Building a predictive structure activity relationship (SAR) tool to mitigate ion channel related seizure liability in novel compounds

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INTRODUCTION

- Seizure liability remains a significant cause of attrition throughout drug development. The role of multiple ion channels in seizure provides an opportunity for a new screening paradigm for early prediction and design optimisation to reduce ion channel related liability.
- We have demonstrated the utility of **iSLA, an *in vitro* seizure liability assay** based on 14 seizure-related ion channels (Figure 1) for seizurogenicity prediction. Assessing 15 seizurogenic compounds on cell lines expressing these ion channels via automated electrophysiology revealed distinct inhibition patterns:
  - Potassium (K_V) channels were more sensitive than sodium (Na_V) channels
  - The nicotinic $\alpha_4\beta_2$ ion channel was the most frequently hit
  - CNS active compounds were more promiscuous than other therapies.
- Building on this work, we embarked on a study to develop a predictive structure activity relationship (SAR) model to identify the structural features driving ion channel inhibition. This will help medicinal chemists avoid seizure liability in the development of novel compounds.

METHODS

Human $K_V2.1$, $Na_V1.2$ and GABA-$\alpha_1\beta_2\gamma_2$ ion channel assays

- $K_V2.1$, $Na_V1.2$ and GABA-$\alpha_1\beta_2\gamma_2$ were selected for the initial compound screening (Figure 1).
- 88 compounds (78 from the Enamine REAL database and 10 parent compounds) were assessed by automated electrophysiology (QPatch II, Sophion) using CHO cell lines expressing human GABA-$\alpha_1\beta_2\gamma_2$, $K_V2.1$ or $Na_V1.2$.
- IC_{50} values were generated using 6- ($K_V2.1$, $Na_V1.2$) or 5-point (GABA-$\alpha_1\beta_2\gamma_2$) dose response curves.
- The pharmacophore fingerprint of activity of the 78 enamine compounds was compared to the respective parent compound, specifically:
  - Hydrophobic group
  - Hydrogen bond donors
  - Hydrogen bond acceptors
  - Aromatic ring attached
  - Aliphatic ring attached
  - Halogen bonds
  - Basic group
  - Acidic group

Key for screening data
- IC_{50} >100 µM
- IC_{50} 30 - 100 µM
- IC_{50} <30 µM

Enamine compound selection

- The **E**namine **RE**adily **A**ccesib**L**e (REAL) database is the largest enumerated database of synthetically feasible molecules (>1.2 billion) that can assist in SAR investigations.
- Compounds for testing were selected from the Enamine REAL database based on their pharmacophore features and similarity to the selected parent compounds (amoxapine [n=10], diphenhydramine [n=9], quetiapine [n=10], 4-AP [n=10], linopirdine [n=8], bepridil [n=9], NS1619 [n=9], quinidine [n=9], verapamil [n=2] and XE991 [n=2]).

