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Paulo Cesar de Oliveira Araujo

**EFEITO DO DISSELENETO DE *m*-TRIFLUORMETIL DIFENILA EM  
MODELOS DE DOR INFLAMATÓRIA AGUDA E SUBCRÔNICA EM  
CAMUNDONGOS**

Santa Maria, RS  
2019

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DOR INFLAMATÓRIA AGUDA E SUBCRÔNICA EM CAMUNDONGOS

Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Bioquímica Toxicológica**

Orientador: Prof. Dr. César Augusto Brüning  
Coorientadora: Prof<sup>a</sup> Dr<sup>a</sup> Cristina Wayne Nogueira

Santa Maria, RS  
2019

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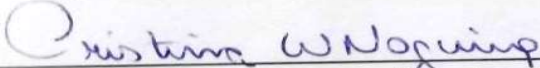
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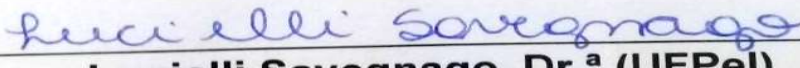
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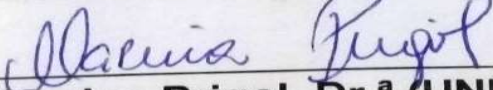
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Aprovado em 21 de fevereiro de 2019:

  
César Augusto Brüning, Dr. (UFPel)  
(Presidente/Orientador)

  
Cristina Wayne Nogueira, Dr.<sup>a</sup> (UFSM)  
(Coorientadora)

  
Lucielli Savegnago, Dr.<sup>a</sup> (UFPel)

  
Marina Prigol, Dr.<sup>a</sup> (UNIPAMPA)

Santa Maria, RS  
2019

*Dedico esta dissertação aos meus amados Pais: Teresinha e Pedro Cezar; à minha irmã Gabriela, meu cunhado Fagner e ao meu amado sobrinho Gustavo. Dedico ao Marcel, irmão de existência e aos meus orientadores César e Cristina por todos os ensinamentos, formação e conduta.*

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***“Mãe me disse quando eu era mais jovem [...]***

***não tenha pressa, não viva rápido demais  
dificuldades virão e passarão [...]  
e não esqueça, filho, existe alguém lá em cima***

***e seja um tipo simples de homem...”***

*(Simple Man – Lynyrd Skynyrd)*

## RESUMO

### EFEITO DO DISSELENETO DE *m*-TRIFLUORMETIL DIFENILA EM MODELOS DE DOR INFLAMATÓRIA AGUDA E SUBCRÔNICA EM CAMUNDONGOS

AUTOR: Paulo Cesar de Oliveira Araujo  
ORIENTADOR: César Augusto Brüning  
COORIENTADORA: Cristina Wayne Nogueira

A inflamação é um processo multicelular e complexo que tem um papel protetor essencial no corpo. No entanto, pode tornar-se patológico quando age com intensidade excessiva e prolongada resistência. Além disso, as opções terapêuticas disponíveis para combater a inflamação apresentam algumas questões relacionadas à eficácia e à segurança. Neste contexto, o desenvolvimento de novas moléculas com perfil farmacológico melhorado é necessário. O disseleneto de *m*-trifluormetil difenila [(*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>] é um composto orgânico de selênio que possui propriedades biológicas promissoras, incluindo ação antinociceptiva em modelos experimentais de nocicepção. Assim, o objetivo principal desta dissertação foi avaliar a ação anti-inflamatória do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> em modelos de inflamação aguda e subcrônica induzida por adjuvante completo de Freund (ACF) em camundongos *Swiss* adultos. O Comitê de Ética em Pesquisa da Universidade Federal de Santa Maria aprovou todos os procedimentos experimentais realizados no presente estudo, que são registrados sob o número **8081170317/2017**. Inicialmente, foi avaliada a estabilidade físico-química do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> em diferentes condições de armazenamento. Nossos resultados demonstraram que independentemente do tempo e condições de armazenamento testadas (freezer [-20 °C], refrigeração [4 °C] ou temperatura ambiente [25 °C]) não foi detectada alterações no conteúdo do composto, sugerindo uma alta estabilidade química do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>. No **protocolo 1**, a inflamação aguda foi induzida nos camundongos por uma injeção intraplantar de ACF e 24 horas depois receberam uma única administração intragástrica (i.g.) de (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>. Uma curva de tempo e dose-resposta foi realizada para avaliar o efeito do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> na hipernocicepção mecânica, usando o filamento de von Frey (FVF), edema e atividade da mieloperoxidase induzida por ACF na pata. O tratamento com (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> reduziu o comportamento hipernociceptivo mecânico (10 e 1 mg / kg, i.g.), bem como diminuiu o edema e a atividade da MPO na pata (10 mg / kg, i.g.). Em seguida, o **protocolo 2** avaliou a efetividade de um regime de tratamento repetido com (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> contra os comprometimentos inflamatórios induzidos pelo ACF em camundongos. A toxicidade potencial de tal esquema de administração também foi avaliada. Os camundongos receberam uma injeção intraplantar de ACF e 14 dias mais tarde foram tratados com (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg / kg, i.g./uma vez por dia / 10 dias). A hipernocicepção mecânica e térmica foi registrada diariamente pelo teste de FVF e teste da chapa quente (52 °C), respectivamente. Os resultados demonstraram que a administração repetida de (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> reduziu a hipernocicepção mecânica e térmica induzida pelo ACF. Além disso, o tratamento repetido com (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> restaurou os comprometimentos nas alterações bioquímicas (edema e atividade da MPO na pata) e moleculares (IL-1β, TNF-α e COX-2, avaliadas em amostras de córtex cerebral contralateral) causados por ACF. Além disso, a administração repetida (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> não desencadeou alterações na atividade locomotora e exploratória (número de cruzamentos, distância e velocidade média), parâmetros bioquímicos plasmáticos (função hepática, renal e cardíaca) e no estado oxidativo tecidual (fígado e rins). Coletivamente, esses dados suportam a ação anti-inflamatória do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>, reforçando o potencial farmacológico do composto.

**Palavras-chave:** Anti-inflamatório.Selênio.Nocicepção.



## ABSTRACT

### EFFECT OF *m*-TRIFLUOROMETHYL-DIPHENYL DISELENIDE IN ACUTE AND SUBCHRONIC INFLAMMATORY PAIN MODEL IN MICE

AUTHOR: Paulo Cesar de Oliveira Araujo

ADVISOR: César Augusto Brüning

CO-ADVISOR: Cristina Wayne Nogueira

The inflammation is a multicelular and complex process that has an essential protective role in the body. However, it can become pathological when it acts with excessive intensity and prolong endurance. In addition, the available therapeutic options to counteract inflammation has some issues regarding efficacy and safety use. In this context, the development of novel molecules with improved pharmacological profile is necessary. The *m*-trifluoromethyl-dyphenil diselenide [(*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>] is an organoselenium compound that has promising biological properties, including antinociceptive action in experimental models of nociception. Thus, the main purpose of this dissertation was to evaluate the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> anti-inflammatory action in models of acute and subchronic inflammation induced by complete Freund's adjuvant (CFA) in *Swiss* adult mice. The Ethical Research Committee of Federal University of Santa Maria approved all experimental procedures carried out in the present study, which are register under the number **8081170317/2017**. Initially, the physicochemical stability of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> in different storage conditions was evaluated. Our results demonstrated that independent of time and storage conditions tested (freezer [-20 °C], refrigeration [4 °C] or room temperature [25 °C]) no alteration in compound content was detected, suggesting a high chemical stability of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>. In the **protocol 1**, the acute inflammation was induced in mice by an intraplantar injection of CFA and 24 h later they received a single intragastric (i.g.) administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>. A time- and dose-response curve was performed to assess the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> effect in the mechanical hypernociception, using the von Frey hair (VFH), paw edema and myeloperoxidase activity induced by CFA. The treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> reduced the mechanical hypernociceptive behavior (10 and 1 mg/kg, i.g.) as well as mitigated the paw thickness and MPO activity (10 mg/kg, i.g.). Following, the **protocol 2** evaluated the effectiveness of a repeated treatment schedule with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> against the inflammatory impairments induced by CFA in mice. The potential toxicity of such administration schedule was also assessed. Mice received an intraplantar injection of CFA and 14 days later they were treated with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg/kg, i.g./once a day/10 days). The mechanical and thermal hypernociception were daily recorded using VFH test and hot-plate test (52 °C), respectively. The results demonstrated that the repeated administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> reduced both mechanical and thermal hypernociception induced by CFA. In addition, the repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> restored the biochemical (edema and MPO activity of paw) and molecular (IL-1β, TNF-α e COX-2, assessed in cerebral contralateral cortex samples) impairments caused by CFA. Furthermore, the repeated (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> administration triggered no alteration in locomotor and exploratory activity (number of crossing, distance and average speed), plasma biochemical parameters (hepatic, renal and cardiac function) as well as in the tissue oxidative status (liver and kidneys). Collectively, these data support the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> anti-inflammatory action, reinforcing the pharmacological potential of the compound.

**Keywords:** Anti-inflammatory.Selenium.Nociception.

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## LISTA DE ABREVIATURAS E SIGLAS

$(m\text{-CF}_3\text{-PhSe})_2$  – Disseleneto de *m*-trifluormetil-difenila;  
(PhSe)<sub>2</sub> – Disseleneto de difenila;  
5-HT – 5-Hidroxitriptamina;  
AEs – Anti-inflamatórios esteroidais;  
ACF – Adjuvante completo de Freund;  
AINEs – Anti-inflamatórios não esteroidais;  
ALT – Alanina aminotransferase;  
AST – Aspartato aminotransferase;  
AR – Artrite reumatóide;  
COX – Ciclo-oxigenase;  
COX-1 – Ciclo-oxigenase-1;  
COX-2 – Ciclo-oxigenase-2;  
CPNC – Constrição parcial do nervo ciático;  
DAMPs – Padrões moleculares associados a danos;  
DMARD – Droga anti-reumática modificadora de doença;  
IL-1 – Interleucina 1;  
IL-6 – Interleucina 6;  
IL-1 $\beta$  – Interleucina 1 $\beta$ ;  
MAO-A – Monoaminoxidase-A;  
MCP – 1 – Proteína quimiotática de monócito tipo 1;  
MPO – Mieloperoxidase;  
Nf $\kappa$ B – Fator nuclear kappa B;  
O – Oxigênio;  
PAMPs – Padrões moleculares associados a patógenos;  
S – Enxofre;  
Se – Selênio;  
SNC – Sistema nervoso central;  
Te – Telúrio;  
TNF- $\alpha$  – Fator de necrose tumoral alfa;

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# 1 INTRODUÇÃO

## 1.1 INFLAMAÇÃO

### 1.1.1 Breve histórico

A inflamação (do latim *inflamare*) teve seus primeiros sinais descritos em papiros egípcios que datam, aproximadamente, de 3.000 a.C. No entanto, foi Celsus, no século I d.C., quem descreveu os quatro sinais cardinais da inflamação: *rubor* (eritema), *calor* (calor), *dolor* (dor) e *tumor* (inchaço) (FULLERTON & GILROY, 2016). Contudo, somente a partir do advento do microscópio (Século XIX) foi possível buscar a compreensão dos mecanismos envolvidos no processo inflamatório, uma vez que este aparato possibilitou a observação da microcirculação e das alterações nos tecidos inflamados (SCOTT et al., 2004). Deste modo as bases fisiológicas dos quatro sinais cardinais puderam ser elucidadas por Augustus Waller (1846) e Julius Cohnheim (1867), no qual se descobriram a emigração de leucócitos dos vasos sanguíneos e outras alterações vasculares características de uma resposta inflamatória aguda (MAJNO & JORIS, 2004).

A partir destas observações microscópicas pode-se verificar a ocorrência de processos distintos, tais como a predominância de leucócitos polimorfonucleares nos estágios iniciais da inflamação aguda, enquanto que em um segundo estágio, a presença em grande parte de monócitos e macrófagos na inflamação crônica (KUSHNER & ANTONELLI, 2017; HEIDLAND et al., 2006). Com base nestas observações, Rudolph Virchow concluiu que não haveria um único estágio, mas sim processos inflamatórios e, posteriormente, adicionou o quinto sinal cardinal: *functio laesa* (perda de função), caracterizando-se desta forma os cinco sinais cardinais da inflamação (FULLERTON & GILROY, 2016).

Embora os quatro sinais cardinais descritos por Celcius aplicarem-se apenas aos processos inflamatórios agudos, podendo-se acrescentar a lesões e infecções, a perda de função é o único sinal universal que acompanha todos os processos inflamatórios, sobretudo, o crônico. Desta maneira, destaca-se a contribuição das pesquisas de Virchow sobre a inflamação para estabelecer a base celular da patologia (MEDZHITOV, 2010).

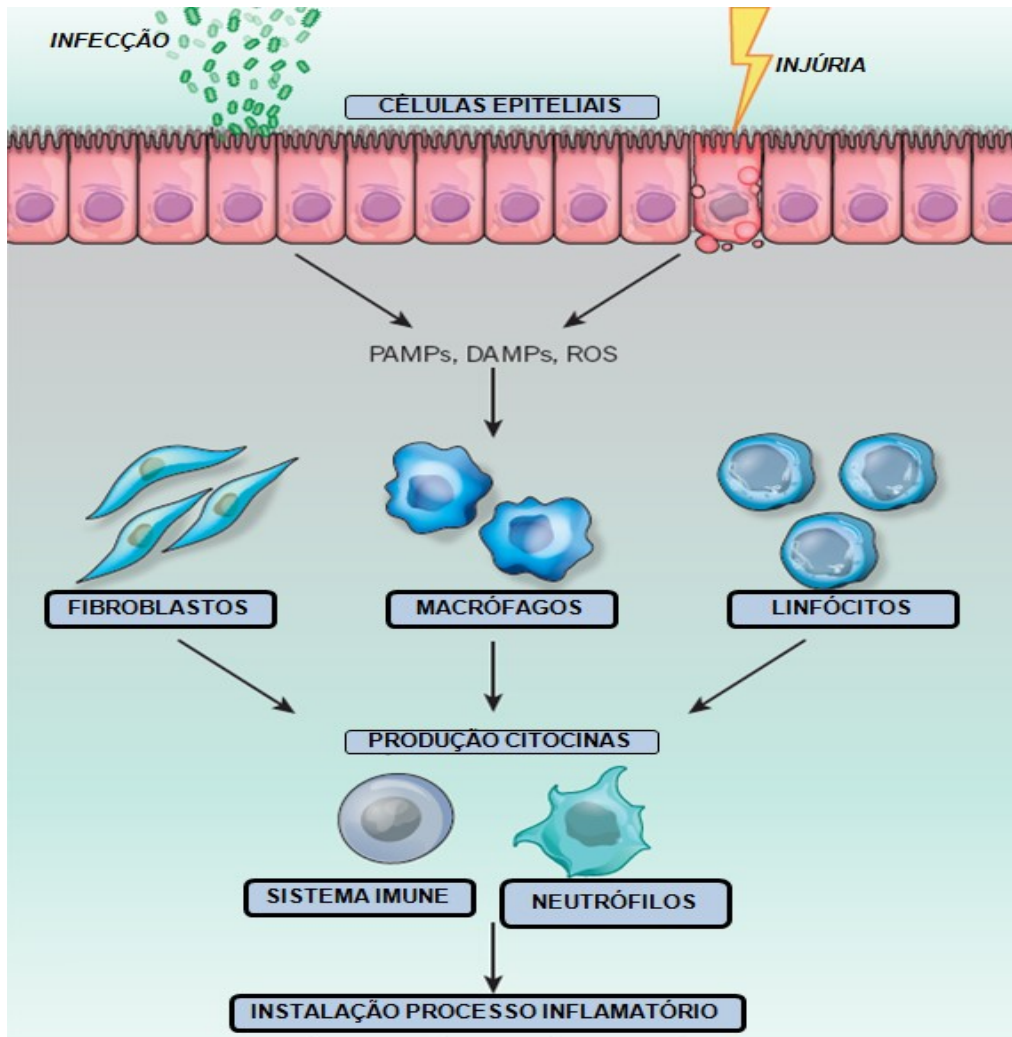
Avanços subsequentes nas últimas décadas expandiram e ampliaram a compreensão dos mecanismos que medeiam o processo inflamatório, destacando-se, por exemplo, que o dano tecidual ativa os sinais que iniciam a resposta imune, com vistas a eliminar o agente nocivo e iniciar o reparo tecidual (GRIVENNIKOV et al., 2010), bem como a atração de leucócitos circulantes a partir de quimiocinas ao tecido inflamado (DMITRIEVA et al., 2016). Ressalta-se a importância desta compreensão, pois o espectro de condições inflamatórias têm-se deslocado de reações inflamatórias agudas a estados inflamatórios crônicos que acompanham doenças como asma, diabetes, aterosclerose e câncer (MEDZHITOV, 2010).

