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Tese de Doutorado

Abordagens farmacológicas e moleculares do efeito do tipo antidepressivo da benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) em camundongos

Camila Simões Pires

Pelotas, 2024

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Em *Capitães da Areia*, Jorge Amado escreveu que certos homens têm estrelas no lugar do coração e, quando morrem, o coração fica no céu. Com toda certeza, posso dizer que minha mãe tinha uma estrela no lugar do coração. Toda minha trajetória é guiada e cuidada por ela. Por isso, dedico esta tese à minha mãe, Gleice (in memoriam), mantendo-a viva em cada linha que escrevi. Dedico também ao meu pai Alvaro, aos meus avós Teresa e Paulo e ao meu amor Herbert por serem as forças que me mantém em pé.

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Resumo

PIRES, Camila Simões. **Abordagens farmacológicas e moleculares do efeito do tipo antidepressivo da benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) em camundongos.** Orientador: César Augusto Brüning. 2024. 117f. Tese (Programa de Pós-Graduação em Bioquímica e Bioprospecção) - Universidade Federal de Pelotas, Pelotas, 2024.

Neste estudo, foi avaliado o efeito do tipo antidepressivo da benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) (CF_3SePB) em camundongos. No artigo 1, os animais foram tratados com CF_3SePB (1–50 mg/kg, via oral) e submetidos ao teste do nado forçado (TNF) ou ao teste de suspensão da cauda (TSC) 30 minutos após a administração. Para explorar o envolvimento dos sistemas serotoninérgico e noradrenérgico, os camundongos foram pré-tratados com p-CPA (depletador de serotonina) ou antagonistas dos receptores serotoninérgicos (WAY100635, cetanserina, ondansetrona, GR110838) ou antagonistas dos receptores noradrenérgicos (prazosina, ioimbina e propranolol). Para verificar a toxicidade aguda de CF_3SePB, os camundongos foram tratados com uma alta dose do composto (300 mg/kg). CF_3SePB apresentou efeito do tipo antidepressivo em ambos os testes, relacionado à modulação do sistema serotoninérgico, especialmente dos receptores 5-HT_{1A} e 5-HT_3, enquanto os antagonistas noradrenérgicos não impediram o efeito antidepressivo do CF_3SePB. O composto demonstrou baixo potencial de induzir toxicidade aguda em camundongos Swiss fêmeas adultas. No manuscrito 1, para investigar o envolvimento do sistema dopaminérgico, camundongos foram pré-tratados com antagonistas dopaminérgicos (haloperidol, SCH 23390 e sulpirida) antes da administração do CF_3SePB. Além disso, foi avaliado o perfil farmacocinético in silico de CF_3SePB. A CF_3SePB apresentou efeito anti-imobilidade no teste de nado forçado, demonstrado pelo aumento da latência até o primeiro episódio de imobilidade e redução da imobilidade total dos camundongos. O pré-tratamento com haloperidol, SCH 23390 e sulpirida impediu esses efeitos, sugerindo que o efeito do tipo antidepressivo do CF_3SePB está relacionado à modulação do sistema dopaminérgico, especificamente dos receptores D_1 e D_2. Além disso, o perfil farmacocinético in silico da CF_3SePB indicou baixa probabilidade de induzir efeitos adversos e a capacidade de atravessar a barreira hematoencefálica (BHE). No manuscrito 2, o composto CF_3SePB foi testado em um modelo de depressão induzida por lipopolissacarídeo (LPS), com pré-tratamento dos camundongos com CF_3SePB, fluoxetina ou veículo, seguido de LPS. Após 24 horas, realizaram-se testes comportamentais para avaliar sintomas depressivos e atividade locomotora. O CF_3SePB foi eficaz na reversão dos comportamentos semelhantes à depressão induzidos por lipopolissacarídeo (LPS) sem afetar a locomoção dos camundongos. O composto também impediu o aumento na expressão dos genes pró-inflamatórios NF-κβ, NLRP3 e COX-2, além dos genes apoptóticos caspase-1, caspase-8 e BAX induzidos por LPS, sugerindo seu potencial para atuar em vias de inflamação e morte neuronal. Adicionalmente, o CF_3SePB reduziu os níveis de espécies reativas e peroxidação lipídica no hipocampo induzidos por LPS, sugerindo efeitos neuroprotetores. Este estudo destaca o potencial inovador do CF_3SePB como um composto inédito com efeito antidepressivo e anti-inflamatório promissor, que pode ser considerado no desenvolvimento de novos antidepressivos.

Palavras-chave: Depressão. Sistema monoaminérgico. Benzamida. Selênio. Neuroinflamação. Camundongos.

Abstract

PIRES, Camila Simões. **Pharmacological and molecular approaches to the antidepressant-like effect of N-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl)benzamide in mice.** Advisor: César Augusto Brüning. 2024. 117p. Thesis (Graduate Program in Biochemistry and Bioprospection) – Federal University of Pelotas, Pelotas, 2024.

In this study, the antidepressant-like effect of the benzamide *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-en-1-yl) (CF_3SePB) was evaluated in mice. In Manuscript 1, the animals were treated with CF_3SePB (1–50 mg/kg, orally) and subjected to the forced swim test (FST) or the tail suspension test (TST) 30 minutes after administration. To explore the involvement of the serotonergic and noradrenergic systems, the mice were pre-treated with p-CPA (serotonin depleter) or antagonists of serotonin receptors (WAY100635, ketanserin, ondansetron, GR110838) or noradrenergic receptor antagonists (prazosin, yohimbine, and propranolol). To assess the acute toxicity of CF_3SePB, the mice were treated with a high dose of the compound (300 mg/kg). CF_3SePB exhibited antidepressant effects in both tests, related to the modulation of the serotonergic system, especially the 5-HT_{1A} and 5-HT_3 receptors, while noradrenergic antagonists did not prevent the antidepressant-like effect of CF_3SePB. The compound showed a low potential to induce acute toxicity in adult Swiss female mice. In Manuscript 1, to investigate the involvement of the dopaminergic system, mice were pre-treated with dopaminergic antagonists (haloperidol, SCH 23390, and sulpiride) before CF_3SePB administration. Additionally, the in silico pharmacokinetic profile of CF_3SePB was evaluated. CF_3SePB exhibited an anti-immobility effect in the forced swim test, demonstrated by an increased latency to the first episode of immobility and a reduction in total immobility of the mice. Pre-treatment with haloperidol, SCH 23390, and sulpiride blocked these effects, suggesting that the antidepressant effect of CF_3SePB is related to the modulation of the dopaminergic system, specifically the D1 and D2 receptors. Furthermore, the in silico pharmacokinetic profile of CF_3SePB indicated a low probability of inducing adverse effects and the ability to cross the blood-brain barrier (BBB). In Manuscript 2, the compound CF_3SePB was tested in a lipopolysaccharide (LPS)-induced depression model, with pre-treatment of the mice with CF_3SePB, fluoxetine, or vehicle, followed by LPS administration. After 24 hours, behavioral tests were performed to assess depressive symptoms and locomotor activity. CF_3SePB was effective in reversing LPS-induced depressive-like behaviors without affecting the locomotion of the mice. The compound also prevented the increase in the expression of the pro-inflammatory genes NF-κB, NLRP3, and COX-2, as well as the apoptotic genes caspase-1, caspase-8, and BAX induced by LPS, suggesting its potential to act on inflammation and neuronal death pathways. Additionally, CF_3SePB reduced the levels of reactive species and lipid peroxidation in the hippocampus induced by LPS, suggesting neuroprotective effects. This study highlights the innovative potential of CF_3SePB as a novel compound with promising antidepressant and anti-inflammatory effects, which could be considered in the development of new antidepressants.

Keywords: Depression. Monoaminergic system. Benzamide. Selenium. Neuroinflammation. Mice.

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Lista de Abreviaturas e Siglas

5-HT	5-hidroxitriptamina
AMMs	Antidepressivos multimodais
BDNF	Fator Neurotrófico Derivado do Cérebro
BHE	Barreira Hematoencefálica
CEUA	Comissão de Ética no Uso de Animais
CF_3SePB	Benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica
CID-10	Classificação Internacional de Doenças 10ª Revisão
DA	Dopamina
DSM-5	Manual de Diagnóstico e Estatístico de Transtornos Mentais da 5ª edição
EROs	Espécies reativas de oxigênio
FLX	Fluoxetina
HPA	Hipotálamo-pituitária-adrenal
IL-1β	Interleucina 1 beta
IL-6	Interleucina 6
IMAOs	Inibidores de Monoamino-oxidase
IRN	Inibidor de Recaptação de Noradrenalina
IRND	Inibidor de Recaptação de Noradrenalina e Dopamina
ISRN	Inibidor Seletivo de Recaptação de Noradrenalina
ISRS	Inibidor Seletivo de Recaptação de Serotonina
LC	Locus Coeruleus
LPS	Lipopolissacarídeo
NA	Noradrenalina
NFκB	Fator nuclear Kappa B
NMDA	N-metil-D-aspartato
OMS	Organização Mundial da Saúde
Se	Selênio
SERT	Transportador de serotonina
SNC	Sistema Nervoso Central
TBS	Teste da Borrifagem de Sacarose
TCA	Teste do Campo Aberto
ATCs	Antidepressivos tricíclicos
TDM	Transtorno Depressivo Maior
TNF-α	Fator de Necrose Tumoral alfa
TSC	Teste da Suspensão da Cauda

SUMÁRIO

1 INTRODUÇÃO

O Transtorno Depressivo Maior (TDM) é uma condição clínica caracterizada por sintomas de tristeza persistente, desânimo, falta de interesse e prazer em atividades prazerosas (KUPFERBERG e HASLER, 2023; KWASNY et al., 2024). O TDM também pode causar distúrbios do sono, bem como cansaço, falta de concentração e apetite (MORSSINKHOF et al., 2020; OPAS, 2024). Esse distúrbio é multifatorial, recorrente, crônico, com alta incidência na população mundial e está presente entre os distúrbios mais debilitantes do mundo (McINTYRE et al., 2023). Estima-se que mais de 300 milhões de pessoas sofram deste distúrbio em todo o mundo (BALANZÁ-MARTÍNEZ e CERVERA-MARTÍNEZ, 2022; KWASNY et al., 2024).

A depressão pode causar grande sofrimento à pessoa acometida e levar à disfunção em suas atividades diárias (GONG et al., 2020). Pode ser de intensidade moderada ou grave e de longa duração, tornando-se uma condição crítica de saúde (OPAS, 2024). As causas da depressão podem ser diferentes para cada indivíduo. Alguns sugerem que a depressão pode ser exclusivamente resultado de fatores genéticos, enquanto outros sugerem que se deve a uma contribuição de fatores genéticos e ambientais, como os principais estressores da vida (GOHAR et al., 2012; BAJ et al., 2023; GUO et al., 2024). Há evidências neurobiológicas que ajudam a entender a depressão, apesar de ainda não serem totalmente elucidadas (LI et al., 2023).

A teoria monoaminérgica define que a via comum para a depressão é a alteração ou déficit de neurotransmissores no sistema nervoso central (SNC), como serotonina (5-HT), dopamina (DA) e noradrenalina (NA) (ŚLIFIRSKI et al., 2021; PASTIS et al., 2024). Apesar de a hipótese das monoaminas auxiliar no entendimento da fisiopatologia da depressão ao longo de várias décadas, esforços têm sido direcionados para identificar outros mecanismos que contribuem para os transtornos depressivos (PASTIS et al., 2024). Entre as diversas teorias, a conexão entre o sistema imunológico e o sistema nervoso central tem ganhado destaque (BURAS et al., 2016; PASTIS et al., 2024). A integridade da barreira hematoencefálica (BHE) pode ser prejudicada por respostas imunológicas inatas e adaptativas, permitindo que sinais inflamatórios da periferia se propaguem para o SNC (BEUREL et al., 2022; HASSAMAL, 2023).

Conforme a hipótese inflamatória, a depressão pode ser desencadeada pela

neuroinflamação, que envolve a ativação do sistema imunológico inato no sistema nervoso central. Essa condição leva a um fenótipo depressivo, caracterizado por sintomas graves e aumento da morbidade e mortalidade (DANESE et al., 2007; HASSAMAL, 2023). A neuroinflamação persistente pode induzir comportamentos semelhantes à depressão ou acelerar o desenvolvimento do TDM. Além disso, os antidepressivos atualmente disponíveis são eficazes na redução dos sintomas depressivos em apenas 37% dos pacientes com TDM (RUSH et al., 2006; WON et al., 2021; WU e ZHANG, 2023).

O selênio (Se) é um oligoelemento amplamente distribuído no corpo humano e essencial para a manutenção da vida, pois está presente no aminoácido essencial selenocisteína, na enzima glutationa peroxidase e na maioria dos tecidos do corpo humano (SANTI e BAGNOLI, 2017; CHUAI et al., 2021; XU-XU et al., 2022; GU e GAO et al., 2022; CHEN et al., 2024). A falta ou deficiência de Se tem sido associada a transtornos de humor, como ansiedade e depressão (WANG et al., 2018). Considerando esses aspectos, os compostos orgânicos de Se demonstram muitas propriedades farmacológicas, incluindo efeitos anti-inflamatórios, antioxidantes, antinociceptivos e antidepressivos (BRÜNING et al., 2015; OLIVEIRA et al., 2016; BAMPI et al., 2020; BIRMANN et al., 2023; LEDEBUHR et al., 2024).

Alguns desses compostos que apresentam efeitos antidepressivos podem ser considerados promissores para o desenvolvimento de novos tratamentos para o TDM (SAVEGNAGO et al., 2007; BRÜNING et al., 2015; BESCKOW et al, 2020). Outros estudos sugerem que compostos orgânicos contendo Se, quando utilizados como tratamento potencial para o TDM e administrados a camundongos, foram eficazes na prevenção de comportamentos depressivos nesses animais (CASARIL et al., 2019). Além disso, a adição de Se a uma benzamida revelou propriedades antidepressivas. A benzamida, uma molécula derivada da amina do ácido carbônico e do ácido benzóico, tem exibido diversas propriedades terapêuticas, incluindo efeitos anticonvulsivantes, analgésicos e antidepressivos (CHIRITA et al., 2010; BESCKOW et al, 2020). Desta forma, este estudo avaliará o efeito do tipo antidepressivo do composto orgânico de Se ligado a benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) (CF_3SePB, figura 1) em camundongos, além de investigar sua relação com o sistema monoaminérgico e neuroinflamação.

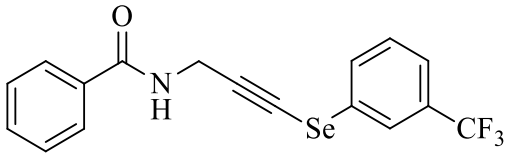

Figura 1. Estrutura química da benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) (CF_3SePB).

2 OBJETIVOS

2.1 Objetivo geral

Investigar o efeito do tipo antidepressivo da benzamida *N*-(3-((3-(trifluorometil)fenil)selenil)prop-2-in-1-ílica) (CF_3SePB) em camundongos, bem como seu envolvimento com o sistema monoaminérgico e imune.

2.2 Objetivos específicos

- Avaliar o efeito do tipo antidepressivo da CF_3SePB nos testes do nado forçado e suspensão da cauda em camundongos;

- Avaliar o mecanismo de ação da CF_3SePB através da utilização de antagonistas de receptores serotoninérgicos, noradrenérgicos, dopaminérgicos;

- Avaliar a toxicidade do composto CF_3SePB;

- Avaliar propriedades farmacológicas e toxicológicas *in sílico*.

- Verificar se o composto CF_3SePB apresentará efeito antidepressivo no modelo de depressão induzida por LPS através do teste da suspensão da cauda, teste do nado forçado e teste de borrifagem de sacarose em camundongos;

- Investigar o mecanismo de ação do composto através da modulação de marcadores oxidativos e inflamatórios em tecido cerebral de camundongos.

3. REVISÃO DE LITERATURA

3.1 TRANSTORNO DEPRESSIVO MAIOR

A depressão é uma doença mental que se apresenta na forma de humor deprimido como sentimento de tristeza, choro e solidão, por exemplo, além de perda de prazer, sentimento de culpa, diminuição de energia, baixa autoestima, sono perturbado ou falta de apetite e concentração (SAMPAIO et al., 2020; RIGA et al., 2020; KUPFERBERG e HASLER, 2023; KWASNY et al., 2024). O TDM é uma condição clínica que afeta mais de 300 milhões de pessoas em todo o mundo (KUPFERBERG e HASLER, 2023; TAYEB et al., 2023). Segundo a Organização Mundial da Saúde (OMS), o TDM é uma das doenças mais incapacitantes da atualidade, com uma prevalência em constante aumento (BALANZÁ-MARTÍNEZ e CERVERA-MARTÍNEZ, 2022; TAYEB et al., 2023). A pandemia de Covid-19 contribuiu para esse crescimento, principalmente devido às medidas drásticas implementadas para conter a disseminação do vírus, como o isolamento social (SANTOMAURO et al., 2021; KUPFERBERG e HASLER, 2023).

A combinação de estresses decorrentes da pandemia pode ter intensificado fatores de risco para condições de saúde mental, como a depressão (OMS, 2022; CHAN et al., 2024). Em pacientes com depressão pré-existente, a priorização governamental no controle da depressão pode ter causado interrupção de serviços não emergenciais e deixado demandas de cuidado em saúde mental sem atendimento (OMS, 2022; CHAN et al., 2024). Um estudo estimou que, em 2020, surgiram 53 milhões de novos casos de depressão, com um aumento de 27,6% na prevalência global (SANTOMAURO et al., 2021; CHAN et al., 2024). Esse aumento foi atribuído a doenças relacionadas à COVID-19 e à redução da mobilidade, afetando pessoas de todas as faixas etárias (HAWES et al., 2022; CHAN et al., 2024).

O perfil do indivíduo com TDM é descrito no Manual Diagnóstico e Estatístico de Transtornos Mentais, 5ª edição (DSM-5) e na Classificação Internacional de Doenças, 10ª revisão (CID-10) (AMERICAN PSYCHIATRIC ASSOCIATION, 2014; BALANZÁ-MARTÍNEZ e CERVERA-MARTÍNEZ, 2022). De acordo com o DSM-5, o paciente deve apresentar cinco dos nove sintomas listados por pelo menos duas semanas (AMERICAN PSYCHIATRIC ASSOCIATION, 2014; OTTE et al., 2016; TAYEB et al., 2023). Já a CID-10 classifica os sintomas conforme a intensidade: leve, moderada ou grave, e esses podem ocorrer de forma única ou recorrente,

caracterizando a recidiva da doença (JAKOBSEN et al., 2020; ŚLIFIRSKI et al., 2021). O TDM exerce um impacto profundo na vida humana, sendo sua etiologia e fisiopatologia ainda um desafio complexo. Tanto a psicoterapia quanto o tratamento farmacológico podem ser eficazes no manejo do TDM, contudo, cerca de 30% dos pacientes não alcançam remissão, mesmo após múltiplas tentativas de tratamento (OTTE et al., 2016).

Os inibidores seletivos da recaptação da serotonina (ISRSs) geralmente são as escolhas de primeira linha para o tratamento da depressão. Entretanto, muitos pacientes não respondem a distintas opções ou possuem intolerância aos efeitos colaterais desses medicamentos (FAQUIH et al., 2019; ANDRADE et al., 2022). Os demais tratamentos farmacológicos disponíveis atualmente para o TDM também apresentam várias limitações, como a ausência de resposta precoce, eficácia reduzida, baixa taxa de remissão no primeiro tratamento com antidepressivos e efeitos colaterais (HUTKA et al., 2021; KRYSTAL et al., 2024). Muitos pacientes com depressão não respondem adequadamente aos tratamentos tradicionais, mesmo após múltiplas tentativas com diferentes classes de medicamentos (SOARES et al., 2024; KRYSTAL et al., 2024). A resistência aos antidepressivos convencionais é uma preocupação crescente no manejo do TDM, com uma parcela significativa de pacientes não respondendo adequadamente aos tratamentos iniciais, e alguns sendo considerados resistentes, mesmo após diversas tentativas com fármacos de diferentes classes (OTTE et al., 2016; KRYSTAL et al., 2024).

Os efeitos da maioria dos antidepressivos, geralmente só se manifestam após várias semanas de tratamento (OTTE et al., 2016). Em geral, a maioria dos antidepressivos leva de quatro a seis semanas para mostrar efeitos terapêuticos completos, expondo os pacientes ao risco de recaída ou agravamento dos sintomas durante esse período (CRUZ et al., 2020). Assim, há uma necessidade crescente de desenvolver novos fármacos que possam tanto modular a resposta inflamatória no sistema nervoso central quanto regular a liberação ou recaptação de monoaminas (OTTE et al., 2016; KRYSTAL et al., 2024). Portanto, é crucial continuar a pesquisa e o desenvolvimento de novos medicamentos para o tratamento da depressão, visando maior eficácia e menos efeitos adversos (RECH et al., 2021; McINTYRE et al., 2023; KRYSTAL et al., 2024).

3.2 FISIOPATOLOGIA DA DEPRESSÃO

Há quase cinco décadas, a hipótese monoaminérgica tem sido uma das principais teorias para explicar a patogênese da depressão (JIANG et al., 2022). Ela sugere que a doença resulta da diminuição da disponibilidade de neurotransmissores monoaminérgicos, como 5-HT, DA e NA no SNC (ŚLIFIRSKI et al., 2021; BAJ at al., 2023; PASTIS et al., 2024; CUI et al., 2024). Essa teoria continua a fornecer uma base neurobiológica significativa para a compreensão da depressão, influenciando o desenvolvimento de terapias que visam aumentar os níveis desses neurotransmissores no cérebro (JIANG et al., 2022; CUI et al, 2024).

A participação da 5-HT no mecanismo do desenvolvimento da depressão tem sido muito estudado nas últimas décadas (CUI et al, 2024). A 5-HT desenvolve um papel importante como neurotransmissor e o seu déficit pode induzir o estado ansioso, obsessivo e compulsões (SONG e KIM, 2021). A 5-HT é um neurotransmissor sintetizado a partir do aminoácido precursor triptofano, nos núcleos da rafe com projeções para várias regiões do SNC (LUCAS-OSMA et al., 2019; QUEVEDO e IZQUIERDO, 2020; SONG e KIM, 2021; CUI et al, 2024). As ações intracelulares da 5-HT são mediadas através de 7 diferentes famílias e 14 subfamílias de receptores (CUI et al, 2024). As famílias dos receptores serotoninérgicos compreendem de 5-HT_1 até 5-HT_7 (CUI et al, 2024). Cada receptor serotoninérgico é diferenciado de acordo com a sua estrutura e origem da sinalização. Todos os receptores serotoninérgicos são acoplados a proteína G com exceção do receptor 5-HT_3 que é um receptor ionotrópico (CUI et al, 2024). O sistema serotoninérgico está ligado a várias funções como sono e vigília, humor, apetite, ritmo circadiano, parte cognitiva como aprendizagem e memória além dos processos autonômicos envolvidos (SONG e KIM, 2021).

Na teoria monoaminérgica, a DA desenvolve seu papel como uma das monoaminas participantes da neurobiologia da depressão (CUI et al, 2024; KARAHODA et al., 2024). A DA é um neurotransmissor pertencente ao grupo das catecolaminas e tem predomínio principalmente no cérebro controlando uma variedade de funções como a atividade locomotora, emoções, prazer, concentração, regulação endócrina e consumo alimentar (MIZUNO et al., 2023; CUI et al, 2024; KARAHODA et al., 2024). A síntese de DA ocorre nos neurônios pré-sinápticos do tronco cerebral a partir de dois aminoácidos: fenilalanina e tirosina (DINIZ et al., 2020). A projeção dos neurotransmissores dopaminérgicos se dá pela via mesolímbica cortical e pela via que se projeta da substância negra para o estriado dorsal

(QUEVEDO IZQUIERDO, 2020; SONG e KIM, 2021). Os efeitos da DA são exercidos nos neurônios pós-sinápticos a partir da interação com os receptores de dopamina. Estes receptores são divididos em duas famílias (D_1 e D_2) e 5 subfamílias (D_1, D_2, D_3, D_4 e D_5). (QUEVEDO IZQUIERDO, 2020; SONG e KIM, 2021).

