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ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E OPIÓIDE NA AÇÃO DO TIPO ANTIDEPRESSIVA DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

César Augusto Brüning

Santa Maria, RS, Brasil
2012

ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E OPIÓIDE NA AÇÃO DO TIPO ANTIDEPRESSIVA DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA EM CAMUNDONGOS

por

César Augusto Brüning

Dissertação apresentada ao Programa de Pós Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Mestre em Bioquímica Toxicológica**

Orientador: Profª Drª Cristina Wayne Nogueira

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Universidade Federal de Santa Maria
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como requisito parcial para obtenção do grau de
Mestre em Bioquímica Toxicológica

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“A mente que se abre a uma nova idéia

jamais voltará a seu tamanho original.”

(Albert Einstein)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO E OPIÓIDE NA AÇÃO DO TIPO ANTIDEPRESSIVA DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA EM CAMUNDONGOS

AUTOR: CÉSAR AUGUSTO BRÜNING
ORIENTADORA: CRISTINA WAYNE NOGUEIRA

Local e Data da Defesa: Santa Maria, 10 de agosto de 2012

Os sistemas serotoninérgico e opióide estão envolvidos na patogênese da depressão assim como no mecanismo de ação dos principais antidepressivos atualmente comercializados. Dados da literatura demonstram que o composto orgânico de selênio disseleneto de *m*-trifluormetil-fenila (*m*–CF_3–PhSe)$_2$ apresenta atividade antioxidante e ansiolítica e é um inibidor seletivo da atividade da enzima monoamino oxidase A (MAO A). O presente estudo teve por objetivo investigar a ação do tipo antidepressiva do (*m*–CF_3–PhSe)$_2$ em camundongos fêmeas, empregando o teste do nado forçado. O envolvimento dos sistemas serotoninérgico e opióide nessa ação também foi avaliado. O composto (*m*–CF_3–PhSe)$_2$ nas doses de 50 e 100 mg/kg (p.o.) apresentou ação do tipo antidepressiva. Esta ação do (*m*–CF_3–PhSe)$_2$ (50 mg/kg p.o.) foi bloqueada pelo pré-tratamento dos camundongos com WAY100635 (0.1 mg/kg, s.c., um antagonista seletivo do receptor 5-HT_{1A}), ritanserina (4 mg/kg, i.p., um antagonista não-seletivo dos receptores 5-$HT_{2A/2C}$), ondansetrona (1 mg/kg, i.p., um antagonista seletivo do receptor 5-HT_3) e naloxona (1 mg/kg, i.p., um antagonista não-seletivo dos receptores opióides). A administração de (*m*–CF_3–PhSe)$_2$ e a interação dos antagonistas com (*m*–CF_3–PhSe)$_2$ não prejudicou a atividade locomotora dos animais, observado no teste do campo aberto. Esses resultados sugerem que o (*m*–CF_3–PhSe)$_2$ produz ação do tipo antidepressiva em camundongos no teste do nado forçado e essa ação é possivelmente mediada através de uma interação com os sistemas serotoninérgico e opióide.

Palavras-chave: Composto orgânico de selênio, antidepressivo, serotonina, opióide, depressão, selênio.

ABSTRACT

Dissertation of Master's Degree
Federal University of Santa Maria, RS, Brazil

INVOLVEMENT OF SEROTONERGIC AND OPIOID SYSTEMS IN THE ANTIDEPRESSANT-LIKE EFFECT OF *m*-TRIFLUOROMETHYL-DIPHENYL DISELENIDE IN MICE

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Serotonergic and opioid systems have been implicated in major depression and in the action mechanism of antidepressants. The organoselenium compound *m*-trifluoromethyl-diphenyl diselenide (*m*–CF_3–PhSe)$_2$ shows antioxidant and anxiolytic activities and is a selective inhibitor of monoamine oxidase A activity. The present study was designed to investigate the antidepressant-like effect of (*m*–CF_3–PhSe)$_2$ in female mice, employing the forced swimming test. The involvement of the serotonergic and opioid systems in the antidepressant-like effect of (*m*–CF_3–PhSe)$_2$ was appraised. (*m*–CF_3–PhSe)$_2$ at doses of 50 and 100 mg/kg (p.o.) exhibited antidepressant-like action in the forced swimming test. The effect of (*m*–CF_3–PhSe)$_2$ (50 mg/kg p.o.) was prevented by pretreatment of mice with WAY100635 (0.1mg/kg, s.c. a selective 5-HT_{1A} receptor antagonist), ritanserin (4 mg/kg, i.p., a nonselective $5HT_{2A/2C}$ receptor antagonist), ondansetron (1 mg/kg, i.p., a selective 5-HT_3 receptor antagonist) and naloxone (1 mg/kg, i.p., a non-selective antagonist of opioid receptors). The administration of (*m*–CF_3–PhSe)$_2$ and the interaction of antagonists with (*m*–CF_3–PhSe)$_2$ did not cause any change in the locomotor activity assessed in the open-field test. These results suggest that (*m*–CF_3–PhSe)$_2$ produced an antidepressant-like effect in the mouse forced swimming test and this effect seems most likely to be mediated through an interaction with serotonergic and opioid systems.

Keywords: Organoselenium, antidepressant-like, serotonin, opioid, depression, selenium.

