

# Towards an MIE Atlas

The roads not taken: avoiding adverse outcome pathways



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# Toxicity Testing



Modern humans are exposed to hundreds of chemicals in their everyday lives.

To ensure the safety of these chemicals **toxicity testing must be carried out.**

In the past 50 years the *in vivo* approach using laboratory animals has changed little – particularly in the pharmaceutical industry.

These tests are **expensive, time consuming, ethically unsound and animal models do not effectively reflect human responses.**

# Toxicity Testing



In 2007 the NRC published a report highlighting these drawbacks and setting in motion the TT21C drive.

Advances in *in silico* and *in vitro* methods pave the way for greater understanding of the mechanisms behind toxicological effects, moving toxicology away from a predominantly observational craft towards a science based on understanding.

# The Adverse Outcome Pathway



One such approach is the Adverse Outcome Pathway (AOP) framework for risk assessment.

The aim is to build understanding of a compounds effects across all levels of biological organisation.

The **Molecular Initiating Event (MIE)** can be thought of as the gateway to the AOP – the initial chemical interaction.

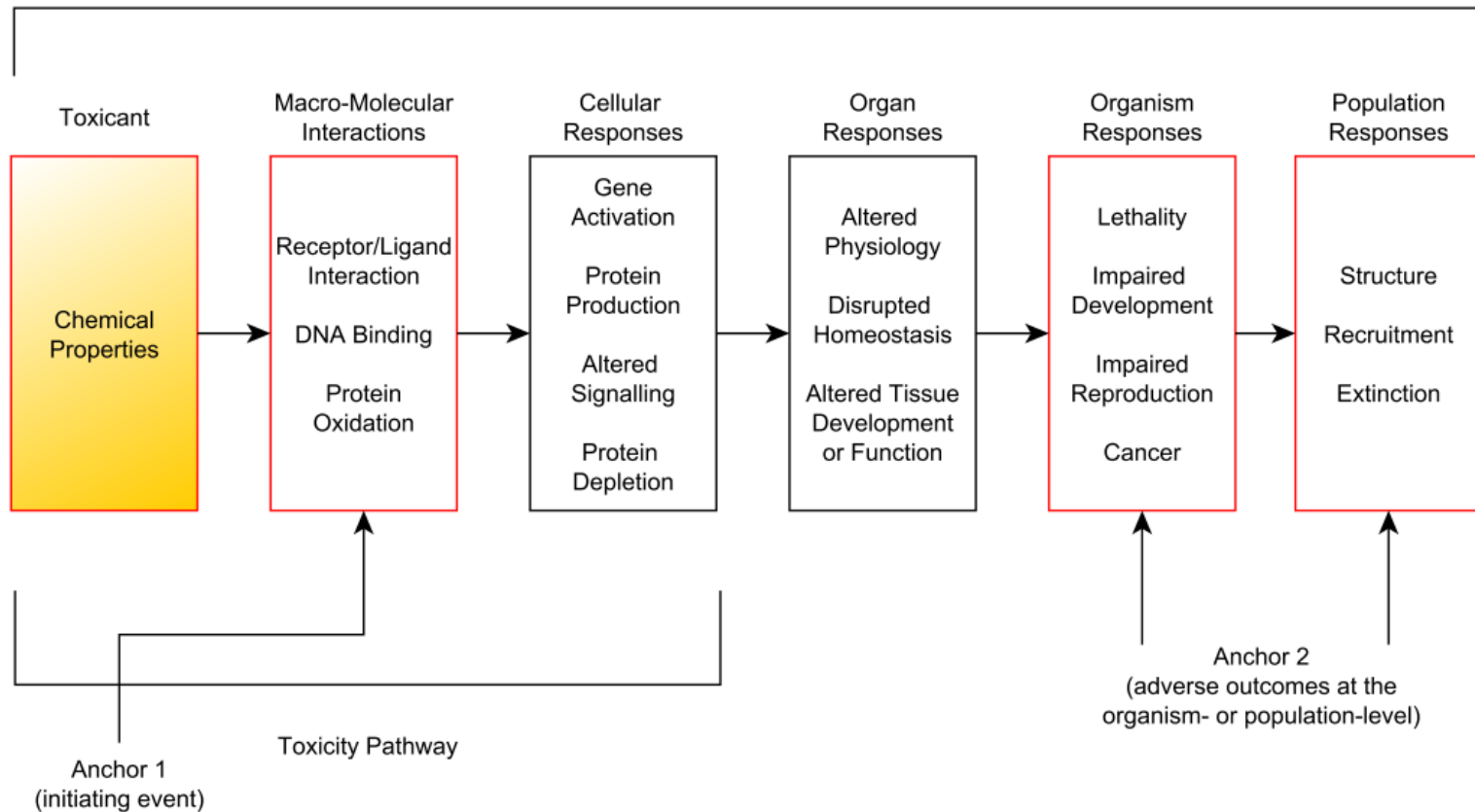
**Chemistry is key to understanding the MIE** – What is it about these molecules that allow them to do this?

Using knowledge of the chemical characteristics that govern these interactions, a **greater understanding of why chemicals cause toxic effects can be gained.**

# The Adverse Outcome Pathway



## Adverse Outcome Pathway



# Finding the MIE



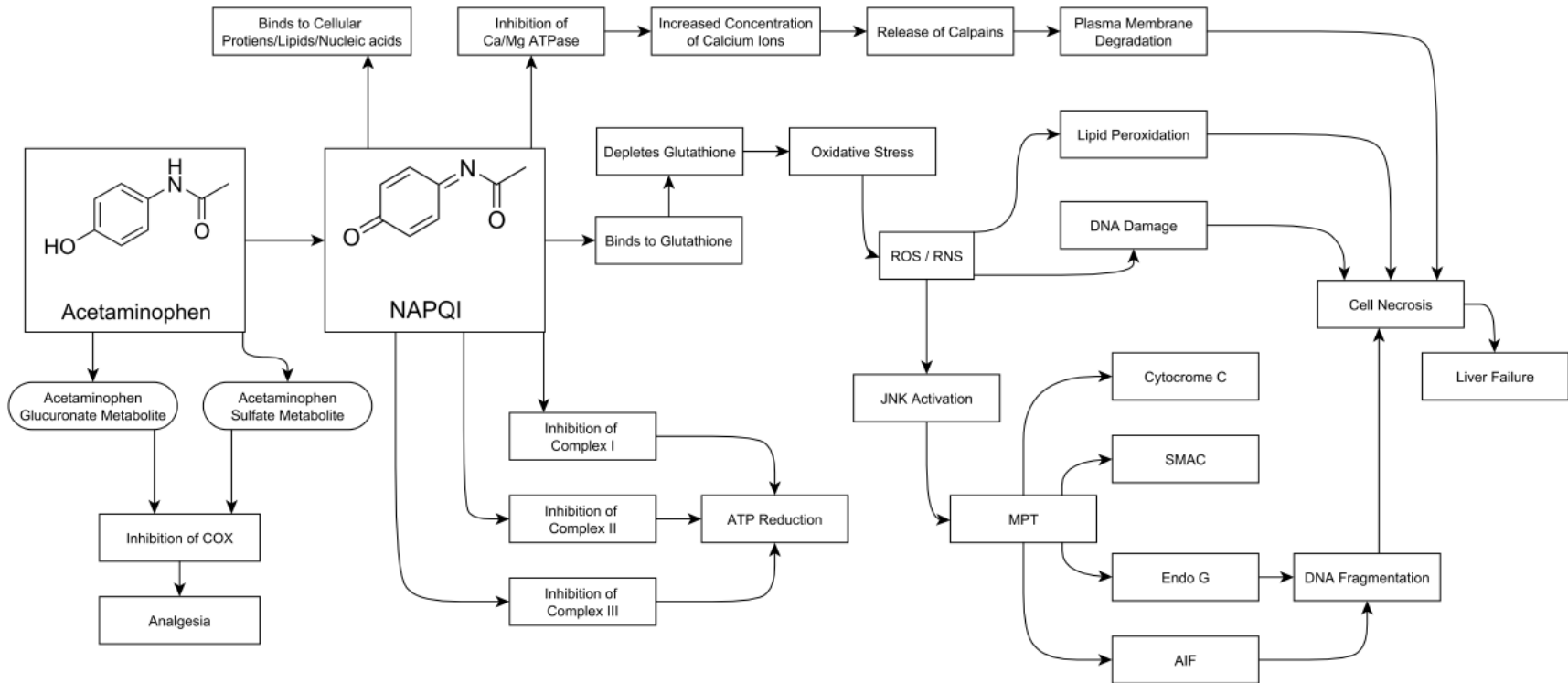
The beginning of the project focused on **searching for existing information on MIEs** to see what could be pieced together.

Searching toxicological databases provided little information as these were frequently poorly populated, and did not contain information relevant to our study.

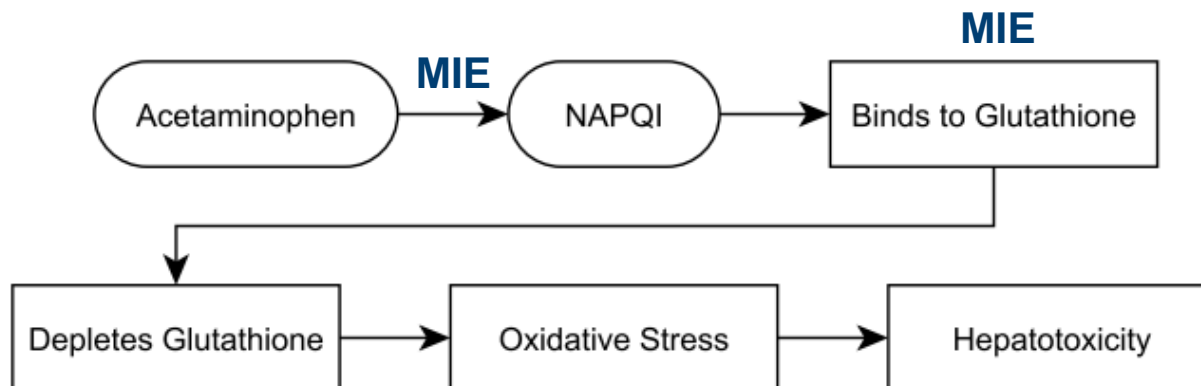
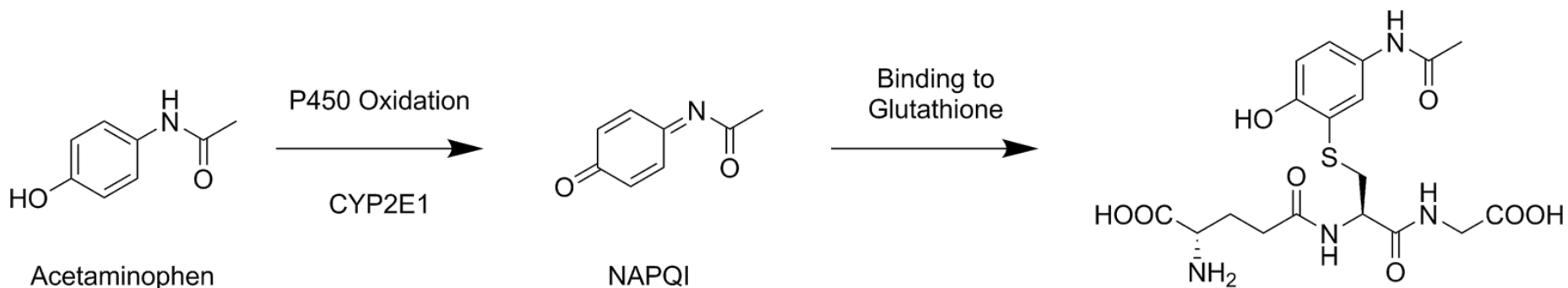
As such **a literature search was performed** for well understood molecules to gain a detailed picture of their toxicity.

Several structurally and toxicologically diverse molecules were chosen for this search.

