The Drug Design and Discovery of Novel Enaminone Derivatives as Potential Anti-seizure Agents

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RATIONALE

Figure 1. Definition of Epilepsy and Statistics[1]

- Seizures can be defined as self-sustained episodes of abnormal neuronal hyperactivity that originates in the site where damage has occurred, known as epileptogenic focus. In America, one in 26 individuals will eventually develop epilepsy in their life **(Figure 1)**.[1,2]
- Despite the success of several ASDs with improved side effect profiles, there are still 30-40% of patients that suffer from uncontrolled seizures. Thus, there is an unmet medical need for new therapeutics that lead to full seizure control with reduced side effects for patients. Research efforts in our lab involve early drug design and development of novel anti-seizure analogs as potential agents for the treatment of drug-resistant and generalized epilepsy.
- T-type Ca^{2+} channels are an important target for anti-seizure medications.[3] We have developed several N-aryl amide enaminone analogs and evaluated their effectiveness at inhibiting the excitation of T-type Ca^{2+} channels.

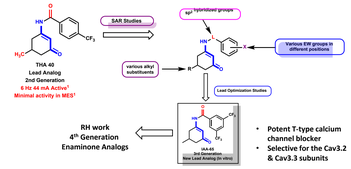

Figure 2. Previous Hit-to-Lead Optimization Studies

METHOD(S)

1) Enaminone derivatives were synthesized, purified and characterized prior to *in vitro* and *in vivo* analysis.
2) Electrophysiology techniques were performed to conduct whole-cell patch clamp experiments on neuronal targets related to epilepsy.[3]
3) For the *in vivo* study, behavioral studies utilizing adult zebrafish and larvae are employed to determined the target compounds neuroprotective effect against a chemically induced seizure. Active compounds are sent to the Epilepsy Screening Program for testing in acute rodent seizure models.

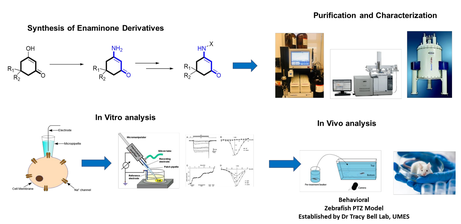

Figure 3. Methodology for Target-Based Drug Design Strategy

RESULTS

Table 1. Physicochemical Properties

Identifier	ClogP	BBB_Filter	Rule of 5	Mol Wt	Pgp Inh	HBD	HBA
RHB analogs	2.96	High (98%)	0	325.07	No	1	3
RHB-59	2.78	High (99%)	0	257.33	Yes	1	3
RHB-77[a]	3.92	High (99%)	0	419.24	No	1	3

[a] = compound predicted to be toxic; ADME codes: **Kow, Sw, rat, Xr+, Xm, HEPX, 3A4**

Table 2. Physicochemical Properties

Compound ID	Percent Yield (%)	Melting Point Range
RHB-59	**62.5%**	**160.4-161.5ºC**
RHB-62	**58.0%**	**186.9-188.0ºC**
ETSP 578001	**43.5%**	**151.0-151.5ºC**
RHB-95	**52.7%**	**130.1-131.2ºC**
RHB-99	**46.3%**	**165.4-166.2ºC**
RHB-103	**20.2%**	**147.1-148.3ºC**
RHB-107	**54.0%**	**170.2-170.8ºC**
RHB-111	**49.5%**	**137.0-138.3ºC**
RHB-115	**61.6%**	**148.3-149.2ºC**
RHB-123	**48.5%**	**138.6-139.2ºC**
RHB-127	**42.3%**	**170.3-171.2ºC**
RHB-133	**54.6%**	**135.0-137.3ºC**
RHB-137	**53.8%**	**177.1-177.9ºC**
RHB-146	**32.0%**	**171.9-172.8ºC**
RHB-158	**32.3%**	**133.4-134.5ºC**

Figure 4. *In Vitro* Electrophysiological Preliminary Results

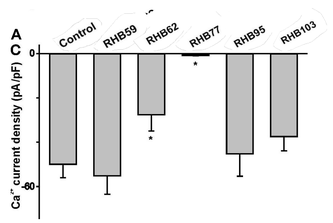

Figure 4. **(A).** Comparison of target analogs (50 μM) effect on the current densities of Cav3.2-stable transfected HEK 293 cells.

