

Identification of Novel Anticonvulsant Drugs using a Larval Zebrafish Model

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The
University
Of
Sheffield.

The Bateson centre

Understanding Life



Epilepsy



Our assay



Screening of kinase inhibitors



Future work

Epilepsy

- Estimated 50 million cases worldwide
- A neurological syndrome with many clinically distinct subtypes, all of which are characterised by *seizures*
- Seizures are periods of acute, dysregulated synaptic activity in the brain

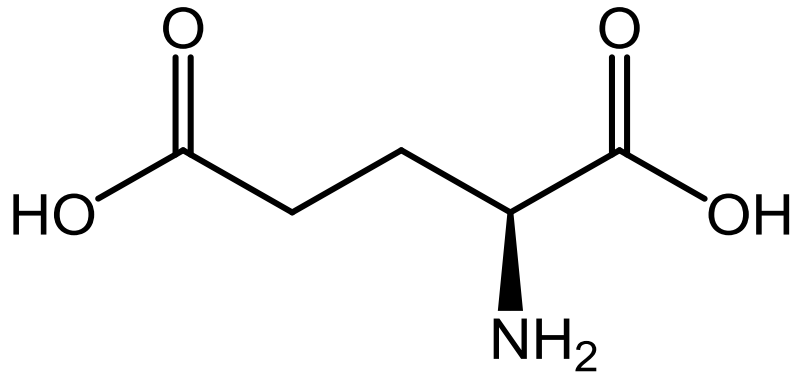
Epilepsy

- The currently available anti-epileptic drugs are not effective enough
- 30% of people with epilepsy fail to respond satisfactorily to first line treatments
- Those drugs that are affective have unwanted side effects

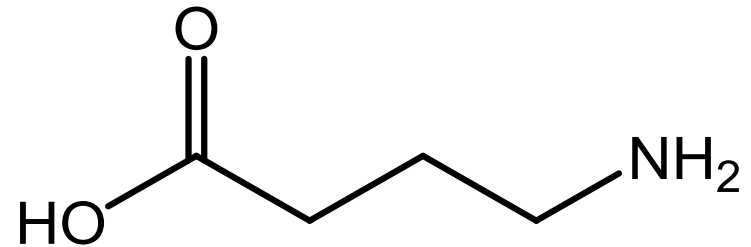


Epilepsy

Seizures are attributed to imbalances of excitatory and inhibitory neurotransmitter activities in the brain; glutamate and γ -amino butyric acid (GABA) respectively



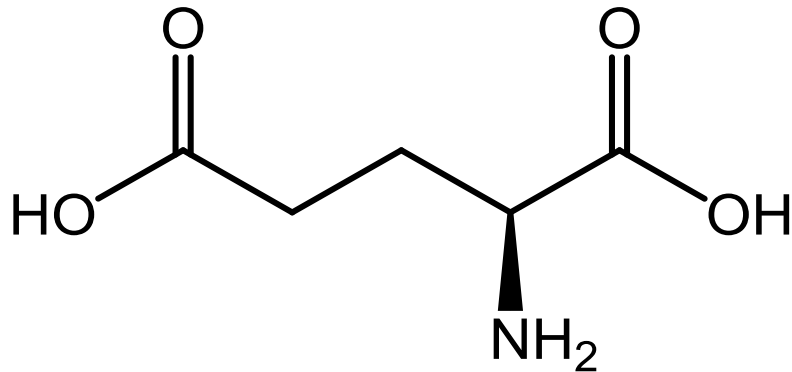
Glutamate (+)



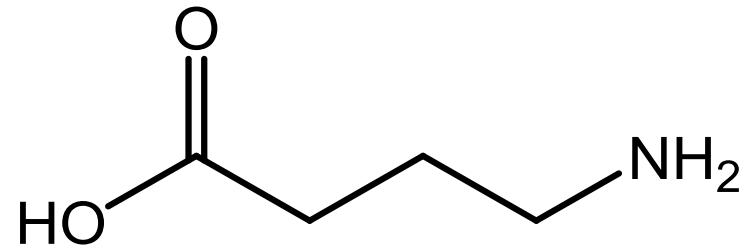
GABA (-)

Epilepsy

Seizures are attributed to imbalances of **excitatory** and inhibitory neurotransmitter activities in the brain; **glutamate** and γ -amino butyric acid (GABA) respectively



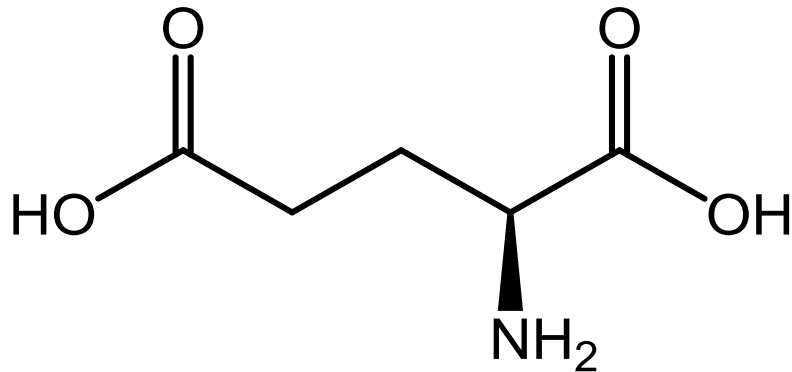
Glutamate (+)



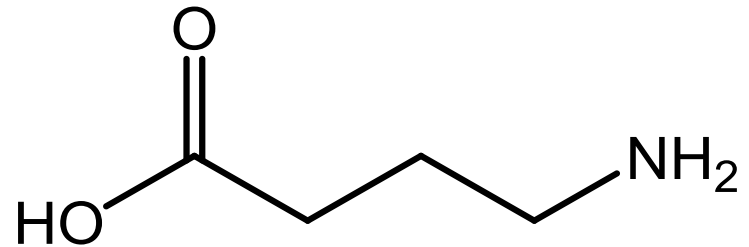
GABA (-)

Epilepsy

Seizures are attributed to imbalances of **excitatory** and **inhibitory** neurotransmitter activities in the brain; **glutamate** and **γ -amino butyric acid (GABA)** respectively



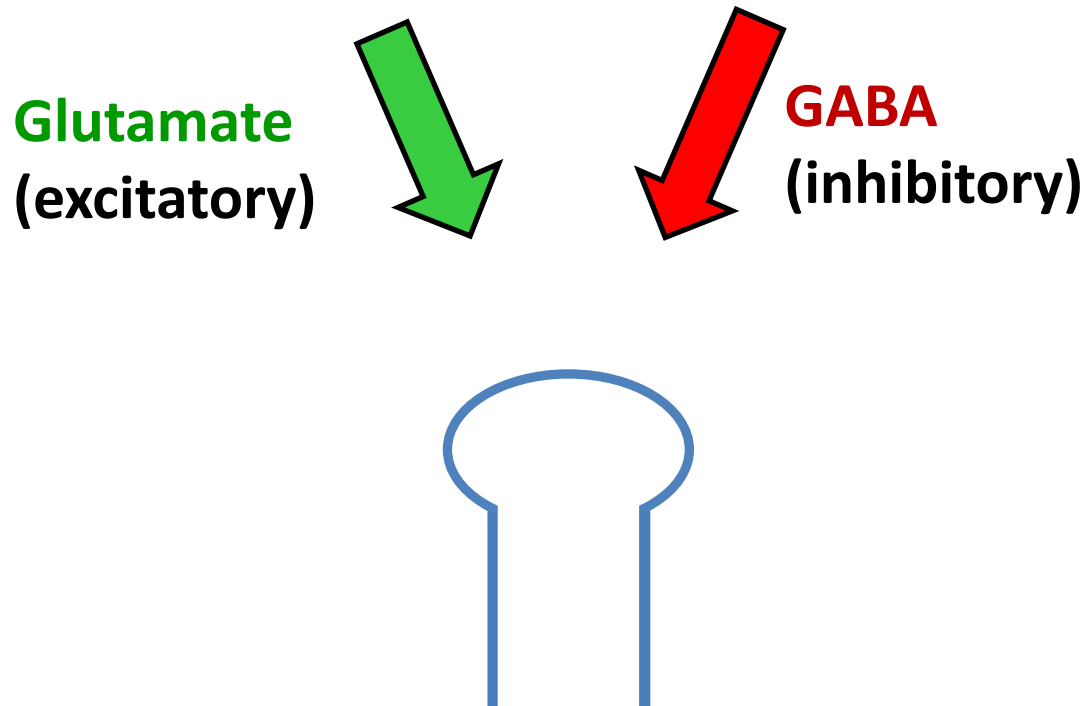
Glutamate (+)



GABA (-)

