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SAÚDE**



**Fernanda Feuerharmel Soares da Silva**

**Avaliação da resposta de  
derivados benzimidazólicos em  
modelo animal de epilepsia  
infantil**

**UFCSPA**  
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# **Avaliação da resposta de derivados benzimidazólicos em modelo animal de epilepsia infantil**

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Orientadora: Prof<sup>a</sup> Dr<sup>a</sup> Helena M. T. Barros

Coorientadores: Prof<sup>a</sup> Juliana F. M.  
Schneider e Prof Cleber R. A. da Silva

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## RESUMO

Epilepsia é um dos problemas neurológicos mais comuns na infância e associa-se a atraso do desenvolvimento neuropsicomotor, assim como a dano neuronal. Sua etiologia envolve um desequilíbrio entre neurotransmissores excitatórios e inibitórios que pode aumentar o estresse oxidativo. Tratamentos atuais não são capazes de controlar completamente crises epiléticas e as medicações utilizadas na população pediátrica tem sua eficácia baseada em estudos com adultos, desconsiderando potenciais efeitos adversos deletérios ao desenvolvimento. Portanto, a busca por novas drogas antiepiléticas é necessária, especialmente focada na faixa etária pediátrica e na neuroproteção. Moléculas benzimidazólicas e seus substituintes são potenciais alvos de pesquisa para este fim. Objetivo: Avaliar o efeito antiepilético de cinco derivados selenobenzimidazólicos inéditos em modelo de epilepsia neonatal em comparação à fenitoína. Métodos: Crises epiléticas foram induzidas com pentilenotetrazol em 132 ratos Wistar entre 10 e 15 dias de vida 30 minutos antes da administração dos novos compostos. Indivíduos foram observados por 60 minutos para avaliação de latência para início de crises, gravidade e duração das mesmas. Resultados: Quatro das cinco drogas testadas mostraram correlação positiva entre o aumento da latência e das doses administradas. A dose de 8mg/kg do composto 2-(2-clorobenzilselenil)-1*H*-benzo[d]imidazol mostrou-se eficaz em elevar o tempo de latência em relação ao controle negativo. Já o composto 2-((3-(trifluormetil)fenil)selenil)-1*H*-benzo[d]imidazol provocou uma redução significativa de crises convulsivas graves em comparação ao controle negativo, além de 100% de redução do tempo de duração de crises graves. Conclusão: Os novos compostos selenobenzimidazólicos podem permitir um novo campo de pesquisa para medicações antiepiléticas, especialmente devido ao Selênio presente como seu substituinte, com potencial antioxidante.

**Palavras-chave:** epilepsia, crises neonatais, benzimidazol, Selênio.

## ABSTRACT

Epilepsy is one of the most common neurologic disorders in childhood and is associated to developmental impairments as well as neurological damage. Its etiology involves an imbalance of excitatory and inhibitory neurotransmitters which enhance oxidative stress. Current treatments are not able to completely control seizures and the drugs used by children have their efficacy based in studies with adults, disregarding potential deleterious effects on development. Therefore, the research for new antiepileptic drugs is needed, especially focused in pediatric population and neuroprotection. Benzimidazole molecules and its substituents are a potential anticonvulsant target for discovering new drugs. Objective: We aim to investigate the antiepileptic effect of five new selenobenzimidazoles compounds in comparison to phenytoin in neonatal seizures model. Methods: Seizures were induced in 132 Wistar rats between 10 and 15 days with pentylenetetrazol 30 minutes before administration of the new compounds. They were evaluated throughout 60 minutes for seizure latency, duration and severity. Results: Four of the five drugs showed a positive correlation between dose and latency enhance. The dose of 8mg/kg of the compound 2-(2-chlorobenzylselenyl)-1*H*-benzo[d]imidazole showed significant difference between test group and negative control group and no difference in comparison to phenytoin group. The compound 2-((3-(trifluoromethyl)phenyl)selenyl)-1*H*-benzo[d]imidazole showed a significant reduction of severe seizures in comparison to negative control as well as a 100% reduction of the duration of these seizures. Conclusion: These new selenobenzimidazoles compounds may be a new field of research for antiepileptic drugs development, especially because its selenium substituent, which may provide antioxidative properties to the molecule.

**Keywords:** Epilepsy, Neonatal Seizures, Benzimidazole, Selenium.

## LISTA DE ABREVIATURAS

2-OG – 2-oxoglutarato

AAT – Aspartato aminotransferase

Aco2 – Aconitase

A-KGDH – Alfa-cetoglutarato desidrogenase

AMPA – Ácido alfa-amino-3-hidroxi-5-metil-4isoxazólico-propiónico

Aralar – Carreador aspartato-glutamato

Asp – Aspartato

BZlSe – Selenobenzimidazol

DNPM – Desenvolvimento neuropsicomotor

EAAT – Transportador de aminoácido excitatório

EO – Estresse oxidativo

EROs – Espécies reativas de oxigênio

FNT - Fenitoína

GABA – Ácido gama-aminobutírico

GABA-T – GABA transaminase

GAD – Descarboxilase ácido glutâmico

GAT – Transportador GABA

GDH – Glutamato desidrogenase

GSH – Glutathiona reduzida

GPx – Glutathiona-peroxidase

GR – Glutathiona redutase

GSSG – Glutathiona oxidada

i.p. – intraperitoneal

LAMOCA-UFRGS – Laboratório de Catálise Molecular da Universidade Federal do Rio Grande do Sul

MES – Máximo eletrochoque transcorneal

NMDA – N-metil-D-aspartato

NOS – Óxido nítrico

Prx<sub>ox</sub> – Peroxirredoxina oxidada

PRX<sub>red</sub> – Peroxirredoxina reduzida

PTZ – Pentilenotetrazol

R – Radical

RLO – Radicais livres de oxigênio

Se – Selênio

SNC – Sistema nervoso central

SOD – Superóxido dismutase

SSA – Succinil semialdeído

TCA – Ciclo do ácido tricarboxílico

Trx – Tiorredoxina

TrxR – Tiorredoxina redutase

vGAT – Transportador vesicular de GABA

vGlut – Transportador vesicular de glutamato

V.O. – Via oral

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## 1. Introdução

### 1.1. Epilepsia na infância

Epilepsia é uma doença do sistema nervoso central (SNC) caracterizada pela ocorrência de crises epiléticas de diversas etiologias e padrões (FISHER et al., 2014; REED, 2012) e representa um dos problemas neurológicos mais comuns da infância (REED, 2012) já que crises convulsivas chegam a acometer de 4% a 10% das crianças pelo menos uma vez (AGARWAL; FOX, 2018). Crises convulsivas também são a principal emergência neurológica no período neonatal afetando 1-5:1000 nascidos vivos a termo, com maior incidência em menores de 1500g (DONOVAN et al., 2016) e relacionam-se a um aumento do risco de morbidades como paralisia cerebral, déficit intelectual e a própria epilepsia (PISANI et al., 2015). Aproximadamente 60% das crises neste período deve-se à encefalopatia hipóxico-isquêmica e os demais casos correlacionam-se com hemorragia intracraniana, malformações cerebrais, infecções, distúrbios metabólicos e/ou hidroeletrólíticos (DONOVAN et al., 2016).

A principal crise convulsiva observada no período da lactância é a crise convulsiva febril que cursa com apresentação benigna, bom prognóstico e, na grande maioria dos casos, tratamento medicamentoso anticonvulsivante desnecessário (REED, 2012). Nos casos em que evolui para o quadro de *status epilepticus* febril, todavia, pode induzir lesões em regiões do SNC, como observado por Lewis et al (2014). Este evidenciou alterações agudas em hipocampo após *status epilepticus* febril que se associaram a imagens compatíveis com esclerose hipocampal após cerca de 1 ano do episódio (LEWIS et al., 2014).

Ainda na mesma faixa etária destacamos outras síndromes mais graves, como a Síndrome de West, considerada uma epilepsia catastrófica (PERRET et al., 2018), podendo cursar com até 200 crises ao dia e associada a prognóstico sombrio, posto que muitas crianças evoluem com epilepsia grave e refratária além de até 90% apresentar atraso do desenvolvimento neuropsicomotor (DNPM) (GALANOPOULOU; MOSHE, 2015; HANCOCK; OSBORNE; EDWARDS, 2013). Ressalta-se também a síndrome de Dravet, que se inicia normalmente no primeiro ano de vida e apresenta crises focais mioclônicas que podem evoluir para

generalizadas, geralmente com duração prolongada (CONNOLLY, 2016). A síndrome ainda pode cursar com prejuízo em relação a atenção, percepção visual e funções executivas (CONNOLLY, 2016).

Na fase pré-escolar e escolar a ocorrência de síndromes epiléticas ditas idiopáticas é mais comum (REED, 2012) e, dentre elas, pode-se citar a epilepsia de ausência, predominante entre 5 e 10 anos e caracterizada por curtos períodos de perda de consciência (AGARWAL; FOX, 2018; SZLAM; MEREDITH, 2013). Também é o período de ocorrência da epilepsia benigna da infância com descargas centro-temporais, presente usualmente entre 3 e 13 anos (SZLAM; MEREDITH, 2013), e da síndrome de Lennox-Gastaut, em crianças entre 3 e 5 anos, caracterizada por crises de difícil controle e de vários padrões, associadas a frequentes *status epilepticus* e atraso do DNPM, especialmente quando pouco controladas (REED, 2012; SZLAM; MEREDITH, 2013).

O diagnóstico de crises convulsivas e início do tratamento precoces visando seu controle são de extrema importância, já que proporcionam ao paciente um desfecho mais favorável (HANCOCK; OSBORNE; EDWARDS, 2013). Sabe-se, entretanto, que uma parcela significativa dos pacientes não chega ao controle adequado das crises ou não responde a tratamento algum (HANCOCK; OSBORNE; EDWARDS, 2013; LUX et al., 2004). Cerca de 10% das crianças com epilepsia apresenta quadros intratáveis e o mau controle das crises é um fator de risco importante para morte súbita (AGARWAL; FOX, 2018), além de gerar pior qualidade de vida (GOLYALA; KWAN, 2017). Portanto, avaliar novas possibilidades de tratamento e de controle das crises é de fundamental importância para o prognóstico clínico, neurológico e sobrevida de pacientes pediátricos.

#### *1.1.1. Glutamato e ácido gama-aminobutírico (GABA) na epilepsia*

Postula-se que alterações no equilíbrio dos principais neurotransmissores excitatórios e inibitórios – glutamato e GABA respectivamente – estejam relacionadas à origem das crises convulsivas (BRADFORD, 1995; BRUNTON, 2011; DINUZZO et al., 2014). O glutamato é um aminoácido que não ultrapassa a barreira hemato-encefálica, portanto é sintetizado no SNC a partir de precursores como a glutamina após sua liberação pelas células da glia e posterior metabolização através da glutaminase mitocondrial (PURVES, 2001) (Figura 1).