### 1.1.2 Processo Inflamatório

Fisiologicamente, a inflamação consiste em uma reação complexa e que envolve uma série de eventos moleculares, celulares e imunológicos. Esta reação ocorre como uma resposta do organismo frente a estímulos possivelmente danosos, como a infecção por patógenos ou sinais endógenos de lesões teciduais (NETEA et al., 2017; FULLERTON & GILROY, 2016). Deste modo, segundo MEDZHITOV (2010) o processo inicial de uma resposta inflamatória típica consiste em quatro componentes: os indutores, sensores, mediadores e tecido-alvos.

A partir de um estímulo potencialmente nocivo ao organismo ou tecido, tem-se o processo de indução que desencadeia o início da resposta inflamatória. Esta resposta é observada por padrões de detecção moleculares, podendo ser associado a patógenos (PAMPs), que são especificamente direcionados às moléculas expressas por patógenos, bem como padrões moleculares associados a danos (DAMPs), os quais são moléculas endógenas que sinalizam danos ou necrose (JANEWAY et al., 2005). Esta sinalização dá-se a partir de macrófagos residentes, células dendríticas e monócitos que produzem rapidamente mediadores, incluindo citocinas e quimiocinas para alertar o sistema imune e os neutrófilos circulantes. Deste modo, os neutrófilos fazem transmigração e ao chegarem ao local do estímulo inflamatório, liberam os conteúdos dos seus grânulos favorecendo a instalação do processo inflamatório (SOEHNLEIN & LINDBOM, 2010) (**Figura 1**).

Figura 1 - Representação esquemática do processo inflamatório.



Fonte: Adaptado de KARIN & CLEVERS (2016).

Assim, torna-se relevante a importância dos mediadores inflamatórios, uma vez que regulam os fenômenos vascular, celular e bioquímico durante a inflamação (MCCONENELL, 2007; SHERWOOD & TOLIVER-KINSKY, 2004). Dentre os principais mediadores do processo inflamatório, podem-se exemplificar as citocinas, o óxido nítrico, as espécies reativas, as prostaglandinas, os leucotrienos e o fator de ativação plaquetária (MCCONENELL, 2007). Alguns destes mediadores são pré-formados e estão estocados em vesículas ou compartimentos específicos, enquanto que outros são gerados no momento do estímulo inflamatório (RANKIN, 2008). Esses mediadores atuam nos tecidos-alvos, incluindo os vasos sanguíneos locais, para induzir vasodilatação, extravasamento de plasma e neutrófilos para o tecido (PECCHI et al., 2009).

Desta forma, o processo inflamatório consiste em um mecanismo protetor do organismo para defender e restaurar as funções fisiológicas quando os mecanismos de homeostase são insuficientes (KOTAS & MEDZHITOV, 2015). No entanto, esta resposta é bem-sucedida quando é seguida pela fase de restauração, caso contrário a resposta inflamatória pode assumir um perfil prejudicial ao organismo, o que ocorre devido à intensa e descontrolada amplificação dos eventos no processo de defesa e às constantes lesões às biomoléculas celulares (ESCH & STEFANO, 2002).

### **1.1.3 Inflamação Aguda**

Neste contexto, a resposta inflamatória aguda consiste em uma sequência coordenada de alterações moleculares, celulares e fisiológicas que pode ocorrer nas primeiras horas após o estímulo lesivo, no qual eventos vasculares encontram-se na base desta resposta (FULLERTON & GILROY, 2016). Desta forma, tem-se um aumento da permeabilidade capilar, adesão de leucócitos polimorfonucleares e agregação de plaquetas com a finalidade de fagocitar e eliminar fragmentos de tecidos e micro-organismos através de mecanismos intracelulares (por exemplo, espécies reativas, mieloperoxidases e proteases) e/ou mecanismos extracelulares (redes de fibras extracelulares de neutrófilos) (FULLERTON & GILROY, 2016; SERHAN & WARD & GILROY, 2010; OKOLI et al., 2007).

Na fase inicial da inflamação aguda, tem-se como mediadores a histamina e a bradicinina as quais dilatam o lúmen do vaso e aumentam o fluxo vascular, responsável pelos sinais clínicos de calor e rubor (ALBERTINI et al., 2004). Posteriormente, em uma segunda fase do processo agudo, há maior predominância de estímulos celulares, os quais permitem a marginação, aderência endotelial, diapedese e migração de leucócitos decorrentes dos estímulos quimiotáticos (KUMAR & ABBAS & ASTER, 2015).

O término da resposta inflamatória aguda ocorre quando o insulto/infecção desencadeante é eliminado e o tecido danificado retorna à sua homeostase. Dentre os mecanismos que corroboram para a transição do estado inflamatório para o de homeostasia, pode-se citar a substituição de prostaglandinas pró-inflamatórias por lipoxinas anti-inflamatórias e recrutamento de neutrófilos para monócitos, resultando



na depuração das células e dando início ao reparo tecidual (SERHAN & SAVILL, 2005).

No entanto, se o estímulo indutor persistir ou não for eliminado pela resposta inflamatória aguda, predispõe o surgimento de processos inflamatórios crônicos e, conseqüentemente, a condições de doenças inflamatórias (FREIRE & DYKE, 2013).

#### **1.1.4 Inflamação Crônica**

Diferentemente da inflamação aguda que se manifesta por alterações vasculares, edema e infiltração principalmente de neutrófilos, na resposta inflamatória crônica tem-se um prolongamento e deslocamento progressivo do tipo de células presentes no local da inflamação e, frequentemente, caracterizado pela substituição de neutrófilos por macrófagos e células imunológicas (MEDZHITOV, 2008). Nesses casos, a inflamação crônica está tipicamente localizada no local onde o indutor inflamatório está presente resultando em remodelação tecidual (MEDZHITOV, 2010).

Durante esse processo evidencia-se uma lesão persistente com destruição tecidual devido à presença das células inflamatórias numa tentativa frustrada de eliminar o agente nocivo e de restaurar a homeostasia tecidual. Outro fator importante deste processo é a cicatrização, com a finalidade de substituir o tecido lesado por tecido conjuntivo, devido à neovascularização acompanhado por fibrose (NATHAN, 2002; TOBY & WILLOUGHBY & GILROY, 2002).

Nestes casos de inflamação crônica, pode-se descrever como um ciclo vicioso que ligam os processos inflamatórios a processos patológicos, uma vez que está associada a uma variedade de doenças como cardiovasculares, metabólicas, neurodegenerativas e câncer (ASHLEY et al., 2012; ELTZSCHIG & CARMELIET, 2011).

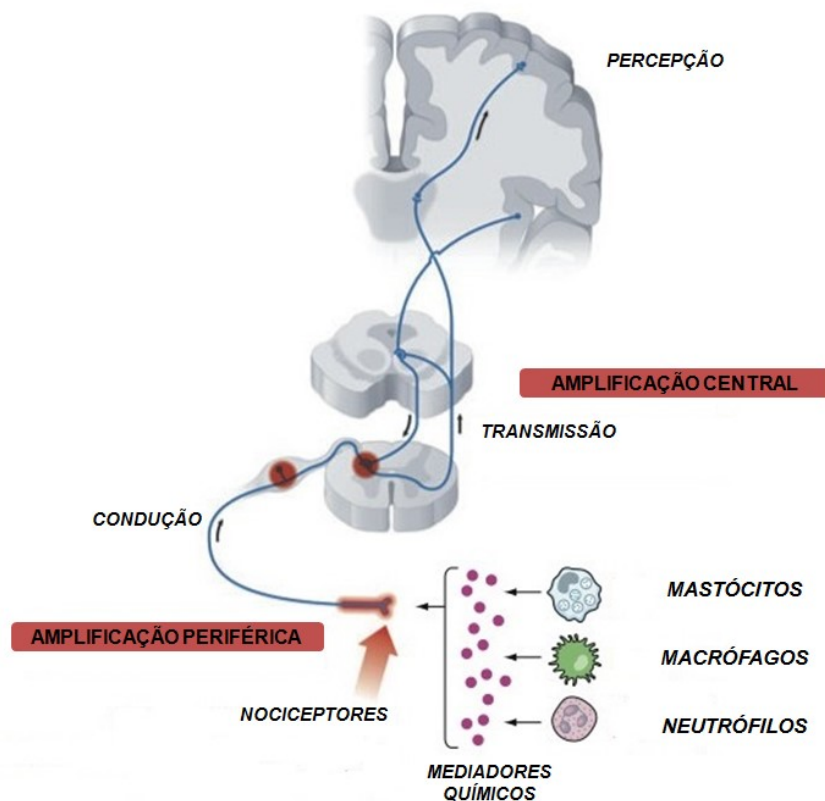
#### **1.1.5 Dor Inflamatória**

Assim como o processo inflamatório, a dor, fisiologicamente, possui efeito protetor e também há a modulação de diversos mecanismos periféricos e centrais. Apesar dos mecanismos de modulação biologicamente complexa, convém ressaltar

que a dor tem uma característica estritamente pessoal, subjetiva e psicológica (OLESEN et al., 2012).

A resposta nociceptiva, ou seja, resultado final da ação do conjunto de componentes químicos envolvidos no processo da dor, resulta de diferentes estímulos traduzidos em impulsos elétricos por nociceptores, terminações nervosas livres que conduzem a nível de sistema nervoso central (SNC), onde esses estímulos serão processados e respondidos (BASBAUM et al., 2009; MILLAN, 2002). Desta forma, a percepção de estímulos nocivos inicia-se através dos nociceptores (**Figura 2**) e sua ativação configura o processo de transdução do estímulo e por apresentarem limiares mais elevados do que os demais receptores, estes se limitam à existência de estímulos intensos e potencialmente prejudiciais (DUBIN & PATAPOUTIAN, 2010).

Figura 2 - Representação do processo de nocicepção inflamatória.



Fonte: Adaptado de COSTIGAN & SCHOLZ & WOOLF (2009).

Esse evento é acompanhado pela liberação local de mediadores químicos que facilitam a gênese e a transmissão do estímulo nociceptivo, tais como

degranulação de mastócitos, ativação de células inflamatórias e expressão de enzimas como a ciclo-oxigenase 2 (COX-2). Destaca-se também, as citocinas pró-inflamatórias como o fator de necrose tumoral alfa (TNF- $\alpha$ ) e interleucina 1 (IL-1) (SCHAFERS & SORKIN, 2008) e quimiocinas como proteína quimiotática de monócito tipo 1 (MCP-1) que podem agir como moléculas chaves na sinalização da dor (WOOLF & MA, 2007).

Não obstante, ressalta-se que, na maioria das situações, o processo inflamatório pode estar relacionado aos mecanismos que envolvem a nocicepção, levando-se em conta que dor e inflamação apresentam mediadores e efetores em comum (LIBBY,2007). Além do mais, reforçando tal raciocínio, a dor está entre os sinais clássicos que caracterizam o processo inflamatório (LAWRENCE et al., 2002). Tal relação intrínseca se explica devido ao fato de que muitos dos mediadores inflamatórios podem estimular, direta ou indiretamente, os nociceptores, contribuindo para o aparecimento da dor (LIBBY,2007).

Do ponto de vista clínico, um dos aspectos mais problemáticos da dor de origem inflamatória é a possibilidade da progressão de um estado agudo para um estado prolongado, aumentando consideravelmente a susceptibilidade do desenvolvimento de um quadro de dor inflamatória crônica e sua conseqüente evolução para situações ainda mais severas, como é o caso das neuropatias (GLASS et al., 2010).

#### **1.1.6 Modelo experimental com adjuvante completo de Freund**

O modelo de nocicepção inflamatória induzido por adjuvante completo de Freund (ACF) é amplamente utilizado em pesquisas biomédicas para determinação do efeito anti-inflamatório de novas moléculas (FEHRENBACHER et al., 2012). Assim, após a injeção de ACF há uma intensa liberação de mediadores que causam o processo inflamatório, assim como alterações bioquímicas e morfológicas no tecido (BILLIAU & MATTHYS, 2011). Essas alterações desenvolvem-se rapidamente, perdurando por semanas a meses, contribuindo na avaliação do potencial de novas moléculas em seus estados agudos e crônicos (RAGHAVENDRA et al., 2004). Outrossim, além da estimulação periférica o modelo contempla uma sensibilização em áreas específicas do sistema nervoso central como, por exemplo,

a região do córtex cerebral, somando-se assim a importância do modelo no estudo de novas intervenções terapêuticas (LI et al., 2017; CHEN et al., 2012).

### **1.1.7 Doenças associadas ao processo inflamatório**

A cerca do desenvolvimento dos processos patológicos tem-se a inflamação como uma das principais causas no progresso das doenças, na qual se estima que 15% dos cânceres humanos estejam relacionados aos processos crônicos infecciosos e, sobretudo, os inflamatórios (HE & KARIN, 2011). Neste sentido, as lesões teciduais mediada pela inflamação são observadas em muitos órgãos como, por exemplo, o coração, pâncreas, fígado, pulmões e cérebro (CHEN et al., 2018).

#### *1.1.7.1 Doenças Neurodegenerativas*

Processos inflamatórios no cérebro podem ser relacionados a doenças no SNC, incluindo as doenças auto-imunes, doenças neurodegenerativas como a Doença de Alzheimer (DA) e a Doença de Parkinson (DP). Essas condições inflamatórias podem aumentar a excitabilidade neuronal, lesionar as células e aumentar a permeabilidade da barreira sangue-cérebro (NELSON et al., 2002; BLOCK et al., 2007). Esse quadro também é observado em lesões traumáticas, acidente vascular cerebral, bem como prejuízos neuropsiquiátricos. Todas essas condições apresentam como característica comum a ativação crônica da microglia que pode desencadear processos de neurotoxicidade, levando à degeneração progressiva (AMOR et al., 2014).

As respostas inflamatórias no SNC também podem ser desencadeadas por ligantes endógenos (DAMPS, PAMPS), proteínas de choque térmico e moléculas de adesão que acessam o cérebro devido ao comprometimento da barreira sangue-cérebro. Estas respostas relacionadas à neurodegeneração incluem a expressão aumentada de citocinas e o da geração de espécies reativas resultando numa ativação da microglia (WALKER & WHETZEL & LUE, 2015).

Nesse sentido, ressalta-se desses processos de ativação microglial o estado de neuroinflamação associado à depressão, uma vez que estudos reportam uma

interação entre os processos de inflamação crônica com mecanismos periféricos e centrais nesta patologia (ROMAN et al., 2013).

#### *1.1.7.2 Doenças Cardiovasculares*

A doença cardiovascular e sua patologia subjacente, a aterosclerose, é uma condição inflamatória crônica sendo a principal causa de morte e incapacidade em todo o mundo (SOFI et al., 2016). Estima-se que até o ano de 2030, 23 milhões de pessoas tenham comprometimentos anuais com as doenças cardiovasculares (LLOYDJONES et al., 2010). Desta forma, destaca-se que os mediadores inflamatórios desempenham funções importantes na aterosclerose, desde o recrutamento inicial de leucócitos até o processo de ruptura da placa aterosclerótica (LIBBY, 2012).

Salienta-se que a inflamação é um dos processos iniciais no estresse cardíaco, haja vista que há o aumento dos níveis de moléculas de adesão endotelial e o aumento da produção e liberação de citocinas e quimiocinas inflamatórias nos tecidos cardíacos afetados (GLEZEVA & BAUGH, 2014).

#### *1.1.7.3 Câncer*

O câncer pode ser definido como uma doença muito complexa, produzida por células que perderam o controle sobre seu crescimento (PAL et al., 2014). Neste processo, evidencia-se que as citocinas pró-inflamatórias induzem o crescimento de células de câncer de mama e na formação do tumor, bem como o recrutamento de células de câncer metastático (BHATELIA et al., 2014; GENG et al., 2013).

#### *1.1.7.4 Artrite*

A artrite é uma doença inflamatória crônica, de cunho autoimune e etiologia ainda não totalmente compreendida, na qual há a superprodução sistêmica de citocinas pró-inflamatórias, destacando-se, por exemplo, TNF- $\alpha$  e IL-6, as quais também podem estar relacionadas a complicações cardiovasculares (AMBRIZ-PÉREZ et al., 2016). A artrite afeta, aproximadamente, 0,5 a 1% da população

mundial e convém frisar o impacto negativo que esta patologia promove na qualidade de vida dos pacientes (QUAN et al., 2008).

## 1.2 TRATAMENTO FARMACOLÓGICO

O tratamento da inflamação visa amenizar a intensidade do processo, no sentido da prevenção de grandes lesões teciduais, assim como promover o conforto e a melhora na qualidade de vida dos pacientes. No entanto, apesar dos recursos terapêuticos disponíveis, a ocorrência de efeitos adversos é bastante comum, especialmente durante tratamento prolongado. Juntamente com tais limitações, a baixa eficácia terapêutica relacionada a algumas doenças reduz o benefício e também a adesão ao tratamento (PAYNE, 2000).

Deste modo, destacam-se abaixo os principais agentes farmacológicos utilizados para o alívio e controle da inflamação:

### 1.2.1 Anti-inflamatórios não esteroidais (AINEs)

Os AINEs são o grupo de medicamentos mais prescritos mundialmente para o tratamento da inflamação e da dor (GOSWAMI et al., 2016). Estima-se que em torno de 29 milhões de adultos nos Estados Unidos tenha usado AINEs regularmente no ano de 2010 (SONDERGAARD et al., 2016). O mecanismo de ação desta classe está relacionado com a inibição da atividade da enzima ciclo-oxigenase (COX), o que causa a diminuição de endoperóxidos cíclicos, tais como prostaglandinas, prostaciclina e tromboxanos, que além de exercerem função fisiológica, também podem agir como agentes inflamatórios (PAYNE, 2000). A única exceção são os fenamatos, que agem como antagonistas dos receptores de prostaglandinas (MONCADA et al., 1978).