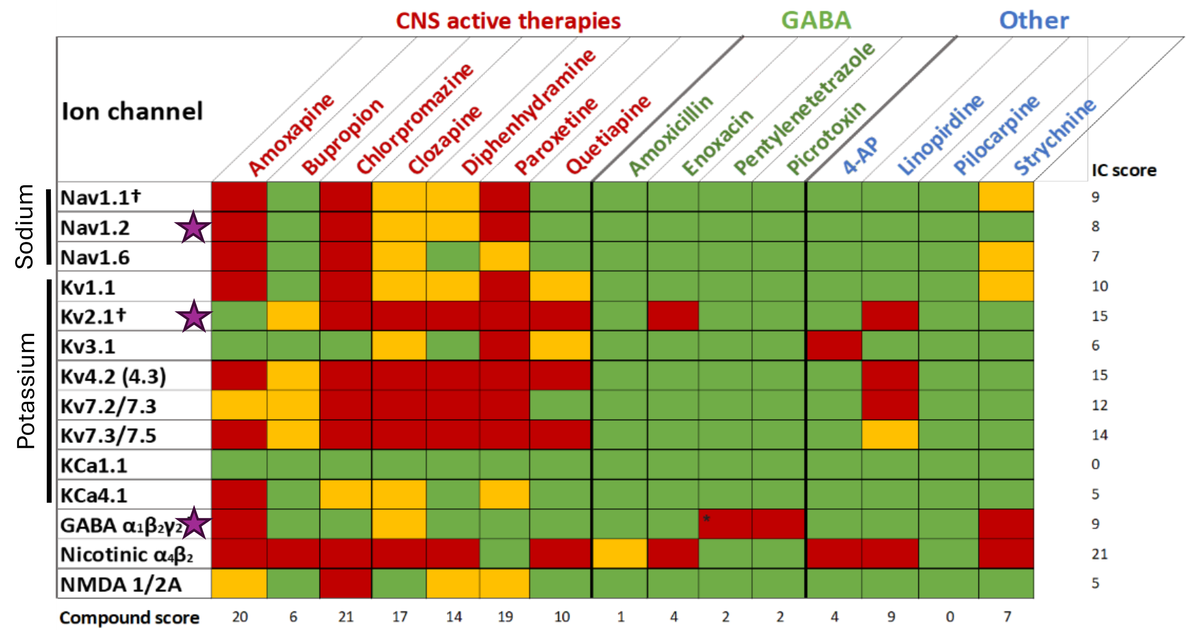


Figure 1. Initial selection of ion channels for automated electrophysiology screening (purple star indicates selected ion channels). Cumulative compound and IC scores calculated using high- (red = 2), intermediate- (yellow = 1) and low- (green = 0) risk hits. Abbreviations: 4-AP (4-aminopyridine), IC (ion channel). Reproduced from Rockley *et al.*, 2023 (PMID: 37632788).

RESULTS

Enamine compounds exhibit a range of activity across seizure-related ion channels

Example IC_{50} curves

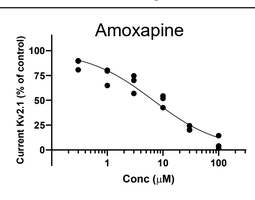
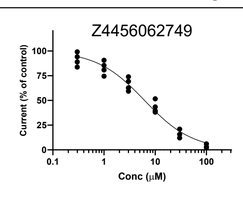
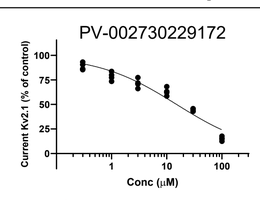
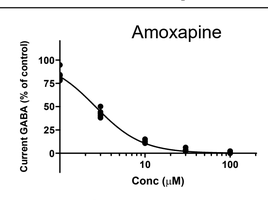
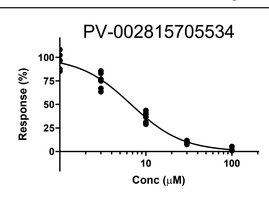
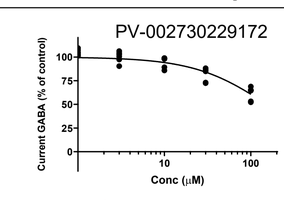
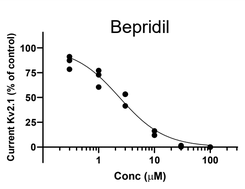
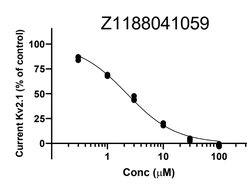
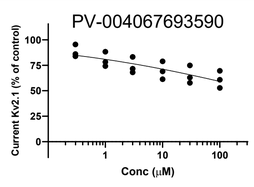
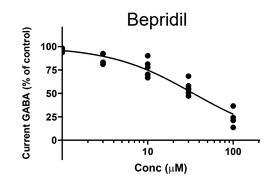
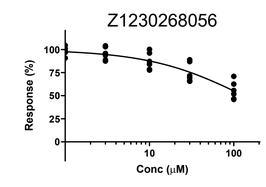
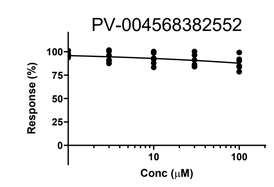
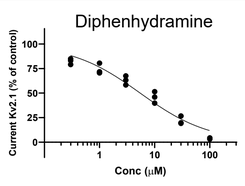
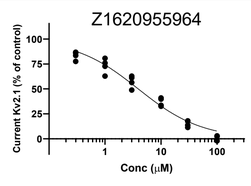