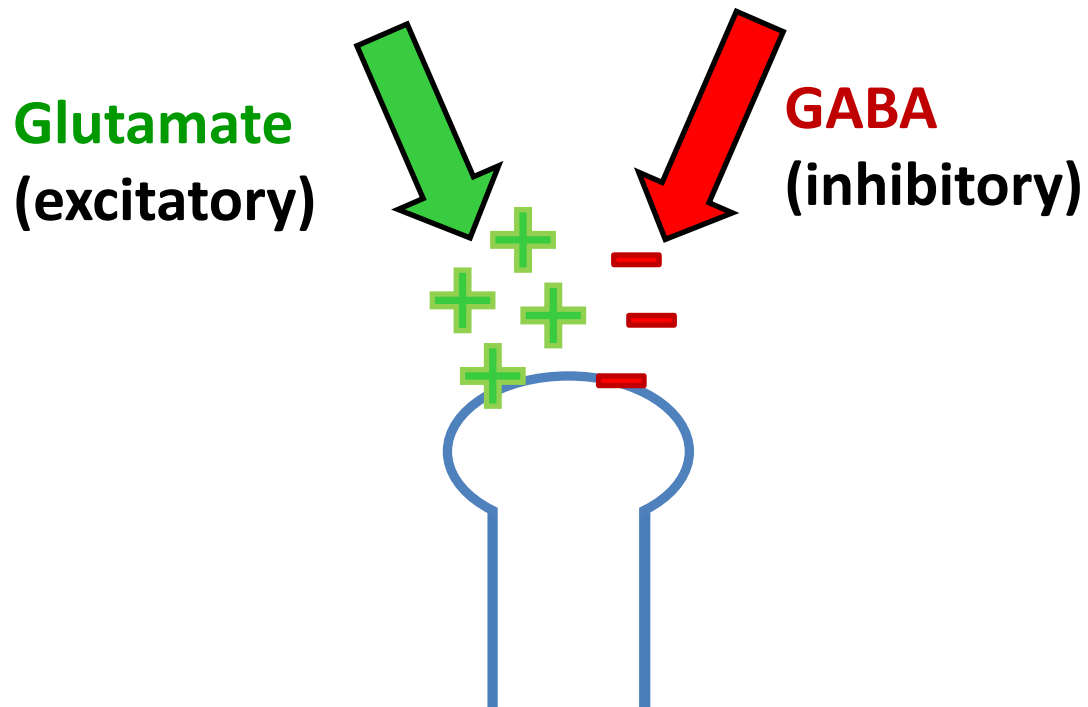
Epilepsy

Normal neurotransmission



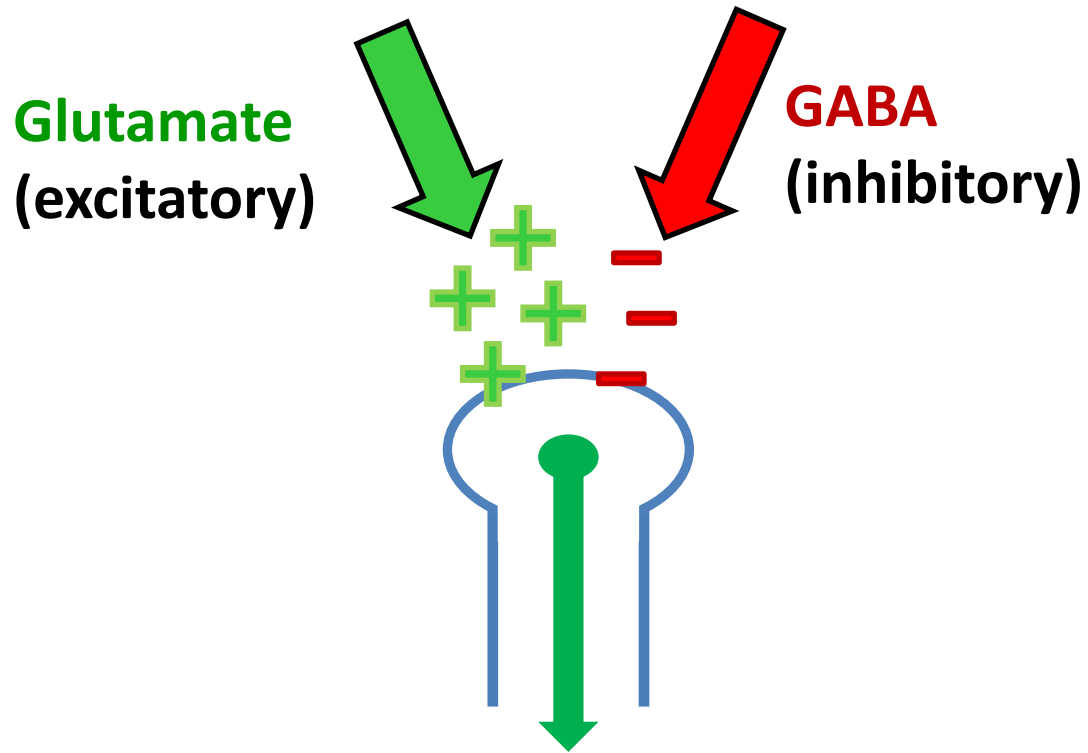
Epilepsy

Normal neurotransmission



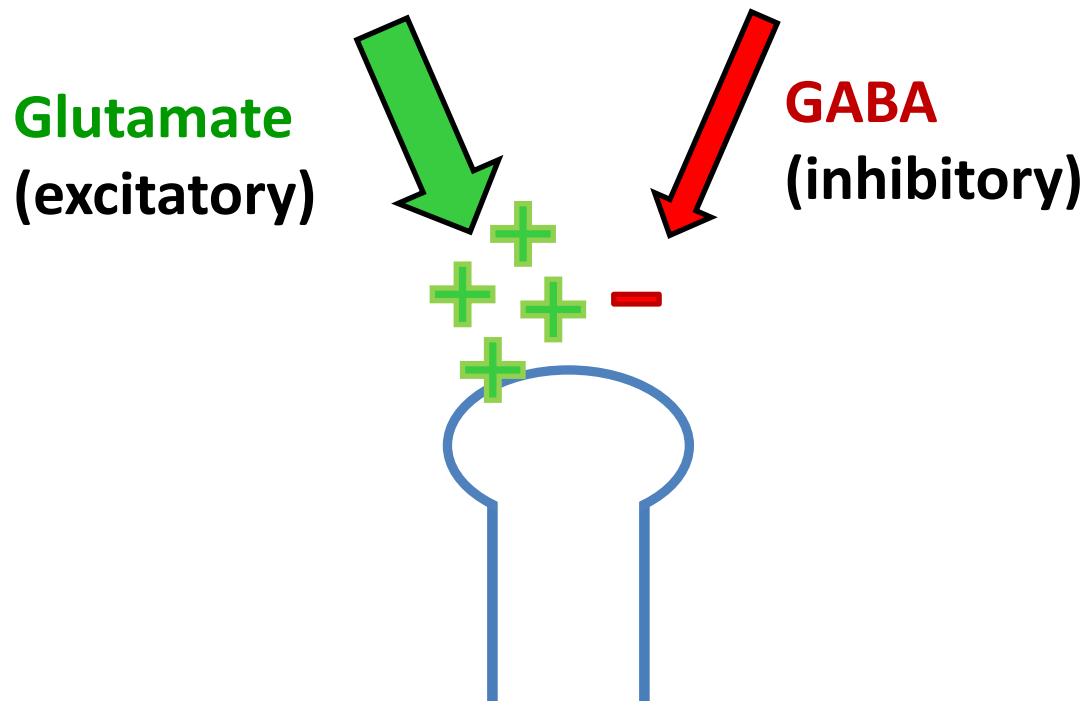
Epilepsy

Normal neurotransmission



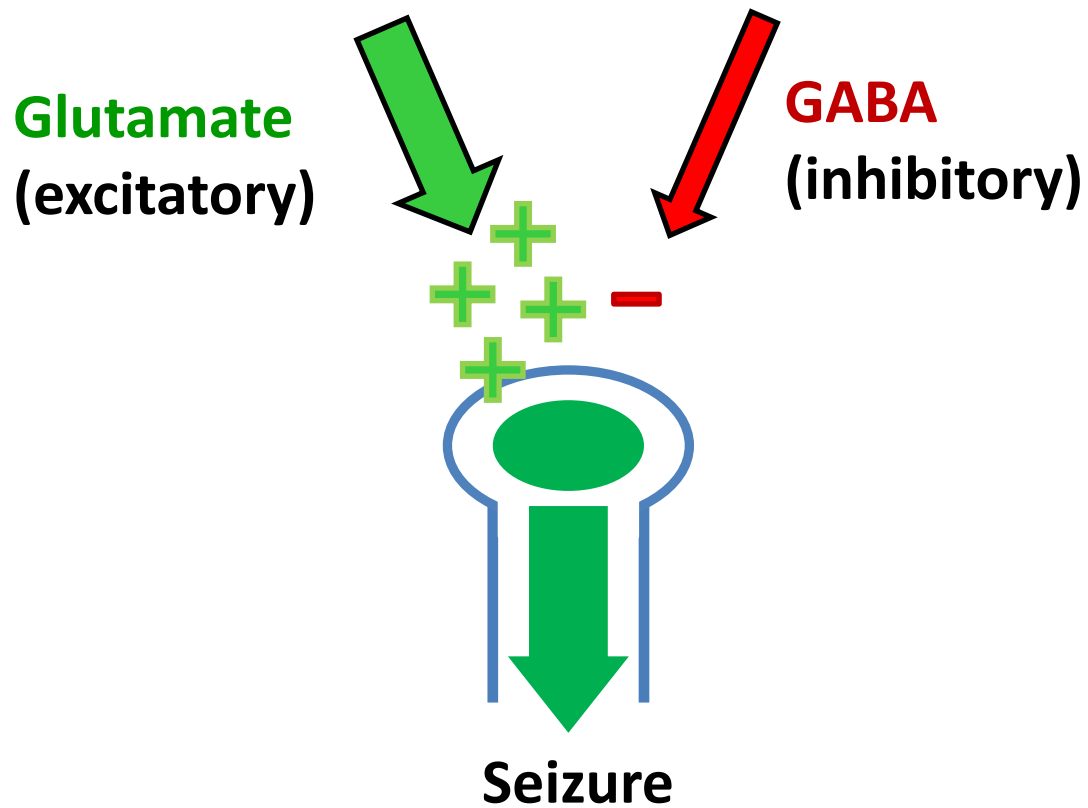
Epilepsy

In a seizure...



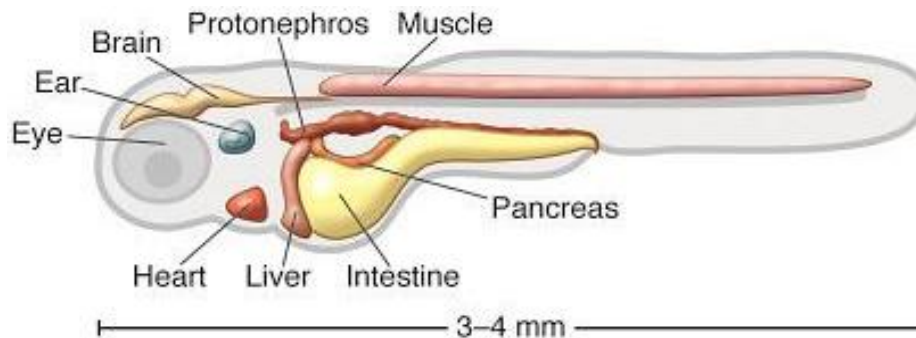
Epilepsy

In a seizure...

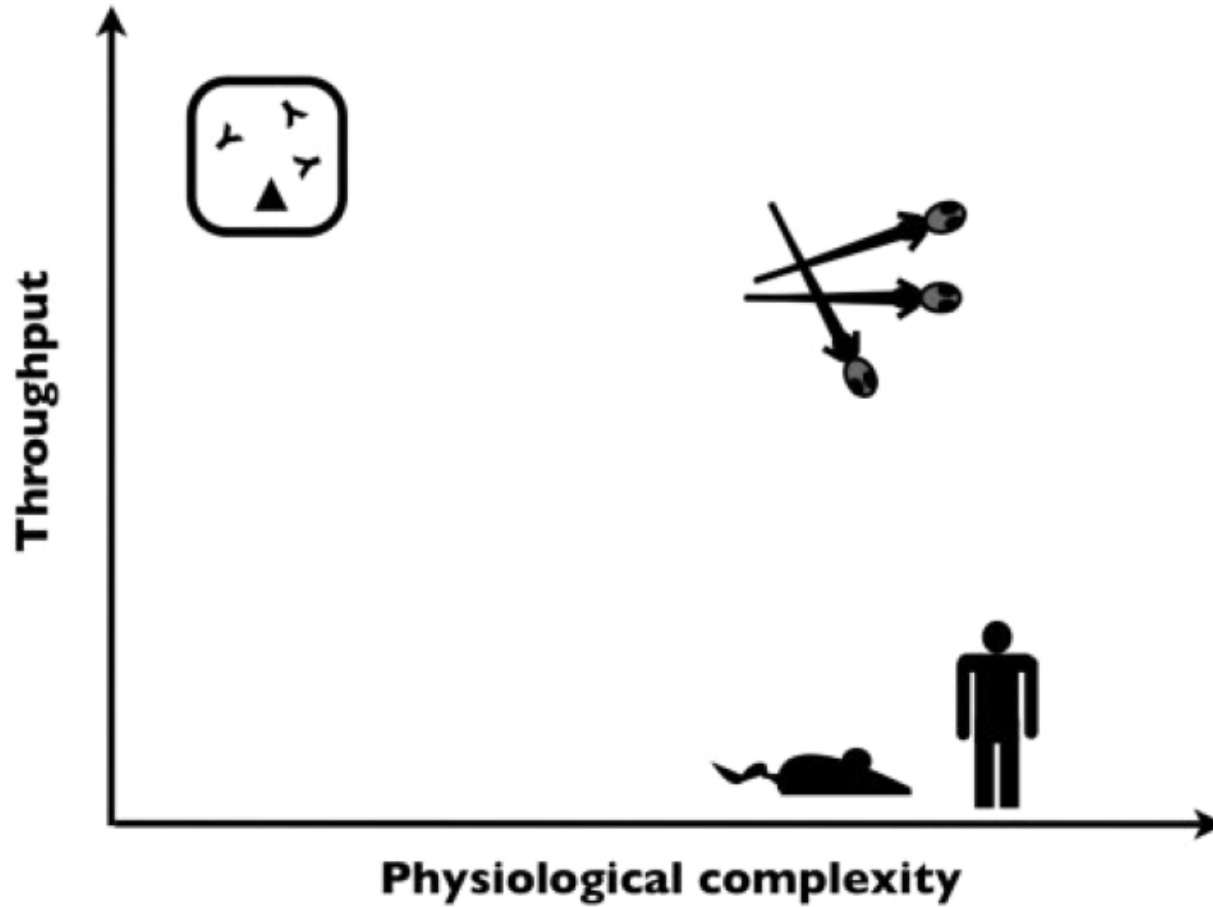


Zebrafish model

- Mapped genome
- Rapid development
- High through-put vertebrate model
- Compounds can be absorbed through aqueous media
- Transparency allows whole mount visualisation of gene expression via *in situ* hybridisation

