

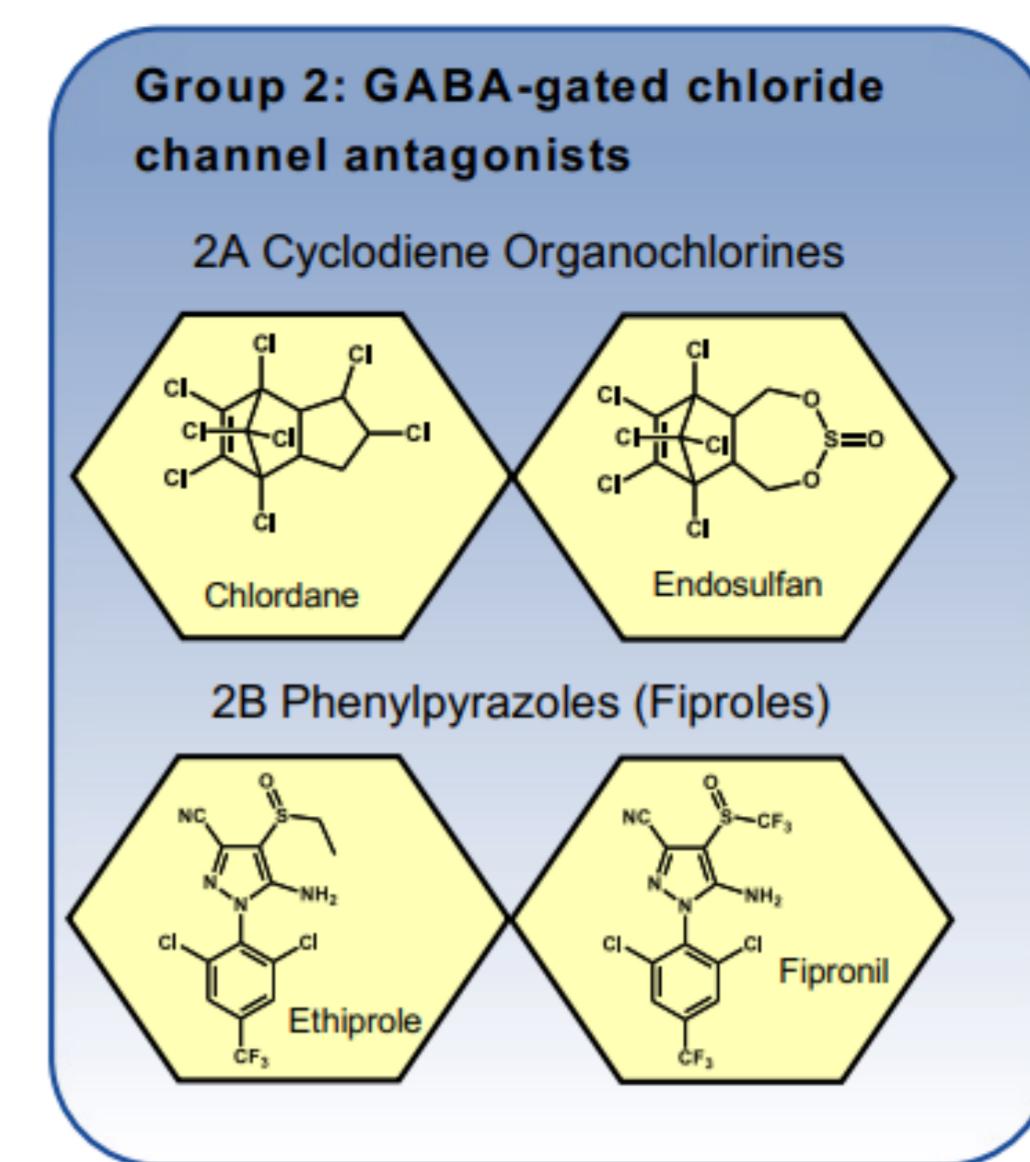
# Cell-based fluorescent assay for invertebrate $\gamma$ -aminobutyric acid (GABA) receptor and characterisation of a novel isoxazoline insecticide

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## Introduction:

GABA is the major inhibitory neurotransmitter in invertebrates, and its receptor is composed of subunits encoded by the Rdl gene<sup>1</sup>. Until 2019 the only commercial insecticides acting at the GABA receptor were the Phenylpyrazoles (e.g. fipronil) and the cyclodiene organochlorines (e.g. endosulfan). Although structurally distinct chemical classes, both share a related mode of action through channel blockade (IRAC group 2)<sup>2</sup>. Resistance due particularly to the A301S mutation and declining environmental acceptability means that organochlorines are no longer used in any capacity for crop protection.