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LISTA DE ABREVIATURAS

(*m*–CF_3–PhSe)$_2$ – Disseleneto de *m*-trifluormetil-fenila
$(PhSe)_2$ – Disseleneto de difenila
5-HT– Serotonina
ANVISA – Agência Nacional de Vigilância Sanitária
BHE – Barreira Hemato-encefálica
INSS – Instituto Nacional de Seguro Social
ISRN – Inibidores seletivos da recaptação de norepinefrina
ISRS – Inibidores seletivos da recaptação de serotonina
MAO – Monoamino oxidase
OMS – Organização Mundial da Saúde
SNC – Sistema nervoso central
TNF – Teste do nado forçado
WAY100635 – N - [2-[4- (2-metoxifenil)-1-piperazinil] etil] - N - 2-piridinilciclohexano carboxamida

SUMÁRIO

1 INTRODUÇÃO

A depressão é uma desordem mental comum, caracterizada por sintomas emocionais e físicos, entre eles humor deprimido, perda de interesse e prazer, sentimentos de fracasso ou baixa autoestima, distúrbios de sono e dificuldade de concentração. Esses problemas podem se tornar crônicos ou recorrentes levando a substanciais prejuízos na vida do paciente. Ademais, a depressão pode levar ao suicídio, uma fatalidade associada à perda de 850000 vidas a cada ano. Segundo estimativas da Organização Mundial da Saúde (OMS), esta desordem psiquiátrica afeta cerca de 121 milhões de pessoas mundialmente e será a segunda principal enfermidade ao final desta década, superada apenas pelas doenças cardiovasculares (McKenna et al., 2005; WHO, 2011).

Os aspectos patofisiológicos das desordens depressivas ainda não são completamente conhecidos. A hipótese de maior relevância data de 1967, onde uma deficiência do sistema monoaminérgico seria a causa do surgimento dos sintomas depressivos, ou seja, diminuição dos níveis cerebrais dos neurotransmissores serotonina (5-HT), norepinefrina e dopamina (Coppen, 1967). Diversas observações fornecem suporte a esta teoria. Certos fármacos que apresentam efetividade no tratamento da depressão agem bloqueando a recaptação de neurotransmissores monoaminérgicos ou inibindo a atividade da enzima monoamino oxidase (MAO), responsável pelo catabolismo de 5-HT e norepinefrina, consequentemente aumentando os níveis destes na fenda sináptica (Mann, 2005; Belmaker and Agam, 2008). De acordo, a depleção experimental do triptofano, precursor da síntese de 5-HT, causa uma recidiva no estado depressivo em pacientes tratados com inibidores seletivos da recaptação de 5-HT (ISRS). Semelhantemente, a depleção da tirosina hidroxilase, necessária à síntese de norepinefrina, causa recidiva nos pacientes tratados com inibidores seletivos da recaptação de norepinefrina (ISRN) (Ruhe et al., 2007). Por fim, as monoaminas são amplamente utilizadas em diversas regiões do sistema nervoso central (SNC), o que pode explicar a diversidade de patologias classificadas como depressão (Mann, 2005; Belmaker and Agam, 2008).

Com base na teoria monoaminérgica, os principais antidepressivos prescritos atualmente agem inibindo a recaptação de 5-HT ou norepinefrina, no entanto, cerca de 1/3 dos pacientes falham ao responder a este tipo de tratamento (Hegadoren, 2009; Dupuy et al, 2011). Desta forma os pesquisadores têm voltado a sua atenção para hipóteses alternativas das desordens depressivas. Inúmeros estudos têm sugerido que o sistema opióide também possa

ser um importante alvo terapêutico no tratamento da depressão (Schreiber et al., 2002; Zomkowski et al., 2005), em adição ao seu papel nos processos de dor. Esses estudos relacionam um prejuízo no sistema opióide com a patofisiologia da depressão. Isso tem sido sugerido, entre outras razões, por que os receptores opióides μ e os opióides endógenos endorfinas, são densamente distribuídos em diversas regiões cerebrais implicadas na resposta a agentes estressores e a estímulos emocionais, como o sistema límbico (septo, núcleo accumbens, amígdala), núcleo talâmico, locus coeruleus e tronco cerebral (Drevets, 1998; Martin-Schild et al, 1999; Zadina, 2002; Sheline, 2000). Algumas evidências demonstram que pacientes com depressão apresentam níveis diminuídos de β-endorfina (Darko et al., 1992; Djurovic et al., 1999) e uma pronunciada redução na disponibilidade de receptores opióides μ no tálamo posterior e no córtex cingulado anterior (Kennedy et al., 2006). Dados clínicos indicam a efetividade de agonistas de receptores opióides μ, oxicodona e oximorfona, e do agonista parcial buprenorfina em pacientes com depressão refratária a outros tratamentos (Bodkin et al., 1995; Stoll and Rueter, 1999). Além disso, Cravezic e colaboradores (2011), utilizando roedores, demonstraram que bloqueadores da degradação de endorfinas causam efeito do tipo antidepressivo. O receptor δ opióide em particular também tem sido associado às desordens de humor (Filiol et al., 2000; Broom et al., 2002; Saitoh et al., 2005). Saitoh e colaboradores (2011) demonstraram que o agonista dos receptores δ, KNT-127, produz ação do tipo antidepressiva em camundongos. Interessantemente, este receptor encontra-se concentrado nas principais regiões cerebrais envolvidas na modulação do humor (Madar et al., 1996). Convém salientar que o envolvimento do sistema opióide na depressão não é necessariamente independente da teoria monoaminérgica, uma vez que inúmeros estudos demonstram que as endorfinas modulam as transmissões serotoninérgicas (Tao and Auerbach, 2002; Hung et al, 2003), dopaminérgicas (Bujdoso et al, 2003; Huang et al, 2004) e noradrenérgicas (Al-Khrasani et al, 2003; Hung et al, 2003).

Apesar dos avanços na compreensão da psicofarmacologia da depressão e a introdução de diversas novas classes de antidepressivos, somente 50 % dos pacientes recebem algum tipo de tratamento, e dentre estes, apenas 60 – 70 % respondem à terapia com antidepressivos típicos, como os ISRS (Joffe et al., 1996; Ward and Irazoqui, 2010). Em relação às drogas que possuem alguma efetividade clínica na depressão, todas elas apresentam inúmeras limitações, como lento início de ação e muitos efeitos colaterais (Páez-Pereda, 2005). Resultado disso, de acordo com OMS, a depressão é umas das doenças que mais gera custos sociais e econômicos para os governos, devido aos gastos com o tratamento da população e às perdas de produção. Estima-se que cerca de 17 milhões de brasileiros sofram

de desordens depressivas e de acordo com um levantamento feito pelo Instituto Nacional de Seguro Social (INSS), em torno de 74000 trabalhadores foram afastados de suas atividades no ano de 2008 em decorrência dessa doença. Segundo o Ministério da Previdência, só em junho de 2008 foram destinados cerca de R$ 12 milhões no pagamento de benefícios aos afastados por depressão. Desta forma a busca por novos compostos e estratégias terapêuticas efetivas para o tratamento das desordens depressivas torna-se de grande importância.