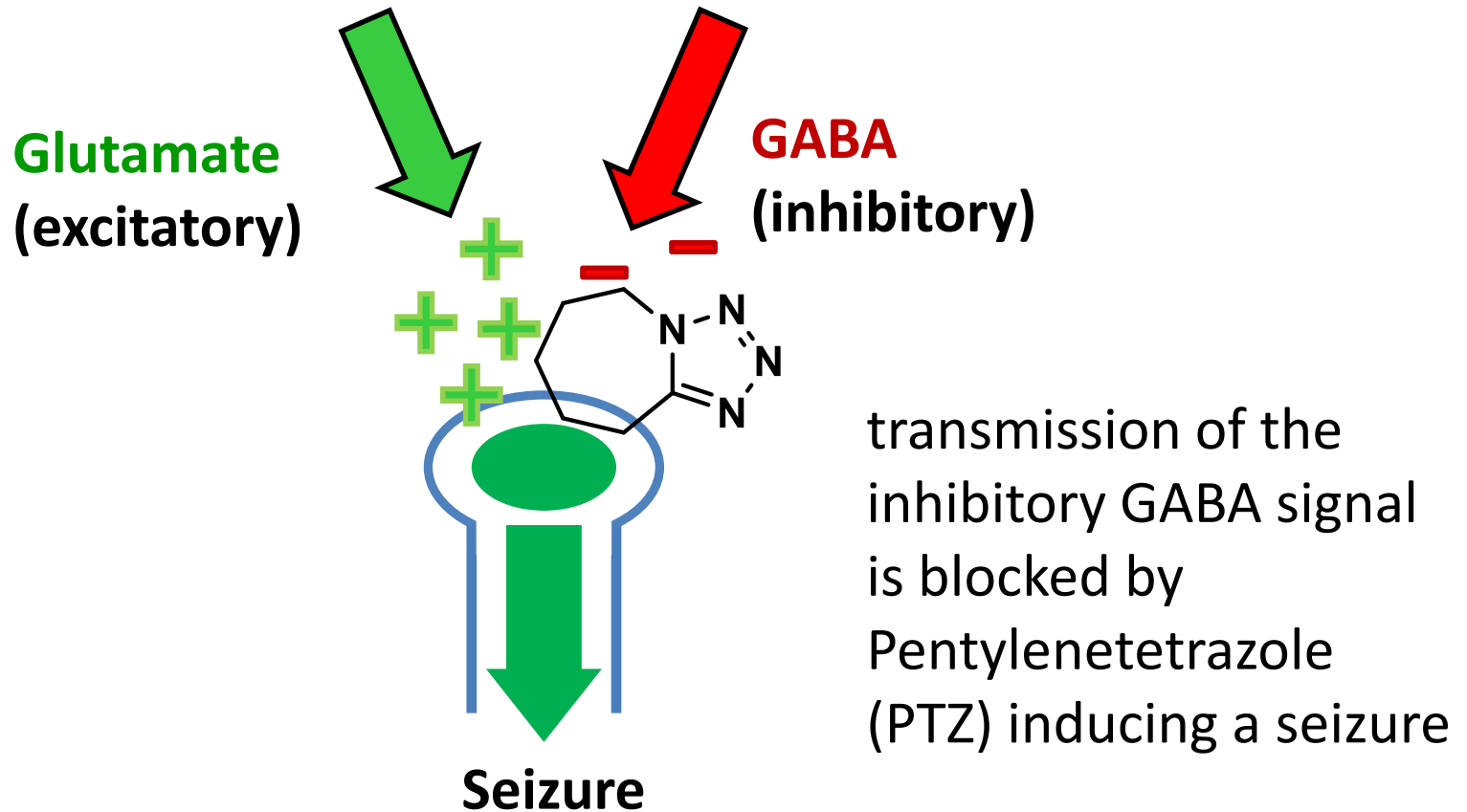


The Assay



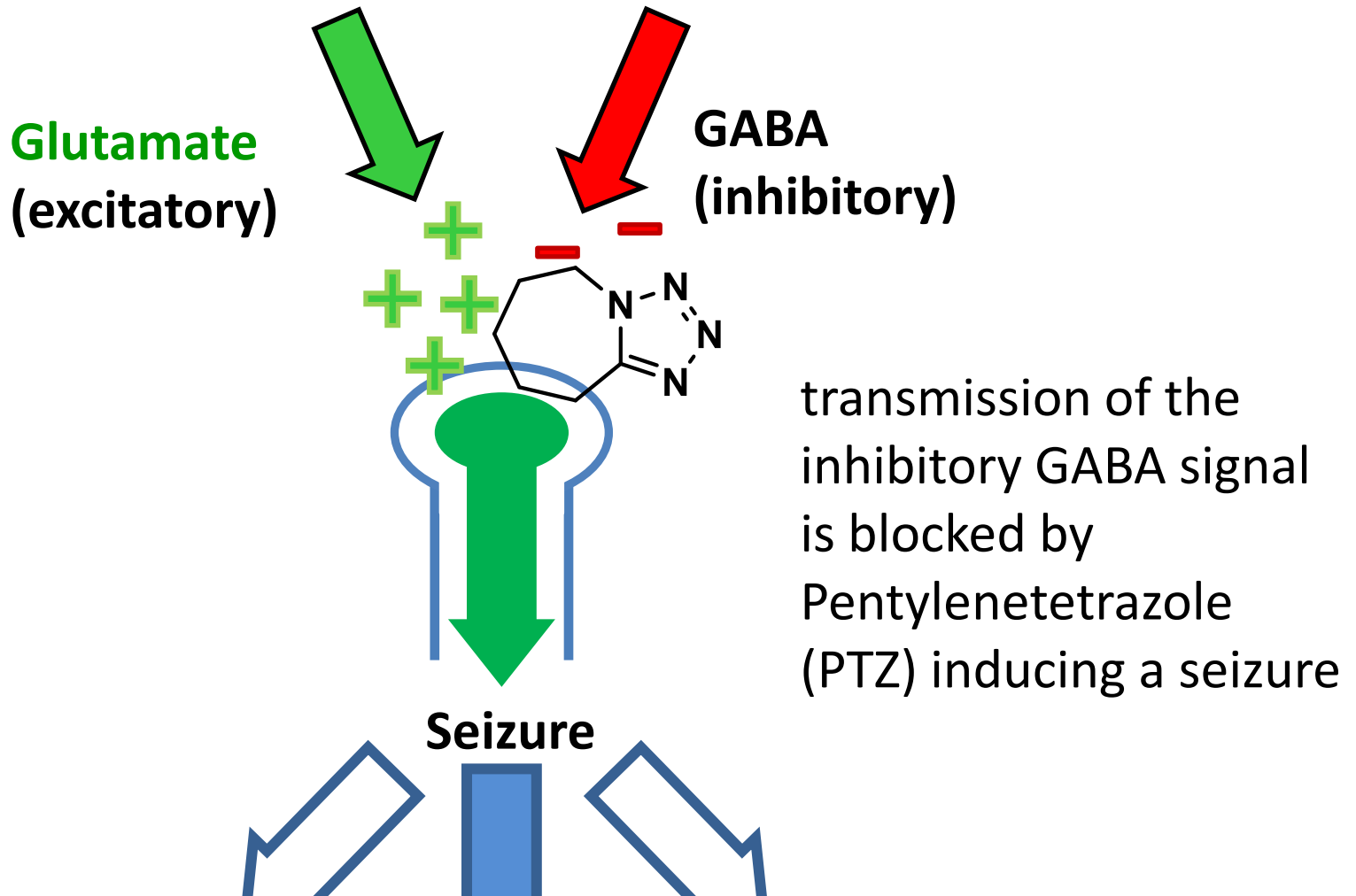
The Assay

In a PTZ-induced seizure...



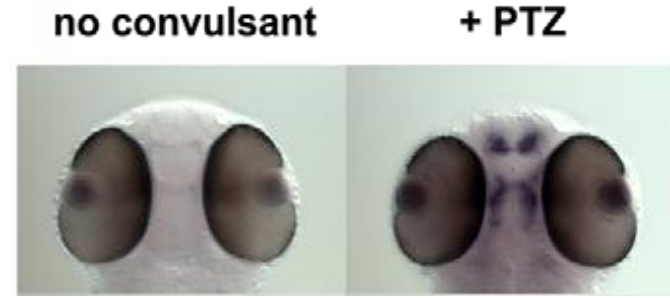
The Assay

In a PTZ-induced seizure...



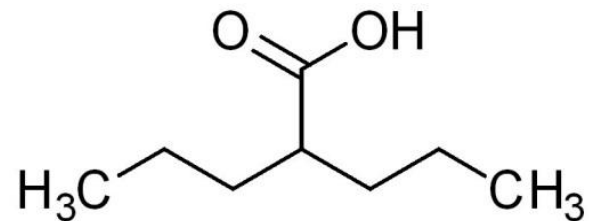
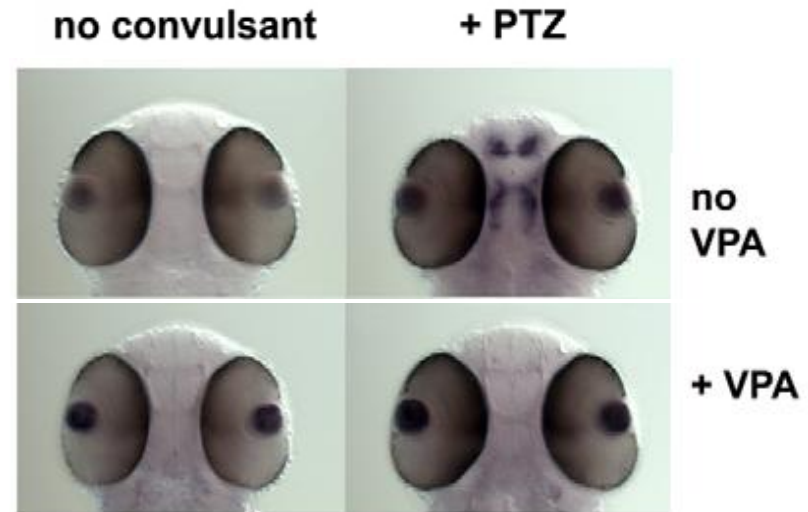
Screening for Anticonvulsants

- A seizure “switches on” particular pattern of gene expression in the brain e.g. *cfos* gene (right)



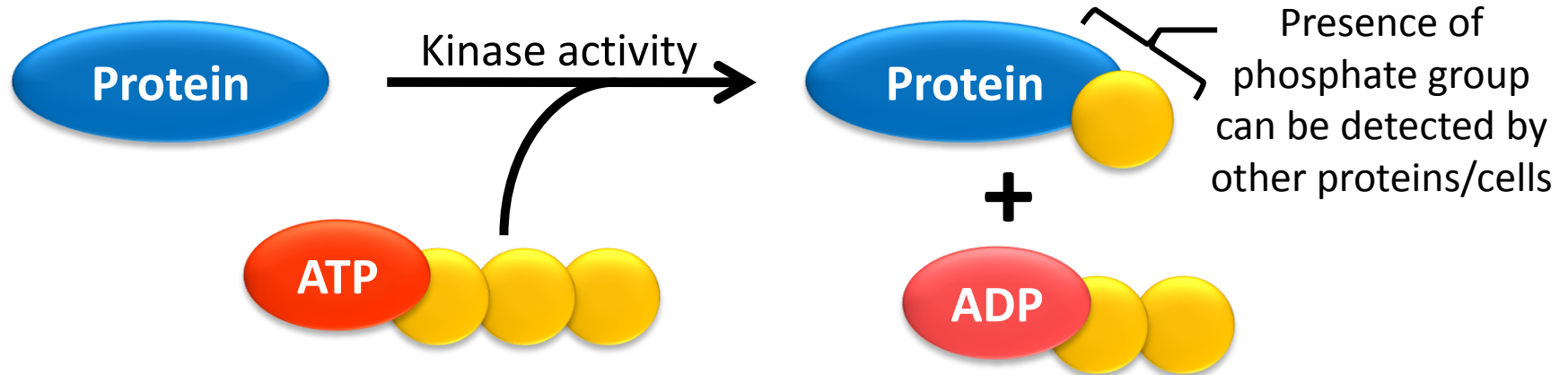
Screening for Anticonvulsants

- A seizure “switches on” particular pattern of gene expression in the brain e.g. *cfos* gene (right)
- *cfos* expression after PTZ exposure is not observed if an effective anticonvulsant is administered
- Positive control is the known anticonvulsant Valproic Acid (VPA)



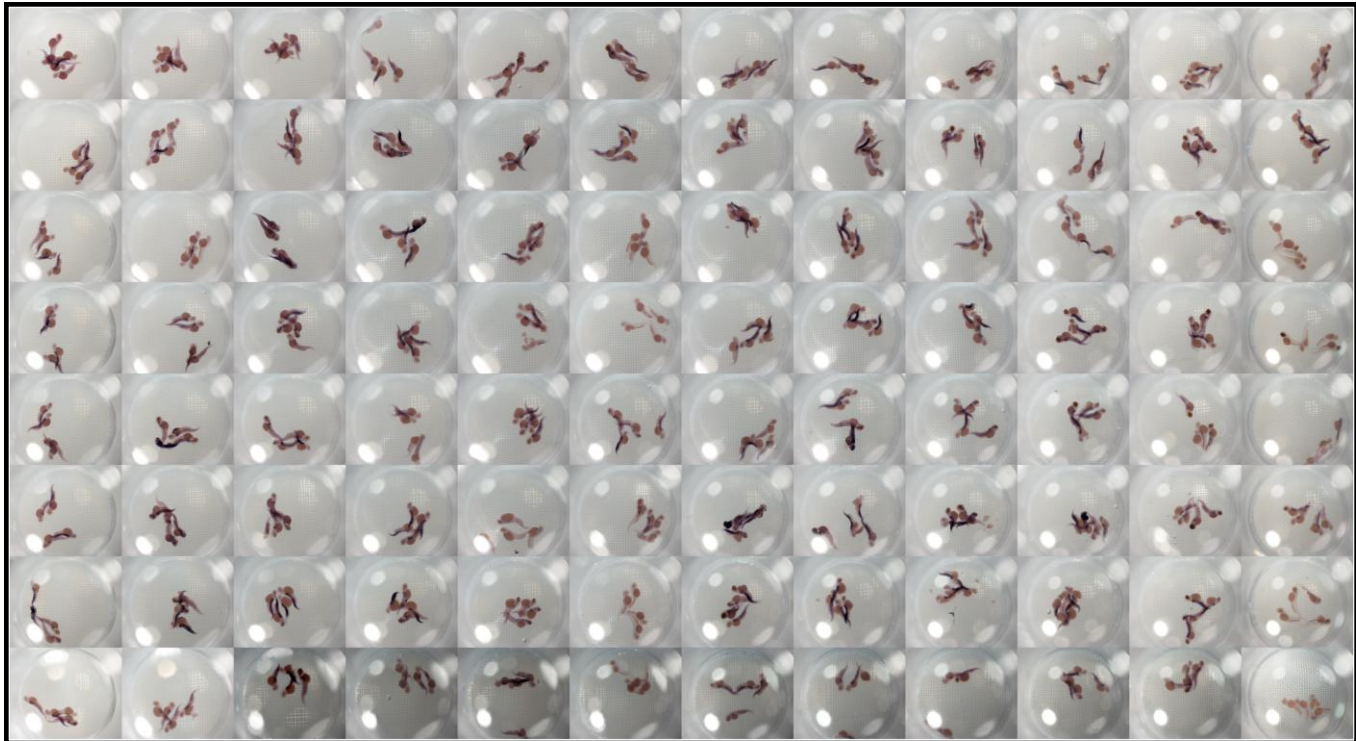
Valproic acid (VPA)

Screening of Kinase Inhibitors



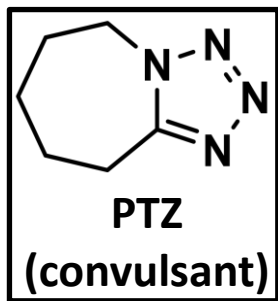
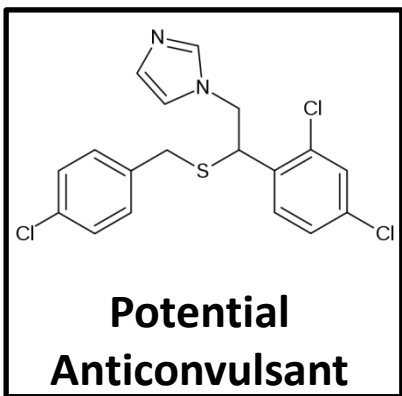
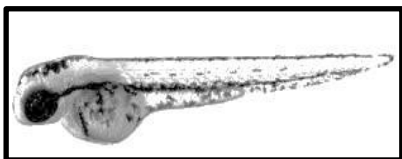
- The specific pathways involved in seizures are still largely unknown, but are likely to include kinase enzymes
- Therefore, we tested the hypothesis that kinase inhibition is a valid anticonvulsant strategy
- Target focussed library screening of over 1000 kinase inhibitors

Screening of Kinase Inhibitors



Compounds screened in a 96 well plate, initially at 25 μ M

In each well...



Fixation

In Situ



or



t0

90 mins

150 mins

A few days
later...

Screening of Kinase Inhibitors

A hit in the *in situ*
screen tells us...

Compound has prevented the
seizure



or



Screening of Kinase Inhibitors

A hit in the *in situ*
screen tells us...