A teoria monoaminérgica expõe também que a NA é componente desta teoria (CUI et al, 2024). A NA é um neurotransmissor pertencente ao grupo das catecolaminas e é sintetizada a partir do aminoácido tirosina (KARAHODA et al., 2024). Este neurotransmissor é amplamente distribuído pelo cérebro além de que possui também distribuição periférica visto que a NA é o principal neurotransmissor nos neurônios pós-ganglionares do sistema nervoso autônomo simpático (DINIZ et al., 2020; BRETON-PROVENCHER et al., 2022). A norepinefrina liberada pelo locus coeruleus (LC) pode estar envolvida na regulação de diversas funções neurais, incluindo olfato, movimento e percepção sensorial (BRETON-PROVENCHER et al., 2022; CUI et al., 2022).

Outra teoria da depressão é a que relaciona-se ao eixo hipotálamo-pituitária-adrenal (HPA), sugerindo que a disfunção deste eixo de resposta ao estresse contribui de forma significativa para a etiologia da depressão (KELLER et al., 2017; HANTSOO et al., 2023; MBIYDZENYUY e QULU, 2024). O eixo HPA tem como papel central a resposta do corpo ao estresse e em condições normais, responde a estímulos estressores ativando a liberação de cortisol pelas glândulas adrenais, ajudando o organismo a lidar com situações de ameaça (KELLER et al., 2017; HANTSOO et al., 2023). Porém, em indivíduos depressivos, o eixo HPA apresenta disfunções caracterizadas com frequência pela hiperatividade do eixo HPA, além de níveis elevados de cortisol (KELLER et al., 2017; HANTSOO et al., 2023; MBIYDZENYUY e QULU, 2024). A hiperatividade do eixo HPA associa-se a diversas alterações neuroquímicas em regiões do cérebro como o hipocampo, córtex pré-frontal e amígdala, sendo essenciais para a regulação do humor e das emoções (KELLER et al., 2017; HANTSOO et al., 2023; MBIYDZENYUY e QULU, 2024).

Altos níveis de cortisol, decorrentes da desregulação do eixo HPA, causam neurotoxicidade, principalmente ao hipocampo, levando a danos neuronais, causando degeneração e comprometendo a neuroplasticidade, além de contribuir para a perpetuação de sintomas depressivos (KELLER et al., 2017; HANTSOO et al., 2023; MBIYDZENYUY e QULU, 2024). Outra consequência da hiperatividade do eixo HPA é a sua ativação excessiva que aumenta a resposta inflamatória, liberando citocinas pró-inflamatórias que também causam danos neuronais (KELLER et al., 2017;

MBIYDZENYUY e QULU, 2024). O processo inflamatório pode intensificar os sintomas depressivos como perda de interesse, fadiga, alterações no apetite, disturbios do sono, dentre outros (KELLER et al., 2017; MBIYDZENYUY e QULU, 2024). A teoria da depressão do eixo HPA destaca a importância de compreendermos a hiperatividade deste eixo e a inflamação associada a ele, como potenciais alvos para intervenções terapêuticas para o tratamento da depressão (KELLER et al., 2017; HANTSOO et al., 2023; MBIYDZENYUY e QULU, 2024).

Tem-se também a hipótese glutamatérgica da depressão que sugere que disfunções no sistema glutamatérgico, como a hiperativação destes neurônios, estão intimamente relacionadas à fisiopatologia da depressão (MENG et al., 2024; CUI et al., 2024). O glutamato, que é o principal neurotransmissor excitatório do SNC, desempenha papel essencial na manutenção da homeostase cerebral e quando ocorre uma disfunção na neurotransmissão glutamatérgica, isto pode resultar em hiperexcitabilidade neuronal contribuindo para a neurotoxicidade, perda sináptica e alterações estruturais em neurônios e regiões do cérebro como hipocampo e córtex pré-frontal, estando associados ao surgimento de sintomas depressivos e déficits cognitivos (MENG et al., 2024; CUI et al., 2024; BAEK et al., 2024).

Outro fator importante é a relação do sistema glutamatérgico com a resposta inflamatória na depressão, onde é proposto que a neuroinflamação, causada por fatores como estresse oxidativo e a ativação de células imunológicas do cérebro, contribui significativamente para o surgimento da depressão (CUI et al., 2024; BAEK et al., 2024). A neuroinflamação afeta a função do glutamato, pois as espécies reativas de oxigênio (EROs) e as citocinas inflamatórias, como (IL)-1β e TNF-α, estimulam a liberação excessiva deste neuotransmissor, aumentando o risco de excitação neuronal e contribuindo para a neurotoxicidade (CUI et al., 2024). Este ciclo leva a ativação de vias inflamatórias e disfunção glutamatérgica, agravando os sintomas depressivos e promovendo alterações estruturais e funcionais no SNC (CUI et al., 2024).

Há muito tempo, grupos de pesquisa se dedicam ao estudo da fisiopatologia do TDM mas sua etiologia ainda não é completamente compreendida (OTTE et al., 2016). Diversas teorias tentam explicar a depressão, incluindo a teoria monoaminérgica e a teoria glutamatérgica. No entanto, a teoria inflamatória sugere que processos inflamatórios, tanto periféricos quanto centrais, desempenham um papel crucial no desenvolvimento e na manutenção do TDM (GUO et al., 2024). Essa teoria tem recebido crescente apoio à medida que pesquisas demonstram que muitos

pacientes com depressão apresentam níveis elevados de marcadores inflamatórios, tanto no sangue quanto no sistema nervoso central (KOUBA et al., 2023; GUO et al., 2024). Segundo a hipótese inflamatória, a depressão pode ser causada por neuroinflamação (DANESE et al., 2007; HASSAMAL, 2023).

A relação entre neuroinflamação e depressão tem se tornado um campo de estudo de grande interesse, especialmente na busca por uma compreensão mais aprofundada das causas biológicas da depressão e pelo desenvolvimento de tratamentos mais eficazes (WU e ZHANG, 2023). A neuroinflamação é caracterizada pela ativação do sistema imunológico inato central, resultando no desenvolvimento de um fenótipo depressivo com sintomas intensos e aumento da morbidade e mortalidade (DANESE et al., 2007; HASSAMAL, 2023). Embora a neuroinflamação seja uma resposta natural a infecções ou lesões, sua ativação crônica ou desregulada tem sido associada a várias condições neurológicas e psiquiátricas, incluindo a depressão (PASTIS et al., 2024).

A neuroinflamação é mediada pela ativação das células da microglia que ativam o fator nuclear kappa B (NF-κB) e induz a produção de citocinas inflamatórias (SUNESON et al., 2021; ZHENG et al., 2021). A sinalização de citocinas desencadeia uma série de efeitos biológicos no monoaminérgico e estresse oxidativo (SUNESON et al., 2021). O aumento dos níveis séricos de citocinas inflamatórias, como interleucina (IL)-1β, IL-6 e fator de necrose tumoral-α (TNF-α) foram observados em pacientes com transtorno depressivo (ZHENG et al., 2021; KOUBA et al., 2023). Essas citocinas podem levar a danos neuronais, resultando no progresso da depressão ou outros transtornos de humor (SUNESON et al., 2021; ZHENG et al., 2021; KOUBA et al., 2023). A neuroinflamação proporciona uma nova perspectiva para compreender e tratar a depressão, especialmente em casos crônicos e resistentes ao tratamento. Ela afeta até 27% dos pacientes com TDM e está associada a uma forma mais grave, crônica e refratária à terapia (HASSAMAL, 2023).

Os mecanismos moleculares da relação entre inflamação e depressão têm sido extensivamente estudados em modelos animais de depressão induzida pela administração periférica de LPS, para mimetizar infecção bacteriana. A administração periférica de LPS desencadeia a diminuição dos níveis do fator neurotrófico derivado do cérebro (BDNF) no cérebro através de altas concentrações de citocinas pró-inflamatórias (DANTZER et al., 2011; KOHMAN e RHODES, 2013). Dessa forma, é possível notar que a inflamação sistêmica induzida pela injeção periférica de LPS, um componente importante da membrana externa de bactérias gram-negativas, ativa significativamente o sistema imunológico induzindo a ativação da microglia que,

consequentemente, produz respostas neuroinflamatórias (HOOGLAND et al., 2015; GUAN et al., 2020). Portanto, o uso do LPS em modelo animal é amplamente aceito para investigar a relação entre neuroinflamação e sintomas depressivos (ZHENG et al., 2021).

A ativação crônica do sistema imunológico no cérebro, um fenômeno também observado em estados inflamatórios persistentes, pode desencadear uma série de sintomas depressivos, incluindo déficits cognitivos e desregulação emocional (HASSAMAL, 2023). Essa resposta inflamatória é frequentemente vinculada ao estresse oxidativo, a redução de fatores neurotróficos, como o BDNF e à apoptose, os quais são apontados como fatores que contribuem para o desenvolvimento do TDM (DWIVEDI, 2009; AFRIDI e SUK, 2021; LIU et al., 2022; ZHANG et al., 2023; TAYEB et al., 2023). O estresse oxidativo ocorre quando há um desequilíbrio na geração de radicais livres, e o organismo não consegue neutralizar essa produção de forma eficaz (BARBOSA et al., 2010; SIES e JONES, 2020; TAYEB et al., 2023). Esse processo prejudicial pode danificar células e tecidos cerebrais, agravando disfunções neuroanatômicas que estão fortemente ligadas ao desenvolvimento e à progressão da depressão (TAYEB et al., 2023).

O acúmulo de espécies reativas de oxigênio (ROS) pode causar danos a lipídios, proteínas e DNA, resultando em lesão celular e apoptose (ZHANG et al., 2023; TAYEB et al., 2023). Os danos provocados pelo estresse oxidativo são amplamente reconhecidos na fisiopatologia do TDM (TAYEB et al., 2023). Pesquisadores sugerem que o estresse oxidativo no cérebro desempenha um papel importante nos transtornos depressivos, uma vez que pode comprometer a função neuronal e a neurotransmissão (MAES et al., 2000). Esses achados sugerem que intervenções terapêuticas direcionadas para modular os processos inflamatórios pode ser essencial para a melhoria das condições clínicas de pacientes com TDM (LIU et al., 2022; ZHANG et al., 2023; TAYEB et al., 2023).

A apoptose, ou morte celular programada, desempenha um papel importante no contexto de distúrbios neurológicos e psiquiátricos, como o TDM, especialmente quando associada à neuroinflamação (NEWTON et al., 2024; YUAN e OFENGEIM, 2024; HALL et al., 2024). No TDM, estudos mostram que fatores inflamatórios, como citocinas pró-inflamatórias, podem ativar a apoptose em células cerebrais, especialmente neurônios e células gliais (ZHENG et al., 2021; HASSAMAL, 2023; KOUBA et al., 2023; CERQUEIRA et al., 2024; HALL et al., 2024). Isso leva a uma redução da neuroplasticidade e neurogênese, causando a perda de células neurais em áreas do cérebro como o hipocampo por exemplo, que é essencial para a

regulação do humor e da cognição, podendo agravar os sintomas depressivos (HALL et al., 2024).

3.3 CLASSES DE FÁRMACOS ANTIDEPRESSIVOS E PROBLEMAS QUE APRESENTAM

Antidepressivos são medicamentos psicoativos empregados no tratamento de transtornos mentais caracterizados por sintomas predominantemente depressivos (SALWAN et al., 2022; ZHENG et al., 2023). Os antidepressivos são frequentemente utilizados no tratamento da depressão e atuam de maneiras diferentes conforme a classe em que se encontram direcionando-se a neurotransmissores específicos para regular humor e comportamento (HARMER et al., 2017; SHEFFLER et al., 2024). Acredita-se que a maioria dos antidepressivos existentes e aprovados atualmente, aumentem os níveis dos neurotransmissores durante as sinapses e apesar de os mecanismos para esse aumento variarem, a maioria dos antidepressivos agem bloqueando a recaptação desses neurotransmissores nos terminais nervosos (HARMER et al., 2017; SHEFFLER et al., 2024).

Mais de 50 anos se passaram desde a descoberta da hipótese monoaminérgica da depressão e mais de 60 anos desde o surgimento do primeiro tratamento farmacológico para o TDM (ROSS e RENYI, 1969; HILLHOUSE e PORTER, 2015). Embora várias classes de medicamentos baseados em monoaminas tenham sido desenvolvidas e aprovadas para tratar o TDM, as taxas de remissão permanecem baixas, havendo um período de latência até que se alcance a remissão total dos sintomas depressivos (TIAN et al., 2022). Os antidepressivos atualmente disponíveis para o tratamento farmacológico da depressão estão divididos em algumas principais classes com base em seus mecanismos de ação (FASIPE, 2018).

Os antidepressivos clássicos são representados pelos tricíclicos (TCAs) e pelos inibidores da monoamino-oxidase (IMAOs) (PEÑA et al., 2016; TIAN et al., 2022). Os TCAs, foram desenvolvidos nos anos 1950 para melhorar o humor de pacientes deprimidos e como mecanismo de ação, eles inibem a recaptação de NA e 5-HT, porém, interagem com receptores adrenérgicos, muscarínicos e histamínicos sendo responsáveis principalmente pelos efeitos colaterais de tontura, comprometimento da memória e sonolência (PEÑA et al., 2016; TIAN et al., 2022). Os IMAOS foram um dos primeiros antidepressivos licenciados para o tratamento da depressão, agindo de forma a inibir a enzima monoamino-oxidase (MAO), responsável pela degradação dos neurotransmissores monoaminérgicos e aumentando a disponibilidade sináptica de neurotransmissores como 5-HT, DA e NA (HILLHOUSE e PORTER, 2015; PEÑA et al., 2016; TIAN et al., 2022). Entretanto, essa classe possui interações medicamentosas e alimentares potencialmente perigosas, incluindo crises

hipertensivas e síndrome serotoninérgica e dessa forma são prescritos, como última opção, apenas quando outros antidepressivos falham no tratamento da depressão (HILLHOUSE e PORTER, 2015; PEÑA et al., 2016; TIAN et al., 2022).

Entre os antidepressivos não clássicos estão os mais prescritos, que são os inibidores seletivos de recaptação de serotonina (ISRS), estes são os mais comuns como antidepressivos de primeira linha para o tratamento da depressão (FASIPE, 2018; TIAN et al., 2022). No final dos anos 1960, surgiram indícios apontando a importância da serotonina no TDM (HILLHOUSE e PORTER, 2015). O mecanismo de ação desta classe envolve a inibição seletiva do transportador de serotonina (SERT), aumentando a disponibilidade de 5-HT nas sinapses (FASIPE, 2018; TIAN et al., 2022). Apesar de os ISRS serem mais seguros do que os TCAs e os IMAOs, eles também apresentam efeitos adversos como insônia, náuseas e disfunção sexual, bem como o efeito terapêutico é retardado devido a dessensibilização dos receptores 5-HT_{1A} quando o uso dos ISRS é prolongado (HILLHOUSE e PORTER, 2015; FASIPE, 2018; TIAN et al., 2022).

Outras classes de antidepressivos existentes são os inibidores de recaptação de serotonina e noradrenalina (IRSN), inibidores de recaptação de noradrenalina (IRN) e os inibidores de recaptação de noradrenalina e dopamina (IRND) e antidepressivos multimodais (AMMs), tendo seus mecanismos de ação agindo sobre o sistema monoaminérgico, entretanto, apresentam efeitos colaterais como náuseas, boca seca, insônia, dentre outros Por fim, os agonistas/antagonistas inverso/agonista parcial do receptor iônico de N-metil-D-aspartato (NMDA)-glutamatérgico, agindo diretamente sobre o sistema de neurotransmissão glutamatérgica excitatória e podem causar efeitos colaterais como tontura, sedação, aumento da pressão arterial e alteração na percepção (HILLHOUSE e PORTER, 2015; FASIPE, 2018; TIAN et al., 2022). Todos os antidepressivos disponíveis, apesar de sua eficácia, estão associados a uma variedade de efeitos colaterais, que impactam não só bem-estar dos pacientes, mas também afetam a adesão ao tratamento. Dessa forma, a busca por novas alternativas com maior eficácia e menores efeitos colaterais tem sido uma constante, visando proporcionar opções de tratamento mais seguras e toleráveis, otimizando os resultados terapêuticos e melhorando a qualidade de vida dos pacientes (MOAGAR-POLADIAN et al., 2017; GRATTAN et al., 2019; CHUAI et al., 2021).

3.4 BENZAMIDA

Entre os novos compostos com potencial terapêutico para o tratamento da depressão, as benzamidas se destacam devido à sua ampla gama de atividades farmacológicas, incluindo efeitos anticonvulsivantes, anti-inflamatórios, analgésicos e antidepressivos (RANA et al., 2008; SIDDIQUI et al., 2008; PERIN et al., 2018). A benzamida é uma molécula derivada da amina do ácido carbônico, que por sua vez é originária do ácido benzoico (Figura 2), descoberto por meio da simplificação molecular da cocaína (CHIRITA et al., 2010; BESCKOW et al., 2020; LEDEBUHR et al., 2024).

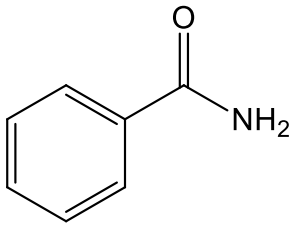

Figura 2. Estrutura química da benzamida.

Dependendo do composto, as benzamidas podem exibir atividades antidepressiva, anticonvulsivante, anti-inflamatória, analgésica, além de influenciar a 5-HT, e apresentar propriedades antitumorais e antimicrobianas (WANG et al., 2010; SUCHETAN et al., 2016; CHIRIȚĂ et al., 2017). São amplamente reconhecidas como uma classe de agentes farmacológicos com várias aplicações no tratamento de distúrbios neurológicos e psiquiátricos. As atividades biológicas observadas em estudos sobre estruturas orgânicas estão diretamente associadas à classe das benzamidas (CHIRIȚĂ et al., 2017).

Essas atividades geralmente são obtidas por meio de modificações na estrutura química da benzamida. Muitas benzamidas substituídas demonstram propriedades bioativas, o que as torna potenciais candidatos para desenvolvimento farmacológico (CHIRIȚĂ et al., 2017). Moléculas que apresentam núcleo benzamida, que é caracterizado por uma estrutura de amida associada ao anel aromático benzênico, são cruciais devido ao seu potencial perfil farmacológico, o que favorece sua aplicação como agentes antibacterianos, antifúngicos, imunomoduladores, antipsicóticos, entre outros (LEAL et al., 2009).

Um estudo apresentou uma série de moléculas sintetizadas contendo núcleo benzamida e todas foram avaliadas quanto ao seu potencial como antipsicóticos

multireceptores (YANG et al., 2016). As benzamidas sintetizadas foram desenvolvidas para atuar nos receptores de dopamina D_2 e serotonina 5-HT_{1A} e 5-HT_{2A}, visando tratar sintomas positivos, negativos e cognitivos da esquizofrenia com menor incidência de efeitos colaterais (YANG et al., 2016). Das benzamidas sintetizadas para este estudo, foram identificadas duas benzamidas com excelentes perfis farmacológicos (YANG et al., 2016).

Além disso, esses compostos reduziram a hiperatividade induzida por PCP (um modelo para sintomas psicóticos) em modelos animais sem provocar catalepsia, o que indica baixa propensão a causar efeitos motores extrapiramidais (YANG et al., 2016). Os resultados deste estudo sugeriram que esses novos compostos têm potencial para avançar em estudos pré-clínicos, oferecendo um perfil de segurança e eficácia promissor para o tratamento da esquizofrenia (YANG et al., 2016). Essas características sugerem um perfil favorável para o uso como antipsicóticos, com menos riscos de efeitos adversos motores, comparado a fármacos convencionais.

Em outro estudo, moléculas com núcleo benzamida foram descritas como antagonistas do receptor de dopamina D_2, com foco no tratamento de sintomas neuropsiquiátricos, como isolamento social que é um sintoma comum em transtornos como esquizofrenia e depressão (IKE et al., 2024). Um dos benefícios das benzamidas, mostrados neste estudo, é o seu papel na modulação do receptor D_2 que regula a liberação de dopamina impactando diretamente no comportamento social (IKE et al., 2024). Ao inibir o receptor D_2, essas moléculas contendo o núcleo benzamida, promovem a liberação da dopamina aumentando a sociabilidade e melhorando o isolamento social patológico (IKE et al., 2024).

Dessa forma, as benzamidas são investigadas como agentes promissores para o tratamento de distúrbios que envolvem sintomas de retraimento social (IKE et al., 2024). As benzamidas substituídas possuem a capacidade ampla de modular as vias neuronais e esta classe possui diversos agentes antipsicóticos atípicos que demonstraram eficácia no tratamento de sintomas negativos de esquizofrenia, devido a combinação de efeitos antipsicóticos e antidepressivos (RACAGNI et al., 2004; GRATTAN et al., 2019). Aliás, as benzamidas substituídas apresentam boa tolerabilidade, devido à sua menor propensão de causarem efeitos extrapiramidais quando comparado aos antipsicóticos tradicionais (RACAGNI et al., 2004; GRATTAN et al., 2019).

Isso reforça o valor das moléculas sintetizadas contendo o núcleo benzamida como agentes complexos e com alto potencial terapêutico, oferecendo abordagens

abrangentes para o tratamento de sintomas de transtornos depressivos, psiquiátricos, dentre outros. Entretanto, a falta de tratamentos eficazes para várias doenças psiquiátricas e a complexidade dos efeitos colaterais dos medicamentos disponíveis ressaltam a necessidade de pesquisas para descobrir novas moléculas e alternativas, sugerindo novas aplicabilidades de novos fármacos a partir de modificações estruturais da benzamida (MOAGAR-POLADIAN et al., 2017; CHIRIȚĂ et al., 2017; GRATTAN et al., 2019).

3.5 COMPOSTOS ORGÂNICOS DE SELÊNIO

O Se, um elemento químico descoberto há mais de 180 anos pelo sueco Jöns Jacob Berzelius, tem sido objeto de numerosos estudos sobre suas propriedades químicas e atividades biológicas (SURAI, 2022). Durante muito tempo, acreditava-se que a principal atividade biológica dos compostos de Se estava relacionada exclusivamente à sua toxicidade, e algumas teorias até associavam eventos históricos a envenenamentos por Se (SANTI et al., 2017; SONEGO et al., 2023). No entanto, a partir da década de 1950, embora estudos atuais confirmem que alguns distúrbios em animais e humanos são causados pela bioacumulação de Se, ficou claro que esse elemento é essencial para a vida (SANTI e BAGNOLI, 2017; KIELISZEK e BANO, 2022; SONEGO et al., 2023).

Atualmente, reconhece-se o Se como um micronutriente essencial, cuja presença em determinadas selenoproteínas desempenha um papel crucial na manutenção do equilíbrio redox nos sistemas biológicos (SANTI e BAGNOLI, 2017; CHUAI et al., 2021; XU-XU et al., 2022; GU e GAO et al., 2022; CHEN et al., 2024). Dada a sua importância no SNC, manter níveis adequados de Se é crucial para a saúde e o bem-estar geral, pois níveis baixos de Se têm sido associados a diversos problemas como um aumento do risco de mortalidade, comprometimento da função imunológica e declínio cognitivo, bem como TDM (BAJ et al., 2023).

Estudos pré-clínicos em animais sugerem que o Se pode exercer efeitos antidepressivos ao influenciar os sistemas dopaminérgico e serotoninérgico (ISLAM et al., 2018; BAJ et al., 2023). Além disso, a relação entre o Se e os níveis do fator neurotrófico derivado do cérebro (BDNF) pode impactar a neuroplasticidade e desempenhar um papel na fisiopatologia da depressão (SHIMIZU et al., 2003; BAJ et al., 2023). Nesse contexto, os compostos orgânicos de Se vêm despertando crescente interesse devido ao seu potencial biológico e terapêutico (CHUAI et al., 2021; XU-XU et al., 2022). Esses compostos possuem uma ampla gama de aplicações, muitas das quais estão associadas às suas propriedades farmacológicas (BARBOSA et al., 2017).

Os compostos orgânicos de Se têm se destacado devido a suas propriedades terapêuticas promissoras, incluindo efeitos antidepressivos, anti-inflamatórios e antinociceptivos (BRÜNING et al., 2015; OLIVEIRA et al., 2016; BAMPI et al., 2020; BESCKOW et al., 2020; BIRMANN et al., 2023; LEDEBUHR et al., 2024). Além disso, tanto compostos naturais quanto sintéticos contendo Se demonstraram atividades antitumorais, antioxidantes, antifibrolíticas, antiparasitárias, antibacterianas, antivirais,

antifúngicas e neuroprotetoras (CHUAI et al., 2021). O Se desempenha também um papel significativo na redução de inflamações devido as suas propriedades anti-inflamatórias e imunomoduladoras importantes, atuando em diversos processos celulares e auxiliando na regulação da resposta imunológica (MAL'TSEVA et al., 2022).