A triagem de novas drogas que possam ser efetivas no tratamento da depressão geralmente é realizada em testes com animais de laboratório, principalmente roedores (Willner e Mitchell, 2002; Duman, 2010). Embora seja difícil avaliar a depressão em animais, existe a hipótese de que os mesmos possam apresentar comportamento do tipo depressivo, como o apresentado pelos humanos. Isso é frequentemente alcançado avaliando o comportamento do animal frente a um evento estressante inescapável (Duman, 2010). Existem inúmeros modelos animais validados para o estudo do comportamento depressivo, entre eles o teste do nado forçado (TNF). Este teste foi originalmente descrito por Porsolt e colaboradores em 1977, sendo amplamente utilizado na triagem de novas drogas antidepressivas, por possuir alto valor preditivo, devido à resposta aos medicamentos já existentes, como os antidepressivos tricíclicos, ISRS, inibidores da MAO e antidepressivos atípicos (Porsolt et al., 1977; Cryan and Lucki, 2000; Cryan et al., 2002).

O selênio, elemento químico descoberto por Jöns Jakob Berzelius em 1817, nutricionalmente essencial aos mamíferos, possui papéis fisiológicos importantes, como componente estrutural de diversas enzimas antioxidantes envolvidas na decomposição de peróxidos (Ursini and Bindoli, 1987; Rayman et al., 2000). A ANVISA recomenda que a ingestão diária de selênio para adultos seja de 70 μg, e o mesmo pode ser encontrado em alimentos como o alho, castanha-do-pará, cebola, brócolis, cogumelos, cereais, pescados, ovos e carnes (Dumont et al., 2006). Sabe-se que este elemento desempenha importante papel para o cérebro. Estudos demonstram que quando há depleção de selênio no organismo, o cérebro recebe uma oferta prioritária deste elemento em relação aos outros órgãos (Buckman et al., 1993; Whanger, 2001). O baixo nível de selênio no cérebro tem efeitos negativos sobre seu funcionamento e está associado a déficit cognitivo, podendo agravar condições neurodegenarativas, como a Doença de Alzheimer (Schweizer et al., 2004; Corrigan et al., 1991), além de alterar a taxa de *turnover* de neurotransmissores (Castano et al., 1997).

Em foco, inúmeros estudos têm demonstrado o papel do selênio nos transtornos de humor (Benton and Cook, 1991; Hawhes and Hornbostel, 1996; Benton 2002). Níveis diminuídos deste elemento na dieta (< 40 μg/dia) estão associados com um aumento na

incidência de ansiedade, depressão e agressividade (Finley e Penland, 1998). Ao contrário, o aumento dos níveis de selênio na dieta ou a suplementação estão associados à melhora do humor e estado depressivo (Benton and Cook, 1991; Rayman et al., 2006; Rayman 2008), embora altos níveis deste elemento possam ser tóxicos (Moxon e Rhian, 1943). O selênio existe na natureza sob a forma orgânica (selenocisteína, selenocistina e selenometionina) e inorgânica (selenito e selenato) (Nakamuro et al., 2000). As formas orgânicas são menos tóxicas e apresentam maior biodisponibilidade (Narajji et al., 2007). Diante disso, a pesquisa científica tem dado atenção aos efeitos farmacológicos de compostos orgânicos de selênio, principalmente no que se refere aos seus efeitos sobre o SNC (Narajji et al., 2007; PATAI, 2012).

Nosso grupo de pesquisa tem amplamente estudado as propriedades farmacológicas e toxicológicas de compostos orgânicos de selênio, especialmente do protótipo disseleneto de difenila $(PhSe)_2$ (**Figura 1A**) (Nogueira and Rocha, 2004; 2010; PATAI, 2012), o qual apresenta diversos efeitos farmacológicos em roedores, como antioxidante (Luchese et al., 2007), anti-inflamatório (Nogueira et al., 2003a; Luchese et al., 2012) e antinociceptivo (Savegnago et ., 2007a). Este composto lipofílico é capaz de atravessar a barreira hemato-encefálica (BHE) e alcançar o SNC (Prigol et al., 2010), onde pode exercer seus efeitos farmacológicos ou toxicológicos. Dessa forma, também foi demonstrado que o $(PhSe)_2$ apresenta ação ansiolítica e antidepressiva, as quais estão relacionadas à interação com receptores $GABA_A$ e com o sistema monoaminérgico (Savegnago et al., 2007b; Ghisleni et al., 2008). Por outro lado este composto também apresenta efeitos tóxicos. A exposição crônica a altas doses de $(PhSe)_2$ pode causar efeitos centrais e há evidências de teratogenicidade em camundongos (Nogueira and Rocha, 2004; Weis et al., 2006; Rosa et al., 2007). Diante disso, alterações na molécula do $(PhSe)_2$ têm sido realizadas com o objetivo de neutralizar seus efeitos tóxicos e manter ou potencializar seus efeitos farmacológicos (Nogueira et al., 2003b; Savegnago et al., 2009). Nogueira e colaboradores (2003b) demonstraram que quando administrado pela via intraperitoneal (i.p.), o $(PhSe)_2$ causa convulsão e morte em camundongos, e o disseleneto de *m*-trifluormetil-fenila $(m\text{–}CF_3\text{–}PhSe)_2$ (**Figura 1B**), um análogo do $(PhSe)_2$ dissubstituído com o grupamento CF_3, não apresenta essa atividade pró-convulsivante nas mesmas doses que o $(PhSe)_2$ induz convulsão, sugerindo que o $(m\text{–}CF_3\text{–}PhSe)_2$ possa ser menos tóxico.