Compound has prevented the
seizure

OR

The compound interferes
with gene expression



or



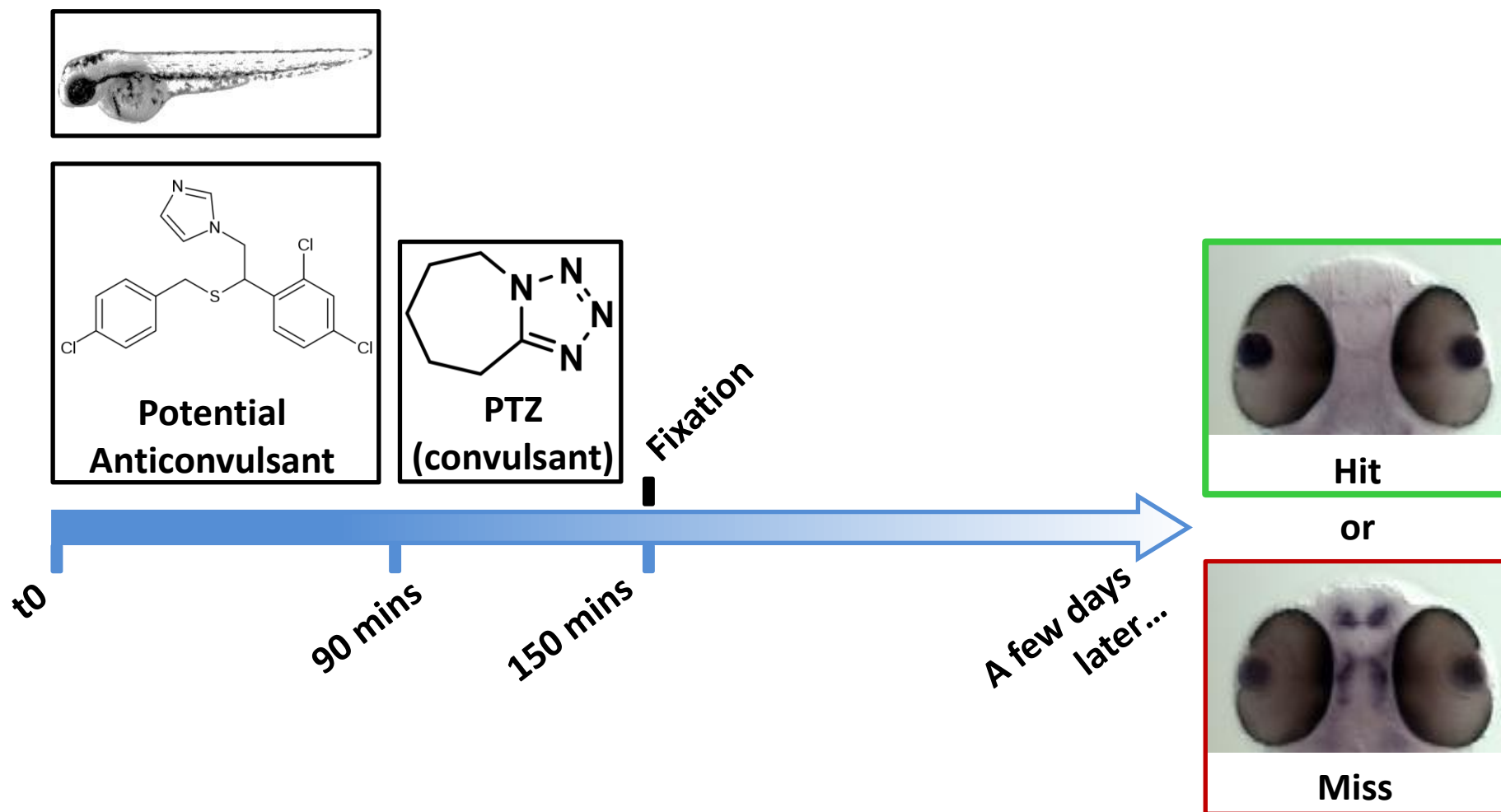
Screening of Kinase Inhibitors

42 hits confirmed by *in situ* repeat screens

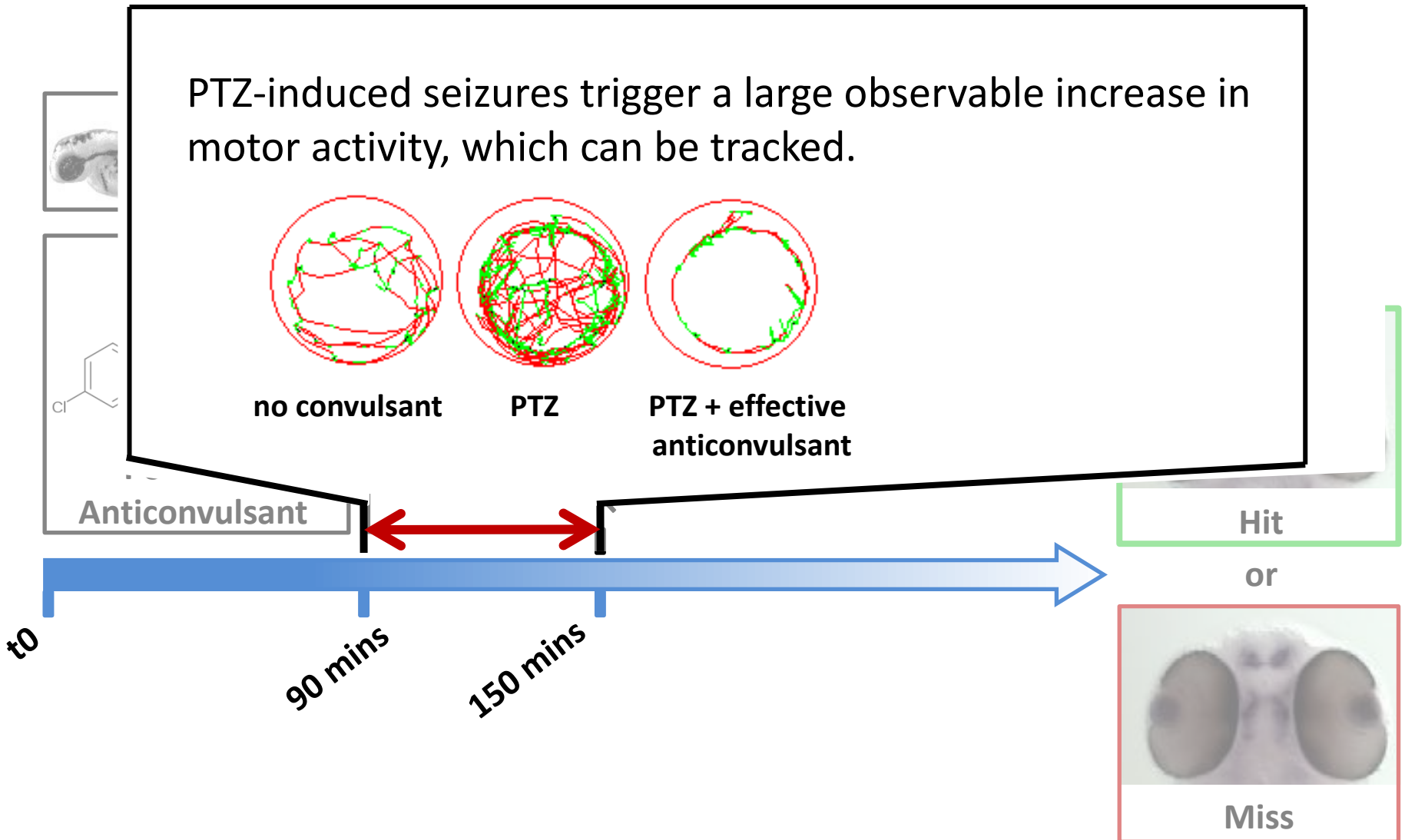
As *cfos* expression can be repressed independently of stopping a seizure (false positive by *in situ*) anticonvulsant activity must be confirmed independently

10 of the most potent were bought in to test in **locomotor assays**

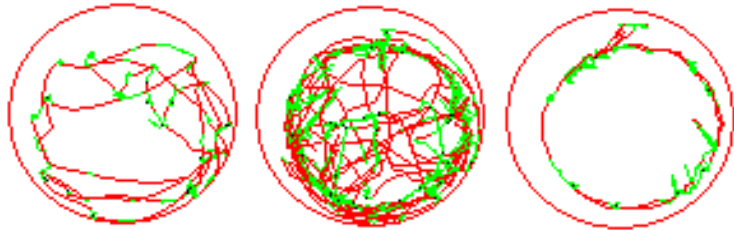
Screening of Kinase Inhibitors



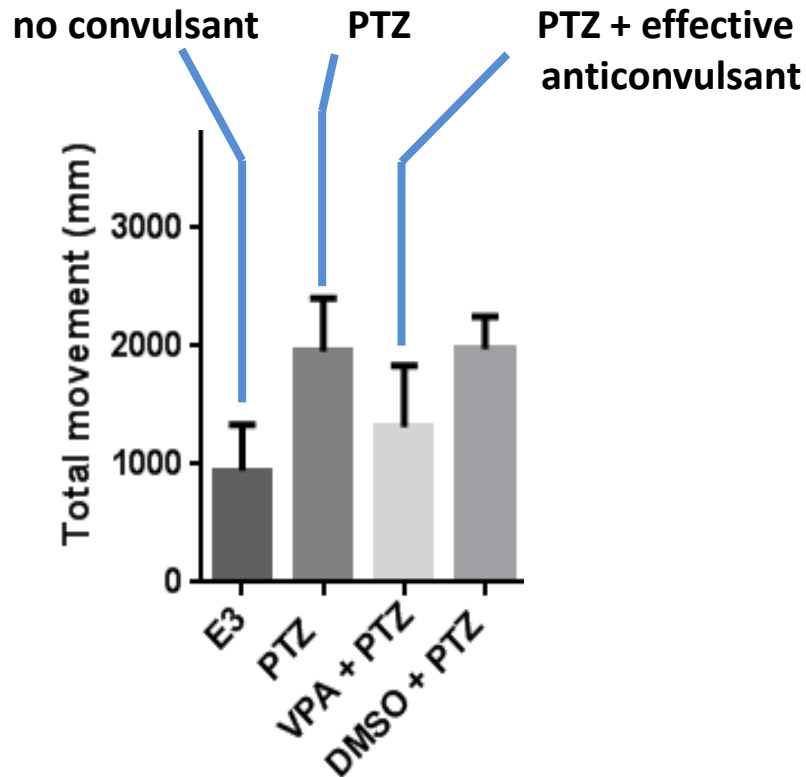
Screening of Kinase Inhibitors



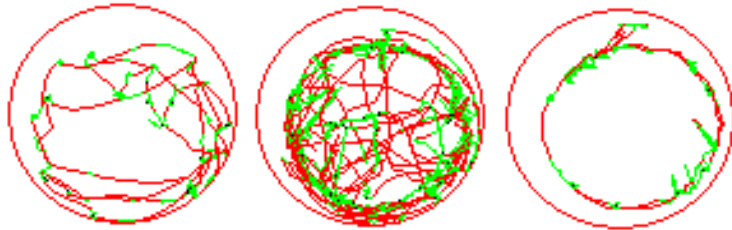
Screening of Kinase Inhibitors



Effective anticonvulsant compounds reduce the distance travelled after PTZ exposure

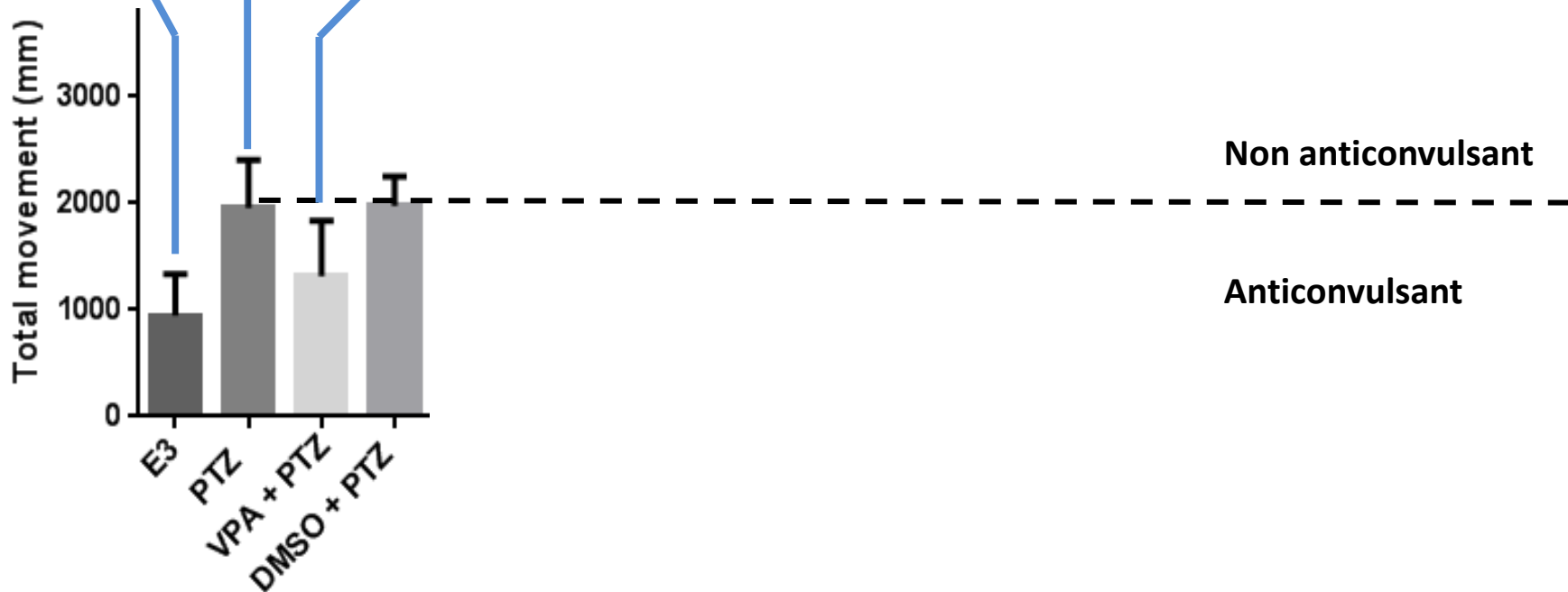


Screening of Kinase Inhibitors

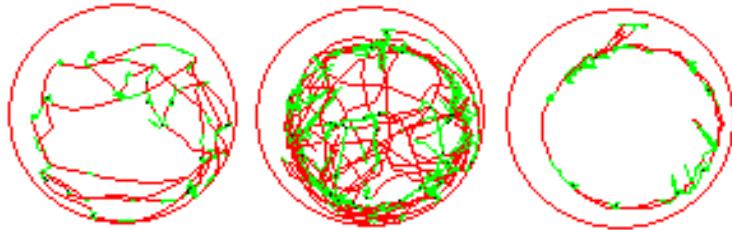


Effective anticonvulsant compounds reduce the distance travelled after PTZ exposure