Historicamente, os primeiros AINES desenvolvidos atuavam inibindo indistintamente a enzima COX, ou seja, tanto a isoforma 1 (COX-1), a qual é constitutiva e sintetiza mediadores citoprotetores, quanto a COX-2, isoforma induzível, cuja atividade e expressão se tornam bastante aumentadas em quadros inflamatórios (YEDGAR et al., 2007; BOTTING, 2006). É justamente à falta de seletividade que se atribuem os efeitos adversos decorrentes do uso dessa classe de fármacos (PAYNE, 2000), tais como danos na mucosa gástrica, alterações na

homeostase sanguínea e renal (PATINO et al., 2003). Os AINES de última geração, apesar de atuarem de forma mais seletiva, com maior especificidade para inibir a COX-2 (VANE e BOTTING, 1998), além do custo elevado, apresentam certas limitações relacionadas aos riscos de efeito adverso associados ao sistema cardiovascular (KUKANICH et al., 2012).

### **1.2.2 Anti-inflamatórios esteroidais (AEs)**

Os fármacos caracterizados como AEs, também conhecidos como glicocorticoides, são moléculas análogas aos esteroides endógenos, com os quais compartilham muitas de suas ações biológicas (STREETEN, 1975). O uso dos AEs está principalmente relacionado ao tratamento de patologias de cunho inflamatório intenso, como doenças auto-imunes, reações de hipersensibilidade e dor inflamatória crônica e neuropática (RIJSDIJK et al., 2016).

No geral, o início de ação destes fármacos pode ser demorado, uma vez que seus principais efeitos estão relacionados à modificação da transcrição de determinados genes envolvidos com a resposta inflamatória (BLOOM et al., 1980). Contudo, o tratamento a longo prazo com AEs está associado com efeitos adversos como retenção hídrica, alteração no metabolismo, principalmente hiperglicemia, imunossupressão severa e até osteoporose (SHAIKH et al., 2012).

### **1.2.3 Imunomoduladores**

Dentre as doenças que possuem origem inflamatória crônica, os distúrbios auto-imunes são alvo de grandes pesquisas na tentativa de elucidar seus mecanismos de geração e progressão, além da busca por tratamentos eficazes na sua remissão (SCOTT et al., 2010). Neste contexto, a artrite reumatóide (AR) possui grande destaque e a droga anti-reumática modificadora de doença (DMARD) é a base do tratamento da AR, podendo ser fármacos biológicos e não biológicos. Os não biológicos incluem a cloroquina e sua forma hidroxilada, hidroxicloroquina, metotrexato, sulfassalazina e leflunomida. Os biológicos são: infliximabe, etanercepte, golimumabe e abatacepte, onde apenas o abatacepte é modulador de IL-1 e os demais de TNF- $\alpha$  (SINGH & CAMERON, 2012).

No entanto, assim como as demais classes de medicamentos, os DMARD não biológicos apresentam efeitos adversos desde náuseas a casos mais sérios como hepatotoxicidade, discrasias sanguíneas e doença pulmonar intersticial (SALLIOT & VAN DER HEIJDE, 2009). Enquanto que os biológicos possuem efeitos adversos que abrangem a reações e a infecções nos locais de infusão e injeção, assim como há o aumento do risco de tuberculose para os pacientes que façam uso de inibidores do TNF- $\alpha$  (DIXON et al., 2010). O risco associado ao uso prolongado destes medicamentos biológicos está nos processos infecciosos, tais como, por exemplo, sépsis, abscessos, candidíase e herpes zoster (STRANGFELD et al., 2009).

Neste sentido, fica evidente que o arsenal terapêutico atualmente disponível para o tratamento dos processos inflamatórios ainda apresenta consideráveis limitações. Devido aos efeitos adversos dos anti-inflamatórios, tanto dos AINEs e AEs quanto dos imunomoduladores, novas substâncias com potencial anti-inflamatório, com baixos custos e associados a uma menor ocorrência de efeitos adversos têm sido pesquisadas para representarem alternativa aos medicamentos disponíveis (YEOMANS, 2011).

### 1.3 SELÊNIO

#### 1.3.1 Características gerais e propriedades biológicas

O elemento químico selênio (Se) foi descoberto em 1817 pelo químico sueco Jöns Jacob Berzelius, sendo este elemento não-metal pertencente à família dos calcogênios da tabela periódica, mesmo grupo que pertence também os elementos oxigênio (O), enxofre (S) e Telúrio (Te) (COMASSETO, 2010). Fisiologicamente, o selênio (Se) é um elemento traço amplamente distribuído pelo corpo humano, fazendo parte da composição química de selenoproteínas como a glutathione peroxidase, tioredoxina redutase e selenoproteína P, as quais são essenciais para o crescimento, metabolismo, desenvolvimento, sistema imune e, sobretudo, defesa antioxidante de organismos vivos (MOCCHIGIANI et al., 2014; STAZI & TRINTI, 2008).

Os efeitos do Se no organismo apresentam perfil concentração dependente, ou seja, variando de elemento traço essencial e antioxidante em concentrações na faixa de nano-micromolar a potencialmente pró-oxidante em concentrações acima



do necessário para a síntese de selenoproteínas. Desta forma, em concentrações muito elevadas, o Se pode acumular-se e reagir com os grupos tióis intracelulares, promovendo aumento exacerbado de espécies reativas e dando origem ao estresse oxidativo e dano a componentes celulares (VICENTI et al., 2001).

Na natureza, o Se pode ser encontrado tanto na forma orgânica (selenocisteína, selenocistina e selenometionina), a qual apresenta maior biodisponibilidade e, geralmente, menor toxicidade em relação à outra forma (YOUNG et al., 1982), que é a inorgânica (selenito e selenato) (NAKAMURO et al., 2000).

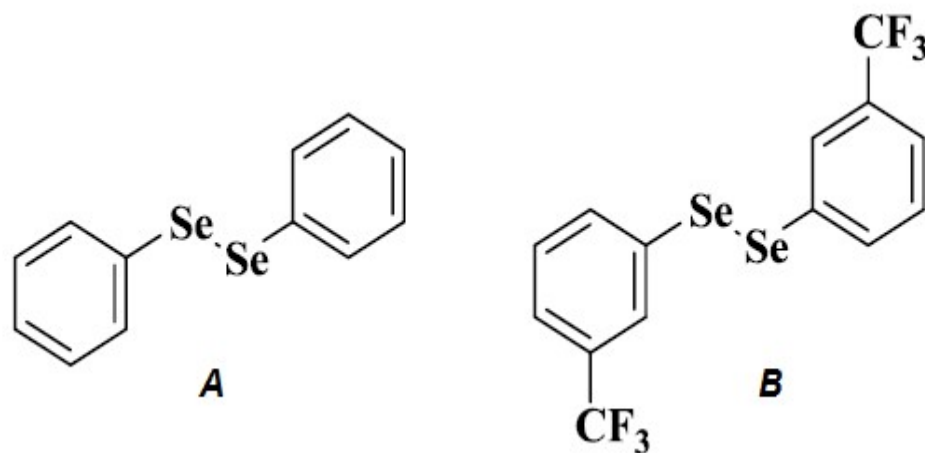
### 1.3.2 Compostos orgânicos de Selênio

Nas últimas décadas, os compostos orgânicos sintéticos de Se vêm sendo foco de pesquisas científicas tanto em síntese orgânica, devido à aplicação dessas moléculas como intermediário de reações (PROCHNOW et al., 2016), como para suas ações em sistemas biológicos, uma vez que várias propriedades farmacológicas e toxicológicas já foram relatadas a essa classe (NOGUEIRA e ROCHA, 2011; NOGUEIRA et al., 2004). Reforçando o potencial desses compostos, a literatura traz inúmeras evidências das propriedades biológicas destes em diversos modelos pré-clínicos, tanto *in vitro* quanto *in vivo* (NOGUEIRA e ROCHA, 2011), e também em estudos realizados em humanos (NOGUCHI, 2016; SINGH et al., 2016).

Entre as moléculas orgânicas de Se, deve-se destacar os disseleneto de diorganoila, sobretudo, o disseleneto de difenila [(PhSe)<sub>2</sub>] (**Figura 3A**), uma vez que é considerado a matriz da classe dos disselenetos e por apresentar importantes propriedades farmacológicas, tais como atividade antiviral (SARTORI et al., 2016), ação do tipo antidepressiva e ansiolítica (SAVEGNAGO et al., 2008), efeito anti-inflamatório (LUCHESE et al., 2012) entre outros. Não obstante, a substituição de grupos funcionais na estrutura do diaril disseleneto tem gerado também compostos com atividades farmacológicas. Nesse sentido, a inserção do grupamento trifluormetila (CF<sub>3</sub>) na posição *meta* na molécula do diaril disseleneto, tem-se o derivado disseleneto de *m*-trifluormetil difenila [(*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>] (**Figura 3B**) o qual se destaca por apresentar muitos efeitos farmacológicos já reportados. Dentre as propriedades já descritas, o composto apresenta efeito do tipo antidepressivo observado no teste do nado forçado (TNF), o qual envolve múltiplos mecanismos de

ação tanto do sistema opioide através da ativação dos receptores  $\mu$  e  $\delta$  e do bloqueio do receptor  $\kappa$ , quanto do sistema serotoninérgico (ROSA et al., 2017; BRÜNING et al., 2015; BRÜNING et al., 2011). Em relação ao sistema serotoninérgico, o  $(m\text{-CF}_3\text{-PhSe})_2$  apresenta efeito ansiolítico em camundongos e inibe seletivamente a atividade da monoaminoxidase A (MAO-A) cerebral *ex vivo*, responsável pelo catabolismo da 5-hidroxitriptamina (5-HT) (BRÜNING et al., 2009).

Figura 3 - (A) Estrutura química do disseleneto de difenila  $(\text{PhSe})_2$  e do (B) disseleneto de *m*-trifluorometil-difenila  $(m\text{-CF}_3\text{-PhSe})_2$ .



Fonte: Adaptado de NOGUEIRA & ROCHA (2011).

Convém ressaltar, sobretudo, o efeito antinociceptivo do  $(m\text{-CF}_3\text{-PhSe})_2$  em diferentes testes de nocicepção aguda em camundongos, tais como: na contorção abdominal induzida por ácido acético, no teste da chapa quente e na injeção de capsaicina na pata e no teste da retirada da cauda após estímulo térmico, no qual é possível relacionar o efeito antinociceptivo à interação com os receptores opioides centrais  $\mu$  e  $\delta$  (BRÜNING et al., 2010). Além disso, o  $(m\text{-CF}_3\text{-PhSe})_2$  apresenta efeito antinociceptivo no teste do glutamato no qual evidencia-se relação com o sistema serotoninérgico uma vez que antagonistas farmacológicos aboliram o efeito antinociceptivo do  $(m\text{-CF}_3\text{-PhSe})_2$  e também da recaptação de 5-HT *ex vivo* (BRÜNING et al., 2014). Ainda, no modelo de constrição parcial do nervo ciático (CPNC) em camundongos, a administração do  $(m\text{-CF}_3\text{-PhSe})_2$  reduziu a hipernocicepção mecânica, assim como normalizou o nível de citocinas pró-inflamatórias no soro e amostras de córtex cerebral e hipocampo contralateral

(BRÜNING et al., 2015a). Destaca-se também o seu efeito do tipo antidepressivo no modelo de depressão induzido TNF- $\alpha$  (BRÜNING et al., 2015b). Esses achados sugerem que a ação antinociceptiva da molécula pode estar relacionada com a modulação do processo inflamatório, hipótese esta que ainda requer mais estudos em modelos experimentais específicos de inflamação e dor inflamatória para confirmação.

Com relação às propriedades toxicológicas do composto (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>, o mesmo apresenta baixa toxicidade em camundongos em doses agudas, com DL<sub>50</sub> (dose letal em 50% dos animais) de 278 mg/kg. A administração de doses de 10 a 300 mg/kg não causa alterações em parâmetros bioquímicos de função hepática, como a alanina aminotransferase (ALT), a aspartato aminotransferase (AST), e renal, como ureia e a creatinina (SAVEGNAGO et al., 2009). Recentemente, Rosa e colaboradores (2017) demonstraram que, diferentemente da morfina, a modulação do sistema opioide exercida pelo composto não causa tolerância farmacológica e nem síndrome de retirada, reforçando o potencial terapêutico do (*m*-CF<sub>3</sub>-PhSe). Ressalta-se também, que após um regime de tratamento repetido com o (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>, nas doses de 5 a 50 mg/kg pela via intragástrica em camundongos, durante oito dias, não se observou toxicidade sistêmica, evidenciando-se que o uso contínuo do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> seja seguro nestas condições de exposição (ROSA et al., 2017).

Sendo assim, levando-se em conta a necessidade de desenvolver novas abordagens farmacológicas que apresentem baixo custo e elevada eficácia e segurança, e considerando as propriedades do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> acima descritas, este composto torna-se um interessante candidato a agente terapêutico aos processos inflamatórios.

## 2 OBJETIVOS

### 2.1 OBJETIVO GERAL

Avaliar o efeito anti-inflamatório do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> em modelos de nocicepção inflamatória em camundongos.

### 2.2 OBJETIVOS ESPECÍFICOS

No modelo de nocicepção inflamatória aguda e subcrônica induzido pelo adjuvante completo de Freund em camundongos:

- Determinar em uma curva tempo/dose resposta a dose efetiva do composto (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> administrado pela via intragástrica;
- Avaliar em um tratamento agudo com o composto (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>: o edema e a atividade da enzima mieloperoxidase na pata;
- Estudar o efeito do tratamento repetido com o composto (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>: na atividade exploratória e na hipernocicepção térmica e mecânica;
- Em um tratamento repetido com o composto (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>: avaliar o edema e a atividade da enzima mieloperoxidase na pata;
- Determinar o nível das proteínas IL-1β, TNF-α e COX-2 no córtex cerebral contralateral;
- Analisar os parâmetros do tônus oxidativo tecidual (fígado e rim) e marcadores plasmáticos de função renal, hepática e cardíaca;

Na estabilidade físico-química do composto:

- Analisar a estabilidade físico-química do (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> em diferentes condições de armazenamento;

### **3 DESENVOLVIMENTO**

O desenvolvimento dessa dissertação está apresentado na forma de um manuscrito científico. Os itens Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se descritos no próprio manuscrito. Em anexo, desta dissertação, encontra-se o parecer de aprovação do projeto de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Santa Maria.

## 1 3.1 MANUSCRITO

2

3 ***Anti-inflammatory action of m-trifluoromethyl-diphenyl diselenide on acute and***  
4 ***subchronic animal models of inflammatory pain: behavioral, biochemical and***  
5 ***molecular insights***

6

7 Paulo Cesar Oliveira Araujo<sup>a</sup>, Marcel Henrique Marcondes Sari<sup>b</sup>, Natália Silva  
8 Jardim<sup>a</sup>, Juliano Ten Kathen Jung<sup>a</sup> and César Augusto Brüning<sup>c,\*</sup>

9

10 <sup>a</sup>Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de  
11 Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de  
12 Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP  
13 97105-900, RS, Brazil14 <sup>b</sup>Laboratório de Tecnologia Farmacêutica, Departamento de Farmácia Industrial,  
15 Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria,  
16 CEP 97105-900, RS, Brazil17 <sup>c</sup>Laboratório de Bioquímica e Neurofarmacologia Molecular, Centro de Ciências  
18 Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas,  
19 CEP 96010-900, RS, Brazil

20

21 \*Correspondence should be sent to:

22 César Augusto Brüning

23 Laboratório de Bioquímica e Neurofarmacologia Molecular, Centro de Ciências  
24 Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas,  
25 CEP 96010-900, RS, Brazil

26 Phone: + 55 53 3275-7355

27 E-mail: cabruning@yahoo.com.br

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## 1 **Abstract**

2 *m*-Trifluoromethyl-diphenyl diselenide [(*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>] is an organoselenium  
3 molecule that displays multiple pharmacological actions, including antinociceptive  
4 effect. The current study investigated the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> restorative properties in  
5 models of acute and chronic inflammatory pain induced by complete Freund's  
6 adjuvant (CFA). Male adult Swiss mice received an intraplantar administration of CFA  
7 in the hindpaw 24 h (acute) or 14 days (subchronic) later they were treated with a  
8 single or repeated (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> schedule via intragastric route, respectively. The  
9 mechanical and thermal hypernociceptive behavior was assessed by von Frey hair  
10 test and hot plate test. Samples of injected-paw were collected to assess the tissue  
11 edema and myeloperoxidase (MPO) activity; samples of cerebral contralateral cortex  
12 were used to determine the content of inflammatory proteins. The acute (*m*-CF<sub>3</sub>-  
13 PhSe)<sub>2</sub> administration (10 mg/kg and 1 mg/kg) reduced the hypernociceptive  
14 behavior and both paw thickness and MPO activity induced by CFA injection. In the  
15 subchronic protocol, the repeated administration with a effective dosage of (*m*-CF<sub>3</sub>-  
16 PhSe)<sub>2</sub> reduced the mechanical and thermal hypernociception and restored the  
17 edema and MPO activity in paw samples. In addition, the repeated treatment  
18 schedule mitigated the increase in TNF- $\alpha$ , IL-1 $\beta$  and COX-2 content in cerebral  
19 contralateral cortex induced by CFA injection. Collectively, these data suggest that  
20 the anti-inflammatory action exerted by (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> are mediated by an interplay  
21 between peripheral and central mechanisms of action without toxicological  
22 impairment, reinforcing the potential biological properties of the compound.