Compostos contendo Se tem sido associados a efeitos protetores contra o estresse oxidativo e a inflamação (DUNTAS et al., 2009; MAL'TSEVA et al., 2022; MAHMOODPOOR et al., 2022). Estes compostos conseguem reduzir a expressão de citocinas pró-inflamatórias, como a IL-6 e TNF-α, e atenuar a ativação de vias de sinalização inflamatórias, como a NF-κB (DUNTAS et al., 2009; MAL'TSEVA et al., 2022; MAHMOODPOOR et al., 2022). Assim, o Se contribui para a diminuição de processos inflamatórios em condições patológicas, além de oferecer suporte ao sistema imunológico frente a infecções, sendo considerado um elemento importante para o controle da inflamação, além de ser um potencial agente adjuvante terapêutico na prevenção e tratamento de doenças associadas a processos inflamatórios (DUNTAS et al., 2009; MAL'TSEVA et al., 2022; MAHMOODPOOR et al., 2022).

Um estudo mostrou um composto orgânico de selênio, contendo o grupamento CF_3 em uma porção da molécula ($(m\text{-}CF_3\text{-}PhSe)_2$), como um multialvo que mostrou efeitos terapêuticos promissores na prevenção de comportamentos depressivos e hipersensibilidade à dor em modelos animais de dor e depressão (BRÜNING et al., 2011; BRÜNING et al., 2015). Este trabalho indicou que a molécula $(m\text{-}CF_3\text{-}PhSe)_2$ possui propriedades antinociceptivas e antidepressivas, associadas a uma potente ação anti-inflamatória reduzindo níveis de citocinas pró-inflamatórias, como TNF-α e IL-1β, além de modular a ativação da proteína quinase p38 (p38 MAPK) e normalizar o BDNF (BRÜNING et al., 2015).

Nesta molécula, a presença do grupamento CF_3 na estrutura da molécula potencializou a ação deste composto, aumentando a sua lipofilicidade, permitindo-lhe atravessar a BHE e elevando a biodisponibilidade no SNC (BRÜNING et al., 2011; BRÜNING et al., 2015). Além disso, o grupamento CF_3 contribuiu para a estabilidade do composto, permitindo maior interação com os sistemas biológicos, ampliando sua eficácia no aumento da disponibilidade de 5-HT na fenda sináptica, o que é um diferencial no tratamento de sintomas depressivos (BRÜNING et al., 2011; BRÜNING et al., 2015). A combinação de efeitos anti-inflamatórios e de regulação do sistema serotoninérgico e glutamatérgico tornou esta molécula promissora para o tratamento da dor neuropática associada a depressão (BRÜNING et al., 2015).

No entanto, há poucos estudos que exploram o desempenho biológico e o

mecanismo de ação de compostos que combinam benzamidas e Se. Assim, o desenvolvimento de moléculas que integram essas estruturas representa uma estratégia promissora no tratamento de pacientes com TDM, oferecendo potencial terapêutico significativo (SIDDIQUI et al., 2008; CHUAI et al., 2021).

4. RESULTADOS

Os resultados desta tese de doutorado são apresentados sob a forma de um artigo científico e dois manuscritos. Os ítens dos materiais e métodos, resultados, discussão e referências estão incluídos nos próprios artigos, os quais foram estruturados conforme as normas das revistas onde o artigo foi publicado e os dois manuscritos submetidos para publicação. Em anexo a esta tese, encontram-se as aprovações dos projetos de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Pelotas.

4.1 ARTIGO

_N_-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl) benzamide induces antidepressant-like effect in mice: involvement of the serotonergic system.

Camila Simões Pires, Marcia Juciele da Rocha, Marcelo Heinemann Presa, Narryman Pinto Zuge, Natália Emanuele Biolosor Kuntz, Benhur Godoi, Cristiani Folharini Bortolatto, César Augusto Brüning.

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ORIGINAL INVESTIGATION

N-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl) benzamide induces antidepressant-like effect in mice: involvement of the serotonergic system

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Abstract

Rationale Major Depressive Disorder (MDD) significantly impairs the quality of life for those affected. While the exact causes of MDD are not fully understood, the deficit of monoamines, especially serotonin and noradrenaline, is widely accepted. Resistance to long-term treatments and adverse effects are often observed, highlighting the need for new pharmacological therapies. Synthetic organic compounds containing selenium have exhibited pharmacological properties, including potential antidepressant effects.

Objective To evaluate the antidepressant-like effect of *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl) benzamide (CF_3SePB) in mice and the involvement of the serotonergic and noradrenergic systems.

Methods Male Swiss mice were treated with CF_3SePB (1–50 mg/kg, i.g.) and 30 min later the forced swimming test (FST) or tail suspension test (TST) was performed. To investigate the involvement of the serotonergic and noradrenergic systems in the antidepressant-like effect of CF_3SePB, mice were pre-treated with p-CPA (a 5-HT depletor, 100 mg/kg, i.p.) or the receptor antagonists WAY100635 (0.1 mg/kg, s.c., a 5-HT_{1A} receptor antagonist), ketanserin (1 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist), ondansetron (1 mg/kg, i.p., a 5-HT_3 receptor antagonist), GR110838 (0.1 mg/kg, i.p., a 5-HT_4 receptor antagonist), prazosin (1 mg/kg, i.p., an α_1-adrenergic receptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenergic receptor antagonist) and propranolol (2 mg/kg, i.p., a non-selective beta-adrenergic receptor antagonist) at specific times before CF_3SePB (50 mg/kg, i.g.), and after 30 min of CF_3SePB administration the FST was performed.

Results CF_3SePB showed an antidepressant-like effect in both FST and TST and this effect was related to the modulation of the serotonergic system, specially the 5-HT_{1A} and 5-HT_3 receptors. None of the noradrenergic antagonists prevented the antidepressant-like effect of CF_3SePB. The compound exhibited a low potential for inducing acute toxicity in adult female Swiss mice.

Conclusion This study pointed a new compound with antidepressant-like effect, and it could be considered for the development of new antidepressants.

Keywords Depression · Serotonergic system · Noradrenergic system · Benzamide · Selenium · Mice

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Introduction

Major Depressive Disorder (MDD) is a clinical condition characterized by symptoms of persistent sadness, discouragement, and lack of interest in pleasurable activities. MDD can also cause sleep disturbances, tiredness and lack of concentration and appetite (WHO 2022) as well a great distress to the affected person and lead to dysfunction in their daily activities (Gong et al. 2020). It can be of moderate or severe intensity and of long duration, becoming a critical health condition (OPAS 2021). Some studies suggest that depression may have a genetic cause, while others suggest that it is due to a contribution of genetic and environmental factors, such as major life stressors (Gohar et al. 2012).

There is neurobiological evidence that helps to understand depression. The monoaminergic theory is currently the most accepted one to explain the etiology of this disorder. This theory defines that the common pathway for depression is the deficit of neurotransmitters in the central nervous system (CNS), mainly serotonin (5-hydroxytryptamine, (5-HT) and noradrenaline (NA) (Coppen 1967), and most of currently available antidepressants act on one or more mechanisms compatible with the monoamine hypothesis. The subfamilies of serotonin receptors assist in regulating physiological functions, mood changes, emotions, memory, among others. Besides, NA is also implicated in the regulation of emotions (Moret and Briley 2011; Herrmann et al. 2004; Kandemir 2023).

The effectiveness of current antidepressants is limited for many people with MDD. This limitation predominantly arises from inadequate adherence to treatment due to the presence of adverse effects or concerns about tolerability. Moreover, the delayed commencement of therapeutic benefits, typically manifesting only during the second to fourth, or subsequent weeks of usage, adds to the diminished impact of these medications (Faquih et al. 2019). Consequently, it becomes imperative to explore novel pharmaceutical solutions that not only yield improved results but also come with fewer accompanying side effects for the purpose of treating depression.

Selenium is a trace element widely distributed in the human body. This element is essential for the maintenance of the human life because it is present in the essential aminoacid selenocysteine, in the enzyme glutathione peroxidase, and in most tissues of human body with the function of catalyzing the breakdown of hydrogen peroxide from the highly reactive metabolite (Steinbrenner and Sies 2013). Interestingly, organic compounds containing selenium demonstrate many pharmacological properties such as anti-inflammatory, antioxidant, antinociceptive, and antidepressant (Besckow et al. 2020; Nogueira and Rocha 2011; da Silva Teixeira Rech et al. 2021; Birmann et al. 2023; Anghinoni et al. 2023). In addition, benzamide is a molecule derived by from carbonic acid amine that is derived by from benzoic acid. Many compounds that have benzamide in their molecules have shown many properties such as anticonvulsivant, analgesic, and antidepressant (Besckow et al. 2020; Chirita et al. 2010).

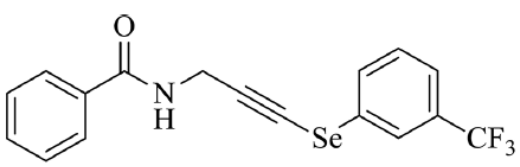

Fig. 1 Chemical structure of *N*-(3-((3-(trifluoromethyl)phenyl)selanyl) prop-2-yn-1-yl)benzamide (CF_3SePB)

Thus, this study evaluated the antidepressant-like effect of *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide (CF_3SePB) (Fig. 1), an organoselenium compound containing benzamide, in mice. As some organoselenium compounds or compounds containing benzamide showed pharmacological properties via modulation of the serotonergic and noradrenergic systems (Asif 2016; Brüning et al. 2014; Brüning et al. 2011; Tully and Donohue 2017; da Silva Teixeira Rech et al. 2021; Ledebuhr et al. 2024), the involvement of these systems in the antidepressant effect of CF_3SePB was also investigated.

Methodology

Animals

Adult Swiss mice (25–30 g) from the Central Biotery of the Federal University of Pelotas were used in this study. All the behavioral tests were performed with male mice, using them only once in each test ($n = 7$ to 12 animals/group, totalizing 374 male mice), with exception of open-field test (OFT), that was performed before the tail suspension test (TST) or the forced swimming test (FST) with the same animals. For the toxicity test, 6 female Swiss mice were used per group, totalizing 12 female mice. The animals were maintained at a temperature of 22 ± 2 °C with a 12 h light/12 hour dark cycle, and light at 7 a.m. The mice were placed in separate boxes ($20 \times 30 \times 13$, 5 animals per cage) with diet ad libitum that consisted of commercial feed and fresh water. The animals did not receive food during the experimental protocol. The animals spent 1 h acclimatizing before starting the experiment. All tests were timed and watched by a blinded observer. The experiments were done in accordance with the rules of the Ethics Committee on Animal Experimentation at UFPel (CEEA 28,792 – 2020) and is according to the National Institutes of Health Guide for the care and use of laboratory animals (NIH publications Nº 8023, revised

1978). All efforts were made to minimize the number of animals used and to alleviate animal suffering.

Drugs

The synthesis of the CF_3SePB (Fig. 1) occurred in the Nucleus of Synthesis and Application of Organic and Inorganic Compounds (NUSAACOI), from the Federal University of Fronteira Sul (UFFS).

According to Balbom et al. (2019), the analysis of ^{13}C and ^{1}H NMR spectra revealed that the analytical and spectroscopic data were in complete agreement with the assigned structure. Additionally, the chemical purity of CF_3SePB was determined using GC/MS. Initially, the CF_3SePB was solubilized in canola oil for intragastric administration at doses of 1, 10 and 50 mg/kg. Fluoxetine was used intraperitoneally (i.p.) at a dose of 20 mg/kg as a positive control to validate the data obtained in the behavioral tests assessing antidepressant-like effects. Other drugs used were *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2pyridinylcyclohexanecarboxamide (WAY100635), 1-[2-[(methylsulfonyl)-amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate (GR113808), ketanserin (+)-tartrate salt, ondansetron hydrochloride, p-chlorophenylalanine methyl ester (p-CPA), prazosin, yohimbine, an propranolol. The drug WAY100635 was administered subcutaneously (s.c.) and the other drugs were administered intraperitoneally (i.p.). Fluoxetine, p-CPA, and the antagonists WAY100635, ketanserin, ondansetron, prazosin, yohimbine, and propranolol were diluted in 0.9% saline. The antagonist GR113808 was suspended in 0.5% carboxymethyl cellulose (CMC) with 0.1% tween 80. All drugs were administered to mice in a constant volume of 10 mL/kg of body weight and obtained from Sigma-Aldrich (St. Louis, MO, USA). Fluoxetine was purchased locally.

Experimental design

Dose-response curve of the antidepressant-like effect of CF_3SePB in mice

To obtain the dose-response curve of the antidepressant-like action of CF_3SePB, mice were treated with CF_3SePB at doses of 1, 10 and 50 mg/kg (i.g.), fluoxetine 20 mg/kg (i.p.) or canola oil (control, 10 mL/kg, i.g.) 30 min before TST or FST. The open-field test (OFT) was performed 4 min before the TST with the same animals that underwent the TST. The doses were selected according to previous studies on the antidepressant-like action of organic selenium compounds (Besckow et al. 2020; Gall et al. 2020; da Rocha et al. 2023; Presa et al. 2023). Fluoxetine was used as the reference antidepressant drug to validate the behavioral tests (Shakiba et al. 2019).

The TST and FST are commonly used tests for screening of new drugs with antidepressant-like effect (Cryan and Slattery 2007; Chatterjee et al. 2012). The OFT is used to analyze the exploratory and locomotor activity of mice that are used ahead of the administration of new drugs (Valvassori et al. 2017), ruling out non-specific effects in screening tests of antidepressant-like action.

Evaluation of the involvement of the serotonergic system in the antidepressant-like effect of CF_3SePB

To evaluate the hypothesis that the antidepressant-like effect of CF_3SePB is related to the modulation of the serotonergic system, FST and the dose of 50 mg/kg of CF_3SePB were chosen. Multiple depression phenotypes can be replicated and assessed in animals (Hasler et al. 2004). An animal model of depression should closely resemble clinical symptoms (face validity). In this regard, one of the most frequently utilized tests for evaluating depressive-like behavior in rodents is the FST test (Steru et al. 1985).

Animals were pre-treated with p-CPA (100 mg/kg, i.p.) or saline, a selective inhibitor of the enzyme tryptophan hydroxylase that causes depletion of 5-HT, or vehicle once a day, for 4 consecutive days. Twenty-four hours after the last p-CPA injection, mice were treated with CF_3SePB (50 mg/kg, i.g.) or canola oil (vehicle, 10 mL/kg, i.g.) and, 30 min later, passed through the FST. Four minutes before the FST, the same animals performed the OFT (Besckow et al. 2020; Gall et al. 2020).

To assess the hypothesis that the antidepressant-like effect of CF_3SePB is involved with specific receptors of the serotonergic system, male mice were pretreated with WAY100635 (0.1 mg/kg, s.c., a $5\text{-}HT_{1A}$ receptor antagonist), ketanserin (1 mg/kg, i.p., a $5\text{-}HT_{2A/2C}$ receptor antagonist), ondansetron (1 mg/kg, i.p., a $5\text{-}HT_3$ receptor antagonist), or GR113808 (0.1 mg/kg, i.p., a $5\text{-}HT_4$ receptor antagonist). Animals were then treated with CF_3SePB (50 mg/kg, i.g.) or canola oil (vehicle, 10 ml/kg, i.g.) after 15 min of administration with the antagonists WAY100635, ketanserin, and ondansetron or 30 min after administration with GR113808. After 30 min following the administration of the compound or vehicle, the animals were subjected to FST for 6 min. The OFT was performed 4 min before the FST with the same animals to check the locomotor activity. The doses and times chosen for treatment with serotonergic receptor antagonists were in accordance with previous studies (Jesse et al. 2010; Castello et al. 2018; Gall et al. 2020).

Evaluation of the involvement of the noradrenergic system in the antidepressant-like effect of CF_3SePB

To evaluate the hypothesis that the antidepressant-like effect of CF_3SePB is related to the modulation of the noradrenergic system, FST and the dose of 50 mg/kg of CF_3SePB were also chosen. Mice were pretreated with prazosin (1 mg/kg, i.p., an α_1-adrenergic receptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenergic receptor antagonist), and propranolol (2 mg/kg, i.p., a non-selective beta-adrenergic receptor antagonist) (da Silva Teixeira Rech et al. 2021), and after 15 min they were treated with CF_3SePB (50 mg/kg, i.g.) or canola oil (vehicle, 10 ml/kg, i.g.). After 30 min following the administration of the compound or vehicle, the animals were subjected to FST for 6 min. To check the locomotor activity of the animal, OFT was performed for 4 min before the FST with the same animals (Besckow et al. 2020; Gall et al. 2020).

Behavioral tests

Open field test

To assess whether there was any possible change in the locomotor and exploratory activity of the animals, the mice were placed individually in the center of a wooden box (30 cm x 40 cm x 40 cm) with nine squares of the same size. Each mouse was observed for 4 min. During that period, it was counting the number of crossings among the squares and the number of elevations of animals on the two paws (rearing) (Walsh and Cummins 1976). At the end of each test, the box was cleaned with 20% alcohol to receive the next animal and leave no olfactory traces.

Tail suspension test

The animals were suspended by the tail and fixed with the aid of an adhesive tape in an apparatus 50 cm high. The mice were observed over a 6-minute period. In this time, immobility time and latency to the first episode of immobility were determined (Steru et al. 1985). Animals were considered immobile when they only hung motionless, indicating an absence of attempt-to-snap behavior escape. The longer the animals remain immobile, the greater the depressive-like behavior.

Forced swimming test

In this test, the animal is directly exposed to stress without the possibility of escape (Berton et al. 2021). The animals are placed in a 29 cm high cylindrical container containing 1.5 L of water at 25 ± 1 °C for 6 min. During this time, the latency to the first episode of immobility and the total immobility time were determined. The mice, when placed in the cylinder with water, try to escape this unfavorable situation by opting for swimming or floating. The longer the animals are floating, the greater the depressive-like behavior.

Acute oral toxicity

To assess toxicity in female mice, CF_3SePB was administered in 3 oral doses of 100 mg/kg, reaching the dose of 300 mg/kg, or vehicle, as indicated by Organization for Economic Cooperation and Development Guidelines (OECD 423, December 17, 2001). Females were fasted for 4 h before administration and with a maximum variation of 20% of body weight, as recommended by the OECD. Animals were observed every 30 min for the first 4 h and periodically during the first 24 h, and then daily for 14 days (OECD) to determine the lethal potential of benzamide. Food and water intake were monitored in the 2 cages/group containing 3 animals in each cage. On the 14th day, the OFT was performed to assess the locomotor activity of mice. After the OFT, the mice were anesthetized for the cardiac puncture procedure and the blood was collected in tubes containing heparin. Plasma was obtained after centrifugation and used for biochemical assays using commercial kits. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma were used as indicators of liver function (Reitman and Frankel 1957). Renal function was assessed by plasma urea levels (Mackay and Mackay 1927). Hemolyzed samples were discarded.

Statistical analysis

Experimental data are expressed as mean ± standard error of mean (S.E.M.). The normal distribution of data was evaluated by the D'Agostino and Pearson test. Comparisons between the experimental groups were performed using one-way (CF_3SePB dose-response curves) or two-way ANOVA (experiments with p-CPA and the antagonists) analysis of variance, followed by the Newman-Keuls post hoc test. After the two-way ANOVA, the post hoc test was performed only if there was a significant interaction between CF_3SePB and the antagonist. The unpaired t test was used to analyze the toxicity tests. Values of p lower than 0.05 ($p < 0.05$) were considered as statistically significant. All analyzes were performed using the GraphPad Software (GraphPad, San Diego, CA, USA).

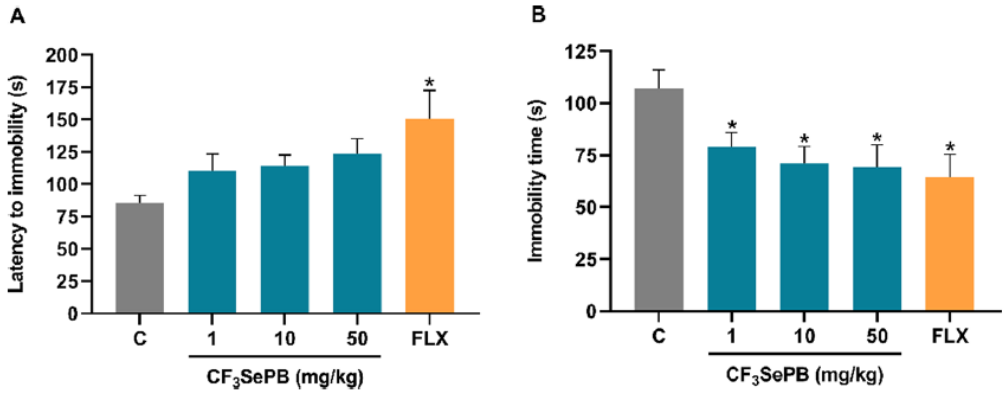

Fig. 2 Effect of CF_3SePB (1, 10 and 50 mg/kg, i.g.) and fluoxetine (20 mg/kg, i.p.) treatments on TST in mice. (**A**) Latency time for the first episode of immobility and (**B**) total immobility time. Fluoxetine was used as a reference drug. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 10–12$ animals/group). (*) $p < 0.5$ when compared with the control group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; C: control; FLX: fluoxetine; i.g.: intragastric; i.p.: intraperitoneally; TST: tail suspension test

Table 1 Effect of CF_3SePB administration on the OFT

Groups	Number of crossings	Number of rearings
Vehicle (canola oil)	64.5 ± 4.1	26.5 ± 1.9
CF_3SePB 1 mg/kg	79.3 ± 2.3	33.4 ± 2.1
CF_3SePB 10 mg/kg	70.1 ± 3.2	30.6 ± 2.3
CF_3SePB 50 mg/kg	72.5 ± 4.7	29.9 ± 2.6
Fluoxetine 20 mg/kg	74.8 ± 5.4	30.1 ± 3.3

Results

Antidepressant-like effect of CF_3SePB on TST

CF_3SePB demonstrated an antidepressant-like effect in mice on TST. The doses of 1, 10 and 50 mg/kg (i.g.) of CF_3SePB did not promote significant changes in relation to the control group in the latency to the first episode of immobility ($F_{(4,49)} = 3.212$, $p = 0.0203$, Fig. 2A). However, the immobility time of the animals in the TST was significantly reduced when CF_3SePB was administered at all doses, compared with the control group ($F_{(4,49)} = 3.473$, $p = 0.0142$, Fig. 2B). Fluoxetine, used as a positive control, promoted a reduction in immobility time and an increase in latency time, validating the results that demonstrate an antidepressant-like effect of the compound on TST in mice (Fig. 2). No change in locomotor activity was observed in OFT on crossing ($F_{(4,49)} = 0.8250$, $p = 0.5155$) and rearing ($F_{(4,49)} = 0.1922$, $p = 0.9414$) (Table 1).

The results represent the means ± standard error of the mean (S.E.M.) ($n = 10–12$ animals/group). Data analyses were conducted through one-way ANOVA. Abbreviation: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; OFT: Open field test.

Antidepressant-like effect of CF_3SePB on FST

The antidepressant-like effect of the CF_3SePB compound in mice was also evaluated in the FST. The doses of 10 and 50 mg/kg of CF_3SePB significantly increased the latency time to the first episode of immobility ($F_{(4,35)} = 7.973$, $p = 0.0001$, Fig. 3A) and all doses reduced the immobility time ($F_{(4,35)} = 15.19$, $P < 0.0001$, Fig. 3B) when compared with the control group. Fluoxetine, used as a positive control at a dose of 20 mg/kg, generated a reduction in immobility time and caused an increase in latency time when compared with the control group. In this way, it validated the results that showed an antidepressant-like effect of the compound (Fig. 3).

5-HT depletion blocked the effect of CF_3SePB

The impacts of 5-HT depletion through p-CPA treatment on the antidepressant-like effect of the CF_3SePB in mice are showed in the Fig. 4. Two-way ANOVA was performed to evaluate the interaction of CF_3SePB treatment on the latency time for the first episode of immobility (Fig. 4A, $F_{(1,38)} = 9.191$, $p = 0.0044$) and on the total immobility time (Fig. 4B, $F_{(1,38)} = 7.985$, $p = 0.0075$) of the animal in the FST. The treatment with the serotonin depletor p-CPA prevented the antidepressant-like effect of CF_3SePB, administered at a dose of 50 mg/kg in the FST.