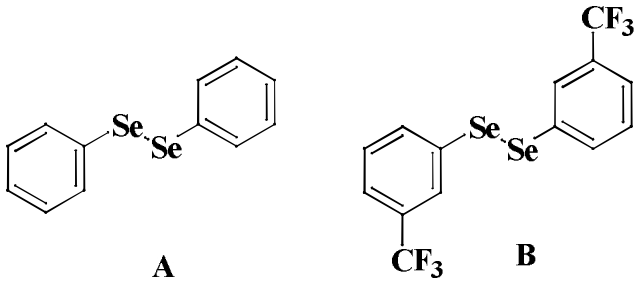

Figura 1. Estrutura química do disseleneto de difenila $(PhSe)_2$ (A) e do disseleneto de *m*-trifluormetil-fenila (*m*–CF_3–$PhSe)_2$ (B).

Neste contexto, as pesquisas em torno do composto (*m*–CF_3–$PhSe)_2$ acentuaram-se recentemente. Além de seu efeito antioxidante (Prigol et al., 2009a), o (*m*–CF_3–$PhSe)_2$ possui ação antipsicótica, por atenuar o comportamento estereotipado induzido por apomorfina em camundongos (Machado et al., 2006), e também ação anticonvulsivante em um modelo com pentilenotetrazol (Prigol et al., 2009b). De especial interesse, foi demonstrado que o (*m*–CF_3–$PhSe)_2$ possui ação do tipo ansiolítica em camundongos, possivelmente relacionada com o sistema serotoninérgico, uma vez que essa ação foi bloqueada por antagonistas de receptores deste sistema, a saber, WAY100635 (antagonista seletivo de receptores 5-HT_{1A}), ritanserina (antagonista não-seletivo de receptores 5-$HT_{2A/2C}$) e ondansetrona (antagonista seletivo de receptores 5-HT_3). Além disso, o (*m*–CF_3–$PhSe)_2$ inibiu seletivamente a atividade da MAO A *ex vivo*, subtipo da enzima MAO, responsável principalmente pela degradação da 5-HT (Brüning et al., 2009). Também foi demonstrado recentemente que o (*m*–CF_3–$PhSe)_2$ possui ação antinociceptiva em modelos químicos e térmicos de nocicepção e este efeito foi relacionado à interação com receptores opióides, mais especificamente os receptores μ e δ, uma vez que o efeito do (*m*–CF_3–$PhSe)_2$ foi bloqueado por antagonistas específicos destes receptores, naloxonazina e naltrindol, respectivamente (Brüning et al., 2010).

Diante do exposto, considerando a necessidade do desenvolvimento de novos tratamentos para a depressão, o envolvimento do composto (*m*–CF_3–$PhSe)_2$ com os sistemas serotoninérgico e opióide e o papel destes na patogênese das desordens depressivas, o (*m*–CF_3–$PhSe)_2$ torna-se um interessante candidato à agente terapêutico da depressão.

2 OBJETIVOS

2.1 Objetivo geral

Identificar a possível ação do tipo antidepressiva do composto (*m*–CF_3–PhSe)$_2$ em camundongos e o envolvimento dos sistemas serotoninérgico e opióide nessa ação.

2.1 Objetivos específicos

Considerando os aspectos mencionados, os objetivos específicos deste estudo compreendem:

- Avaliar a possível ação do tipo antidepressiva do (*m*–CF_3–PhSe)$_2$ em camundongos;
- Verificar se o sistema serotoninérgico (receptores 5-HT_{1A}, 5-$HT_{2A/2C}$ e 5-HT_3) e opióide estão envolvidos na ação do tipo antidepressiva do (*m*–CF_3–PhSe)$_2$.

3 RESULTADOS

Os resultados que fazem parte dessa dissertação estão apresentados na forma de um artigo científico. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas do artigo estão dispostos de acordo com a recomendação do periódico científico no qual foi publicado.

3.1 Artigo

O efeito do tipo antidepressivo do disseleneto de *m*-trifluormetil-fenila no teste do nado forçado em camundongos envolve os sistemas serotoninérgico e opióide

ANTIDEPRESSANT-LIKE EFFECT OF *m*-TRIFLUOROMETHYL-DIPHENYL DISELENIDE IN THE MOUSE FORCED SWIMMING TEST INVOLVES OPIOID AND SEROTONERGIC SYSTEMS

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Antidepressant-like effect of *m*-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems

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Selenium

ABSTRACT

Serotonergic and opioid systems have been implicated in major depression and in the action mechanism of antidepressants. The organoselenium compound *m*-trifluoromethyl-diphenyl diselenide (m–CF_3–$PhSe)_2$ shows antioxidant and anxiolytic activities and is a selective inhibitor of monoamine oxidase A activity. The present study was designed to investigate the antidepressant-like effect of (m–CF_3–$PhSe)_2$ in female mice, employing the forced swimming test. The involvement of the serotonergic and opioid systems in the antidepressant-like effect of (m–CF_3–$PhSe)_2$ was appraised. (m–CF_3–$PhSe)_2$ at doses of 50 and 100 mg/kg (p.o.) exhibited antidepressant-like action in the forced swimming test. The effect of (m–CF_3–$PhSe)_2$ (50 mg/kg p.o.) was prevented by pretreatment of mice with WAY100635 (0.1 mg/kg, s.c. a selective 5-HT_{1A} receptor antagonist), ritanserin (4 mg/kg, i.p., a non-selective $5HT_{2A/2C}$ receptor antagonist), ondansetron (1 mg/kg, i.p., a selective 5-HT_3 receptor antagonist) and naloxone (1 mg/kg, i.p., a non-selective antagonist of opioid receptors). These results suggest that (m–CF_3–$PhSe)_2$ produced an antidepressant-like effect in the mouse forced swimming test and this effect seems most likely to be mediated through an interaction with serotonergic and opioid systems.