23

24 **Keywords:** selenium, inflammation, pain, toxicity

25

## 1 **1. Introduction**

2

3 Inflammation is a biological response to harmful stimuli, such as pathogens,  
4 damaged cells or toxic molecules [1] that acts by removing injurious stimuli and  
5 restoring the homeostasis process [2]. Thus, inflammation is an important protective  
6 response that has a crucial role in the regeneration of injured tissues [3]. However,  
7 depending on the situation, the uncontrolled inflammation may become chronic,  
8 contributing to the development of a variety of inflammatory diseases, which is no  
9 longer beneficial [4]. In addition, the available pharmacological therapy to counteract  
10 the inflammation has several issues regarding adverse effects and/or poor efficacy.  
11 Indeed, the non-steroidal anti-inflammatory drugs (NSAIDs), which comprise the  
12 most prescribed class of pharmacological agents, are associated with gastrointestinal  
13 injury development, nephrotoxicity and hematological damage, mainly in long-lasting  
14 treatment schedules [5]. Thus, there is an urgent need for developing novel  
15 pharmacological approaches that could better supply the clinical management of  
16 inflammation.

17 Considering the complex pathophysiology of the inflammatory process, an  
18 optimal and desired drug candidate would be effective in modulating as many targets  
19 as possible [6]. In this sense, the organoselenium compounds are emerging as an  
20 important source of novel biologically active agents [7]. The diaryl diselenides are a  
21 class of selenium-containing compounds that displays an array of important  
22 pharmacological actions, such as the potential antinociceptive and anti-inflammatory  
23 properties. Among the compounds, the *m*-trifluoromethyl-diphenyl diselenide [(*m*-  
24 CF<sub>3</sub>-PhSe)<sub>2</sub>] is highlighted because of its specific pharmacological modulation of the  
25 opioid system [8-10]. It seems that the interaction with such neurotransmitter system



1 mediates at least in parts its antidepressant-like and antinociception actions [10, 11].  
2 In addition, differently from morphine, the repeated administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>  
3 caused pharmacological effects without developing tolerance and addiction [8].  
4 Despite these data, a study assessing the potential anti-inflammatory action of (*m*-  
5 CF<sub>3</sub>-PhSe)<sub>2</sub> has not been reported.

6 Therefore, the aim of this study was to evaluate the anti-inflammatory action of  
7 (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> in acute and subchronic animal models of inflammatory pain induced  
8 by complete Freund's adjuvant (CFA). The potential (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> restorative  
9 effects were assessed by behavioral tests of thermal and mechanical  
10 hypernociception as well as via biochemical and molecular inflammatory markers.  
11 Additionally, the physicochemical stability of the compound and a toxicological study  
12 were carried out.

13

## 14 **2. Material and methods**

15

### 16 *2.1. Drugs and reagents*

17

18 (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> was synthesized in our laboratory following the method of [12]  
19 and accurately evaluated before its use. The chemical purity (99.9%) was determined  
20 by gas chromatography-mass spectrometry and high-performance liquid  
21 chromatography (Shimadzu QP2010PLUS). The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra  
22 analysis (400 MHz, Bruker Avance™ III HD) showed analytical and spectroscopic  
23 data in full agreement with its assigned molecular structure. The CFA, diclofenac  
24 sodium (DCF), N, N, N', N'-tetramethylbenzidine, bovine serum albumin (BSA),  
25 protease inhibitor cocktail, tris(hydroxymethyl)aminomethane, acrylamide, bis-

1 acrylamide, sodium dodecyl sulfate (SDS), pre-stained molecular weight standard  
2 and bromophenol blue were obtained from Sigma-Aldrich (São Paulo, Brazil). All  
3 other chemicals were of analytical grade and obtained from standard commercial  
4 suppliers.

5

## 6 *2.2. Stability studies*

7

8 The physicochemical stability of  $(m\text{-CF}_3\text{-PhSe})_2$  was evaluated considering the  
9 influences of time and the storage condition. Initially, a  $(m\text{-CF}_3\text{-PhSe})_2$  oily solution  
10 was prepared with canola oil at compound concentration 1 mg/mL. The samples  
11 were stored in amber flasks and at different storage conditions: room temperature  
12 (25 °C), refrigeration (4 °C) and freezer (-20 °C). At predetermined time periods (soon  
13 after the preparation and 60 days later) the compound content was determined using  
14 a high-performance liquid chromatography (HPLC). The  $(m\text{-CF}_3\text{-PhSe})_2$   
15 quantification was performed on a LC-10A HPLC system (Shimadzu, Japan), which  
16 was equipped with: a LC-20AT pump, an UV-VIS SPD-M20A detector, a CBM-20A  
17 system controller and a SIL-20A HT autosampler valve. To molecule separation, a  
18  $\text{C}_{18}$  column (Phenomenex Gemini reversed phase, 5  $\mu\text{m}$ , 110 Å, 150 mm x 4.60 mm)  
19 was used at room temperature (25 °C). The analytical conditions were set based on  
20 previous developed HPLC methodologies for organoselenium compounds  
21 quantification [13, 14]: an isocratic mobile phase composed by methanol and  
22 ultrapure water (90:10, v/v), a 1 ml/min flow rate, a 20  $\mu\text{L}$  injection volume and a 243  
23 nm detection wavelength. Peak areas were measured and integrated employing a  
24 Shimadzu LC solution software program. The methodology was linear in a

1 concentration range of 5.0-25.0 µg/ml ( $r=0.998$ ). The experiment was performed in  
2 triplicate.

3

### 4 *2.3. Animals*

5

6 This study was carried out using male adult Swiss mice (25-35 g) obtained  
7 from the Federal University of Santa Maria breeding colonies. Animals were housed  
8 in polypropylene cages, under a 12 h light/dark cycle (7 AM to 7 PM), controlled  
9 temperature ( $22 \pm 2^\circ\text{C}$ ) and humidity (60%), receiving standard food (GUABI, RS,  
10 Brazil) and water *ad libitum*. This study was approved by the Ethical Research  
11 Committee of Federal University of Santa Maria (**#8081170317/2017**) affiliated to the  
12 Council for Control of Animal Experiments (CONCEA) and in accordance with the  
13 NIH Guide for the Care and Use of Laboratory Animals. The number of animals used  
14 in the current study was the minimum necessary to demonstrate consistent effects of  
15 drug treatment and all efforts were made to minimize their suffering.

16

### 17 *2.4. Pharmacological and toxicological assessments*

18

#### 19 *2.4.1. General procedures and experimental design*

20

21 This study was divided in two protocols (**Fig. 2**). The first set of experiments  
22 was carried to assess the possible restorative effects of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> against  
23 behavioral and biochemical impairments in a model of acute inflammatory pain  
24 induced by CFA (**Fig. 2A and 2B**). Following, the protocol 2 (**Fig. 2C**) investigated if  
25 a repeated administration schedule using a effective (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> dosage (first

1 protocol) would mitigate the behavioral and molecular changes caused by CFA-  
2 induced subchronic inflammatory pain model in mice. Furthermore, parameters of  
3 general toxicity were also evaluated.

4 Mice received the treatment via intragastric route (i.g.), in single or repeated  
5 administration regiment and in a 10 mL/kg constant volume of body weight. The  
6 dosage, administration route and treatment schedule were designed considering  
7 previous studies, which demonstrated (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> pharmacological actions, such  
8 as antinociceptive effect [10].

9

#### 10 2.4.2. Experiment 1

11

12 The acute (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> anti-inflammatory action was investigated using the  
13 animal model of inflammatory pain induced by CFA in mice. The induction was  
14 performed by a single subcutaneous intraplantar (i.pl.; 20 µL) injection of CFA (1  
15 mg/mL of heat killed *Mycobacterium tuberculosis* in 85% paraffin oil and 15%  
16 mannide monooleate) in right hindpaw of mice [15]. Before the induction, the basal  
17 behavioral response was assessed by paw withdrawal frequency in the von Frey hair  
18 (VFH) test (Section 2.5.2). Following, CFA or saline (control) was injected (i.pl.) and  
19 24 h later the animals performed the VFH test. The time- and dose-response curves  
20 of mechanical anti-hypernociceptive action were performed after a single (*m*-CF<sub>3</sub>-  
21 PhSe)<sub>2</sub> administration, using distinct groups of animals to each tested dose. The  
22 animals received vehicle (canola oil, i.g., 10 mL/kg) or (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (0.1, 1 or 10  
23 mg/kg; i.g. in canola oil; N: 10/group) and the withdrawal response frequency in VFH  
24 was recorded over time after treatment (0.5, 1, 2, 3, 4, 5 and 6 h). The diclofenac  
25 sodium, a non-steroidal anti-inflammatory drug (DCF, 5 mg/kg; i.g. in saline; N:

1 10/group) was used as positive control [16] (**Fig. 2A**). Following, to further investigate  
2 the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> anti-inflammatory action, another set of animals was treated to  
3 investigate the potential pharmacological action of the compound against paw  
4 inflammatory impairments induced by CFA. The animals received (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (10  
5 mg/kg, i.g.) or DCF and at predetermined time after treatment (between 2 and 3 h  
6 after treatment, which were selected based on the time- and dose-response curves)  
7 they were anesthetized and then euthanized to collect samples of the injected paw  
8 (cut below the knee joint) for *ex vivo* evaluations (Section 2.6.) (**Fig. 2B**).

9

#### 10 2.4.3. Experiment 2

11

12 The second part of this study investigated if a repeated administration of  
13 effective dose of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> would restore the behavioral and molecular  
14 impairments in a subchronic inflammatory pain model induced by the CFA. On day 0,  
15 the basal behavior of the animals was evaluated on locomotor activity monitor (LAM)  
16 (Section 2.5.1.), mechanical hypernociception using the VFH test in both paws  
17 (Section 2.5.2.) and thermal hyperalgesia using the hot plate test (Section 2.5.3.)  
18 (Fig.1C). Following, CFA or saline (control) was injected (i.pl.) in the right hindpaw of  
19 mice. Beginning from the 14<sup>th</sup> day after injection until the 23<sup>rd</sup>, the animals received  
20 vehicle (canola oil), (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> at a dose of 1 mg/kg (once a day, i.g.) or DCF at  
21 a dose of 5 mg/kg (twice a day, i.g.) and thermal and mechanical hypernociception  
22 were daily assessed. The animals were periodically monitored to record mortality,  
23 behavioral pattern changes, ptosis, tremors, diarrhea, salivation, piloerection,  
24 tremors, locomotor alterations or seizures. Furthermore, before receiving the  
25 treatment, the body weight gain was daily recorded as well. Twenty-four hours after

1 the last treatment (24<sup>th</sup> day), the animals performed the LAM, the VFH test and the  
2 hot plate test and then were slightly anesthetized prior to blood collection by cardiac  
3 puncture. The blood samples were collected with heparin tubes and centrifuged at  
4 2,500 xg during 10 min to obtain the plasma fraction, which were used for the  
5 biochemical assays (Section 2.6.3.). After this procedure, some selected tissues  
6 (paw, kidney and liver) were removed and then frozen and stored at – 80 °C to  
7 further evaluations (Section 2.6.2.). Samples of brain were also collected and the  
8 contralateral cortex was dissected, flash-frozen and stored at - 80 °C until the  
9 western blot analysis (Section 2.6.4.).

10

## 11 *2.5. Behavioral assessment*

12

### 13 *2.5.1. Spontaneous locomotor activity*

14

15 To discard non-specific effects of treatments, spontaneous locomotor behavior  
16 of mice was evaluated using a video tracking software coupled with an open field  
17 plexiglas chamber. The apparatus is a clear acrylic plastic open field (45 x 45 x 45  
18 cm<sup>3</sup>) surrounded by a frame consisting of 32 photocells placed on opposite walls  
19 (16L x 16W, 2 cm apart from each other) that continuously tracks the animal. Data  
20 were collected in the photobeam breaks as an activity indication in different  
21 predertermined areas in the open field, which were recorded by a computer, using  
22 the Monitor Activity<sup>®</sup> software (Insight). During the test, the animals were placed in  
23 the center of the apparatus, and for 4 min the following parameters were recorded:  
24 Number of crossings and rearings, average speed (mm/s) and total distance traveled  
25 (cm).

1    2.5.2. *Mechanical hypernociception evaluation*

2

3           The mechanical hypernociception was measured by the paw withdrawal  
4 frequency using the VFH test (Stoelting, Chicago, IL) according to Bortolanza [17].  
5 The animals were first acclimatized during 1 h in individual plexiglas chambers (9 x 7  
6 x 11 cm) with wire mesh bottoms to allow the access the plantar surface of paws.  
7 The results were expressed as percentage of response frequency, which were  
8 determined after ten manual applications of a calibrated nylon VFH filament of 1.0 g  
9 (duration of 1-2 s each) [18] perpendicular to the right hindpaw plantar surface  
10 (Experiment 1) or both ipsilateral and contralateral hind paws (right and left hind  
11 paws respectively – Experiment 2).

12

13    2.5.3. *Thermal hypernociception*

14

15           The thermal hypernociception was measured by the hot plate test according to  
16 the method previously described [19]. The animals were placed in an acrylic cylinder  
17 (20 cm in diameter) onto the hot plate apparatus, maintained at  $52 \pm 1$  °C. The time  
18 (s) between the placement onto the heated surface and licking of their hindpaws or  
19 jumping was recorded as the response latency. A 60 s cut-off was used to prevent  
20 tissue damage.

21

22

23

24

25

1 2.6. *Ex vivo studies*

2

3 2.6.1. Paw samples evaluation

4

5 2.6.1.1. *Edema determination*

6

7 The paw edema was quantified by the weight difference between the injected  
8 (saline or CFA) and the opposite paw (no injected paw) of each animal. The weight  
9 difference was estimated on analytical laboratory scales thereafter their collection.  
10 The results are expressed as weight difference (mg).

11

12 2.6.1.2. *Myeloperoxidase (MPO) assay*

13

14 The MPO activity was evaluated according to a previously published  
15 methodology [20]. The paw samples were homogenized in potassium phosphate  
16 buffer (20 mmol/L, pH 7.4) containing ethylenediaminetetracetic acid (0.1 mmol/L)  
17 and centrifuged at 2000 x g for 10 min at 4 °C to yield a low-speed supernatant  
18 fraction (S<sub>1</sub>). The S<sub>1</sub> fraction was further centrifuged at 20,000 x g for 15 min at 4°C  
19 to obtain a final pellet that was resuspended in a medium containing potassium  
20 phosphate buffer (50 mmol/L, pH 6.0) and hexadecyltrimethylammonium bromide  
21 (0.5%). The samples were frozen and thawed three times to cause total cell lysis  
22 before the enzymatic assay. The activity of MPO enzyme was performed by adding  
23 an aliquot of re-suspended final pellet to a medium containing the re-suspension  
24 buffer and N,N,N',N'-tetramethylbenzidine (1.5 mmol/L). The MPO activity kinetic  
25 analysis started after the H<sub>2</sub>O<sub>2</sub> (0.01%) addition to the system and the color reaction



1 was measured at 655 nm at 37 °C during 2 min. The results are expressed as optic  
2 density (O.D.)/mg protein/min.

3

#### 4 *2.6.2. Biochemical parameters of tissue oxidative status*

5

6 The tissue samples (liver and kidney) were homogenized in 50 mM Tris HCl at  
7 pH 7.5, 1:10 (w/v) and then centrifuged at 2,500 x g during 10 min at 4 °C to yield a  
8 low-speed supernatant fraction (S<sub>1</sub>). Excepting for carbonyl protein content  
9 determination (Section 5.2.3), in which the pure homogenates were used, freshly  
10 prepared S<sub>1</sub> was applied for determining some parameters of tissue oxidative status.  
11 Protein concentration of each sample was quantified by the Coomassie blue method  
12 using BSA (1 mg/mL) as the analytical standard [21].

13

##### 14 *2.6.2.1. Non-protein thiol levels*

15

16 To determine the non-protein thiol (NPSH) quantity in the samples, an aliquot  
17 of S<sub>1</sub> was mixed with 10% trichloroacetic acid solution (1:1, v/v) and the clear  
18 supernatant (S<sub>2</sub>) was obtained through centrifugation. The S<sub>2</sub> contains the free thiol  
19 groups, which reacts with 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) generating an  
20 yellow complex measured at 412 nm [22]. The results were expressed as nmol  
21 NPSH/g tissue.