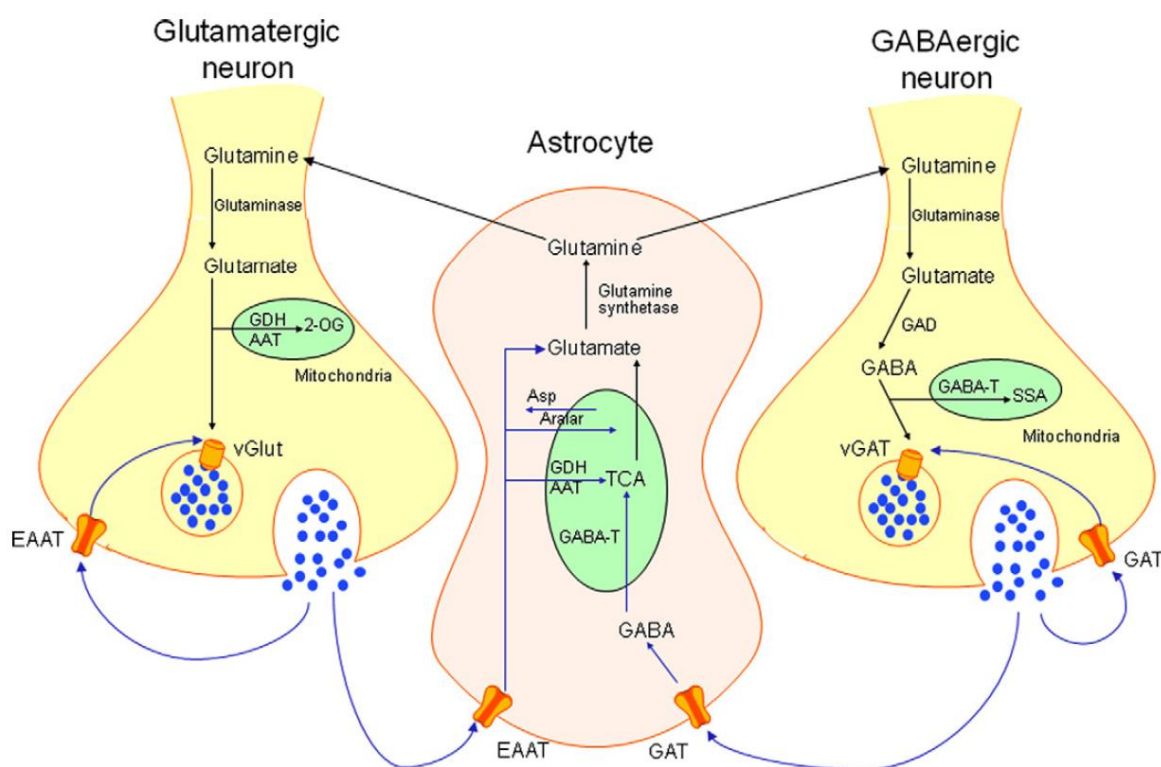
Também pode ser produzido a partir da transaminação do intermediário do ciclo do ácido tricarboxílico, 2-oxoglutarato, durante a produção de piruvato e/ou oxaloacetato (MCKENNA, 2007; NELSON & COX, 2014). Após sua liberação na fenda sináptica é recaptado por astrócitos onde pode ser metabolizado novamente em glutamina ao associar-se à amônia livre – que, em excesso, é tóxica para o SNC – através da glutamina sintetase (NELSON & COX, 2014). A glutamina em excesso é transportada para outros órgãos onde é novamente convertida em glutamato e amônio (NELSON & COX, 2014). O glutamato também pode dar origem à glutathione que atua como tampão redox ao remover peróxidos tóxicos, reação catalisada pela selenoproteína glutathione-peroxidase (GPx), uma enzima antioxidante que tem em sua composição, um átomo de selênio na forma de selênio-cisteína (NELSON & COX, 2014; PITTS et al., 2014).

Sabe-se que o neurotransmissor glutamato em concentrações elevadas está relacionado a diversos tipos de epilepsia assim como quadros de *status epilepticus* (LEVITE, 2014). Durante a neurotransmissão a concentração de glutamato na fenda sináptica aumenta e são os astrócitos os responsáveis por sua recaptação (PINTO et al., 2017). Na eventualidade do aumento excessivo do neurotransmissor, essa recaptação pode ser insuficiente e, portanto, provocar excitotoxicidade (MCKENNA, 2007) que, por sua vez, leva a dano neuronal irreversível e morte neuronal (LEVITE, 2014; SZCZUROWSKA; MARES, 2013).

A toxicidade do glutamato deve-se à hiperestimulação de seus receptores que provoca um aumento importante de cálcio intracelular que, por sua vez, ativa enzimas que danificam membranas, DNA, entre outras estruturas, levando os neurônios à morte (LEVITE, 2014). Dentre seus receptores ionotrópicos e metabotrópicos podemos citar os receptores N-metil-D-aspartato (NMDA), ácido alfa-amino-3-hidroxi-5-metil-4isoxazólico-propiónico (AMPA) e 2-carboxi-3-carboximetil-4-isopropenil-pirrolidina (Cainatos) (LEVITE, 2014). Os receptores NMDA estão distribuídos por todo o cérebro, especialmente no hipocampo e tálamo, e são encontrados nos astrócitos, na membrana pré-sináptica e, majoritariamente, na pós-sináptica dos neurônios (LEVITE, 2014; SZCZUROWSKA; MARES, 2013). Os receptores AMPA relacionam-se à neuroplasticidade e facilitam a abertura dos receptores NMDA através da redução do bloqueio  $Mg^{+2}$  dependente (LEVITE, 2014), enquanto os receptores cainatos

estão presentes em membranas pré e pós-sinápticas e podem levar à excitação ou inibição neuronal ao reduzir a liberação de GABA (LEVITE, 2014).

Estudos prévios identificaram em 30% dos pacientes com diferentes tipos de epilepsia a presença de autoanticorpos contra receptores AMPA que foram responsáveis pela ativação destes receptores e pela indução de excitotoxicidade *in vitro*, enquanto *in vivo* provocaram agravamento de crises epiléticas (LEVITE, 2014). Além disso observou-se que medicações agonistas NMDA provocavam crises convulsivas no início da vida de ratos e antagonistas tanto de receptores NMDA quanto AMPA eram capazes de suprimi-las (SZCZUROWSKA; MARES, 2013).



**Figura 1.** Síntese, armazenamento, liberação, transporte e metabolismo do glutamato e GABA. 2-OG: 2-oxoglutarato; AAT: aspartato aminotransferase; Aralar: carreador aspartato-glutamato; Asp: aspartato; EAAT: transportador de aminoácido excitatório; GABA-T: GABA transaminase; GAD: descarboxilase ácido glutâmico; GAT: transportador GABA; GDH: glutamato desidrogenase; SSA: succinil semialdeído; TCA: ciclo do ácido tricarboxílico; vGAT: transportador vesicular de GABA; vGlut: transportador vesicular de glutamato. Retirado de Rowley et al (2012).

O neurotransmissor GABA é sintetizado a partir do glutamato através da sua descarboxilação pela enzima descarboxilase ácido glutâmica (ROWLEY et al., 2012) (Figura 1). É degradado pela GABA-transaminase em succinil-semialdeído que, por sua vez, pode ser oxidado em succinato ou reduzido em ácido gama-OH-butírico (ROWLEY et al., 2012). Este neurotransmissor também age em receptores ionotrópicos e metabotrópicos que induzem hiperpolarização da membrana e inibição da atividade neuronal em algumas regiões cerebrais (DINUZZO et al., 2014).

Estudos mostraram relações entre síndromes epiléticas e o mau funcionamento de receptores GABA<sub>A</sub> (DINUZZO et al., 2014). Foram descritas alterações de RNAm de receptores GABA secundárias a crises convulsivas reduzindo inclusive a eficácia de medicações antiepiléticas com atuação nestes receptores (BROOKS-KAYAL et al., 1998) e também alterações em subunidades dos receptores prévias às crises que poderiam contribuir para o início da epilepsia (SCHOUSBOE et al., 2014). Além disso, há descrição de morte de interneurônios GABAérgicos inibitórios secundária ao aumento de sinapses excitatórias durante as crises convulsivas (SZCZUROWSKA; MARES, 2013) e de *downregulation* de receptores GABA provocada pela ativação de receptores NMDA (NAYLOR, 2014).

### 1.1.2. Epilepsia e estresse oxidativo

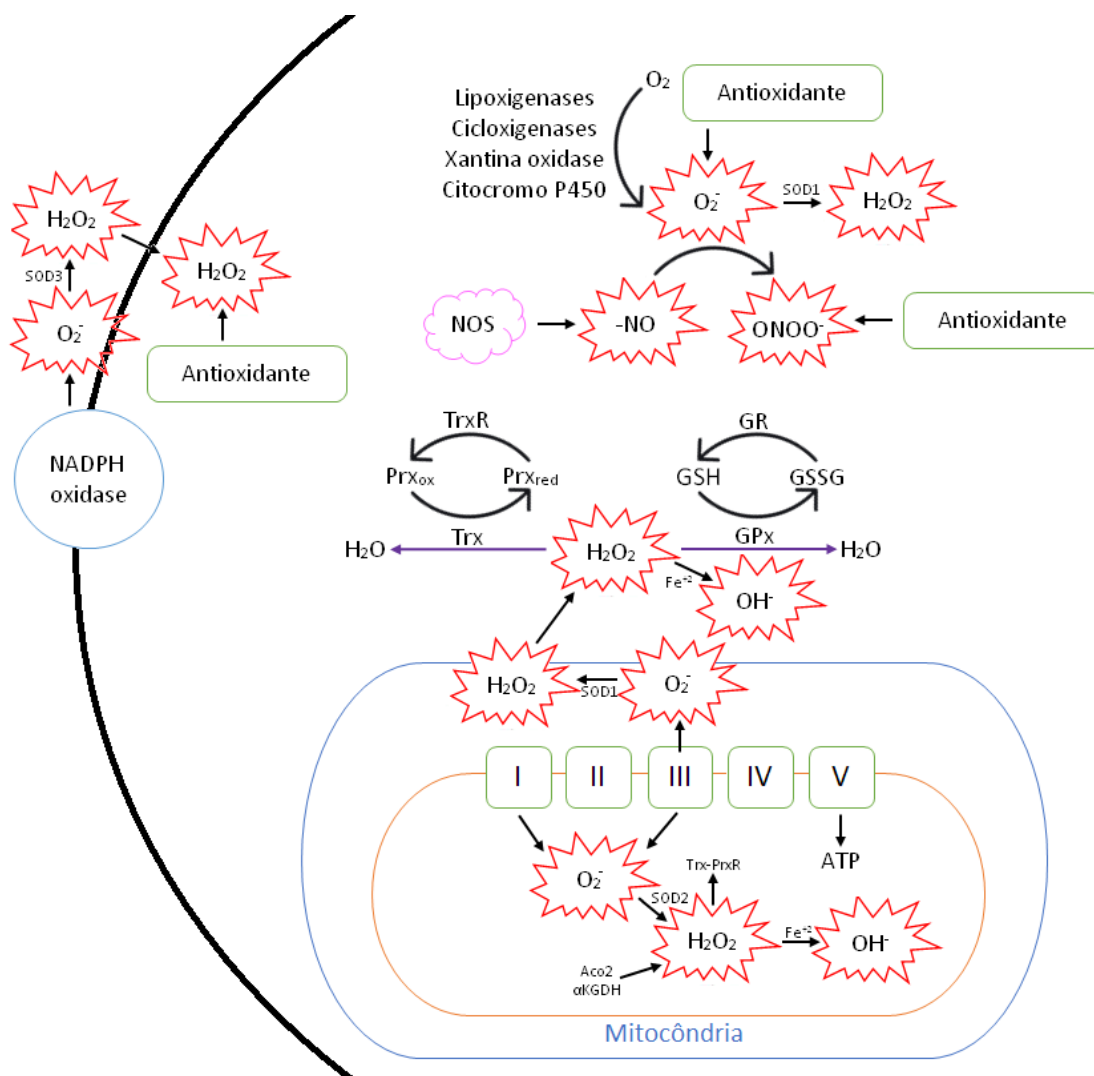
Radicais livres caracterizam-se por espécies com elétrons não pareados em sua superfície e, como exemplo, pode-se citar as espécies reativas de oxigênio (EROs) (PEARSON-SMITH; PATEL, 2017) que são produzidas tanto através do metabolismo aeróbico mitocondrial quanto por reações enzimáticas citoplasmáticas, podendo estas serem ativadas por crises convulsivas (PEARSON-SMITH; PATEL, 2017) (Figura 2). Células do SNC são bastante sensíveis aos efeitos do estresse oxidativo (EO) e estudos recentes têm mostrado uma relação entre EO, disfunção mitocondrial e morte neuronal em alguns tipos de epilepsia, assim como entre EO e a excitotoxicidade causada pelo glutamato (MENDEZ-ARMENTA et al., 2014). Seus receptores, através da atividade do Ca<sup>2+</sup>, permitem que a fosfolipase A2 libere ácido araquidônico que gera O<sup>2-</sup> através de seu metabolismo para formação de eicosanoides (MENDEZ-ARMENTA et al., 2014). O aumento do Ca<sup>2+</sup> intracelular também ativa enzimas como proteína-quinase C,

xantina-oxidase, fosfatases e fosfolipases que produzem EROs através do ácido araquidônico e, por fim, o aumento de  $\text{Ca}^{2+}$  na mitocôndria facilita a abertura do poro de transição dependente de  $\text{Ca}^{2+}$  que pode induzir à apoptose celular (MENDEZ-ARMENTA et al., 2014; SINGH; GROVER; PAL PATHAK, 2010). O estresse oxidativo ainda pode acometer a oligodendróglia e alterar a produção de mielina exercida por ela ao longo do desenvolvimento (PINTO et al., 2017).