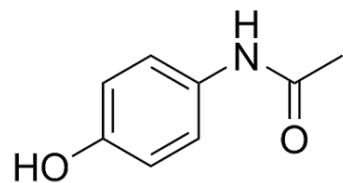
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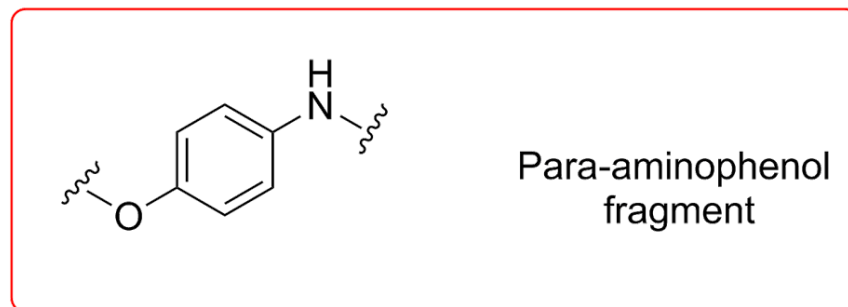
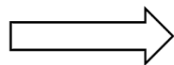
# Acetaminophen



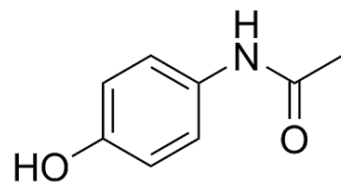
# Acetaminophen SAR



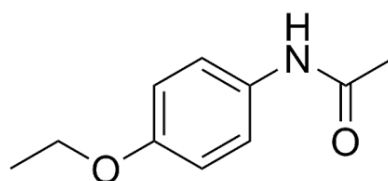
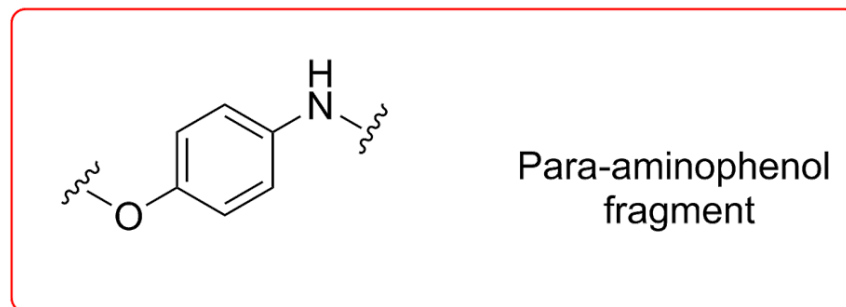
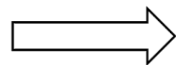
Acetaminophen



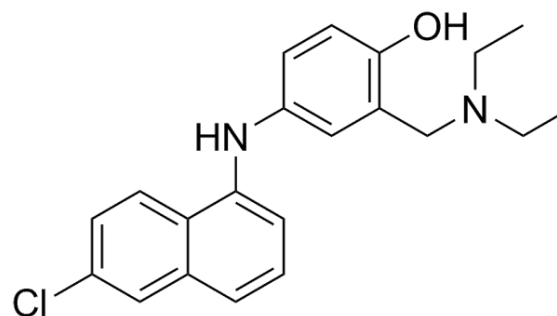
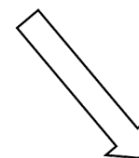
# Acetaminophen SAR



Acetaminophen

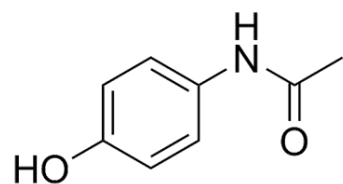


Phenacetin

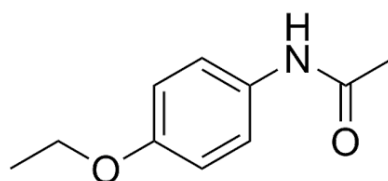
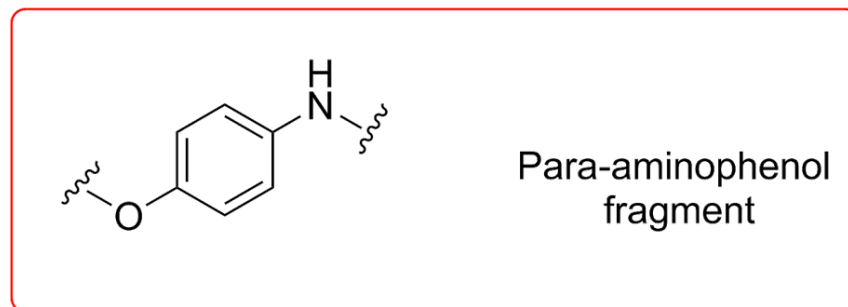
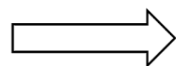


Amiodiaquine

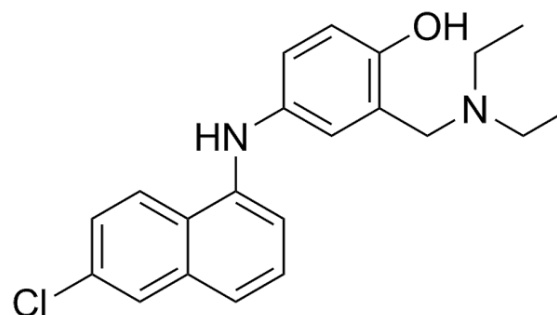
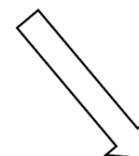
# Acetaminophen SAR



Acetaminophen



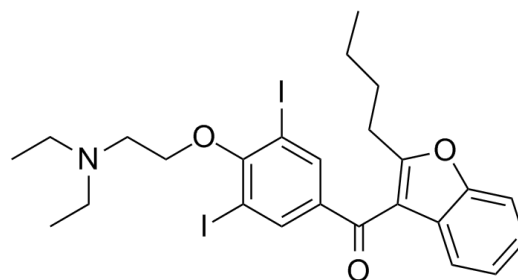
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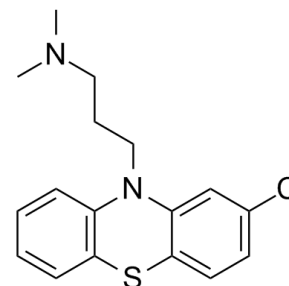
Amiodiaquine



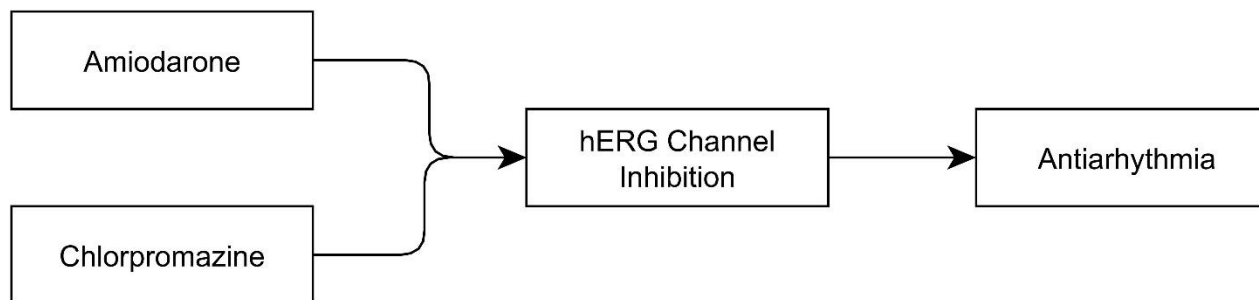
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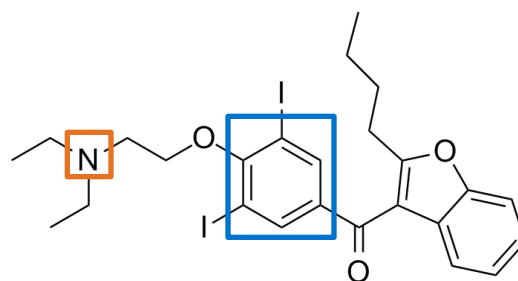
Amiodarone



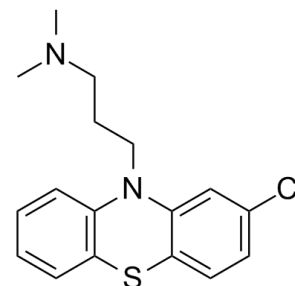
Chlorpromazine



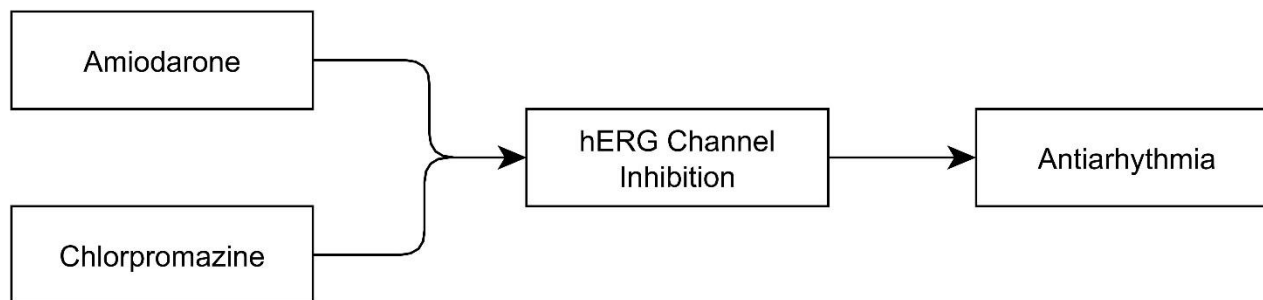
# Antiarrhythmia SAR



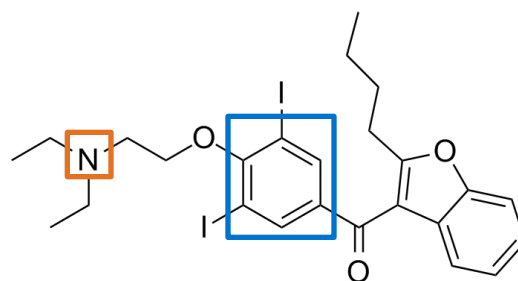
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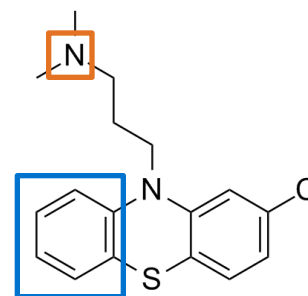
Chlorpromazine