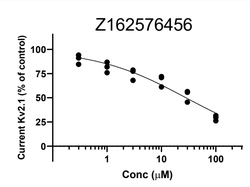
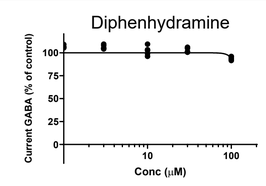
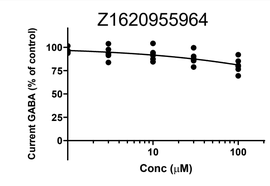
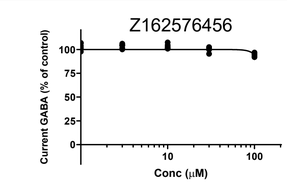
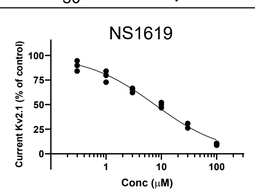
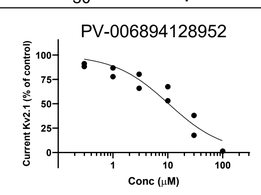
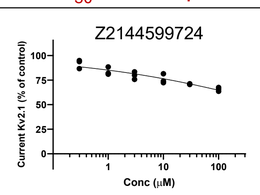
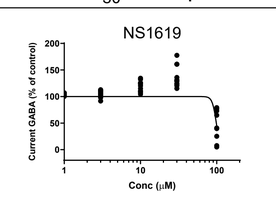
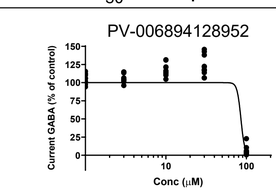
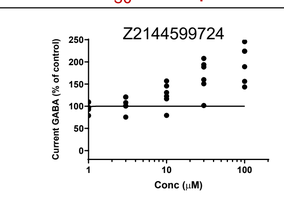
Compound	$K_V2.1$			GABA-$\alpha_1\beta_2\gamma_2$		
	Parent compound	Enamine with *similar* potency	Enamine with *lower* potency	Parent compound	Enamine with *similar* potency	Enamine with *lower* potency
Amoxapine	 IC_{50} = 6.678 µM	 IC_{50} = 6.189 µM	 IC_{50} = 15.06 µM	 IC_{50} = 2.6 µM	 IC_{50} = 6.7 µM	 IC_{50} >100 µM
Bepridil	 IC_{50} = 2.363 µM	 IC_{50} = 2.229 µM	 IC_{50} >100 µM	 IC_{50} = 34.13 µM	 IC_{50} >100 µM	 IC_{50} >100 µM
Diphenhydramine	 IC_{50} = 5.461 µM	 IC_{50} = 3.918 µM	 IC_{50} = 28.32 µM	 IC_{50} >100 µM	 IC_{50} >100 µM	 IC_{50} >100 µM
NS1619	 IC_{50} = 7.602 µM	 IC_{50} = 10.32 µM	 IC_{50} >100 µM	 Possible PAM / agonist	 Possible PAM / agonist	 Possible PAM / agonist