According to Table 2, when the animals were treated with p-CPA and CF_3SePB, no changes were observed in the number of crossings and rearings in the OFT.

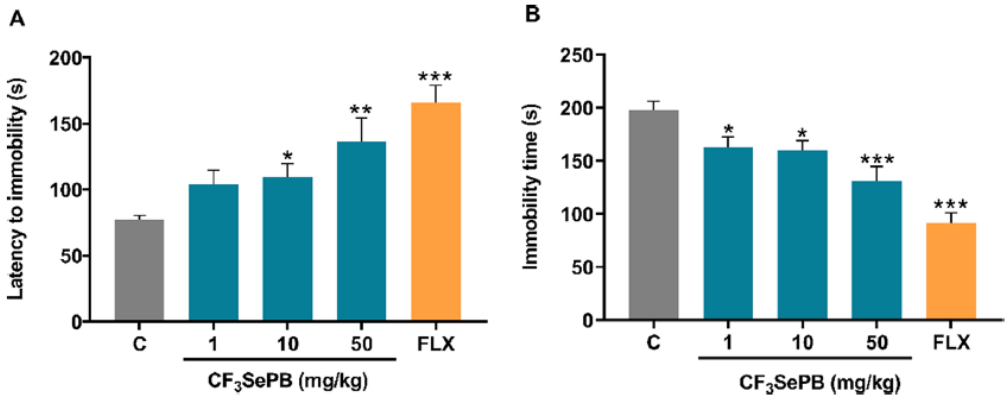

Fig. 3 Effect of CF_3SePB (1, 10 e 50 mg/kg, i.g.) and fluoxetine (20 mg/kg, i.p.) treatments on FST in mice. (**A**) Latency time for the first episode of immobility and (**B**) total immobility time. Fluoxetine was used as a reference drug. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 8$ animals/group). (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ when compared with the control group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl) prop-2-yn-1-yl)benzamide; C: control; FLX: fluoxetine; FST: forced swim test

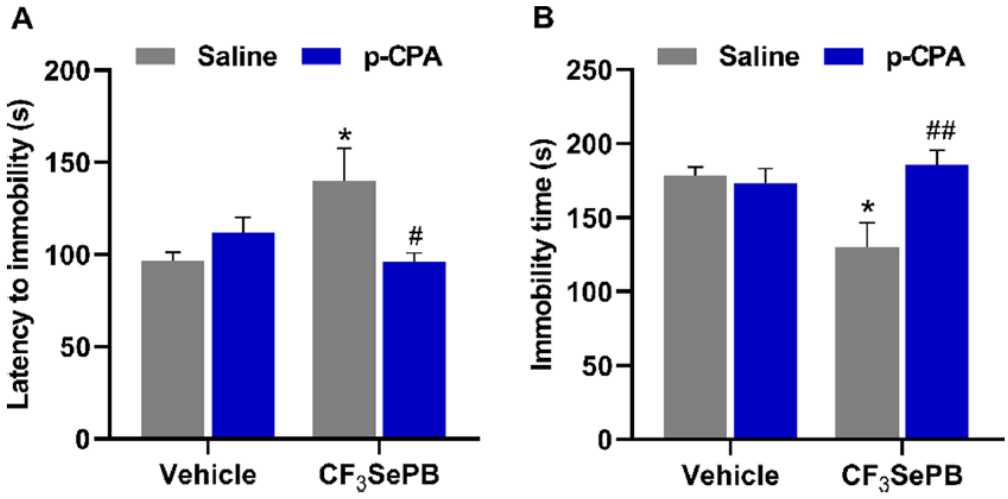

Fig. 4 Effect of pretreatment with p-CPA (100 mg/kg, i.p) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in the mouse FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 10$–11 animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. (*) $p < 0.05$, compared with the control group; (#) $p < 0.05$ and (##) $p < 0.01$ compared with the CF_3SePB group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; p-CPA: p-chlorophenylalanine; FST: Forced swim test

Involvement of serotonergic receptors in the CF_3SePB antidepressant-like effect in mice subjected to the FST

The results shown in Figs. 5to 8 show the analysis of the involvement of the serotonergic receptors in the antidepressant-like effect of CF_3SePB in mice on FST. From the data obtained in the FST, CF_3SePB demonstrated an antidepressant-like effect in mice at all doses tested. However, at the dose of 50 mg/kg, the effect was more significant. Thus, this was the dose chosen to perform the tests with antagonists of the serotonergic system.

Figures 5 A and 5B show that pre-treatment with WAY100635, which is a selective 5-HT_{1A} receptor antagonist, blocked the effect of CF_3SePB (50 mg/kg, i.g.). Data analysis showed that WAY100635 blocked the antidepressant-like effect of CF_3SePB as shown in the latency time for the first episode of immobility ($F_{(1,37)} = 9.332$, $p = 0.0042$) and in the total immobility time ($F_{(1,37)} = 6.017$, $p = 0.0190$) of the mice. The number of crossings as well as the number of rearings in the OFT were not altered by treatment with the compound CF_3SePB and/or WAY100635 (Table 2)

On the other hand, in the pre-treatment of mice with the 5-$HT_{2A/2C}$ receptor antagonist ketanserin, the increase in the latency time (Fig. 6A) and the reduction in the total immobility time (Fig. 6B) induced by CF_3SePB (50 mg/kg, i.g.) in the FST were not blocked by this antagonist. In the two-way ANOVA, the main effect of the CF_3SePB compound it was verified in the latency time for the first episode of immobility ($F_{(1,28)} = 21.66$, $p < 0.0001$) and in the total immobility

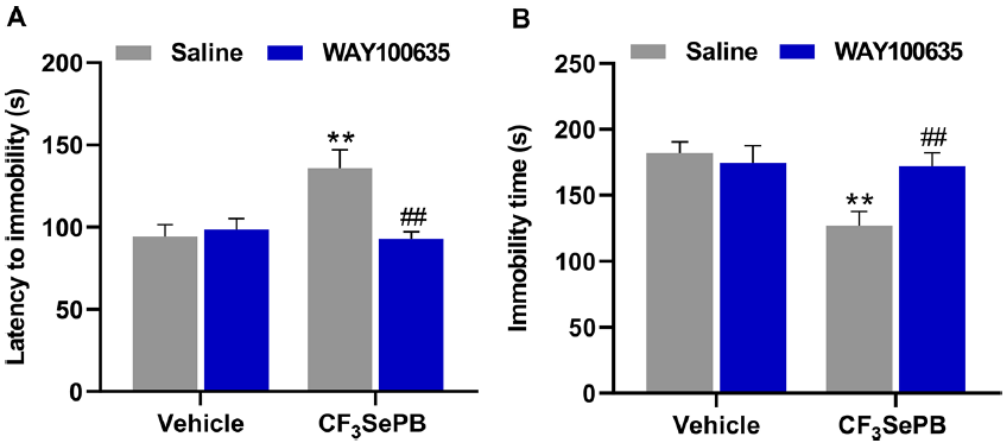

Fig. 5 Effect of pretreatment with WAY100635 (0.1 mg/kg, s.c.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 10–11$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. (**) $p < 0.01$ compared with the control group; (##) $p < 0.01$ compared with the CF_3SePB group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test

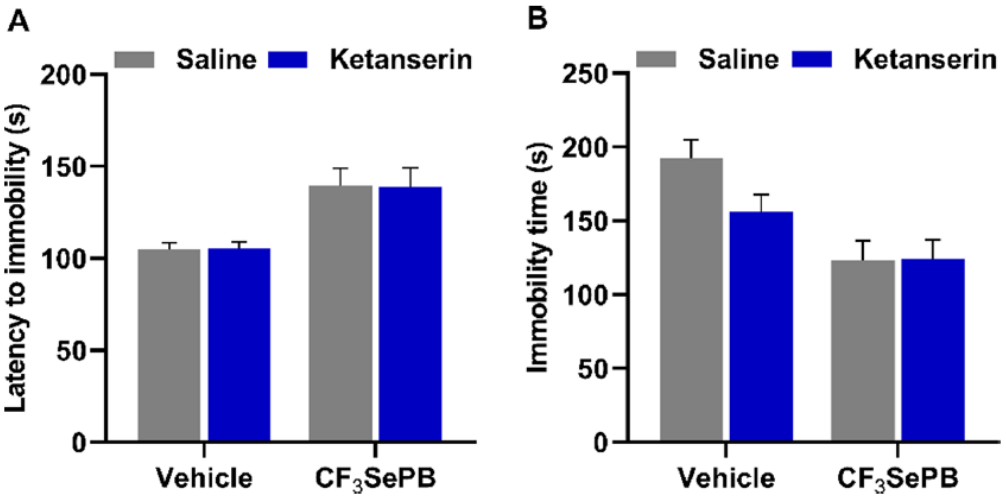

Fig. 6 Effect of pretreatment with ketanserin (1 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 8$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA). Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test

time ($F_{(1,28)} = 16.38$, $p = 0.0004$). As seen in Table 2, the treatment with ketanserin and/or CF_3SePB did not produce any change in the number of crossings and rearings of mice in the OFT.

Figure 7A and B describe the results that were obtained from the pre-treatment with the 5-HT_3 receptor antagonist ondansetron and the compound CF_3SePB. Ondansetron administration blocked the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) on FST in mice. Two-way ANOVA analysis showed the interaction between CF_3SePB and ondansetron pre-treatment on latency time for the first episode of immobility ($F_{(1,33)} = 4.830$, $p = 0.0351$) and on total immobility time ($F_{(1,33)} = 13.62$, $p = 0.0008$) in FST. In the OFT, no differences were observed in the number of crossings and rearings in mice that received treatment with ondansetron and CF_3SePB (Table 2).

The pretreatment with GR113808, a 5-HT_4 receptor antagonist, shown in Fig. 8A and B, did not prevent the antidepressant-like action of the CF_3SePB (50 mg/kg, i.g.). Two-way ANOVA showed main effect of CF_3SePB on latency to the first immobility episode ($F_{(1,34)} = 28.28$, $p < 0.0001$) and total immobility time ($F_{(1,34)} = 21.34$, $p < 0.0001$) in FST in mice. In the OFT, the number of crossings and rearings were not altered in animals that were treated with GR 113,808 and/or CF_3SePB (Table 2).

The results represent the means ± standard error of the mean (S.E.M.) ($n = 8–11$ animals/group). Data analyses were conducted through two-way ANOVA. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; p-CPA: p-chlorophenylalanine; CMC: carboxymethylcellulose; OFT: open field test.

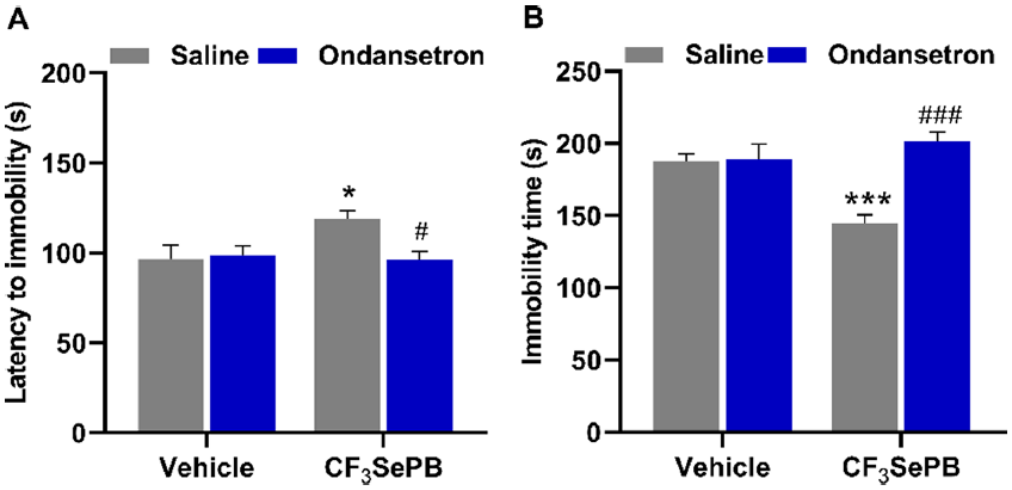

Fig. 7 Effect of pretreatment with ondansetron (1 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 9–10$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. (*) $p < 0.05$ and (***) $p < 0.001$ compared with the control group; (#) $p < 0.05$ and (###) $p < 0.001$ compared with the CF_3SePB group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl) prop-2-yn-1-yl)benzamide; FST: Forced swim test

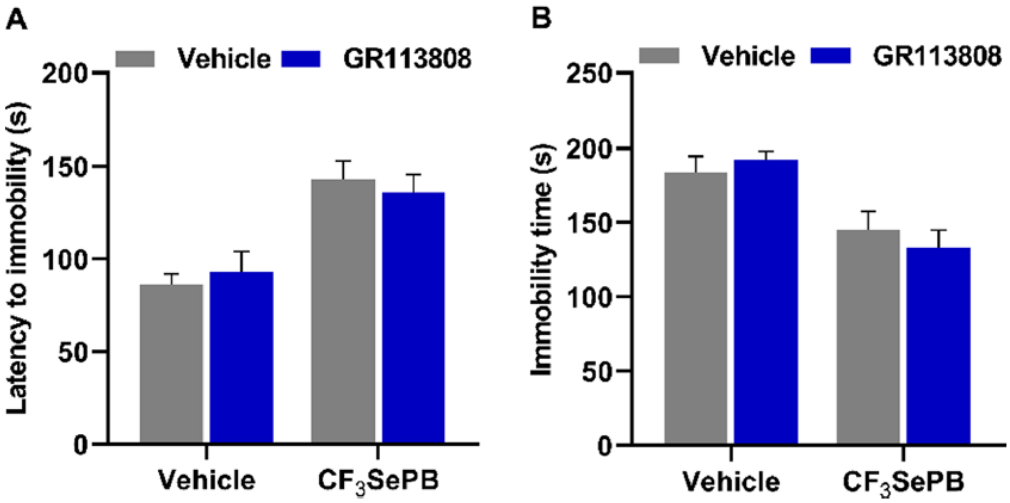

Fig. 8 Effect of pretreatment with GR113808 (0.1 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 9–10$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA). Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test

Noradrenergic receptors are not involved in the CF_3SePB antidepressant-like effect in mice subjected to the FST

The results shown in Figs. 9, 10 and 11 show the analysis of the involvement of the noradrenergic receptors in the antidepressant-like effect of CF_3SePB in mice on FST. Based on the data acquired from the FST, the dose of 50 mg/kg for the compound CF_3SePB was also selected to conduct tests involving antagonists of the noradrenergic system. Figure 9 shows that pre-treatment with prazosin, which is a selective α_1-adrenergic receptor antagonist, did not block the anti-immobility effect of CF_3SePB (50 mg/kg, i.g.) in the FST. Two-way ANOVA showed main effect of CF_3SePB in the latency time for the first episode of immobility ($F_{(1,25)} = 5.544$, $p = 0.0267$) (Fig. 9A) and in the total immobility time ($F_{(1,25)} = 7.46$, $p = 0.0114$) (Fig. 9B) of the mice. The number of crossings as well as the number of rearings in the OFT were not altered by treatment with the compound CF_3SePB and/or prazosin (Table 3).

The pretreatment with yohimbine, an α_2-adrenergic receptor antagonist, shown in Fig. 10, did not prevent the antidepressant-like action of the CF_3SePB (50 mg/kg, i.g.). Two-way ANOVA showed main effect of CF_3SePB on latency to the first episode of immobility ($F_{(1,28)} = 23.10$, $p < 0.0001$) (Fig. 10A) and total immobility time ($F_{(1,28)} = 18.88$, $p = 0.0002$) (Fig. 10B) in FST in mice. In the OFT, the number of crossings were not altered in animals that were treated with yohimbine and/or CF_3SePB (Table 3).

Pre-treatment of mice with the non-selective beta-adrenergic receptor antagonist, propranolol, did not prevented the anti-immobility effect of CF_3SePB (50 mg/kg, i.g.) in the FST (Fig. 11). The two-way ANOVA showed a main effect of CF_3SePB in the latency to immobility ($F_{(1,25)} = 4.268$,

Table 2 Effect of CF_3SePB, and/or 5-HT antagonists or p-CPA administration on the OFT behavioral parameters in mice

Experiment	Groups	Number of crossing	Number of rearing
p-CPA	Vehicle	73.9 ± 6.4	24.9 ± 2.1
	p-CPA (100 mg/kg)	78.4 ± 6.6	24.3 ± 3.0
	CF_3SePB (50 mg/kg)	67.3 ± 3.3	25.3 ± 3.1
	p-CPA + CF_3SePB	76.2 ± 6.6	26.7 ± 3.2
WAY100635	Vehicle	74.8 ± 7.9	35.0 ± 3.6
	WAY100635 (0.1 mg/kg)	74.8 ± 6.0	35.2 ± 2.8
	CF_3SePB (50 mg/kg)	74.2 ± 4.5	34.7 ± 1.8
	WAY100635 + CF_3SePB	75.4 ± 5.5	33.7 ± 2.9
Ketanserin	Vehicle	75.9 ± 9.5	29.0 ± 5.5
	Ketanserin (1 mg/kg)	75.6 ± 6.5	27.7 ± 1.9
	CF_3SePB (50 mg/kg)	85.2 ± 5.1	30.9 ± 3.9
	Ketanserin + CF_3SePB	69.6 ± 6.5	26.5 ± 2.7
Ondansetron	Vehicle	73.2 ± 5.0	33.2 ± 2.9
	Ondansetron (1 mg/kg)	69.8 ± 4.0	32.4 ± 3.9
	CF_3SePB (50 mg/kg)	69.1 ± 4.7	34.3 ± 2.8
	Ondansetron + CF_3SePB	75.0 ± 8.0	33.0 ± 3.9
GR 113808	Vehicle	81.9 ± 5.8	34.1 ± 2.4
	GR113808 (0.1 mg/kg)	71.3 ± 5.0	32.8 ± 1.9
	CF_3SePB (50 mg/kg)	60.9 ± 4.4	35.7 ± 3.7
	GR113808 + CF_3SePB	69.6 ± 5.3	34.7 ± 4.0

$p = 0.04$) and in the total immobility time ($F_{(1,25)} = 14.66$, $p = 0.0008$). The treatment with propranolol and/or CF_3SePB did not produce any change in the number of crossings and rearings of mice in the OFT (Table 3).

CF_3SePB has low potential to cause acute toxicity in mice

The administration of CF_3SePB (300 mg/kg, i.g.) did not cause any death during the 14 days in which the experimental protocol was performed and did not induce other signs of general toxicity (salivation, diarrhea, piloerection, lethargy, ptosis, and tremors/seizures) within the first 24 h after treatment. In relation to food consumption (Fig. 12A, df = 2, t = 2.037, $p = 0.1786$) and water consumption (Fig. 12B, df = 2, t = 1.550, $p = 0.2612$), there were no statistical differences between vehicle and CF_3SePB treated animals. Both vehicle and the CF_3SePB treated group gained weight (Fig. 12C, df = 10, t = 1.217, $p = 0.2516$). The number of crossings (Fig. 12D, df = 10, t = 0.199, $p = 0.8460$) and rearings (Fig. 12E, df = 10, t = 1.841, $p = 0.0955$) were not altered by CF_3SePB in relation to vehicle group. The treatment with CF_3SePB (300 mg/kg, i.g.) did not alter any of the enzymatic activities of AST (Fig. 12F, df = 10, t = 1.422, $p = 0.1855$) and ALT (Fig. 12G, df = 10, t = 0.3391, $p = 0.7416$) and plasma urea levels (Fig. 12H, df = 10, t = 1.984, $p = 0.0753$) compared to the vehicle group.

Discussion

In the present study, selenopropargyl benzamide CF_3SePB demonstrated a significant antidepressant-like effect in male Swiss mice. This activity was shown to be dependent on the interaction with the serotonergic system through the involvement of 5-HT_{1A} and 5-HT_3 receptors and not related to modulation of α_1-, α_2-, and β-adrenergic receptors. In addition, the treatment did not change the locomotor and exploratory activities of the animals when they were subjected to the OFT.

The antidepressant effect of CF_3SePB was verified through the FST and the TST, where the parameters evaluated were the total time in which the animals were immobile and the latency time to the first episode of immobility. Those tests are largely used in preclinical screening to identify the efficacy of new compounds with antidepressant properties and have strong predictive validity with a wide applicability to different compounds (Steru et al. 1985; Wang et al. 2017; Hao et al. 2019; Domingues et al. 2022). FST has a

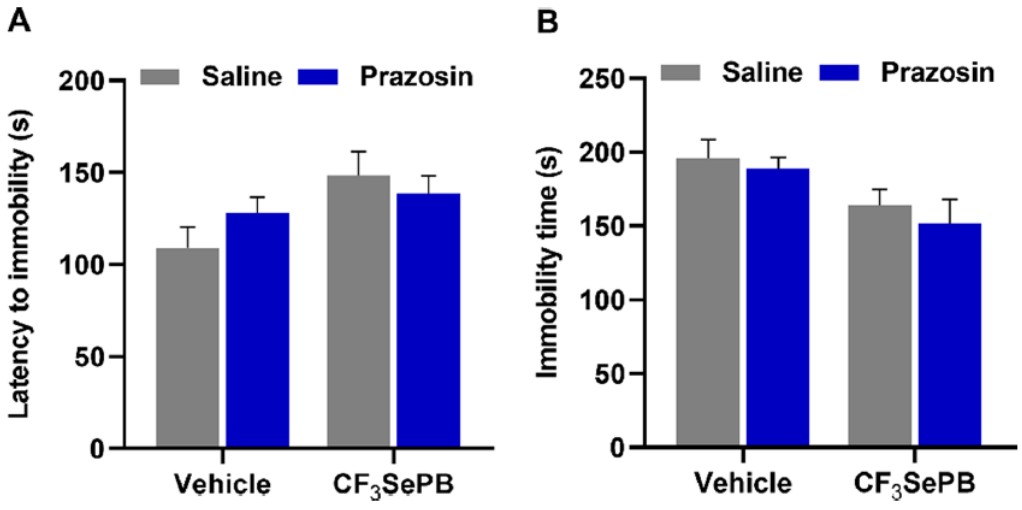

Fig. 9 Effect of pretreatment with prazosin (1 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 7$–8 animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA). Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test

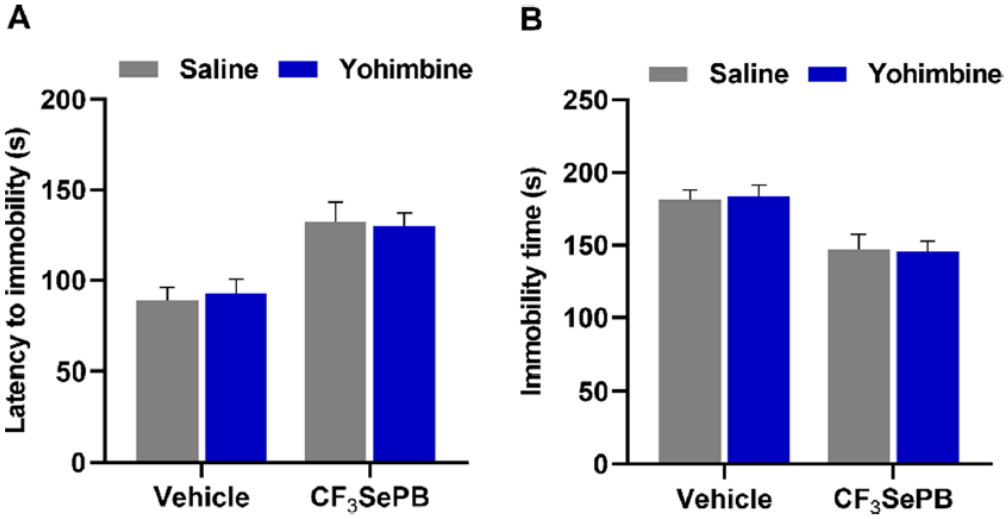

Fig. 10 Effect of pretreatment with yohimbine (1 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 8$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA). Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl) prop-2-yn-1-yl)benzamide; FST: Forced swim test

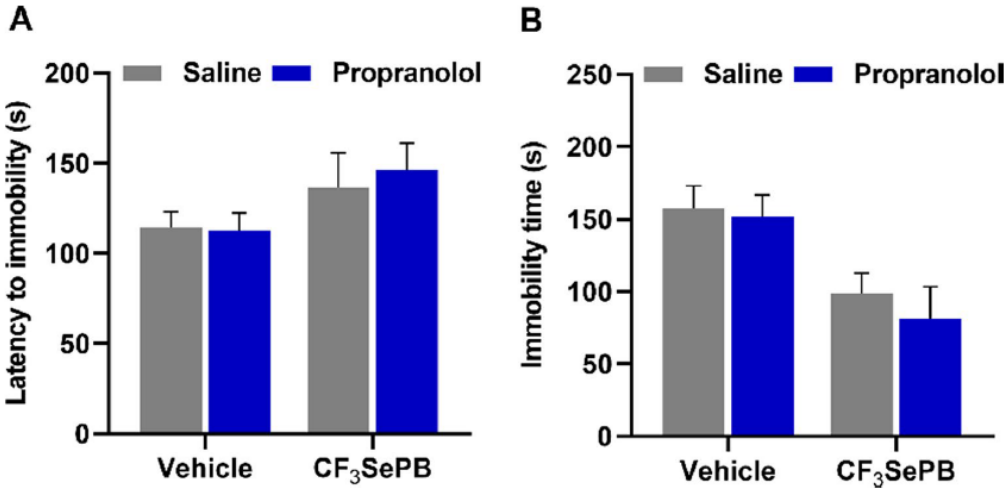

Fig. 11 Effect of pretreatment with propranolol (2 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (**A**) Latency to first episode of immobility and (**B**) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) ($n = 7–8$ animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA). Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl) prop-2-yn-1-yl)benzamide; FST: Forced swim test

Table 3 Effect of CF_3SePB, and/or 5-HT antagonists administration on the OFT behavioral parameters in mice

Experiment	Groups	Number of crossing	Number of rearing
Prazosin	Vehicle	60.7 ± 5.2	28.7 ± 3.9
	Prazosin (1 mg/kg)	59.5 ± 5.7	21.4 ± 2.7
	CF_3SePB (50 mg/kg)	62.5 ± 5.8	31.2 ± 4.6
	Prazosin + CF_3SePB	62.1 ± 4.7	28.6 ± 3.4
Yohimbine	Vehicle	73.6 ± 5.3	34.7 ± 2.6
	Yohimbine (1 mg/kg)	65.0 ± 4.6	30.7 ± 2.4
	CF_3SePB (50 mg/kg)	79.0 ± 4.9	34.3 ± 2.8
	Yohimbine + CF_3SePB	70.6 ± 2.7	35.3 ± 3.0
Propranolol	Vehicle	78.1 ± 3.6	43.8 ± 2.7
	Propranolol (2 mg/kg)	78.4 ± 2.0	43.1 ± 1.8
	CF_3SePB (50 mg/kg)	82.7 ± 5.6	43.5 ± 2.4
	Propranolol + CF_3SePB	79.5 ± 7.6	44.8 ± 2.5

The results represent the means ± standard error of the mean (S.E.M.) ($n = 7–8$ animals/group). Data analyses were conducted through two-way ANOVA. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl) phenyl)selanyl)prop-2-yn-1-yl)benzamide; OFT: open field test

broad-spectrum for testing the efficiency of antidepressants and TST has been considered a supplementary method for FST. Considering the importance of researching new treatments for major depressive disorder, an effective reversal of the immobility state of the animals is sought, since the antidepressant effect is reflected by the low immobility behavior of the mice. In this work, CF_3SePB presented antidepressant-like in both tests, from the dose of 1 mg/kg.

