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AVALIAÇÃO DOS EFEITOS FARMACOLÓGICO E TOXICOLÓGICO DE 4-ORGANOCALCOGENO-ISOQUINOLINAS

DISSERTAÇÃO DE MESTRADO

Tuane Bazanella Sampaio

Santa Maria, RS, Brasil

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AVALIAÇÃO DOS EFEITOS FARMACOLÓGICO E TOXICOLÓGICO DE 4-ORGANOCALCOGENO-ISOQUINOLINAS

Tuane Bazanella Sampaio

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Orientadora: Prof.ª Dr.ª Cristina Wayne Nogueira

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elaborada por
Tuane Bazanella Sampaio

como requisito parcial para obtenção do grau de
Mestre em Bioquímica Toxicológica

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Santa Maria, 12 de março de 2014

“Enquanto houver você do outro lado,

Aqui do outro eu consigo me orientar...”

O anjo mais velho – O Teatro Mágico

Dedico esta dissertação aos meus pais, Paulo e Vera, e a minha irmã, Daiane, por serem a luz e a terra firme do meu caminho!

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Primeiramente, agradeço à Deus, por sempre estar comigo, pelo dom da vida, pelos obstáculos, por proteger meu caminho e guiar minha jornada.

Aos meus pais, por serem meus conselheiros, pela compreensão nos momentos de ausência, pelo amor, apoio e confiança. À minha irmã, por ser minha melhor amiga, meu porto seguro, meu maior orgulho e o exemplo de tudo que eu almejo ser. À vocês toda a minha gratidão e amor!

Às minhas amigas, de Cruz Alta e da Farmácia, que comemoram as minhas conquistas como suas: essa daqui, também é nossa. Amo vocês, obrigada por tudo!

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“Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.”

Antonio Machado

RESUMO

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

AVALIAÇÃO DOS EFEITOS FARMACOLÓGICO E TOXICOLÓGICO DE 4-ORGANOCALCOGENO-ISOQUINOLINAS

AUTORA: TUANE BAZANELLA SAMPAIO
ORIENTADORA: CRISTINA WAYNE NOGUEIRA
Data e Local da Defesa: Santa Maria, 12 de março de 2014.

A monoamina oxidase (MAO) é uma enzima alvo no tratamento de diversas patologias, sendo que novas moléculas que a inibam de maneira seletiva, potente, reversível, e ausente de efeitos adversos suas isoformas são procuradas. Neste sentido, o primeiro manuscrito desta dissertação avaliou o potencial inibitório dos 4-organocalcogeno-isoquinolinas na atividade cerebral da MAO-A e B *in vitro*, elucidando seus perfis cinéticos e a interação composto e enzima. Os resultados demonstram que todos os compostos apresentam inibição seletiva da MAO-B, sendo o composto 3-fenil-4-(selenofenil) isoquinolina o mais potente. O perfil cinético revelou inibição do tipo mista e reversível da enzima, coerente aos resultados do *docking* molecular. Sabe-se que tanto compostos orgânicos de selênio quanto isoquinolinas relacionam-se a situações pró-oxidantes, deste modo, investigou-se o efeito *in vitro* dos 4-organoseleno-isoquinolinas na atividade cerebral das enzimas δ-aminolevulinato dehidratase (δ-ALA-D) e Na^+, K^+-ATPase, as quais possuem resíduos de cisteína facilmente oxidáveis. Os dados demonstram que os compostos substituídos com cloro, flúor e trifluormetil no anel aromático ligado ao átomo de Se do composto 3-fenil-4-(selenofenil) isoquinolina inibem ambas as enzimas sulfidrílicas, o que não foi observado com o composto substituído com metil e com o composto não substituído. Além disso, visto que a inibição das enzimas δ-ALA-D e Na^+, K^+-ATPase foi revertida por ditiotreitol é possível propor o envolvimento da oxidação dos resíduos de cisteína pelos compostos. Devido à inibição seletiva e reversível da MAO-B e ao baixo potencial toxicológico demonstrado, o composto 3-fenil-4-(selenofenil) isoquinolina torna-se um candidato a mais estudos que possuam esta enzima como alvo terapêutico.

Palavras-chave: δ-ALA-D. Isoquinolina. MAO. Na^+, K^+-ATPase. Selênio.

ABSTRACT

Dissertation of Master's Degree
Postgraduate Programme in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria

EVALUATION OF PHARMACOLOGYC AND TOXICOLOGYC EFFECTS OF 4-ORGANOCHALCOGEN-ISOQUINOLINES

AUTHOR: TUANE BAZANELLA SAMPAIO
ADVISOR: CRISTINA WAYNE NOGUEIRA
Date and Place of the Defense: Santa Maria, March 12, 2014.

Monoamine oxidase (MAO) is a target enzyme in the treatment of several pathologies, being that new molecules which inhibit of a selective, potent and reversible manner their isoforms and without adverse effects are searched. In this way, the first manuscript of this dissertation evaluated the *in vitro* inhibitory potential of the 4-organochalcogen-isoquinolines on cerebral MAO-A and B activities, elucidating their kinetics profile and the interaction compound x enzyme. The results demonstrated that all compounds were selective inhibitors of MAO-B, being compound 3-phenyl-4-(selenophenyl) isoquinoline the most potent. The kinetics profile revealed a mixed and reversible inhibition of enzyme, consistent to the results of molecular docking. It is known that both organic selenium compounds and isoquinolines are linked to pro-oxidants situations, thus, it was investigated the *in vitro* effect of 4-organoseleno-isoquinolines on cerebral activities of the enzymes δ-aminolevulinate dehydratase (δ-ALA-D) e Na^+, K^+-ATPase, which have easily oxidized cysteine residues. Data demonstrated that compounds substituted with chloro, fluoro and trifluoromethyl in the aromatic ring bonded to the selenium atom of compound 3-phenyl-4-(selenophenyl) isoquinoline inhibited both sulfhydryl enzymes, which was not observed in the compound substituted with methyl and in a non-substituted compound. Furthermore, since the inhibition of enzymes δ-ALA-D and Na^+, K^+-ATPase was restored by dithiothreitol it is possible to propose the oxidation of cysteine residues by compounds. The selective and reversible inhibition of MAO-B and the low toxicological potential demonstrated by compound 3-phenyl-4-(selenophenyl) isoquinoline become this compound a candidate for more studies, which aim this enzyme as a therapeutic target.

Keywords: δ-ALA-D. Isoquinoline. MAO. Na^+, K^+-ATPase. Selenium.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

1-Metil-TIQ – 1-metil-1,2,3,4-tetrahidroisoquinolina

ALA – ácido aminolevulínico

AS-101 – telurato de tricloro amônio-dioxoetileno –o,o’

ATP - trifosfato de adenosina

DHAR – deidroascorbato redutase

DIO – iodotironina deiodinase

DTT – ditiotreitol

FAD – dinucleotídeo de flavina e adenina

FAO – organização das nações unidas para a agricultura e alimentação

GPx – glutationa peroxidase

GST – glutationa s-transferase

IDR – ingestão diária recomendada

MAO- monoamina oxidase

MPTP - 1-metil-4-fenil-1,2,3,6-tetrahidropiridina

NPS – nitroprussiato de sódio

OMS – organização mundial de saúde

PBG – porfobilinogênio

SPS2 – selenofosfato sintetase 2

TIQ - 1,2,3,4-tetrahidroisoquinolina

TRXR – tiorredoxina redutase

δ-ALA-D - δ-aminolevulinato dehidratase

SUMÁRIO

1 INTRODUÇÃO

1.1 A família dos calcogênios

A família dos calcogênios (16 ou 6A) é composta pelos elementos químicos não metálicos oxigênio (O), enxofre (S), selênio (Se) e telúrio (Te), bem como pelos metais Polônio (Po) e Ununhexium (Uuh). Estes elementos possuem como característica o término da configuração eletrônica em ns^2np^4, exibindo 6 elétrons na camada de valência e número de oxidação -2 (WIESER, 2011).

Dentre os elementos pertences a esta família, S, Se e Te destacam-se por estarem presentes em importantes intermediários e reagentes utilizados em síntese orgânica (BRAGA, A. L. Z., G.; ANDRADE, L. H.; SILVEIRA, C. C., 1997), e ainda, pelas diversas atividades biológicas reportadas (GRINBERG et al., 2005; NOGUEIRA et al., 2004).

O S é o elemento químico de número atômico 16 e massa molecular 32 u. A temperatura ambiente encontra-se no estado sólido na forma de cristais amarelos que ocorrem em diversos minerais de sulfito e sulfato. Utilizado em diversos processos industriais, como produção de ácido sulfúrico para baterias, fungicida, na forma de sulfito para conservar bebidas alcóolicas e também como laxante no caso do sulfato de magnésio; o enxofre também possui importante papel biológico. Este elemento está presente nos aminoácidos metionina e cisteína, os quais formam pontes dissulfeto entre si, importantes para a formação da estrutura terciária de proteínas. Somado a isto, moléculas como tiamina, biotina e coenzima A, grupos prostéticos essenciais para a atividade catalítica de algumas enzimas, possuem S em sua estrutura (INGENBLEEK e KIMURA, 2013).

De forma semelhante, o Se, elemento de número atômico 34 e massa molecular igual a 79 u, é estabelecido como um mineral traço essencial para a saúde humana, constituindo o aminoácido selenocisteína, o qual está presente em no mínimo 25 proteínas (KRYUKOV et al., 2003). Entre essas proteínas, destaca-se a presença deste aminoácido em enzimas como a glutationa peroxidase (GPx), a

tiorredoxina redutase (TrxR), iodotironina deiodinases (DIO) e selenofosfato sintetase 2 (SPS2) (PAPP et al., 2007).

Devido à importância biológica do Se, a Organização Mundial de Saúde (OMS) preconiza uma ingestão diária recomendada (IDR) de 34-35 μg deste elemento para adultos (FAO/OMS, 2002). Sabe-se que uma ingestão inferior a 10 μg/dia está relacionada ao aparecimento da doença de Keshan (LI et al., 1985), assim como concentrações elevadas, superiores a 400 μg/dia, podem ocasionar perda de cabelo, desenvolvimento de pele icteroide e distúrbios gastrointestinais (FAO/OMS, 2002). A ingestão de Se ocorre através do consumo de plantas e animais, sendo as principais fontes a castanha-do-pará, cebola, cogumelos, brócolis, cereais, pescados, carnes e ovos, ou ainda através da suplementação dietética (DUMONT et al., 2006). Este elemento encontra-se no solo em sua forma inorgânica de selenito (Se^{6+}) e selenato (Se^{4+}), e ao ser metabolizado, devido a sua semelhança química com o S, o substitui nas moléculas de aminoácidos sulfurados, formando os selenoaminoácidos (selenometionina e selenocisteína) (YOUNG et al., 1982).

Outro calcogênio, utilizado no meio industrial e presente em compostos orgânicos com atividades biológicas reportadas, é o Te. Este elemento, de número atômico 52 e massa molecular equivalente a 128 u, apresenta-se em diferentes estados de oxidação: Te^{6+} (telurato), Te^{4+} (Telurito), Te^{2+} (telureto) e Te^{0} (telúrio elementar) (SCANSETTI, 1992). Na indústria, o Te é utilizado como componente de ligas metálicas, na produção de microchips, vidro e aço, bem como na produção de explosivos, soluções oxidantes para polir metais e na indústria petroquímica (ABDEL AZIZ, 2006; TAYLOR, 1996; YAREMA e CURRY, 2005). Como é prontamente absorvido pelo organismo, tanto na forma inorgânica de teluritos e teluratos quanto como compostos orgânicos de Te, e sua exposição ocupacional é crescente, esse elemento pode causar intoxicação aguda, induzindo dores de cabeça, sonolência, enjoo e alteração da frequência cardíaca (MULLER et al., 1989; TAYLOR, 1996). Entretanto, a partir da década de 70 as propriedades farmacológicas de compostos orgânicos de Te despertaram o interesse de pesquisadores, aumentando as pesquisas de seus possíveis efeitos benéficos e tóxicos.

1.2 Atividade Farmacológica de Organocalcogênios

Nas últimas décadas, compostos contendo átomos de calcogênios têm despertado o interesse da comunidade científica devido a estes apresentarem diversas propriedades bioquímicas e farmacológicas. Estudos demonstram que compostos orgânicos contendo S, Se ou Te são potentes antioxidantes (GRINBERG et al., 2005; NOGUEIRA et al., 2004) e também atuam na oxidação de grupos tióis a dissulfetos (FARINA et al., 2001; GOEGER e GANTHER, 1994).

Em relação às propriedades farmacológicas dos compostos orgânicos de S, Meotti et al. (2003) reportaram o potencial antioxidante de uma classe de tiofenos (Figura 1A), que, somada a redução da peroxidação lipídica alguns compostos demonstraram ação anti-inflamatória e antinoceptiva em roedores. Outro tiofeno acetilênico também apresentou ação anti-inflamatória no teste do edema da pata induzido por carragenina (ZENI et al., 2001) (Figura 1B).

O disseleneto de ditienila é um composto que apresenta tanto S quanto Se em sua estrutura (Figura 1C). Este composto possui atividade antioxidante e inibe de forma não seletiva a monoamina oxidase (MAO) *in vitro*, além disso, apresenta ação pró-oxidante contra bactérias e fungos e protege da neurotoxicidade induzida por ácido caínico em ratos (BORTOLATTO et al., 2013; BORTOLATTO et al., 2011; PESARICO et al., 2013). Prigol et al. (2008), também testaram dicalcogenetos *in vitro* e *in vivo* (Figura 1D), demonstrando o potencial antioxidante *in vitro* e a proteção contra o dano oxidativo cerebral causado por nitroprussiato de sódio (NPS) em camundongos.