22

23

24

25

#### 1 2.6.2.2. *Delta-aminolevulinic acid dehydratase activity*

2

3 The Delta-aminolevulinic acid dehydratase ( $\delta$ -ALA-D) activity was determined  
4 according to Sassa [23]. The samples were incubated at 37 °C in a medium  
5 containing 45 mM phosphate buffer, pH 6.8 and the enzymatic reaction was initiated  
6 by adding the substrate (5-aminolevulinate; 2.2 mM final concentration). The  
7 incubation was stopped by adding trichloroacetic acid solution (10% TCA) with 10  
8 mM HgCl<sub>2</sub>. The reaction product, porphobilinogen, was spectrophotometrically  
9 measured at 555 nm with modified Erlich's reagent. Results are expressed as nmol  
10 of porphobilinogen/h/mg protein.

11

#### 12 2.6.2.3. *Protein carbonyl content*

13

14 The carbonyl proteins reaction with dinitrophenylhydrazine (DNPH) is the  
15 method to measure carbonyl content [24]. The samples were diluted with 10 mM Tris  
16 HCl at pH 7.4, 1:10 (v/v). Aliquots of 1 mL of these dilutions were placed in tubes with  
17 200  $\mu$ L of 10 mM DNPH in 2M HCl or only 200  $\mu$ L of 2M HCl. Afterwards all tubes  
18 were incubated for 1 h at room temperature and under light protection. The tubes  
19 were shaken using vortex mixer every 15 min. After that, 0.5 mL of denaturation  
20 buffer (sodium phosphate buffer, containing 3% SDS, pH 6.8), 1.5 mL of ethanol and  
21 1.5 mL of hexane were added to all tubes. Immediately tubes were vortexed for 40 s  
22 and centrifuged for 15 min at 2400 x g. Supernatants were discarded and obtained  
23 pellet was separated, washed twice with 1 mL of a mixture of ethanol:ethyl acetate  
24 (1:1, v/v) and dried at room temperature for 2 min. The pellet was immediately

1 dissolved in 1 mL of denaturation buffer. Absorbance was measured at 370 nm.  
2 Results are expressed as nmol carbonyl content/mg protein.

3

#### 4 *2.6.3. Plasma biochemical parameters*

5

6 The plasma samples were used to estimate some liver, kidney and cardiac  
7 biochemical parameters of tissue functionality and cellular damage using commercial  
8 kits (Labtest, Minas Gerais, Brazil). The alanine (ALT) and aspartate  
9 aminotransferase (AST) activities were measured to evaluate hepatic function, urea  
10 levels to assess renal function and creatine kinase (CK) to determine the cardiac  
11 muscle tissue health. The results of ALT, AST and CK activities were expressed as  
12 U/dL and urea levels were expressed as mg/dL.

13

#### 14 *2.6.4. Western blotting analysis*

15

16 To estimate the content of some inflammatory-related proteins, samples of  
17 control (saline + vehicle) and CFA + treatments groups were assayed by western  
18 blotting technique. No further evaluations were performed with both *per se* groups  
19 because they do not elicit any pharmacological action. The cerebral contralateral  
20 cortex samples were homogenized in commercial RIPA (Radio-Immunoprecipitation  
21 Assay) buffer (300  $\mu$ L) with protein inhibitor cocktail (Sigma-Aldrich Co., 1:100). The  
22 content of protein in the samples were assessed using the bicinchoninic acid (BCA)  
23 assay. Prior the western blotting analyses, protein concentration of each sample was  
24 adjusted to 2  $\mu$ g/ $\mu$ L using a sample buffer (consisting essentially of 500 mM Tri HCl  
25 pH 6.8, glycerol, 10% SDS, 2- $\beta$ -mercaptoethanol and 2% bromophenol blue, which

1 was applied as a marker of the electrophoresis process). The samples (20 µg  
2 protein/well) were separated on an SDS-polyacrylamide gel by electrophoresis.  
3 Following, the proteins were transferred to a nitrocellulose membrane (0.45 µm, Bio-  
4 Rad) using the Transfer-Blot® Turbo™ Transfer System (1.0 A, 45 min, Bio-Rad).  
5 After blocking with 3% BSA solution for 1 h, the blots were incubated overnight at 4  
6 °C with rabbit *anti*-COX-2 (74 kDa/Rabbit/1:1000, Cell Signaling Technology,  
7 Danvers, USA), *anti*-IL-1β (31 kDa/Mouse/1:1000, Santa Cruz Biotechnology, Santa  
8 Cruz, CA, USA), *anti*-TNF-α (26 kDa/Mouse/1:1000, Santa Cruz Biotechnology,  
9 Santa Cruz, CA, USA). *Anti*-β-actin (42 kDa/Mouse/1:1000, Cell Signaling  
10 Technology, Danvers, USA) was stained as protein loading control. After incubation  
11 with the primary antibodies, the membranes were washed and incubated with the  
12 respective peroxidase-conjugated secondary antibodies for 1 h at room temperature.  
13 A chemiluminescence kit was used for protein detection and the signals were  
14 captured with Amersham Imager 600 (GE Healthcare Life Sciences). The optical  
15 density (O.D.) bands were quantified using Image J (NIH, Bethesda, MD, USA)  
16 software for Windows. Each value was derived from the ratio between arbitrary units  
17 obtained by the protein band and the respective β-actin band. The results were  
18 expressed as % of control.

19

## 20 2.7. Statistical evaluation

21

22 All results are given as the mean ± standard error of mean (S.E.M.) of the “η”  
23 observation. The data normality was evaluated using the D’Agostino and Pearson  
24 omnibus normality test. A difference was accepted as significant when  $p < 0.05$ . Data  
25 of compound stability evaluation were analyzed using paired Student’s *t* test.

1 Statistical comparisons among experimental groups of the protocol experimental 1  
2 were performed using repeated or ordinary One-Way analysis of variance (ANOVA)  
3 followed by the Newman-Keuls' multiple range test when appropriate. Statistical  
4 differences among experimental groups of the experimental protocol 2 were  
5 performed using repeated or ordinary Two-Way ANOVA followed by the Newman-  
6 Keuls' multiple range test when appropriate. Main effects of first-order interactions  
7 are show only when interactions were not statistically significant. The GraphPad  
8 Prism<sup>®</sup> statistical software version 6 (GraphPad, USA) was used for the statistical  
9 analysis of experimental data.

10

### 11 **3. Results**

12

#### 13 *3.1. Stability study*

14

15 The stability study demonstrated that the compound content had no alteration  
16 independent of the storage condition applied (room temperature (25 °C), refrigeration  
17 (4 °C) and freezer (- 20 °C) over the experimental time. The paired Student's *t* test  
18 did not reveal significant statistical difference between the storage time at room  
19 temperature ( $t_{(6)} = 0.004$ ,  $p > 0.05$ ), refrigeration ( $t_{(6)} = 0.094$ ,  $p > 0.05$ ) and freezer  
20 ( $t_{(6)} = 0.634$ ,  $p > 0.05$ ) (Fig. 1).

21

22

23

24

1 3.2. Time- and dose-response curves of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> effect on the CFA- induced  
2 mechanical hypernociceptive behavior

3  
4 The results of the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> time- and dose-response curves in the  
5 mechanical hypernociception induced by CFA injection are depicted in Fig. 3A. The  
6 One-way ANOVA of repeated measures revealed that the CFA injection caused an  
7 increase in the percentage of response to VFH stimulation, which persisted during all  
8 the experiment. The treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> reduced this inflammatory  
9 nociceptive behavior in a distinct time profile depending on the compound dose. The  
10 Newman-Keuls' post hoc test showed that the dose 10 mg/kg reduced the  
11 percentage of response to VFH stimulation starting at 0.5 h and remained significant  
12 until 5 h after treatment reaching its action peak at 3 h ( $F_{(1,432)} = 13.35, p < 0.001$ ). In  
13 addition, the treatment with dose 1 mg/kg caused a statistically significant decrease  
14 in the mechanical hypernociception only 2 h after treatment, while the dose 0.1  
15 mg/kg had no significant pharmacological effect in the mechanical hypernociception.  
16 As expected, the group of animals that received DCF had a reduction in the  
17 mechanical hypernociception, which started at 1 h and remaining significant up to 3 h  
18 after treatment.

19 The results of area under the curve (AUC) corroborate with the data obtained  
20 in the behavioral evaluation (Fig. 3B). The ordinary One-way ANOVA showed a  
21 statistically significant difference among the experimental groups. The results of post  
22 hoc analysis revealed a statistical difference in the AUC values between saline- and  
23 CFA-injected groups. Treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> at a dose 10 mg/kg reduced the  
24 AUC values in comparison to those of the group that received CFA + vehicle ( $F_{(5,59)} =$

1 155.5,  $p < 0.001$ ). The treatment with DCF reduced the AUC values in comparison to  
2 those of CFA group.

3

### 4 3.3. *(m-CF<sub>3</sub>-PhSe)<sub>2</sub> treatment acute reduce the paw inflammatory alterations caused* 5 *by CFA injection*

6

7 Considering the results obtained in the time- and dose-response curves, the  
8 intervals between 2 and 3 h after *(m-CF<sub>3</sub>-PhSe)<sub>2</sub>* treatment (10 mg/kg) and 1 and 2 h  
9 later DCF (5 mg/kg) administration were selected to perform the following steps of  
10 the protocol 1. The results of paw edema and MPO activity are depicted in Fig. 4.  
11 The CFA injection triggered an increase in the paw edema and in the plantar MPO  
12 activity in comparison to the control group. Treatment with *(m-CF<sub>3</sub>-PhSe)<sub>2</sub>* was  
13 effective against both inflammatory alterations, reducing the paw edema ( $F_{(3,31)} =$   
14  $43.20$ ,  $p < 0.001$ ) and MPO activity ( $F_{(3,31)} = 38.65$ ,  $p < 0.001$ ) as well. The DCF  
15 administration also reduced both parameters.

16

### 17 3.4. *Repeated treatment of (m-CF<sub>3</sub>-PhSe)<sub>2</sub> in the behavioral parameters in a* 18 *subchronic inflammatory pain model induced by CFA*

19

#### 20 3.4.1. *Spontaneous locomotor activity (LAM)*

21

22 There was not a statistically significant difference among experimental groups  
23 in the parameters of LAM (number of crossings and rearings, total distance traveled  
24 and average speed) ( $p > 0.05$ ; Supplementary material; Table S1).

25

### 1 3.4.2. Mechanical hypernociception

2

3 The results of repeated treatment with 1 mg/kg of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> restored the  
4 mechanical hypernociception in the CFA-induced subchronic inflammation (Fig. 5A).  
5 The two-way ANOVA of repeated measures revealed a significant treatment (*m*-CF<sub>3</sub>-  
6 PhSe)<sub>2</sub> and time interaction ( $F_{(33,348)} = 7.720, p < 0.001$ ). The Newman-Keuls' post  
7 hoc test demonstrated that CFA injection in the paw produced an increase in the  
8 percentage of response to VFH stimulation, which persisted over the experimental  
9 evaluation. The (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> administration elicited an anti-hypernociceptive action  
10 started on the 2<sup>nd</sup> day of treatment (15<sup>th</sup> day) and remained significant until the end of  
11 the experiment. Regarding the DCF treatment, the two-way ANOVA of the  
12 mechanical hypernociception data revealed that there was a significant treatment  
13 and time interaction ( $F_{(33,336)} = 8.140, p < 0.001$ ). The DCF administration attenuated  
14 the mechanical hypernociception, starting on the 16<sup>th</sup> day of treatment and remained  
15 significant until the end the study.

16 Confirming these results, the two-way ANOVA yielded a statistically significant  
17 interaction to (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> ( $F_{(1,29)} = 76.85, p < 0.001$ ) and DCF in the AUC data  
18 ( $F_{(1,29)} = 40.33, p < 0.001$ ) (Fig. 5B). The results of post hoc analysis revealed the  
19 treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> caused a reduction in the AUC values in comparison  
20 to CFA group. The DCF group showed a partial reduction in the AUC values in this  
21 condition.

22

23

24

25



### 1 3.4.3. Thermal hypernociception

2

3 Fig. 6 depicts the effect of repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg/kg) in  
4 the thermal hypernociception (Fig. 6A). The two-way ANOVA of repeated measures  
5 showed a significant treatment (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> and time interaction ( $F_{(33,336)} = 1.860$ ,  
6  $p < 0.001$ ). Pos hoc test demonstrated that the CFA injection caused a decrease  
7 around 50% of the paw withdrawal response latency in comparison to the control  
8 group. The (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> treatment was effective in reducing the thermal  
9 hypernociceptive behavioral since the 2<sup>nd</sup> day of treatment until the end of the  
10 experiment, increasing around 53% the paw withdraw latency in comparison to CFA  
11 + Vehicle group at the end of treatment. Corroborating with these results, the two-  
12 way ANOVA of AUC data showed a significant interaction between treatment (*m*-  
13 CF<sub>3</sub>-PhSe)<sub>2</sub> and time ( $F_{(1,28)} = 4.591$ ,  $p < 0.05$ ) (Fig. 6B). The compound  
14 administration to mice increased the respective AUC values in comparison with the  
15 CFA + vehicle group.

16 The two-way ANOVA demonstrated significative main effect of DCF treatment  
17 to thermal hypernociceptive behavior ( $F_{(33,336)} = 1.715$ ,  $p < 0.001$ ) and AUC data  
18 ( $F_{(1,28)} = 46.87$ ,  $p < 0.001$ ). Pos hoc test revealed that DCF administration attenuated  
19 the reduction in paw withdrawal latency over the days of treatment (around 28% in  
20 comparison to CFA + Vehicle group on the last day of behavioral test) as well as  
21 caused a partial increase in the AUC values.

22

23

24

1 3.5. *(m-CF<sub>3</sub>-PhSe)<sub>2</sub> repeated treatment reduces the paw inflammatory injuries in a*  
2 *subchronic inflammatory pain model induced by CFA*

3  
4 The results of paw edema and MPO activity are demonstrated in Fig. 7. The  
5 two-way ANOVA of data revealed a significant CFA x *(m-CF<sub>3</sub>-PhSe)<sub>2</sub>* interactions for  
6 paw edema ( $F_{(1,29)} = 9.650$ ,  $p < 0.01$ ) and MPO activity ( $F_{(1,29)} = 4.038$ ,  $p < 0.05$ ). The  
7 CFA injection induced an increase in paw thickness and MPO activity. Repeated  
8 treatment with *(m-CF<sub>3</sub>-PhSe)<sub>2</sub>* was effective in reverting these inflammatory  
9 alterations in comparison to the CFA + vehicle group.

10 The two-way ANOVA evaluation yielded a significant CFA x DCF interactions  
11 for paw edema ( $F_{(1,28)} = 8.018$ ,  $p < 0.01$ ) and MPO activity ( $F_{(1,28)} = 5.082$ ,  $p < 0.05$ ).  
12 The pos hoc test showed that DCF administration restored both parameters.

13  
14 3.6. *(m-CF<sub>3</sub>-PhSe)<sub>2</sub> repeated treatment attenuated the increase in cerebral*  
15 *contralateral cortex inflammatory protein levels*

16  
17 The representative protein bands and TNF- $\alpha$ , IL-1 $\beta$  and COX-2 levels are  
18 depicted in Fig. 8. The ordinary One-way ANOVA revealed a significant difference  
19 among the groups. The Newman-Keuls' post hoc test showed that CFA injection  
20 caused an increase in the TNF- $\alpha$ , IL-1 $\beta$  and COX-2 levels in comparison to the  
21 control group. The repeated treatment with subeffective dose of *(m-CF<sub>3</sub>-PhSe)<sub>2</sub>* as  
22 well as the DCF administration mitigated the CFA-induced augmentation in the TNF-  
23  $\alpha$  ( $F_{(3-15)} = 118.8$ ,  $p < 0.001$ ; Fig. 8B), IL-1 $\beta$  ( $F_{(3-19)} = 10.05$ ,  $p < 0.001$ ; Fig. 8C) and  
24 COX-2 ( $F_{(3-19)} = 7.77$ ,  $p < 0.01$ ; Fig. 8D).

25

### 1 3.7. General toxicity and biochemical parameters

2

3 The repeated administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> triggered neither clinical  
4 abnormality signs nor body weight gain alterations to mice over the experimental  
5 protocol. However, the animals treated with DCF had a reduction in the body weight  
6 gain (Supplementary material; Fig. 2S).

7

8 The results of tissue oxidative stress markers (Table 2S) and plasma  
9 biochemical parameters (Table 3S) are depicted in the supplementary material. The  
10 repeated treatment caused no alterations in the δ-ALA-D activity as well as in the  
11 protein carbonyl and NPSH levels in the liver and kidney samples in comparison to  
12 the control group ( $p > 0.05$ ). Besides, urea levels, ALT, AST and CK activities did not  
13 statistically differ among the experimental groups ( $p > 0.05$ ).

13

## 14 4. Discussion

15

16 The present study evaluated the physicochemical stability and the anti-  
17 inflammatory effect of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> on the behavioral, biochemical and molecular  
18 impairments induced by CFA injection in mice. The results showed that the  
19 compound has high chemical stability independent of the storage condition tested.  
20 Acute and repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> effectively reduced the  
21 mechanical and thermal hypernociception behavior as well as the paw inflammatory  
22 injuries in both animal models of inflammation. The repeated treatment with (*m*-CF<sub>3</sub>-  
23 PhSe)<sub>2</sub> 1 mg/kg (i.g.) mitigated the increase in inflammatory protein levels at the  
24 central nervous system induced by CFA. Moreover, the repeated treatment schedule

1 with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> caused nor general toxicity neither alterations in locomotor and  
2 exploratory behaviors and in the biochemical parameters.