We used *Drosophila* S2 cells stably expressing homo-oligomeric wild type and A301S mutant *Drosophila* Rdl GABA receptors with the FLIPR membrane potential (FMP) assay kit to develop a fluorescent assay in the Hamamatsu FDSS7000EX screening system. This assay was used extensively to support projects that led to the discovery of isocycloseram, a novel isoxazoline insecticide acting via inhibition of the GABA receptor at a different site to that of the fiproles and organochlorines.



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## Method and Materials

*Drosophila* cell lines stably expressing the cloned *Drosophila* Rdl GABA receptor and the cloned *Drosophila* Rdl A301S mutation were generated at University College London<sup>3</sup>.

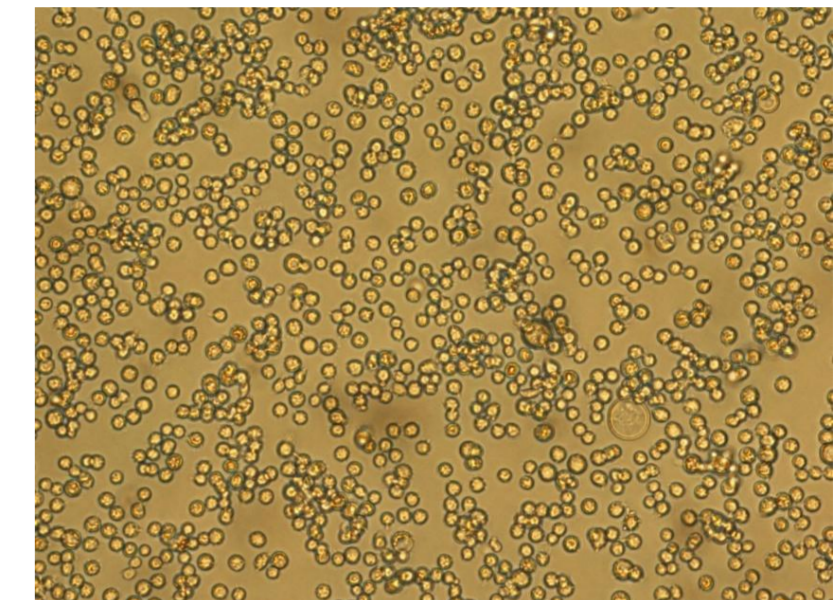
*Drosophila* S2 cells were harvested on the day of the assay and re-suspended at  $2.6 \times 10^6$  cells/ml in HBSS buffer, pH7. 25 $\mu$ l cell suspension was dispensed into black PDL coated 384-well clear-bottomed plates and the plates spun at 3000rpm for 4 min to attach the non-adherent cells to the bottom of the plate. 25 $\mu$ l of FMP-dye (blue) in HBSS buffer (1X) was added to each well and plates incubated at room temperature for 60min. In antagonist format, test compounds were diluted (3X) in assay buffer, 25 $\mu$ l dispensed into each well and the plates incubated at RT for 30min. To activate the ion channel, 25 $\mu$ l of GABA (10 $\mu$ M final concentration, representing EC<sub>95</sub>) in HBSS was added and the fluorescence measured for 2min at 1s intervals.

### Settings used to record signal in the FDSS7000EX:

- 2 Xenon lamps
- Excitation wavelength 480nm, emission wavelength 565nm (FMP-1).
- SP filter = 495
- Dispenser control:
  - ✓ Volume 25 $\mu$ l
  - ✓ Aspirate speed = 25 $\mu$ l/s, height = 1.00mm
  - ✓ Dispense speed = 25 $\mu$ l/s, height = 3.50mm – 1<sup>st</sup> addition (agonist)
  - ✓ Dispense speed = 25 $\mu$ l/s, height = 4.00mm – 2<sup>nd</sup> addition (antagonist)
- No titration, No shaking
- Wash cycles 10 during measurements
- Export Max ratio at 50sec.