Já os compostos orgânicos de Se, além da atividade antioxidante *in vitro* e *in vivo* bem reportada, destacam-se devido a este elemento estar presente no sítio ativo de enzimas com função antioxidante. Deste modo, os compostos orgânicos de Se apresentam atividade mimética as enzimas GPx, deidroascorbato redutase (DHAR) e glutationa-S-transferase (GST), e agem como substrato para a TrxR (LUCHESE e NOGUEIRA, 2010; NOGUEIRA et al., 2004; SAUSEN DE FREITAS et al., 2010).

Organoselênios, como o ebselen (Figura 1E) e os disselenetos de diarila (Figuras 1F-H), apresentam diversas atividades farmacológicas. Estes compostos demonstram ação anti-inflamatória e antinoceptiva (CHAGAS et al., 2013b; NOGUEIRA et al., 2003), do tipo antidepressiva e ansiolítica (BRUNING et al.,

2009b; GAY et al., 2010; POSSER et al., 2009) e insulino mimética (BARBOSA et al., 2006). Ainda conferem proteção contra os danos induzidos por diferentes substâncias ao fígado (ROCHA et al., 2005), rins (BRANDAO et al., 2009; CHANDER et al., 2004), estômago (INEU et al., 2008; TABUCHI et al., 1995) e coração (DA ROCHA et al., 2009; SAAD et al., 2006). Em relação à neuroproteção, o ebselen foi utilizado em testes clínicos para o tratamento de neuropatologias associadas ao estresse oxidativo (SAITO et al., 1998). Tanto o ebselen quanto os disselenetos, possuem atividade neuroprotetora em diferentes modelos animal (BORTOLATTO et al., 2011; KONDOH et al., 1999; PINTON et al., 2013).

Esses compostos orgânicos de selênio parecem possuir múltiplos sítios de ação, pois exercem seus efeitos farmacológicos através da modulação de diversos sistemas, como o serotoninérgico, dopaminérgico, noradrenérgico, GABAérgico e glutamatérgico (NOGUEIRA e ROCHA, 2011). Brüning et al. (2010) observaram a interação do disseleneto de diarila disubstituído com trifluormetil, *m*-trifluormetil disseleneto de difenila, com sistema opióide central, interagindo com receptores μ e δ. Este mesmo disseleneto apresentou inibição seletiva da atividade da MAO-A em córtex de camundongos (BRUNING et al., 2009b), enquanto que o disseleneto de difenila inibiu a atividade da MAO-total em ratos (SAVEGNAGO et al., 2007).

Os compostos orgânicos de telúrio tiveram sua primeira propriedade farmacológica descrita por Sredni et al. (1987), na qual foi demonstrada a ação imunomoduladora do AS-101 (telurato de tricloro amônio-dioxoetileno-O,O') em camundongos (Figura 1I). Atualmente, este composto encontra-se em ensaios de fase clínica II, por mediar efeitos antitumorais (FREI et al., 2008; FRIEDMAN et al., 2009).

Após o AS-101, outros compostos contendo Te passaram a ser estudados, como o ditelureto de difenila (Figura 1J), exibindo potencial antioxidante frente à peroxidação lipídica, carbonilação e nitrosilação de proteínas, além de atuar como *scavenger* de espécies reativas de oxigênio e nitrogênio (AVILA et al., 2008; JACOB et al., 2000; PINTON et al., 2011), somado a isto, estes compostos mimetizam a atividade da enzima GPx, importante contra o dano oxidativo (ANDERSSON et al., 1993; BRAGA, A. L. et al., 2009). Estudos recentes do nosso grupo de pesquisa mostraram que o 2-feniletinilbutil-telúrio (Figura 1L), um teluroacetileno, protegeu do dano oxidativo cerebral induzido por NPS (SOUZA et al., 2009), bem como possui efeito notrópico na tarefa da esquiva inibitória (SOUZA et al., 2012), no déficit

cognitivo induzido por escopolamina e pelo peptídeo beta amiloide 25-35 (SOUZA et al., 2013a; SOUZA et al., 2013b).

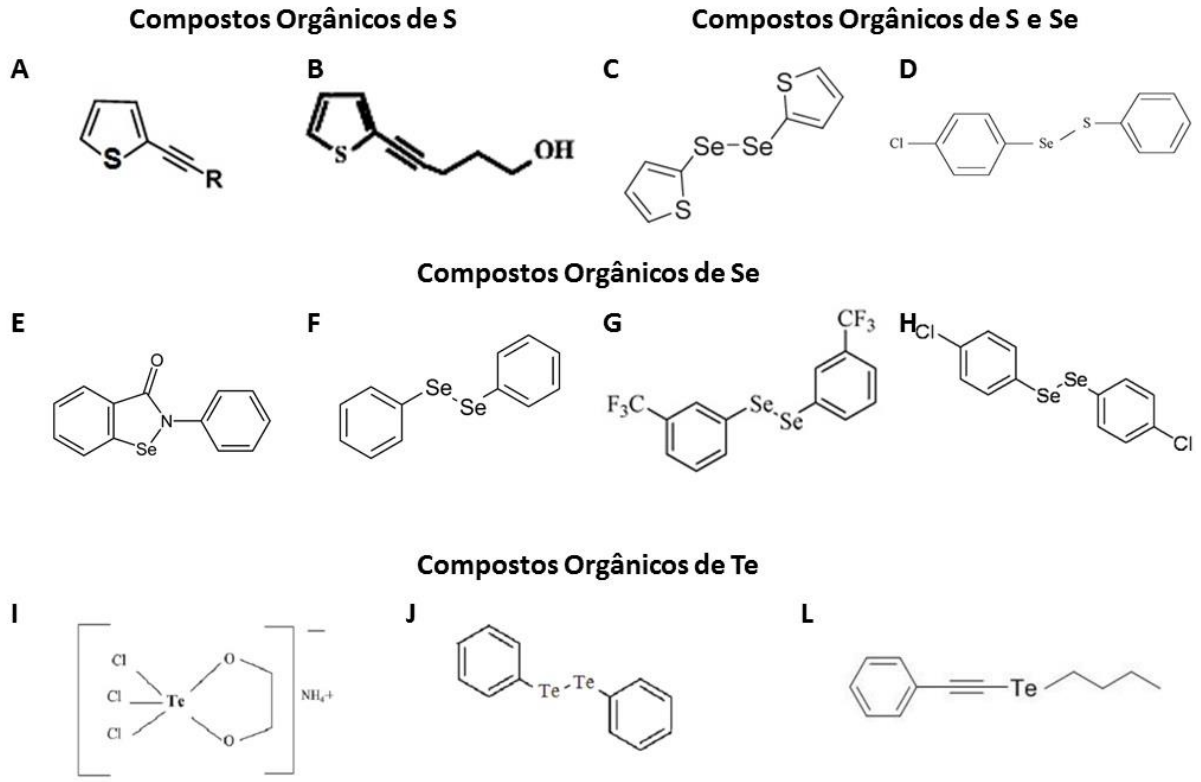

Figura 1 - Estrutura química de alguns organocalcogênios com atividade farmacológica descrita. Compostos orgânicos de S: (A) Estrutura dos tiofenos, (B) Tiofeno acetilénico. Compostos orgânicos de S e Se: (C) Disseleneto de Ditienila (D) Dicalcogeneto assimétrico substituído com cloro na fenila ligada ao Se. Compostos orgânicos de Se: (E) Ebselen, (F) Disseleneto de Difenila, (G) *m*-Trifluormetil Disseleneto de Difenila, (H) *p*-Cloro Disseleneto de Difenila. Compostos orgânicos de Te: (I) Telurato de Tricloro Amônio-dioxoetileno-O,O', (J) Ditelureto de Difenila e (L) 2-Feniletinilbutil-telúrio.

1.3 As Enzimas Sulfidrílicas e os Organocalcogênios

Como descrito acima, os organocalcogênios apresentam uma série de atividades farmacológicas em diversos modelos animais, entretanto, há alguns relatos de intoxicação aguda após a ingestão de suplementos dietéticos e vitamínicos contendo esses compostos, além de efeitos tóxicos causados pela exposição ocupacional (CLARK et al., 1996; VINCETI et al., 2009). O mecanismo pelo qual os calcogênios exercem sua toxicidade não está completamente

elucidado, porém há fortes indícios que sugerem a oxidação de grupos tióis endógenos, que no caso de enzimas sulfidrílicas, reduzem sua atividade catalítica (NOGUEIRA et al., 2003). O Se na forma de selenol (R-SeH/R-Se-) apresenta-se como um potente nucleófilo, agindo como um “super S”, com poder redutor maior que o análogo tiol (NOGUEIRA e ROCHA, 2011), da mesma forma, a reatividade do Te é superior a do Se (NOGUEIRA et al., 2003).

A δ-aminolevulinato dehidratase (δ-ALA-D) é uma enzima sulfidrílica, que catalisa a condensação de duas moléculas do ácido aminolevulínico (ALA), formando o monopirrol porfobilinogênio (PBG) (JAFFE e LAWRENCE, 2012). Essa enzima é um ponto chave na síntese de tetrapirrólicos, como o grupo heme, o qual exerce várias funções conforme a apoproteína a que se associa, gerando hemoglobina, citocromos, peroxidases e outras proteínas. Essa enzima possui resíduos de cisteína (grupamentos tióis) essenciais para sua atividade catalítica, sendo assim quando estes resíduos são oxidados, sua atividade é reduzida, levando ao acúmulo do substrato da sua reação, o ALA, que por sua vez possui ação pró-oxidante quando em concentração acima da fisiológica (ROCHA et al., 2012).

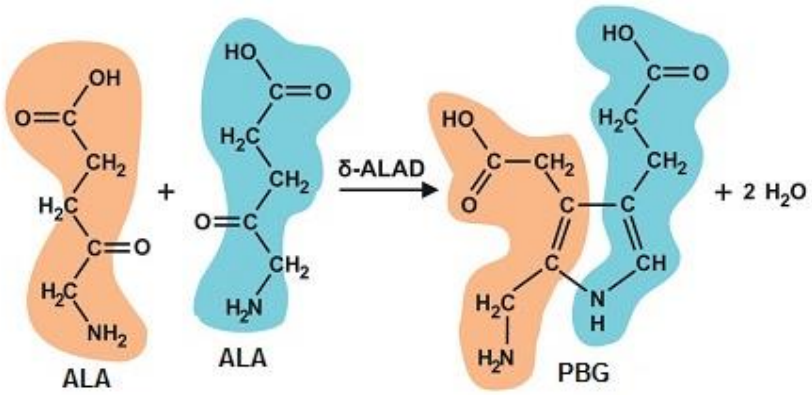

Figura 2 – Reação catalisada pela enzima δ-ALA-D. Síntese de PBG a partir da condensação assimétrica de duas moléculas de ALA. Adaptado de ROCHA et al., 2012.

A δ-ALA-D é uma enzima oligomérica, com o sítio ativo em formato de um octâmero, no qual os resíduos de cisteína são encontrados coordenados à íons de Zn (II), os quais auxiliam na estabilização desses resíduos. Para que haja a formação do PBG é necessário que o sítio ativo da enzima esteja reduzido, sendo

assim a oxidação desses aminoácidos ou a aceleração do processo de auto-oxidação pela retirada do Zn^{2+} por agentes quelantes, levam a redução de sua atividade enzimática (BEBER et al., 1998; EMANUELLI et al., 1998). Sabe-se que quando exposta a situações pró-oxidantes, a δ-ALA-D apresenta sua atividade inibida devido a oxidação dos resíduos de cisteína, o que a torna um importante biomarcador em intoxicação por metais, por exemplo (ROCHA et al., 2012).

Semelhantemente a δ-ALA-D, a Na^+, K^+-ATPase é uma enzima que possui grupamentos tióis essenciais para sua atividade e que são suscetíveis a agentes oxidantes. Esta enzima possui como função regular a concentração intracelular dos íons Na^+ e K^+, e consequentemente seus gradientes através da membrana plasmática (KAPLAN, 2002). Para isto, utiliza o trifosfato de adenosina (ATP) como fonte energética para o transporte ativo de três cargas positivas de Na^+ para o meio extracelular, e duas de K^+ para o meio intracelular (BERTORELLO, 1995). Quando a Na^+, K^+-ATPase está inativa, ocorre a despolarização parcial da membrana, permitindo a entrada excessiva de Ca^{2+} dentro dos neurônios. O aumento dos níveis intracelulares de Ca^{2+} pode induzir diversos eventos tóxicos, incluindo uma maior liberação de glutamato, neurotransmissor envolvido nos processos de excitotoxicidade, podendo levar a morte neuronal (VELDHUIS et al., 2003).