1. Introduction

Depression is characterized by a wide range of debilitating emotional and physical symptoms. Numerous neural pathways are involved in the pathophysiology of depression. Therefore, a great number of monoamine neurotransmitters participate in the underlying mechanisms of antidepressants (Palucha and Pile, 2002). The causes of depression have been, in part, attributed to the dysregulation of one or all of these monoamine neurotransmitters at the synapse (Prange et al., 1974), principally the serotonin (5-HT) (Nutt, 2008), which plays important role in mediating behavioral effects of antidepressant drugs (Millan, 2004; Papakostas, 2006). Most of the prescribed antidepressants directly affect 5-HT turnover in the brain (Kreiss and Lucki, 1995), inhibit 5-HT reuptake and also interact with 5-HT_{1A} and 5-HT_2 receptors (Cryan et al., 2005).

Besides the well-known involvement of the monoaminergic system in the mechanism of action of classical antidepressants, substantial evidence supports the theory that the activation of the opioid system is implicated in the mechanisms underlying the effect of antidepressants (Devoize et al., 1984; Tejedor-Real et al., 1995; Zomkowski et al., 2005), although opioid compounds are mainly used for the treatment of pain. In this way, early studies showed that chronic administration of the opioid antagonist naltrexone induces a depression-like syndrome, indicating a general role for opioid system in depression (Hollister et al., 1981). Other studies have shown that patients with severe depression coupled with anxiety display decreased serum β-endorphin levels (Darko et al., 1992; Djurovic et al., 1999) and decreased μ-opioid receptor availability (Kennedy et al., 2006). Furthermore, some clinical reports describe the effectiveness of the μ-opiate agonists, oxycodone and oxymorphone, and the partial agonist, buprenorphine in patients with refractory major depression (Bodkin et al., 1995; Stoll and Rueter, 1999).

Selenium is an essential trace element nutritionally important to mammals, with physiological roles as a structural component of several antioxidant enzymes involved in the peroxide decomposition (Ursini and Bindoli, 1987; Rayman, 2000). It has been reported that insufficient selenium intake affects some psychological parameters and that selenium supplementation is associated with an improvement in mood and depression status (Benton and Cook, 1991; Benton, 2002).

m-Trifluoromethyl-diphenyl diselenide [(m–CF_3–$PhSe)_2$] is an organoselenium compound, which displays some pharmacological properties, such as antioxidant (Prigol et al., 2009a) and antipsychotic (Machado et al., 2006). Moreover, (m–CF_3–$PhSe)_2$ produces an anxiolytic effect (Brüning et al., 2009) which is related to the interaction with 5-HT receptors and selective inhibition of monoamine oxidase A (MAO-A), a key enzyme implicated in 5-HT

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metabolism. In addition, we recently showed that $(m\text{–}CF_3\text{–}PhSe)_2$ has antinociceptive action by interacting with μ and δ opioid receptors (Brüning et al., 2010).

In view of the above considerations, the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$ was investigated in the mouse forced swimming test. The hypothesis that serotonergic and opioid systems are involved in the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in the forced swimming test was tested.

2. Materials and methods

2.1. Experimental animals

Behavioral experiments were conducted using Swiss female mice (25–35 g). Female mice were randomly selected without monitoring the estrous cycle (Duarte et al., 2007). Animals were maintained at 22–25 °C with free access to water and food, under a 12:12 hour light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, of the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

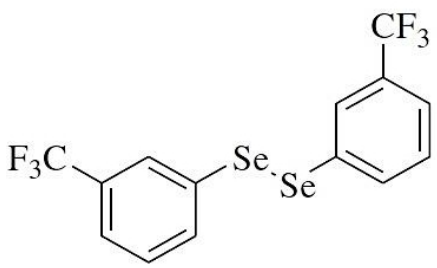
2.2. Drugs

$(m\text{–}CF_3\text{–}PhSe)_2$ (Fig. 1) was prepared and characterized in our laboratory by the method previously described (Paulmier, 1986). Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(m\text{–}CF_3\text{–}PhSe)_2$ (99.9%) was determined by GC/MS. N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2pyridinylcyclohexanecarboxamide (WAY100635), naloxone hydrochloride and ritanserin were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers. All drugs were dissolved in saline, except $(m\text{–}CF_3\text{–}PhSe)_2$ that was dissolved in canola oil and ritanserin that was dissolved in saline with 1% of Tween 80. Mice received all drugs in a constant volume of 10 ml/kg body weight. Appropriate vehicle-treated groups were also assessed simultaneously. Pretreatment time of 30 min for administration of $(m\text{–}CF_3\text{–}PhSe)_2$ was based on previously published reports (Brüning et al., 2009; Prigol et al., 2009b).

2.3. Behavioral tests

2.3.1. Forced swimming test

The forced swimming test, as originally described by Porsolt et al. (1977a,b), is the most widely used model to screen new antidepressant drugs. This test is quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, monoamine oxidase inhibitors, and atypical (Porsolt et al., 1977b; Cryan and Lucki, 2000; Cryan et al., 2002). All the mechanisms of action of treatments could be determined in the forced swimming test, but clinical correlations should be considered very carefully (Petit-Demouliere et al., 2005).



m-trifluoromethyl-diphenyl diselenide

Fig. 1. Chemical structure of *m*-trifluoromethyl-diphenyl diselenide $(m\text{–}CF_3\text{–}PhSe)_2$.

In this test, 30 min after the p.o. administration of $(m\text{–}CF_3\text{–}PhSe)_2$ (1–100 mg/kg) or canola oil (vehicle), mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C. The total duration of immobility was recorded during 6 min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1977a,b). Paroxetine (8 mg/kg, i.p., a selective serotonin reuptake inhibitor, SSRI) (Gay et al., 2010) and morphine (5 mg/kg, s.c., a nonselective opioid agonist) (Zomkowski et al., 2005), administered 45 and 30 min before the forced swimming test, respectively, were used as positive controls.