Estudos prévios evidenciaram aumento do EO desencadeado por crises convulsivas em humanos e modelos animais associado a efeito citotóxico possivelmente secundário à lesão direta dos EROs a macromoléculas, à propagação da reação em cadeia de radicais livres de oxigênio (RLO), à oxidação do DNA e à peroxidação de lipídeos alterando a estrutura das membranas celulares (RUMIA et al., 2013), o que pode culminar em alterações de liberação e recaptação de neurotransmissores e da manutenção do equilíbrio hidroeletrolítico (PEARSON-SMITH; PATEL, 2017). O dano ao DNA causado pelo EO pode provocar erros de incorporação de bases nitrogenadas, ruptura de filamentos, alteração da metilação de bases, entre outras consequências (MENDEZ-ARMENTA et al., 2014). Quando esses danos acumulam-se superando a capacidade de reparação, ocorre a disfunção proteica seguida da disfunção celular e, finalmente, a apoptose (BHRIGU et al., 2012). Crowe *et al* (2011) mostrou que, após crises convulsivas repetidamente provocadas por ácido caínico, o cérebro de ratos mostrava aumento da ruptura de filamentos duplos de DNA (CROWE et al., 2011). Cantafora *et al* (2014), ao analisar células de hipocampo após indução de crises convulsivas focais límbicas com generalização secundária, também evidenciou aumento do dano de DNA (CANTAFORA et al., 2014). Em uma avaliação de tecido neurológico ressecado de pacientes com epilepsia refratária encontrou-se um aumento de 284% da 8-oxo-7,8-diidro-2-deoxiguanosina (um produto da oxidação de DNA) em relação aos controles provenientes de bancos de cérebros (RUMIA et al., 2013). Em pacientes com epilepsia refratária, a ressecção do foco epileptogênico provocou normalização de marcadores séricos de EO (FREY et al., 2017).

Portanto é possível que a excitotoxicidade presente durante as crises convulsivas, especialmente as prolongadas, aumente o EO e este contribua e/ou seja um dos grandes responsáveis pelo dano cerebral secundário à epilepsia e

evolução de muitos pacientes para quadros de atraso do DNPM evidenciados em várias síndromes epiléticas infantis.



**Figura 2.** Possíveis origens de espécies reativas no neurônio. NOS: óxido nítrico; GSH: glutatona reduzida; GR: glutatona redutase; GSSG: glutatona oxidada; GPx: glutatona peroxidase; SOD: superóxido dismutase; Trx: tioredoxina; TrxR: tioredoxina redutase; Prx<sub>ox</sub>: peroxirredoxina oxidada; Prx<sub>red</sub>: peroxirredoxina reduzida; Aco2: aconitase; A-KGDH: alfa-cetoglutarato desidrogenase

Adaptado de Pearson-Smith (2017).

### 1.1.3. Selênio e epilepsia

O Selênio (Se) é um elemento essencial para o corpo humano e seu papel em processos biológicos é conhecido desde a década de 50 (PITTS, 2014). Ele é convertido ao aminoácido selenocisteína (PILLAI; UYEHARA-LOCK; BELLINGER, 2014) que é incorporado ao grupo de enzimas antioxidantes glutatona peroxidases

(ROMANOWSKA, 2006), cujos principais componentes encontrados no SNC são a GPx-1 e GPx-4 (PILLAI; UYEHARA-LOCK; BELLINGER, 2014). A GPx-1 localiza-se nos neurônios e células da glia, enquanto a GPx-4, em condições normais, somente em neurônios e, em situações patológicas, também nos astrócitos (PILLAI; UYEHARA-LOCK; BELLINGER, 2014; PITTS et al., 2014). A GPx-4 encontra-se em maior quantidade em cérebros em desenvolvimento, reduzindo conforme o crescimento (SOLOVYEV, 2015) e atua utilizando hidroperóxidos fosfolipídicos, destruindo-os, além de suprimir a peroxidação lipídica (PILLAI; UYEHARA-LOCK; BELLINGER, 2014; SOLOVYEV, 2015) e alterar a sinalização intracelular, reduzindo a ação das lipoxidades e cicloxigenases, responsáveis pela geração de metabólitos do ácido araquidônico (ROMANOWSKA et al., 2007). Já a GPx-1 tem seu papel relacionado a peróxidos de hidrogênio e peróxidos orgânicos solúveis (ROMANOWSKA et al., 2007).

Também atua no SNC, entre outras, a família das selenoproteínas tiorredoxina redutases (TrxR) na redução de peróxido de hidrogênio e EO, além de nos mecanismos de controle da transcrição celular (PILLAI; UYEHARA-LOCK; BELLINGER, 2014). O Se ainda pode modular o influxo de  $Ca^{2+}$  e atuar especificamente contra a excitotoxicidade e na ativação da glia (SOLOVYEV, 2015).

A deficiência do elemento está relacionada à piora da função cognitiva e motora, além deste ter sido encontrado em menor quantidade em pacientes com doenças de Alzheimer, de Parkinson, Huntington e epilepsia (NEVITT et al., 2017), sendo sua reposição responsável pela redução de crises convulsivas em alguns indivíduos (FORCELLI et al., 2012; JOKEIT; KRAMER; EBNER, 2005). Foi evidenciado também que, em modelos animais, epilepsia refratária provocava aumento da peroxidação de lipídeos e a administração de Se concomitantemente a topiramato provocava sua redução e aumento de proteínas como as da família GPx (YUREKLI; NAZIROGLU, 2013). Outros estudos mostraram uma correlação inversa entre a concentração de Se e crises convulsivas febris e que, durante crises epiléticas intratáveis e idiopáticas, ocorria a redução sérica do elemento, podendo indicar seu consumo na tentativa de prevenir a excitotoxicidade (PILLAI; UYEHARA-LOCK; BELLINGER, 2014). Em modelos animais, ratos *knockout* para GPx-4 apresentaram maior risco de crises epiléticas no período neonatal (PILLAI;

UYEHARA-LOCK; BELLINGER, 2014). Pesquisas prévias foram capazes de corroborar funções antioxidantes advindas também de compostos organosselênicos (SHINGALAPUR et al., 2010; TAMIR; MECHOULAM; MEYER, 1980).

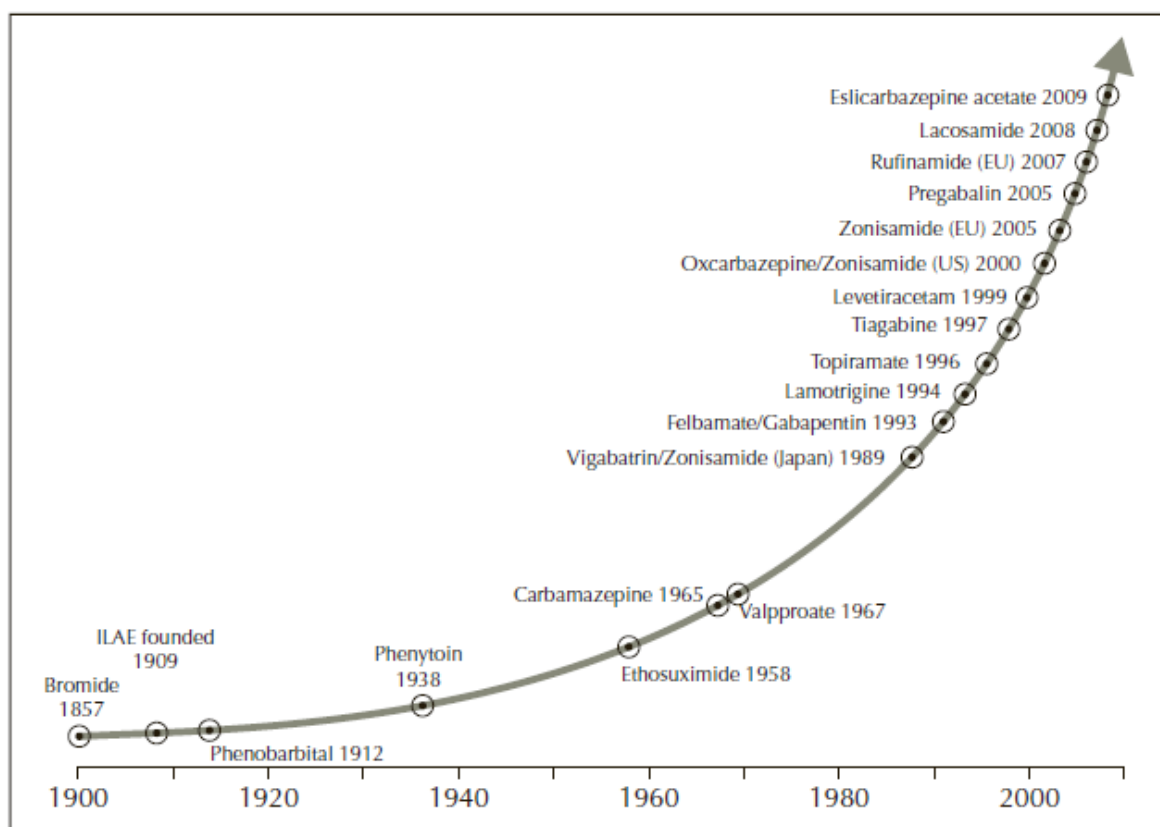
## **1.2. Desenvolvimento de novos antiepilépticos**

O tratamento farmacológico da epilepsia teve início em 1857, quando o brometo de potássio, possivelmente um agonista GABA (LOSCHER et al., 2013), foi utilizado com sucesso em pacientes do sexo feminino que apresentavam crises convulsivas (ARZIMANOGLU et al., 2010). Em 1912 foi descoberta atividade anticonvulsivante do fenobarbital e, posteriormente em 1937, a atividade da fenitoína, eficaz em mais pacientes e menos sedativa (ARZIMANOGLU et al., 2010). Em 1953 a carbamazepina deu início a uma nova geração de anticonvulsivantes e foi seguida pelo ácido valpróico em 1962 (ARZIMANOGLU et al., 2010). Desde então, vários novos fármacos antiepilépticos têm sido disponibilizados (Figura 3), entretanto não suficientes para controlar crises em cerca de 30% dos pacientes ou para reverter quadros resistentes à medicação, indicando pouco progresso nos últimos 50 anos (LOSCHER et al., 2013). Sabe-se também que o uso da maioria das medicações na população pediátrica ocorreu por extrapolação dos seus efeitos em adultos e que até por volta dos 2 anos de idade essa extrapolação, quando cuidadosa, pode ocorrer (WADSWORTH et al., 2016). Entretanto efeitos no crescimento, no desenvolvimento cognitivo, motor e de linguagem, tal qual efeitos na aprendizagem e comportamento são de extrema importância e não podem ser extraídos de estudos em uma população de adultos (WADSWORTH et al., 2016). Ademais, indivíduos recém-nascidos raramente são investigados em estudos pré-clínicos ou ensaios clínicos (WADSWORTH et al., 2016).