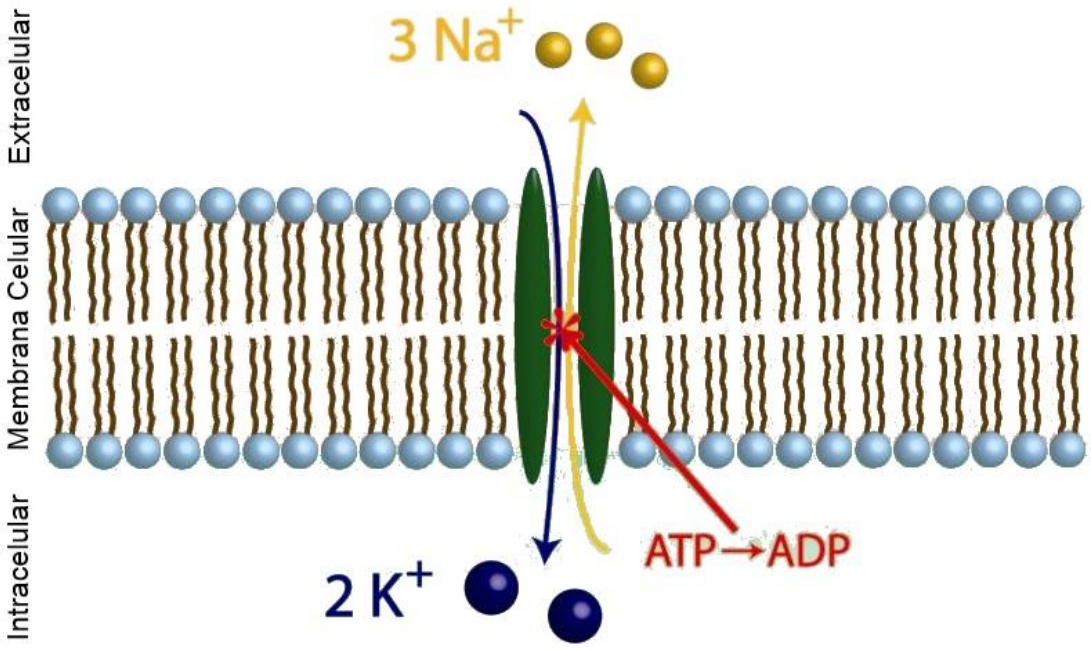

Figura 3 – Reação catalisada pela enzima Na^+, K^+-ATPase. Transporte ativo de íons Na^+ e K^+, utilizando energia proveniente da hidrólise do ATP.

Estudos demonstraram que a inibição dessas enzimas sulfidrílicas por organocalcogênios era revertida através do uso de agentes redutores, relacionando a toxicidade desses compostos com a oxidação dos resíduos de cisteína. No caso da interação da δ-ALA-D com os compostos orgânicos de Se pode ocorrer ainda a formação de complexos do Se com o Zn^{2+}, contribuindo para o processo oxidativo, e consequentemente, para a inibição enzimática (BRANDAO et al., 2008; BRUNING et al., 2009a; CHAGAS et al., 2013a).

1.4 Isoquinolinas

Isoquinolina é uma molécula orgânica heterocíclica aromática formada por um anel de benzeno fundido na face (*c)* a um anel de piridina (MCNAUGHT et al., 1998) (Figura 4).

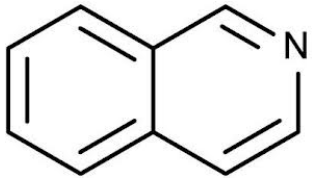

Figura 4 – Estrutura química da isoquinolina.

Derivados de isoquinolina fazem parte de um grande grupo de alcaloides derivados do aminoácido tirosina, mas também podem ser obtidos de forma sintética. Muitas isoquinolinas obtidas de fontes naturais possuem atividades farmacológicas descritas, é o caso da morfina derivada da *Papaver somniferum* L. e sua atividade analgésica, do antigota – colchicina - extraído da *Colchicum autumnale* L., do antiemético emetina derivada da *Cephaelis ipecacuanha* e dos antimicrobianos berberina e sanguinarina de plantas das espécies *Berberis* spp. e *Sanguinaria* spp (FACCHINI, 2001; SATO e KUMAGAI, 2013).

Outros derivados de isoquinolina são formados endogenamente como metabólitos de neurotransmissores no cérebro de humanos, como por exemplo, a 1,2,3,4-tetrahidroisoquinolina (TIQ), que possui ação neurotóxica e parece estar

envolvida na Doença de Parkinson (NIWA et al., 1989; YAMAKAWA e OHTA, 1997). Estes compostos são alvos de estudo por possuírem estruturas semelhantes a 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP), uma toxina exógena com ação seletiva em neurônios dopaminérgicos e que, após ser metabolizada pela MAO-B, produz Parkinsonismo (LANGSTON et al., 1983; PIENAAR et al., 2012). No entanto, outra isoquinolina endógena,1-metil-TIQ, apresenta ação neuroprotetora contra anormalidades comportamentais induzidas pelo MPTP e pela TIQ em roedores (KOTAKE et al., 1995; TASAKI et al., 1991), uma vez que essa isoquinolina inibe a atividade da MAO-B, impedindo o metabolismo dessas neurotoxinas.

1.5 Monoamina Oxidase

A MAO é uma enzima mitocondrial responsável pela desaminação oxidativa dos neurotransmissores monoaminérgicos e aminas exógenas. As isoformas A e B catalisam a desaminação oxidativa de monoaminas através da redução do cofator FAD (dinucleotídeo de flavina e adenina), o qual se liga covalentemente aos resíduos de cisteína, presentes em ambas as isoformas, através de uma ligação tioéster (WOUTERS, 1998).

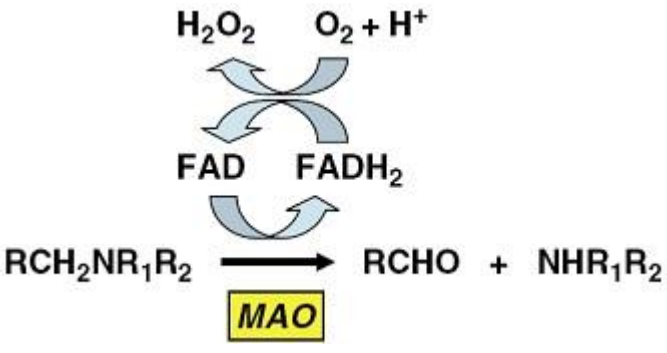

Figura 5 – Desaminação oxidativa de monoaminas realizada pela MAO, através da redução do FAD. $RCH_2NR_1R_2$: amina primária; RCHO: derivado aldeído; NHR_1R_2: amina substituída ou amônia. Adaptado de YOUDIM e BAKHLE, 2006.

Apesar de catalisar a mesma reação, as isoformas da MAO diferenciam-se entre si pela seletividade a inibidores e substratos e distribuição tecidual, além de distinguirem-se pela expressão durante o desenvolvimento, no qual a MAO-A é mais expressa, e durante o processo de envelhecimento, aonde ocorre o aumento da expressão da isoforma B. Esta enzima encontra-se amplamente distribuída nos tecidos de mamíferos, entretanto as proporções das suas isoformas variam de tecido para tecido. A MAO-A é mais expressa em tecidos periféricos, como o intestino, enquanto que a MAO-B possui maior expressão no sistema nervoso central. Além disto, em humanos ocorre alteração na atividade da MAO dependendo da estrutura aonde a enzima está expressa, sendo assim os gânglios da base e o hipotálamo apresentam maior atividade da MAO que o observado no cerebelo e neocórtex (YOUDIM, M. B. e BAKHLE, 2006). Sabe-se que a MAO-A é seletivamente inibida por clorgilina e possui alta afinidade pelos substratos 5-hidroxitriptamina e norepinefrina, enquanto a MAO-B é seletivamente inibida por selegilina e metaboliza preferencialmente a feniletilamina (GAWESKA e FITZPATRICK, 2011). Contudo, ambas as isoformas podem metabolizar o mesmo substrato como, por exemplo, a dopamina e a tiramina (BENEDETTI e DOSTERT, 1985). Entretanto, em humanos, a dopamina é principalmente metabolizada pela MAO-B (YAMADA e YASUHARA, 2004).

As isoformas apresentam homologia em torno de 70 %, sendo que a principal diferença entre elas encontra-se na estrutura do sítio oposto ao cofator FAD. Na MAO-A este sítio é constituído apenas por uma grande cavidade, enquanto que na MAO-B, ele encontra-se bipartido (BINDA et al., 2011). Devido a estas diferenças estruturais, inibidores seletivos da MAO-A geralmente são compostos planares substituídos por funções hidroxilas, já inibidores da isoforma B irão depender do tamanho da molécula planar e sua densidade eletrônica, para inibir a enzima seletivamente (MEDVEDEV et al., 1996; WOUTERS, 1998).

Inibidores da MAO são utilizados no tratamento de distúrbios depressivos – incluindo depressão atípica, dupla e crônica (ROBINSON, 2002) – bem como em doenças neurodegenerativas, como as Doenças de Alzheimer e Parkinson (FERNANDEZ e CHEN, 2007; RIEDERER et al., 2004). Entretanto, seu uso é restrito devido às crises hipertensivas agudas causadas pela administração concomitante de um inibidor da MAO e alimentos quem contenham tiramina, como

o queijo (COSTA et al., 2012). Apesar da desaminação da tiramina ser realizada por ambas as isoformas, no trato gastrointestinal esta molécula sofre ação, principalmente, da MAO-A (YOUDIM, M. B. H., 1995). Sendo assim, inibidores não seletivos ou seletivos para MAO-A tendem a causar esse efeito adverso, tendo que ser recomendado ao paciente uma dieta restritiva durante o tratamento.

Desta forma, a pesquisa de novas moléculas que interajam com as isoformas da MAO, inibindo-as e possuindo poucos efeitos adversos, torna-se relevante, visto que inibidores dessas enzimas têm sido utilizados na clínica para o tratamento de muitas doenças.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os possíveis efeitos farmacológicos e toxicológicos de 4-organocalcogeno-isoquinolinas.

2.2 Objetivos específicos

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

- Avaliar o potencial inibitório *in vitro* dos 4-organocalcogeno-isoquinolinas na atividade cerebral das isoformas A e B da MAO de ratos, elucidando a cinética e a reversibilidade da inibição;
- Esclarecer a maneira pela qual os derivados de isoquinolina interagem com a MAO-B através de modelos *in silico*;
- Estudar a cinética e reversibilidade da inibição da MAO-B nos compostos com maior potência inibitória;
- Determinar o potencial toxicológico *in vitro* dos 4-organoseleno-isoquinolinas através da atividade das enzimas δ-ALA-D e Na^+, K^+-ATPase em homogeneizado de cérebro de ratos;
- Investigar a influência do ditiol DTT sobre a inibição das enzimas sulfidrílicas causada por alguns derivados de isoquinolina testados, bem como do Zn^{2+} sobre a inibição da δ-ALA-D.

3 DESENVOLVIMENTO

Os resultados que fazem parte dessa dissertação estão apresentados na forma de dois manuscritos em fase de redação. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas estão dispostos de acordo com a recomendação dos periódicos científicos aos quais estes foram e serão enviados.

3.1 Manuscrito 1

4-Organochalcogen-isoquinolines selective and reversibly inhibit the cerebral monoamine oxidase B activity: *in vitro* and *in silico* studies

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Abstract

Isoquinolines are formed endogenously as metabolites of neurotransmitters and are studied because they have structures similar to neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and selegiline, a selective inhibitor of MAO-B. The present study investigated a possible *in vitro* inhibitory activity of new 4-organochalcogen-isoquinoline derivatives, containing sulfur **1**, selenium **2** or tellurium **3** on MAO-A and B activities. Considering that the non-substituted selenoisoquinoline derivative **2** showed the best inhibitory profile (IC_{50} = 36.41 ± 12.15 μM), new compounds were synthesized by adding substituents (methyl **2a**, fluorine **2b**, chloro **2c** and trifluoromethyl **2d**) to the aromatic ring bonded to the selenium atom of compound **2**. All tested compounds were selective MAO-B inhibitors. Compounds **2** and **2b** were chosen to study the enzymatic kinetic and the reversibility of inhibition. Molecular docking studies were used to further understand the interactions of isoquinoline derivatives with the enzyme. These compounds demonstrated reversible and mixed inhibition by decreasing apparent V_{max} and increasing apparent K_m. Molecular docking studies showed that compounds **2** and **2b** interact with the residues Tyr 398 and Ile 199 in the active site, which are important for the selective inhibition of MAO-B. In conclusion, selenoisoquinolines **2** and **2b** fit in the profile of third generation MAO inhibitors (selective and reversible), which are promising alternatives for treatment of several disorders. Nonetheless, more studies are necessary in order to clearly elucidate a possible use of these compounds as therapeutic alternatives.

Keywords: isoquinoline, kinetics profile, MAO (MAO-B), organochalcogen, selenium, molecular docking.

Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) is a mitochondrial enzyme present in mammalian tissues responsible for the oxidative deamination of monoamine neurotransmitters as well as exogenous amines. MAO-A and MAO-B isoforms catalyze the oxidative deamination of monoamines with the reduction of the flavin adenine dinucleotide (FAD) cofactor. FAD cofactor is covalently bonded to the cysteine, present in both isoforms, by a thioester bond . However, despite the similarities there are differences between the two isoforms, such as the amines metabolized and their selective inhibitors. MAO-A is selectively inhibited by clorgyline and has 5-hydroxytryptamine and norepinephrine as preferred substrates, whereas MAO-B is selectively inhibited by selegiline and metabolizes phenylethylamine preferentially . Dopamine and tyramine are deaminated by both isoforms in most animals . However in humans dopamine is metabolized by MAO-B mainly .

Isoquinoline derivatives are heterocyclic aromatic organic compounds constituted of a benzene ring fused at face (*c*) to a pyridine ring . These compounds are formed endogenously as metabolites of neurotransmitters and become target of study because they have similar structures to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective dopaminergic exogenous pro-toxin that targets mainly nigral neurons producing symptoms of Parkinson's disease after oxidation by MAO-B . Isoquinolines have also structural similarities with selegiline, a known selective inhibitor of MAO-B .