3         The physicochemical stability of a biologically active compound it is an  
4 important feature in the field of novel drugs discovery [25]. Predicting such  
5 characteristic provides advantages in view of optimizing experimental conditions to  
6 study the compound in distinct assays (*in vitro* and *in vivo*) [26]. It is interesting to  
7 highlight that the majority of scientific studies about the *in vivo* pharmacological and  
8 toxicological effects of organoselenium compounds are carried out using inert oils  
9 (canola oil, soybean oil or mineral oil) as the solvent to solubilize these molecules [7].  
10 In this sense, the current study assessed the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> physicochemical  
11 stability by evaluating the influence of different conditions of storage in the compound  
12 content. Our results showed that independent of the storage condition tested  
13 (freezer, refrigeration or room temperature) no alterations were observed in the  
14 compound content in the canola oil solution over the 60 days of experiment. These  
15 data are in accordance to previous studies of our research group in which the  
16 physicochemical stability of *p*-methoxyl-diphenyl diselenide [(OMePhSe)<sub>2</sub>], a (*m*-CF<sub>3</sub>-  
17 PhSe)<sub>2</sub> structural analogue, were also assessed using similar methodology [13].  
18 Thus, our data suggest that (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> has high physicochemical stability in the  
19 experimental conditions tested.

20         The class of organoselenium molecules comprises several examples with  
21 potential pharmacological properties [7]. Of particular importance, preclinical studies  
22 demonstrated that these compounds have potential antioxidant, antinociceptive, and  
23 anti-inflammatory actions [10, 27-29]. The (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> is a diaryl diselenide class  
24 representative that has multiple pharmacological actions, including the  
25 antinociceptive effect in distinct animal models of acute [9, 10] and chronic pain [11].

1 It was already demonstrated that an interplay between the serotonergic system and  
2 endogenous opioid system possibly mediates the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> antinociceptive  
3 property [9, 10]. In addition, a recent study of our research group demonstrated that  
4 the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> repeated administration to mice did not cause tolerance and  
5 physical signals of withdrawal [8]. Thus, the promising pharmacological profile of this  
6 selenium-containing molecule supports further studies to better elucidate its possible  
7 application as a therapeutic agent in the management of clinical pain and/or  
8 inflammatory-related diseases.

9 The CFA induced inflammatory pain is a widely used protocol to study  
10 mechanisms associated with inflammation as well as possible acute and chronic  
11 effects of anti-inflammatory drugs [30, 31]. In the current study, the CFA injection in  
12 mice triggered hypernociception behaviors in both protocols (acute and subchronic)  
13 in addition to the local inflammatory process and modification in the content of  
14 inflammatory proteins at central nervous system. Administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> to  
15 mice effectively restored the altered nociceptive behaviors. Regarding these results,  
16 the scientific literature reported that others diaryl diselenide compounds, such as  
17 diphenyl diselenide [(PhSe)<sub>2</sub>] and (OMePhSe)<sub>2</sub>, also are effective in reducing the  
18 mechanical hypernociception induced by CFA injection but in distinct time- and dose-  
19 response profiles [15, 27].

20 The (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (10 mg/kg, i.g.) elicited a rapid onset of action (0.5 h later  
21 the administration), which is maintained up to 5 h after administration, while both  
22 (PhSe)<sub>2</sub> (10 mg/kg, i.g.) and (OMePhSe)<sub>2</sub> (25 mg/kg, i.g.) had inferior duration of anti-  
23 hypernociceptive action [17, 27]. Interestingly, the pharmacologically active dose of  
24 (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> that elicits antinociception in acute chemical/thermal-induced animal  
25 models of pain is lower (1 mg/kg, i.g.) than the others diaryl diselenides [10].

1 Besides, the results of current study demonstrated that the administration schedule  
2 of repeated dosage (1 mg/kg, i.g.) reverted the mechanical and thermal  
3 hypernociception induced by the model of subchronic inflammation. Reinforcing  
4 these data, previous reports also showed the efficacy of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> administered  
5 in similar protocols (0.1 and 0.01 mg/kg daily, during 14 days), such as the mouse  
6 comorbid pain and depression model [11], while others diaryl diselenides, requires  
7 superior doses to elicit pharmacological action in the same animal model [32]. Then,  
8 our results further support an important structure-activity relationship regarding the  
9 selenium-containing molecules in which the chemical modifications performed on  
10 diaryl diselenides could strongly modify the pharmacological potency of compounds  
11 [7].

12 The CFA injection triggers the release of several mediators causing  
13 biochemical, morphological and molecular inflammatory events in the paw [31] as  
14 well as sensitization at specific central nervous systems areas. In the current study,  
15 in both acute and subchronic protocols the CFA caused an increase in the paw  
16 thickness and MPO activity. At the site of injection, CFA promotes cellular events,  
17 such as infiltration and proliferation of polymorphonuclear leukocytes, and vascular  
18 events, generating the paw edema [1]. Our results demonstrated that the acute and  
19 repeated administration of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> attenuated the paw edema formation and  
20 decreased the MPO activity, a marker of polymorphonuclear infiltration whose activity  
21 is directly related to the amount of leukocytes [33]. Thus, it is possible to hypothesize  
22 that (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> elicited an anti-inflammatory action through a leukocytes  
23 infiltration inhibition, demonstrated by the suppression of MPO activity, which in turns  
24 reduced the paw edema formation. In addition, corroborating with these data, the  
25 restorative effect of others organoselenium compounds against paw inflammatory

1 injuries induced by CFA [27, 28, 33] or other irritant agents (carrageenan, aradonic  
2 acid, croton oil, formalin) [29, 34-36 ] was already demonstrated by previous studies,  
3 reinforcing our results and the potential pharmacological properties of selenium-  
4 containing molecules [7, 37].

5 The peripheral inflammation induced by the injection of CFA triggered a  
6 modification in the levels of inflammatory proteins at the central nervous system [38].  
7 Accordingly, the results of subchronic protocol of inflammation demonstrated that the  
8 levels of TNF- $\alpha$ , IL-1 $\beta$  and COX-2 were higher in the CFA + vehicle group than those  
9 of control animals. The repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> downregulated the  
10 increased content of inflammatory proteins at the cerebral contralateral cortex,  
11 suggesting that the compound could act by modulating both peripheral and central  
12 targets to its anti-inflammatory action. Indeed, previous studies demonstrated the (*m*-  
13 CF<sub>3</sub>-PhSe)<sub>2</sub> effectiveness in restoring the neuroinflammatory injuries induced by a  
14 model of neuropathic pain and by TNF- $\alpha$  injection [11, 39], reinforcing that the  
15 modulation of cellular pathways related to the inflammatory proteins could contribute  
16 at least in parts to the compound anti-inflammatory action.

17 Besides the pharmacological efficacy of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>, it is important the  
18 evaluate the possible occurrence of toxicological effects as an indication of safety in  
19 the use of the compound. Our results revealed that (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> repeated  
20 administration did not change the locomotor and exploratory activity, the plasma  
21 parameters of hepatic and renal function as well as no imbalance of oxidative status  
22 in the liver and kidney of mice were triggered. These data are in accordance with  
23 previous studies in which no histological and functional injuries were observed in  
24 liver, kidney and brain tissues after a repeated administration schedule of (*m*-CF<sub>3</sub>-  
25 PhSe)<sub>2</sub> to mice [8]. Interestingly, while no toxic effects were observed in plasma

1 biochemical markers and oxidative parameters, the repeated treatment with DCF  
2 caused a reduction in body weight gain of mice, which was not observed to (*m*-CF<sub>3</sub>-  
3 PhSe)<sub>2</sub>. Thus, the results suggest that under the experimental conditions used in the  
4 current study the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> elicited pharmacological action without triggering  
5 any toxicological impairment, discarding some possible pitfalls or artifacts.

6 In conclusion, the results demonstrated that acute or repeated treatment with  
7 (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> elicited an anti-inflammatory action in the inflammatory pain model  
8 induced by CFA injection. Our data showed the compound effectiveness in reducing  
9 the mechanical and thermal hypernocicepcion as well as by modulating the paw  
10 edema formation and MPO activity. Besides, the (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> administration  
11 downregulated the content of inflammatory proteins in the cerebral cortex  
12 contralateral, suggesting that its global anti-inflammatory action could be associated  
13 to an interplay between peripheral and central mechanisms. Nevertheless, further  
14 studies are needed to understand the molecular mechanisms whereby this  
15 compound leads to modulation of inflammatory process.

16

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18

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22



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1 **Figure captions**

2

3 **Fig. 1** Evaluation of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> content in the oily solution stored at 25 °C,  
4 4 °C and – 20 °C over a 60-day period of stability evaluation.

5

6 **Fig. 2** Schematic view of the experimental acute inflammatory pain model. **(A)**  
7 Time- and dose-response curves of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> anti-hypernociceptive effect  
8 using the von Frey hair (VFH) test. **(B)** Another set of animals were treated in  
9 order to evaluate the effect anti-inflammatory of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub>. **(C)** Schematic  
10 view of the experimental subchronic inflammatory pain model. Abbreviation: C –  
11 confirmatory of mechanical hypernociceptive induction with CFA in the VFH  
12 test.

13

14 **Fig. 3** Time- and dose-response curves of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> treatment effect in  
15 the mechanical hypernociception induced by an inflammatory pain model **(A/B)**.  
16 The mean of each animal was determined 24h earlier and after CFA-injection  
17 (Basal and C) and then the animals received the treatment accordingly to the  
18 groups and the mechanical hypernociception response was measured at  
19 determined times after the administration (0.5; 1; 2; 3, 4, 5, and 6 h). The  
20 results represent the means ± S.E.M. of 10 animals/group. One-way ANOVA of  
21 repeated or ordinary measures followed by Newman Keuls' test was performed  
22 to time/dose-response curve and the area under curve (AUC), respectively. The  
23 (\*) denotes significant difference in comparison to the control group, \*\*\**p*<0.001;  
24 the (#) denotes significant difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> 10 mg/kg group in  
25 comparison to the CFA group, #*p*<0.05, ###*p*<0.01, ####*p*<0.001; the (\$) denotes

1 significant difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> 1 mg/kg group in comparison to the CFA  
2 group, \$\$\$*p*<0.001; and (&) denotes significant difference of DCF group in  
3 comparison to the CFA group, &&*p*<0.01 and &&&*p*<0.001.

4

5 **Fig. 4** Effect of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> treatment in the paw edema **(A)** and MPO  
6 activity **(B)** induced by CFA injection to mice. Data are reported as mean ±  
7 S.E.M. of 8 animals/group. One-way ANOVA of ordinary measures followed by  
8 Newman Keuls' test was performed. The (\*) denotes significant difference in  
9 comparison to the control group, \*\*\**p*<0.001; the (#) denotes significant  
10 difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> 10 mg/kg group in comparison to the CFA group,  
11 #*p*<0.05, ##*p*<0.01, ###*p*<0.001; and (&) denotes significant difference of DCF  
12 group in comparison to the CFA group, &&*p*<0.01 and &*p*<0.05.

13

14 **Fig. 5** Effect of repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg/kg) in the **(A)**  
15 mechanical hypernociception induced by a subchronic inflammatory pain model  
16 and **(B)** area under curve (AUC - 14<sup>th</sup> to 24<sup>th</sup> day). The results represent the  
17 means ± S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way  
18 ANOVA of repeated or ordinary measures followed by Newman Keuls' multiple  
19 comparison test when appropriate. The (\*) denotes significant difference in  
20 comparison to the control group, \*\*\**p*<0.001; the (#) denotes significant  
21 difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> group in comparison to the CFA group, ###*p*<0.001;  
22 and (&) denotes significant difference of DCF group in comparison to the CFA  
23 group, &&&*p*<0.001 and &&*p*<0.01.

24

1 **Fig. 6** Effect of repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg/kg) in the **(A)**  
2 thermal hypernociception induced by a subchronic inflammatory pain model and  
3 **(B)** area under curve (AUC - 14<sup>th</sup> to 24<sup>th</sup> day). The results represent the means  
4 ± S.E.M. of 8 animals/group. The results were analyzed by two-way ANOVA of  
5 repeated or ordinary measures followed by Newman Keuls' multiple comparison  
6 test when appropriate. The (\*) denotes significant difference in comparison to  
7 the control group, \*\*\**p*<0.001, \*\**p*<0.01 and \**p*<0.05; the (#) denotes significant  
8 difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> group in comparison to the CFA group, ####*p*<0.001,  
9 ##*p*<0.01 and #*p*<0.05; and (&) denotes significant difference of DCF group in  
10 comparison to the CFA group, &&*p*<0.01 and &*p*<0.05.

11

12 **Fig. 7** Effect of repeated treatment with (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> (1 mg/kg) in the **(A)** paw  
13 edema and **(B)** MPO activity induced by a subchronic inflammatory pain model.  
14 The results represent the means ± S.E.M. of 7 - 9 animals/group. The results  
15 were analyzed by two-way ANOVA of ordinary measures followed by Newman  
16 Keuls' multiple comparison test when appropriate. The (\*) denotes significant  
17 difference in comparison to the control group, \*\*\**p*<0.001 and \**p*<0.05; the (#)  
18 denotes significant difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> group in comparison to the CFA  
19 group, ####*p*<0.001 and ##*p*<0.01; and (&) denotes significant difference of DCF  
20 group in comparison to the CFA group, &&&*p*<0.001 and &*p*<0.05.

21

22 **Fig. 8** The (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> repeated treatment effect on the inflammatory protein  
23 levels TNF-α **(B)**, IL-1β **(C)** and COX-2 **(D)** in the cerebral contralateral cortex  
24 samples of mice subjected to subchronic inflammatory pain model. The  
25 representative blot bands are depicted in **(A)**. The results represent by means ±

1 S.E.M. of 4-5 animals/group. The results were analyzed by One-way ANOVA of  
2 ordinary measures followed by Newman Keuls' test. The (\*) denotes significant  
3 difference in comparison to the control group, \*\*\* $p < 0.001$  and \* $p < 0.05$ ; the (#)  
4 denotes significant difference of (*m*-CF<sub>3</sub>-PhSe)<sub>2</sub> group in comparison to the CFA  
5 group, ### $p < 0.001$  and # $p < 0.05$ ; and (&) denotes significant difference of DCF  
6 group in comparison to the CFA group, &&& $p < 0.001$  and && $p < 0.01$ .