Selenium is an element distributed throughout the human body and is essential for the maintenance of human life, presenting several health benefits. Selenium is part of the selenoproteins that protect against oxidative stress, in addition to improving thyroid and immune functions, helping to improve mental illnesses and mood disorders (Steinbrenner and Sies 2013; Hossain et al. 2021). Interestingly, the lack or deficiency of selenium has been linked to mood disorders such as anxiety and depression (Pasco et al. 2012). Previous studies have demonstrated pharmacological properties of selenium-containing compounds, including antidepressant-like effects (Brüning et al. 2014, 2015; Birmann et al. 2019; Gall et al. 2020; Besckow et al. 2020; da Silva

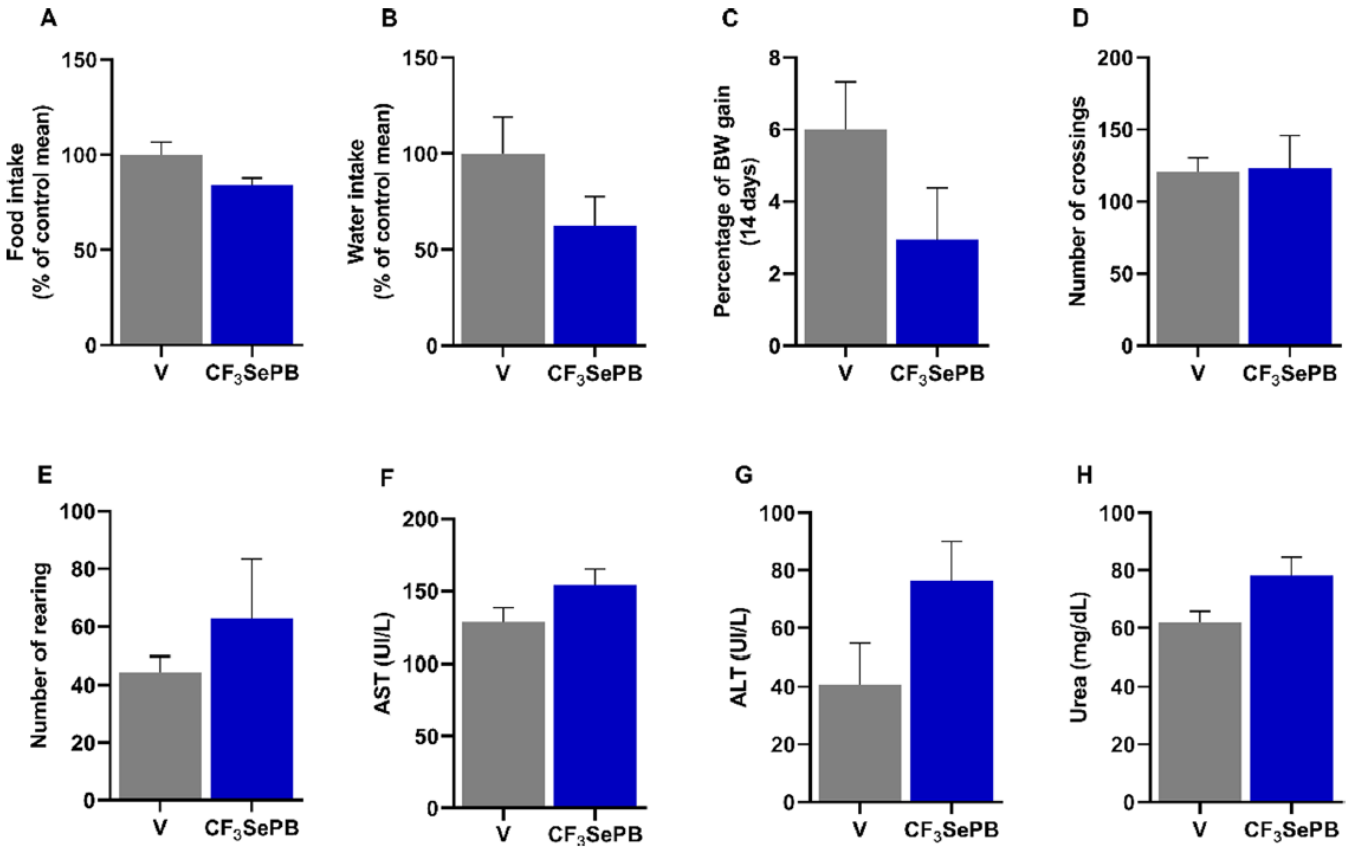

Fig. 12 Effect of CF_3SePB treatment (300 mg/kg, i.g.) on acute oral toxicity parameters in female mice. (**A**) Food and (**B**) water intake for 14 days mean for 2 cages per group (3 animals per cage) and (**C**) percentage of weight gain. (**D**) Number of crossings and (**E**) rearings in the OFT. Activities of (**F**) AST and (**G**) ALT, and (H) urea levels in the plasma ($n = 6$ animals/group). Values were expressed as mean ± standard error of mean (S.E.M.). Data were analyzed by the unpaired t test. Abbreviations: V: vehicle; CF_3SePB: *N*-(3-((3-(trifluoromethyl) phenyl)selanyl)prop-2-yn-1-yl)benzamide; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BW: body weight. OFT: open field test

Teixeira Rech et al. 2021; Ramli et al. 2022; Veloso et al. 2021). Furthermore, many compounds that have benzamide in their structure have shown various properties such as anticonvulsant, analgesic, and antidepressant (Chirita et al. 2010). Therefore, the combination of these molecules in a single compound has potential pharmacological advantages that need to be investigated and CF_3SePB is consistent with the compounds studied by our research group, which have already demonstrated antidepressant properties (Gall et al. 2020; Besckow et al. 2020; da Silva Teixeira Rech et al. 2021).

Serotonin is a neurotransmitter synthesized from the precursor amino acid tryptophan in the raphe nuclei with projections to various regions of the CNS (Quevedo and Izquierdo 2020). The serotonergic system is linked to various functions such as sleep and wakefulness, mood, appetite, circadian rhythm, and cognitive parts such as learning and memory, in addition to the autonomic processes (Jonathan, Sherin and Nemeroff 2011). Serotonin deficit has been related to the development of pathologies such as anxiety, eating and depressive disorders. The participation of serotonin and its derivatives in the mechanism of depression development has been extensively studied in recent decades (Gałecki and Talarowska 2018) and despite the high number of MDD cases, the pathophysiology of this disease is still not fully understood (Malhi and Man 2018). However, the development of resistance to long-term treatments is increasingly frequent (Akil et al. 2018), which demonstrates the need to seek new pharmacological therapies for this pathology. In general, antidepressants that act through the serotonergic system seek to increase the extracellular concentrations of serotonin, acting on different subtypes of serotonergic receptors, via the reuptake of the neurotransmitter in the synaptic cleft or its degradation (Hao et al. 2019).

To verify the influence of the serotonergic system on the activity of the CF_3SePB compound, a protocol was implemented in which serotonin was depleted in the animals' brains, inhibiting its synthesis with p-CPA. Tryptophan hydroxylase is the enzyme responsible for serotonin synthesis in the CNS and p-CPA is an irreversible inhibitor of this enzyme, which drastically reduces the level of serotonin in

the noradrenergic system, more specifically α_1-, α_2-, and β-adrenergic receptors.

In this study, the acute oral toxicity of CF_3SePB at a high dose (300 mg/kg) in female mice was also evaluated and it was evidenced that there was no animal mortality or signs of general toxicity. The locomotor activity of mice was not altered, as evidenced by the number of crossings and rearings in the OFT. The activities of AST and ALT, as well as the plasma levels of urea, were not significantly altered, demonstrating that CF_3SePB did not cause acute hepatic or renal toxicity. In addition to these parameters, water and food intake were not significantly altered, although they tended to be reduced in mice that received CF_3SePB compared to control animals. These results show that CF_3SePB has a low potential to induce toxic effects. Despite of this, considering that antidepressant drugs are usually administered chronically, the toxicity of CF_3SePB should be also evaluated during chronic administration in future studies.

Conclusion

Overall, the results reported in this study showed for the first time the antidepressant-like effect of oral administration of CF_3SePB in mice. We also illustrate the involvement of the serotonergic system in this effect, specifically the modulation of 5-HT_{1A} and 5-HT_3 receptors and no involvement with the noradrenergic system. The findings of this study may contribute to the development of new strategies in the treatment of depression. However, further studies are needed to understand other mechanisms of action involved in the antidepressant-like effect of CF_3SePB.

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Author contributions Conceptualization: C.S.P and C.A.B; Data curation: C.S.P., M.J.R., M.H.P., N.P.Z., N.E.B.K.; Formal analysis; C.S.P., M.J.R., M.H.P., N.P.Z., and N.E.B.K.; Funding acquisition: B.G., C.F.B. and C.A.B.; Investigation: C.S.P., M.J.R., M.H.P., N.P.Z., and N.E.B.K.; Methodology: C.S.P., M.J.R., M.H.P., N.P.Z., and N.E.B.K.; Project administration: C.S.P., B.G., C.F.B., and C.A.B.; Resources: B.G., C.F.B. and C.A.B.; Supervision: B.G., C.F.B. and C.A.B.; Validation: C.S.P., B.G., C.F.B., and C.A.B.; Visualization: C.S.P., B.G., C.F.B., and C.A.B. Roles/Writing - original draft: C.S.P., M.J.R., M.H.P., N.P.Z.: Writing - review & editing: B.G., C.F.B., and C.A.B.

Data availability Data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

The authors declare that there are no conflicts of interest. The experiments were done in accordance with the rules of the Ethics Committee on Animal Experimentation at UFPel (CEEA 28792 – 2020) and is according to the National Institutes of Health Guide for the care and use of laboratory animals (NIH publications Nº 8023, revised 1978). All efforts were made to minimize the number of animals used and to alleviate animal suffering.

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4.2 MANUSCRITO 1

Dopaminergic receptors involvement in the antidepressant effect of *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl) benzamide in mice

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Abstract

Major Depressive Disorder (MDD) directly impacts the lives of countless individuals worldwide, yet its causes remain incompletely understood. However, it is recognized that a deficiency in monoamines, including dopamine, may contribute to this disorder. *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl) (CF_3SePB) is an organoselenium compound that presented antidepressant-like effect in mice related to modulation of serotonergic, but not noradrenergic system. To expand the knowledge about CF_3SePB mechanisms of action, this study aimed to evaluate the involvement of dopaminergic system in its antidepressant-like effect. Male Swiss mice were pre-treated with the haloperidol (0.05 mg/kg, i.p., a non-selective dopaminergic receptor antagonist), SCH 23390 (0.01 mg/kg, s.c., a D_1 receptor antagonist), and sulpiride (50 mg/kg, i.p., a D_2 receptor antagonist) 15 min before CF_3SePB (50 mg/kg, i.g.), and after 30 min of CF_3SePB administration the forced swimming test (FST) was performed. CF_3SePB presented an anti-immobility effect in the FST, demonstrated by increase in the latency to first episode of immobility and reduction of total immobility of mice, and the pre-treatment of mice with haloperidol, SCH 23390 and sulpiride prevented these effects, showing that the antidepressant-like effect of CF_3SePB is related to the modulation of the dopaminergic system, specifically the D_1 and D_2 receptors. In addition, in silico pharmacokinetic profiling of CF_3SePB predicted its low likelihood of inducing adverse effects and potential to cross the blood-brain barrier. These results expand the understanting of CF_3SePB mechanisms for its antidepressant-like effect, reinforcing the potential of this organonoselenium compound for the development of a new antidepressants.

Keywords: Depression. Dopaminergic system. Benzamide. Selenium. Forced swimming test. Mice.

1. Introduction

Major depressive disorder (MDD) is characterized as a chronic psychiatric disorder where the bearer has depressed mood, cognitive dysfunction, inability to feel pleasure, anhedonia, guilt, decreased energy, low self-esteem, disturbed sleep or lack of appetite and concentration (Rehm and Shield, 2019; Sampaio et al., 2020; Riga et al., 2020). This disorder not only presents high morbidity but also a significant recurrence rate, making its management challenging. Additionally, studies suggest that depression may have a genetic basis, while others propose that depression has biological, psychosocial, personality-based, among other, playing important roles in the development of this disorder (Gohar et al., 2012; Shao and Zhu, 2020). A comprehensive understanding of these factors is essential for an effective approach to the treatment and prevention of major depressive disorder.

Despite the fact that the pathophysiological mechanisms underlying depression have not yet been fully elucidated (Pitsillou et al., 2020), the monoaminergic theory of depression is one of the most influential explanatory perspectives for understanding the pathophysiology of this psychiatric disorder. This theory suggests that depression is related to imbalances in monoaminergic neurotransmitters, particularly dopamine (DA), serotonin (5-HT), and noradrenaline (NA), in the brain, thereby triggering the signs and symptoms of depressive disorders (Delmondes et al., 2023). Given the complexity of the pathological mechanism of MDD, many antidepressant medications, precise diagnostic options, and pharmacological therapeutic strategies are quite restricted in terms of their selectivity and therapeutic efficacy (Cui et al., 2024; Delmondes et al., 2023).

The adequate presence of selenium, a trace element, is crucial for the well-being and proper functioning of the human body; its insufficiency or absence has been associated with mood disorders such as anxiety and depression (Pasco et al., 2012; Wang et al., 2018). Recent studies have highlighted the potential of synthetic organic selenium compounds in animal tests and depression models (Gall et al., 2020; Besckow et al., 2020; Rech et al., 2021; Birmann et al., 2023), which has sparked greater interest due to their pharmacological properties (Nogueira et al., 2004; Nogueira and Rocha, 2011, Anghinoni et al., 2023) such as anticonvulsant, analgesic, and antidepressant (Besckow et al., 2020; Ledebuhr et al., 2024; Chirita et al., 2010). A previous study demonstrated that CF_3SePB exhibited an antidepressant-like effect in male Swiss mice through modulation of the serotonergic system, but not the noradrenergic system. Additionally, the compound at a high dose did not cause acute toxicity in female mice (Pires et al., 2024). Thus, the aim of this study was to broaden the understanding of the mechanism of action of CF_3SePB (Figure 1) by assessing the involvement of the dopaminergic system in the antidepressant-like effect exhibited by CF_3SePB in mice. Additionally, the potential toxicity and pharmacokinetic profile of CF_3SePB were assessed through in silico ADMET analysis.

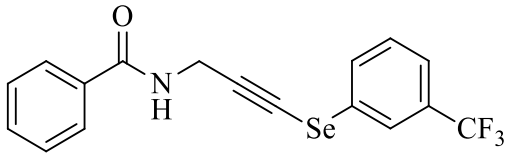

Fig. 1. Chemical structure of *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide (CF_3SePB)

2. Materials and methods

2.1 Animals

In this study, adult male Swiss mice (25-30 g) from the Central Biotery of the Federal University of Pelotas were used. All behavioral tests were conducted with each animal used only once in each test (n = 8 to 10 animals/group, totaling 135 male mice), except for the open field test (OFT), which was performed before the forced swimming test (FST) with the same animals. The animals were maintained at a temperature of 22 ± 2 ºC with a 12-hour light/12-hour dark cycle, with lights on at 7 a.m. Housed in separate cages (20 × 30 × 13, 5 animals per cage), the mice had access to commercial feed and fresh water ad libitum, without receiving food during the experimental protocol. After one hour of acclimatization, the tests were initiated and monitored by a blinded observer. The study followed the guidelines of the Ethics Committee on Animal Experimentation at UFPel (CEUA 28,792 - 2020), which are in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publications No. 8023, revised in 1978), aimed at minimizing the number of animals used and mitigating animal suffering.

2.2 Drugs

The compound CF_3SePB (Fig. 1) was synthesized at the Nucleus of Synthesis and Application of Organic and Inorganic Compounds (NUSAACOI) at the Federal University of Fronteira Sul (UFFS). The ^{13}C and ^{1}H NMR spectra were consistent with the assigned structure when analytical and spectroscopic data were analyzed (Balbom et al., 2019). The chemical purity of the compound was verified using GC/MS. Canola oil was used to dissolve CF_3SePB for subsequent intragastric administration at a dose of 50 mg/kg. Other drugs used included haloperidol (0.05 mg/kg, intraperitoneal), SCH 23390 (0.01 mg/kg, subcutaneous) and sulpiride (50 mg/kg, intraperitoneal). Haloperidol and SCH 23390 were dissolved in saline solution. Sulpiride was dissolved in saline solution plus 5% DMSO. All drugs were administered to mice at a constant volume of 10 mL/kg of body weight and were acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Experimental design

2.3.1 Evaluation of the involvement of the dopaminergic system in the antidepressant-like effect of CF_3SePB

To evaluate the hypothesis that the antidepressant effect of CF_3SePB is related to the modulation of the dopaminergic system, the forced swim test (FST) was conducted using a dose of 50 mg/kg of CF_3SePB (Pires et al., 2024). The FST is frequently used for screening new compounds with potential antidepressant properties (Yankelevitch-Yahav et al., 2015; Armario, 2021). Male mice were pre-treated with haloperidol (0.05 mg/kg, i.p., a non-selective dopaminergic receptor antagonist), SCH 23390 (0.01 mg/kg, s.c., a D_1 receptor antagonist) and sulpiride (50 mg/kg, i.p., a D_2 receptor antagonist) to assess involvement with specific receptors of the dopaminergic system. Fifteen minutes after the administration of the antagonists, CF_3SePB (50mg/kg, i.g.) or vehicle was administered. Thirty minutes after the administration of the compound or vehicle, the animals underwent the FST for 6 minutes, and the open field test (OFT) was conducted 4 minutes prior (Figure 2). The timing and doses of the dopaminergic antagonists used in the animal treatments were chosen in accordance with previous studies (Donato et al., 2013; da Silva Teixeira Rech et al., 2021).

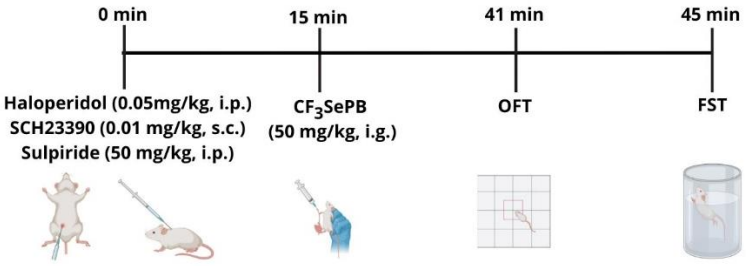

Fig. 2. Experimental design for evaluating the involvelment of the dopaminergic system in the antodepressant-like effect of CF_3SePB.

2.3.2 Behavioral tests

2.3.2.1 Open field test (OFT)

In this test, mice were individually placed in the center of a wooden box (30 cm x 40 cm x 40 cm) divided into nine equally sized squares to investigate potential alterations in the animals' locomotor and exploratory activity. Each animal was observed for 4 minutes, during which the number of crossings between squares and the number of times the animals reared up on their hind legs (rearing posture) were recorded (Walsh and Cummins, 1976). After each test, the box was sanitized with 20% alcohol to eliminate any olfactory traces and prepare it for the next animal.

2.3.2.2 Forced Swim Test (FST)

In the Forced Swim Test (FST), animals were placed in a cylindrical container measuring 29 cm in height containing 1.5 L of water at 25 ± 1 ºC for 6 minutes, subjecting them to a stressful situation from which they cannot escape (Yankelevitch-Yahav et al., 2015; Berton et al., 2021). During the time they were inside the cylindrical container, the latency to the first episode of immobility and the total time of immobility were recorded, and the mice attempt to escape from this unfavorable situation by swimming or floating. The longer the animals remain floating without trying to escape, the greater the depressive-like behavior.

2.3 The Pharmacokinetics and Toxicity Prediction

In accordance with the methodology proposed by Pires, Blundell, and Ascher (2015), an in silico prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) was conducted for CF_3SePB. To begin, the two-dimensional structure of CF_3SePB was drawn using ChemDraw 19.0.0 software and then converted into a SMILE sequence. The SMILES codes were then loaded into the pkCSM software (<http://biosig.unimelb.edu.au/pkcsm>) to predict the ADMET parameters.

2.4 Statistical analysis

The experimental data are presented as mean ± standard error of the mean (SEM). The normality of data was assessed using the D'Agostino and Pearson test. Comparisons among experimental groups were conducted using two-way analysis of variance, followed by the Newman-Keuls post hoc test. p-values less than 0.05 ($p < 0.05$) were considered statistically significant. All analyses were conducted using GraphPad Software (GraphPad, San Diego, CA, USA).

3. Results

3.1 Involvement of dopaminergic receptors in the CF_3SePB antidepressant-like effect in mice subjected to the FST

The results of the involvement of dopaminergic receptors in the antidepressant-like effect of the compound CF_3SePB in mice in the FST are shown in Figures 3, 4, and 5. The data obtained in the FST indicated the antidepressant-like effect of the compound CF_3SePB in mice at the tested dose. Figures 3A and 3B show that pretreatment with haloperidol, a non selective dopaminergic receptor antagonist, blocked the effect of CF_3SePB (50 mg/kg, i.g.) as demonstrated by the latency time for the first episode of immobility ($F_{(1, 34)} = 11.99$, $p = 0.0015$) and the total immobility time ($F_{(1, 34)} = 4.332$, $p = 0.0450$) of the mice. The number of crossings as well as the

number of rearings in the OFT were not altered by treatment with the compound CF_3SePB and/or haloperidol (Table 1).