Isoquinoline derivatives may be found in a variety of foods, such as milk, bananas and cocoa, and even in the brain of some animal species . These compounds can exhibit both neurotoxic and neuroprotective actions. 1,2,3,4-Tetrahydroisoquinoline and 1-benzyl-1,2,3,4-tetrahydroisoquinoline are examples of endogenous neurotoxic isoquinolines that appear to be involved in Parkinson's disease . Moreover, another endogenous isoquinoline, 1-methyl-1,2,3,4-tetrahydroisoquinoline showed neuroprotective, anti-addictive, antidepressant and MAO-inhibiting properties . This neuroprotective isoquinoline inhibits both MAO-A and MAO-B activities, interacts as an agonist with dopamine receptors and increases the levels of monoamines in the brain . In addition, a series of isoquinolines, as N-methyl-isoquinolinium ions, demonstrated reversible MAO-A inhibition, an important target for the therapeutic of major depression, aggression and neurodegenerative

conditions . These findings suggest that new isoquinoline derivatives which could effectively inhibit MAO activity might be useful for treatment of several disorders.

Compounds containing chalcogen atoms have been studied because of their diverse pharmacological properties. Organochalcogen compounds containing sulfur (S), selenium (Se) and tellurium (Te) showed promising antioxidant activity and can oxidize thiol groups to disulfides . Among these chalcogens, Se is distinguished by being present in compounds that affect MAO activity, for example *m*-trifluoromethyl-diphenyl diselenide and diphenyl diselenide , which inhibit MAO-A and total MAO in rodents, respectively. Furthermore, Tang *et al.* demonstrated that both inorganic and organic selenium supplementation caused an inhibition in MAO-B activity in rats.

Therefore, the present study aimed to investigate a possible inhibitory effect of new 4-organochalcogen-isoquinoline derivatives on cerebral MAO-A and MAO-B activities *in vitro*. In addition, the enzymatic kinetics, selectivity and reversibility, by dialysis, of 4-organochalcogen-isoquinoline derivatives inhibitory effect were investigated. *In silico* molecular docking studies were used to further understand the interactions of compounds with the enzyme.

Materials and Methods

Chemicals

4-Organochalcogen-isoquinoline derivatives were prepared and characterized in our laboratory by the method previously described . Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that isoquinoline derivatives obtained presented analytical and spectroscopic data in full agreement with their assigned structures. The chemical purity of compounds (99.9%) was determined by GC/MS. Isoquinoline derivatives were dissolved in dimethylsufoxide (DMSO) for the experiments. All other chemical reagents utilized for biochemistry assays were obtained from standard commercial suppliers.

The tested 4-organochalcogen-isoquinoline derivatives are shown in Figure 1: 3-phenyl-4-(phenylthio) isoquinoline **1**; 3-phenyl-4-(phenylseleno) isoquinoline **2**; 3-phenyl-4-(phenyltelluro) isoquinoline **3**; 4-(4-methylphenylseleno)-3-phenylisoquinoline **2a**; 4-(4-fluorophenylseleno)-3-phenylisoquinoline **2b**; 4-(4-chlorophenylseleno)-3-phenylisoquinoline **2c**, and 4-(3-trifluoromethylphenylseleno)-3-phenylisoquinoline **2d**.

Animals

Male adult Wistar rats (200-300 g) from our own breeding colony were used. The animals were kept in a separate animal room, on a 12 h light/dark cycle, at a room temperature of 22 ± 2 °C, with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animals Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments. The animal care was according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Mitochondrial Preparation

Animals were killed by decapitation and whole brains were quickly removed and washed in ice-cold isolation medium (pH 7.4, Na_2PO_4 0.0168 M/KH_2PO_4 0.0106 M isotonized with sucrose 0.32 M). Preparations of cerebral mitochondria were used for MAO assay as previously described by Soto-Otero *et al.* . Briefly, after removing blood vessels and pial membranes, brains were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900 *x* g at 4 ^{0}C for 5 min. The supernatant was centrifuged at 12,500 *x* g at 4 ^{0}C for 15 min. The mitochondria pellet was then washed once with isolation medium and recentrifuged under the same conditions. Finally, the mitochondrial pellet was reconstituted in a buffer solution (Na_2PO_4 0.0168 M/KH_2PO_4 0.0106 isotonized with KCl 0.0036 M, pH 7.4) and stored in aliquots at -20 ^{0}C for up to 48 h before enzyme assay.

MAO Activity Assay

MAO activity was determined as described by Krajl with some modifications of Soto-Otero *et al.* . Aliquots of samples were incubated at 37 ^{0}C for 5 min in a medium containing buffer solution (Na_2PO_4/ KH_2PO_4 isotonized with KCl, pH 7.4), specific inhibitors, pargyline (a MAO-B inhibitor, 250 nM) or clorgyline (a MAO-A inhibitor, 250 nM) and non-substituted 4-organochalcogen-isoquinoline derivatives **1**, **2** and **3** (final concentrations of 1 to 100 μM) dissolved in DMSO.

Then kynuramine dihydrobromide (final concentration of 90 μM to MAO-A and 60 μM to MAO-B assay) was added to the reaction mixture as substrate. Samples

were then incubated at 37 ^{0}C for 30 min. After incubation, the reaction was terminated by adding of 10% trichloroacetic acid (TCA). After cooling and centrifugation at 16000 *x* g for 5 min, an aliquot of supernatant was added to 1 M NaOH. The fluorescence intensity was measured spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO-A and MAO-B activities were expressed as nmol of 4-hydroxyquinoline formed/mg protein/min.

After carrying out MAO activity assays, the IC_{50} values (half maximal inhibitory concentration) for the non-substituted isoquinoline derivatives **1**, **2**, and **3** were calculated.

Considering that the non-substituted isoquinoline derivative **2** had the best inhibitory profile, the logical next step in our investigation was to further clarify whether the introduction of substituents (methyl **2a**, fluorine **2b**, chloro **2c** and trifluoromethyl **2d**) in the aromatic ring bonded to the selenium atom could alter its inhibitory effect on the enzyme activity. The IC_{50} values were determined for these new 4-organochalcogen-isoquinoline derivatives, at protein concentration about 1.0 mg/ml.

Molecular docking procedures

In order to predict the energies and the mode of interaction between isoquinoline derivatives and MAO-B an *in silico* molecular docking study was carried out. The crystal structure of human MAO-B (in complex with the selective inhibitor 7-(3-chlorobenzyloxy)-4-(methylamino) methyl-coumarin, C18) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/) with PDB code 2V61 . Previously, water molecules from MAO-B crystal structure were removed, followed by addition of missing hydrogen atoms and calculation of AM1-BCC partial charges . After that, the geometry of the enzyme structure was optimized using the program UCSF Chimera using AM1-BCC partial charges (1000 steps). The structure of ligands (isoquinoline derivatives) were constructed using the program Avogadro 0.9 followed by geometry optimization with the Universal Force Field (UFF) partial charges and, subsequently, PM6 semi-empirical quantum method from MOPAC2012 . Partial charges for FAD cofactor were also calculated using PM6 method.

For docking procedure, ligands and macromolecules were previously prepared using AutoDock Tools 4.2 . In this first step, nonpolar hydrogens were merged and the charges previously calculated for MAO-B structure and ligands during optimization were maintained. Molecular flexible docking simulations were carried out using AutoDock Vina 1.1.1 program . All rotatable bonds within the ligands were allowed to rotate freely. The side chain from residues Ile199, Ile316, Tyr326, Phe168, Gln206 and Trp119 were maintained flexible while the others residues were kept rigid. The grid was centered in the active site of human MAO-B at coordinates x = 50.916; y = 155.621 and z = 28.872, with dimensions of the grid box: 20 Å × 20 Å × 20 Å. The spacing was consisted of 1 Å and the exhaustiveness was set of 200. All other docking parameters were used as defaults. For each docking result, the conformation with the lowest binding free energy was accepted as the most probable model of interaction. The conformations and ligand-enzyme interactions were analyzed using the programs Accelrys Discovery Studio Visualizer v.3.5.

Compounds **2** and **2b** were chosen to carry out the kinetic studies based on the results obtained with MAO-B activities and molecular docking.

Inhibition Kinetics

The isoquinoline derivatives **2** and **2b** at concentrations equal to their IC_{50} values**,** and different concentrations of kynuramine dihydrobromide (5-160 μM) were incubated with mitochondrial fraction for evaluating of the MAO-B activity , the type of inhibition exhibited by compounds was determined by measuring the apparent V_{max} and apparent K_m values. These were subsequently compared to the apparent V_{max} an apparent K_m values recorded in the absence of inhibitor.

Reversibility of MAO-B Inhibition by Dialysis

The reversibility of MAO-B inhibition by isoquinoline derivatives **2** and **2b** were determined by dialysis according to Harfenist *et al.* with some modifications proposed by Sant'Anna *et al.* . The membrane of dialysis was pretreated as follows: first, the membranes were washed in distilled water at 80 ^{0}C for 15 min. Subsequently, they were washed in 10 mM $NaHCO_3$ for 30 min, followed with four washes in 10 mM ethylenedinitrilo-tetra acetic acid disodium salt (EDTA) for 30 min each. Finally, the membranes were again washed in distilled water at 80 ^{0}C for 30 min and then stored in 40% ethanol solution under refrigeration.

On the day of the experiment, the membranes were washed with distilled water and buffer solution (Na_2PO_4 0.0168 M/KH_2PO_4 0.0106 isotonized with KCl 0.0036 M, pH 7.4). The mixtures of mitochondrial preparation with or without isoquinoline derivatives **2** or **2b** (at IC_{50} concentrations) were dialyzed at room temperature. A 1 ml portion of each mixture was then dialyzed with shaking vs. 40 ml outer buffer (Na_2PO_4 0.0168 M/KH_2PO_4 0.0106 isotonized with KCl 0.0036 M added of dithiotreitol 1 mM, pH 7.4). Samples were collected for testing at 2, 4, 6, and 24 hours after starting dialysis. The outer buffer was changed at 2, 4, and 6 hours after the start, and dialysis was finished at 24 hours. The reversibility of MAO-B inhibition was defined based upon the report by Harfenist *et al.* .

Protein Determination

The protein concentration was measured by the method of Bradford , using bovine serum albumin as the standard.

Statistical Analysis

Statistical analysis of data from MAO activity and inhibition kinetics were performed using one-way ANOVA (analysis of variance), followed by post hoc Student-Newman-Keuls multiple comparison test. Data from the reversibility of MAO inhibition were analyzed by repeated measures two-way (Time x Isoquinoline) followed by post hoc Bonferroni test. Apparent K_m and apparent V_{max} values were calculated by nonlinear regression using a logarithmic function of the type sigmoid. Data were expressed as means ± S.E.M. Values of $P < 0.05$ were considered statistically significant. All analyses were performed using the “GraphPad Software” (GraphPad, San Diego, CA, USA).

IC_{50} values were calculated by linear regression from individual experiments using “GraphPad Software” (GraphPad software, San Diego, CA, USA). The IC_{50} values were reported as means accompanied by their 95% confidence limits.

Results

MAO Activity

Non-substituted 4-organochalcogen-isoquinoline derivatives **1**, **2** and **3** did not inhibit MAO-A activity. By contrast, isoquinoline derivative **3** at concentrations equal

or greater than 10 μM increased MAO-A activity (Table 1). Non-substituted isoquinoline derivatives **1**, **2** and **3** at concentrations equal to or greater than 25, 10, and 100 μM, respectively, inhibited MAO-B activity. The IC_{50} values (**1** = 72.80 ± 11.21 μM; **2** = 36.41 ± 12.15 μM; **3** did not inhibit 50% of the enzyme activity up to concentration of 100 μM) demonstrated that compound **2** had the highest potency when compared to the other non-substituted isoquinoline derivatives tested (Table 1).

The obtained data showed that the introduction of substituent groups in the aromatic ring bonded to the selenium atom did not affect MAO-A activity (Table 2). Besides, none of substituents added to the aromatic ring bonded to the selenium atom improved the inhibitory effect demonstrated by the non-substituted isoquinoline derivative **2** on MAO-B activity. In contrast, the presence of substituents in the aromatic ring bonded to the selenium atom seems to decrease the potency of inhibition since only the substituted isoquinoline derivative **2b** showed IC_{50} lower than the concentration of 100 μM (81.64 ± 24.65) (Table 2).

In silico molecular docking

AutoDock Vina is a free-academic docking program that operates by pairing an empirically-weighted scoring function containing terms for values such as hydrogen bonding, van der Waals interactions, rotatable bond penalties, and a sophisticated gradient-based local search as a global optimization algorithm . In order to ensure the efficiency of AutoDock Vina program in proposing a reliable molecular model, a previous redocking of the co-crystalized ligand C18 in the active site of MAO-B (PDB code 2V61) was performed. The best conformation of the crystal ligand C18 proposed by redocking was almost identical with the original conformation from human MAO-B 2V61, showing a root mean square deviation (RMSD) of 2.0 Å (lowest ΔG_{bind} = –6.6 kcal.mol^{-1}). Here we can infer that AutoDock Vina program could be used in this study for an accurate prediction of enzyme-ligand interactions, since a RMSD value ≤ 2.0 Å is a condition often used for the correct bound structure prediction .