Testes para avaliação de novos antiepilépticos utilizam classicamente vários modelos animais de crises convulsivas para triagem pré-clínica (RHODEN; RHODEN, 2006; KEHNE et al., 2017), sendo o da administração de pentilenotetrazol (PTZ) considerado um dos de primeira linha, apesar do risco da produção de resultados falso-negativos (GOLYALA; KWAN, 2017; LOSCHER et al., 2013). O modelo de PTZ é utilizado desde 1944 para avaliação da resposta a

antiepilépticos e é considerado padrão para pesquisa de novos fármacos que atuem contra crises convulsivas mioclônicas e caracterizadas por padrão eletroencefalográfico de ponta-onda (DHIR; CHOPRA, 2015; GOLYALA; KWAN, 2017; RHODEN; RHODEN, 2006; PETERSON, 1998).

Löscher (2013) cita 3 estratégias pré-clínicas úteis para o desenvolvimento de novas medicações antiepilépticas: a triagem aleatória de novas moléculas de diferentes categorias estruturais, mas sem mecanismo de ação elucidado; a avaliação de moléculas cuja estrutura seja derivada de antiepilépticos já conhecidos; e o *design* de drogas baseado em hipóteses sobre sítio de ação. Entretanto, relata que poucos fármacos foram provenientes desta última estratégia de desenvolvimento baseado especificamente no alvo terapêutico (LOSCHER et al., 2013).



**Figura 3.** Linha do tempo de desenvolvimento de medicações antiepilépticas (Retirada de: Arzimanoglou et al (2010).

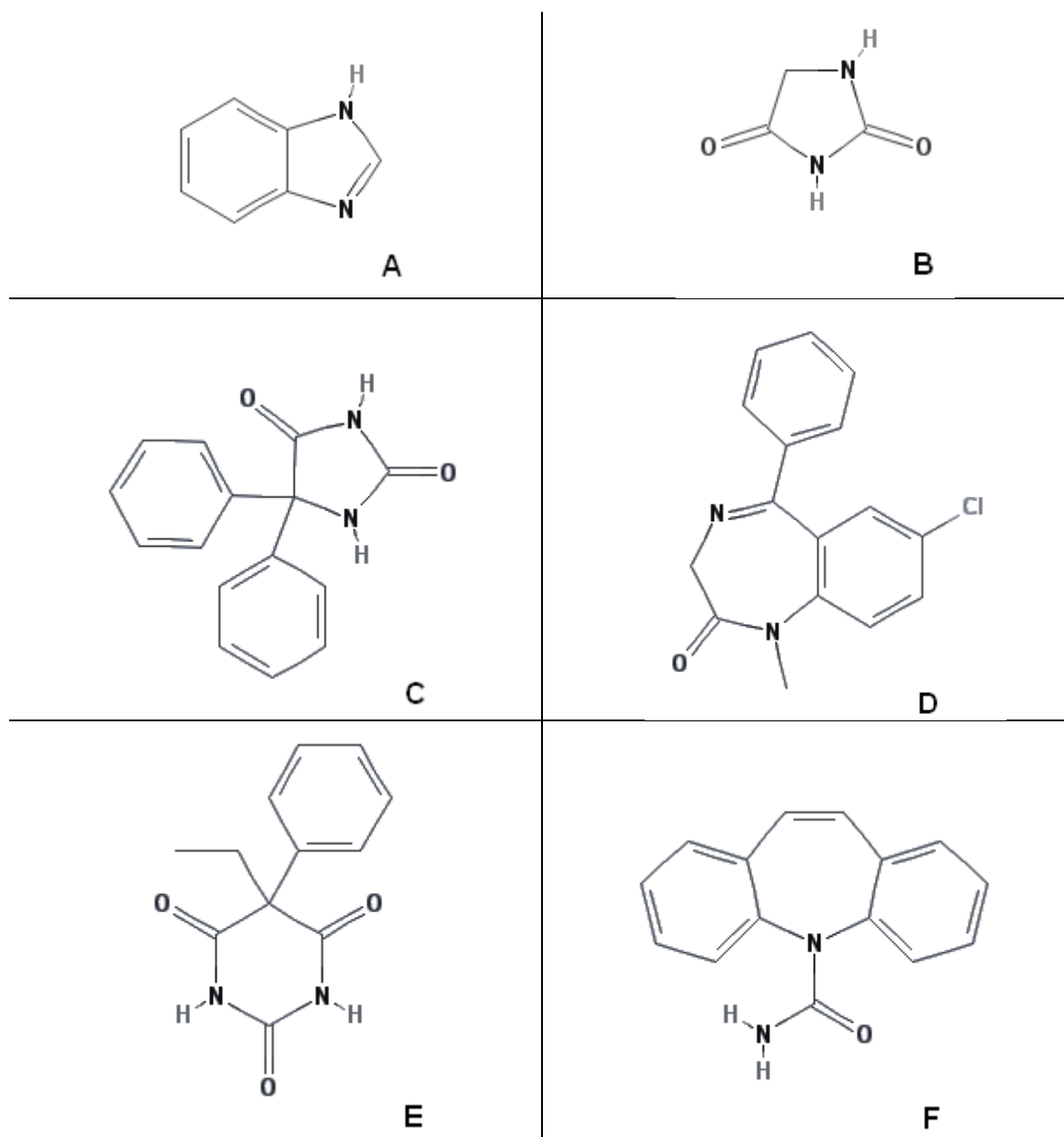
### 1.2.1. Derivados imidazóis e atividade antiepiléptica

A hidantoína (imidazolidina-2,4-diona) (Figura 4B), desde sua descoberta em 1861, tem se provado uma estrutura heterocíclica de grande importância farmacológica (BARVE, 2015). Em 1908 foi sintetizada pela primeira vez a difenil-hidantoína (2,4-diceto-tetra-hidro-imidazol) (Figura 4C), mais conhecida na prática médica como fenitoína (FNT), cujo primeiro uso clínico, citado previamente, foi descoberto em 1937 (SMITH, 1988). A atividade antiepiléptica da FNT deve-se essencialmente à presença de uma unidade hidrofóbica, um grupo doador de elétrons e um domínio de ligação com hidrogênio (SHINGALAPUR et al., 2010; TAMIR; MECHOULAM; MEYER, 1980) e sua estrutura química guarda semelhanças com outros fármacos de ação anticonvulsivante em decorrência da presença de anéis heterocíclicos nitrogenados como, por exemplo, o diazepam (Fig. 4D), fenobarbital (Fig. 4E), carbamazepina (Fig. 4F), entre outros (LOPEZ, 2011; BRUNTON, 2011). Seu mecanismo de ação não foi totalmente elucidado, mas foram descritas previamente ações de estabilização de membrana, alterações de canais iônicos e de transporte ativo (LOPEZ et al., 2011) que envolvem a redução de disparos de potenciais de ação evocados (BRUNTON, 2011). Há também relatos na literatura, em modelos animais, de aumento da conversão de glutamato e glutamina em GABA, elevação da concentração de GABA em SNC, aumento da condução sináptica inibitória e prolongamento dos potenciais pós-sinápticos inibitórios, além de alterações em outros neurotransmissores como norepinefrina, acetilcolina, serotonina e dopamina (SMITH, 1988).

Ao longo dos anos novas moléculas heterocíclicas contendo anéis benzimidazólicos – composto oriundo da fusão entre um benzeno e um anel imidazol com dois átomos de hidrogênio não adjacentes (SILVA, 2014) – foram descobertas e observou-se que algumas possuíam afinidade pelos receptores glutamatérgicos NMDA provocando sua inibição e decorrente proteção à excitotoxicidade, além de reduzir a morte neuronal *in vitro* (CHOPRA, 2004; WEE et al., 2010). Já outros compostos, por sua vez, foram eficazes em reduzir e até abortar crises convulsivas em modelos animais (BHRIGU et al., 2012; KALUSALINGAM et al., 2018).

As atividades biológicas observadas nas substâncias heterocíclicas também são decorrentes, em grande parte, dos compostos substituintes acoplados a seus

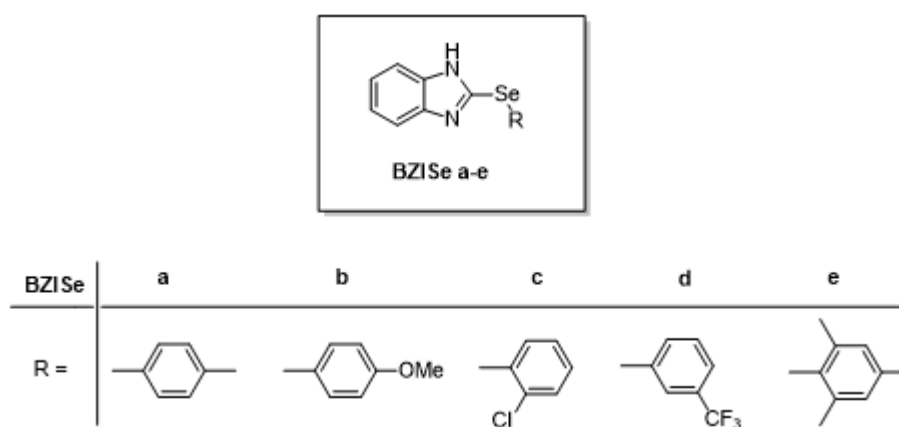
núcleos (SMIT et al., 2015). Nesse contexto cabe retomar o importante papel do Se como neuroprotetor e a necessidade de busca de novos fármacos que possuam tanto um efeito antiepiléptico quando de neuroproteção.



**Figura 4.** Estrutura química de: A) Anel benzimidazólico; B) Hidantoína; C) Fenitoína; D) Diazepam; E) Fenobarbital; F) Carbamazepina. Estruturas retiradas de PubChem – *Open Chemistry Database*.

No ano de 2014 foram sintetizados pelo grupo do professor Paulo H. Schneider, no Laboratório de Catálise Molecular da Universidade Federal do Rio Grande do Sul (LAMOCA-UFRGS) compostos benzimidazólicos inéditos contendo o calcogênio selênio (SILVA, 2014) e com grande potencial terapêutico.

Após revisão da literatura concluiu-se que seria possível que as moléculas imidazólicas desenvolvidas atuassem regulando a interação GABA-glutamato, além de modular a resposta imunológica e de hiperexcitabilidade em SNC levando ao controle de crises convulsivas em quadros de epilepsia. Portanto, propôs-se o estudo dos efeitos anticonvulsivantes dos novos compostos benzimidazólicos disponibilizados pelo LAMOCA-UFRGS (Figura 5) que contam com um átomo de selênio conectando o sistema benzimidazólico a substituintes ligados a um anel benzênico que possuem variância em relação à posição de ligação no anel e também à natureza (doador ou aceitor de elétrons), em modelo animal de epilepsia neonatal e de início precoce já consagrado na literatura.



**Figura 5.** Estrutura das moléculas benzimidazólicas disponibilizadas pelo LAMOCA-UFRGS para teste.

R: radical; Se: Selenio; BZlSe: Selenobenzimidazol; “a”: 2-(p-toluil-selenil)-1H-benzo[d]imidazol, “b”: 2-((4-metoxifenil)selenil)-1H-benzo[d]imidazol, “c”: 2-((2-clorofenil)selenil)-1H-benzo[d]imidazol, “d”: 2-((3-(trifluormetil)fenil)selenil)-1H-benzo[d]imidazol, “e”: 2-(mesitil-selenil)-1H-benzo[d]imidazol

### 1.2.2. Escolha das doses dos fármacos

Os derivados benzimidazólicos disponibilizados não foram avaliados em nenhum estudo prévio. Em revisão bibliográfica foram encontrados estudos que avaliaram outros derivados benzimidazólicos no controle de crises convulsivas, porém as doses foram bastante divergentes assim como o padrão de resposta e via de administração (Tabela 1). Além disso nenhuma pesquisa avaliou modelos neonatais, portanto as doses foram escolhidas visando formulação de curva dose-resposta com início em doses mais baixas em relação à literatura.