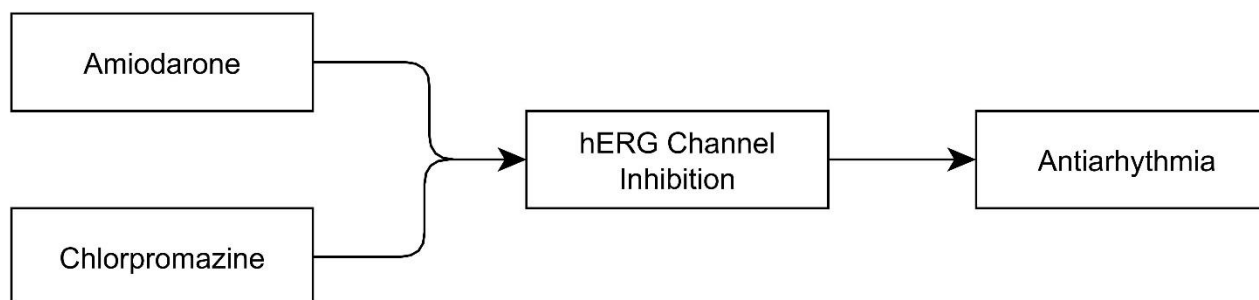
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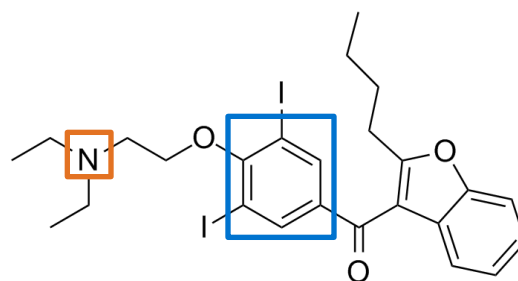
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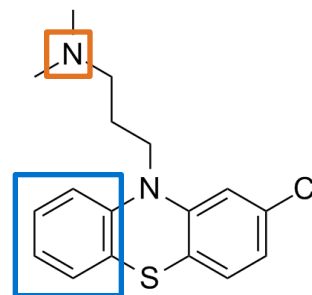
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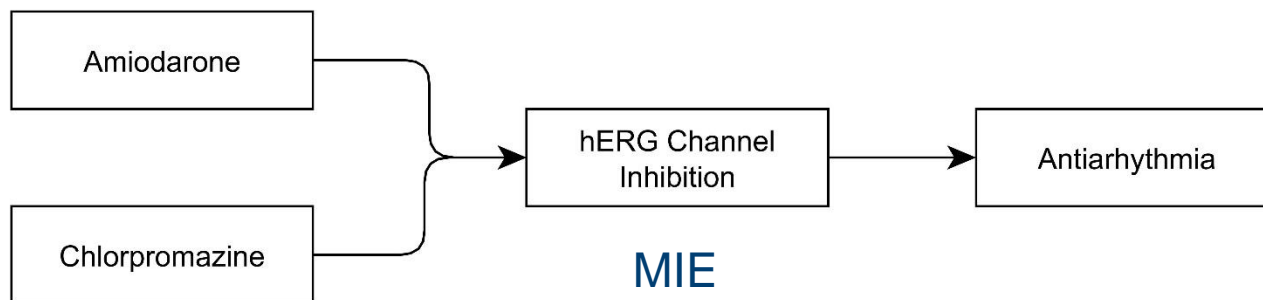
# Antiarrhythmia SAR



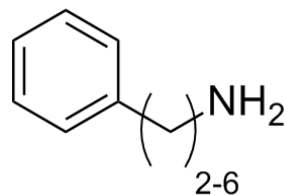
Amiodarone



Chlorpromazine

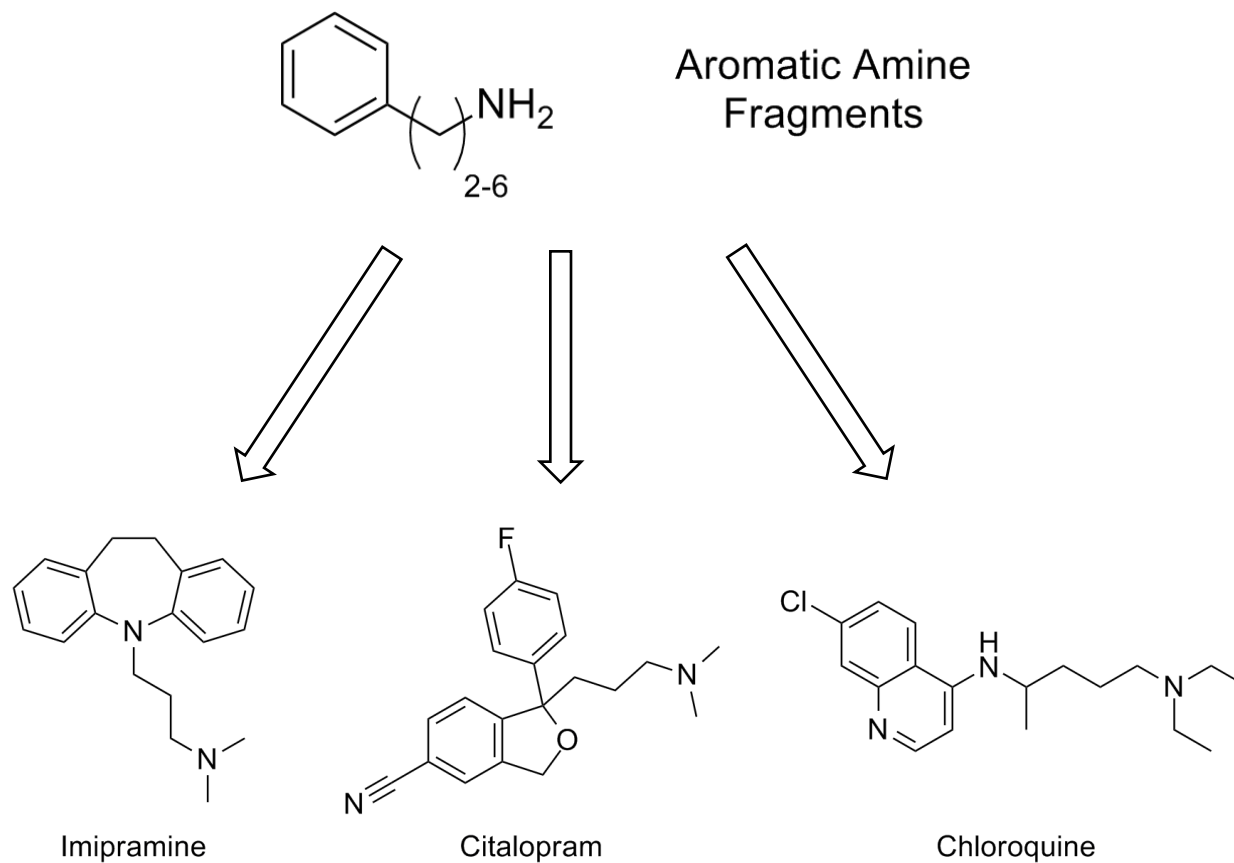


# Antiarrhythmia SAR

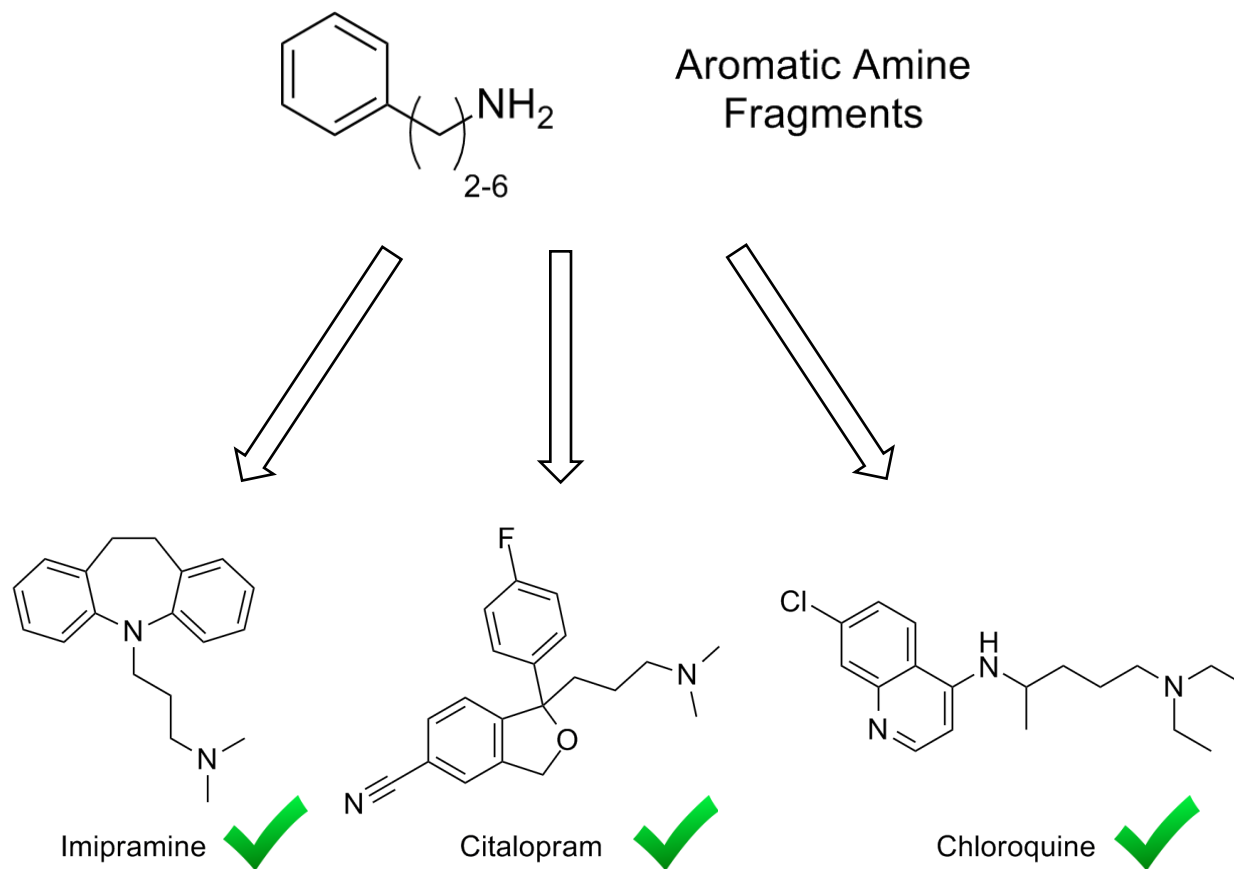


Aromatic Amine  
Fragments

# Antiarrhythmia SAR



# Antiarrhythmia SAR



# Defining the MIE



A single definition of the MIE has yet to reach acceptance. Different definitions stem from different fields that have focused on a specific type of interaction.

With experience from the atlas of many interactions we were able to combine the best features of existing definitions to form a unified definition.

*The MIE is the initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway.*

This definition focuses on the **initial interaction**, **relates the interaction to a measurable outcome**, **includes a multitude of different interactions**, and **does not focus the term entirely in toxicological research**.

# The MIE Principles



To prove the concept of predictive toxicology using the chemistry of molecules, models needed to be built and tested using knowledge we had gained so far.

As such the principles of the MIE Atlas were used to design a model approach.

**Characteristics of molecules that are associated with a certain MIE** will be used to build the models. While many characteristics can be evaluated, black box models with little mechanistic or theoretical background lead to confusion and fail to provide new understanding.

*As such we aim to build clear models using **fragment structural alerts in 2D**, and a range of **chemically sound molecular descriptors**.*

This will allow additional understanding to be gained about the MIE itself and help our models at a regulatory stage.

# NIH Tox Data Challenge



The NIH Tox Data Challenge 2014 was initiated by the US NIH over the summer to “crowdsource” data analysis and **evaluate computational models for toxicity prediction**.

Data on several receptors and *in vitro* tests were provided as training sets and test sets will be evaluated by the NIH to determine a score for submitted models.

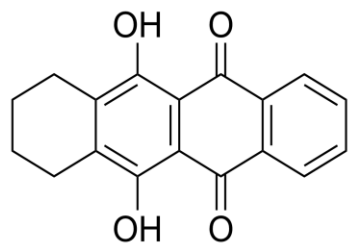
The **Nuclear Receptor binding data provided a good dataset for MIE based models** to be developed and tested.

Receptors included are the Androgen Receptor (AR), the Estrogen Receptor (ER), the Aryl hydrocarbon Receptor (AhR), Aromatase, and PPAR-gamma. There are also ligand binding domains for the Androgen and Estrogen receptors (AR-LBD and ER-LBD).