Figure 2. Representative IC_{50} plots for amoxapine, bepridil, diphenhydramine and NS1619 compared to enamines with similar (± 20% change in IC_{50} from parent; black text) or lower (>20% increase in IC_{50} from parent; red text) potency screened against seizure-related $K_V2.1$ and GABA-$\alpha_1\beta_2\gamma_2$ ion channels. Where enamines with similar or lower potency were not identified during screening, alternative representative compounds have been shown. Abbreviation: PAM (positive allosteric modulator).

Enamine compounds exhibit a range of activity across seizure-related ion channels

Table 1. IC_{50} values for 88 compounds screened against $K_V2.1$, $Na_V1.2$ and GABA-$\alpha_1\beta_2\gamma_2$ ion channels. The number of enamines with similar (± 20% change in IC_{50} from parent; ~), lower (>20% increase in IC_{50} from parent; ↓) or higher (>20% decrease in IC_{50} from parent; ↑) potency are presented for each channel. Abbreviation: PAM (positive allosteric modulator).

Compound	$K_V2.1$				$Na_V1.2$				GABA-$\alpha_1\beta_2\gamma_2$			
	IC_{50}	No. ~ potency	No. ↓ potency	No. ↑ potency	IC_{50}	No. ~ potency	No. ↓ potency	No. ↑ potency	IC_{50}	No. ~ potency	No. ↓ potency	No. ↑ potency
4-AP	>100 µM	9	0	1	>100 µM	10	0	0	>100 µM	10	0	0
Amoxapine	6.68 µM	4	4	2	26.21 µM	1	9	0	2.6 µM	0	10	0
Bepridil	2.36 µM	1	6	2	9.36 µM	0	9	0	34.13 µM	0	9[a]	0
Diphenhydramine	5.46 µM	1	3	5	>100 µM	6	0	3	>100 µM	9	0	0
Linopirdine	8.81 µM	0	5	3	>100 µM	7	0	1	>100 µM	6	0	2
NS1619	7.60 µM	0	9	0	56.89 µM	1	8	0	Possible PAM or agonist	3[b]	6[c]	0
Quetiapine	16.76 µM	2	2	6	>100 µM	9	0	1	>100 µM	10	0	0
Quinidine	1.23 µM	0	9	0	>100 µM	9	0	0	>100 µM	7	0	2
Verapamil	1.54 µM	0	2	0	35.59 µM	0	2	0	>100 µM	2	0	0
XE991	19.21 µM	0	0	2	>100 µM	2	0	0	>100 µM	2	0	0

[a.] 1 compound possible PAM/agonist. [b.] 3 compounds possible PAM/agonists. [c.] 6 compounds inactive (IC_{50} >100 µM).

Pharmacophore dyad groups have significantly different potencies at $K_V2.1$

Table 2. Mean IC_{50} values for 88 compounds screened against $K_V2.1$ ion channel for pharmacophore dyads of interest. P <0.05 considered significant (2-tailed t-test, with Bonferroni correction). Abbreviations: AR (aromatic group), BG (basic group), HA (hydrogen bond acceptor), Hal (halogen), HD (hydrogen bond donor), N= (number of compounds with or without pharmacophore), RR (aliphatic ring). Black box indicates representative pharmacophore dyads shown in Figure 3.

Pharmacophore	Mean with (N=)	Mean without (N=)	delta	p-value
HD-2 bond-Hal	3.3 (7)	4.8 (81)	-1.5	1.52E-11
HD-direct link-AR	3.7 (21)	4.9 (67)	-1.3	1.35E-08
AR-4 bond-BG	5.1 (34)	4.3 (54)	0.8	1.47E-07
HA-2 bond-BG	5.1 (35)	4.3 (53)	0.8	9.58E-07
AR-direct link-RR	5.1 (25)	4.5 (63)	0.6	1.54E-05
AR-1 bond-BG	5.0 (37)	4.4 (51)	0.7	4.72E-05
BG-2 bond-BG	5.2 (11)	4.6 (77)	0.7	5.27E-05
HA-same group-RR	5.1 (17)	4.5 (71)	0.6	6.75E-05
RR-same group-BG	5.1 (17)	4.5 (71)	0.6	6.75E-05
HD-2 bond-BG	5.2 (13)	4.5 (75)	0.6	0.00015714

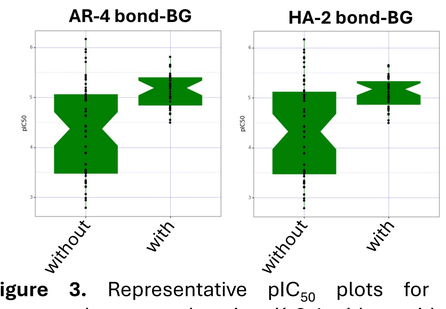


Figure 3. Representative pIC_{50} plots for 88 compounds screened against $K_V2.1$ with or without pharmacophore dyads. Increased pIC_{50} indicates higher potency (positive delta value).

DISCUSSION AND CONCLUSIONS

- The 88 compounds tested show a range of activities at seizure-related ion channels ($K_V2.1$, $Na_V1.2$ and GABA-$\alpha_1\beta_2\gamma_2$) with enamine compounds exhibiting increased and decreased potency at these channels compared to the respective parent compounds.
  - Most compounds were inactive (IC_{50} >100 µM) at GABA-$\alpha_1\beta_2\gamma_2$
  - Amoxapine and Bepridil parent compounds were the most potent across all 3 channels. The majority of respective enamine compounds showed a decreased potency compared to the parent compounds indicating SAR can be a useful tool to eliminate seizurogenic risk at these channels.
- Initial screening using $K_V2.1$ shows individual pharmacophore dyads extracted have distinct chemical groups that may present a significant risk. This initial analysis provides evidence that some chemical groups may be more likely to inhibit $K_V2.1$, which may extend to other ion channels.
- Future testing of the compound set across more ion channels will provide additional evidence to build a predictive SAR tool. This will help medicinal chemists avoid seizure liability in novel compound development.

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