2.3.2. The role of the serotonergic system in the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$ in the forced swimming test

To address the role of the serotonergic system in the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$ on the forced swimming test, distinct groups of animals were treated with different classes of drugs. For this purpose, mice were pretreated with WAY100635, a selective 5-HT_{1A} receptor antagonist (0.1 mg/kg, s.c.) (Savegnago et al., 2007), ritanserin, a non-selective anatagonist of 5-$HT_{2A/2C}$ receptors (4 mg/kg, i.p.) (Wang et al., 2008) or ondansetron, a selective 5-HT_3 receptor antagonist (1 mg/kg, i.p.) (Savegnago et al., 2007). 15 min after WAY100635, ritanserin or ondansetron administration, $(m\text{–}CF_3\text{–}PhSe)_2$ (50 mg/kg, p.o.) or canola oil was administered, and 30 min later the forced swimming test was carried out.

2.3.3. The role of the opioid system in the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$ in the forced swimming test

To investigate the possible contribution of the opioid system to the effect of $(m\text{–}CF_3\text{–}PhSe)_2$ on reducing the immobility time in the forced swimming test, animals were pretreated with naloxone (1 mg/kg, i.p., a non-selective antagonist of opioid receptors) (Kaster et al., 2007) or vehicle and after 30 min they received $(m\text{–}CF_3\text{–}PhSe)_2$ (50 mg/kg) or vehicle and were tested in the forced swimming test 30 min later.

2.3.4. Open-field test

The locomotor and exploratory behavior was assessed in the open-field test. The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open-field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 9 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) (Walsh and Cummins, 1976). We have previously demonstrated that $(m\text{–}CF_3\text{–}PhSe)_2$ (0.1–100 mg/kg) has no effect on locomotor and exploratory behavior of mice in the open-field test (Brüning et al., 2009). In addition, the interaction of $(m\text{–}CF_3\text{–}PhSe)_2$ (100 mg/kg) with WAY100635 (0.1 mg/kg) (Brüning et al., 2009) or naloxone (1 mg/kg) (Brüning et al., 2010) did not cause any change in the locomotor activity assessed in the open-field test.

To verify whether the administration of $(m\text{–}CF_3\text{–}PhSe)_2$ with ritanserin or ondansetron impairs motor abilities mice were pretreated with ritanserin (4 mg/kg, i.p.) or ondansetron (1 mg/kg i.p.) and 15 min after $(m\text{–}CF_3\text{–}PhSe)_2$ (50 mg/kg, p.o.) or canola oil was administered. Thirty minutes later, the open-field test was carried out.

2.4. Statistical analysis

Results are represented as the mean ± S.E.M. The statistical significance difference of groups was calculated by means of one-way or two-way analysis of variance (ANOVA) followed by Newman–Keuls test when appropriate. Probability values less than 0.05 ($P<0.05$) were considered as statistically significant.

3. Results

3.1. Effect of (m–CF_3–PhSe)$_2$ on the forced swimming test

The immobility time in the forced swimming test of animals treated with (m–CF_3–PhSe)$_2$ is shown in Fig. 2. *Post hoc* analysis showed that doses of 50 and 100 mg/kg of (m–CF_3–PhSe)$_2$ decreased the immobility time of mice in the forced swimming test. The dose of 50 mg/kg of (m–CF_3–PhSe)$_2$ was chosen for all further studies. The positive controls, paroxetine (8 mg/kg) and morphine (5 mg/kg) significantly decreased the immobility time in the forced swimming test.

3.2. The role of the serotonergic system in the antidepressant-like effect of (m–CF_3–PhSe)$_2$ in the forced swimming test

Pretreatment of mice with WAY100635 (0.1 mg/kg, s.c., a 5-HT_{1A} receptor antagonist) (Fig. 3A), ritanserin (4 mg/kg, i.p., an antagonist of 5-$HT_{2A/2C}$ receptors) (Fig. 3B) or ondansetron (1 mg/kg, i.p., a 5-HT_3 receptor antagonist) (Fig. 3C) prevented the antidepressant-like effect of (m–CF_3–PhSe)$_2$ (50 mg/kg, p.o.) in the forced swimming test.

3.3. The role of the opioid system in the antidepressant-like effect of (m–CF_3–PhSe)$_2$ in the forced swimming test

The anti-immobility effect caused by (m–CF_3–PhSe)$_2$ (50 mg/kg, p.o.) was significantly prevented by pretreatment of mice with naloxone (1 mg/kg, i.p., a non-selective opioid antagonist) (Fig. 4).

3.4. Effect caused by interaction of (m–CF_3–PhSe)$_2$ and 5-HT antagonists on the open-field test

The administration of ritanserin (4 mg/kg, i.p.) or ondansetron (1 mg/kg i.p.) with (m–CF_3–PhSe)$_2$ (50 mg/kg, p.o.) did not cause any change in the locomotor activity assessed in the open-field test (data not shown).

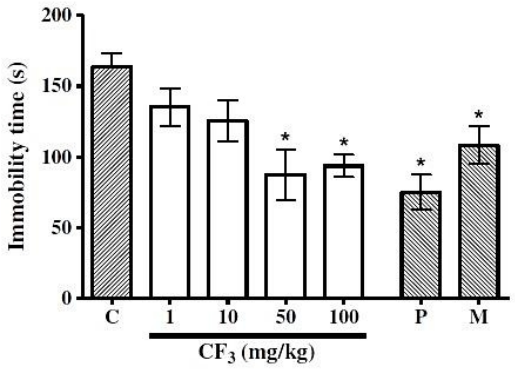


Fig. 2. Effect of (m–CF_3–PhSe)$_2$ administration in the mouse forced swimming test. (m–CF3–PhSe)2 (1–100 mg/kg) was administered, p.o., 30 min before the test. Paroxetine (8 mg/kg, i.p.) and morphine (5 mg/kg, s.c.), administered 45 and 30 min before the forced swimming test, respectively, were used as positive controls. Each column represents mean ± S.E.M. from 6 to 8 animals for group. Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls test. (*) $P<0.05$ when compared to the control group. Abbreviation: CF_3 — (m–CF_3–PhSe)$_2$; P — paroxetine; M — morphine.