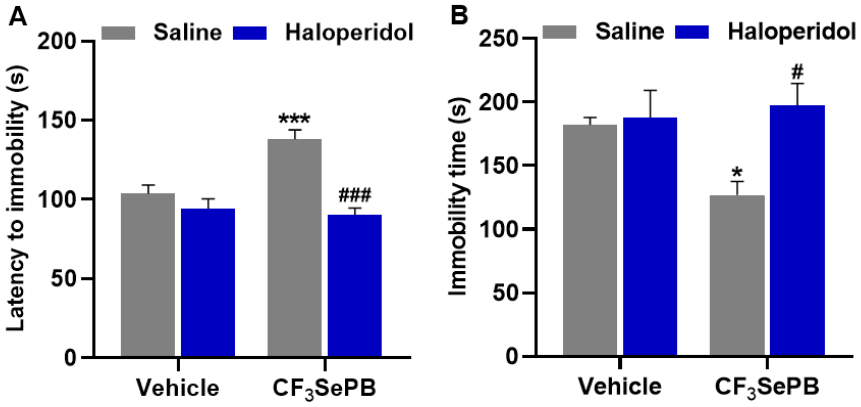

Fig. 3 Effect of pretreatment with haloperidol (0.05 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (A) Latency to first episode of immobility and (B) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) (n = 10–11 animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. (*) $p < 0.05$ and (***) $p < 0.001$ compared with the control group; (#) $p < 0.05$ and (###) $p < 0.001$ compared with the CF_3SePB group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test.

The data analysis also showed that SCH 23390, a D_1 receptor antagonist, blocked the increase in latency time for the first episode of immobility (Figure 4A, $F_{(1, 33)} = 7.843$, $p = 0.0085$) and the reduction in total immobility time of mice in the FST after treatment with CF_3SePB (Figure 4B, $F_{(1,33)} = 6.875$, $p = 0.0131$). The locomotor activity of the animals, as well as exploratory activity, in the OFT, were not altered by CF_3SePB treatment (Table 1).

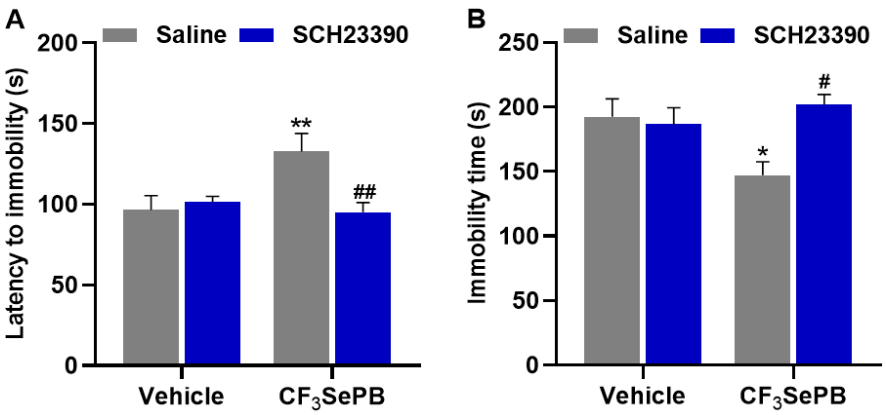

Fig. 4 Effect of pretreatment with SCH 23390 (0.01 mg/kg, s.c.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (A) Latency to first episode of immobility and (B) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) (n = 10–11 animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. (*) $p < 0.05$ and (**) $p < 0.01$ compared with the control group; (#) $p < 0.05$ and (##) $p < 0.01$ compared with the CF3SePB group. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test.

The pre-treatment with sulpiride, a D_2 receptor antagonist, prevented the antidepressant-like action of the CF_3SePB (50 mg/kg, i.g.) (Figures 5A and 5B). Two-way ANOVA showed interaction of CF_3SePB and sulpiride on latency to the first immobility episode ($F_{(1,36)} = 9.426$, $p = 0.0041$) and total immobility time ($F_{(1,36)} = 9.667$, $p = 0.0037$) in FST in mice. In the OFT, the number of crossings and rearings were not altered in animals that were treated with sulpiride and/or CF_3SePB (Table 1).

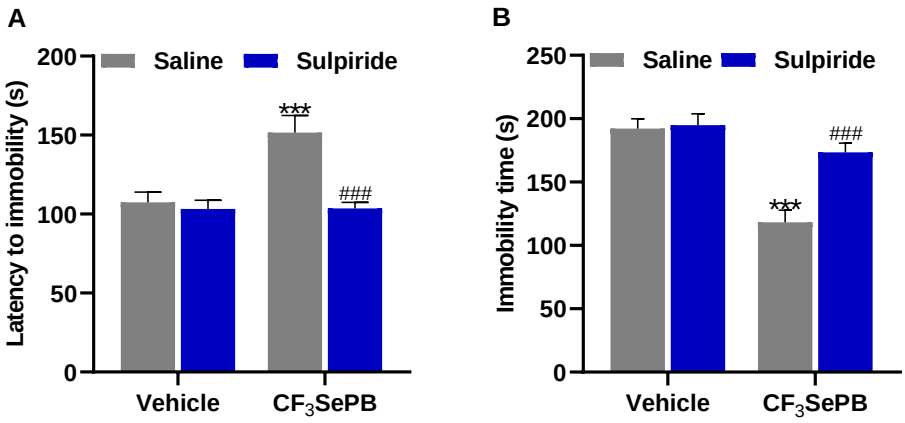

Fig. 5 Effect of pretreatment with sulpiride (50 mg/kg, i.p.) on the antidepressant-like effect of CF_3SePB (50 mg/kg, i.g.) in mice on FST. (A) Latency to first episode of immobility and (B) total immobility time. Values were expressed as the mean ± standard error of the mean (S.E.M.) (n =10 animals/group). Data were analyzed by two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls test. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; FST: Forced swim test.

Table 1: Effect of CF_3SePB, and/or dopaminergic antagonists on the OFT behavioral parameters in mice.

Experiment	Groups	Number of crossing	Number of rearing

Haloperidol	Vehicle	74.2 ± 3.5	30.1 ± 2.3
	Haloperidol (0.05 mg/kg)	73.3 ± 1.8	28.6 ± 2.5
	CF_3SePB (50 mg/kg)	70.7 ± 4.7	29.9 ± 1.8
	Haloperidol + CF_3SePB	70.8 ± 6.3	28.9 ± 2.7
SCH-23390	Vehicle	82.4 ± 4.1	32.6 ± 2.0
	SCH-23390 (0.01 mg/kg)	83.2 ± 3.8	33.2 ± 1.8
	CF_3SePB (50 mg/kg)	81.2 ± 3.5	34.3 ± 2.1
	SCH-23390 + CF_3SePB	83.3 ± 2.6	33.5 ± 1.3
Sulpiride	Vehicle	63.2 ± 2.1	28.5 ± 2.9
	Sulpiride (50 mg/kg)	66.2 ± 2.6	29.4 ± 2.2
	CF_3SePB (50 mg/kg)	71.0 ± 2.8	34.2 ± 2.4
	Sulpiride + CF_3SePB	70.5 ± 2.5	33.8 ± 3.1

The results represent the means ± standard error of the mean (S.E.M.) (n = 10-11 animals/group). Data analyses were conducted through two-way ANOVA. Abbreviations: CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; OFT: open field test.

3.2 The Pharmacokinetics and Toxicity Prediction

The application of in silico techniques to predict ADMET and bioavailability parameters using software is advantageous for assessing the pharmacokinetic and toxicological profiles of synthetic compounds (Pires et al., 2015). Table 2 presents the outcomes of the pkCSM platform's predictions for ADMET and bioavailability parameters.

Table 2. *In Silico* ADMET parameters of CF_3SePB

Parameters	CF_3SePB **results**	**Unit**
Absorption		
Water solubility	-4.72	Numeric (log mol/L)
Caco2 permeability	1.305	Numeric (log Papp in 10^{-6} cm/s)
Intestinal absorption	94.068	Numeric (%Absorbed)
P-glycoprotein substrate	No	Categorical (Yes/No)

P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution		
VDssa	0.093	Numeric (log L/kg)
BBB permeability	0.548	Numeric (log BB)
Metabolism		
CYP2D6 substrate	No	Categorical (Yes/No)
CYP3A4 substrate	Yes	Categorical (Yes/No)
CYP1A2 inhibitor	Yes	Categorical (Yes/No)
CYP2C19 inhibitor	Yes	Categorical (Yes/No)
CYP2C9 inhibitor	Yes	Categorical (Yes/No)
CYP2D6 inhibitor	No	Categorical (Yes/No)
CYP3A4 inhibitor	No	Categorical (Yes/No)
Excretion		
Total clearance	1.934	Numeric (log mL/min/kg)
Toxicity		
AMES toxicity	No	Categorical (Yes/No)
Maximum tolerated dose	0.284	Numeric (log mg/kg/day)
hERG I inhibitor	No	Categorical (Yes/No)
hERG II inhibitor	Yes	Categorical (Yes/No)
Hepatotoxicity	Yes	Categorical (Yes/No)
T. Pyriformis toxicity	1.755	Numeric (log µg/L)
Minnow toxicity	-0.325	Numeric (log mM)

Regarding water solubility, CF_3SePB had a value of -4.72, indicating low solubility in water (Bergstrom & Larsson, 2018). Caco-2 cell permeability (human colorectal adenocarcinoma cells) and intestinal absorption values indicated that CF_3SePB has moderate intestinal permeability. The results suggest that CF3SePB does not interact with P-glycoprotein, but demonstrates the potential to inhibit one of its isoforms, P-glycoprotein I.

In relation to the distribution of the compound, the value of the volume of distribution (VDss) of CF_3SePB was 0.093, indicating a better distribution in the tissues (Pires et al., 2015). Furthermore, the value of 0.48 for the permeability in the blood-brain barrier (BBB) suggests that the compound has the potential to easily cross this barrier (Suenderhauf, Hammann, & Huwyler, 2012).

Regarding metabolism, CF_3SePB appears to act as a substrate for the CYP3A4 enzyme and has inhibitory potential on the activities of CYP1A2, CYP2C19, and CYP2C9 enzymes. Concerning excretion, CF_3SePB had a low total clearance value, suggesting that the compound may have a slow elimination rate.

The in silico toxicological results for CF_3SePB indicated non-mutagenic characteristics, suggesting low toxicity. These observations obtained through computational simulations indicate the promising potential of CF_3SePB as a new pharmaceutical candidate.

4. Discussion

The results obtained in this work showed that the dopaminergic system is involved in the antidepressant-like effect of the selenopropargyl benzamide CF_3SePB in mice, in addition to the modulation of the serotonergic system but not the noradrenergic system observed in a previous study (Pires et al., 2024). The non-selective dopaminergic antagonist haloperidol, and the selective antagonists of D_1 and D_2 receptors, SCH 23390 and sulpiride, respectivey, prevented the anti-immobility effect of CF_3SePB in the FST without alteration of locomotor activity in the OFT. The FST is one of the most widely used animal models for evaluating the activity of compounds with potential antidepressant properties (Porsolt et al, 1978).

The dysfunctions in the monoaminergic neurotransmitters, including DA, has been considered a crucial element in the pathophysiology of depression (Wang et al., 2019). DA is a neurotransmitter belonging to the catecholamine group and is predominantly present in the brain, where it regulates a variety of functions, including locomotor activity, emotions, pleasure, concentration, endocrine regulation, and food intake. The synthesis of DA occurs in the presynaptic neurons of the brainstem, from two amino acids: phenylalanine and tyrosine (Diniz, Nevez, Vieira, 2020). The projection of dopaminergic neurotransmitters occurs through two distinct pathways. The first is the mesolimbic cortical pathway, which extends from the ventral tegmental area to the nucleus accumbens. The second pathway projects from the substantia nigra to the dorsal striatum (Quevedo, Izquierdo, 2020). The effects of DA are manifested in postsynaptic neurons through interaction with dopamine receptors, which are classified into two families, D_1 and D_2 (Diniz, Nevez, Vieira, 2020).

The modulation of the monoamines, including DA, is the main mechanism of action of many antidepressants (Wang et al., 2019). Drugs that modulate the dopaminergic system exhibit antidepressant profiles in animal models of depression (Muscat et al., 1992; Wang et al., 2019) and have shown efficacy in treating depression in humans (Wang et al., 2019). Dopamine receptor agonists have been shown to augment antidepressant-like effects observed in animal behavioral despair models, while dopamine receptor antagonists have been shown to reverse them (Renard et al., 2001; Joca et al., 2000). Pharmacological experiments suggest that D_1/D_2 receptors play a key role in the response to biological antidepressant treatments (Jesse et al., 2010).

This study demonstrated that the antidepressant-like effect of CF_3SePB is dependent on the modulation of the D_1 receptor, as the antagonist of this receptor, SCH 23390, as well as haloperidol, a non-selective antagonist of dopaminergic receptors, blocked the anti-immobility effect of CF_3SePB in the FST. Animal studies

have shown that manipulation of dopamine D1 receptors can affect depression-related behavior, where D1 receptor agonists can induce antidepressant-like effects in rodents (Desormeaux, et al., 2020). Impaired social interaction is a prominent feature of various psychiatric disorders, including MDD. The D1 receptor has been associated with social behavior in such a way that activating this receptor increases social behavior (Plavén-Sigray et al., 2014).

Many findings indicate that D_1 receptor agonists may represent promising targets for the development of new antidepressant treatments. Activation of the D_1 receptor also contributes to other beneficial effects, including improved learning and memory in rodents (Puig et al., 2014; Hansen and Manahan-Vaughan 2014; Caragea et al, 2024). D_1 receptors are linked to cognitive function and are involved in the regulation of depression- and anxiety-like behaviors (Goldman-Rakic et al., 2004; McNab et al., 2009; Hare and Duman, 2020; Tong et al, 2023).

It has also been shown that the antidepressant-like effect of CF_3SePB depends on the modulation of the D_2 receptor, as the antagonist of this receptor, sulpiride, blocked the anti-immobility effect of CF_3SePB in the FST. The results obtained from this study indicate that the dopaminergic system is involved in the antidepressant-like effect of the compound CF_3SePB in the FST. Drugs that activate D_2 dopamine receptors help reduce symptoms produced in depression, such as anhedonia and apathy, which are seen as primary social symptoms (Bonci and Hopf, 2005). Clinical investigations have suggested the use of D_2 receptor agonists for the treatment of depression (Waehrens and Gerlach, 1981; Abbasi-Maleki and Mousavi, 2017). The sensitization of D_2 dopamine receptors in the mesolimbic dopaminergic system may represent a pathway in antidepressant action (Willner et al, 2005). Studies have shown that when activated, the D_2 receptor mediates motivational behaviors and counteracts anhedonia (Amiri et al., 2016). Thus, the behavioral changes observed in animals induced by the compound are consistent with previous studies demonstrating the antidepressant action of other organoselenium compounds (Pires et al., 2024). Therefore, we hypothesize that CF_3SePB could facilitate dopaminergic neurotransmission, leading to activation of dopamine receptors in the brains of mice.

Lastly, this research performed a projection of pharmacokinetic parameters and bioavailability of CF_3SePB using the pkCSM software. For satisfactory absorption, a compound must be water-soluble and have intestinal permeability. CF_3SePB has a solubility value of -4.72, which indicates low solubility in water (Bergstrom & Larsson, 2018); however, our in vivo results indicate that this does not hinder the absorption of the compound. Furthermore, CF_3SePB showed high intestinal absorption (94,068%) and appeared to be permeable to the blood-brain barrier, which is a critical pharmacokinetic characteristic for the evaluation and development of antidepressant drugs (Suenderhauf et al., 2012). Cytochrome P450 enzymes play a fundamental role in the metabolism of a wide range of drugs (Zhao et al. 2021). Co-administration of drugs that are substrates, inducers, or inhibitors of these enzymes can cause significant changes in the plasma concentrations of the compounds,

resulting in decreased or increased therapeutic effects, in addition to possible adverse events (McDonnell & Dang, 2013). The knowledge that CF_3SePB acts as a substrate for the CYP3A4 enzyme and has inhibitory potential on the activities of CYP1A2, CYP2C19, and CYP2C9 allows for a better understanding of the possible interactions that may arise when this compound is used in conjunction with other drugs. In relation to excretion, a low total clearance value was recorded, implying a slow elimination rate for the compound. The in silico toxicological results of CF_3SePB indicate that the compound does not exhibit mutagenic properties but suggests hepatotoxicity. Nevertheless, an acute toxicity protocol conducted with the compound did not reveal hepatotoxicity (Pires et al., 2024). This discrepancy may be attributable to the fact that the computational analyses did not account for the dose of the compound in their evaluation. The findings from computational simulations indicate that the CF_3SePB compound exhibits low toxicity, suggesting its potential as a promising candidate for a novel therapeutic agent.

5. Conclusion

The findings of this study demonstrated the involvement of the dopaminergic system in the antidepressant-like effect presented by CF_3SePB, as specified by the modulation of D_1 and D_2 receptors. Additionally, CF_3SePB demonstrated a low likelihood of inducing adverse effects. In silico ADMET predictions suggested high intestinal absorptivity and the capacity to cross the blood-brain barrier. The results of this study encourage us to continue investigating the therapeutic potential of the compound CF_3SePB, a better understanding of other mechanisms of action and to contribute to the development of new therapies and strategies for the treatment of depression.

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4.3 MANUSCRITO 2

_N_-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl benzamide ameliorates lipopolysaccharide-induced depression-like behavior in mice targeting inflammatory and apoptotic genes

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Abstract

The organoselenium compound CF_3SePB has previously shown promise as an antidepressant due to its modulation of the serotonergic system. This study aimed to investigate the potential of CF_3SePB to ameliorate depressive-like behaviors induced by lipopolysaccharide (LPS) in mice. Male Swiss mice were pretreated with CF_3SePB (10 mg/kg, i.g.), vehicle (10 mg/kg, i.p.), or fluoxetine (10 mg/kg, i.p.), and 30 minutes later, they were treated with LPS (0.83 mg/kg, i.p.) or vehicle (10 mg/kg, i.g.). Twenty-four hours after LPS treatment, the behavioral tests tail suspension test, sucrose splash test, and forced swim test were conducted to assess depressive-like behaviors and open-field test was conducted to evaluate the locomotor activity of mice. Additionally, mice were euthanized, and the hippocampal tissue was removed and analyzed for biochemical parameters and gene expression. CF_3SePB effectively reversed LPS-induced depressive-like behaviors without affecting locomotion. Furthermore, CF_3SePB prevented the increase in the expression of the pro-inflammatory genes nuclear factor kappa B (NF-κB), NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, and cyclooxygenase-2 (COX-2) and the apoptotic genes caspase-1, caspase-8, and BCL-2-Associated X Protein (BAX) induced by LPS, indicating its potential to target inflammation and neuronal death pathways. It also reduced reactive species (RS) and lipid peroxidation levels in hippocampus induced by LPS, suggesting neuroprotective effects. These findings highlight the potential of CF_3SePB as a novel therapeutic agent for depression, particularly in models involving inflammation-mediated mechanisms.

Keywords: Depression. Lipopolysaccharide. Benzamide. Selenium. Mice. Neuroinflammation

INTRODUCTION

The compound *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl) benzamide (CF_3SePB, Figure 1) is a synthetic organic compound containing a selenium atom and a benzamide core in its structure (Balbom et al., 2019; Pires et al., 2024). Organic selenium compounds exhibit various pharmacological properties such as anticonvulsant, anti-inflammatory, analgesic, and antidepressant-like effects (Caliendo et al., 2001; Chirita et al., 2010; Birmann et al., 2023; Ledebuhr et al., 2024). CF_3SePB demonstrated antidepressant-like effects via modulation of the serotonergic system, but not the noradrenergic system, when tested in mice. Additionally, at high doses, it exhibited a low potential to induce acute toxicity in female mice (Pires et al., 2024).

Major Depressive Disorder (MDD) is a psychiatric disorder affecting approximately 300 million people worldwide, with its prevalence steadily increasing. The World Health Organization (WHO) predicts that MDD will become the leading psychiatric disorder by 2030 (Cui et al., 2024). Many patients with MDD find limited efficacy in current antidepressant medications (Hutka et al., 2021; Krystal et al, 2024). Furthermore, therapeutic benefits often emerge gradually, typically within two to six weeks of treatment initiation, due to potential decreases in adherence caused by side effects and low tolerability. Consequently, there is a pressing need for research exploring novel therapeutic alternatives that can enhance the pharmacological treatment of depression by not only minimizing side effects but also demonstrating promising outcomes (Rech et al., 2021; McIntyre et al., 2023; Krystal et al., 2024).

The pathophysiological mechanisms of depression are not yet fully understood; however, the monoaminergic theory suggests that depression may be caused by deficits in the neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NA) in the brain, leading to depressive symptoms (Pastis et al., 2024). In recent years, efforts have been made to identify other mechanisms involved in depressive disorders. Among various theories, the connection between the immune system and the central nervous system is increasingly highlighted (Buras et al., 2016). The integrity of the blood-brain barrier (BBB) can become compromised by innate and adaptive responses, leading to the propagation of inflammatory signals from the periphery to the central nervous system (CNS) (Medina-Rodriguez and Beurel, 2022; Hassamal, 2023).

Chronic activation of the immune system in the brain can lead to depressive symptoms, including cognitive impairments and emotional dysregulation (Hassamal, 2023). This inflammatory response is often associated with oxidative stress (Liu et al., 2022), decreased neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) (Dwivedi, 2009), and apoptosis (Zhang et al., 2023), which are suggested as contributing factors to the occurrence of MDD (Dwivedi, 2009; Afridi and Suk, 2021; Liu et al., 2022).

Oxidative stress occurs when there is an imbalance during the production of free radicals, and the human body is unable to neutralize this production (Barbosa et al., 2010; Sies and Jones, 2020). The excess of reactive

oxygen species damages lipids, proteins, and DNA, potentially leading to cellular damage and death (apoptosis). Some researchers consider oxidative stress in the brain to be a significant contributor to depressive disorders, as it can disrupt neuronal function and neurotransmission (Maes et al., 2000).

BDNF is the most abundant neurotrophin in the CNS, with high concentrations in the hippocampus (Trombetta et al., 2020). BDNF plays a crucial role in maintaining synaptic plasticity and hippocampal neurogenesis, a region essential for memory acquisition and consolidation (Martinho Jr et al., 2012; Trombetta et al., 2020). Studies have indicated that low levels of BDNF may lead to a reduction in hippocampal volume and are considered a potential cause of memory deficits and depression (Syafrita et al., 2020).

These elements together provide a broad understanding of the mechanisms that may underlie neurological and psychiatric disorders, particularly depression. Understanding these connections and identifying drugs that can effectively modulate them could contribute to the development of more effective therapeutic strategies for treating depression. Therefore, this study aims to investigate the antidepressant efficacy of the compound CF_3SePB in a neuroinflammatory model of depression induced by lipopolysaccharide (LPS) in mice.

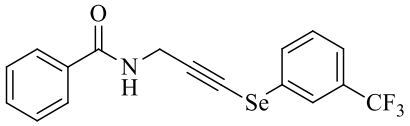

Figure 1. Chemical structure of *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide (CF_3SePB)

RESULTS AND DISCUSSION

To mimic a neuroinflammatory condition, the LPS-induced depression model was used. The animals were pretreated with CF_3SePB or fluoxetine and then with LPS. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), was used to validate the behavioral tests. The depressive behavior induced by LPS was evaluated by measuring the duration of immobility in mice during the tail suspension test (TST) and the forced swim test (FST). The locomotor activity of the animals was assessed using the open field test (OFT), and grooming behavior and motivation were evaluated using the sucrose splash test (ST). After the behavioral tests, the mice were euthanized, and the hippocampus was removed for biochemical analysis and expression of genes related to neuroinflammation, apoptosis and neurogenesis.

In Figure 2A, the results showed that the treatments with CF_3SePB and/or LPS injection did not cause any changes in the the number of crossings of mice ($F_{(4, 55)} = 0.5578$, $p = 0.6942$). Similarly, as showed in the

Figure 2B, the treatments with CF_3SePB and/or LPS did not alter the number of rearings ($F_{(4, 55)}$ = 0.3260, p = 0.8594). These results show that the administration of CF_3SePB and/or LPS did not cause changes in the animals' locomotor activity, excluding any interference of locomotor alterations in behavioral tests accesing the depressive phenotype. The administration of fluoxetine also did not cause any changes in the locomotor activity of the mice compared to control group.