**Tabela 1.** Doses de derivados benzimidazólicos avaliados previamente para controle de crises convulsivas em modelos animais

| Referência                | Composto                                                                                        | Espécie | Doses (mg/kg) | Via de adm | Modelo animal | Grupo amostral |
|---------------------------|-------------------------------------------------------------------------------------------------|---------|---------------|------------|---------------|----------------|
| <b>Singh, 2010</b>        | Derivados 1,2,5-benzimidazóis trisubstituídos                                                   | Wistar* | 40            | i.p.       | PTZ           | N=6            |
| <b>Kalusalingam, 2010</b> | Derivados de 2-mercaptopbenzimidazol                                                            | Wistar* | 20            | i.p.       | MES           | N=6            |
| <b>Shaharyar, 2011</b>    | Derivados de 2-[2-(fenoximetil)-1H-benzimidazol-1-il]-N0-[(Z)-fenilmetilideno] acetil-hidrazida | -*      | 30, 100, 500  | i.p.       | PTZ e MES     | N=10           |
| <b>Bhrigu, 2012</b>       | 2-[(1-feniletilidina substituída) hidrazina]-N-fenil-1H-benzo[d]imidazol -1-carbo-tioamidas     | Wistar* | 30, 60, 100   | v.o        | PTZ e MES     | N=6            |
| <b>Shingalapur, 2010</b>  | 4-tiazolidinonas 5 e 1,3,4-oxadiazóis com 2-mercaptop benzimidazol                              | Wistar* | 20 - 85       | i.p.       | MES           | -              |

Adm: Administração. V.O: via oral. I.P: intraperitoneal. PTZ: Pentilenotetrazol. MES: máximo eletrochoque transcorneal. N: quantidade de sujeitos por grupo experimental. \*Adultos.

## 2. Justificativa

Considerando a necessidade de novos fármacos antiepilépticos que visem controle mais eficaz de crises convulsivas e que, simultaneamente, previnam os danos neuronais ocorridos em decorrência do quadro e, considerando a falta de estudos de fármacos destinados à população pediátrica, especialmente neonatal, se faz imperativa a triagem de novos fármacos em modelos pré-clínicos neonatais de epilepsia que possam contribuir para as duas áreas supracitadas: tratamento da epilepsia e neuroproteção.

O presente estudo também cogitou a possibilidade de que as novas moléculas benzimidazólicas atuem em situações de hiperexcitabilidade e modulação tanto da resposta imune em SNC quanto da excitação neuronal e, portanto, possam favorecer o controle de crises convulsivas em quadros de epilepsia.

A proposta de investigação é original e não encontrada em revisão da literatura realizada, posto que, além de fazer uso de moléculas inéditas, poucos estudos foram encontrados com modelos animais não adultos.

A evidência do benefício terapêutico nesta pesquisa permitirá possibilidades de ensaios garantindo novas direções para o tratamento de síndromes epiléticas em humanos que venham a interferir na morbidade dos indivíduos, assim como melhorar a qualidade de vida.

### **3. Objetivos**

#### **3.1. Objetivo geral**

Avaliar a eficácia de novas moléculas benzimidazólicas em modelo animal de epilepsia neonatal.

#### **3.2. Objetivos específicos**

- Avaliar a latência para início das crises no modelo de pentilenotetrazol de epilepsia neonatal e comparar em relação aos grupos controles positivo (FNT) e negativo (NaCl0,9%) frente às novas moléculas benzimidazólicas;
- Avaliar a gravidade das crises apresentadas pelos indivíduos e comparar em relação aos grupos controles positivo (FNT) e negativo (NaCl0,9%) frente às novas moléculas benzimidazólicas.

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## 5. Artigo científico

**Title:** Antiepileptic properties of new selenobenzimidazole derivatives: a pre-clinical study in neonatal model of epilepsy

**Running title:** Selenobenzimidazole derivatives as new antiepileptic drugs.

**Authors:** Fernanda Feuerharmel, MD<sup>1,2</sup>; Rodrigo Borges da Silva, MSc<sup>3</sup>; Paulo Henrique Schneider PhD<sup>3</sup>; Juliana Maria Forain Miolo Schneider, PhD<sup>4</sup>; Helena Tannhauser Barros MD, PhD<sup>1</sup>.

1 – Laboratory of Neuropsychopharmacology, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil.

2 – Department of Neurology, *Santa Casa de Misericórdia* Hospital, Porto Alegre, RS, Brazil.

3 – Laboratory of Molecular Catalysis, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

4 – Department of Pharmacosciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil.

### Corresponding author:

Dr. Fernanda Feuerharmel, MD.

Laboratory of Neuropsychopharmacology, Department of Pharmacology, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil.

Street Sarmiento Leite, number 245, Zipcode: 90050-170, Porto Alegre, RS, Brazil.

Phone: +55 51 3303-8793 or 3303-9000

Email: fer\_feuer@hotmail.com

**Abstract:**

**Background:** Epilepsy is a common neurologic disorder in childhood and is associated to neurological damage and developmental impairments. Current treatments are not able to completely control seizures, therefore the research for new drugs is needed. Benzimidazole molecules and its substituents are a potential anticonvulsant target for discovering new drugs. **Objective:** We aim to investigate the antiepileptic effect of new selenobenzimidazoles compounds in comparison to phenytoin in neonatal seizures model. **Methods:** Seizures were induced in 132 Wistar rats between 10 and 15 days with pentylenetetrazol 30 minutes before administration of the new compounds. They were evaluated throughout 60 minutes for seizure latency and severity. **Results:** 4 of the 5 drugs showed a positive correlation between dose and latency enhance. The dose of 8mg/kg of the compound 2-(2-chlorobenzylselenyl)-1*H*-benzo[d]imidazole showed significant difference between test group and negative control group and no difference in comparison to phenytoin group. The compound 2-((3-(trifluoromethyl)phenyl)selenyl)-1*H*-benzo[d]imidazole showed a significant reduction of severe seizures in comparison to negative control as well as a 100% reduction of the duration of these seizures.

**Conclusion:** These new selenobenzimidazoles compounds may be a new field of research for antiepileptic drugs development, especially because its selenium substituent, which may provide antioxidative properties to the molecule.

**Keywords:** Epilepsy, Neonatal Seizures, Benzimidazole, Selenium.

## Introduction

Epilepsy is among the most common chronic neurologic disorders of childhood affecting about 1% of children and presenting with the highest incidence rate during infancy (Aaberg *et al*, 2017; Russ *et al*, 2012). Seizures affects 1-5:1000 live births, especially the ones with less than 1500g (Donovan *et al*, 2016) and may provoke great neurological and other physical impairments (Chen *et al*, 2017; Strzelczyk *et al*, 2017). Furthermore, children with seizures are at increased risk for developmental and behavioral comorbidities, increasing needs for specialized health care (Russ *et al*, 2012). Around 10% of children with epilepsy present intractable seizures, which increase the risk of sudden death (Agarwal and Fox, 2018). Considering the above, new treatment for epilepsy, especially in neonatal epilepsy are needed.

Benzimidazoles are well known heterocyclic compounds with a large range of uses (Bansal *et al*, 2017). Another example of heterocyclic compound is phenytoin (**Figure 1A**), that has its antiepileptic activity partially explained by the presence of a hydrophobic unit and a hydrogen-bonding domain (Shingalapur *et al*, 2010) as well as other benzimidazoles derivatives (Shingalapur *et al*, 2010). It was demonstrated that similar heterocyclic molecules present affinity for NMDA receptors, inhibiting them and therefore the glutamate provoked excitotoxicity (Fuchigami *et al*, 2010; Wee *et al*, 2010). The biological activity of the heterocycles can be also explained by the substituents attached to it (Smit *et al*, 2015), such as selenium that has demonstrated, as an organoselenium compound, antioxidant activity (Azad and Tomar, 2014).

Current pharmacological treatments may be associated with morbidity (Nevitt *et al*, 2017) and are ineffective for around 30% of the patients (DiNuzzo *et al*, 2014). These are some of the reasons why new drugs need to be developed. The aim of this study was to investigate the anti-epileptic effect of five new heterocyclic compounds developed by the Laboratory of Molecular Catalysis at Federal University of Rio Grande do Sul (LAMOCA-UFRGS), characterized by a benzimidazole ring binded to a benzene through selenium (**Figure 1B**).

## Material and Methods

Seizures were elicited in 132 Wistar albino rats with pentylenetetrazol (PTZ, Sigma, St. Louis, MO, USA). They were kept in optimum environmental conditions under a 12h/12h alternating light/dark cycle, from 22 to 24 °C of temperature, with food and water *ad libitum*. All efforts were made to minimize the number of animals used and reduce their suffering. All animals that persisted with epileptic activity at the end of the experiment were treated with phenytoin 20mg/kg. The experimental procedures were in accordance with ethical principles of the Brazilian College of Animal Experimentation (COBEA) and were approved by the local Ethics Commission on Animal Experimentation of Federal University of Health Sciences of Porto Alegre. The heterocyclic compounds were kindly provided by IC-UFRGS and prepared in a solution with DMSO (2mg/mL).

Animals in postnatal date (PD) between 10 and 15 (day of birth=0) were divided in 7 groups and injected with the five new compounds *via* intraperitoneal (ip) route at the doses of 1mg/kg (n=6), 2mg/kg (n=6), 4mg/kg (n=6) and 8mg/kg (n=6) for each substance; NaCl 0,9%(n=6) as a negative control and phenytoin (n=6; 20mg/kg) as positive control. After 30 minutes were injected with pentylenetetrazol (PTZ; 60 mg/kg) ip to induce seizures and noticed for a period of 60 minutes according to the works of Dhir and Chopra (2015) and Velisek *et al* (1992). Pilot study was made and the dose of 60mg/kg was chosen instead of 80mg/kg of PTZ, because of the high percentage of mortality. Evaluation of seizure latency started immediately after PTZ administration up to the star of seizures. After that we evaluated each animal every 5 minutes for 30 seconds for seizure characterization and duration. Seizure latency was evaluated with Kruskal-Wallis chi-squared test followed by a multiple comparison test with Wilcoxon rank sum test with continuity correction ( $p<0,05$ ). We also performed the correlation with Spearman's rank test and checked the normality of data with Shapiro-Wilk test. We evaluated, using Fisher's exact test, the presence or not of severe seizures among the test's groups and negative control (NaCl0,9%). The characterization of severity was made according to Klioueva *et al* (2001): 0 - no change in behavior; 0.5 - atypical behavior; 1 - isolated myoclonic jerks, ear and facial twitching; 2 - atypical minimal seizures, convulsive wave through the body; 3 - fully developed minimal seizures, clonus of the head muscles and forelimbs; 4 - major seizures (generalized without the tonic

phase); 5 – generalized tonic-clonic seizures (**Table 1**). Severe seizure was defined by the presence of either major seizures or generalized tonic-clonic seizures.

## Results

In the analysis of latency time to seizures of the five new compounds, Kruskal Wallis showed significant difference for all of them. However, after post hoc correction for multiple comparison, only the drug 2-(2-chlorobenzylselenenyl)-1*H*-benzo[d]imidazole (**Figure 1B.2**) in dose 8mg/kg remained significant when compared to negative control ( $p = 0.01$ ) and, as expected, the same happened with phenytoin ( $p = 0.02$ ). There was no significant difference of latency time of this drug in dose 8mg/kg when compared to phenytoin ( $p > 0.5$ ). The other 4 compounds showed no significant difference between doses and negative control for latency to seizures after post hoc. These results are summarized in **Figure 2** and **Supplementary Table 1**.

Despite that, the Spearman's test indicated a positive correlation between the doses of 4 drugs and the enhance of latency, especially for drug 2-(2-chlorobenzylselenenyl)-1*H*-benzo[d]imidazole ( $\rho = 0.73$ ,  $p < 0,0001$ ). All correlations are summarized in **Figure 3**.

We were also able to find a difference among the presence of severe seizures between negative control and 2-((3-(trifluoromethyl)phenyl)selenenyl)-1*H*-benzo[d]imidazole (**Figure 1B.4**) at the dose of 1mg/kg ( $p = 0.015$ ). The results are shown in **Figure 4**. And, when comparing the duration of severe seizures, there was a reduction of 38.51% between the 2-(mesitylselenenyl)-1*H*-benzo[d]imidazole (**Figure 1B.1**) at the dose of 8mg/kg (166s) and phenytoin (270s) as well as a reduction of 100% between 2-((3-(trifluoromethyl)phenyl)selenenyl)-1*H*-benzo[d]imidazole at the dose of 1mg/kg (0s) and phenytoin (270s).