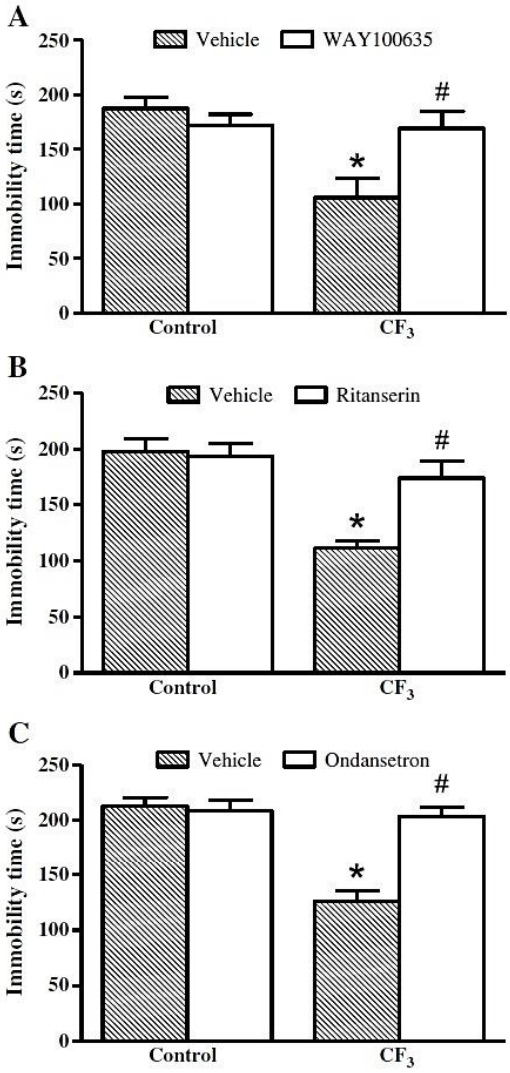


Fig. 3. Effect of WAY100635 (0.1 mg/kg, s.c.) (A), ritanserin (4 mg/kg, i.p.) (B), ondansetron (1 mg/kg, i.p.) (C) and/or (m–CF_3–PhSe)$_2$ (50 mg/kg, p.o.) on the mouse forced swimming test. WAY100635, ritanserin or ondansetron were administered 15 min before (m–CF_3–PhSe)$_2$ and the forced swimming test was performed 30 min after (m–CF_3–PhSe)$_2$ administration. Each column represents mean ± S.E.M. from 6 animals for group. Statistical analysis was performed by two-way ANOVA followed by Newman–Keuls test. (*) $P<0.05$ as compared with the vehicle-treated control. (#) $P<0.05$ as compared with the same group pretreated with vehicle. Abbreviation: CF_3 — (m–CF_3–PhSe)$_2$.

4. Discussion

In the present study, we demonstrated that (m–CF_3–PhSe)$_2$ administered by oral route produces a significant antidepressant-like action in the mouse forced swimming test. Moreover, an involvement of serotonergic (5-HT_{1A}, 5-$HT_{2A/2C}$ and 5-HT_3 receptors) and opioid systems in the antidepressant-like effect of (m–CF_3–PhSe)$_2$ was evidenced.

An important theory for the formation of depression is the monoamine hypothesis which suggests that there is a decreasing effect of biological amines like 5-HT, noradrenaline and dopamine in depression (Schildkraut, 1965). It is well known that the 5-HT system plays an important role in the neural regulation of mood (Duman et al., 1997) and that an enhancement of 5-HT neurotransmission underlies in the therapeutic response to different class of antidepressant treatment. In this study, the involvement of the serotonergic system in the antidepressant-like effect

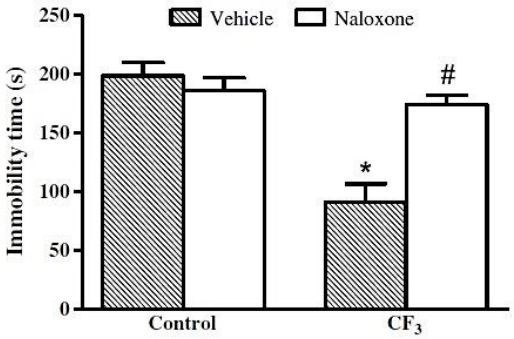


Fig. 4. Effect of naloxone (1 mg/kg, i.p.) and/or $(m\text{–}CF_3\text{–}PhSe)_2$ (50 mg/kg, p.o.) on the mouse forced swimming test. Naloxone was administered 30 min before $(m\text{–}CF_3\text{–}PhSe)_2$ and the forced swimming test was performed 30 min after $(m\text{–}CF_3\text{–}PhSe)_2$ administration. Each column represents mean ± S.E.M. from 6 to 7 animals for group. Statistical analysis was performed by two-way ANOVA followed by Newman–Keuls test. (*) $P<0.05$ as compared with the vehicle-treated control. (#) $P<0.05$ as compared with the same group pretreated with vehicle. Abbreviation: CF_3 — $(m\text{–}CF_3\text{–}PhSe)_2$.

elicited by $(m\text{–}CF_3\text{–}PhSe)_2$ was evidenced by the demonstration that selective antagonists of 5-HT_{1A}, 5-$HT_{A/2C}$ and 5-HT_3 receptors consistently reversed the $(m\text{–}CF_3\text{–}PhSe)_2$ action in the forced swimming test. Accordingly, the modulation of serotonergic receptors has been implicated in the mechanism of action of several classes of antidepressant drugs. The 5-HT_{1A} receptors are located pre-synaptically in the raphe nuclei, and post-synaptically in limbic and cortical regions. These receptors act by inhibiting the firing rate of 5-HT neurons and are particularly relevant to the antidepressant responses (Blier and Ward, 2003); besides that, major depressive disorder is associated with a widespread reduction in 5-HT_{1A} receptor binding (Sargent et al., 2000). The 5-HT_2 receptors are also widely distributed throughout the brain, in a pattern that suggests that their activation may be implicated in the regulation of mood disorders (Celada et al., 2004). The role of 5-HT_3 receptors in depressive disorders is much less studied than 5-HT_{1A} and 5-$HT_{2A/2C}$. Here, we showed that the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ was blocked by pretreatment of mice with ondansetron, a selective 5-HT_3 receptor antagonist. These data are consistent with results of the previous study showing a blockade of the antidepressant-like effect of diphenyl diselenide, a parent compound of $(m\text{–}CF_3\text{–}PhSe)_2$, by ondansetron (Savegnago et al., 2007).