Hamamatsu FDSS7000EX



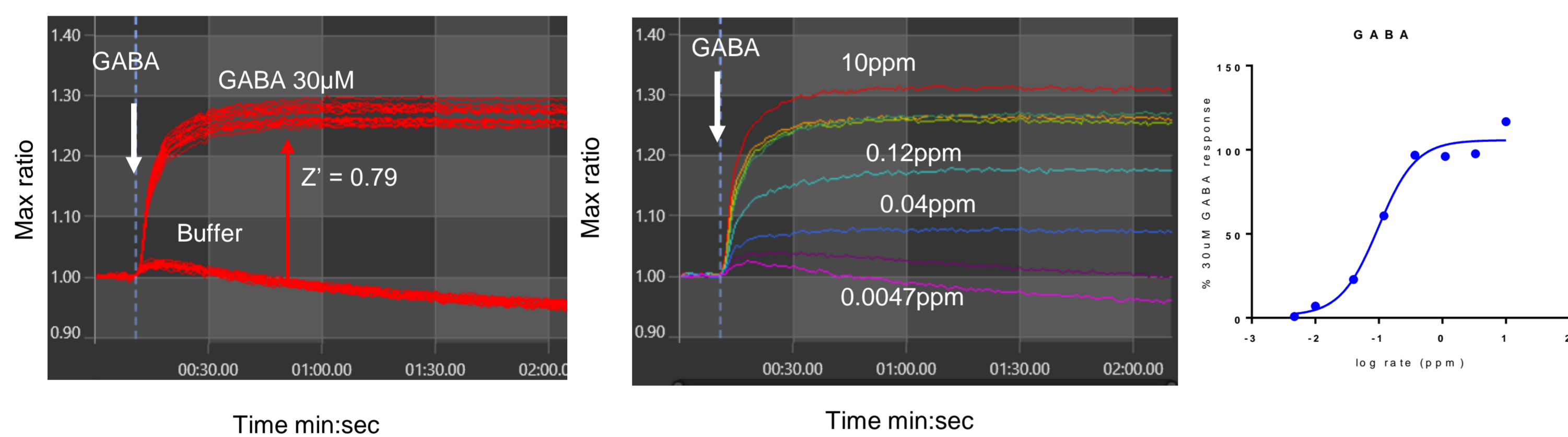
*Drosophila* S2 cells\_ Dm Rdl GABA receptor