# AR-LBD Results



## Positive

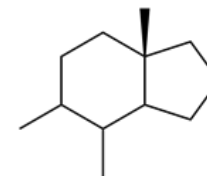


CID 371180

Hg

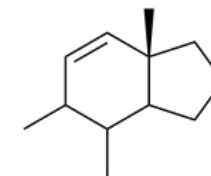
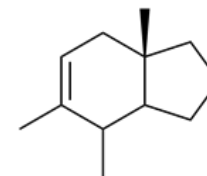
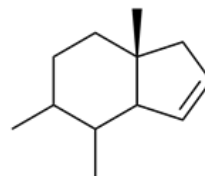


Trimethyl Tin

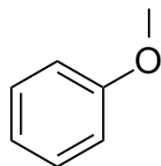


Steroidal Core

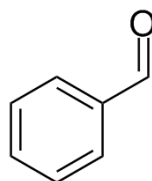
Mercury



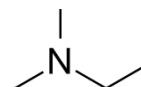
## Negative



Anisole



Benzaldehyde

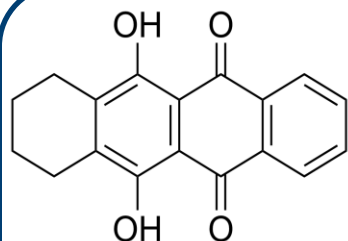


Dimethylethylamine

# AR-LBD Results



## Positive



CID 371180

Hg

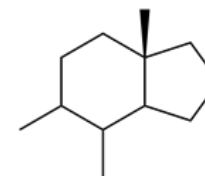
Mercury

Set A

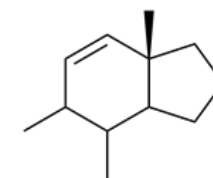
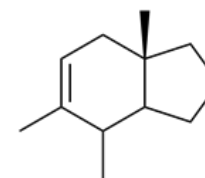
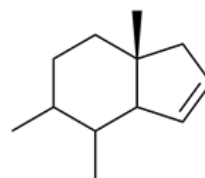


Trimethyl Tin

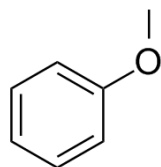
Set B



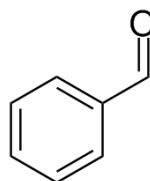
Steroidal Core



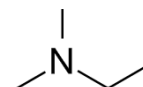
## Negative



Anisole



Benzaldehyde

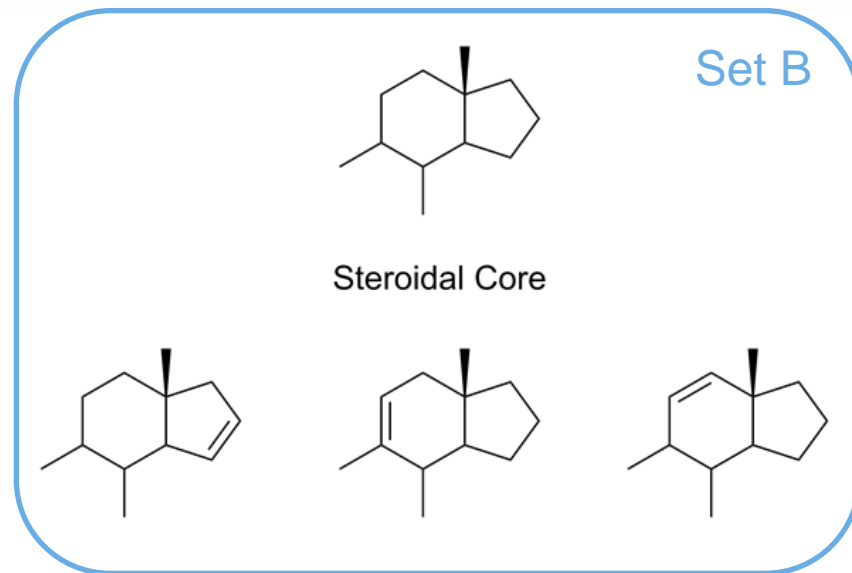
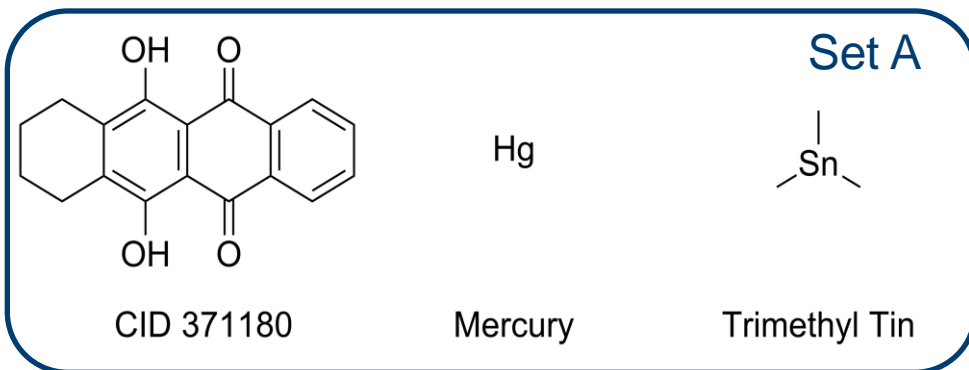


Dimethylethylamine

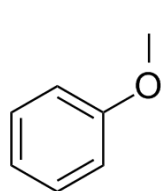
# AR-LBD Results



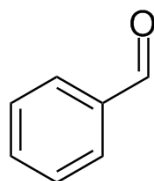
## Positive



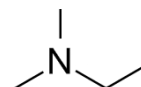
## Negative



Anisole



Benzaldehyde



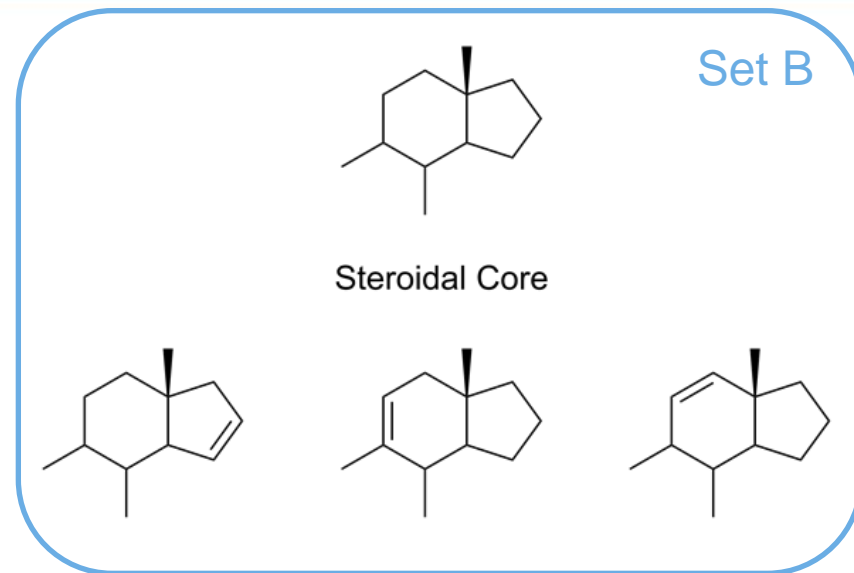
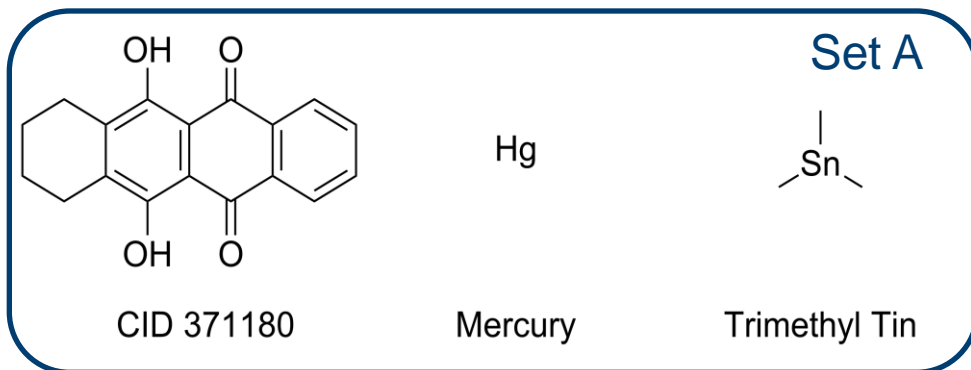
Dimethylethylamine

Set	Model	TP	FN	TN	FP	SE	SP	Q	MCC
Train	(SET A) Or (NO NEG + SET B)	188	115	8223	73	62.0	99.1	97.8	0.657
Test (int)	(SET A) Or (NO NEG + SET B)	12	7	816	8	63.2	99.0	98.2	0.606

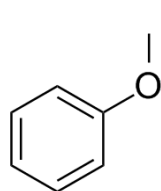
# AR-LBD Results



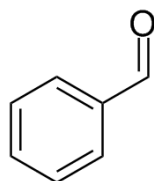
## Positive



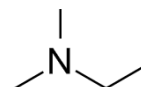
## Negative



Anisole



Benzaldehyde



Dimethylethylamine

Set	Model	TP	FN	TN	FP	SE	SP	Q	MCC
NIH Test Set	(SET A) Or (NO NEG + SET B)	1	3	247	1	25.0	99.6	98.4	0.346

Balanced Accuracy: 62.3

Best Score: 65.0

2<sup>nd</sup> Best in Top 10

# Application to a Large Dataset



In order to apply our approach to a large dataset, a number of appropriate receptors were chosen, based on our previous research and a paper on pharmacological anti-targets (Bowes 2012).

**This set includes a wide variety of targets**, including Enzymes, GPCRs, Ion Channels, Nuclear Receptors, and Transporters.

Receptor binding data has been obtained for analysis from the open source database ChEMBL.

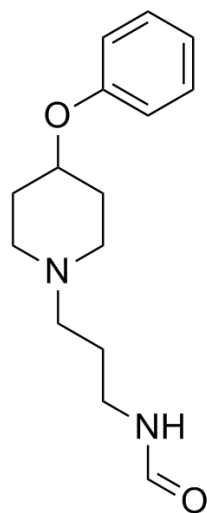
This data is being used to elucidate 2D fragments associated with toxicity, **to be built into MIE reports**.

# Histamine Receptors

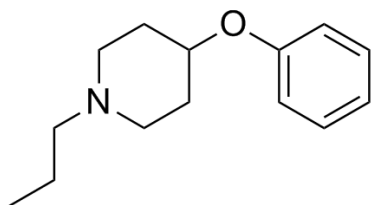


## Histamine H1 Receptor Fragments

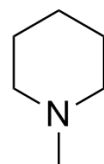
(Train 499, Test 173)