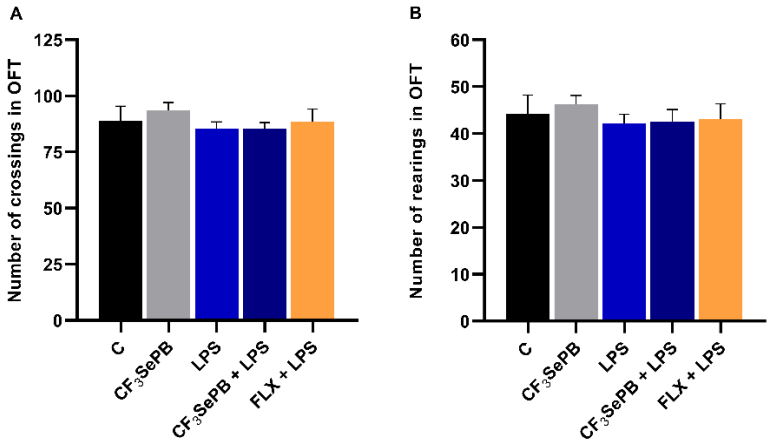

Figure 2. Effect of pre-treatment with LPS (0.83 mg/kg, i.p.) and CF_3SePB (10 mg/kg, i.g.) in (A) number of crossings and (B) rearings of mice in the OFT. Fluoxetine was used as the reference medication. The results represent the means ± standard error of the mean (S.E.M.) (n = 12 animals/group). Data analyses were conducted using one-way ANOVA. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selanyl)prop-2-yn-1-yl)benzamide; C: Control; FLX: Fluoxetine; i.g.: Intragastric; i.p.: Intraperitoneal; OFT: Open field test.

In the FST (Figure 3A), the results showed that the latency time to the first immobility episode in animals treated with LPS was significantly reduced compared to the control group. The pretreatment with CF_3SePB prevented this reduction ($F_{(4, 55)}$ = 7.885, p < 0.0001). The administration of fluoxetine also prevented the effect of LPS, leading to an increase in the latency time to the first immobility episode in mice compared to the LPS group. In Figure 3B, the LPS group showed a significant increase in the total immobility time compared to the control group, and the pre-treatment with CF_3SePB prevented this effect ($F_{(4, 55)}$ = 8.498, p < 0.0001). The administration of fluoxetine also prevented the increase in the immobility time caused by LPS.

FST is a behavioral method used to assess the efficacy of new antidepressant compounds in rodents (Yankelevitch-Yahav et al., 2015; Armario, 2021). In this behavioral method, it is assumed that when placing the animal in a cylinder with water, it will attempt to escape from the unpleasant situation and will eventually exhibit

immobility. This can be considered a behavior of despair, leading to stress in the animals, and indicating a predisposition to major depression (Yankelevitch-Yahav et al., 2015; Armario, 2021). The compound CF_3SePB prevented the increase in immobility time induced by LPS in the FST, showing an antidepressant-like effect.

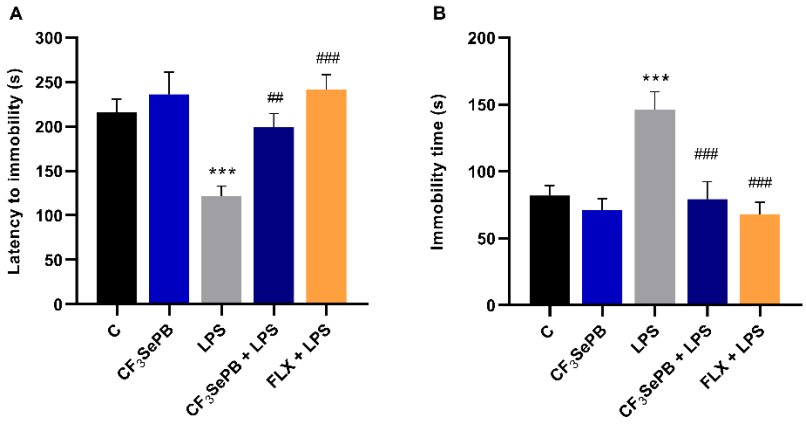

Figure 3. Effect of treatment with LPS (0.83 mg/kg, i.p.) and CF_3SePB (10 mg/kg, i.g.) on the FST in mice. (A) Latency time to the first episode of immobility and (B) total immobility time. Fluoxetine was used as a reference drug. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 12 animals/group). (***) $p < 0.001$ compared to the control group. (##) $p < 0.01$ and (###) $p < 0.001$ compared to the LPS group. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl)benzamide; C: Control; FLX: Fluoxetine; i.g.: Intragastric; i.p.: Intraperitoneal; FST: Forced swim test.

The compound CF_3SePB demonstrated an antidepressant-like effect in mice in the TST against LPS. In figure 4A, the results showed that LPS injection significantly reduced the latency time to the first immobility episode in the animals compared to the control group, while the compound CF_3SePB prevented this effect ($F_{(4, 55)} = 3.654$, $p = 0.0104$). Pretreatment with fluoxetine also prevented the effect of LPS, increasing the latency time to the first episode of immobility in mice compared to the LPS group. In Figure 4B, there was a significant increase in the total immobility time of the LPS group compared to the control group, and the pre-treatment with CF_3SePB significantly reduced the total immobility time of the mice compared to the LPS group ($F_{(4, 55)} = 8.498$, $p < 0.0001$), showing its protective effect against LPS-induced depression-like behavior. The administration of fluoxetine also reduced the effect of LPS when compared to the LPS group.

The TST is based on the time the animal remains immobile in an environment from which it cannot escape. This immobility time in mice reflects despair-like behavior, which is an indicator of depressive psychomotor retardation within a depression framework (Unal and Canbeyli, 2019; Yin et al., 2023). The TST has strong predictive validity and wide applicability for different compounds when conducting preclinical screening

of new compounds with antidepressant properties (Abelaira et al., 2013). The increase in immobility time in animals induced by LPS was prevented by the compound CF_3SePB in the TST, showing an antidepressant-like effect in this test.

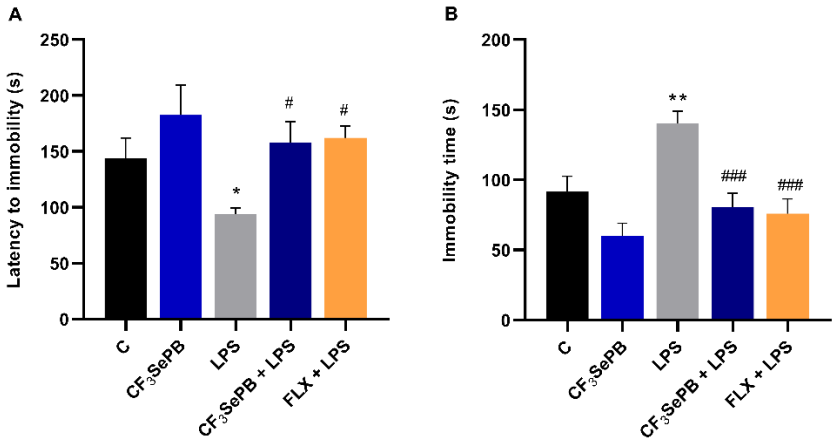

Figure 4. Effect of treatments with LPS (0.83 mg/kg, i.p.) and/or CF_3SePB (10 mg/kg, i.g.) in the TST in mice. (A) Latency time to the first immobility episode and (B) total immobility time. Fluoxetine was used as a reference drug. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 12 animals/group). (*) $p < 0.05$, (**) $p < 0.01$ compared to the control group. (#) $p < 0.05$ and (###) $p < 0.001$ compared to the LPS group. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-in-1-yl)benzamide; C: Control; FLX: Fluoxetine; i.g.: Intragastric; i.p.: Intraperitoneal; TST: Tail suspension test.

The results of the ST are shown in figure 5. In Figure 5A, the latency time to the first grooming episode in mice treated with LPS was increased compared to the control group. Pre-treatment with the compound CF_3SePB significantly decreased the latency time to the first grooming episode in the animals compared to the LPS group ($F_{(4, 55)} = 5.270$, $p = 0.0011$). The injection of fluoxetine also reduced the latency time to the first grooming episode in the animals compared to the LPS group.

In figure 5B, mice pre-treated with LPS showed a reduction in the total grooming time compared to the control group, and the group pre-treated with CF_3SePB showed a significant increase in total grooming time compared to the LPS group ($F_{(4, 55)} = 9.118$, $p < 0.0001$). The administration of fluoxetine significantly reversed the effect of LPS on the total grooming time in mice when compared to the LPS group.

ST is a valid marker of apathy behavior and self-care in depressed mice, being used in depression models (Yin et al., 2023). In rodents, signs of apathy manifest, among other things, through dishevelment due to lack of self-care, being considered a depressive phenotype (Cathomas et al., 2015; Becker et al., 2021). In the ST, when a

10% sucrose solution is sprayed on the animal's back, it forms a viscous layer that mimics dirt and triggers the rodent's desire to clean its fur through licking or scratching (Becker et al., 2021). The delay in starting the fur cleaning process or the decrease in the frequency of cleaning is considered reduced self-care behavior, being associated with the depressive phenotype (Planchez et al., 2019). The compound CF_3SePB increased the self-care time of animals treated with LPS, with can also be considered an antidepressant-like effect.

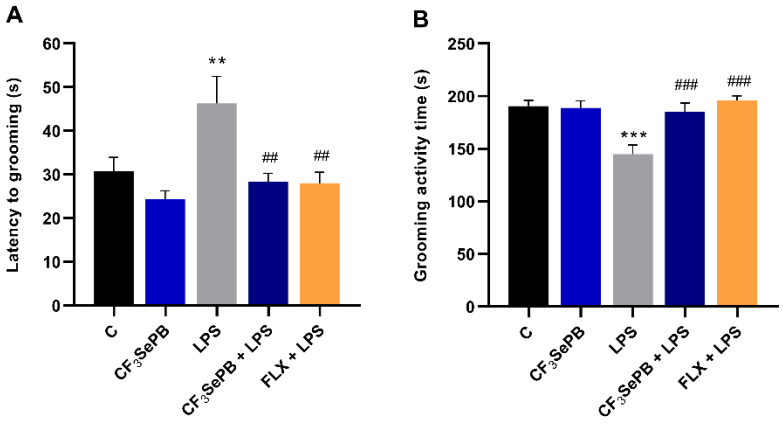

Figure 5. Effect of treatments with LPS (0.83 mg/kg, i.p.) and CF_3SePB (10 mg/kg, i.g.) on the ST in mice. (A) Latency time to the first cleaning episode and (B) total cleaning time of the mice. Fluoxetine was used as a reference drug. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 12 animals/group). (**) $p < 0.01$ and (***) $p < 0.001$ compared to the control group. (##) $p < 0.01$ and (###) $p < 0.001$ compared to the LPS group. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl)benzamide; C: control; FLX: fluoxetine; i.g.: intragastric; i.p.: intraperitoneal; ST: splash test.

LPS administration significantly increased the expression of the pro-inflammatory gene nuclear factor kappa B (NF-κB) (Figure 6A), which is closely involved in the pathogenesis of depression (Glezer et al., 2000). CF_3SePB effectively counteracted LPS-induced NF-κB upregulation in the hippocampus of mice ($F_{(3, 10)} = 29.71$, $p < 0.0001$). NF-κB regulates the expression of genes involved in inflammation, cell survival, and development. Activation of NF-κB by LPS from gram-negative bacteria can lead to structural and functional changes in the brain, such as altered neuroplasticity, synaptic function, and impaired neurogenesis, all of which are associated with depression (Glezer et al., 2000; Koo and Duman, 2008; Caviedes et al., 2017). Studies indicate that antidepressants, including SSRIs and other types, can decrease NF-κβ activity, thereby reducing brain inflammation and, consequently, improving depressive symptoms (Capuron et al., 2002; Wang et al., 2009). The modulation of NF-κB expression may be related to the antidepressant-like effect of the CF_3SePB compound.

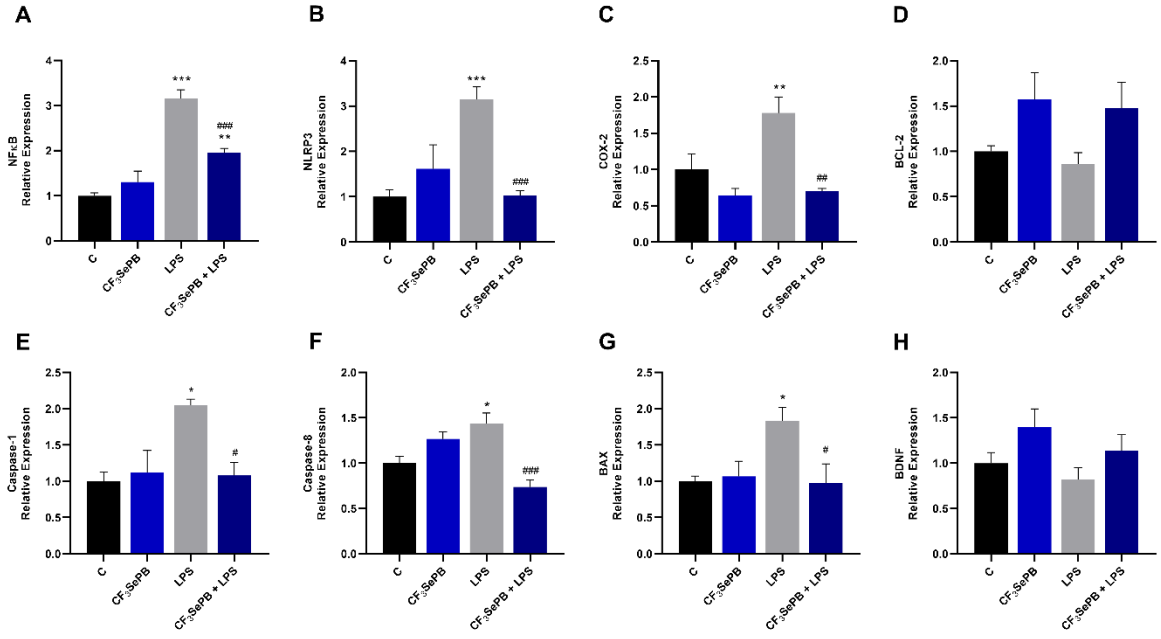

Figure 6. Effect of treatments with LPS (0.83 mg/kg, i.p.) and/or CF_3SePB (10 mg/kg, i.g.) on the gene expression of (A) NF-κB, (B) NLRP3 inflammasome, (C) COX-2, (D) BCL-2, (E) caspase-1, (F) caspase-8, (G) BAX, and (H) BDNF. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 3-4 animals/group). (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ compared to the control group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ compared to the LPS group. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl)benzamide; C: control; i.g.: intragastric; i.p.: intraperitoneal**.**

LPS significantly increased NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome expression in the hippocampus, and CF_3SePB pretreatment prevented this effect ($F_{(3, 11)} = 14.83$, $p = 0.0004$). The NLRP3 inflammasome is a multiprotein complex closely involved in neuroinflammation. Activation of the NLRP3 inflammasome can lead to caspase-1-dependent release of inflammatory cytokines (Alcocer-Gómez, 2014; Kouba et al., 2023). NLRP3 inflammasome activation, driven by neuroinflammation linked to peripheral inflammation, is closely associated with the severity of MDD (Kouba et al., 2023). Studies revealed that mRNA levels of NLRP3 were significantly elevated in patients with MDD compared to healthy controls, while antidepressants reduce NLRP3 levels (Alcocer-Gómez, 2014; Inserra et al., 2019). In this context, the antidepressant-like effect of CF_3SePB observed in the LPS-model may be linked to the modulation of NLRP3 expression in the hippocampus of the animals.

As shown in Figure 6C, LPS significantly increased the expression of cyclooxygenase-2 (COX-2), a key enzyme involved in the production of inflammatory prostaglandins (Müller et al., 2006; Strekalova et al., 2022).

CF_3SePB pretreatment effectively prevented LPS-induced COX-2 upregulation ($F_{(3, 11)} = 9.988$, $p = 0.0018$). COX-2 is typically expressed at low levels in healthy tissues but can be induced by inflammatory stimuli (Müller et al., 2006). Depression has been linked to increased COX-2 expression in the brain, particularly in the hippocampus, where it plays a crucial role in synaptic activity and memory consolidation (Müller et al., 2006; Guo et al., 2009; Strekalova et al., 2022). Some studies suggest that COX-2 inhibitors may have antidepressant effects by reducing neuroinflammation (Müller et al., 2006; Majd et al., 2015). The modulation of COX-2 activity has been explored as a potential target for the treatment of depression, especially in cases where inflammation is a significant factor (Müller et al., 2006; Majd et al., 2015). The observed inhibition of COX-2 expression by CF_3SePB in the hippocampus of mice may contribute to its antidepressant-like effects.

The BCL-2 protein is a key regulator of apoptosis (programmed cell death) and is involved in controlling the balance between cell survival and death (Arantes-Gonçalves and Coelho, 2006). From a pharmacological perspective, the expression of BCL-2 can be upregulated by antidepressant medications, which is specifically related to the response to depression treatment. This fact underscores the potential role of BCL-2 in the etiology of depression and positive outcomes in antidepressant treatment (Kosten et al., 2008; Zhang et al., 2014). Studies have shown that many antidepressants can increase BCL-2 expression in the hippocampus (Yucel et al., 2016). This is relevant because, by increasing BCL-2, antidepressants help protect neurons from apoptosis and promote neuronal survival (Yucel et al., 2016; Kubera et al., 2011). Antidepressant treatment can reverse hippocampal volume reduction and improve neuroplasticity by increasing BCL-2 expression and other neurotrophic factors (Kubera et al., 2011). These effects may contribute to the recovery of neuronal function and the improvement of depressive symptoms (Yucel et al., 2016). In this study, BCL-2 expression was not significantly altered by any treatment ($F_{(3, 11)} = 2.382$, $p = 0.1251$). However, the treatment with CF_3SePB, alone and in combination with LPS, showed a strong tendency to increase the levels of this anti-apoptotic gene, which could be associated with the antidepressant-like effect of CF_3SePB.

Caspase-1 and caspase-8 are proteolytic enzymes involved in the processes of apoptosis and inflammation, and they have been studied in relation to depression. Caspase-1 is particularly involved in the activation of pro-inflammatory cytokines, such as IL-1β and IL-18, through the inflammasome (Roy et al., 2023). The activation of caspase-1 in the brain can lead to chronic inflammation, which is associated with neuronal dysfunction and reduced neuroplasticity, common features in depression (Franchi et al., 2009; Inserra et al., 2019). Some studies suggest that antidepressants may modulate caspase-1 activity, thereby reducing brain inflammation and improving depressive symptoms (Li et al., 2018). Inhibition of caspase-1 is seen as a potential therapeutic pathway for treating depression (Roy et al., 2023). LPS administration induced a significant increase in caspase-1 expression in the hippocampus of mice and CF_3SePB prevented this effect ($F_{(3, 12)} = 6.556$, $p = 0.0071$) (Figure

6E), in accordance with the expression profile of NLRP3 inflammasome (Figure 6B). This effect of CF_3SePB in the LPS-induced caspase-1 upregulation could be also closely related with its antidepressant-like effect observed in the behavioral tests.

LPS also induced a significant increase in caspase-8 expression (Figure 6F). This protein is a key enzyme in the extrinsic pathway of apoptosis, initiating the process of cell death in response to extracellular signals, such as death ligands (TNF-α) (Luchs and Pantaleão, 2010; Orning and Lien, 2021; Jiang et al, 2021). Caspase-8 activation is also a central part of the inflammatory response, and the dysregulation of caspase-8 has been implicated in several diseases, including the development of depressive symptoms (Inserra et al., 2019), making it a promising target for therapeutic intervention. CF_3SePB pretreatment prevented LPS-induced hippocampal caspase-8 expression in this study ($F_{(3, 12)} = 11.68$, $p = 0.0007$) (Figure 6F), suggesting that the modulation of caspase-8 is part of the mechanism of action of this organoselenium compound.

The BCL-2-associated X protein (BAX) is a pro-apoptotic protein crucial in regulating apoptosis, or programmed cell death (Hamnér et al., 1999; Luchs and Pantaleão, 2010). As part of the BCL-2 protein family, which includes both pro- and anti-apoptotic members, BAX facilitates the release of cytochrome C from the mitochondria, leading to the activation of caspases that initiate the apoptotic process (Luchs and Pantaleão, 2010). In depression, there is evidence that the balance between pro-apoptotic proteins like BAX and anti-apoptotic proteins like BCL-2 may be disrupted, resulting in increased vulnerability to cell death in brain regions associated with mood regulation, such as the hippocampus (Deckwerth et al., 1996; Kosten et al., 2008). Studies show that BAX expression may be increased in depression, suggesting that a heightened propensity for apoptosis could contribute to the neurodegeneration observed in these states (Kosten et al., 2008). Dysregulation in the balance between BAX and BCL-2 can lead to the loss of neurons, particularly in the hippocampus, a crucial area for mood regulation and memory (Deckwerth et al., 1996; Kosten et al., 2008). Some studies indicate that antidepressant treatment may modulate BAX activity and reduce BAX expression, potentially helping to restore apoptotic balance, reduce cellular damage, and thereby promote neuronal survival (Kosten et al., 2008). Hippocampal BAX expression was significantly increased by LPS treatment in this study, and the pre-treatment with CF_3SePB prevented this alteration ($F_{(3, 11)} = 4.718$, $p = 0.0237$) (Figure 6G), which probably account for its antidepressant-like effect.

BDNF is a critical neurotrophin for brain development and function. In the CNS, BDNF plays a pivotal role in neurodevelopment, neural network formation, and neuronal plasticity (Martinho Jr et al., 2012). BDNF is particularly important for structural plasticity in the hippocampus. It promotes the growth and branching of developing neurons and regulates the morphology of mature neurons (Ji et al., 2010; Yang et al., 2020). Reduced levels of BDNF in the hippocampus have been linked to depression (Woelfer et al., 2018). Additionally, BDNF

may increase the expression of the antiapoptotic protein BCL-2, involved in cell survival (Arantes-Gonçalves and Coelho, 2006). Many antidepressants have been shown to increase BDNF levels in the brain. This increase is considered one of the mechanisms through which these medications alleviate depressive symptoms (Arantes-Gonçalves and Coelho, 2006; Banasr et al., 2011). In this study, no significant alterations were observed in hippocampal BDNF expression among the groups ($F_{(3, 11)} = 2.096$, $p = 0.1589$) (Figure 6H). However, LPS showed a slight tendency to reduce BDNF expression, and the pre-treatment with CF_3SePB showed a tendency in prevent this effect. CF_3SePB alone also showed a tendency in increase BDNF expression in relation to control group.

The administration of LPS also increased the levels of reactive species (RS) and lipid peroxidation, demonstrated by thiobarbituric acid reactive substances (TBARS) levels, in the mice hippocampus (Figure 7). Oxidative stress results from an imbalance between the production of RS and the antioxidant defense system (Barbosa et al., 2010). This imbalance generates highly reactive molecules that damage cellular structures, cause DNA breakage, and harm cell membranes, such as through lipid peroxidation (Stocker and Keaney, 2004; Dringen et al., 2005). Lipid peroxidation can be described as a sequence of biochemical events that leads to the oxidation of polyunsaturated lipids in cell membranes, making them an easy target for reactive oxygen species (Urso and Clarkson, 2003). Lipid peroxidation causes changes in membrane structure and permeability, which can lead to the destruction of the membrane's integrity and even cell death (Dal-Pizzol et al., 2000). The actions of RS destroy neurons and are closely associated with psychiatric disorders (Maes et al., 2000; Tsuboi et al., 2006; Valko et al., 2007). The brain is rich in polyunsaturated fatty acids, making it particularly susceptible to damage caused by RS (Valko et al., 2007; Mangialasche et al, 2009). Regions of the limbic system, such as the hippocampus, which are involved in the pathophysiology of depression, are affected by this imbalance, playing a crucial role in the pathogenesis of depression (Frodl et al., 2002). CF_3SePB pretreatment effectively prevented LPS-induced hippocampal increase of RS ($F_{(3, 28)} = 5.459$, $p = 0.0044$) and lipoperoxidation ($F_{(3, 44)} = 11.13$, $p < 0.0001$) in this study (Figure 7), showing an antioxidant effect. This propertie could be, at in least in part, responsible for its antidepressant-like effect.

