated Channel KQT 1	Alert 1a	60	20	75.00
Serotonin 3A Receptor	Alert 1 OR 2	19	67	22.09
Sodium Channel V Subunit Alpha	Alert 1b, 2, 3 OR 4	28	14	66.67
Voltage Gated K Channel Subunit Kv7.1	Alert 1	7	0	100.00

Target		TP	FN	SE
Nuclear Receptors				
Androgen Receptor	Alert 1a, 1b OR 1c	230	107	68.25
Glucocorticoid Receptor	Alert 1a, 1b, 1c OR 1d	76	376	16.81
Transporters				
Dopamine Transporter	Alert 1 OR 2	164	271	37.70
Norepinephrine Transporter	Alert 1a, 1b, 1c, 1d OR 2	490	148	76.80
Serotonin Transporter	Alert 1, 2, 3 OR 4	637	190	77.03

Table 4. Results for models of combined structural alerts for each receptor. TP=True Positive, FN=False Negative, SE=Sensitivity.