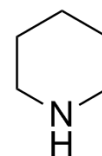
HH1R Frag 1  
12%



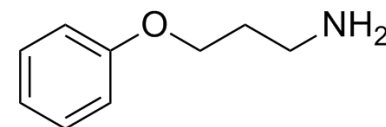
HH1R Frag 2  
13%



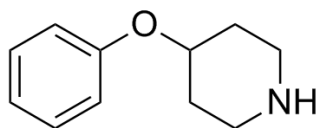
1-Methylpiperidine  
37%



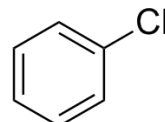
Piperidine  
38%



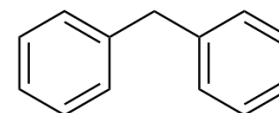
3-Phenoxy-1-propanamine  
20%



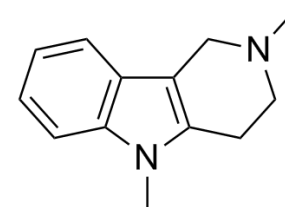
4-Phenoxypiperidine  
13%



Monochlorobenzene  
30%



Diphenylmethane  
16%



CID 587118  
9%

# Histamine Receptors

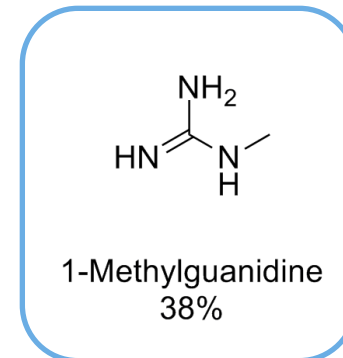
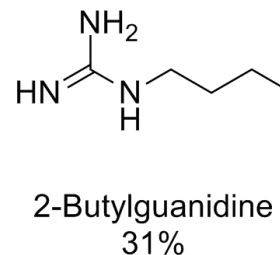
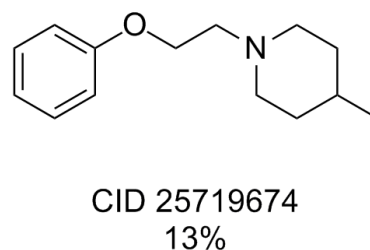
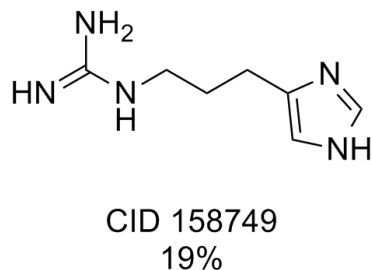
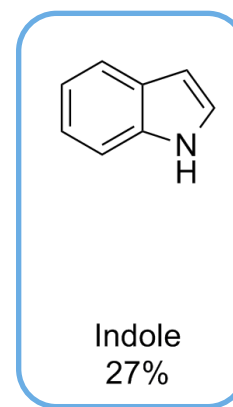
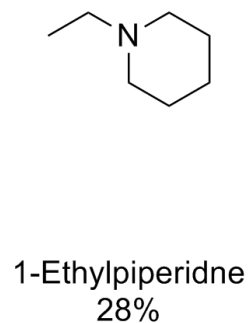
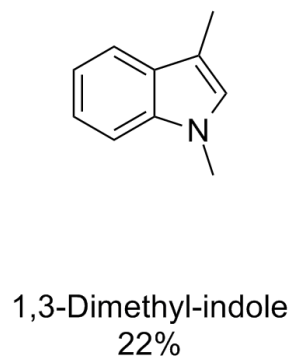
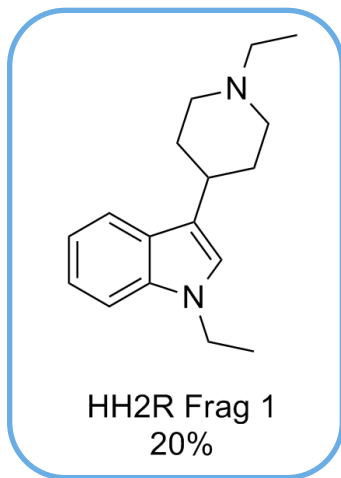
Compound

Molecular Initiating  
Event

Adverse Outcome  
Pathway

## Histamine H2 Receptor Fragments

(Train 141, Test 50)



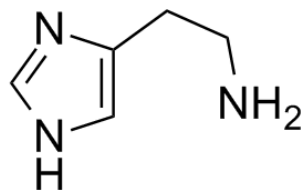
# Histamine Receptors

Compound

Molecular Initiating  
Event

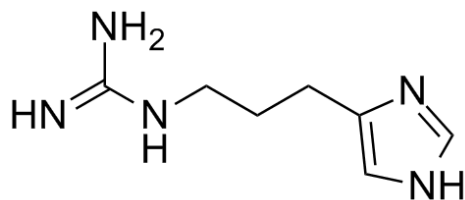
Adverse Outcome  
Pathway

## The Endogenous Ligand – Histamine

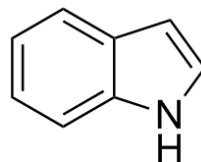


Histamine

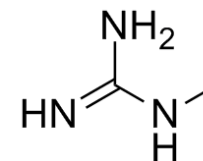
## Greater Structural Similarity to H2 Fragments than H1.



CID 158749



Indole

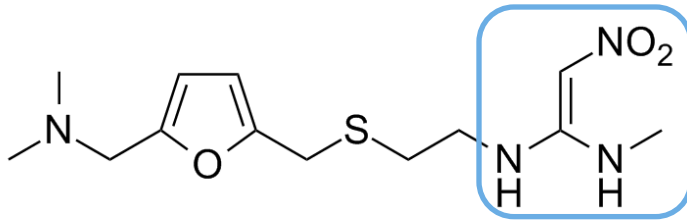


1-Methylguanidine

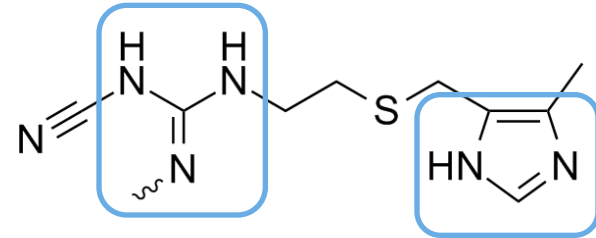
# Histamine Receptors



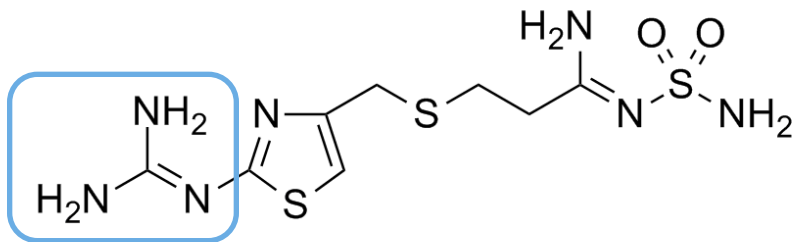
## Histamine H2 Receptor Specific Antagonists



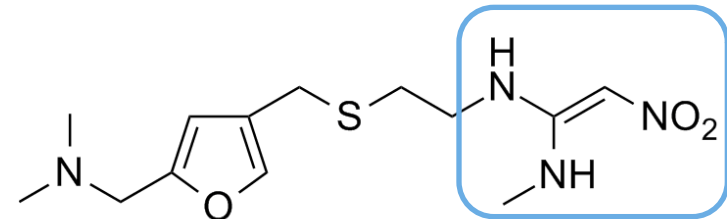
Ranitidine



Cimetidine



Famotidine

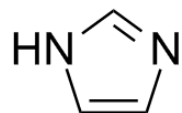


Nizatidine

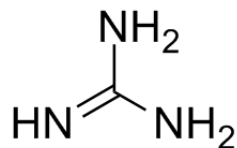
# Histamine Receptors



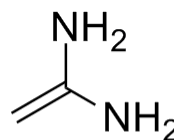
## Histamine H2 Receptor: Histamine-like MIE



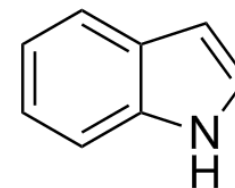
Imidazole



Guanidine



Diamino ethylene

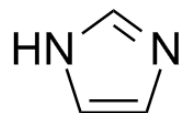


Indole

# Histamine Receptors

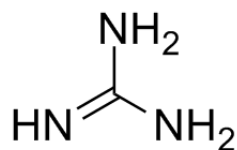


## Histamine H2 Receptor: Histamine-like MIE



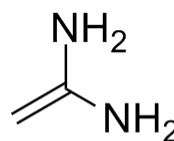
Imidazole

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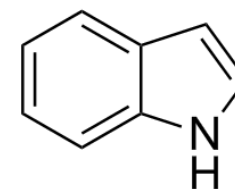
Guanidine

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Diamino ethylene

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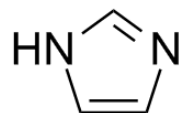
Indole

38/141

# Histamine Receptors

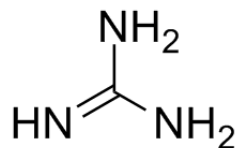


## Histamine H2 Receptor: Histamine-like MIE



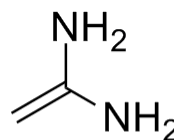
Imidazole

48/141



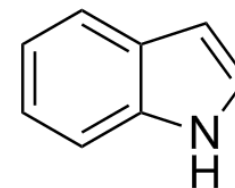
Guanidine

54/141



Diamino ethylene

~~1/141~~



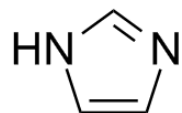
Indole

38/141

# Histamine Receptors

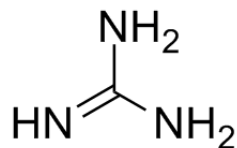


## Histamine H2 Receptor: Histamine-like MIE



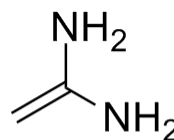
Imidazole

48/141



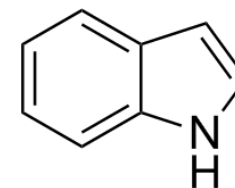
Guanidine

54/141



Diamino ethylene

1/141



Indole

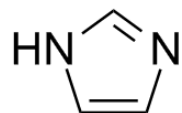
38/141

36/141

# Histamine Receptors

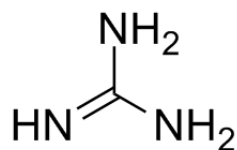


## Histamine H2 Receptor: Histamine-like MIE



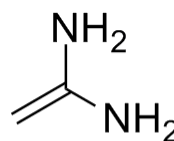
Imidazole

48/141



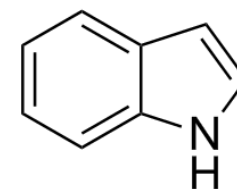
Guanidine

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Diamino ethylene

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Indole

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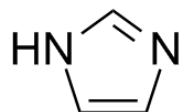


5/141

# Histamine Receptors

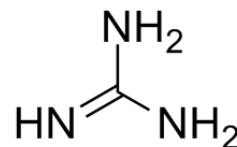


## Histamine H2 Receptor: Histamine-like MIE



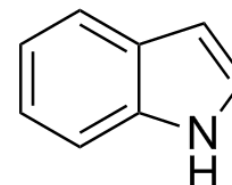
Imidazole

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Guanidine

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Indole

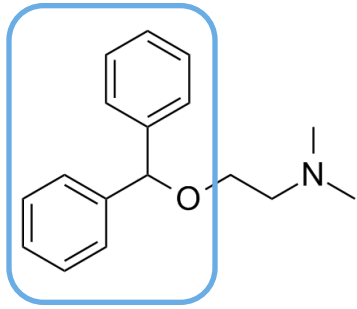
38/141

<b>MW</b>	299	390	422
<b>3DSA</b>	504	637	628
<b>3DVol</b>	489	618	612
<b>ALogP</b>	1.36	1.80	2.19

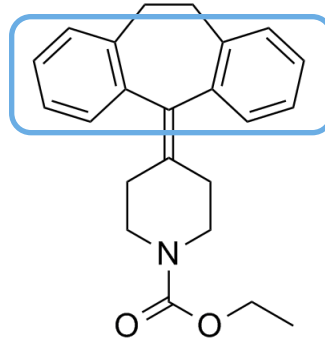
# Histamine Receptors



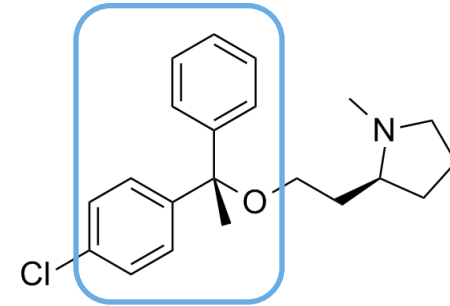
## Histamine H1 Receptor Specific Antagonists