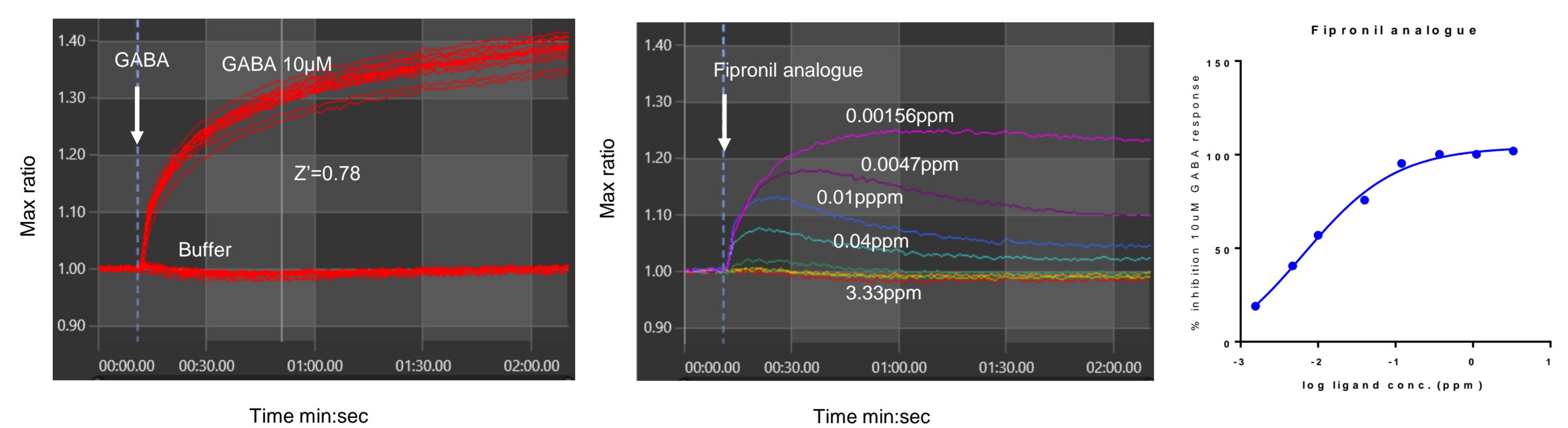
## Results

### 1. GABA signal on FDSS7000Ex

GABA activated the *Drosophila* homomeric Rdl GABA-gated Cl<sup>-</sup> channel giving a reproducible max ratio signal of 1.2-1.3 on FDSS7000Ex with a Z' of 0.79. A GABA potency of 0.85 $\mu$ M was obtained (Fig 1.). Inhibition of the 10 $\mu$ M GABA response was seen with a range of GABA blockers including a fipronil analogue (used as an assay standard) with an IC<sub>50</sub> of 0.02 $\mu$ M (Fig. 2).



**Fig 1:** GABA mediated activation of *Drosophila* homomeric Rdl GABA-gated Cl<sup>-</sup> channel expressed in *Drosophila* S2 cells. GABA gave an EC<sub>50</sub> of 0.85 $\mu$ M.