Figure 5. Results of Target Identification Studies

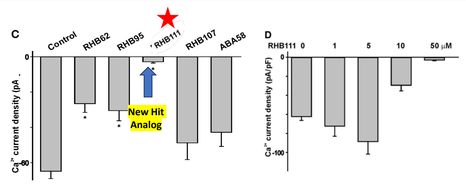

Figure 5. **(C).** Comparison of target analogs (50 μM) effect on the current densities of Cav3.2-stable transfected HEK 293 cells. **(D).** Concentration-dependent effect of RHB 111 on the T-type Ca^{2+} current densities generated in HEK 293-Cav3.2 (n= 9-11).

Table 3. Results of Compound ETSP 578001 in Test 33
Figure 6: Off target Identification Studies of ETSP 578001

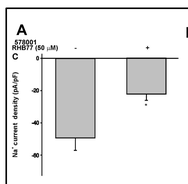

Table 1: Profile of Initial Antiseizure Activity and Rotarod Performance of ETSP# 578001 in Male Mice (Intraperitoneal (IP) administration).

Test	Time(Hours) Dose (mg/kg)	0.25 N / F	0.25 Comment Code	0.5 N / F	0.5 Comment Code	2.0 N / F	2.0 Comment Code
6HZ	30			0 / 4		0 / 4	
	100			0 / 4		0 / 4	
	300			0 / 4		0 / 4	
MES	30			0 / 4	4	0 / 4	
	100			0 / 4	4	0 / 4	4
	300			1 / 4		2 / 4	
Rotarod	30			0 / 8		0 / 8	
	100			0 / 8		0 / 8	
	300			0 / 8		0 / 8	
72 hour mortality	30	0 / 15					
	100	0 / 14					
	300	0 / 16					

(A). Effect of **ETSP 578001** on voltage-gated sodium channels (50 μM). **(B).** Profile of initial anti-seizure activity and rotarod performance of male mice (intraperitoneal injection); N = number of animals protected; F = number of animals tested. For rotarod test, N = number of animals displaying adverse effects; F = number of animals tested. Any adverse effects are noted. Comment code **4** = death

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- Dr. Miguel Martin-Caraballo (UMES)- Collaborator (Electrophysiology)
- Dr. Tracy Bell (UMES) – Collaborator (Zebrafish)

External Collaborators:
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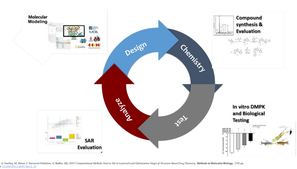
CONCLUSION(S)

- Fifteen target analogs have been designed and synthesized based on prototype IAA65.
- Preliminary *in vitro* studies showed **RHB-111** has ≥ 90% inhibition of T-type voltage-gated calcium channel.
- To validate the biological activity of molecule **ETSP 578001**, was investigated in acute battery rodent seizure models at NINDS ETSP. The preliminary results showed that molecule **ETSP 578001** had low efficacy and safety as an anti-seizure agent.
- A target-based drug design strategy was employed, that led to the discovery of **ETSP 578001**, as a dual VGSC and T-VGCC blocker. Future studies will involve lead optimization and target identification studies on ETSP 578001.

Lead Optimization



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(2) Amaye, I. J.; Haywood, R. D.; Mandzo, E. M.; Wirick, J. J.; Jackson-Ayotunde, P. L. Enaminones as building blocks in drug development: Recent advances in their chemistry, synthesis, and biological properties. Tetrahedron 2021, 83, 131984.

(3) Amaye, I. J.; Jackson-Ayotunde, P. L.; Martin-Caraballo, M. Evaluation of potential anticonvulsant fluorinated N-benzamide enaminones as T-type Ca2+ channel blockers. Bioorganic & Medicinal Chemistry 2022, 116766.

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