The lowest predicted binding free energies (ΔG_{bind}) and the mean obtained by all conformations from each isoquinoline derivative compound binding MAO-B are given in Table 3 and Figure 2. The lowest predicted ΔG_{bind} values for compounds ranged between –6.7 and –3.9 kcal.mol^{-1} (mean of ΔG_{bind} values ranging from –5.04± 0.331 to –2.68 ± 0.415 kcal.mol^{-1}). According to the results of MAO-B inhibition by 4-

organoselenium-isoquinoline derivatives tested, it is reasonable to state that among the non-substituted and substituted to the aromatic ring bonded to the selenium atom compounds, the isoquinolines derivatives **2** (IC_{50} value = 36.41 ± 12.15 μM) and **2b** (IC_{50} = 81.64 ± 24.65 μM), respectively, present the best inhibitory effects. For this reason, the next experiments were conducted only with these compounds.

The representative models of interaction proposed by molecular docking for compounds **2** and **2b** are shown in Figure 3. In a similar manner, a crucial role of van de Waals and hydrophobic interactions with aromatic moieties of both compounds and side chains of residues from the human MAO-B active site (Trp119, Phe168, Ile199, Ile316, Tyr326, Tyr398 and flavin moiety of FAD) can be noted, including a CH…π interaction between the carbon-α of Ile199 and the isoquinoline moiety of compounds (distances: 3.76 Å for compound **2** and 2.64 Å for compound **2b**) and two π-π stacking interactions: one involving the aromatic side chain of the residue Tyr398 and the aromatic ring of 4-phenylselenanyl moiety from compound **2** (distance: 4.03 Å) or the aromatic ring of 4-(*p*-fluorophenyl)selenanyl moiety from compound **2b** (distance: 4.14 Å) and other involving the 3-phenyl moiety of both compounds (Figure 3).

Inhibition kinetics

The kinetic profile of compounds **2** and **2b** tested at their IC_{50} values on MAO-B activity is demonstrated in Figure 4. Compounds **2** and **2b** were mixed inhibitors since they decreased apparent V_{max} and increased apparent K_m for MAO-B (Table 4).

Reversibility of MAO-B Inhibition by Dialysis

Results of dialysis are depicted in Figure 5. These data show that compounds **2** and **2b** reversibly inhibited MAO-B activity. The reversal of MAO-B inhibition was observed at six hours after starting the dialysis for isoquinoline derivative **2** and between six and 24 hours for compound **2b**.

Discussion

The results of this study clearly indicate that all 4-organochalcogen-isoquinoline derivatives tested are selective MAO-B inhibitors. It was also demonstrated that the non-substituted isoquinoline derivative **2** was a better MAO-B

inhibitor than compounds **1** and **3**, indicating that the chalcogen atom influences their inhibitory activities. Furthermore, the introduction of different substituents in isoquinoline derivative **2** seems to decrease its inhibitory potency. On the other hand, none of the tested compounds inhibited MAO-A activity and non-substituted isoquinoline derivative **3** increased the activity of this enzyme isoform.

One plausible explanation for this fact is that despite both MAO-A and MAO-B share approximately 70% sequence identities, they differ considerably in the structures of their sites opposite the flavin cofactor. MAO-A has a monopartite cavity of ~550 $Å^3$, and MAO-B exhibits a bipartite cavity structure with an entrance cavity of 290 $Å^3$ and a substrate cavity of ~400 $Å^3$. MAO-A inhibitors generally are planar molecules substituted by hydroxyl functions, whereas MAO-B inhibition depends on the size of planar molecules and distribution of electron density for the selective inhibition . In addition, site-directed mutagenesis studies and crystallographic data on MAO isoforms have identified the importance of Phe208 in MAO-A and the corresponding Ile199 in MAO-B for substrate selectivity .The residue Ile199, exclusive from MAO-B, plays an essential role as a conformational “gate” separating the two cavities, determinant for the specificity of the MAO-B inhibitors . Moreover, an aromatic cage (or “aromatic sandwich”) formed by the residues Tyr398 and Tyr345 near from flavin moiety of FAD acts as an important recognition site for the substrate amino group .

Regarding the selective activity of isoquinoline derivatives to MAO-B, the molecular docking results demonstrated that the interaction of isoquinoline moiety of compounds close to residue Ile199 via CH…π non-covalent interaction seems to be essential to the specific binding to MAO-B, also including the close contact of 4-phenylchalcogenanyl moiety with the aromatic cage Tyr398 via π- π stacking interaction. The fact that the non-substituted isoquinoline derivative **2** was better MAO-B inhibitor than the compound **1** could be explained by the inductive effect exercised by the chalcogen atom in the quinoline ring. In other words, the cloud of electrons in a sigma bond between two different atoms is not uniform and is slightly shifted toward the more electronegative of the two atoms. Considering that the sulfur atom is more electronegative than the selenium atom, the sulfur atom attracts the cloud of electrons letting the quinoline ring less reactive, which could account for the lower MAO-B inhibitory activity shown by compound **1**. Concerning the lesser MAO-B inhibitory potential of the non-substituted isoquinoline derivative **3**, it could be

explained by the fact that the coupling between MAO-B enzyme and compound **3** could be hindered by steric effects since the tellurium atom shows a large atomic radius than selenium.

The highest inhibitory potential shown by the non-substituted isoquinoline derivative **2** in MAO-B activity motivated us to investigate the effect of the addition of substituents in the aromatic ring bonded to the selenium atom of isoquinoline derivative **2**. The obtained 4-organoselenium-isoquinoline derivatives substituted with methyl **2a**, fluorine **2b**, chloro **2c** and trifluoromethyl **2d** were tested on MAO activity aiming to find a possible better inhibitory profile. Similar to data demonstrated with non-substituted isoquinoline derivatives, MAO-A activity was not altered independent of the nature of the substituent tested. Regarding MAO-B activity, the isoquinoline derivative substituted in the aromatic ring bonded to the selenium atom **2b** was the best inhibitor. However, when comparing the inhibitory effect on MAO-B activity *in vitro*, the non-substituted compound **2** was a more potent inhibitor than the substituted compound **2b** (compare IC_{50} values of **2** = 36.41 ± 12.15 μM and **2b** = 81.64 ± 24.65 μM).

Based on the fact that the compound containing the highest degree of steric hindrance (**2d**) showed the lowest MAO-B inhibitory effect, we believe that changes in MAO-B activity could be due to the stepwise substitution of the hydrogen on the aromatic ring bonded to the selenium atom by fluorine, methyl, chloro, or trifluoromethyl group, which increases gradually the steric hindrance . This suggests that large substituents hinder the interaction between compound and enzyme and consequently, the enzyme inhibition.

Compounds **2** and **2b** were chosen to carry out the profile kinetics studies by present the best inhibitory effects on MAO-B among the non-substituted and substituted to the aromatic ring bonded to the selenium atom compounds, respectively.

Concerning the inhibition kinetics, compounds **2** and **2b** demonstrated a mixed mechanism of inhibition, by decreasing apparent V_{max} and increasing apparent K_m. The mixed inhibition is a type of non-competitive inhibition, in which a compound can interact with the free enzyme and the enzyme-substrate complex at a site other than the active site . Binda *et al.* demonstrated that MAO has an active site for the binding substrate, a membrane domain plus a binding flavine domain, in which the classic inhibitors bind. The kinetics and molecular docking results suggest that these

compounds interact with the enzyme as classic inhibitors. In addition, their binding affinities with MAO-B seem to be similar of known MAO-B inhibitors, such as benzylamine and phenethylamine .

Data from dialysis experiments were analyzed following the protocol established by Harfenist *et al.* . In this protocol, the reversibility of MAO activity up to 24 hours was evaluated as follows: 0-20% recovery of MAO activity indicates irreversible binding; 20-80% represents a partially reversible binding; and values higher than 80% suggest a reversible binding. Therefore, the results obtained by us revealed that compounds **2** and **2b** were MAO-B reversible inhibitors, although differences in the inhibition profile of the tested compounds exist. The non-substituted isoquinoline derivative **2** demonstrated recover of MAO-B activity higher than 80% at six hours after starting dialysis, whereas the derivative substituted in the aromatic ring bonded to the selenium atom **2b** showed reversal of inhibition in the period between six to 24 hours. As previously mentioned, the MAO-B inhibition depends on the distribution of electron density . Accordingly, the fluorine atom is an electron-withdrawing group, which may influence the strength and/or stability of the binding with the enzyme. Compounds **2** and **2b** showed a fundamental feature for a new MAO inhibitor, which suggests the third generation of MAO inhibitors, combining reversibility and selectivity. Reversible and selective MAO-B inhibitors have been reported as compounds that avoid the “cheese effect”, which occurs when an irreversible MAO inhibitor is administered together with foods containing tyramine, leading to an acute hypertensive crisis .

MAO is a therapeutic target for treatment of depressive disorders, including atypical, chronic, and double depressions . Moreover, some authors have reported the involvement of MAO in neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases . However, the use of MAO inhibitors had seen restrict due to reports of acute hypertension after ingestion of tyramine, mainly with the use of irreversible MAO-A inhibitor, and toxic interactions with some drugs. Nowadays, the four MAO inhibitors currently in clinical use in the United States are irreversible inhibitors: tranylcypromine, isocarboxazid, selegiline, and phenelzine . Both MAO-A and MAO-B oxidized tyramine, but the isoform A is responsible for metabolizing the dietary tyramine, in the gastrointestinal tract . Selegiline is an irreversible MAO-B inhibitor at low oral doses, been used with safety and efficacy as adjunct treatment for Parkinson disease and still has antidepressant effect, but its

selectivity is lost at higher doses . In this context, compounds **2** and **2b** demonstrated an advantageous inhibitory profile since they show selective and reversible MAO-B inhibition. Nevertheless, more studies are necessary in order to clearly elucidate a possible use of these 4-organoselenium-isoquinoline derivatives as alternatives for the treatment of depressive and neurodegenerative diseases.

Conclusion

All tested 4-organochalcogen-isoquinoline derivatives inhibited MAO-B isoform activity and none of them inhibited MAO-A activity, pointing to the fact that all tested 4-organochalcogen-isoquinoline derivatives are selective MAO-B inhibitors. Regarding the inhibitory potency *in vitro*, compound **2** showed higher inhibitory potential when compared to the non-substituted isoquinoline derivatives tested **1** and **3**. Additionally, the introduction of different substituents in the aromatic ring bonded to the selenium atom of isoquinoline derivative **2** decreased its inhibitory potency. Both isoquinoline derivatives **2** and **2b** demonstrated mixed and reversible inhibition. The interactions proposed by molecular docking for compounds **2** and **2b** were found to be in full agreement with the selective inhibition of MAO-B *in vitro*. The selenocompounds tested showed as advantage to fit in the profile of third generation MAO inhibitors (selective and reversible), which are promising alternatives for the treatment of several disorders. Nonetheless, considering that these are the first results concerning these 4-organochalcogen-isoquinoline derivatives, more studies are necessary in order to clearly elucidate their possible use as therapeutic alternatives.

Conflict of interest

The authors declare they have no conflicts of interest to disclose.

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References

[1] Wouters J. Structural aspects of monoamine oxidase and its reversible inhibition. Curr Med Chem 1998;5:137-62.

[2] Knoll J, Magyar K. Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv Biochem Psychopharmacol 1972;5:393-408.
[3] Johnston JP. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem Pharmacol 1968;17:1285-97.
[4] Gaweska H, Fitzpatrick PF. Structures and Mechanism of the Monoamine Oxidase Family. Biomol Concepts 2011;2:365-77.
[5] Benedetti MS, Dostert P. Stereochemical Aspects of Mao Interactions - Reversible and Selective Inhibitors of Monoamine-Oxidase. Trends Pharmacol Sci 1985;6:246-51.
[6] Naoi M, Maruyama W, Inaba-Hasegawa K. Type A and B monoamine oxidase in age-related neurodegenerative disorders: their distinct roles in neuronal death and survival. Curr Top Med Chem 2012;12:2177-88.
[7] McNaught KS, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, et al. Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease. Biochemical Pharmacology 1998;56:921-33.
[8] Thull U, Kneubuhler S, Gaillard P, Carrupt PA, Testa B, Altomare C, et al. Inhibition of monoamine oxidase by isoquinoline derivatives. Qualitative and 3D-quantitative structure-activity relationships. Biochem Pharmacol 1995;50:869-77.
[9] Pienaar IS, Lu B, Schallert T. Closing the gap between clinic and cage: sensori-motor and cognitive behavioural testing regimens in neurotoxin-induced animal models of Parkinson's disease. Neurosci Biobehav Rev 2012;36:2305-24.
[10] Gerlach M, Youdim MB, Riederer P. Pharmacology of selegiline. Neurology 1996;47:S137-45.
[11] Katagiri N, Chida S, Abe K, Nojima H, Kitabatake M, Hoshi K, et al. Preventative effects of 1,3-dimethyl- and 1,3-dimethyl-N-propargyl-1,2,3,4-tetrahydroisoquinoline on MPTP-induced Parkinson's disease-like symptoms in mice. Brain Res 2010;1321:133-42.
[12] Makino Y, Ohta S, Tachikawa O, Hirobe M. Presence of Tetrahydroisoquinoline and 1-Methyl-Tetrahydro-Isoquinoline in Foods - Compounds Related to Parkinsons-Disease. Life Sciences 1988;43:373-8.
[13] Niwa T, Yoshizumi H, Tatematsu A, Matsuura S, Nagatsu T. Presence of Tetrahydroisoquinoline, a Parkinsonism-Related Compound, in Foods. J Chromatogr-Biomed 1989;493:347-52.
[14] Niwa T, Takeda N, Sasaoka T, Kaneda N, Hashizume Y, Yoshizumi H, et al. Detection of Tetrahydroisoquinoline in Parkinsonian Brain as an Endogenous Amine by Use of Gas-Chromatography Mass-Spectrometry. J Chromatogr-Biomed 1989;491:397-403.
[15] Kotake Y, Tasaki Y, Makino Y, Ohta S, Hirobe M. A Novel Endogenous Amine, 1-Benzyl-1,2,3,4-Tetrahydroisoquinoline in the Mouse-Brain and Parkinsonian Csf - Relation to Parkinsons-Disease. Journal of Neurochemistry 1995;65:S46-S.
[16] Wasik A, Mozdzen E, Romanska I, Michaluk J, Antkiewicz-Michaluk L. Antidepressant-like activity of the endogenous amine, 1-methyl-1,2,3,4-tetrahydroisoquinoline in the behavioral despair test in the rat, and its neurochemical correlates: a comparison with the classical antidepressant, imipramine. Eur J Pharmacol 2013;700:110-7.
[17] Antkiewicz-Michaluk L, Lazarewicz JW, Patsenka A, Kajta M, Zieminska E, Salinska E, et al. The mechanism of 1,2,3,4-tetrahydroisoquinolines

neuroprotection: the importance of free radicals scavenging properties and inhibition of glutamate-induced excitotoxicity. J Neurochem 2006;97:846-56.