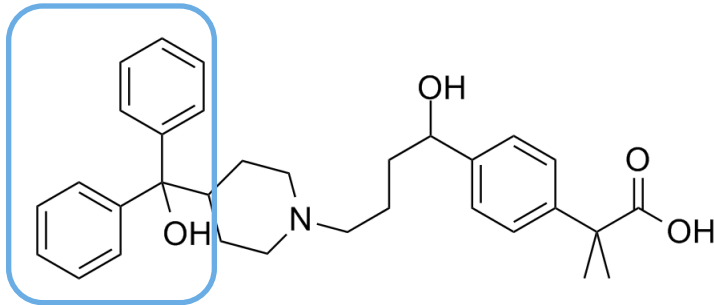
Diphenhydramine



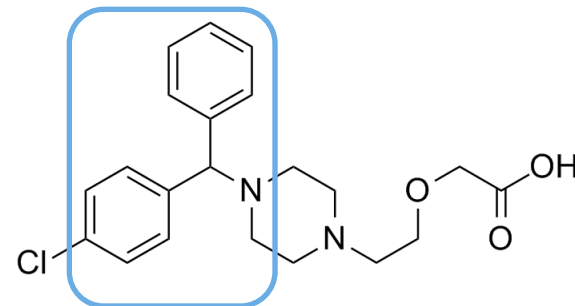
Loratadine



Clemastine



Fexofenadine



Cetirizine

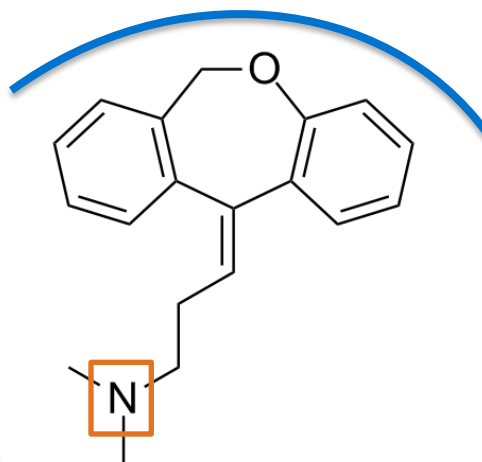
# Histamine Receptors

Compound

Molecular Initiating  
Event

Adverse Outcome  
Pathway

## Histamine H1 Receptor Crystal Structure



Doxepin

A flexibly attached basic nitrogen is protonated to form a charge-charge interaction

A large hydrophobic pocket is occupied by the tricyclic system

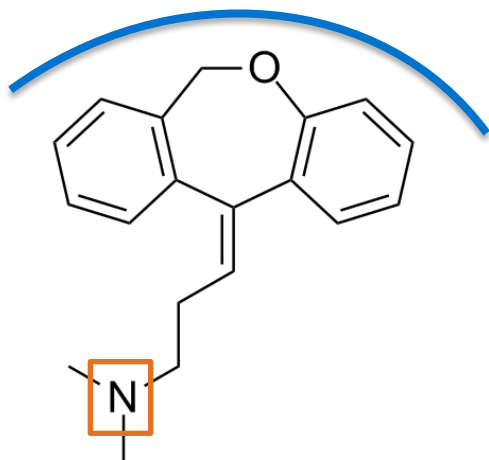
# Histamine Receptors

Compound

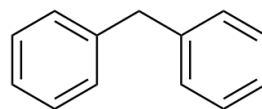
Molecular Initiating  
Event

Adverse Outcome  
Pathway

## Histamine H1 Receptor: Doxepin-like MIE



Doxepin



Diphenylmethane



Tertiary Amine

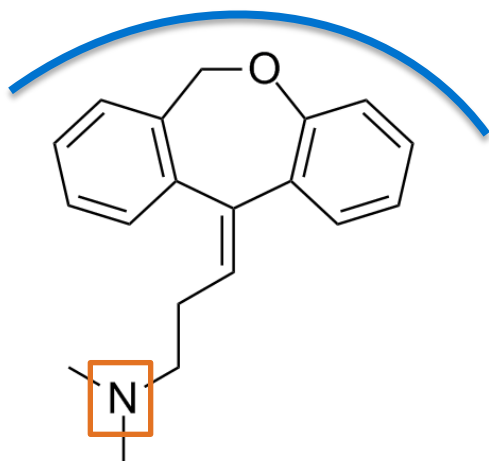
# Histamine Receptors

Compound

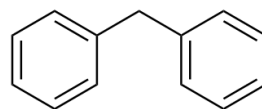
Molecular Initiating  
Event

Adverse Outcome  
Pathway

## Histamine H1 Receptor: Doxepin-like MIE



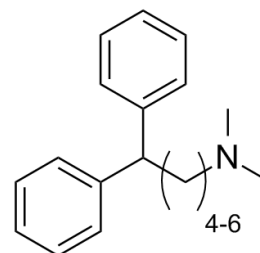
Doxepin



Diphenylmethane



Tertiary Amine



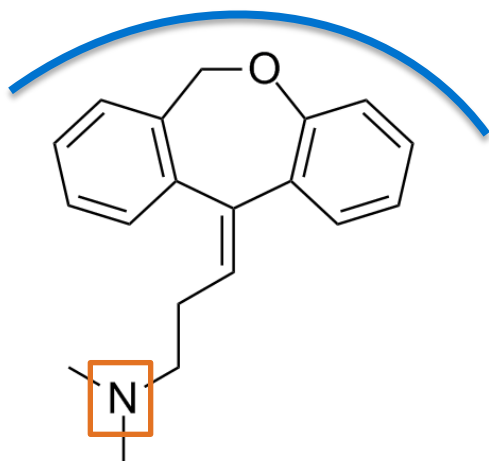
# Histamine Receptors

Compound

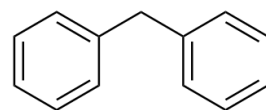
Molecular Initiating  
Event

Adverse Outcome  
Pathway

## Histamine H1 Receptor: Doxepin-like MIE



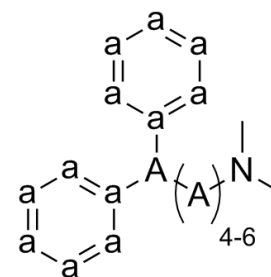
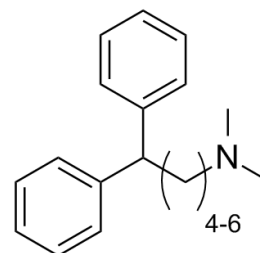
Doxepin



Diphenylmethane



Tertiary Amine



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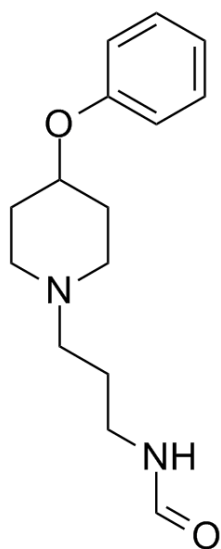
# Histamine Receptors

Compound

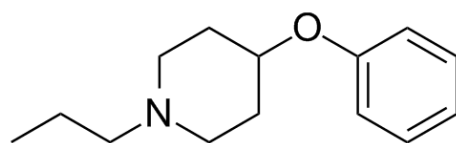
Molecular Initiating  
Event

Adverse Outcome  
Pathway

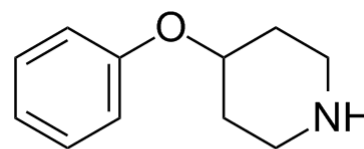
## Histamine H1 Receptor: 4-Phenoxypiperidine-like MIE



HH1R Frag 1



HH1R Frag 2



4-Phenoxypiperidine

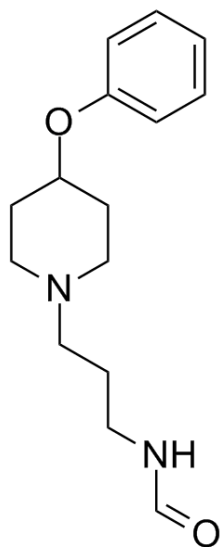
# Histamine Receptors

Compound

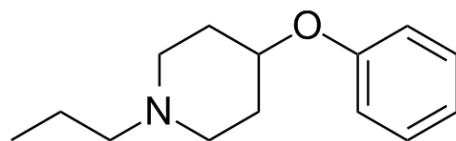
Molecular Initiating  
Event

Adverse Outcome  
Pathway

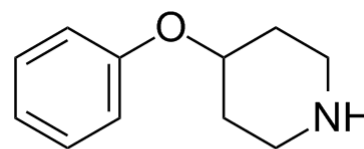
## Histamine H1 Receptor: 4-Phenoxypiperidine-like MIE



HH1R Frag 1



HH1R Frag 2



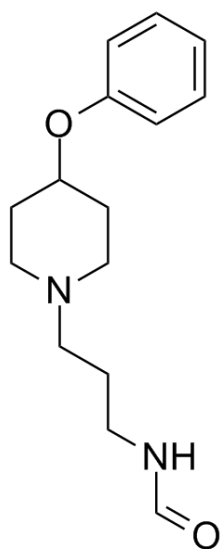
4-Phenoxypiperidine

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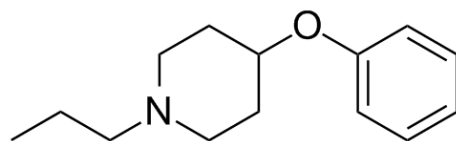
# Histamine Receptors



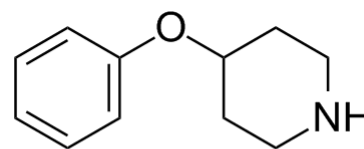
## Histamine H1 Receptor: 4-Phenoxypiperidine-like MIE



HH1R Frag 1



HH1R Frag 2



4-Phenoxypiperidine

67/436

No Overlap with Doxepin MIE

# Thymidylate Synthase

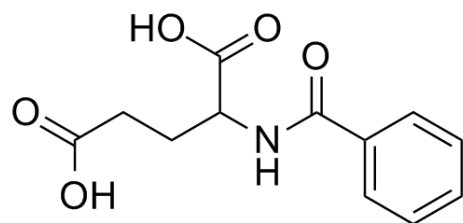
Compound

Molecular Initiating  
Event

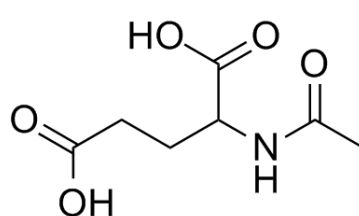
Adverse Outcome  
Pathway

## Thymidylate Synthase Fragments

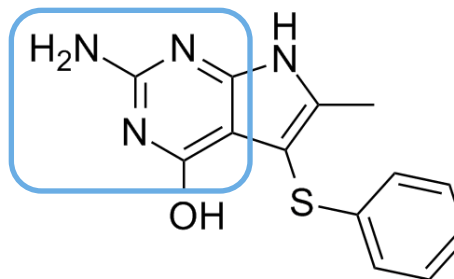
(Train 178, Test 61)