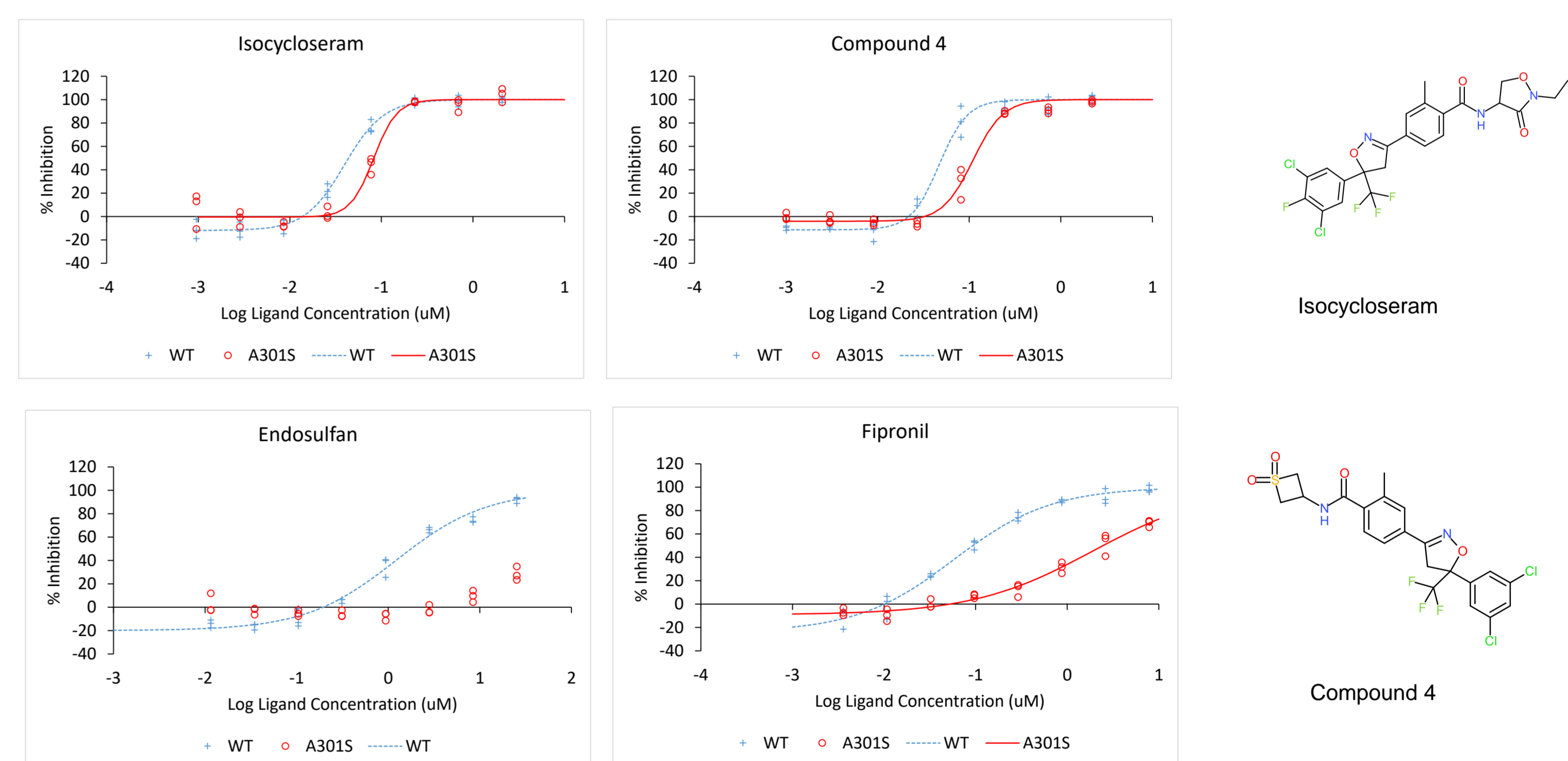


**Fig 2:** Inhibition of GABA response by a fipronil analogue in *Drosophila* S2 cells expressing wild-type Rdl. Fipronil analogue gave an IC<sub>50</sub> of 0.02 $\mu$ M.

### 2. Lack of cross-resistance and differentiation to IRAC group 2 insecticides

Isocycloseram and compound 4 (a close analogue) inhibited GABA-mediated activation of the *Drosophila* homomeric Rdl GABA-gated Cl<sup>-</sup> channel with high potency giving IC<sub>50</sub> values of 40nM and 47nM, respectively. These potencies were comparable to that observed for fipronil (56nM, Fig 3) whereas endosulfan showed lower potency (1.2 $\mu$ M, Fig 3).

The cell line expressing Rdl gene containing A301S mutation showed a high degree of insensitivity to endosulfan and fipronil (resistant factors >21 and 33.8, respectively) whereas isocycloseram and its analogue 4, showed only small differences in potency between the two lines (resistance factor 2 and 2.3, respectively).