[18] Antkiewicz-Michaluk L, Filip M, Michaluk J, Romanska I, Przegalinski E, Vetulani J. An endogenous neuroprotectant substance, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), prevents the behavioral and neurochemical effects of cocaine reinstatement in drug-dependent rats. J Neural Transm 2007;114:307-17.

[19] Patsenka A, Antkiewicz-Michaluk L. Inhibition of rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds - 1,2,3,4-tetrahydro-isoquinoline alkaloids. Pol J Pharmacol 2004;56:727-34.

[20] Grinberg L, Fibach E, Amer J, Atlas D. N-acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. Free Radical Bio Med 2005;38:136-45.

[21] Nogueira CW, Zeni G, Rocha JBT. Organoselenium and organotellurium compounds: Toxicology and pharmacology. Chemical Reviews 2004;104:6255-85.

[22] Farina M, Folmer V, Bolzan RC, Andrade LH, Zeni G, Braga AL, et al. Selenoxides inhibit delta-aminolevulinic acid dehydratase. Toxicology Letters 2001;119:27-37.

[23] Goeger DE, Ganther HE. Oxidation of Dimethylselenide to Dimethylselenoxide by Microsomes from Rat-Liver and Lung and by Flavin-Containing Monooxygenase from Pig-Liver. Archives of Biochemistry and Biophysics 1994;310:448-51.

[24] Bruning CA, Souza ACG, Gai BM, Zeni G, Nogueira CW. Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. European Journal of Pharmacology 2011;658:145-9.

[25] Savegnago L, Jesse CR, Pinto LG, Rocha JBT, Nogueira CW, Zeni G. Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats. Prog Neuro-Psychoph 2007;31:1261-9.

[26] Tang YL, Wang SW, Lin SM. Both inorganic and organic selenium supplements can decrease brain monoamine oxidase B enzyme activity in adult rats. Brit J Nutr 2008;100:660-5.

[27] Stein AL, Bilheri FN, da Rocha JT, Back DF, Zeni G. Application of Copper(I) Iodide/Diorganoyl Dichalcogenides to the Synthesis of 4-Organochalcogen Isoquinolines by Regioselective C?N and C?Chalcogen Bond Formation. Chem-Eur J 2012;18:10602-8.

[28] Soto-Otero R, Mendez-Alvarez E, Hermida-Ameijeiras A, Sanchez-Sellero I, Cruz-Landeira A, Lamas MLR. Inhibition of brain monoamine oxidase activity by the generation of hydroxyl radicals - Potential implications in relation to oxidative stress. Life Sciences 2001;69:879-89.

[29] Krajl M. A Rapid Microflurimetric Determination of Monoamine Oxidase. Biochemical Pharmacology 1965;14:1684-&.

[30] Binda C, Wang J, Pisani L, Caccia C, Carotti A, Salvati P, et al. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. J Med Chem 2007;50:5848-52.

[31] Jakalian A, Jack DB, Bayly CI. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. J Comput Chem 2002;23:1623-41.

[32] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605-12.
[33] Rappe AK, Casewit CJ, Colwell KS, Goddard WA, Skiff WM. Uff, a Full Periodic-Table Force-Field for Molecular Mechanics and Molecular-Dynamics Simulations. J Am Chem Soc 1992;114:10024-35.
[34] Stewart JJ. Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements. J Mol Model 2007;13:1173-213.
[35] Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform 2009;1:15.
[36] Stewart JJP. MOPAC2012, Stewart Computational Chemistry. USA: Colorado Springs, 2012.
[37] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009;30:2785-91.
[38] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.
[39] Sant' Anna GD, Machado P, Sauzem PD, Rosa FA, Rubin MA, Ferreira J, et al. Ultrasound promoted synthesis of 2-imidazolines in water: A greener approach toward monoamine oxidase inhibitors. Bioorg Med Chem Lett 2009;19:546-9.
[40] Harfenist M, Heuser DJ, Joyner CT, Batchelor JF, White HL. Selective inhibitors of monoamine oxidase .3. Structure-activity relationship of tricyclics bearing imidazoline, oxadiazole, or tetrazole groups. Journal of Medicinal Chemistry 1996;39:1857-63.
[41] Bradford MM. Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. Anal Biochem 1976;72:248-54.
[42] Bursulaya BD, Totrov M, Abagyan R, Brooks CL, 3rd. Comparative study of several algorithms for flexible ligand docking. J Comput Aided Mol Des 2003;17:755-63.
[43] Binda C, Mattevi A, Edmondson DE. Structural properties of human monoamine oxidases A and B. Int Rev Neurobiol 2011;100:1-11.
[44] Medvedev AE, Ivanov AS, Veselovsky AV, Skvortsov VS, Archakov AI. QSAR analysis of indole analogues as monoamine oxidase inhibitors. J Chem Inf Comp Sci 1996;36:664-71.
[45] Tsugeno Y, Ito A. A key amino acid responsible for substrate selectivity of monoamine oxidase A and B. J Biol Chem 1997;272:14033-6.
[46] Hubalek F, Binda C, Khalil A, Li M, Mattevi A, Castagnoli N, et al. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. J Biol Chem 2005;280:15761-6.
[47] Binda C, Newton-Vinson P, Hubalek F, Edmondson DE, Mattevi A. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. Nat Struct Biol 2002;9:22-6.
[48] Begue JP, Bonnet-Delpon D. Recent advances (1995-2005) in fluorinated pharmaceuticals based on natural products. J Fluorine Chem 2006;127:992-1012.

[49] Marangoni AG, editor. Enzyme kinetics: a modern approach. New Jersey: Wiley-Interscience, 2003.
[50] Anderson MC, Hasan F, McCrodden JM, Tipton KF. Monoamine oxidase inhibitors and the cheese effect. Neurochem Res 1993;18:1145-9.
[51] Robinson DS. Monoamine oxidase inhibitors: a new generation. Psychopharmacol Bull 2002;36:124-38.
[52] Riederer P, Danielczyk W, Grunblatt E. Monoamine oxidase-B inhibition in Alzheimer's disease. Neurotoxicology 2004;25:271-7.
[53] Fernandez HH, Chen JJ. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. Pharmacotherapy 2007;27:174S-85S.
[54] Costa MJJ, Moyer M, O'Reardon JP. Monoamine Oxidase inhibitors: An important but underutilized treatment Part I: Efficacy. Psychopharm Rev 2012;47:73-80.
[55] Youdim MBH. The Advent of Selective Monoamine-Oxidase-a Inhibitor Antidepressants Devoid of the Cheese Reaction. Acta Psychiat Scand 1995;91:5-7.

Figure Captions

Fig. 1 Chemical structures of 4-organochalcogen-isoquinoline derivatives tested: 3-phenyl-4-(phenylthio) isoquinoline **1**, 3-phenyl-4-(phenylseleno) isoquinoline **2**, 3-phenyl-4-(phenyltelluro) isoquinoline **3**, 4-(4-methylphenylseleno)-3-phenylisoquinoline **2a**, 4-(3-fluorophenylseleno)-3-phenylisoquinoline **2b**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline **2c**, and 4-(4-trifluoromethylphenylseleno)-3-phenylisoquinoline **2d.**

Fig. 2 Box-and-whisker plot with mean of predicted docking binding free energies (ΔG_{bind}) from conformations of isoquinoline-derivative compounds in complex with human MAO-B (PDB id: 2V61). The cross (+) inside the box denotes in y-axis the mean value of ΔG_{bind} and the lowest horizontal bar indicates in y-axis the lowest ΔG_{bind}, according to results performed by AutoDock Vina 1.1 program.

Fig. 3 Representative molecular docking with lowest energy for compounds **2** (A) and **2b** (B) in the active site of human MAO-B (PDB id: 2V61). Green dot lines denote CH…π and π-π stacking interactions. Green ball inside the aromatic structures denotes its centroid. All the amino acid residues which are involved in close contacts to inhibitors are shown in stick drawing. Inhibitors are centered and shown in stick drawing. For a best clarification, non-polar hydrogen atoms are hidden. Atoms in sticks are colored as follows: hydrogen white, carbon gray, oxygen red, fluorine cyan, nitrogen purple, sulfur yellow and selenium orange. Docking results were analyzed and figures were obtained using Accelrys Discovery Studio Visualizer v.3.5 program.

Fig. 4 MAO-B activity inhibition kinetics showed by isoquinoline derivatives **2** and **2b** at IC_{50}. The results were demonstrated as means $\pm$ S.E.M. of 3-4 individual experiments. **2**, 3-phenyl-4-(phenylseleno) isoquinoline; **2b**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline.

Fig. 5 Reversibility by dialysis of MAO-B activity inhibition by isoquinoline derivatives **2** and **2b** at IC_{50}. Data were expressed as means $\pm$ S.D. of 3-4 individual experiments. The values found were considered statistically significant when $*P <$

0.05 (Two-way ANOVA/Bonferroni) compared with the control. **2**, 3-phenyl-4-(phenylseleno) isoquinoline; **2b**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline.

Tables

Table 1: Effect of non substituted isoquinoline derivatives **1**, **2**, and **3** on cerebral MAO-A and B activities.

MAO-A	**Isoquinoline derivatives (µM)**						
	0	**1**	**5**	**10**	**25**	**50**	**100**
1	5.05 ± 0.01	6.04 ± 0.90	5.95 ± 0.58	6.20 ± 0.64	5.85 ± 1.01	5.29 ± 1.20	4.02 ± 0.57
2	6.51 ± 0.01	6.66 ± 0.44	6.61 ± 0.45	7.05 ± 0.36	6.85 ± 0.20	5.90 ± 0.71	6.20 ± 0.45
3	5.06 ± 0.01	5.78 ± 0.32	6.01 ± 0.40	7.91 ± 0.98*	8.25 ± 0.39*	7.93 ± 0.90*	7.39 ± 0.48*
MAO-B							
1	17.84 ± 0.02	17.65 ± 0.83	15.43 ± 1.05	15.71 ± 0.86	13.11 ± 0.58**	11.92 ± 0.80***	6.07 ± 0.77***
2	18.50 ± 0.01	17.54 ± 1.07	15.46 ± 0.67	12.70 ± 1.98**	10.63 ± 0.64***	8.20 ± 0.49***	7.37 ± 0.47***
3	18.84 ± 0.01	17.54 ± 0.81	16.86 ± 0.78	16,19 ± 0.60	14.27 ± 1.82	13.96 ± 2.24	11.67 ± 0.51**

The data were expressed as means ± S.E.M. of 3-4 individual experiments. MAO activity was expressed as nmol of 4-hydroxyquinoline/mg protein/minute. The values found were considered statistically significant when $*P < 0.05$ $**P < 0.01$ $***P < 0.001$ (One – way ANOVA followed of Student-Newman-Keuls test). **1**, 3-phenyl-4-(phenylthio) isoquinoline; **2**, 3-phenyl-4-(phenylseleno) isoquinoline; **3**, 3-phenyl-4-(phenyltelluro) isoquinoline.

Table 2: Effect of isoquinoline derivatives substituted in the aromatic ring bounded to the selenium atom **2a**, **2b**, **2c**, and **2d** on cerebral MAO-A and B activities.