no convulsant PTZ PTZ + effective anticonvulsant



Screening of Kinase Inhibitors

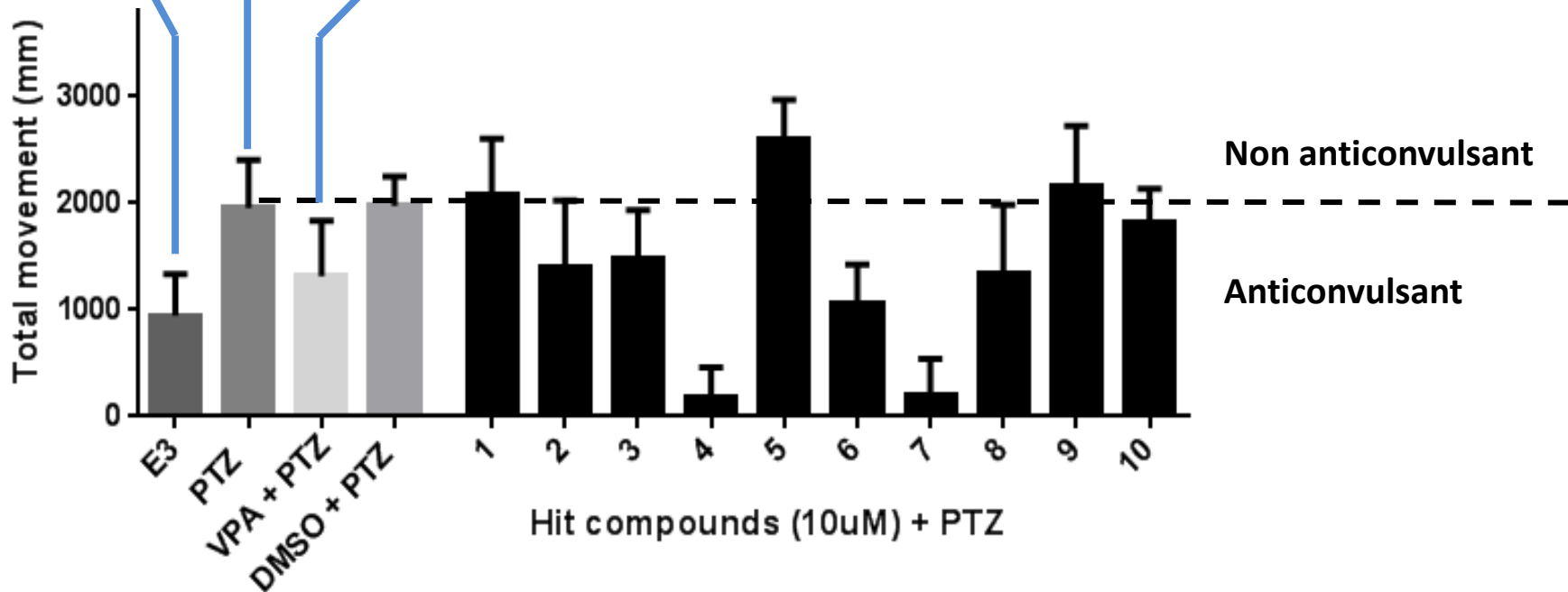


no convulsant

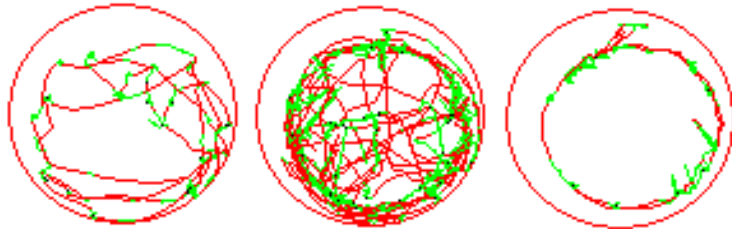
PTZ

PTZ + effective
anticonvulsant

6 of 10 hit compounds show significant dose dependant reduction in locomotor activity after PTZ exposure



Screening of Kinase Inhibitors

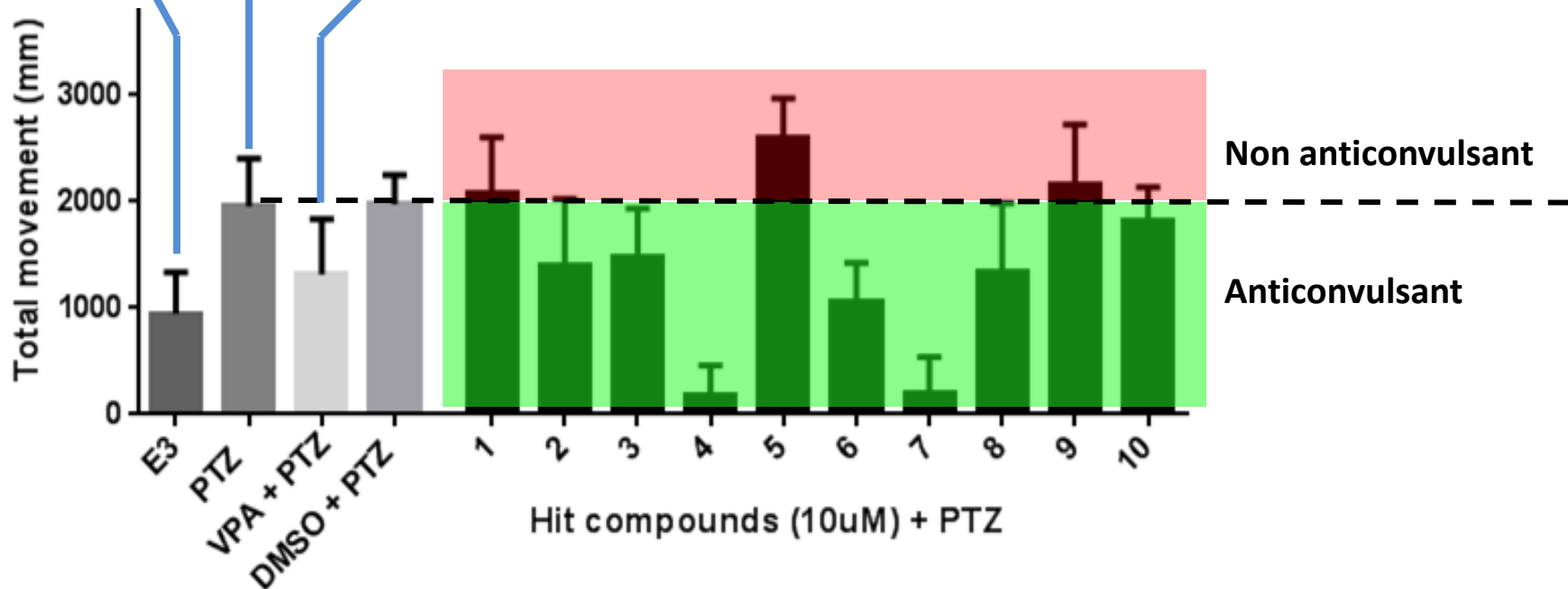


no convulsant

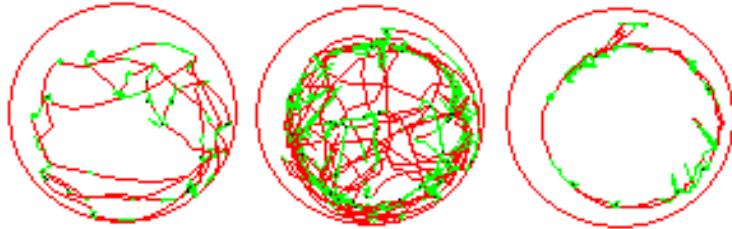
PTZ

PTZ + effective
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6 of 10 hit compounds show
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Screening of Kinase Inhibitors



no convulsant

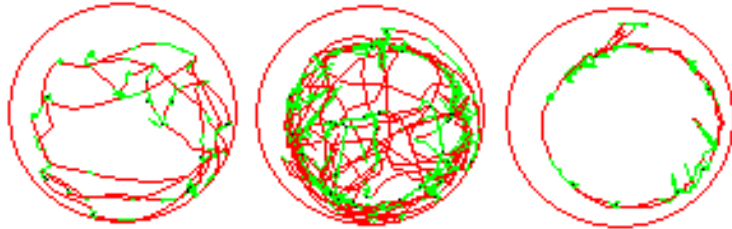
PTZ

PTZ + effective
anticonvulsant

**A hit in the locomotor
screen tells us...**

Compound has prevented the
seizure

Screening of Kinase Inhibitors



no convulsant

PTZ

PTZ + effective
anticonvulsant

**A hit in the locomotor
screen tells us...**

Compound has prevented the seizure

OR

Compound is sedative

OR

Compound paralysed larvae

What does each screen tell us?

A hit in the *in situ*
screen tells us...

Compound has prevented the
seizure

OR

The compound interferes
with gene expression

A hit in the locomotor
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Compound is sedative

OR

Compound paralysed larvae

If a compound stops **both** the transcriptional and behavioural seizure responses then it is likely that that compound is a true hit

What does each screen tell us?

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screen tells us...

Compound has prevented the
seizure

OR

The compound interferes
with gene expression

A hit in the locomotor
screen tells us...

Compound has prevented the
seizure

OR

Compound is sedative

OR

Compound paralysed larvae

OR...

What does each screen tell us?

A hit in the *in situ*
screen tells us...

Compound has prevented the
seizure

OR

The compound interferes
with gene expression

OR

The larvae are dead

A hit in the locomotor
screen tells us...

Compound has prevented the
seizure

OR

Compound is sedative

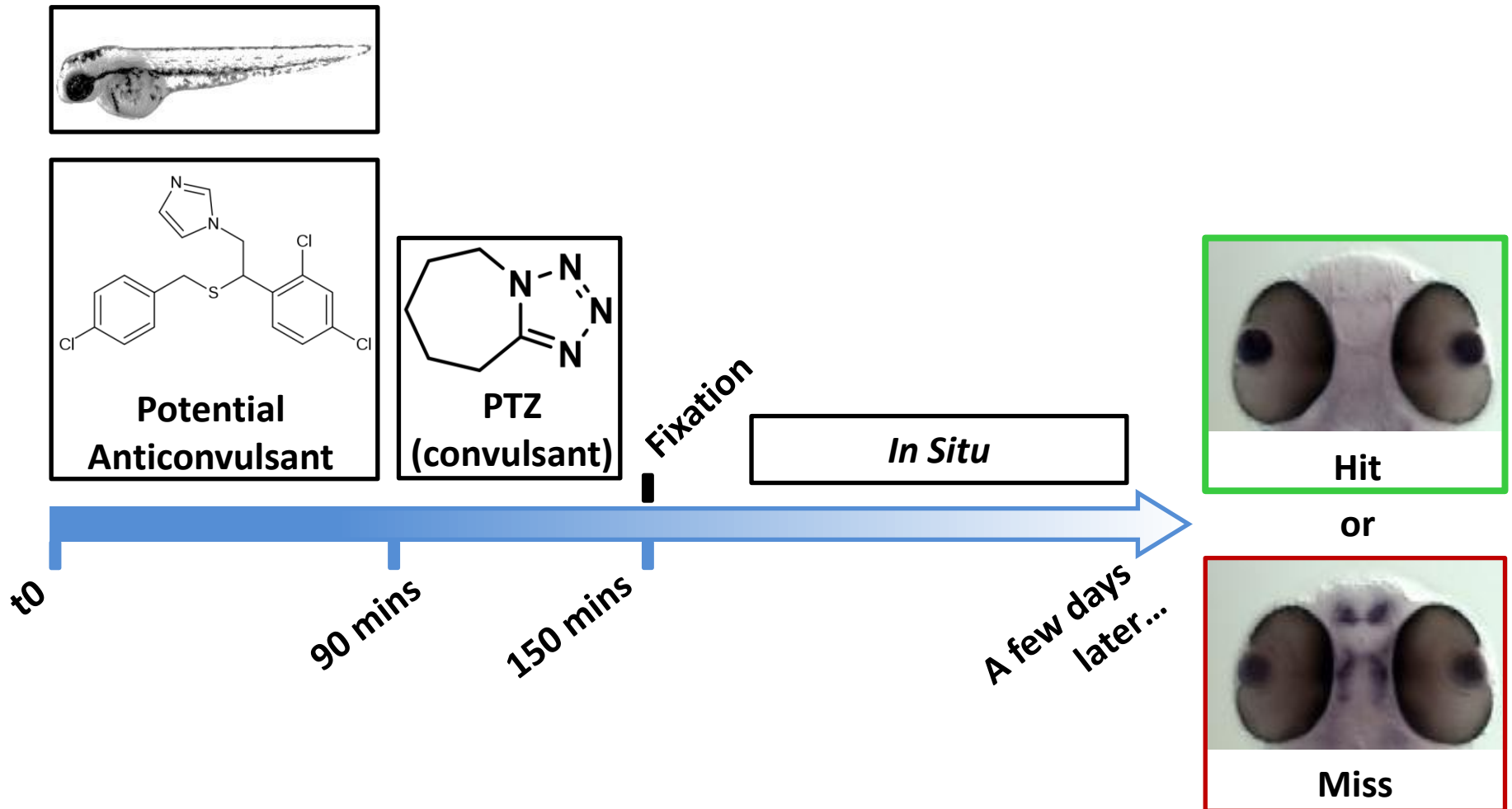
OR

Compound paralysed larvae

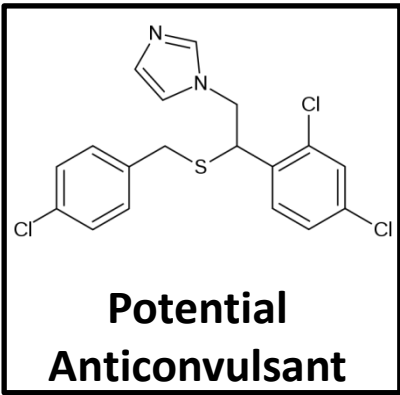
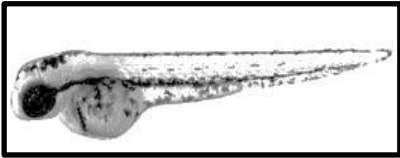
OR