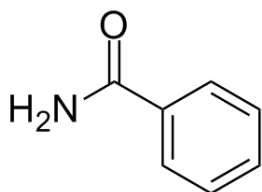
N-Benzoylglutamic acid  
22%



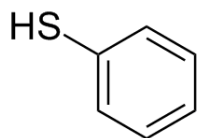
N-Acetyl-DLglutamic acid  
25%



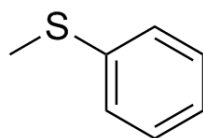
CID 8858953  
11%



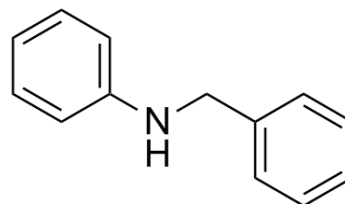
Benzamide  
28%



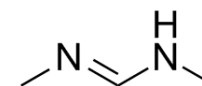
Thiophenol  
49%



Thioanisole  
39%



Benzylaniline  
17%



N,N'-Dimethylimidofornamide  
38%



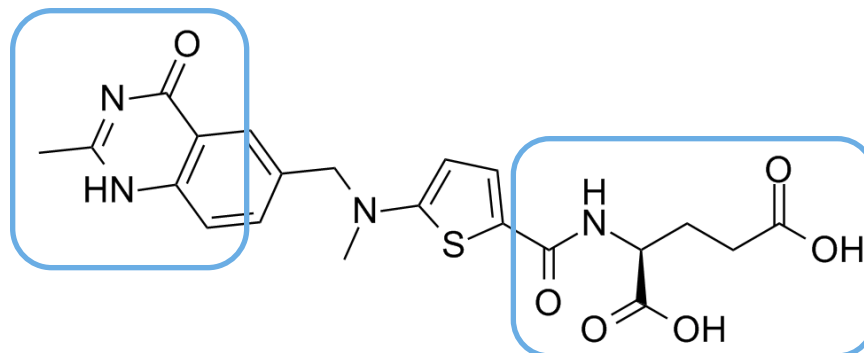
# Thymidylate Synthase

Compound

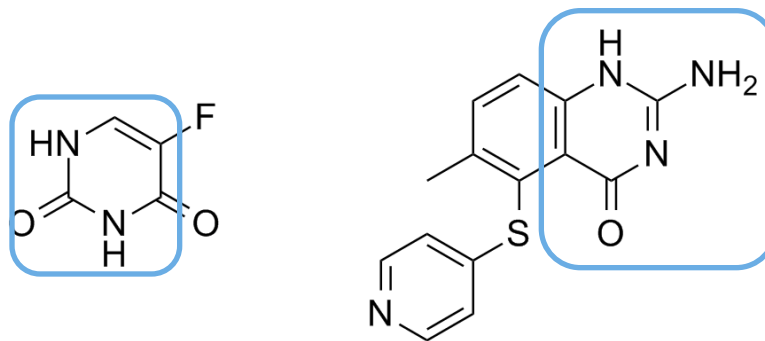
Molecular Initiating Event

Adverse Outcome Pathway

## Known Binders



Raltitrexed



Fluorouracil

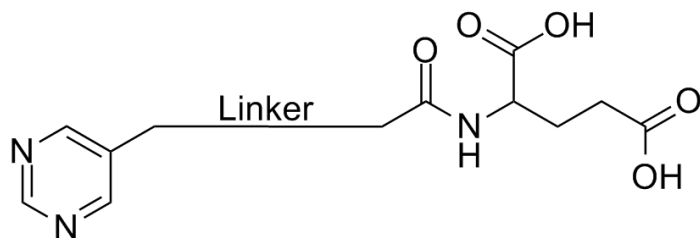
Nolatrexed

# Thymidylate Synthase



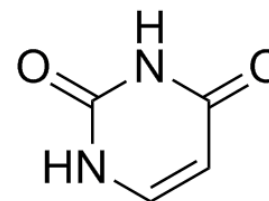
## Two Analogue Compound Classes

### Folate Analogues



Folate Pharmacophore

### Fluoropyrimidines



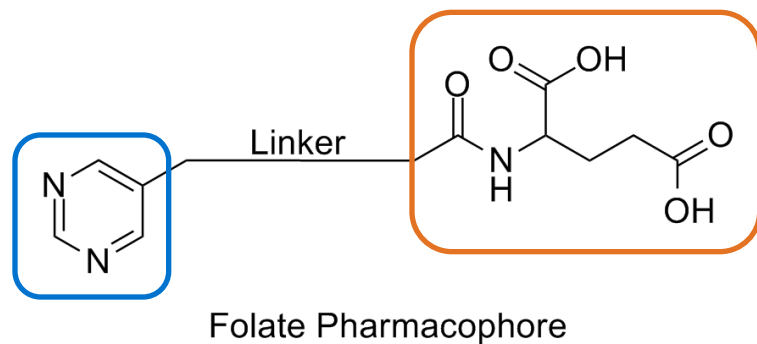
Uracil

# Thymidylate Synthase



## Two Analogue Compound Classes

### Folate Analogues

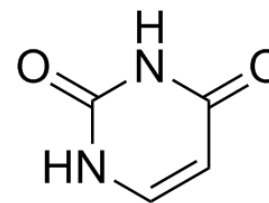


8 Matches

45 Matches

Both:  
4 Matches

### Fluoropyrimidines



Uracil

2 Matches

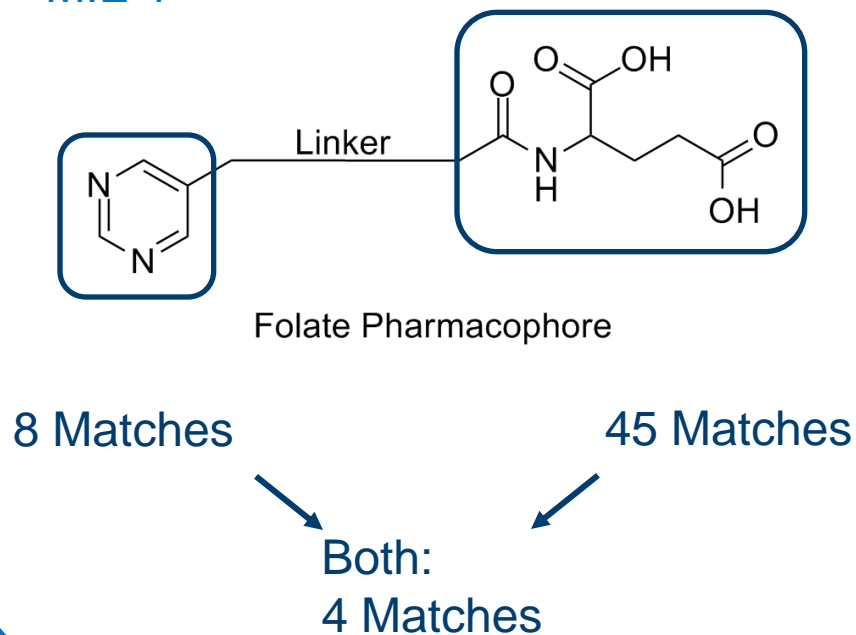
# Thymidylate Synthase



## Two Analogue Compound Classes

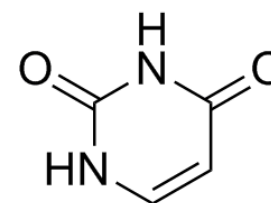
### Folate Analogues

MIE 1



### Fluoropyrimidines

MIE 2



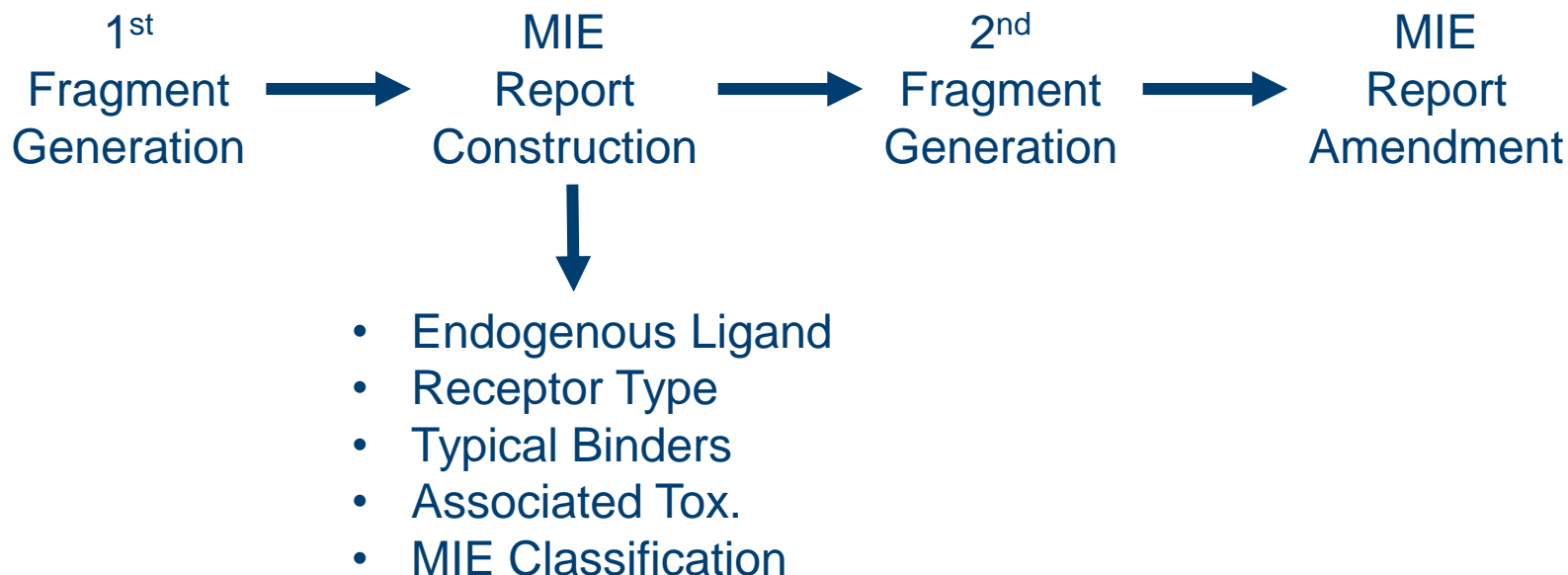
Uracil

2 Matches

# Towards an MIE Atlas



These reports are the initial finding that will lead towards a large number of well characterised MIEs, across a diverse set of important pharmacological receptors, for publication and use in developing SAR tools.



# The Big Question

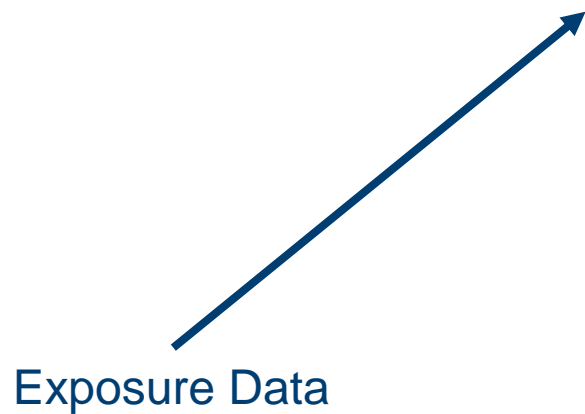


**“Here’s a new molecule – is it safe?”**

# The Big Question



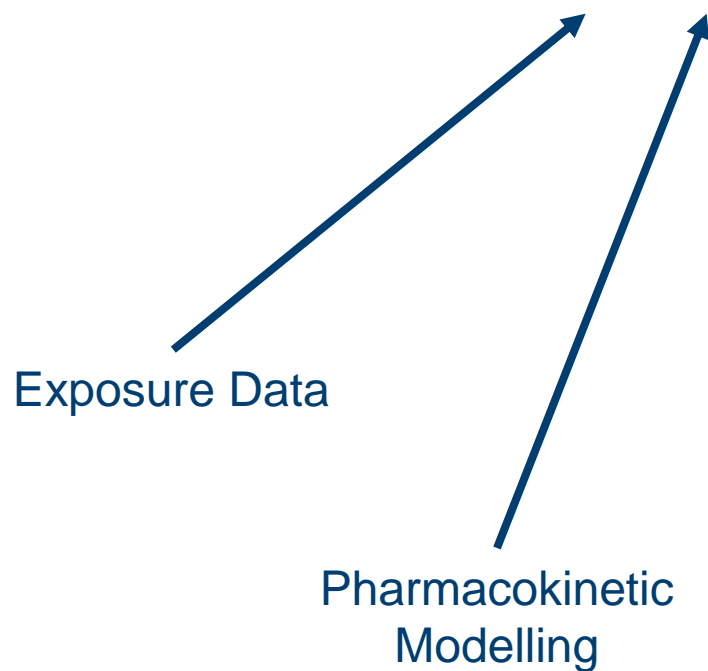
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# The Big Question