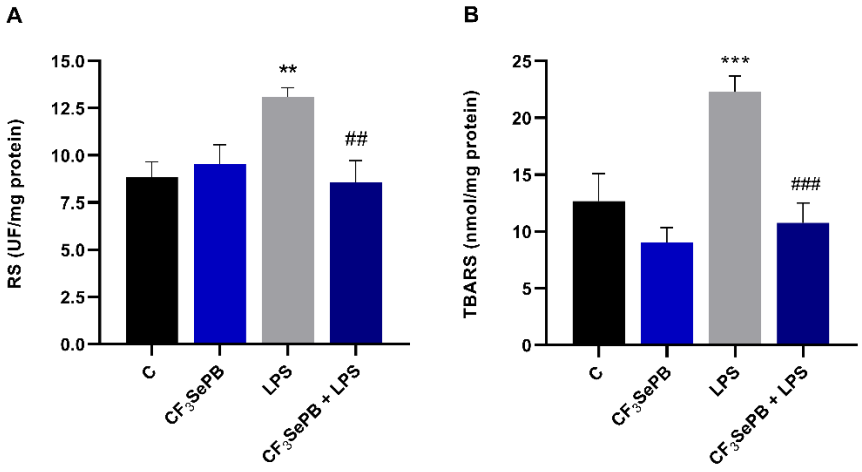

Figure 7: Effect of treatments with LPS (0.83 mg/kg, i.p.) and/or CF_3SePB (10 mg/kg, i.g.) on (A) RS and (B) TBARS levels. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 8-12 animals/group). (**) $p < 0.01$ compared to the control group. (##) $p < 0.01$ and (###) $p < 0.001$ compared to the LPS group. Abbreviations: LPS: Lipopolysaccharide; CF_3SePB: *N*-(3-((3-(trifluoromethyl)phenyl)selenyl)prop-2-yn-1-yl)benzamide; C: control; i.g.: intragastric; i.p.: intraperitoneal.

Conclusion

This study demonstrates the protective effects of the organoselenium compound CF_3SePB against depressive-like behaviors induced by LPS, as well as its anti-inflammatory effect. The antidepressant-like effects of CF_3SePB were mediated, in part, by modulating the expression of the pro-inflammatory genes NF-κB, NLRP3 inflammasome, and COX-2, and the expression of the apoptotic genes caspase-1, caspase-8, and BAX. Additionally, CF_3SePB reduced RS levels and lipid peroxidation, suggesting a neuroprotective role. While further research is required to fully elucidate the underlying mechanisms, the findings of this study provide compelling evidence for the potential of CF_3SePB as a novel therapeutic approach for depression, particularly in models involving inflammation-mediated mechanisms.

MATERIAL AND METHODS

Animals

For this study, adult male Swiss mice (25-30 g) from the Central Animal Facility of the Federal University of Pelotas were used. Each test was conducted in such a way that each animal was used only once per test (n = 12 animals/group, totaling 120 animals), except for the OFT, which was performed before the FST or the TST using

the same animals. The animals were maintained on a 12-hour light/12-hour dark cycle, with lights on at 7 a.m. The ambient temperature was 22 ± 2 ºC. The mice were housed in separate cages (20 × 30 × 13, 5 animals per cage) with access to commercial feed and fresh water ad libitum, without receiving food during the experimental protocol. After one hour of acclimatization, the tests were initiated and monitored by a blinded observer. The study followed the guidelines of the Ethics Committee on Animal Experimentation at UFPel (CEUA 23110.021022/2022-85), which are in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publications No. 8023, revised in 1978), aimed at minimizing the number of animals used and mitigating animal suffering.

Drugs

The synthesis of the CF_3SePB (Fig. 1) occurred in the Nucleus of Synthesis and Application of Organic and Inorganic Compounds (NUSAACOI), from the Federal University of Fronteira Sul (UFFS) (Balbom et al, 2019). CF_3SePB was solubilized in canola oil for intragastric administration at dose of 10 mg/kg. Fluoxetine was purchased locally and used intraperitoneally (i.p.) at a dose of 20 mg/kg as a positive control to validate the data obtained in the behavioral tests assessing antidepressant-like effects. The LPS from Escherichia coli, L-3129, serotype 0127:B8, was purchased from Sigma-Aldrich., St Louis, USA. LPS was dissolved in saline and administered at a dose of 0.83 mg/kg intraperitoneally (i.p.).

Experimental design

The experimental design for the compound CF_3SePB used to evaluate the antidepressant-like effect in a preclinical model of LPS-induced depression in mice is shown in figure 9. The animals were divided into two sets, and in both, at time zero, they were pretreated with the CF_3SePB at a dose of 10 mg/kg (i.g.), vehicle (saline (10 mL/kg, i.p.)), or fluoxetine (20 mg/kg, i.p.). After 30 minutes, 0.83 mg/kg of LPS or vehicle (canola oil (10 mL/kg, i.g.)) was administered intraperitoneally. Behavioral tests were conducted 24 hours after LPS injection. A positive control group was included with the administration of fluoxetine (20 mg/kg, i.p.) and LPS (0.83 mg/kg, i.p.) to validate the experimental protocol.

The behavioral tests performed with the animals in the first set were the TST, followed by the sucrose splash test (SST). For the second set, the behavioral tests conducted were the OFT, followed by the FST. After the behavioral tests of both sets, the mice were euthanized. For the animals in the first set, before euthanasia, cardiac puncture was performed to collect blood for analysis of plasma corticosterone levels, and after euthanasia, the hippocampus was removed for evaluation of lipid peroxidation. For the animals in the second set, after euthanasia, the hippocampus was removed for qRT-PCR analysis.

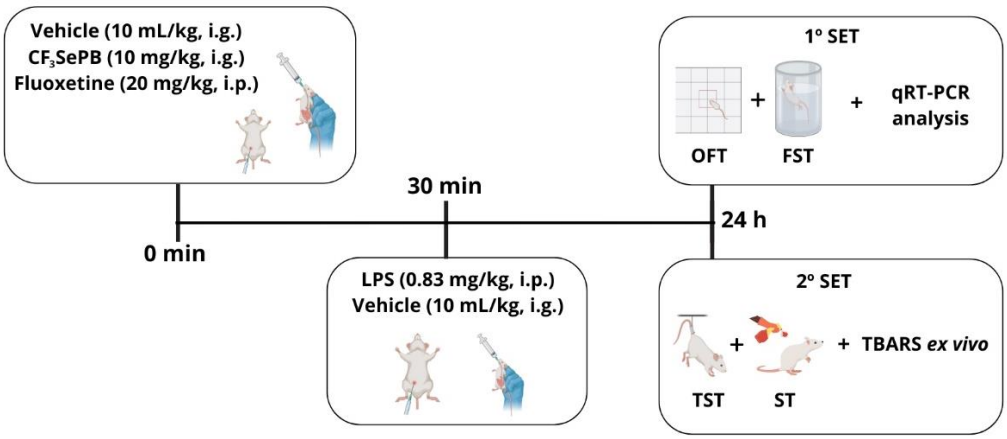

Figure 8: Experimental design for mechanism elucidation related to the antidepressant effect of the compound CF_3SePB in a model of depression induced by LPS in mice.

Behavioral tests

Open Field Test (OFT)

In the OFT, the mice were individually placed in the center of a wooden box (30 cm x 40 cm x 40 cm) divided into nine equally sized squares to investigate potential changes in locomotor and exploratory activity. Each animal was observed for 4 minutes, during which the number of crossings between the squares and the number of times they reared up on their hind legs (rearing posture) were recorded (Walsh and Cummins, 1976). After each test, the box was sanitized with 20% alcohol to eliminate any olfactory traces and prepare it for the next animal.

Forced Swimming Test (FST)

In this test, animals were placed in a cylinder measuring 29 cm in height filled with 1.5 L of water at 25 ± 1 ºC for 6 minutes, exposing them to a stressful situation from which they could not escape (Yankelevitch-Yahav et al., 2015; Berton et al., 2021). During this period, the latency to the first episode of immobility and the total immobility time were recorded as the mice attempted to escape by swimming or floating. The longer the animals floated without attempting to escape, the greater the behavior resembling depression.

Tail suspension test (TST)

The animals were suspended by the tail and secured with adhesive tape to a 50 cm high device. The mice were observed over a 6-minute period. During this time, immobility time and latency to the first immobility episode were recorded (Steru et al., 1985). The animals were considered immobile when they hung motionless, indicating an absence of escape attempt behavior. The longer the animals remained immobile, the more pronounced the depressive-like behavior.

Splash Test (ST)

The ST assesses self-grooming behavior as an indicator of motivational behavior, following the protocol used in other studies (Birmann et al., 2020; Liu et al., 2018). This method involves spraying a 10% sucrose solution onto the dorsal coat of mice in their cages. Due to the viscosity of this solution, the sucrose adheres to the mice's fur, triggering grooming behavior. The test lasts a total of 5 minutes, during which the latency time and total grooming time are recorded. The observer will be blinded to the different treatment groups.

Biochemical assays

Quantitative real-time PCR

Total mRNA was extracted from the prefrontal cortex and hippocampus using TRIzol followed by mRNA quantification. The cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription kit according to the manufacturer's protocol. The amplification was made with the GoTaq® qPCR Master Mix using the Stratagene Mx3005P real-time PCR. Gene expressions were normalized using GAPDH primer as a reference gene. The following genes were analyzed:

Table 1: Primer sequences used in the real-time PCR analysis.

Gene	Sequence 5'-3'
GAPDH	F: GGGTGAGGCCGGTGCTGAG
	R: TGGGGGTAGGAACACGGAAGG
BAX	F: CCACCAGCTCTGAACAGATC
	R: CAGCTTCTTGGTGGACGCAT
BCL2	F: TGGGATGCCTTTGTGGAACT
	R: GAGACAGCCAGGAGAAATCA
BDNF	F: TAACGGCGGCAGACAAAAAGACT
	R: GTGTCTATCCTTATGAATCACCAGCCAA
COX-2	F: TGGGTGTGAAAGGAAATAAGGA
	R: GAAGTGCTGGGCAAAGAATG
CASP-1	F: CGTCTTGCCCTCATTATCTG
	R: TCACCTCTTTCACCATCTCC
CASP-8	F: CTCCGAAAAATGAAGGACAGA
	R: CGTGGGATAGGATACAGCAGA
NFkB	F: GCTTTCGCAGGAGCATTAAC
	R: CCGAAGCAGGAGCTATCAAC
NRLP3	F: GGTCCTCTTTACCATGTGCTTC
	R: AAGTCATGTGGCTGAAGCTGTA

Reactive Species (RS) Measurement

RS levels in mouse hippocampi were quantified using according to Loetchutinat et al. (2005). Briefly, homogenate samples were incubated with Tris-HCl buffer (10 mM, pH 7.4) and dichloro-dihydro-fluorescein diacetate (DCHF-DA, 0.0033 mM). RS-mediated oxidation of DCHF-DA to fluorescent 2',7'-dichlorofluorescein

(DCF) was monitored spectrophotometrically at an excitation wavelength of 488 nm and an emission wavelength of 520 nm. Results were expressed as units (U) of fluorescence per milligram of protein.

Evaluation of lipid peroxidation

Lipid peroxidation in the hippocampus of mice was measured by the formation of malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) technique. An aliquot of the homogenized supernatant was incubated with 8.1% sodium dodecyl sulfate (SDS), 0.8% thiobarbituric acid (TBA), and acetic acid/HCl (pH 3.4) at 95 °C for 1 hour. TBARS levels was measured by spectrophotometry at a wavelength of 532 nm. The results were expressed as nmol TBARS/mg of protein (Ohkawa et al., 1979).

Protein determination

The protein level of the samples was determined according to the methodology of Bradford (1976), which uses bovine serum albumin as a standard (1 mg/mL).

Statistical analysis

Experimental data are presented as mean ± standard error of the mean (S.E.M.). Data normality was evaluated using the D'Agostino and Pearson test. Comparisons between experimental groups were performed using one-way ANOVA, followed by the Newman-Keuls post hoc test. Values of p less than 0.05 ($p < 0.05$) were considered statistically significant. All analyses were conducted using GraphPad software (GraphPad, San Diego, CA, USA).

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5. DISCUSSÃO

O TDM é uma condição psiquiátrica crônica caracterizada por sintomas como humor deprimido, disfunção cognitiva, anedonia, sentimentos de culpa, fadiga, baixa autoestima, distúrbios no sono, perda de apetite, dificuldade de concentração, entre outros (ŚLIFIRSKI et al., 2021; BAJ at al., 2023; PASTIS et al., 2024; CUI et al., 2024). Embora os mecanismos fisiopatológicos subjacentes à depressão ainda não sejam totalmente esclarecidos, a teoria monoaminérgica é amplamente aceita como uma das principais explicações para sua fisiopatologia (PASTIS et al., 2024; CUI et al., 2024). Segundo essa teoria, a depressão decorre de desequilíbrios nos neurotransmissores monoaminérgicos, como DA, 5-HT e NA, no SNC, causando os sintomas típicos do transtorno (DELMONDES et al., 2023). Dada a complexidade dos mecanismos patológicos do TDM, as opções terapêuticas e diagnósticas disponíveis atualmente apresentam limitações em termos de seletividade e eficácia (DELMONDES et al., 2023; CUI et al., 2024).

Este estudo revela que a benzamida CF_3SePB, um composto orgânico de Se inédito, exibe efeito do tipo antidepressivo significativo em camundongos. Os resultados mostrados no **artigo** são associados principalmente à interação com o sistema serotoninérgico, especificamente através dos receptores 5-HT_{1A} e 5-HT_3. A serotonina foi inicialmente o primeiro neurotransmissor associado a depressão na pesquisa (BREMSHEY et al., 2023). A eficácia antidepressiva do CF_3SePB foi validada por meio do TNF e TSC, métodos amplamente aceitos na triagem pré-clínica de novos antidepressivos (YANKELEVITCH-YAHAV et al., 2015; ARMARIO, 2021). Ambos os testes demonstraram que o CF_3SePB reduz o tempo de imobilidade dos camundongos, evidenciando seu potencial antidepressivo. A depleção de serotonina no cérebro dos camundongos, induzida pelo p-CPA, bloqueou o efeito antidepressivo do CF_3SePB, sugerindo que a presença funcional da serotonina é crucial para a manifestação dos efeitos antidepressivos do composto.

Adicionalmente, o antagonista do receptor 5-HT_{1A}, WAY100635, bloqueou o efeito antidepressivo do CF_3SePB, reforçando a ideia de que a modulação desse receptor é essencial para o efeito observado. O papel do Se e de compostos que o incorporam na modulação do humor e da depressão também é relevante, uma vez que a deficiência de Se tem sido associada a distúrbios de humor e a presença de compostos contendo Se tem mostrado propriedades antidepressivas em diversos estudos (WANG et al., 2018). A combinação do Se com outras estruturas químicas,

como a benzamida, pode potencializar essas propriedades, conforme sugerido pelo CF_3SePB. A avaliação da toxicidade aguda do CF_3SePB demonstra que, apesar de sua eficácia antidepressiva, o composto tem um baixo potencial para induzir efeitos tóxicos a altas doses, conforme evidenciado pela ausência de mortalidade e pela manutenção das funções hepáticas e renais normais nos camundongos.

Interessantemente, o composto CF_3SePB mostrou que seu efeito do tipo antidepressivo em camundongos está envolvido na modulação do sistema dopaminérgico, descrito no **manuscrito 1**, além da modulação do sistema serotoninérgico observada no artigo. Os antagonistas não seletivos dopaminérgicos, como o haloperidol, e os antagonistas seletivos dos receptores D_1 e D_2, SCH 23390 e sulpirida, respectivamente, impediram o efeito anti-imobilidade do CF_3SePB no TNF, sem alterar a atividade locomotora dos animais no TCA. O TNF é amplamente utilizado para avaliar a atividade de compostos com potencial antidepressivo (YANKELEVITCH-YAHAV et al., 2015; ARMARIO, 2021). Esses achados corroboram a eficácia do TNF como um modelo para avaliar compostos com potenciais propriedades antidepressivas, além de destacar a importância do sistema dopaminérgico na mediação dos efeitos antidepressivos do CF_3SePB. Isso sugere que o efeito antidepressivo do CF_3SePB pode estar associado à interação com o sistema dopaminérgico, além da sua relação com o sistema serotoninérgico.

A DA desempenha um papel fundamental na fisiopatologia da depressão, influenciando várias funções cerebrais, incluindo emoções, prazer e atividade locomotora (MIZUNO et al., 2023). A disfunção nos neurotransmissores monoaminérgicos, como a DA, é uma característica crítica da depressão (WANG et al., 2019). O estudo confirma que a modulação dos receptores D_1 e D_2 é essencial para o efeito antidepressivo do CF_3SePB, reforçando a hipótese de que a DA influencia significativamente a resposta a tratamentos antidepressivos. A baixa solubilidade em água do CF_3SePB, embora não afete negativamente a absorção, junto com a alta absorção intestinal e a permeabilidade à BHE, indica que o composto possui características farmacocinéticas favoráveis. Apesar da indicação de hepatotoxicidade nos resultados in sílico, a ausência de hepatotoxicidade em estudos agudos sugere que o CF_3SePB é um candidato promissor para o desenvolvimento de novos antidepressivos, apresentando baixa toxicidade e potencial para uso clínico.

A interação entre o sistema imunológico e o SNC tem ganhado destaque crescente, sendo que a ativação do sistema imunológico pode desencadear alterações no cérebro que afetam o humor, o comportamento e influenciam diretamente os circuitos neurais (BURAS et al., 2016; HASSAMAL, 2023; WU e

ZHANG, 2023). Essa conexão é apoiada pela teoria inflamatória da depressão, que sugere que processos inflamatórios crônicos ou agudos podem contribuir para o desenvolvimento e manutenção dos sintomas depressivos (GUO et al., 2024). A inflamação sistêmica, que é a ativação do sistema imunológico periférico, pode contribuir para o desenvolvimento da depressão, de forma que as respostas imunes inatas e adaptativas podem comprometer a integridade da BHE propagando os sinais inflamatórios para o SNC, causando a neuroinflamação (LIU et al., 2022; RICHARDSON et al., 2022; GUO et al., 2024).

Colaborando com os achados do artigo e do manuscrito 1, no **manuscrito 2** foi demonstrado o efeito do tipo antidepressivo do composto CF_3SePB a partir da indução de depressão por LPS. O CF_3SePB foi eficaz na reversão dos comportamentos depressivos induzidos por LPS no TSC, TNF e TBS, sem afetar a locomoção dos camundongos no TCA. Esse achado é relevante, pois sugere que o CF_3SePB pode atuar especificamente em mecanismos relacionados à depressão sem causar efeitos adversos sobre a mobilidade, o que é um aspecto crucial em tratamentos para transtornos depressivos (ABELAIRA et al., 2013; CATHOMAS et al., 2015; BECKER et al., 2021; YIN et al., 2023). Além disso, o CF_3SePB impediu o aumento na expressão dos genes pró-inflamatórios, incluindo o NF-κB, NLRP3 e COX-2, assim como dos genes apoptóticos caspase-1, caspase-8 e BAX induzidos pelo LPS. Esses resultados indicam o potencial do CF_3SePB para modular vias inflamatórias e de morte neuronal, fatores amplamente reconhecidos por seu papel na fisiopatologia da depressão (GLEZER et al., 2000; KOO e DUMAN, 2008; YUCEL et al., 2016; CAVIEDES et al., 2017; INSERRA et al., 2019; STREKALOVA et al., 2022; KOUBA et al., 2023). O controle da expressão desses genes sugere que o CF_3SePB pode reduzir a neuroinflamação, um componente crítico na depressão induzida por estresse inflamatório.

O tratamento também reduziu os níveis de ROS e a peroxidação lipídica no hipocampo induzidos pelo LPS, sugerindo efeitos neuroprotetores. O estresse oxidativo e a peroxidação lipídica são conhecidos por contribuir para a disfunção neuronal e a morte celular, e sua redução sugere que o CF_3SePB pode proteger contra danos celulares que frequentemente acompanham a depressão (FRODL et al., 2002). Os achados deste trabalho evidenciam o potencial inovador do CF_3SePB como um novo agente terapêutico para a depressão. O composto não causou toxicidade nos animais e demonstrou eficácia na modulação dos sistemas serotoninérgico e dopaminérgico, além de agir em modelos que envolvem mecanismos mediados por inflamação. Esses resultados sugerem que o CF_3SePB pode oferecer uma

abordagem terapêutica promissora, abordando múltiplos mecanismos associados à depressão sem provocar efeitos adversos significativos, destacando seu potencial para o desenvolvimento de novas estratégias no tratamento desse transtorno.

A benzamida CF_3SePB, uma molécula inédita com efeito antidepressivo e anti-inflamatório, envolvida na modulação do sistema serotoninérgico e dopaminérgico, mostrou-se promissora no tratamento da depressão em camundongos, devido à sua capacidade de atuar sobre os sistemas serotoninérgico, dopaminérgico e mostrar seu potencial efeito anti-inflamatório, mecanismos chave no desenvolvimento e progressão do transtorno depressivo.

6. CONCLUSÃO

Este estudo forneceu evidências inéditas sobre o efeito do tipo antidepressivo do composto CF_3SePB em camundongos, destacando a modulação do sistema serotoninérgico, especialmente os receptores 5-HT_{1A} e 5-HT_3, e a ausência de interação com o sistema noradrenérgico. A participação do sistema dopaminérgico também foi evidenciada através da modulação dos receptores D_1 e D_2. Adicionalmente, o CF_3SePB apresentou um baixo potencial para induzir efeitos adversos e mostrou alta absorção intestinal e capacidade de atravessar a barreira hematoencefálica, conforme previsto pelas análises in silico de ADMET.

Os efeitos protetores do CF_3SePB contra comportamentos depressivos induzidos por LPS foram associados à modulação da expressão de genes pró-inflamatórios e apoptóticos, além da redução de espécies reativas e peroxidação lipídica, sugerindo um efeito neuroprotetor. Esses resultados não apenas reforçam o potencial terapêutico inovador do composto CF_3SePB para o tratamento da depressão, particularmente em modelos que envolvem mecanismos inflamatórios, mas também destacam a necessidade de mais estudos para explorar plenamente os mecanismos subjacentes e desenvolver novas estratégias terapêuticas para a depressão. Como perspectivas futuras, o composto CF_3SePB será testado em outros modelos clínicos abrangendo outros sistemas, a fim de avaliar sua eficácia terapêutica e identificar potenciais interações com outros sistemas do SNC.

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8. ANEXOS

ANEXO A - Aprovação do Comitê de Ética

PARECER Nº **146/2020/CEEA/REITORIA**
PROCESSO Nº 23110.028792/2020-97

Certificado

Certificamos que a proposta intitulada "**AVALIAÇÃO DO EFEITO DO TIPO ANTIDEPRESSIVO DE BENZAMIDAS SELENOPROPARGÍLICAS SUBSTITUÍDAS EM CAMUNDONGOS**", registrada com o n° 23110.028792/2020-97**,** sob a responsabilidade de **César Augusto Brüning** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **08 de dezembro de 2020**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	01/02/2021 a 01/02/2024
Espécie/linhagem/raça	*Mus musculus*/Swiss
N° de animais	690
Idade	60 dias
Sexo	684 machos e 6 fêmeas
Origem	Biotério Central - UFPel

Código para cadastro nº **CEEA 28792-2020**

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 11/12/2020, às 11:09, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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Referência: Processo nº 23110.028792/2020-97 SEI nº 1152458

ANEXO B - Aprovação do Comitê de Ética

PARECER Nº **170/2022/CEUA/REITORIA**
PROCESSO Nº 23110.021022/2022-85

Certificado

Certificamos que a proposta intitulada **"Avaliação do efeito de uma benzamida selenopropargílica substituída em modelo de depressão induzida por lipopolissacarídeo em camundongos"**, registrada com o n° **23110.021022/2022-85,** sob a responsabilidade de **César Augusto Brüning** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas, em reunião de 30/10/2022.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início: 01/10/2022 Término: 15/12/2024
Espécie/linhagem/raça	*Mus musculus* / Swiss
N° de animais	**182**
Idade	2 meses
Sexo	Machos
Origem	Biotério Central - UFPel

Código para cadastro nº **CEUA 021022/2022-85**

Priscila Marques Moura de Leon

Coordenadora da CEUA

Documento assinado eletronicamente por **PRISCILA MARQUES MOURA DE LEON**, **Professor do Magistério Superior**, em 11/10/2022, às 15:25, conforme horário oficial de Brasília, com fundamento no art. 4º, § 3º, do Decreto nº 10.543, de 13 de novembro de 2020.

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