The larvae are dead

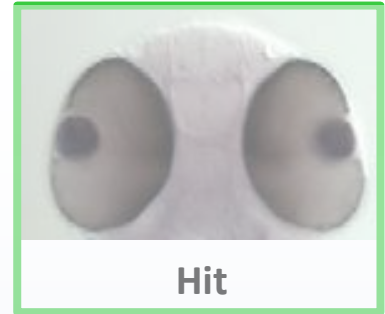
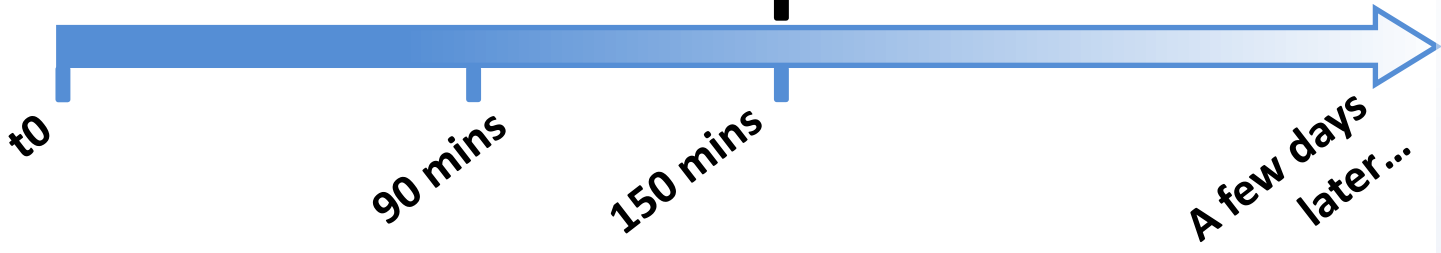
Screening of Kinase Inhibitors



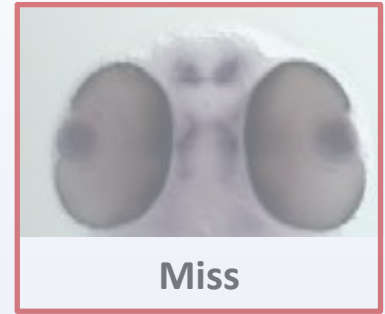
Screening of Kinase Inhibitors



- 48hpf embryos have a functional, visible heart
- Effect of compounds on both heart rate and morphology can be observed under microscope



or



What does each screen tell us?

Normal heart rate
tells us...

Compound is not toxic - thus locomotor and *in situ* results are valid

What does each screen tell us?

Normal heart rate
tells us...

Compound is not
toxic

A hit in the locomotor
screen tells us...

Compound has
prevented the seizure
OR
Compound is sedative
OR
Compound paralysed
larvae

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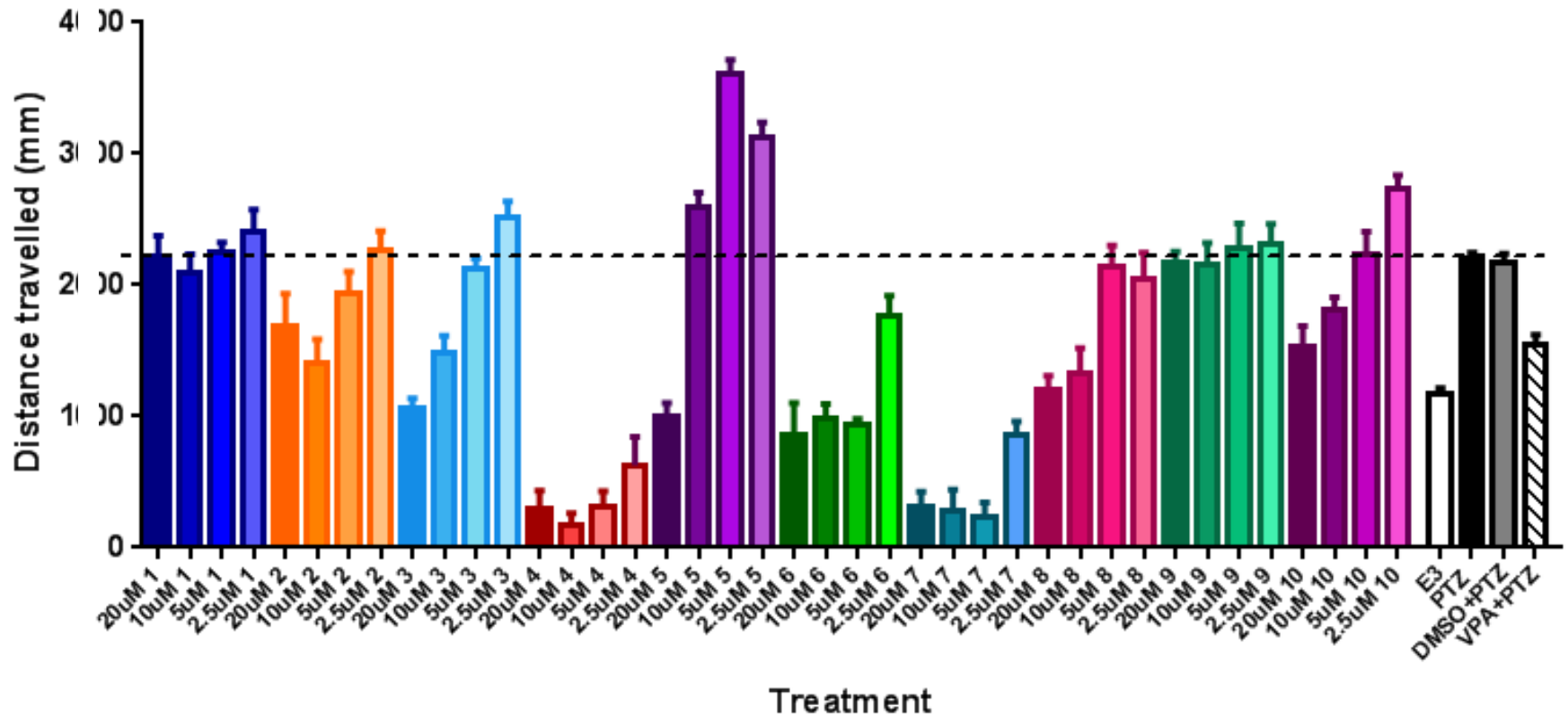
OR

The compound
interferes
with gene expression

A validated hit compound is one that is not toxic and prevents the behavioural and transcriptional responses to a seizure

Locomotor response to PTZ

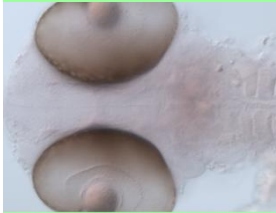
Behavioural response of 4dpf larvae exposed to CK1 Hits for 150mins, then PTZ for 10mins