## Discussion

Our results show that the compound 2-(2-chlorobenzylselenenyl)-1*H*-benzo[d]imidazole at the dose of 8mg/kg was able to change the latency for seizures when compared to negative control group in the neonatal epilepsy model. The dose did not have significant difference comparing to phenytoin. We were also able to

find a positive correlation between dose and seizure latency for four of the five drugs tested. There was also a difference on the number of individuals that presented severe seizures, receiving 1mg/kg of 2-((3-(trifluoromethyl)phenyl)selenyl)-1*H*-benzo[d]imidazole when comparing to negative control. These individuals also presented a wide decrease on seizures duration as well as did the ones receiving 2-(mesitylselenyl)-1*H*-benzo[d]imidazole at the dose of 1mg/kg when comparing to the phenytoin group. According to the International League Against Epilepsy, these results already show superiority of efficacy/effectiveness in comparison to phenytoin, since the seizures reduction was higher than 20% (Glauser *et al*, 2013). Our results are in line with previous evidence of antiepileptic effect of benzimidazoles compounds (Bhrigu *et al*, 2012) and are reinforced by the robust evidence of phenytoin (Nevitt *et al*, 2017). Moreover, this is the first work describing the evidence of antiepileptic properties of selenobenzimidazoles derivatives in a neonatal model of epilepsy.

Despite the evidence discussed above, it is known that phenytoin has important and life threatening adverse effects, such as Stevens-Johnson syndrome and toxic epidermal necrolysis (Frey *et al*, 2017), megaloblastic anemia (Nevitt *et al*, 2017), as well as motor and memory impairment (Forcelli *et al*, 2012; Jokeit *et al*, 2005), and augmented oxidative stress (OS) (Winn and Wells, 1995).

The most accepted origin for seizures are the imbalance between glutamate and GABA (DiNuzzo *et al*, 2014). The excitotoxicity generated by glutamate excess can lead to irreversible neuronal damage (Levite, 2014). It is caused in part, through the rise of intracellular  $\text{Ca}^{2+}$  which allows the phospholipase A2 to release arachidonic acid that will generate  $\text{O}_2^-$  radicals during its metabolism for production of eicosanoids (Mendez-Armenta *et al*, 2014). This is the main reason antiepileptic drugs should have not only the anticonvulsant effect but also a neuroprotective role, especially antioxidant properties.

Our drug, carrying a nitrogen-containing heterocyclic system such as phenytoin, probably elicited the anticonvulsant effect due to reduction in the opening of the voltage gated  $\text{Na}^+$  channels that modulate the action potential. This effect is possibly related to the hydrophobic unit present in benzimidazole ring (Shingalapur *et al*, 2010; Tamir *et al*, 1980) and the presence of a carbon-chloro bond that may enhance the affinity for its biological target (Scholfield *et al*, 2013). The results could

also be partially explained by a trifluoromethyl group binded to a benzene ring which is common in other psychotropic medications as fluoxetine, fluvoxamine and halothane (PubChem) and may provide more molecular stability (Gakh and Shermolovich, 2014).

It is noteworthy that our compounds also have a selenium atom in its structures. Selenium containing organic molecules act as antioxidants through enzymatic reactions responsible for reducing peroxide radicals especially in central nervous system (Naziroglu and Yurekli, 2013). Previous studies showed antioxidant roles from organoselenium compounds (Saluk *et al*, 2013). Moreover, the deficiency of selenium is related to impairment of cognitive and motor function and its low concentration is associated not only with epilepsy, but also with other chronic neurologic disorders such as Alzheimer disease and Parkinson disease (Pillai *et al*, 2014). O que pode ser de interesse em relação à proteção de neurônios lesionados na epilepsia. In addition, its replacement was reported to be responsible for seizures reduction (Pillai *et al*, 2014).

Since the discovery of the antiepileptic role of valproic acid, new drugs have been developed, however insufficient for seizure control in almost 30% of patients and still incapable of reversing drug-resistant epilepsy (Löscher; Klitgaard; Twyman & Schmidt, 2013). It is known also that the use of most drugs in pediatric population is made by extrapolation of efficacy on adults even though the impossibility of evaluation of effects on development (Wadsworth *et al*, 2016). Furthermore, newborns rarely are investigated in preclinical or clinical trials (Wadsworth *et al*, 2016). That's why we suggest that preclinical investigation on potential antiepileptic drugs encompass also newborns and infants' animal models.

Our study has some limitations. First, we did not perform a prospective evaluation to measure long term side effects; second, we did not perform biochemical and histological evaluations to investigate neuronal protection and other molecular alterations. Notwithstanding these limitations, the current study has shed light on a promise field of new pharmacological targets to epilepsy treatment. Therefore, further studies should replicate these findings in larger sample sizes, with different tests and in longitudinal designs. Also, we suggest a higher range of doses to be tested, since lower ones did not produce a significant result, despite the

evidence of a positive correlation. Besides that, the potential antioxidant effect and neuroprotective role of this drug need to be studied.

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The authors claim no financial support or conflict of interest

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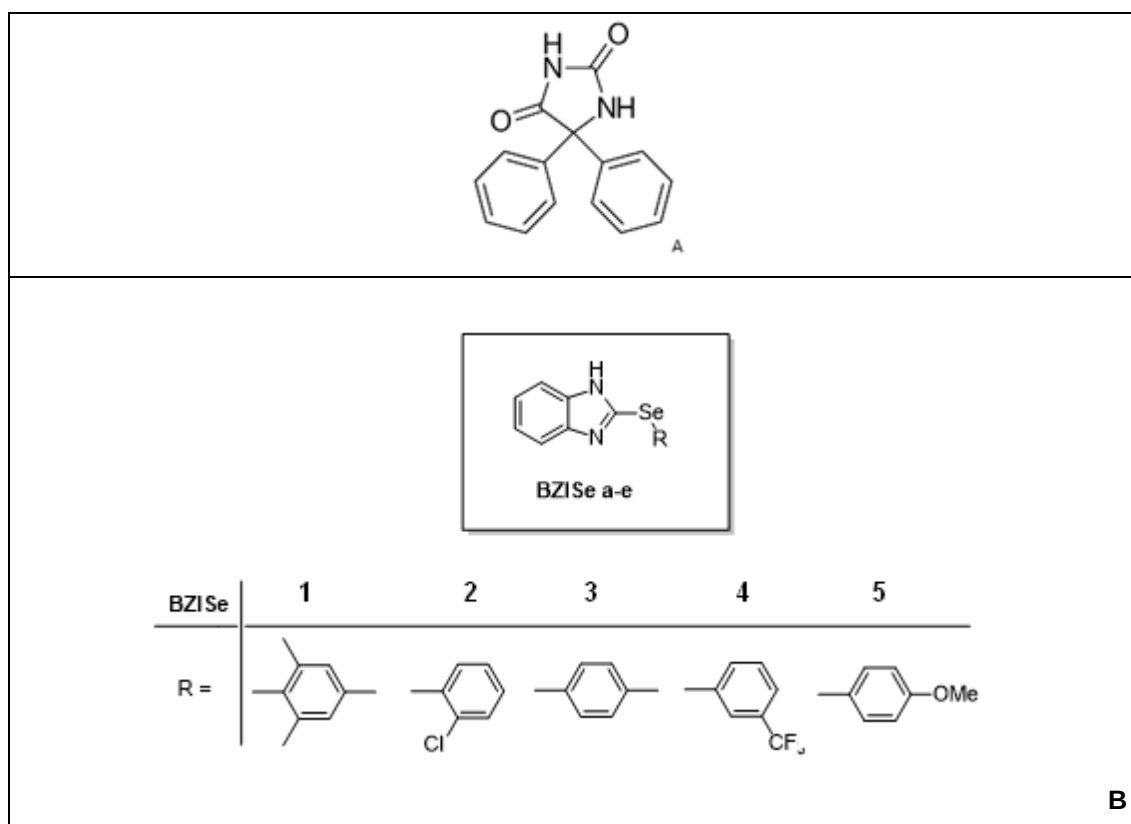
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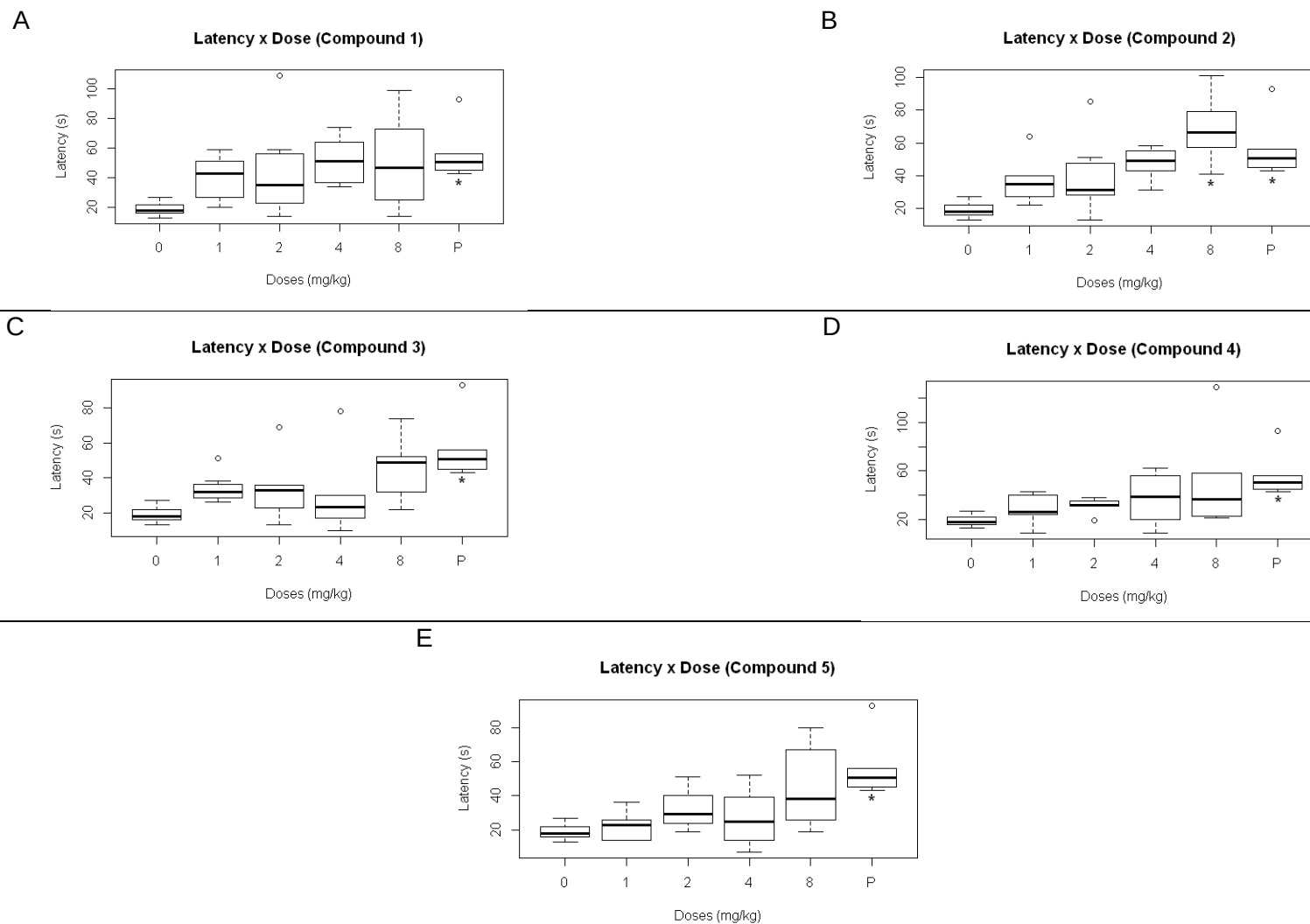
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## Figures