MAO-A	**Isoquinoline derivatives (µM)**						
	0	**1**	**5**	**10**	**25**	**50**	**100**
2a	6.60 ± 0.02	7.61 ± 0.81	7.79 ± 0.50	8.08 ± 0.16	7.65 ± 0.63	7.42 ± 0.58	8.02 ± 0.45
2b	7.51 ± 0.13	8.15 ± 0.21	8.20 ± 0.34	9.00 ± 0.34	8.72 ± 0.40	8.63 ± 0.51	8.48 ± 0.83
2c	7.23 ± 0.12	8.22 ± 0.93	8.69 ± 1.09	7.82 ± 0.45	8.22 ± 0.71	8.92 ± 1.33	7.77 ± 0.84
2d	8.03 ± 0.03	10.08 ± 0.63	10.09 ± 1.67	10.38 ± 2.16	10.53 ± 1.56	8.87 ± 1.47	9.38 ± 2.58
MAO-B							
2a	19.24 ± 0.01	17.21 ± 0.48	17.70 ± 0.67	17.64 ± 0.44	17.04 ± 0.21	15.01 ± 0.32**	12.55 ± 0.24***
2b	19.40 ± 0.01	18.97 ± 0.49	17.63 ± 0.34	16.23 ± 0.50	14.90 ± 0.15***	12.25 ± 0.48***	7.33 ± 1.16***
2c	18.40 ± 0.02	18.47 ± 0.61	19.32 ± 0.12	17.38 ± 0.47	15.72 ± 0.23	13.16 ± 1.47**	12.91 ± 1.31**
2d	17.43 ± 0.01	17.53 ± 1.52	16.91 ± 0.58	15.42 ± 1.06	14.77 ± 0.82	11.79 ± 1.23*	11.94 ± 1.10*

The data were expressed as means ± S.E.M. of 3-4 individual experiments. MAO activity was expressed as nmol de 4-hydroxyquinoline/mg protein/minute. The values found were considered statistically significant when $*P < 0.05$ $**P < 0.01$ $***P < 0.001$ (One – way ANOVA followed by Student-Newman-Keuls test). **2a**, 4-(4-methylphenylseleno)-3-phenylisoquinoline; **2b**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline; **2c**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline; **2d**, 4-(3-trifluoromethylphenylseleno)-3-phenylisoquinoline.

Table 3: Predicted docking binding free energies (ΔG_{bind}) from conformations of isoquinoline derivatives in complex with human MAO-B (PDB id: 2V61).

	Number of conformations	Mean of all predicted ΔG_{bind} (kcal.mol^{-1})[a]	Lowest ΔG_{bind} (kcal.mol^{-1})
1	10	-5.04 ± 0.331	-6.70
2	7	-5.01 ± 0.334	-6.60
2a	11	-5.03 ± 0.280	-6.60
2b	9	-2.97 ± 0.308	-4.50
2c	7	-2.68 ± 0.415	-3.90
2d	7	-3.24 ± 0.401	-4.60

[a] The data are expressed as mean ± SEM. The conformations were chosen according to the error of energy prediction from AutoDock Vina, which is 2.85 kcal.mol^{-1} (Trott and Olson, 2010).

Table 4: Inhibition kinetics showed by isoquinoline derivatives **2** and **2b**.

	Control	2	2b
Apparent $V_{máx}$ [a]	9,444 ± 0,464	7,234 ± 0,314*	7,925 ± 0,464*
Apparent K_m [b]	5,976 ± 1,396	16,400 ± 2,413 *	14,380 ± 2,974*

The results were demonstrated as means ± S.E.M. of 3-4 individual experiments. The values found were considered significant when *P < 0.05 (One-way ANOVA followed by Student-Newman-Keuls test). [a] represents ηmol de 4-hydroxyquinoline/mg protein/minute and [b] represents mM. **2**, 3-phenyl-4-(phenylseleno) isoquinoline; **2b**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline.

Figures

Figure 1

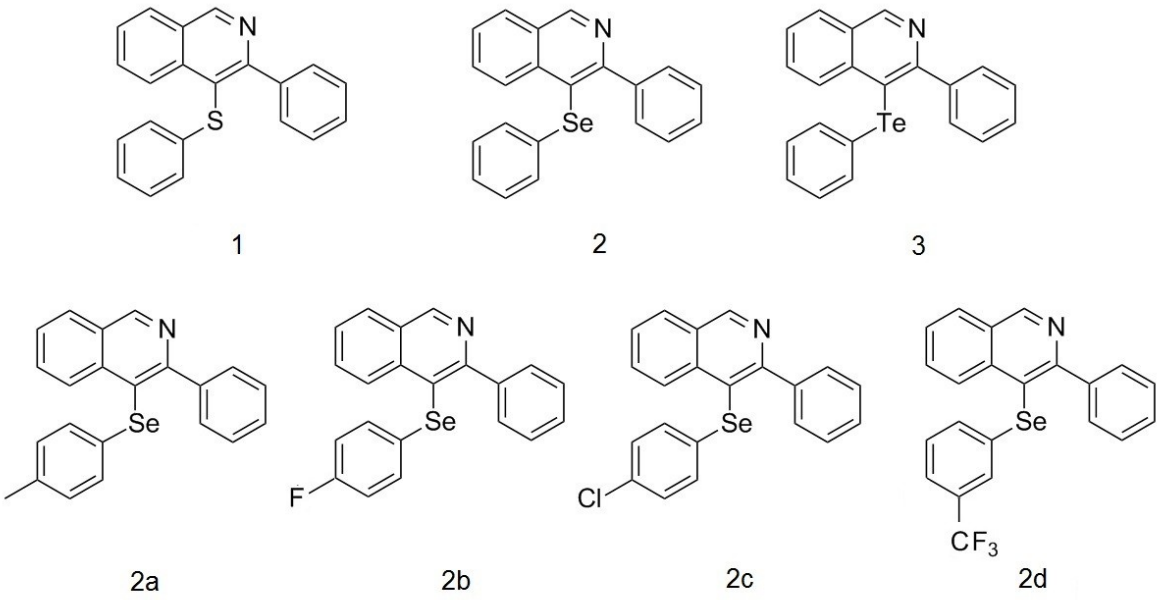


Figure 2

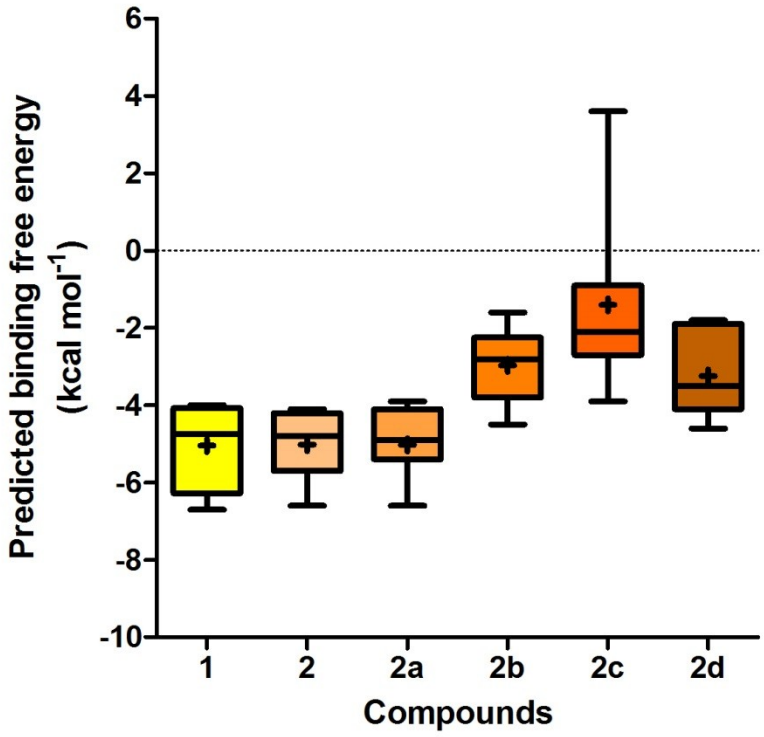


Figure 3

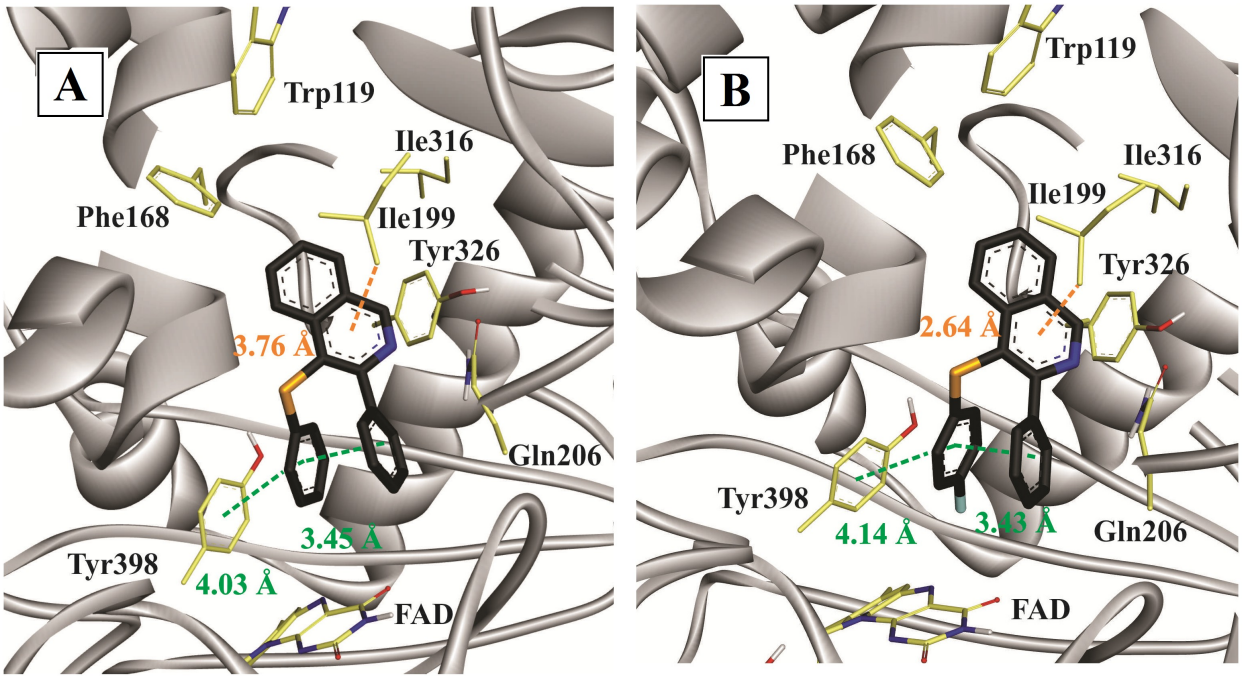


Figure 4

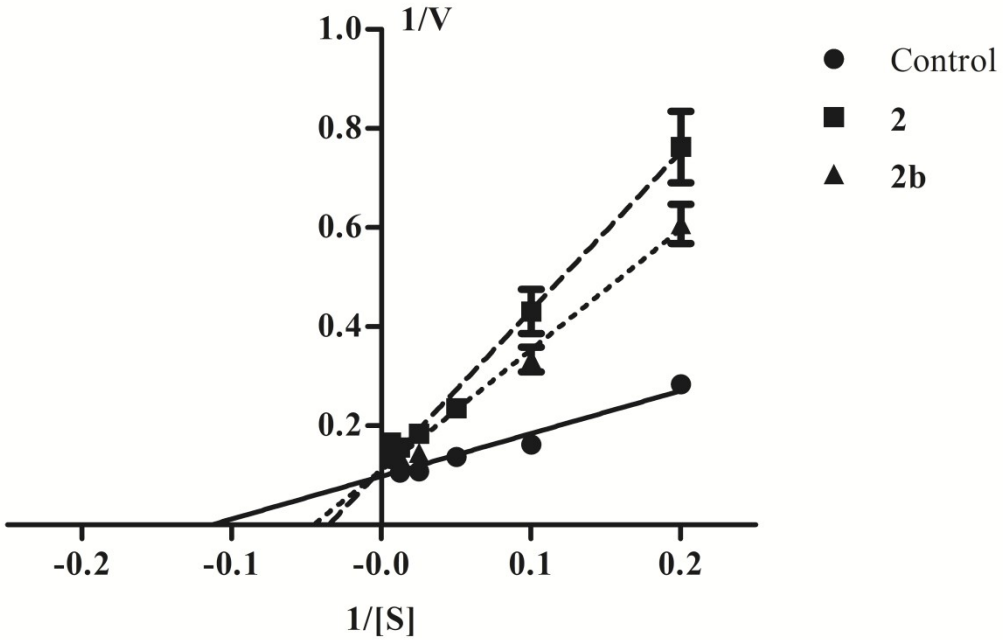
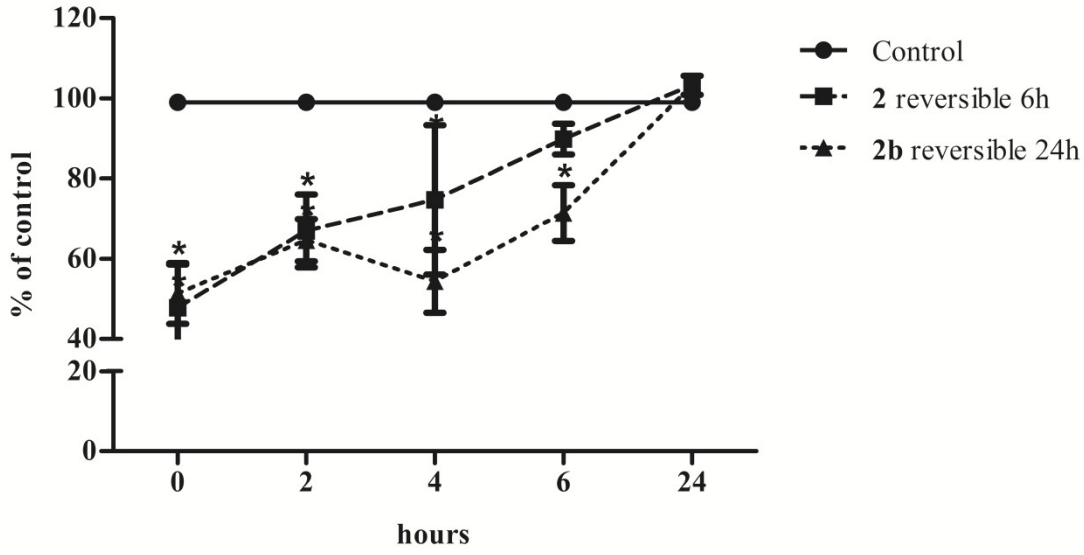