In Situ's

Media
alone

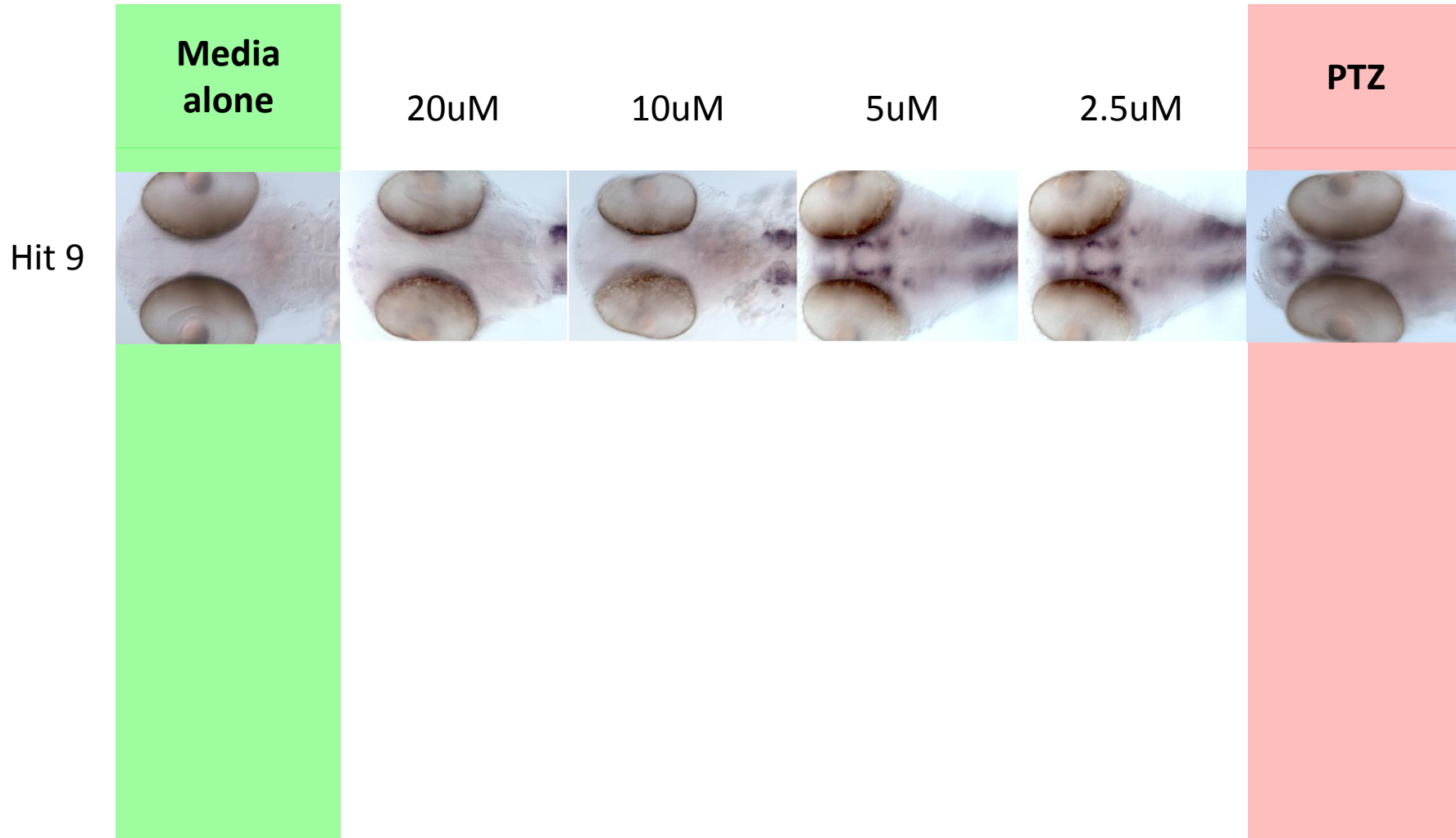
Hit 9



PTZ

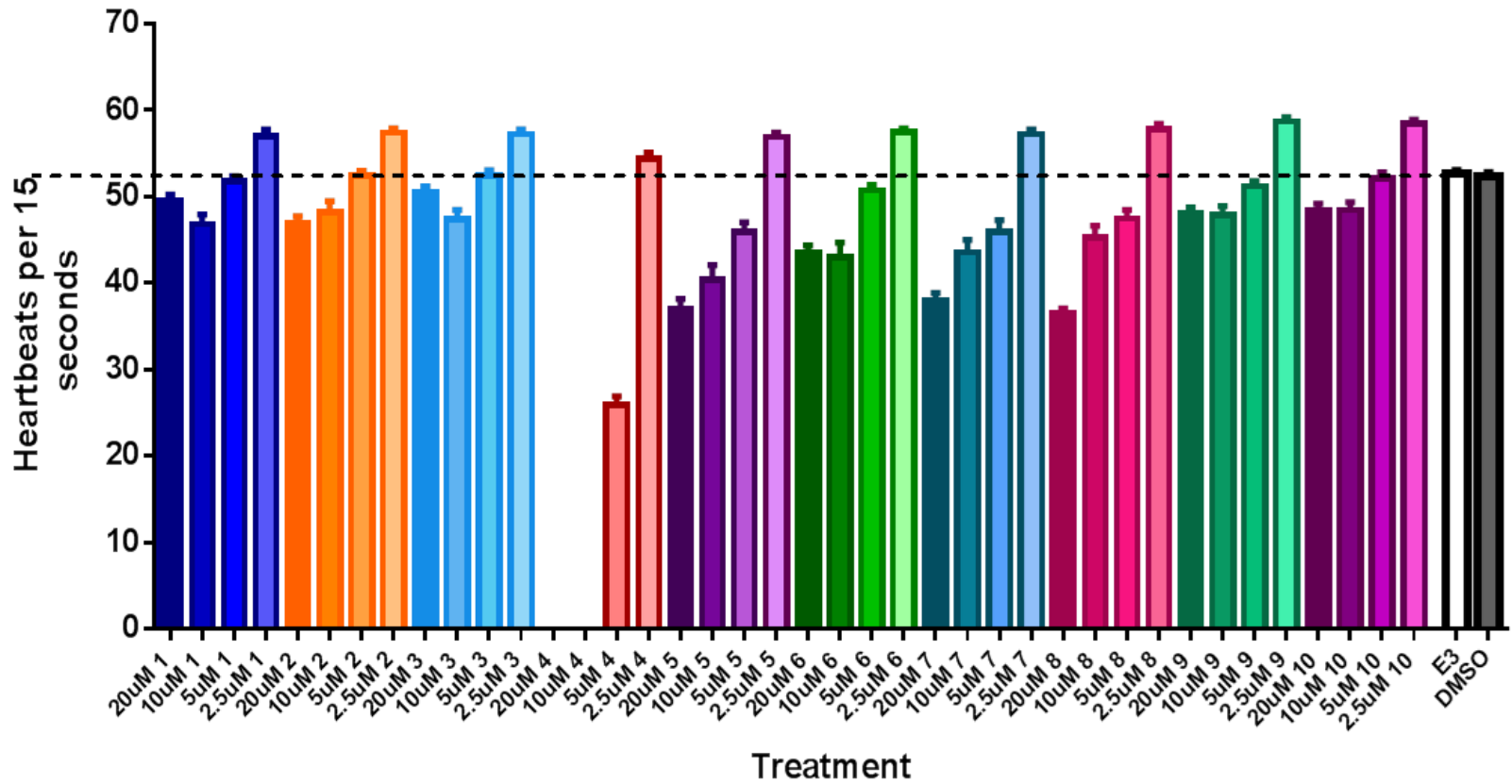


In Situ's



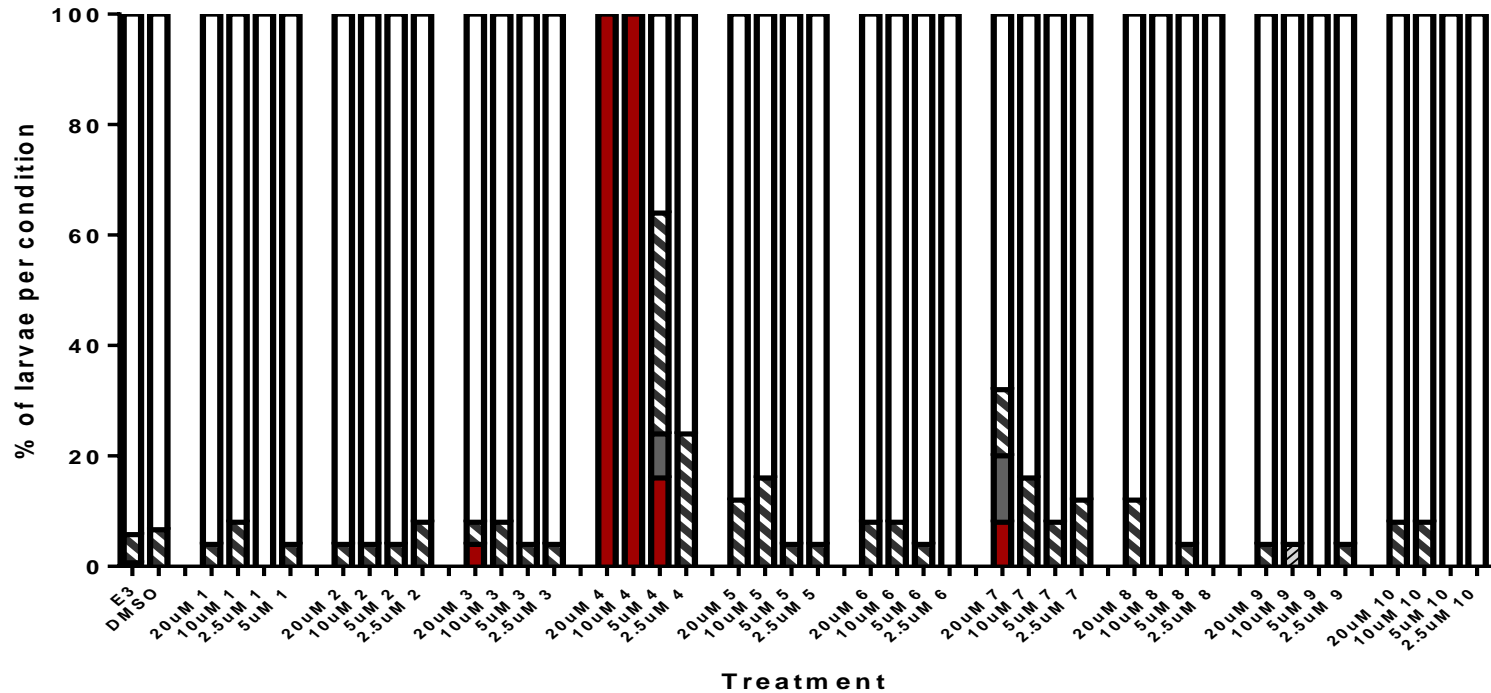
Heart rates






Heart rates / 15seconds of stimulated 3dpf larvae 24h post exposure to CK1 Hits



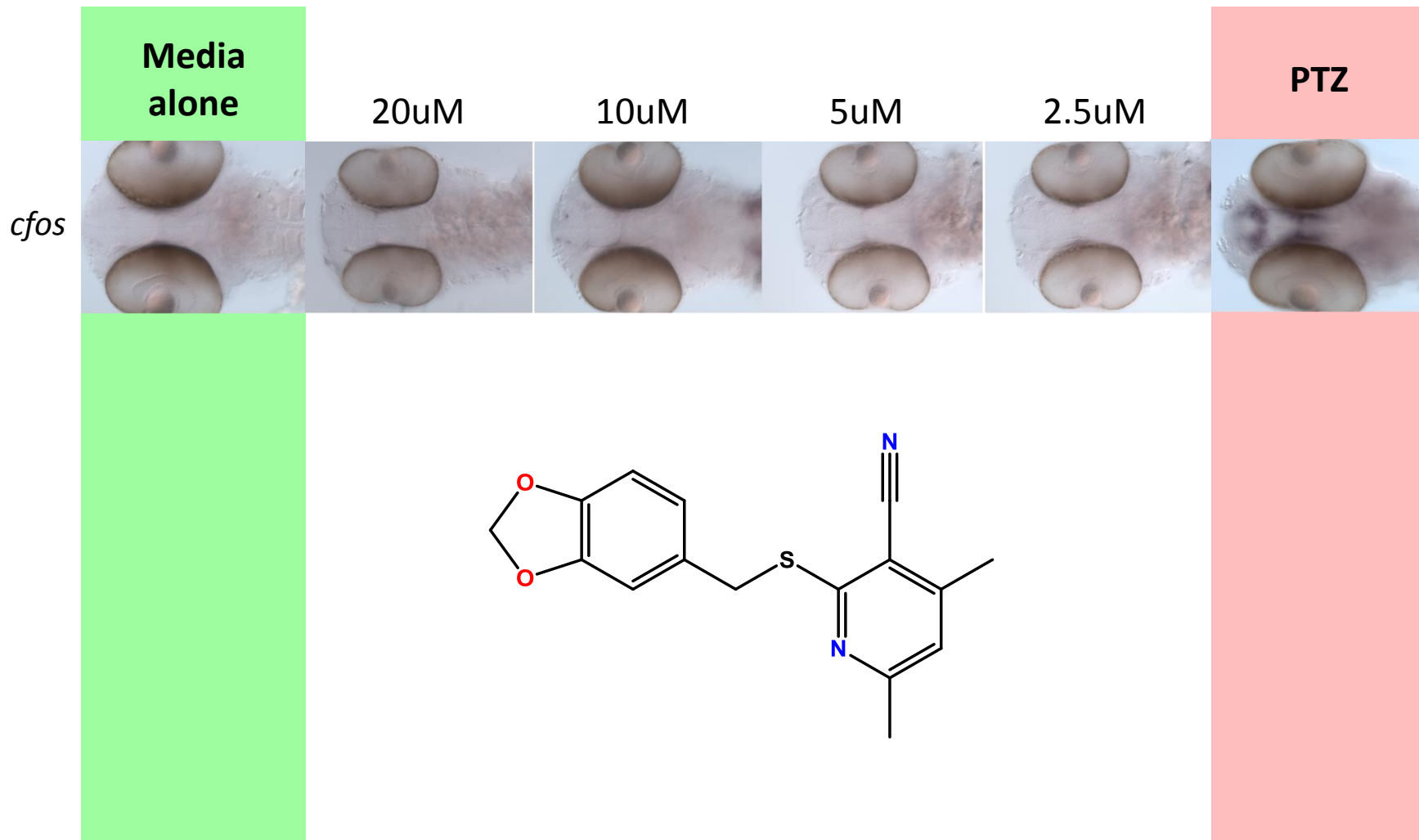
Toxicity

All Ck1 Hits Toxicity- 24h post exposure (3dpf)