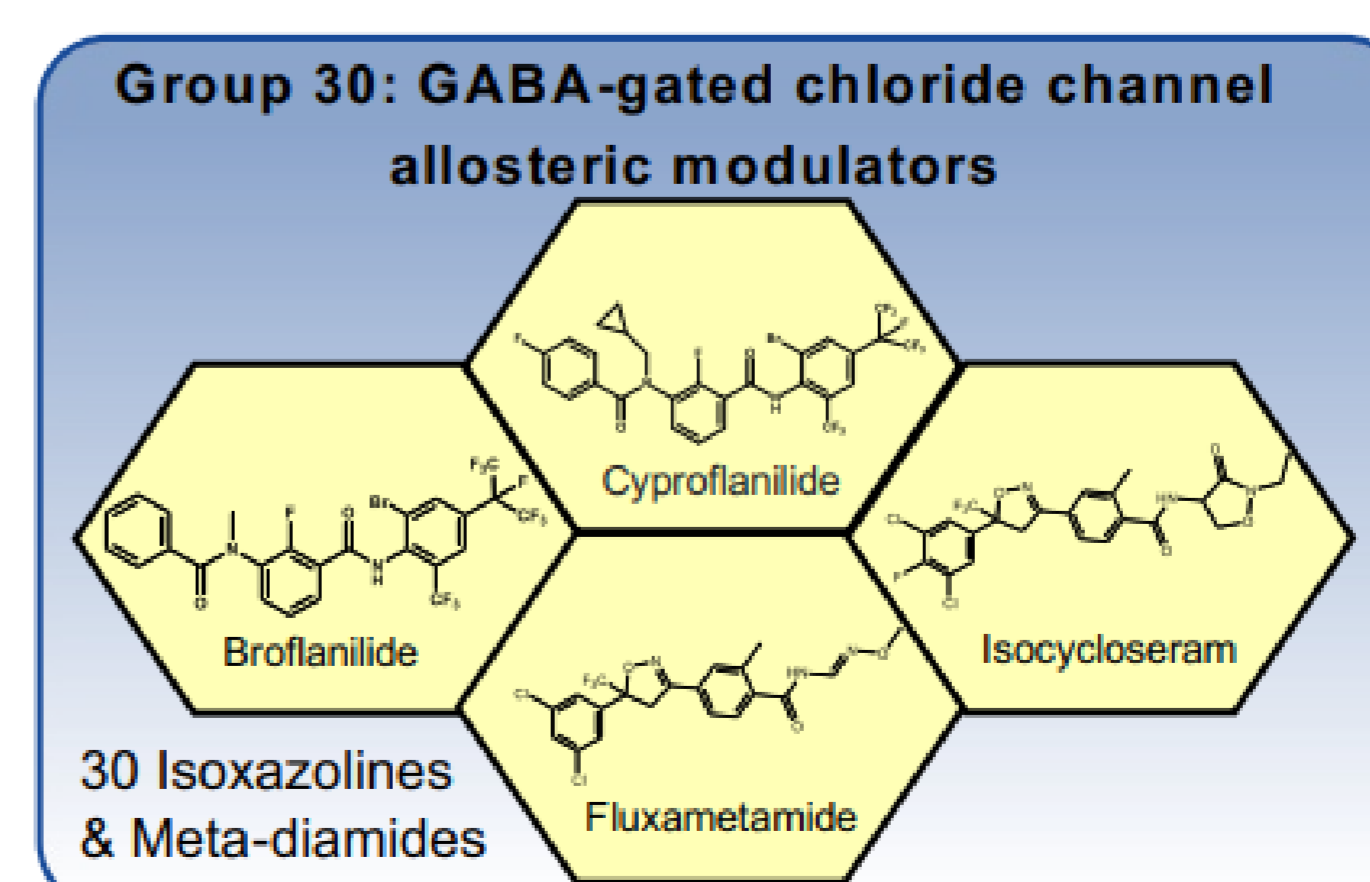
Compound	WT IC <sub>50</sub> ( $\mu$ M) and 95% CI	A301S IC <sub>50</sub> ( $\mu$ M) and 95% CI	RF (A301S / WT) and 95% CI
Isocycloseram	0.040 (0.034 - 0.047)	0.080 (0.068 - 0.095)	2.0 (1.6 - 2.6)
Compound 4	0.047 (0.039 - 0.055)	0.109 (0.092 - 0.129)	2.3 (1.8 - 3.0)
Endosulfan	1.202 (1.018 - 1.419)	>25.28	>21.0 (NA)
Fipronil	0.056 (0.048 - 0.066)	1.903 (1.612 - 2.246)	33.8 (26.7 - 42.7)

**Fig 3:** Inhibition of the GABA response by isocycloseram, compound 4, Endosulfan and Fipronil in *Drosophila* S2 cell lines expressing wild-type and A301S mutant Rdl.

## Summary

The FDSS7000Ex played an important role in our discovery of isocycloseram, a non-competitive antagonist of the invertebrate GABA receptor at a site distinct to that of the fiproles and cyclodienes (IRAC group 2).

The use of mutant cell lines provided one piece of evidence to enable the Insecticide Resistant Action Committee (IRAC) to classify this novel insecticide in IRAC group 30 "GABA-Gated Chloride Channel Allosteric Modulators".



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Insecticide Resistance Action Committee

## References:

1. Buckingham, S.D., Biggin, P.C., Sattelle, B.M., et al., 2005. Insect GABA receptors: splicing, editing, and targeting by antiparasitics and insecticides. *Mol. Pharmacol.* 68,942.
2. IRAC: Insecticide Resistance Action Committee Mode of Action Classification. <https://irac-online.org/>
3. Millar, N.S., Buckingham, S.D., Sattelle, D.B., 1994. Stable expression of a functional homo-oligomeric *Drosophila* GABA receptor in a *Drosophila* cell line. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 258, 307-314.
4. Blythe, J., Earley F.G.P., Piekarska-Hack, K., Firth, L., Bristow, J., Hirst, E.A., Goodchild, J.A., Hillesheim, E., and Crossthwaite A.J., 2022. The mode of action of isocycloseram: A novel isoxazoline insecticide. *Pesticide Biochemistry and Physiology* 187 105217.

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