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## FIGURES

Fig. 1

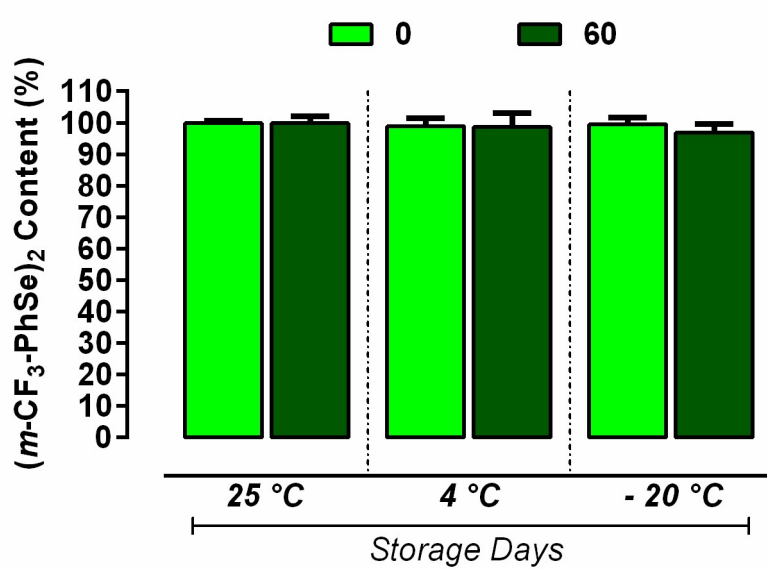




Fig. 2

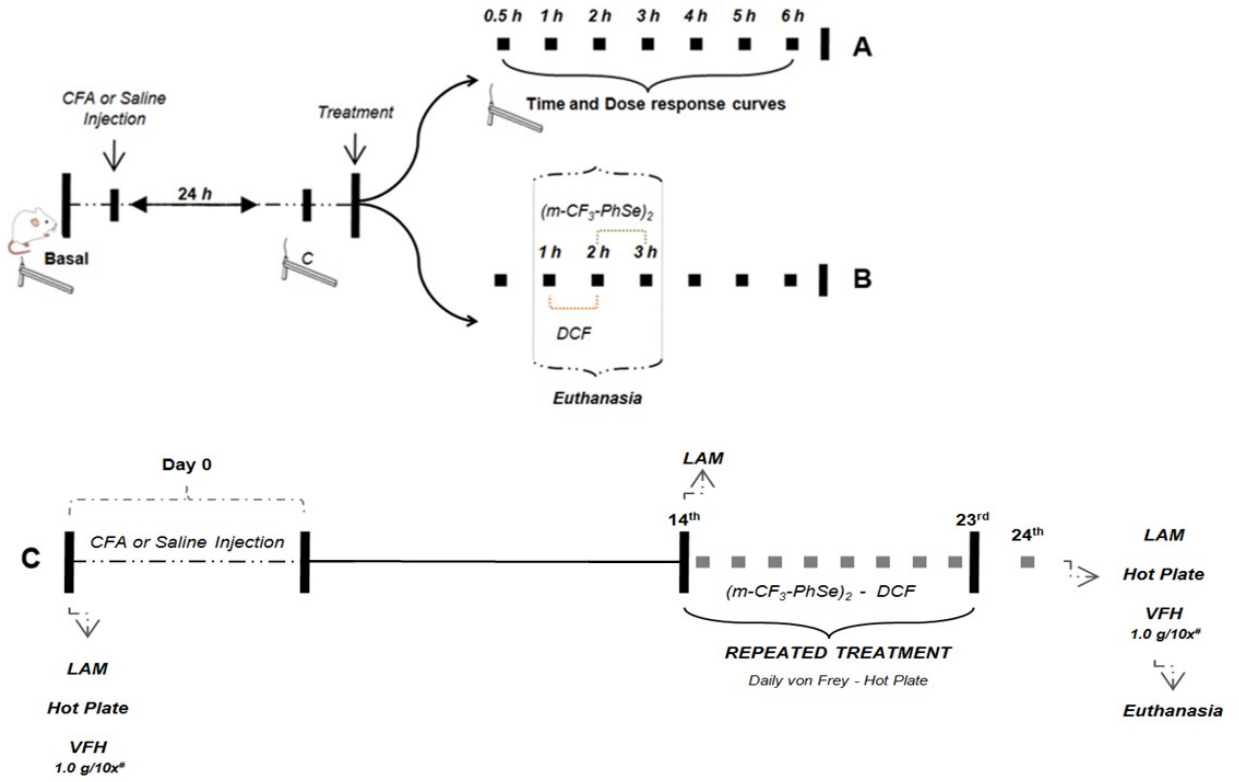


Fig. 3

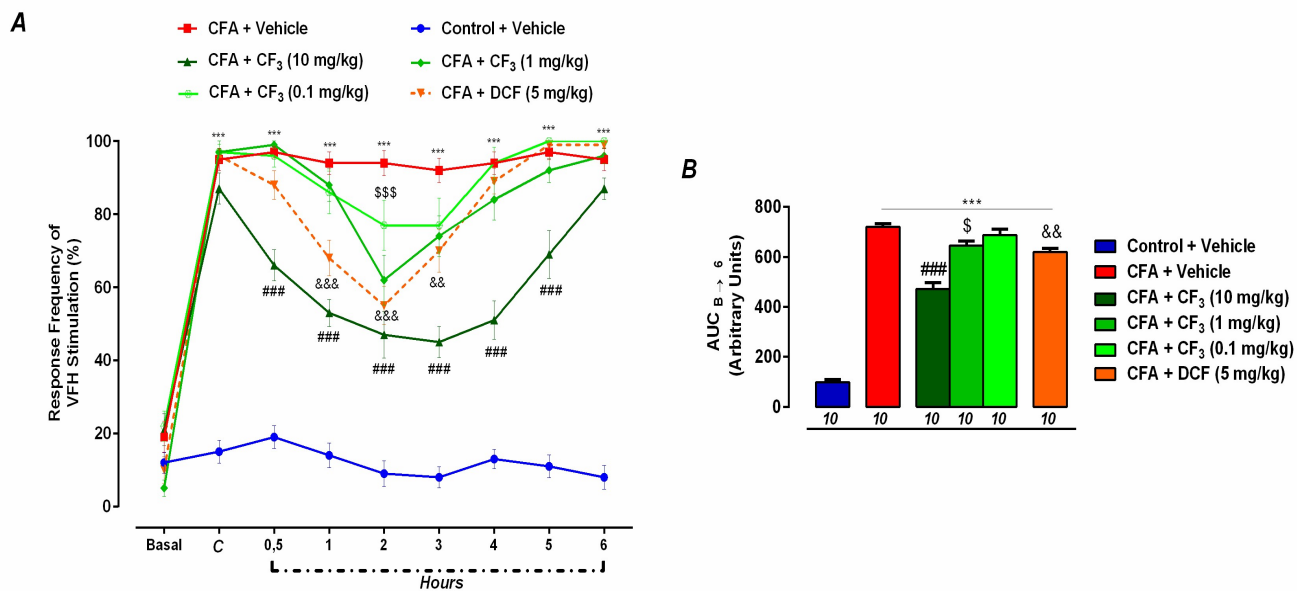


Fig. 4

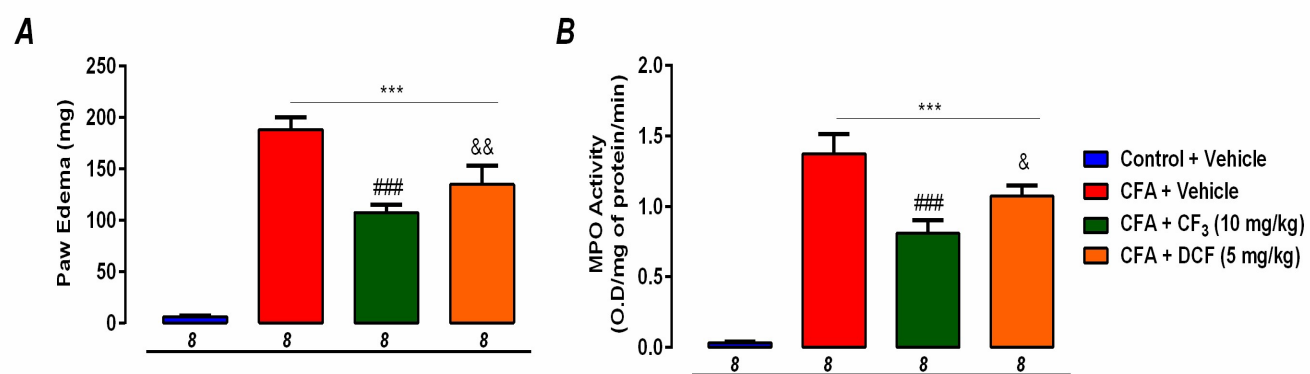


Fig. 5

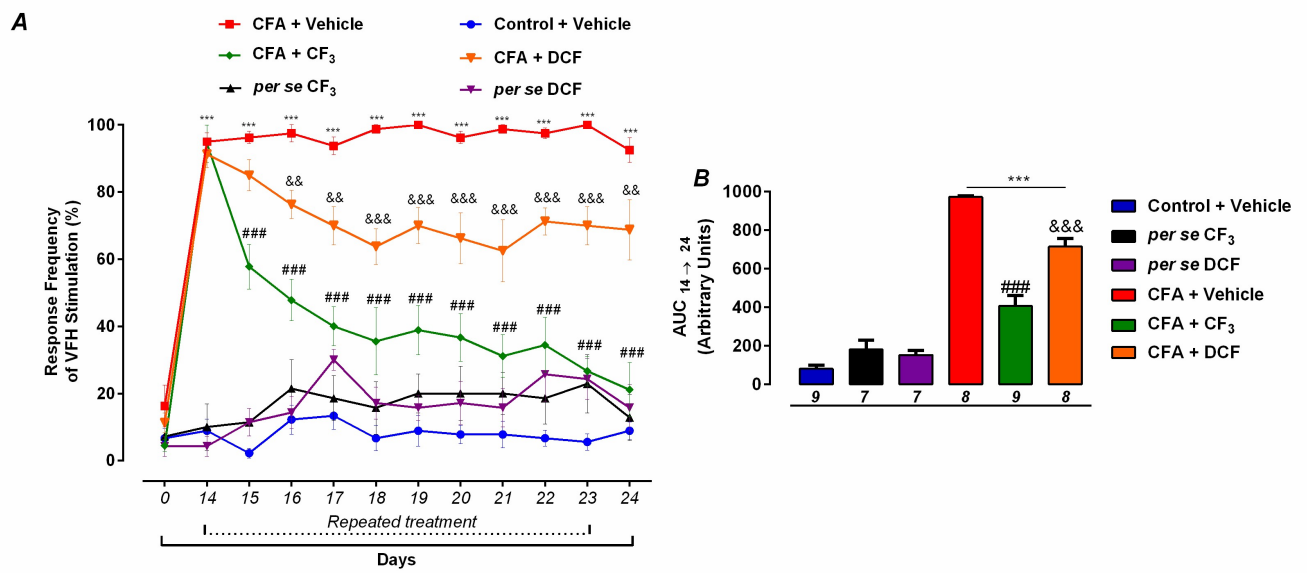


Fig. 6

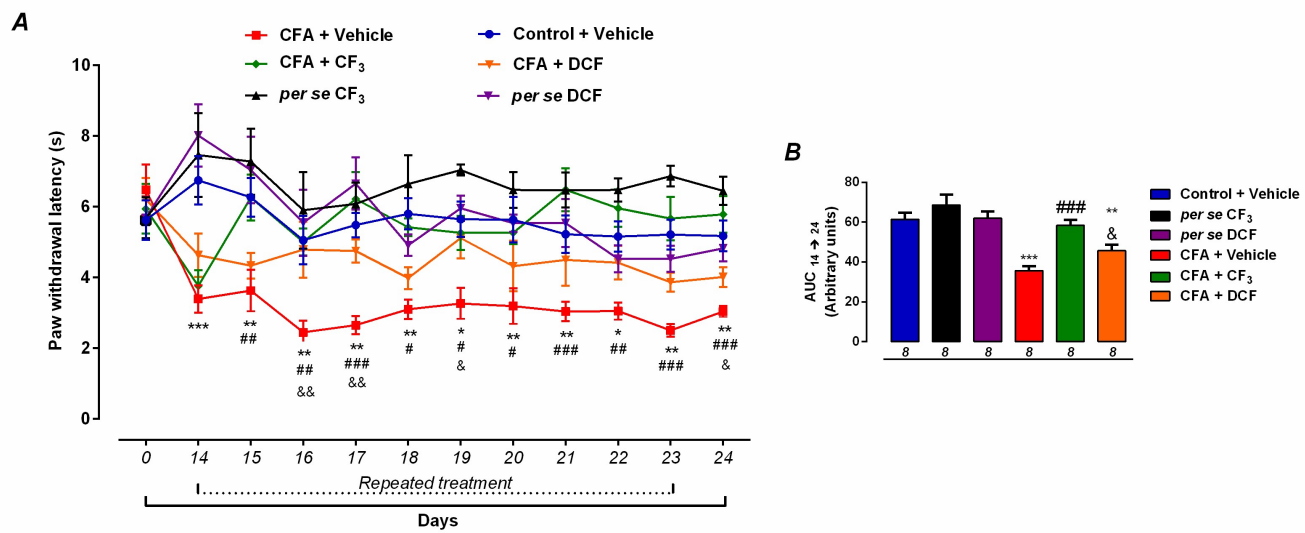


Fig. 7

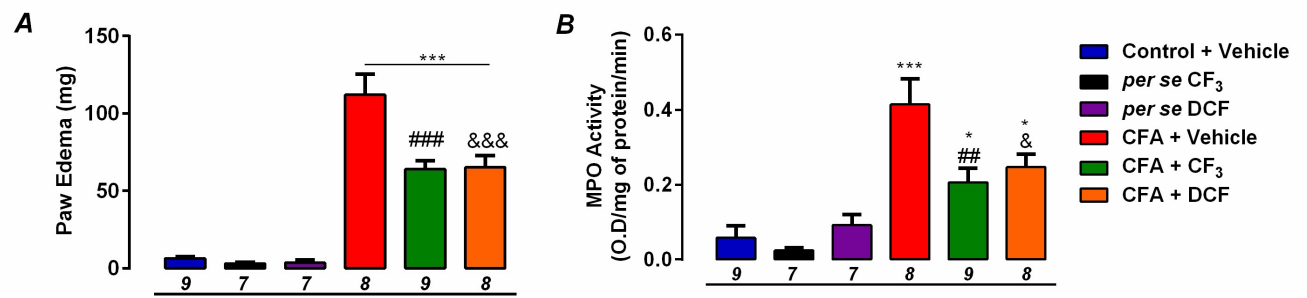
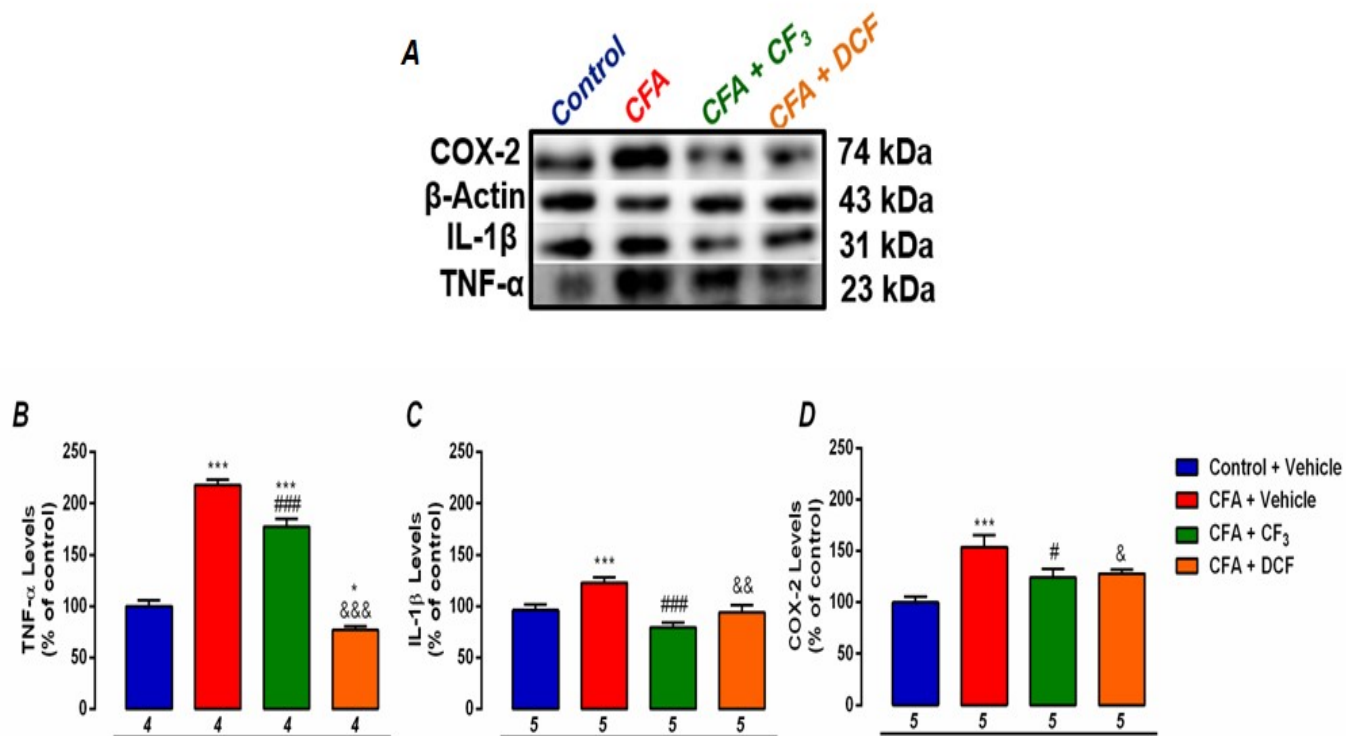


Fig. 8



**SUPPLEMENTARY MATERIAL*****Anti-inflammatory action of m-trifluoromethyl-diphenyl diselenide on acute and subchronic animal models of inflammatory pain: behavioral, biochemical and molecular insights***

Paulo Cesar Oliveira Araujo<sup>a</sup>, Marcel Henrique Marcondes Sari<sup>b</sup>, Natália Silva Jardim<sup>a</sup>, Juliano Ten Kathen Jung<sup>a</sup> and César Augusto Brüning<sup>c,\*</sup>

<sup>a</sup>Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

<sup>b</sup>Laboratório de Tecnologia Farmacêutica, Departamento de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

<sup>c</sup>Laboratório de Bioquímica e Neurofarmacologia Molecular, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, CEP 96010-900, RS, Brazil

\*Correspondence should be sent to:

César Augusto Brüning

Laboratório de Bioquímica e Neurofarmacologia Molecular, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, CEP 96010-900, RS, Brazil

Phone: + 55 53 3275-7355

E-mail: cabruning@yahoo.com.br



## FIGURES

Figure S1

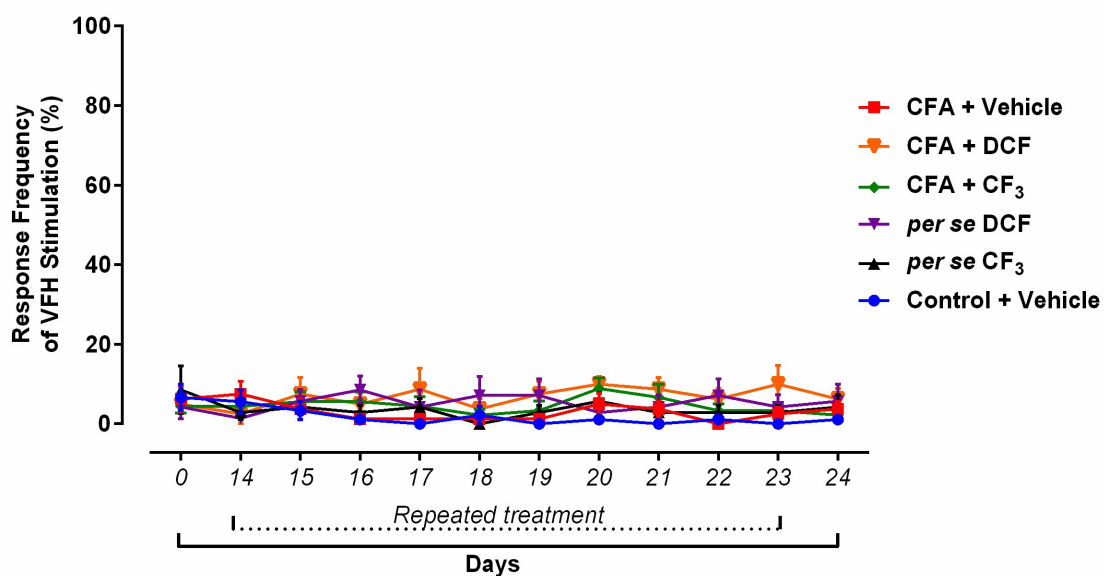


Figure S1. Effect of repeated treatment with  $(m\text{-CF}_3\text{-PhSe})_2$  (1 mg/kg) in the mechanical hypernociception induced by a subchronic inflammatory pain model (Paw contralateral). The results represent the means  $\pm$  S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way ANOVA of repeated measures followed by Newman Keuls' multiple comparison test when appropriate.