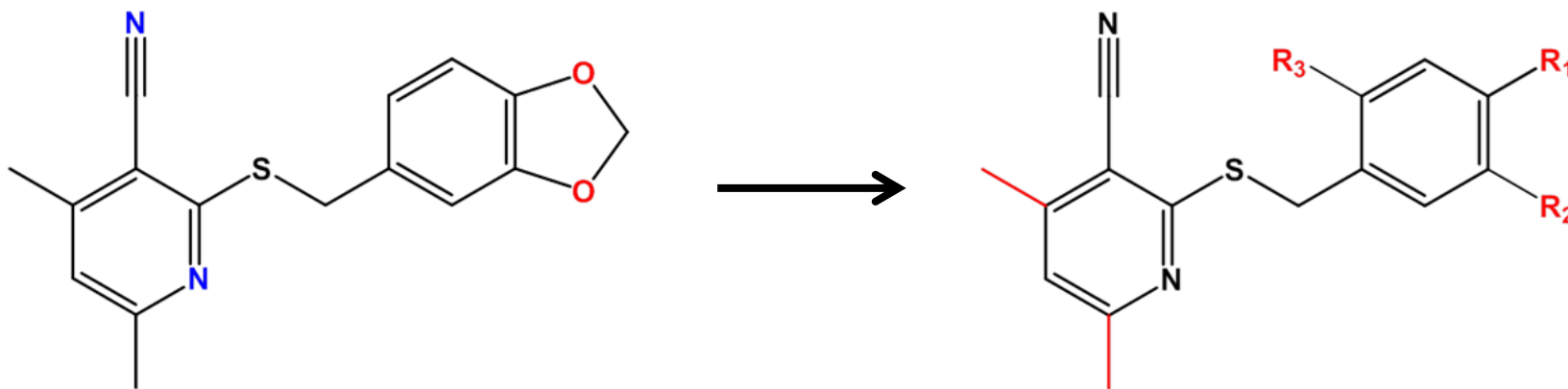


-  Normal
-  Odema
-  Curved Spine
-  Odema + Curved spine
-  No Heartbeat

Best so far – F3394-0676

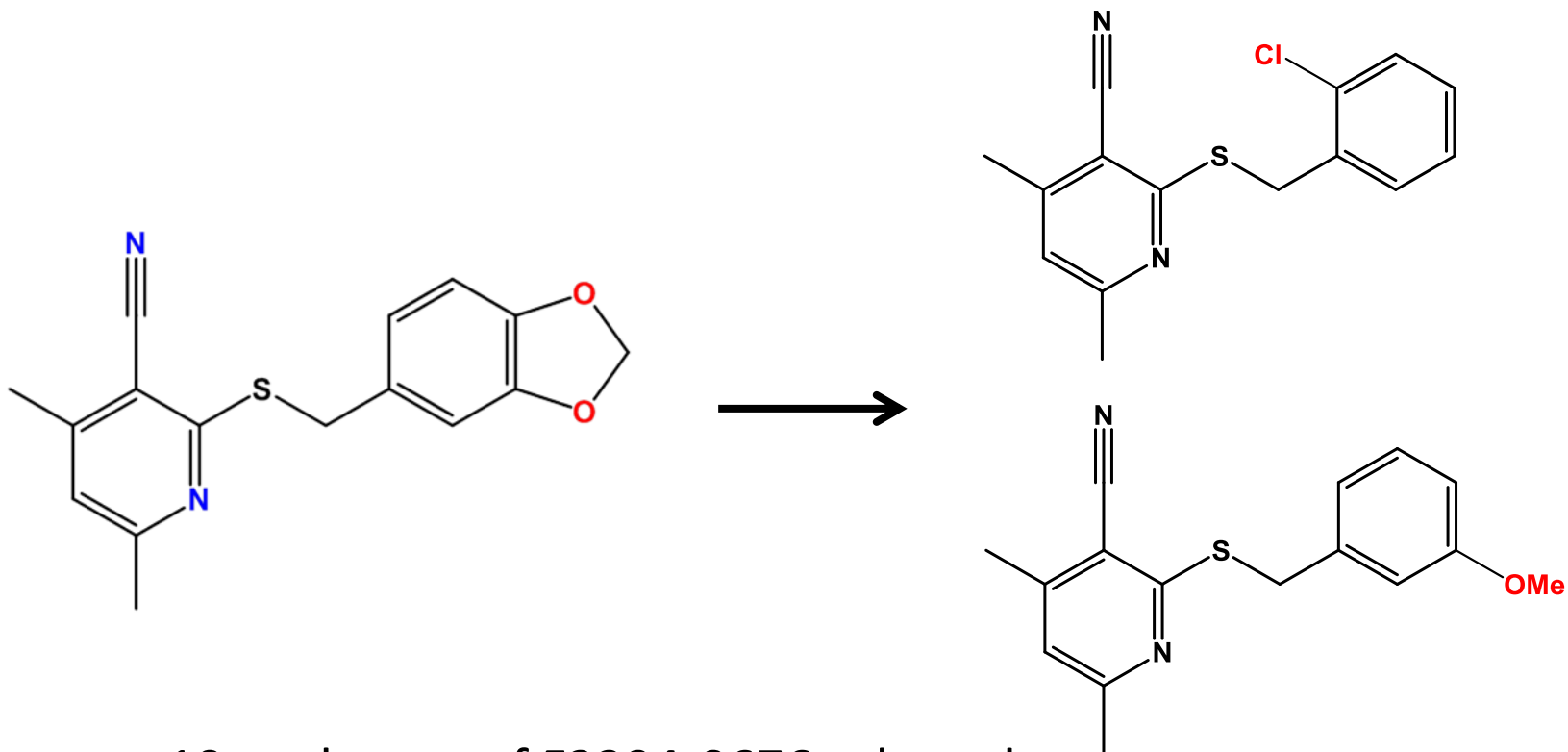


F3394-0676



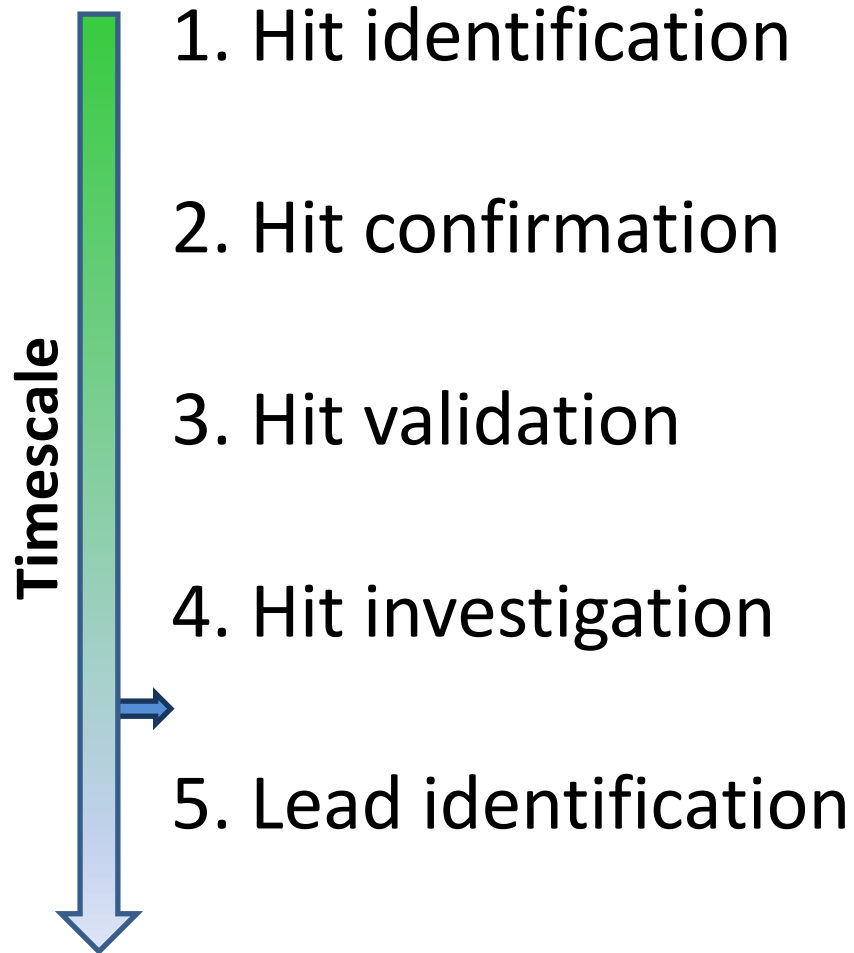
- 10 analogues of F3394-0676 selected
- Tested in *in situ*, locomotor and toxicology assays

F3394-0676

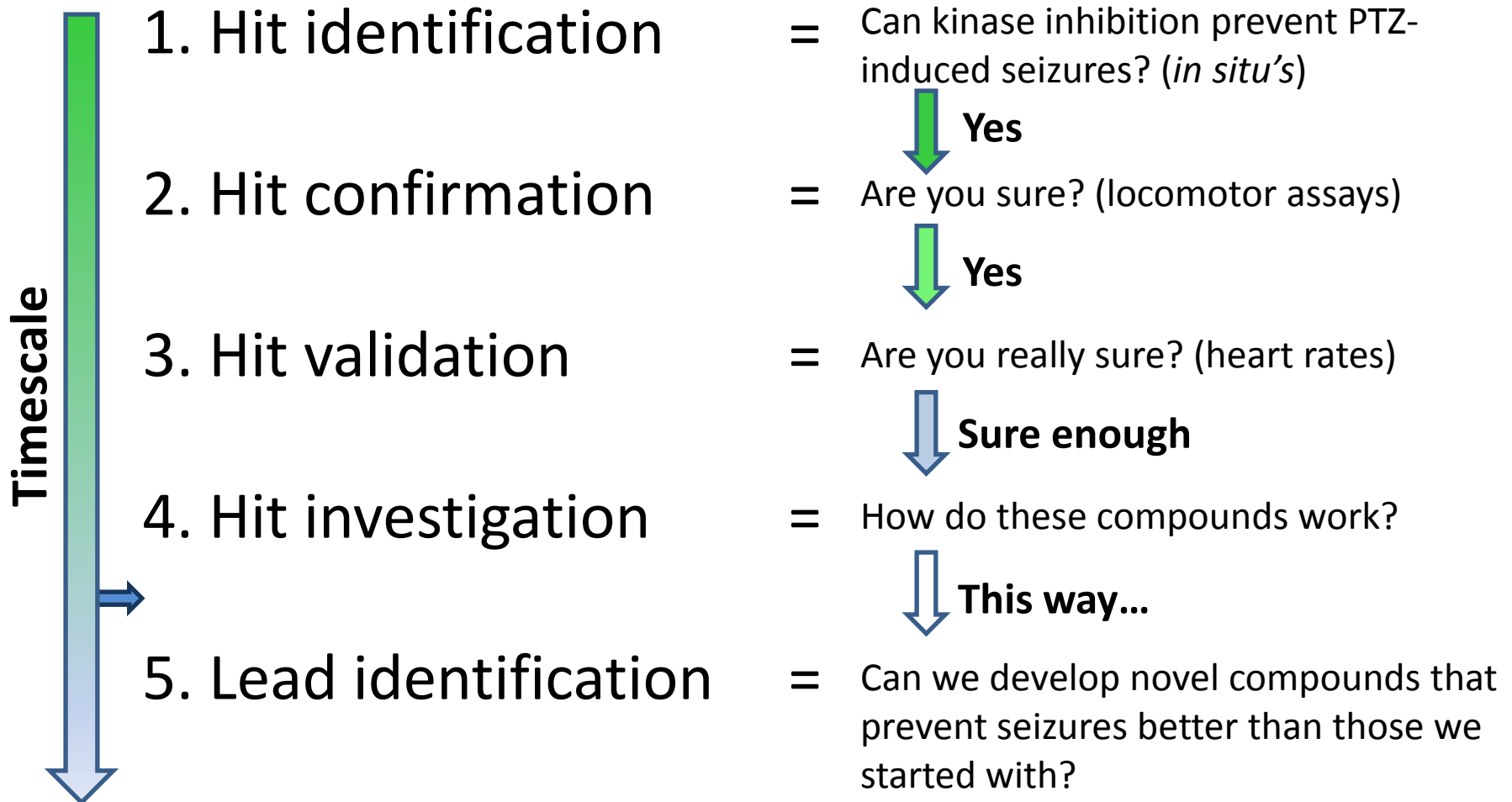


- 10 analogues of F3394-0676 selected
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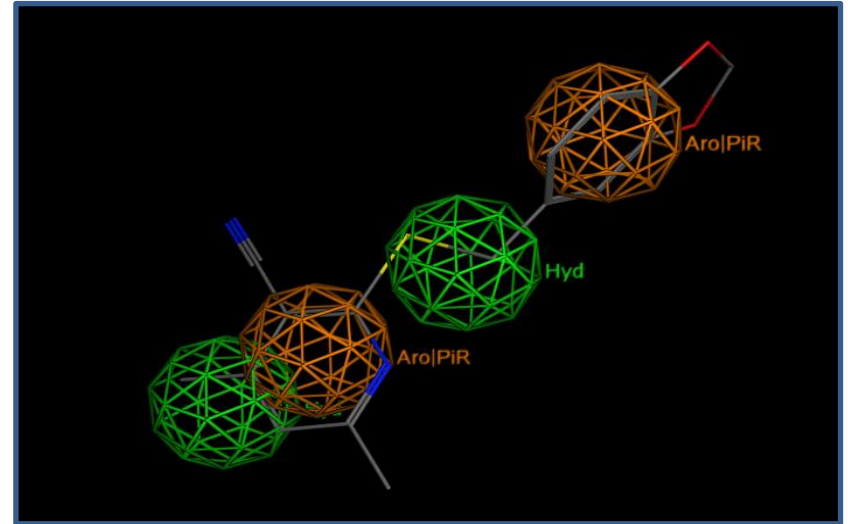
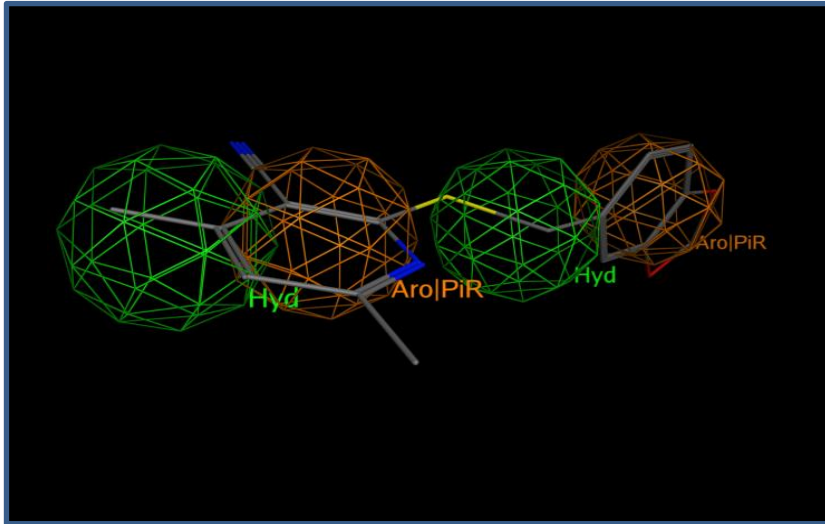
Summary



Summary

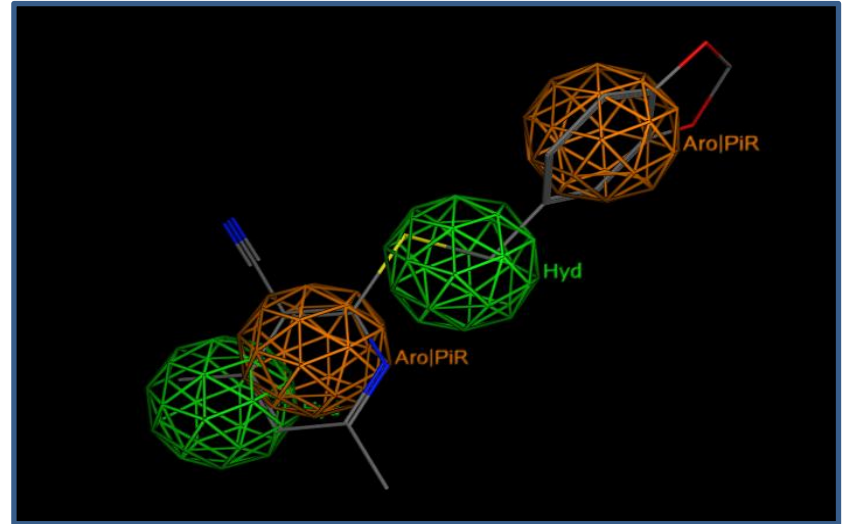
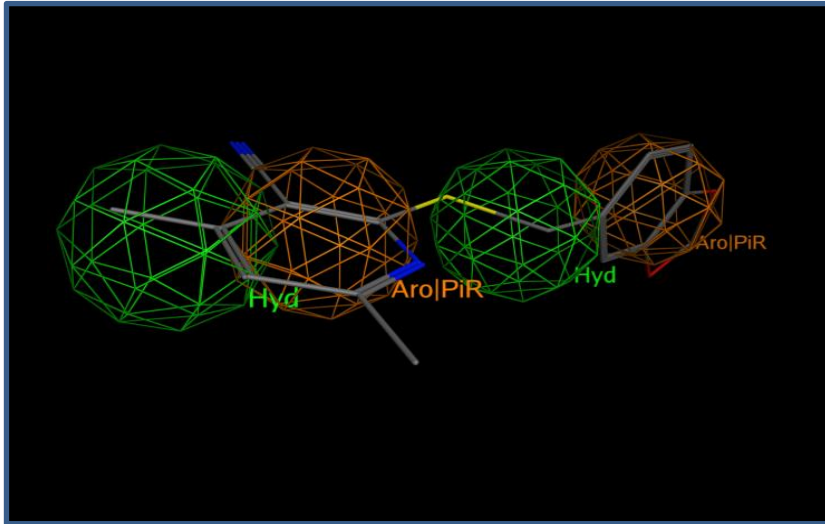


Investigation of F3394-0676



- 10 analogues of F3394-0676
- Currently being tested via *in situ*, locomotor and toxicology assays

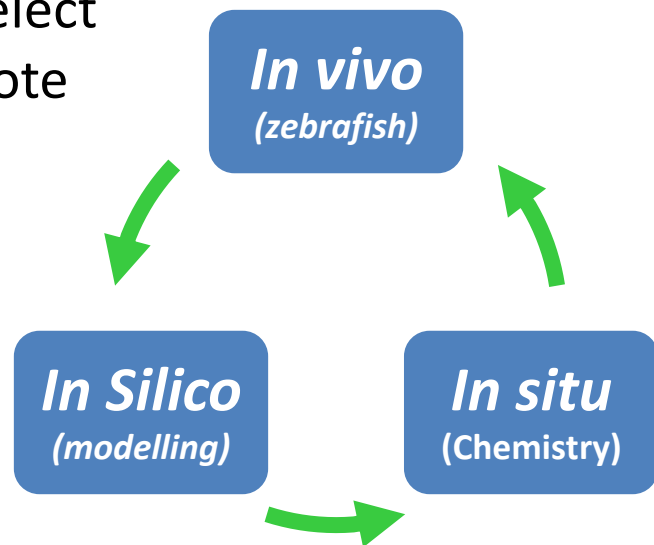
Investigation of F3394-0676



- 10 analogues of F3394-0676
- Currently being tested via *in situ*, locomotor and toxicology assays

Future Work

- ❑ qPCR- to quantify the transcriptional responses
- ❑ F3394-0676 kinase inhibition profiling
- ❑ Searching for compounds that match our pharmacophore of F3394-0676 analogues
 - ❑ Must develop selection criteria to select ligands from, for example, ChEMBL to promote structural diversity



Acknowledgements

Particular thanks to

Vincent Cunliffe

Sarah Baxendale

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...Any Questions?



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Understanding Life

In Situ's