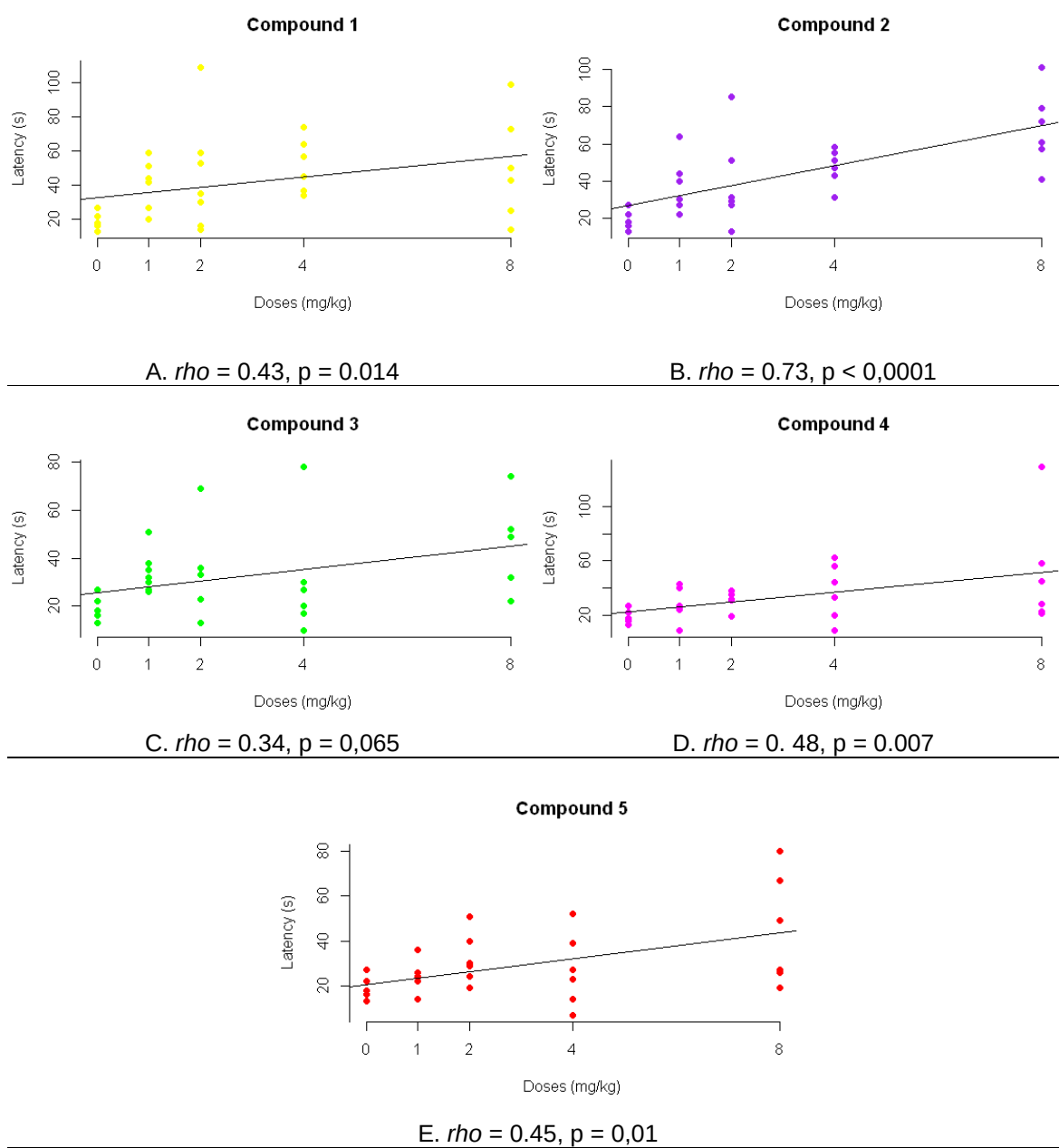


**Figure 1.** A: Phenytoin structure. B: Chemical structure of five benzimidazole compounds provided by the Institute of Chemistry at Federal University of Rio Grande do Sul

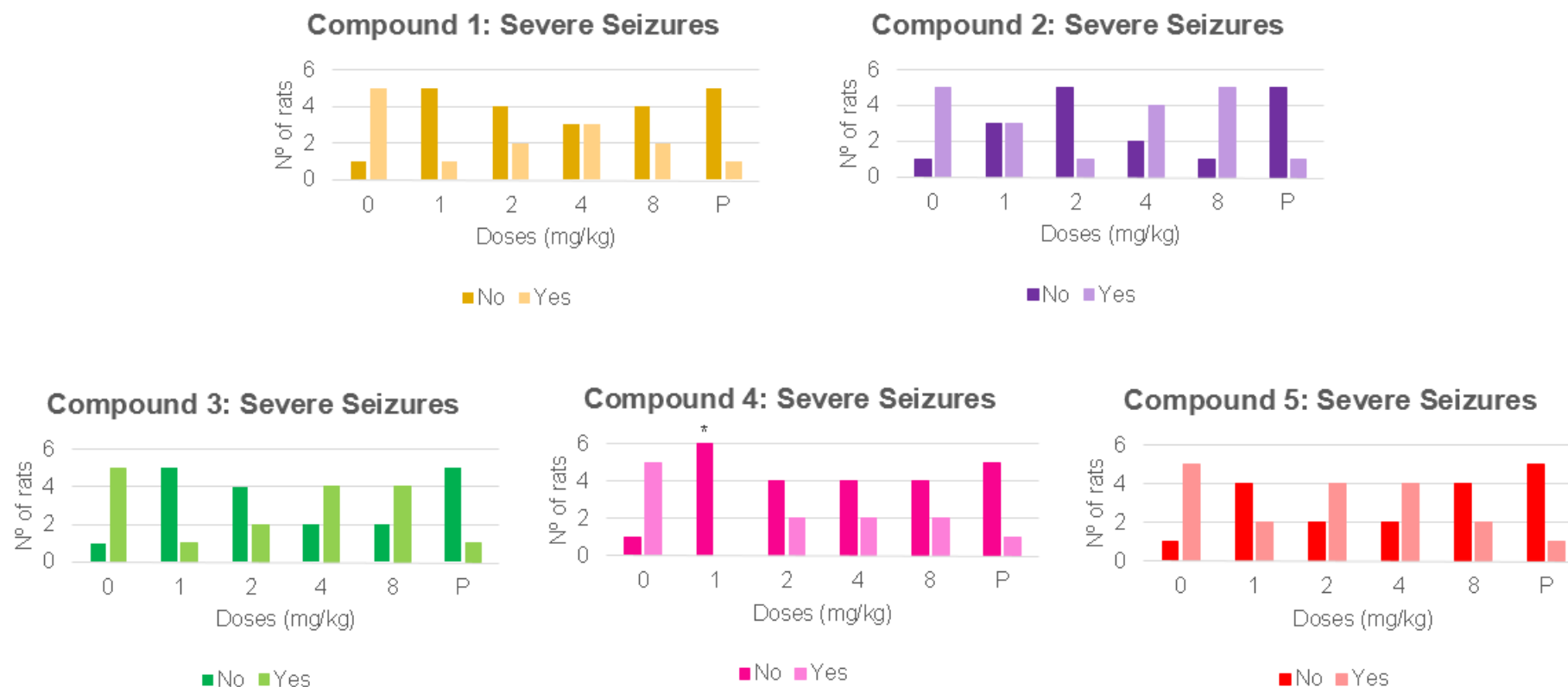
R: Radical. Se: Selenium. BZlSe: Selenobenzimidazole. 1: 2-(mesitylselenyl)-1*H*-benzo[d]imidazole, 2: 2-(2-chlorobenzylselenyl)-1*H*-benzo[d]imidazole, 3: 2-(*p*-tolueneselenyl)-1*H*-benzo [d] imidazole, 4: 2-((3-(trifluoromethyl)phenyl)selenyl)-1*H*-benzo[d]imidazole, 5: 2 - ((4-methoxyphenyl) selenyl) -1*H*-benzo [d] imidazole



**Figure 2.** Boxplots of the tested compounds. \* Remained significant after post hoc. P: Phenytoin (20mg/kg); 0: negative control. 1: 2-(mesitylselenyl)-1*H*-benzo[d]imidazole, 2: 2-(2-chlorobenzylselenyl)-1*H*-benzo[d]imidazole, 3: 2-(p-tolueneselenyl)-1*H*-benzo[d]imidazole, 4: 2-((3-(trifluoromethyl)phenyl)selenyl)-1*H*-benzo[d]imidazole, 5: 2-((4-methoxyphenyl)selenyl)-1*H*-benzo[d]imidazole. Latency given in seconds.



**Figure 3.** Correlation between doses and latency. 0: negative control. 1: 2-(mesitylselenyl)-1H-benzo[d]imidazole, 2: 2-(2-chlorobenzylselenyl)-1H-benzo[d]imidazole, 3: 2-(p-tolueneselenyl)-1H-benzo[d]imidazole, 4: 2-((3-(trifluoromethyl)phenyl)selenyl)-1H-benzo[d]imidazole, 5: 2-((4-methoxyphenyl)selenyl)-1H-benzo[d]imidazole



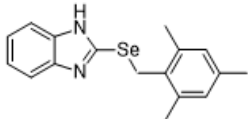
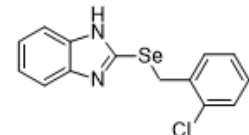
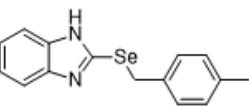
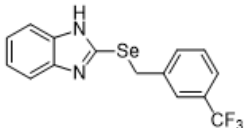
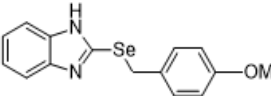
**Figure 4.** Number of animals with severe seizures during experiment. \*Significant after Fisher's test in comparison to negative control ( $p = 0,015$ ). P: Phenytoin (20mg/kg). 1: 2-(mesitylselenenyl)-1H-benzo[d]imidazole, 2: 2-(2-chlorobenzylselenenyl)-1H-benzo[d]imidazole, 3: 2-(p-tolueneselenenyl)-1H-benzo[d]imidazole, 4: 2-((3-(trifluoromethyl)phenyl)selenenyl)-1H-benzo[d]imidazole, 5: 2-((4-methoxyphenyl)selenenyl)-1H-benzo[d]imidazole

## Tables

| Score | Behavior                                                                                                               | Severity          |
|-------|------------------------------------------------------------------------------------------------------------------------|-------------------|
| 0     | No changes of behavior                                                                                                 | -                 |
| 0,5   | Atypical behavior                                                                                                      | Mild seizures     |
| 1     | Isolated myoclonic jerks, ear and facial twitching                                                                     |                   |
| 2     | Atypical minimal seizures, convulsive wave through the body                                                            | Moderate seizures |
| 3     | Fully developed minimal seizures, clonus of the head muscles and forelimbs, righting reflex was present                |                   |
| 4     | Major seizures (generalized without the tonic phase)                                                                   | Severe Seizures   |
| 5     | Generalized tonic-clonic seizures began with running, followed by the lost of righting ability, then short tonic phase |                   |