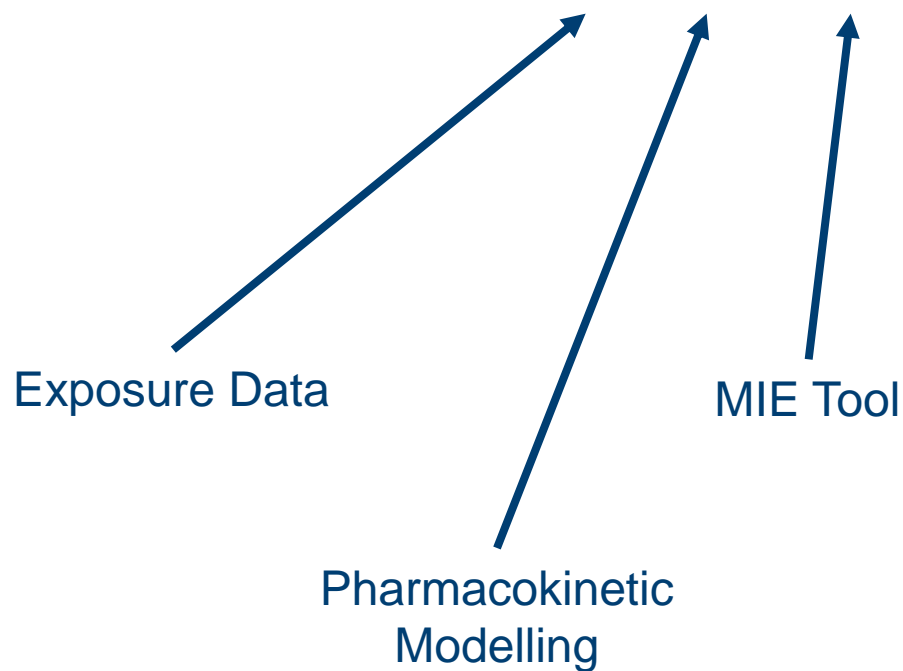
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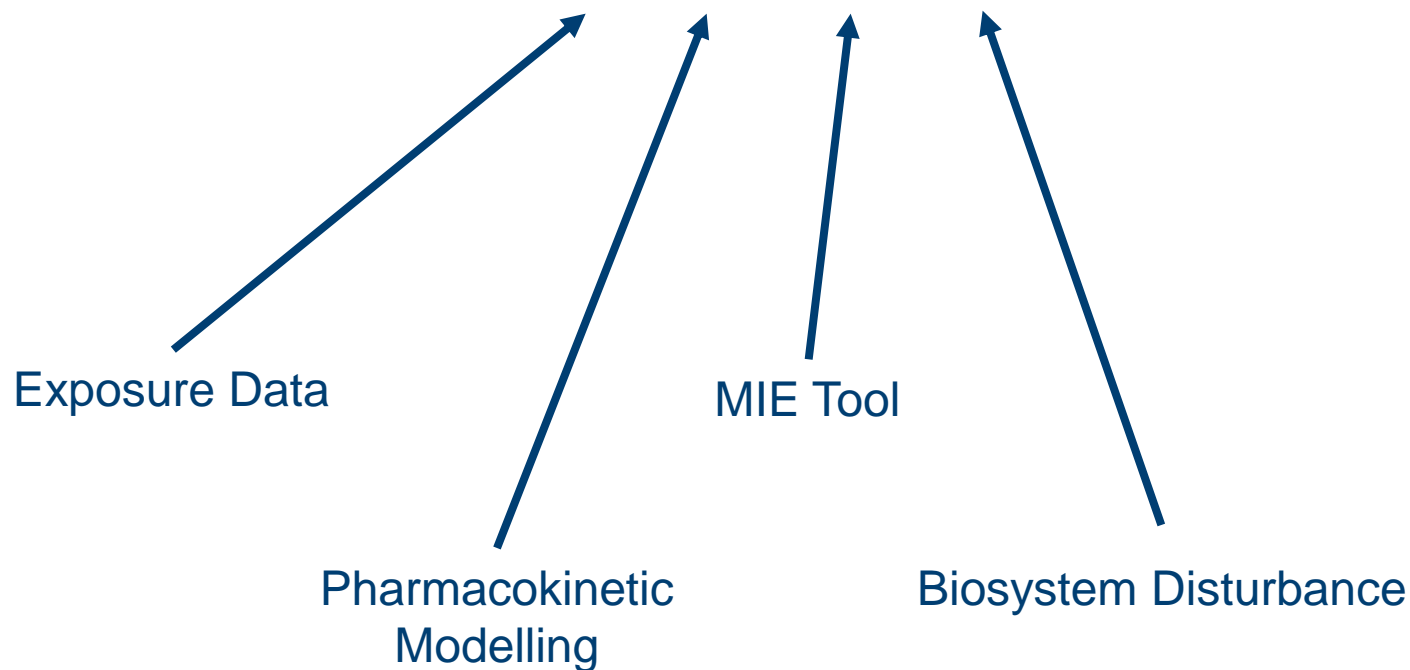
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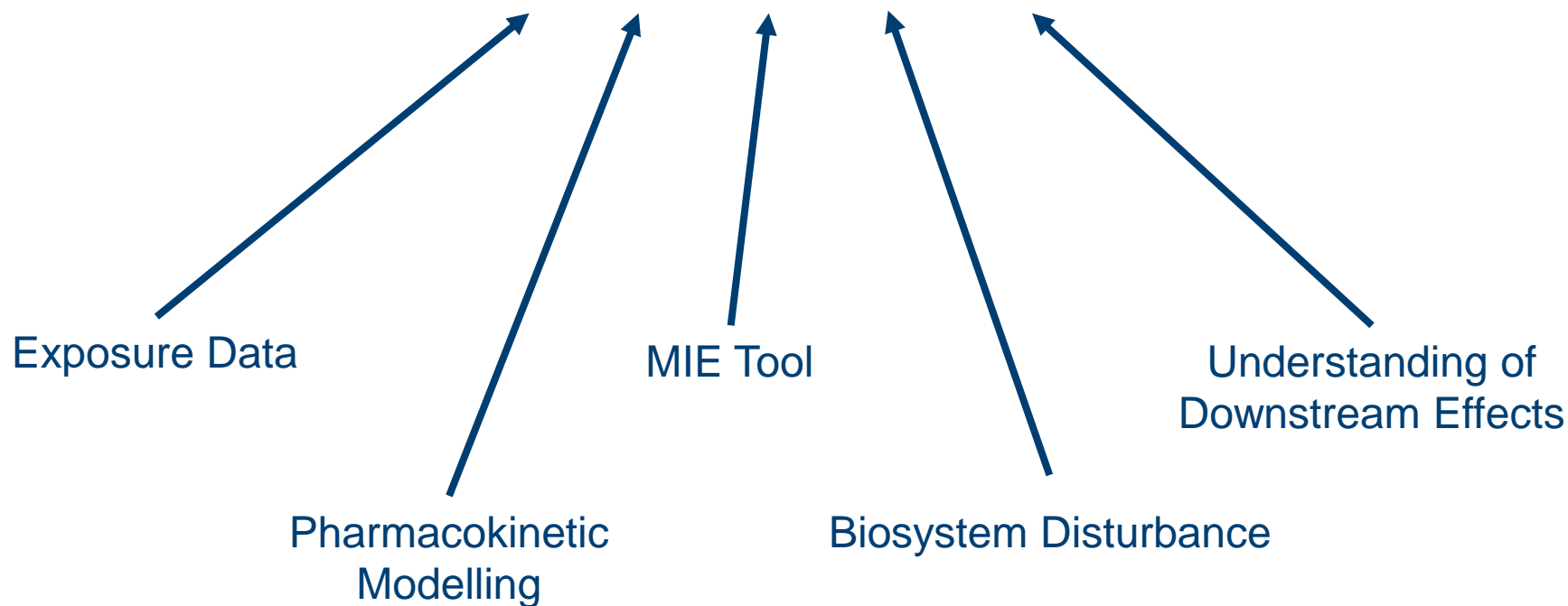
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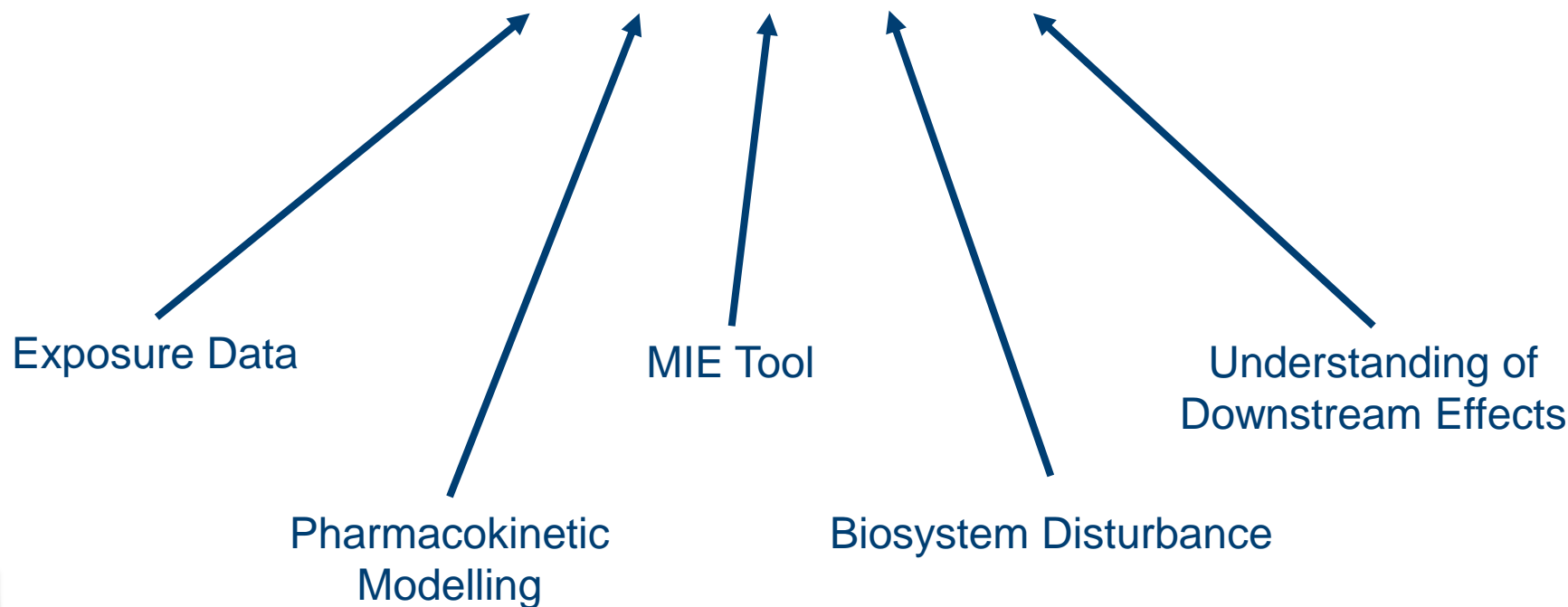
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# The Big Question



“Here’s a new molecule – is it safe?”



**MIE-AOP Research**

# The Big Question



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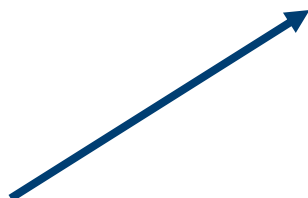
“Here’s a new molecule – what MIEs can it activate?”

# The Big Question

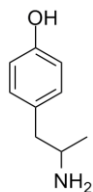


~~“Here’s a new molecule – is it safe?”~~

“Here’s a new molecule – what MIEs can it activate?”



2D Fragments



Hydroxyamphetamine  
48%

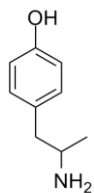
# The Big Question



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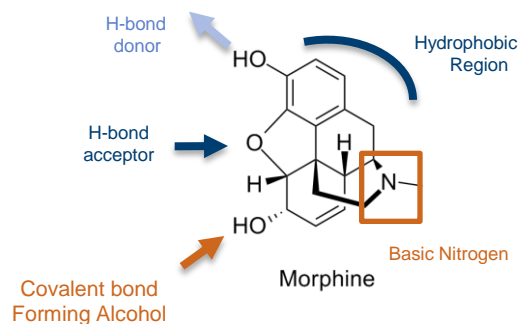
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Literature Knowledge



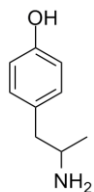
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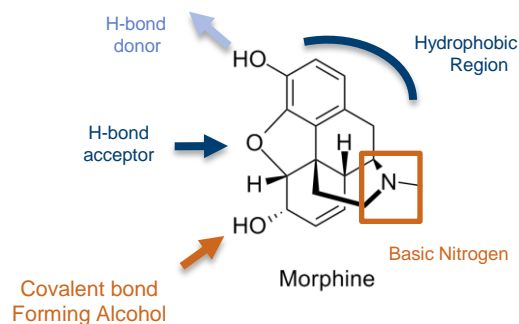
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Hydroxyamphetamine  
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Literature Knowledge



Computational Tools



# Acknowledgements



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- Unilever
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