We previously reported that $(m\text{–}CF_3\text{–}PhSe)_2$ is a selective inhibitor of MAO-A activity, a key enzyme associated with metabolism of 5-HT, in the cerebral cortex of mice (Brüning et al., 2009). Thus, it is possible that the inhibition of MAO-A contributes to the $(m\text{–}CF_3\text{–}PhSe)_2$ antidepressant-like action in the forced swimming test. It is important to point out that although the inhibition of MAO-A provides a direct neurochemical evidence for the involvement of the serotonergic system in this action, the pharmacological evidence suggests that $(m\text{–}CF_3\text{–}PhSe)_2$ could be acting on multiple sites, such as 5-HT_1, 5-HT_2 and 5-HT_3 receptors, rather than on one specific target. Accordingly, multiple mechanisms are likely to be involved in the pharmacological effects of $(m\text{–}CF_3\text{–}PhSe)_2$. Nogueira and Rocha (2010) have suggested that the controlled oxidation of thiol-containing enzymes, receptors or channels could be involved in the pharmacological properties of the organoselenium compounds.

While most currently prescribed antidepressants act by influencing serotonergic neurotransmission, a growing number of studies suggest that the opioid system may also be an important therapeutic target for the treatment of depression (Schreiber et al., 2002), in addition to its role in the pain process. Indeed, substantial evidence supports the belief that the impairment in the opioid system underlies the pathophysiology of depression. This has been suggested, among other reasons, because μ-opioid receptors are densely distributed in several brain regions implicated in the response to stressors and emotionally salient stimuli. Furthermore, it has been shown that there is such a pronounced reduction in μ-opioid receptor availability in the posterior thalamus and anterior cingulate cortex of patients with major depressive disorder (Kennedy et al., 2006). In this study, naloxone, a non-selective antagonist of opioid receptors, reversed the reduction of immobility time elicited by $(m\text{–}CF_3\text{–}PhSe)_2$ in the forced swimming test, indicating that its antidepressant-like action is mediated, at least in part, by an interaction with the opioid system. In this context, we recently reported that μ and δ opioid receptors are involved in the $(m\text{–}CF_3\text{–}PhSe)_2$ antinociceptive action (Brüning et al., 2010), further supporting its capability of modulating the opioid system. It is important to emphasize that diphenyl diselenide, a structural analogue of $(m\text{–}CF_3\text{–}PhSe)_2$, does not modulate the opioid system in any of the pharmacological actions in which it is implicated (Nogueira and Rocha, 2010).

Considering that both, opioid and serotonergic systems, are involved in depressive disorders, new studies related to the molecular mechanisms of $(m\text{–}CF_3\text{–}PhSe)_2$ action are needed in order to strengthen the neurochemical basis of drug targets before envisaging any potential therapeutic use of this organoselenium compound. A remarkable example that a drug which acts on opioid and monoaminergic systems may be useful in depression is (±)-tramadol, which is a weak agonist of μ-opioid receptors and, like antidepressant drugs, is able to inhibit the reuptake of 5-HT and noradrenaline. It is a widely used analgesic, and interestingly, it has shown antidepressant properties, both clinically and pre-clinically (Rojas-Corrales et al., 2002; Shapira et al., 2001).

Furthermore, it is very important to mention that $(m\text{–}CF_3\text{–}PhSe)_2$ did not cause impairment in the locomotor activity assessed in the open-field test (Brüning et al., 2009). In the same way, the administration of $(m\text{–}CF_3\text{–}PhSe)_2$ with the serotonergic and opioid receptor antagonists did not affect the locomotor activity of mice in the open-field test (Brüning et al., 2009; 2010).

Taken together, the results showed pharmacological evidence supporting the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in the forced swimming test, without affecting the locomotor activity. The $(m\text{–}CF_3\text{–}PhSe)_2$ antidepressant-like effect in the mouse forced swimming test involves the modulation of serotonergic and opioid systems.

Acknowledgments

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4 CONCLUSÃO

Os resultados apresentados nesta dissertação permitem concluir que o composto (*m*–CF_3–PhSe)$_2$ apresenta ação do tipo antidepressiva em camundongos no teste do nado forçado e esta ação é possivelmente mediada pelos sistemas serotoninérgico e opióide. Desta forma, este composto orgânico de selênio torna-se um candidato à possível agente terapêutico para o tratamento das desordens depressivas, mediante estudos mais aprofundados em torno desta molécula.

5 PERSPECTIVAS

Tendo em vista os resultados obtidos com esse trabalho, as perspectivas para trabalhos posteriores são:

- Avaliar o efeito do (*m*–CF_3–PhSe)$_2$ sobre a recaptação de serotonina;
- Realizar estudos de união específica (*binding*) entre o (*m*–CF_3–PhSe)$_2$ e os receptores serotoninérgicos e opióides;
- Avaliar o envolvimento do sistema serotoninérgico no efeito antinociceptivo do (*m*–CF_3–PhSe)$_2$;
- Avaliar o efeito do (*m*–CF_3–PhSe)$_2$ em modelos crônicos de depressão e dor (dor neuropática).

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