**Table 1.** Evaluation score according to Klioueva *et al* (2001) and severity graduation used on Fisher's exact test

## Supplementary material

| Drug                                                                                | Latency – Median (IQR) |             |             |                  | Kruskal-Wallis  |           |          |    |         |
|-------------------------------------------------------------------------------------|------------------------|-------------|-------------|------------------|-----------------|-----------|----------|----|---------|
|                                                                                     | 1mg/kg                 | 2mg/kg      | 4mg/kg      | 8mg/kg           | Phenytoin       | NaCl 0,9% | $\chi^2$ | df | P       |
| 1                                                                                   |                        |             |             |                  |                 |           |          |    |         |
|    | 43(18.5)               | 35(33)      | 51(23.25)   | 46.5(37.75)      | 50.5(10.5)<br># | 18(4.5)   | 11.85    | 5  | 0.03    |
| 2                                                                                   |                        |             |             |                  |                 |           |          |    |         |
|    | 40(13.5)               | 30(18.5)    | 49(10)      | 66.5(19.25)<br># | 50.5(10.5)<br># | 18(4.5)   | 20.593   | 5  | < 0.001 |
| 3                                                                                   |                        |             |             |                  |                 |           |          |    |         |
|    | 32(7.5)                | 33(13)      | 23.5(11.5)  | 49(20)           | 50.5(10.5)<br># | 18(4.5)   | 15.875   | 5  | 0.007   |
| 4                                                                                   |                        |             |             |                  |                 |           |          |    |         |
|   | 26(12.5)               | 32(4)       | 38.5(29.75) | 36.5(30.5)       | 50.5(10.5)<br># | 18(4.5)   | 1.912    | 5  | 0.01    |
| 5                                                                                   |                        |             |             |                  |                 |           |          |    |         |
|  | 23(9.5)                | 29,5(12.25) | 25(19.75)   | 38(36.25)        | 50.5(10.5)<br># | 18(4.5)   | 16.934   | 5  | 0.004   |

**Supplementary table 1.** The table shows the result from Kruskal-Wallis of 5 benzimidazole derivatives evaluated in 4 different doses when compared to phenytoin and negative control (NaCl 0,9%). # = Remained statistically significant after post hoc with multiple comparisons. IQR = Interquartile range

## 6. Conclusão

Nossa pesquisa evidenciou que o composto 2-(2-clorobenzilselenil)-1*H*-benzo[d]imidazol mostrou-se eficaz em elevar o tempo de latência para início das crises no modelo de PTZ em relação ao grupo controle negativo e que não houve diferença estatisticamente significativa entre o fármaco e a fenitoína utilizada como controle positivo. Também encontramos uma correlação positiva entre o aumento da dose e o aumento do tempo de latência tanto para o fármaco em questão quanto para outros 3 avaliados dentre os 5 testados ao total. Em relação à gravidade das crises, pudemos observar que houve uma menor incidência de crises graves no grupo referente ao composto 2-((3-(trifluormetil)fenil)selenil)-1*H*-benzo[d]imidazol na dose de 1mg/kg e que houve redução de mais de 20% do tempo de duração das mesmas nos compostos 2-(mesitilselenil)-1*H*-benzo[d]imidazol na dose de 8mg/kg e 2-((3-(trifluormetil)fenil)selenil)-1*H*-benzo[d]imidazol na dose de 1mg/kg. Resultados estes que são compatíveis com estudos encontrados previamente mostrando o efeito antiepiléptico de compostos benzimidazólicos e também com o conhecido efeito da fenitoína como medicação anticonvulsivante.

Apesar da bem documentada eficácia da fenitoína, sabe-se que esta pode cursar com efeitos adversos graves e também que outras medicações desenvolvidas até o momento não foram capazes de tratar crises em uma considerável parcela dos indivíduos. Portanto, novos fármacos que proporcionem uma eficácia antiepiléptica maior associada à neuroproteção são necessários, seja para tratar e prevenir os efeitos deletérios das crises convulsivas em SNC ou para evitar os efeitos indesejados adversos das drogas já existentes, como, entre eles, o atraso do DNPM.

Os compostos 2-(2-clorobenzilselenil)-1*H*-benzo[d]imidazol, 2-((3-(trifluormetil)fenil)selenil)-1*H*-benzo[d]imidazol e 2-(mesitilselenil)-1*H*-benzo[d]imidazol, sendo derivados benzimidazólicos que guardam semelhanças com a fenitoína, podem ter provocado um efeito anticonvulsivante em decorrência da redução da abertura dos canais de Na<sup>+</sup> voltagem-dependente que modulam o potencial de ação. Esse efeito está possivelmente relacionado à unidade hidrofóbica presente no anel benzimidazólico. Entretanto, podemos citar também a presença da ligação Carbono-Cloro e Carbono-Flúor. A ligação entre um carbono

e halogênios, possuidores de alta reatividade, pode gerar um potencial eletrostático positivo oposto à ligação covalente ocorrida entre os 2 átomos, facilitando sua ligação com outros átomos como oxigênio e nitrogênio, o que poderia aumentar a sua afinidade pelo alvo biológico, além de garantir maior estabilidade à molécula.

Não podemos ignorar também a presença do Selênio (Se) como um ligante dos fármacos e seu potencial antioxidante. O Se, na forma de selênio-cisteína, é um dos componentes essenciais da enzima glutathione-peroxidase que catalisa a remoção de peróxidos tóxicos provenientes do metabolismo aeróbico pela glutathione que, por sua vez, é formada a partir do glutamato, entre outros aminoácidos, e atua como um importante tampão redox. Dessa forma, a molécula estudada possuiria potencial ação antioxidante, o que seria de suma importância em casos de epilepsia, especialmente nas de apresentação neonatal, visando melhor desfecho quando a desenvolvimento neuropsicomotor e qualidade de vida.

Considerando a necessidade de criação de novos fármacos, o presente estudo foi capaz de triar 5 moléculas inéditas em modelo de epilepsia neonatal através da administração de pentilenotetrazol, das quais 4 mostraram possuir potencial anticonvulsivante. Como perspectivas futuras, faz-se necessário avaliar as moléculas em doses maiores, analisar função neuroprotetora por meio de marcadores de estresse oxidativo, inflamação e comportamento, assim como impacto a longo prazo no neurodesenvolvimento. Concluímos também que é possível que um grupo amostral mais amplo seja necessário no futuro para realização dos experimentos e avaliação.

## ANEXO I

Normas para submissão do artigo para a revista Neuropsychopharmacology.

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|                                                  |   |                                                 |    |
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*Neuropsychopharmacology* is an international scientific journal and the official publication of the American College of Neuropsychopharmacology (ACNP). This journal focuses upon clinical and basic science contributions that advance our understanding of the brain and behavior, especially as related to the molecular, cellular, physiological and psychological properties of agents acting within the central nervous system and the identification of the new molecular targets for the development of the next generation of drugs. While original reports are preferred, mini-reviews and perspectives are invited by the editorial office. In view of the interdisciplinary nature of the field, particular emphasis is placed on:

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- biologically relevant aspects of the epidemiology, diagnosis and treatment of these disorders
- the basic mechanisms by which psychopharmacological agents exert their effect

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##### Editor-in-Chief

William A. Carlezon Jr., PhD  
Professor of Psychiatry and Neuroscience  
Harvard Medical School  
McLean Hospital  
Belmont, MA  
USA

##### Editorial Office

5034 Thoroughbred Lane  
Brentwood, TN 37027  
USA  
E-mail: [journal@acnp.org](mailto:journal@acnp.org)  
Tel: +1 615 324 2360  
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*Neuropsychopharmacology* publishes:

- Original Articles
- Review Articles
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- Commentaries
- Perspectives
- Editorials
- Research Highlights

#### MANUSCRIPT SPECIFICATIONS BY ARTICLE TYPE

| Article Type         | Abstract | Word Count | Display Items (figures and tables) | References (maximum) |
|----------------------|----------|------------|------------------------------------|----------------------|
| Original Article     | Yes      | 4000       | 5                                  | 50                   |
| Review Article *     | Yes      | 4000       | 5                                  | 50                   |
| Correspondence (LTE) | No       | 500        | 1                                  | 6                    |

|                       |                                                                                |      |   |                                          |
|-----------------------|--------------------------------------------------------------------------------|------|---|------------------------------------------|
| NPPR Review Article * | By invitation only: authors will receive editorial guidelines upon invitation. |      |   |                                          |
| NPPR Hot Topic *      | By invitation only: authors will receive editorial guidelines upon invitation. |      |   |                                          |
| Editorial *           | No                                                                             | 1000 | 1 | 6                                        |
| Commentary *          | No                                                                             | 1000 | 1 | 6                                        |
| Perspective *         | No                                                                             | 1000 | 1 | 6                                        |
| Research Highlight*   | No                                                                             | 1000 | 1 | 6 (one must be article under discussion) |

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Papers should be formatted as follows:

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  - b. Abstract (unstructured)
  - c. Introduction
  - d. Materials (or patients) and Methods
  - e. Results
  - f. Discussion
  - g. Funding and Disclosure (covers all authors and sources of funding listed for the manuscript)
  - h. Acknowledgments (includes special thanks, contributions, or dedications)
  - i. References (listed alphabetically, not numbered)
  - j. Figure legends (Figures should be uploaded as separate attachments.)
4. Tables
5. Figures
6. Supplemental Material (Will be published online only. See "SUPPLEMENTARY INFORMATION" section below.)
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Journal. Barbosa MDFS, Nguyen QA, Tchernev VT, Ashley JA, Deter JC, Blaydes SM et al (1996). Identification of the homologous beige and Chediak-Higashi syndrome genes. *Nature* 382: 262–265.

Book. Reisman RS, Wilson JS, Franks LF (1997). *Fundamentals of Neuropsychopharmacology*, 3rd edn. CRC Press: Boca Raton, FL.

Chapter in edited book. Mully AG Jr (1990). Equity and variability in modern health care. In: Andersen TF, Mooney G (eds). *The Challenges of Medical Practice Variations*. Palgrave: New York, pp 145–286.

Online document. World Health Organization (2001). Summary Report of the Meeting to Revise ICD-10. [http://www.who.int/mental\\_health/advisory\\_group\\_meeting.pdf](http://www.who.int/mental_health/advisory_group_meeting.pdf).

Online publication ahead of print. Meador-Woodruff JH, Davis KL, Haroutunian V (2000). Abnormal kainate receptor expression in pre-frontal cortex in schizophrenia. *Neuropsychopharmacology*, e-pub ahead of print 7 August 2000. doi:10.1038/npp.2010.87.

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|                        |                         |
|------------------------|-------------------------|
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|                                 |                                                                                |
|---------------------------------|--------------------------------------------------------------------------------|
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|            |                                                                                                                                                           |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
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|            |                                                                                                                                                           |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
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Revised February 2018

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## ANEXO II

## Carta de aprovação do projeto pelo CEUA-UFCSPA

## ANEXO I

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COMISSÃO  
PROTOCOLO Nº 188/16  
RECEBIDO EM:  
04/11/16

## 1. PRAZO

Início: 15/08/2016

Término: 01/03/2018

29 v.

## 2. TÍTULO DO PROJETO

Avaliação da resposta de canabidiol e derivados benzimidazólicos em modelos animais  
de epilepsia infantil e Síndrome de West

Área do conhecimento: Neuropsicofarmacologia

## 3. RESPONSÁVEL

|                           |                                             |
|---------------------------|---------------------------------------------|
| Nome completo             | Helena Maria Tannhauser Barros              |
| Instituição               | UFCSPA                                      |
| E-mail                    | helenbar@ufcspa.edu.br                      |
| Departamento / Disciplina | Farmacologia/ Mestrado em Ciências da Saúde |

## Experiência Prévia:

Não ☐  
Sim ☒

Quanto tempo? 30 anos

## Treinamento:

Não ☐  
Sim ☒

Quanto tempo? 30 anos

Edi C. Pulroinik  
Secretária do CEUA/UFCSPA

Poderá ser solicitado o projeto a critério da CEUA respeitando confidencialidade e conflito de interesses.

Quando cabível, anexar o termo de consentimento livre e esclarecido do proprietário ou responsável pelo animal.

## 15. RESOLUÇÃO DA COMISSÃO

15. RESOLUÇÃO DA COMISSÃO

A Comissão de Ética no uso de animais, na sua reunião de 29/11/16, APROVOU os procedimentos éticos apresentados neste Protocolo.

Assinatura: \_\_\_\_\_

Coordenador da Comissão

Edi C. Pulrolnik  
Secretaria do CEP/UFCSA

A Comissão de Ética No Uso de Animais, na sua reunião de \_\_\_\_/\_\_\_\_/\_\_\_\_, emitiu o parecer em anexo e retorna o Protocolo para sua revisão.

Assinatura: \_\_\_\_\_

Coordenador da Comissão

Parecer dado ao protocolo (assinar e carimbar o parecer):

Parecer dado ao protocolo (assinar e carimbar o parecer).