Figure S2

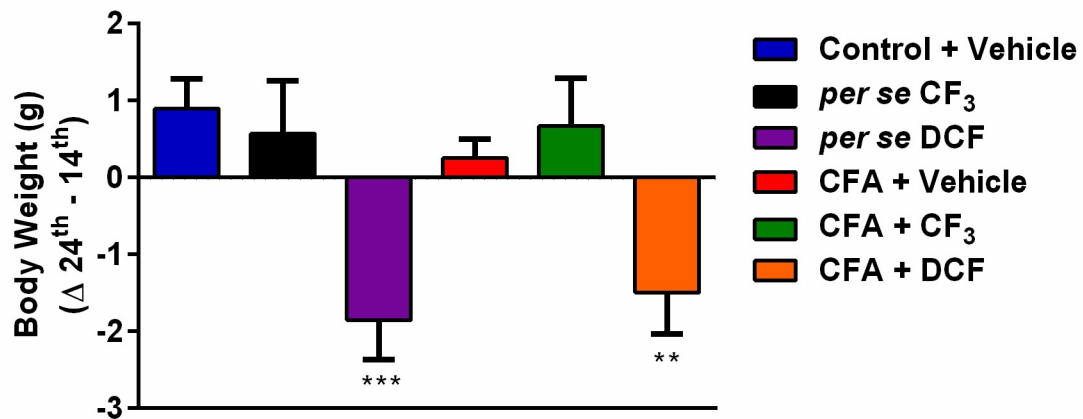


Figure S2. Effect of repeated treatment in the body weight (difference to 14<sup>th</sup> at 24<sup>th</sup>). The results represent the means  $\pm$  S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way ANOVA of ordinary measures followed by Newman Keuls' multiple comparison test when appropriate. The (\*) denotes significant difference in comparison to the control group, \*\*\* $p < 0.001$  and \*\* $p < 0.01$ .

## TABLES

**Table 1.** Effect of repeated treatment on the mouse spontaneous locomotor activity parameters.

<i>Day</i>	<i>Group</i>	<i>Spontaneous locomotor activity</i>			
		<i>Rearings</i>	<i>Crossings</i>	<i>Distance</i>	<i>Average Speed</i>
<b>0</b>	<i>Control + Vehicle</i>	19.00 ± 1.944	616.1 ± 52.34	11220 ± 908.6	47.49 ± 3.662
	<i>per se CF<sub>3</sub></i>	22.57 ± 3.571	737.9 ± 92.24	11843 ± 1329.0	49.70 ± 5.580
	<i>per se DCF</i>	19.57 ± 2.349	644.3 ± 60.62	10822 ± 1164.0	47.20 ± 5.442
	<i>CFA + Vehicle</i>	20.13 ± 2.125	666.6 ± 56.93	11941 ± 830.3	50.07 ± 3.520
	<i>CFA + CF<sub>3</sub></i>	21.11 ± 1.752	680.4 ± 28.48	10957 ± 571.5	46.76 ± 2.404
	<i>CFA + DCF</i>	18.75 ± 1.729	669.0 ± 42.81	11753 ± 843.2	49.36 ± 3.456
<b>14<sup>th</sup></b>	<i>Control + Vehicle</i>	23.89 ± 1.829	602.0 ± 67.37	10051 ± 1162.0	43.80 ± 4.134
	<i>per se CF<sub>3</sub></i>	28.14 ± 2.931	700.6 ± 88.34	10350 ± 1302.0	44.78 ± 5.013
	<i>per se DCF</i>	27.71 ± 3.733	743.4 ± 66.17	11303 ± 926.4	47.95 ± 3.839
	<i>CFA + Vehicle</i>	26.75 ± 2.111	652.1 ± 39.57	10962 ± 499.8	48.13 ± 2.135
	<i>CFA + CF<sub>3</sub></i>	20.00 ± 1.772	552.6 ± 46.70	8284 ± 751.1	35.29 ± 3.211
	<i>CFA + DCF</i>	17.63 ± 2.556	710.1 ± 143.6	10448 ± 1289.0	45.49 ± 5.298
<b>24<sup>th</sup></b>	<i>Control + Vehicle</i>	23.78 ± 3.398	607.7 ± 83.61	10126 ± 1277.0	43.03 ± 5.443
	<i>per se CF<sub>3</sub></i>	31.57 ± 5.145	761.1 ± 96.70	11537 ± 1406.0	48.90 ± 5.881
	<i>per se DCF</i>	22.29 ± 3.483	542.00 ± 80.74	8287 ± 1094.0	35.60 ± 4.816
	<i>CFA + Vehicle</i>	24.50 ± 3.047	678.8 ± 100.9	10772 ± 1233.0	45.64 ± 5.063
	<i>CFA + CF<sub>3</sub></i>	18.11 ± 1.338	503.8 ± 32.94	8058 ± 742.8	34.15 ± 3.171
	<i>CFA + DCF</i>	18.50 ± 3.370	545.5 ± 79.89	8527 ± 1015.0	40.81 ± 3.283

The results represent the means ± S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way ANOVA of repeated measures followed by Newman Keuls' multiple comparison test when appropriate.

**Table 2.** Effect of repeated treatment on the biochemical parameters of tissue oxidative status.

<b>Tissue</b>	<b>Group</b>	<b>Biochemical parameters</b>		
		<b>Protein Carbonyl</b>	<b><math>\delta</math>-ALA-D</b>	<b>NPSH</b>
<b>Liver</b>	<i>Control + Vehicle</i>	2.091 $\pm$ 0.213	6.761 $\pm$ 0.447	0.7961 $\pm$ 0.048
	<i>per se CF<sub>3</sub></i>	1.570 $\pm$ 0.144	6.492 $\pm$ 0.506	0.9075 $\pm$ 0.063
	<i>per se DCF</i>	2.018 $\pm$ 0.259	6.267 $\pm$ 0.547	0.7492 $\pm$ 0.033
	<i>CFA + Vehicle</i>	1.900 $\pm$ 0.234	6.428 $\pm$ 0.673	0.7938 $\pm$ 0.069
	<i>CFA + CF<sub>3</sub></i>	2.147 $\pm$ 0.197	7.044 $\pm$ 0.509	0.8234 $\pm$ 0.069
	<i>CFA + DCF</i>	2.089 $\pm$ 0.265	6.796 $\pm$ 0.633	0.9742 $\pm$ 0.155
<b>Kidney</b>	<i>Control + Vehicle</i>	1.535 $\pm$ 0.144	2.886 $\pm$ 0.168	0.6921 $\pm$ 0.070
	<i>per se CF<sub>3</sub></i>	1.535 $\pm$ 0.182	3.310 $\pm$ 0.169	1.713 $\pm$ 0.260***
	<i>per se DCF</i>	1.180 $\pm$ 0.163	3.268 $\pm$ 0.205	1.586 $\pm$ 0.269***
	<i>CFA + Vehicle</i>	1.484 $\pm$ 0.217	3.421 $\pm$ 0.216	0.6392 $\pm$ 0.124
	<i>CFA + CF<sub>3</sub></i>	1.501 $\pm$ 0.239	3.049 $\pm$ 0.366	1.022 $\pm$ 0.140
	<i>CFA + DCF</i>	1.308 $\pm$ 0.219	2.721 $\pm$ 0.295	0.9326 $\pm$ 0.132

The results represent the means  $\pm$  S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way ANOVA of ordinary measures followed by Newman Keuls' multiple comparison test when appropriate. The (\*) denotes significant difference in comparison to the control group, \*\*\* $p < 0.001$ .

**Table 3.** Effect of repeated treatment on plasma biochemical parameters.

<b>Group</b>	<i>Plasma biochemical parameters</i>			
	<b>ALT (U/dL)</b>	<b>AST (U/dL)</b>	<b>CK (U/dL)</b>	<b>UREA (mg/dL)</b>
<i>Control + Vehicle</i>	52.78 ± 7.58	88.56 ± 10.58	243.5 ± 17.76	26.72 ± 2.50
<i>per se CF<sub>3</sub></i>	58.14 ± 8.92	71.00 ± 5.19	181.1 ± 20.44	29.48 ± 4.40
<i>per se DCF</i>	47.43 ± 6.56	99.57 ± 13.61	258.5 ± 37.01	25.88 ± 1.38
<i>CFA + Vehicle</i>	55.50 ± 4.71	101.5 ± 11.44	262.8 ± 38.41	35.53 ± 4.04
<i>CFA + CF<sub>3</sub></i>	63.89 ± 5.66	75.89 ± 5.84	219.9 ± 29.48	28.26 ± 1.65
<i>CFA + DCF</i>	60.13 ± 6.29	76.88 ± 8.47	199.1 ± 26.60	26.18 ± 2.33

The results represent the means ± S.E.M. of 7 - 9 animals/group. The results were analyzed by two-way ANOVA of ordinary measures followed by Newman Keuls' multiple comparison test when appropriate.

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## 5 ANEXO

### ANEXO A: Parecer de aprovação do projeto de pesquisa pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (UFSM):



Comissão de Ética no Uso de Animais

da  
Universidade Federal de Santa Maria

#### CERTIFICADO

Certificamos que a proposta intitulada "Efeitos do disseleneto de m-trifluorometil difenila (m-CF<sub>3</sub>-PhSe)<sub>2</sub> na dâde dor-depressão e nocicepção inflamatória em modelos animais.", protocolada sob o CEUA nº 8081170317, sob a responsabilidade de **Cristina W. Nogueira** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 07/06/2017.

We certify that the proposal "Effects of m-trifluoromethyl diphenyl (m-CF<sub>3</sub>-PhSe)<sub>2</sub> diselenide on pain-depression and inflammatory nociception in animal models.", utilizing 210 Heterogenics mice (210 males), protocol number CEUA 8081170317, under the responsibility of **Cristina W. Nogueira** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 06/07/2017.

Finalidade da Proposta: **Pesquisa (Acadêmica)**

Vigência da Proposta: de **05/2017** a **05/2018** Área: **Bioquímica E Biologia Molecular**

Origem: **Biotério Central UFSM**

Espécie: **Camundongos heterogênicos**

sexo: **Machos**

idade: **30 a 60 dias**

N: **210**

Linhagem: **Swiss**

Peso: **25 a 35 g**

Resumo: Estudos epidemiológicos demonstram que a dor e a depressão coexistem em mais de 70% dos casos de dor crônica (Bair et al., 2003; Arnow et al., 2006). Esta relação leva-se a teoria que a depressão pode aumentar a percepção da dor e que a depressão é uma consequência comum dos sintomas de dor (Landi et al., 2005). Essa interação tem sido classificada como diade dor-depressão (Bair et al., 2004; Goldenberg, 2010). Embora a relação entre dor e depressão venha atraindo o interesse científico, o mecanismo associado a essa diade não é completamente elucidado. A interação de diversos fatores como neurotransmissores, neuropeptídeos, estresse oxidativo, nitrosativo e inflamação têm sido associada à comorbidade entre dor e depressão (Kramer et al., 1998; Elhwuegi, 2004; Maes et al., 2010; Walker et al., 2014). O papel do estresse oxidativo e nitrosativo na indução da inflamação neurogênica também encontra-se relacionado à diade dor-depressão, como por exemplo, na estimulação da produção de fator nuclear  $\kappa$ -B (NF- $\kappa$ B) que leva ao aumento dos níveis de citocinas pró-inflamatórias como o fator de necrose tumoral- $\alpha$  (TNF- $\alpha$ ), a interleucina 1 $\alpha$  (IL-1 $\alpha$ ), a interleucina 6 (IL-6) e o interferon- $\gamma$  (INF- $\gamma$ ) (Pall, 2007; Dowlati et al., 2010). Estudos tanto em pacientes com depressão maior quanto com dor crônica têm demonstrado elevados níveis de espécies reativas de oxigênio (ERO) e nitrogênio (ERN), bem como produtos de peroxidação lipídica quando comparados a indivíduos saudáveis (Bilici et al., 2001; Bagis et al., 2005; Sarandol et al., 2007). Deste modo, níveis aumentados de espécies reativas de oxigênio e nitrogênio e citocinas pró-inflamatórias também podem ativar a via apoptótica, ativação da micróglia, assim como a ativação da enzima indoleamina 2,3-dioxigenase 1 (IDO-1), responsável pela degradação do triptofano, precursor da serotonina, culminando em um déficit deste neurotransmissor e acúmulos de metabólitos que contribuem para a neurotoxicidade (Joseph and Levine, 2004; Walker et al., 2014; Stone and Perkins, 1981). A inflamação é caracterizada pelos sinais clássicos como calor, rubor, dor, inchaço e consequente perda da função do órgão/local afetado (Lawrence et al., 2002). O processo inflamatório, geralmente é caracterizado como uma resposta de cura a uma injúria local, entretanto este pode evoluir para estados crônicos caso o insulto inicial persista, elevando o risco de uma progressão para uma resposta sistêmica. Em resposta a um insulto, uma ampla rede de sinais químicos, compreendendo a liberação de moléculas derivadas de aminoácidos, peptídeos, proteínas, moléculas gasosas, lipídios, dentre outras substâncias, as quais iniciam, mantêm e terminam a resposta do hospedeiro a um dano tecidual (Medzhitov, 2008). Tanto a dor aguda quanto a dor crônica estão frequentemente associadas a processos inflamatórios, como resultado da lesão tecidual, reatividade imunológica anormal ou lesão nervosa (Stein et al., 2003). Modelos animais que mimetizam a interação entre nocicepção e comportamento do tipo depressivo têm sido empregados para avaliar os mecanismos associados a essa comorbidade e como screening de novas terapias (Nagakura et al., 2009; Arora et al., 2011). Deste modo, a disfunção monoaminérgica e a fibromialgia induzidas por reserpina são consideradas um modelo bioquímico adequado, uma vez que a reserpina bloqueia o transporte vesicular de monoaminas para o armazenamento ou a neurotransmissão, causando auto-oxidação de dopamina e o catabolismo oxidativo pela monoamino oxidase (MAO) podendo resultar em estresse oxidativo e nitrosativo (Lohr et al., 2003; Nagakura et al., 2012). O disseleneto de m-trifluorometil difenila (m-CF<sub>3</sub>-PhSe)<sub>2</sub> é um composto orgânico de selênio que apresenta diversas propriedades



*Comissão de Ética no Uso de Animais*

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farmacológicas que o candidatam à uma possível terapia para a díade dor-depressão. Este composto apresenta tanto efeito antinociceptivo (Brüning et al., 2010; 2014) como do tipo antidepressivo (Brüning et al., 2011), relacionado à interação com receptores serotoninérgicos e opióides. Paralelamente, o (m $\square$ CF3 $\square$ PhSe)<sub>2</sub> apresenta efeito antioxidante (Prigol et al., 2009) e análogos estruturais deste composto apresentam efeito anti-inflamatório (Luchese et al., 2012; Pinton et al., 2013). Diante do exposto, neste projeto pretende-se avaliar os efeitos protetores do (m $\square$ CF3 $\square$ PhSe)<sub>2</sub> em dois modelos experimentais distintos, a saber, de comorbidade dor-depressão e de nocicepção inflamatória em camundongos.

Local do experimento: Laboratório - 2424

Santa Maria, 08 de junho de 2017

Profa. Dra. Daniela Bitencourt Rosa Leal  
Coordenadora da Comissão de Ética no Uso de Animais  
Universidade Federal de Santa Maria

Prof. Dr. Denis Broock Rosemberg  
Vice-Coordenador da Comissão de Ética no Uso de Animais  
Universidade Federal de Santa Maria