Figure 5


120
100
80
60
40
20
0
% of control
0
2
4
6
24
hours
Control
2 reversible 6h
2b reversible 24h

3.2 Manuscrito 2

4-Organoseleno-isoquinolines effects *in vitro* on cerebral δ-aminolevulinate dehydratase and Na^+, K^+-ATPase activities

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Abstract

The toxicity of organoselenium compounds and isoquinoline derivatives is linked to pro-oxidants situations induced by these compounds. δ-Aminolevulinic acid dehydratase (δ-ALA-D) can be considered a marker of oxidative stress, due to the high sensibility to oxidizing agents. Similarly to δ-ALA-D, Na^+, K^+-ATPase has sulfhydryl groups susceptible to oxidation. Therefore, this study aimed to evaluate the *in vitro* toxicology potential of new 4-organoseleno-isoquinoline derivatives. Cerebral δ-ALA-D and Na^+, K^+-ATPase activities were determined and the involvement of suphydryl groups in 4-organoseleno-isoquinoline derivatives effect was investigated. Compounds substituted with fluoro (**3**), chloro (**4**) and trifluoromethyl (**5**) at the selenium-bonded aromatic ring inhibited δ-ALA-D (IC_{50} values: 78.42, 92.27, 44.98 μM, respectively) and Na^+, K^+-ATPase (IC_{50} values: 41.36, 89.43, 50.66 μM, respectively) activities, possibly due to electronic effects induced by these groups. Compounds **1** and **2** did not alter the activity of these enzymes. Dithiothreitol restored the enzymatic activities inhibited by **3**, **4** and **5**, suggesting the involvement of sulfhydryl residues. However, the release of essential zinc seems not to be related to the δ-ALA-D inhibition by compounds **3**, **4** and **5**. According to the *in vitro* data, effect of acute oral administration of compound **1** (300 mg/kg, intragastric) on markers of systemic toxicity in rats was evaluated. None signs of toxicity was observed during or after treatment. In conclusion, the present study suggests that the insertion of electron-withdrawing groups in the aromatic ring bonded to the selenium atom of compound **1** increased its potential inhibitory in sulfhydryl enzymes. Furthermore, toxic signs did not exhibit *in vivo* and *ex vivo* by rats treated with compound **1**. Although more studies are necessary about this specific compound, our findings suggest that compound **1**, that have pharmacological properties, is a safe compound.

Keywords: δ-ALA-D, Isoquinoline, Na^+, K^+-ATPase**,** Selenium, Sulfhydryl groups.

Introduction

Selenium (Se) is an essential trace element for human health. It stands out by presenting both structural and enzymatic roles in selenoproteins, such as glutathione peroxidase, an important antioxidant enzyme . Se is an atom belonging to the chalcogen group, the same as the elements tellurium and sulfur, for this reason they share some physical and chemical properties. In the chemical form of a selenolate (R-Se-) it presents itself as a soft and strong nucleophile. Due this behavior, Se can be considered a kind of “supersulfur”, having a stronger reducing power than the analogue sulfhydryl group . Several organoselenium compounds have demonstrated pharmacological properties as antioxidant , antinociceptive and anti-inflammatory , antidepressant , anti-hyperglycemic , antibacterial and antifungal , antiepileptic and nootropic .

Nevertheless, the long-term overexposure to dietary Se is associated with increased risk to develop diabetes type II , amyotrophic lateral sclerosis and some types of cancer . Moreover, gastrointestinal disturbances, hair and nail changes, and neurologic manifestations can be caused by a high ingest of Se . Although the environmental toxicity of Se in humans is rare, the use of chalcogen compounds in organic synthesis, industry and in agriculture can increase human exposure risk to Se .

The exact mechanism underlying Se toxicity is still not completely elucidated. However, studies suggest that the oxidation of endogenous low- and high-molecular-weight sulphydryl groups catalyzed by organic and inorganic forms of Se and the production of reactive oxygen species (ROS) generated from this reaction could mediate the toxicity of this element . For instance, reduced sulfhydryl residues present in proteins are targets to organoselenium compounds, which can result in sulfhydryl oxidation to disulfides and, in the case of enzymes, to decrease its catalytic activity .

Isoquinolines are heterocyclic aromatic organic compounds formed of a benzene ring fused at face (*c*) to a pyridine ring . These compounds were found in the brain exhibiting both neuroprotective and neurotoxic actions. Endogenous neurotoxins such as 1,2,3,4-tetrahydroisoquinoline (TIQ), 1-benzyl-TIQ and 1-methyl-5,6-dihydroxy-TIQ produce Parkinsonism by initiate apoptotic process in dopaminergic neurons by the generation of free radicals . On the other hand, the 4

-organoseleno-isoquinoline derivatives tested in this study are selective and reversible inhibitors *in vitro* of the isoform B of cerebral monoamine oxidase (MAO-B). In this context, the toxicology of these MAO-B inhibitors represents an important point to be investigated since they could become therapeutic strategies to disorders which have this enzyme as therapeutic target.

δ-Aminolevulinic acid dehydratase (δ-ALA-D) can be considered an marker of oxidative stress, due to high sensibility to pro-oxidants situations . This enzyme presents vicinal cysteinyl residues in its active site coordinate of essential Zn (II) ions and the proximity between them makes the enzyme particularly sensitive to oxidation . Nowadays, δ-ALA-D inhibition has been linked to pathophysiological conditions, such as cervical cancer and diabetes type 1 and 2 . Similarly δ-ALA-D, Na^+, K^+-ATPase has sulfhydryl groups essentials for its activity and susceptible to oxidizing agents. This enzyme regulates the cellular Na^+/K^+ concentrations and hence their gradients across the plasma membrane, which are required for vital functions such as membrane co-transports, cell volume regulation and membrane excitability .

Considering the above mentioned, this study aimed to evaluate the *in vitro* toxicology potential of new 4-organoseleno-isoquinoline derivatives. δ-ALA-D and Na^+, K^+-ATPase activities were determined and the involvement of suphydryl groups in 4-organoseleno-isoquinoline derivatives effect was investigated in rat brain homogenates.

Materials and Methods

Chemicals

4-Organoseleno-isoquinoline derivatives were prepared and characterized by the method previously described . Analysis of the ^1H NMR and ^{13}C NMR spectra showed that obtained isoquinoline derivatives presented analytical and spectroscopic data in full agreement with their assigned structures. The chemical purity of compounds (99.9%) was determined by GC/MS. For the experiments, isoquinoline derivatives were dissolved in dimethylsufoxide (DMSO). Figure 1 shows 4-organoseleno-isoquinoline derivatives tested: 3-phenyl-4-(phenylselenyl) isoquinoline **1**; 4-(4-methylphenylselenyl)-3-phenylisoquinoline **2**; 4-(4-fluorophenylselenyl)-3-phenylisoquinoline **3**; 4-(4-chlorophenylselenyl)-3-phenylisoquinoline **4**, and 4-[(3-trifluoromethyl)phenylselenyl]-3-phenylisoquinoline **5**.

δ-Aminolevulinic acid (δ-ALA), dithiothreitol (DTT), ouabain and adenosine triphosphate (ATP) were obtained from Sigma (St. Louis, MO, USA). All the other chemical reagents utilized for biochemistry assays were obtained from standard commercial suppliers.

Animals

Male and female adult Wistar rats (200-300 g) from our own breeding colony were used for *in vitro* and *ex vivo* experiments, respectively. The animals were kept in a separate animal room, on a 12 h light/dark cycle, at a room temperature of 22 ± 2 ^{0}C, with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animals Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

Tissue Preparation for enzymatic assays

Animals were killed by decapitation and brain was quickly removed and placed on ice. Brain was homogenized in 50 mM Tris/HCl, pH 7.4 1:5 and 1:10 (w/v) for δ-ALA-D and Na^{+}, K^{+}-ATPase activities, respectively. The homogenates were centrifuged at 2.400 ×g for 10 min at 4 ^{0}C. The supernatant (S1) was used for the enzyme assays. For each assay 3-4 independent experiments were performed in duplicate, in different days, using different animals.

δ-ALA-D activity

δ-ALA-D activity was assayed according to the method described by Sassa , with some modifications. The principle of this method is based on the enzyme incubation with an excess of δ-ALA. For the *in vitro* experiment, an aliquot of 200 µl of the S1 was pre-incubated for 10 min at 37 ^{0}C in the presence of 4-organoseleno-isoquinoline derivatives at different concentrations (1-100 µM). Enzymatic reaction was initiated by adding the substrate (δ-ALA) to a final concentration of 2.2 mM in a medium containing 45 mM phosphate buffer, pH 6.8 and incubated for 1 h for liver and kidney and 3 h for brain, at 37 ^{0}C. The incubation was stopped by adding trichloroacetic acid solution (10 % TCA) with 10 mM $HgCl_2$. The product of the reaction, phorphobilinogen (PBG), is mixed with modified Erlich's reagent, and the developed color is measured spectrophotometrically (555 nm) against a blank.

Effect of DTT and $ZnCl_2$ as restoring agents for δ-ALA-D inhibition

Aiming to study the effect of DTT and $ZnCl_2$ on reversing δ-ALA-D inhibition, 4-organoseleno-isoquinoline derivatives, at IC_{50} (half maximal inhibitory concentration) concentration, were pre-incubated with S1 for 10 min at 37 ^{0}C. After this time, the reaction was started by the addition of substrate in the presence of 3 mM DTT or 100 mM $ZnCl_2$ and incubated for 3 h at 37 ^{0}C. The incubation was stopped by adding 10 % TCA with 10 mM $HgCl_2$. The porphobilinogen is mixed with modified Erlich's reagent and measured as described above.

Na^+, K^+-ATPase activity

Na^+, K^+-ATPase activity was determined according to the method described by Wyse et al. , with some modifications. The reaction mixture for cerebral Na^+, K^+-ATPase activity assay contained 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, and 50 mM Tris/HCl, pH 7.4, in a final volume of 500 µl. An aliquot of 50 µl of S1 was pre-incubated at 37 ^{0}C for 10 min in the presence of 4-organoseleno-isoquinoline derivatives at different concentrations (1-100 µM). The reaction was initiated by the addition of ATP to a final concentration of 3 mM and incubated at 37 ^{0}C for 30 min. The same groups were carried out under the same conditions with the addition of 0.1 mM ouabain. Na^+, K^+-ATPase activity assay was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow , with some modifications.

Effect of DTT as restoring agents for Na^+, K^+-ATPase inhibition

Aiming to study the effect of DTT on reversing Na^+, K^+-ATPase inhibition, 4-organoseleno-isoquinoline derivatives, at IC_{50} concentration, were pre-incubated with S1 at 37 ^{0}C for 10 min. After this time, the reaction was started by the addition of 3 mM DTT and 3 mM ATP and incubated at 37 ^{0}C for 30 min. Released inorganic phosphate (Pi) was measured as described above.

Acute oral toxicity

Based on the *in vitro* data, compound **1** was selected to carry out the *in vivo* toxicity. For the experiment, 3-phenyl-4-(phenylselenyl) isoquinoline was dissolved in canola oil and a single dose of 300 mg/kg intragastric (i.g.) was administered in

female Wistar rats, in a constant volume of 1 ml/kg of body weight . The control group received canola oil. The food was withheld in the night before to the treatment, and it was replaced 4 h after the administration of compound. The animals were observed individually during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 h, and daily for a total of 14 days. During this period, the signs of toxicity were observed and the individual body weight gain was recorded and calculated according to the formula [baseline body weight (obtained before the beginning of treatment) - body weight at the end of the experiment].

Water and food consumptions were measured in rats exposed to compound **1**. The averages of water and food consumption were calculated according to the formula [water and food intake/number of animals per cage]. After 14 days, the rats were decapited and plasma samples were collected for dosage of creatinine and urea levels, lactate dehydrogenase (LDH), aspartate (AST) and alanine (ALT) aminotransferases activities using commercial kits (Labtest, Minas Gerais, Brazil). Kidney and liver samples were used to determinate of δ-ALA-D activity. Brain was removed to carry out Na^+, K^+-ATPase and δ-ALA-D activities as well as for histologic evaluation.

Protein determination

The protein concentration was measured by the method of Bradford , using bovine serum albumin as the standard.

Statistical analysis

Statistical analysis of data from δ-ALA-D and Na^+, K^+-ATPase activity *in vitro* were performed using one-way ANOVA (analysis of variance), followed by Student-Newman-Keuls multiple comparison test. The effect of restoring agents was analyzed by two-way ANOVA followed by Bonferroni test. *Ex vivo* experiments were carried out using unpaired Student's *t* test. All analyses were performed using the "GraphPad Software" (GraphPad, San Diego, CA, USA). All data were expressed as means ± S.E.M. Values of $P < 0.05$ were considered statistically significant.

IC_{50} values were calculated by linear regression from individual experiments using "GraphPad Software" (GraphPad software, San Diego, CA, USA). The IC_{50} values were reported as means accompanied by their 95 % confidence limits.

Results

Effect of 4-organoseleno-isoquinolines on cerebral δ-ALA-D activity *in vitro*

4-Organoseleno-isoquinolines substituted with halogen atoms to the selenium-bonded aromatic ring (fluoro (**3**), chloro (**4**) and trifluoromethyl (**5**)) inhibited δ-ALA-D activity at concentrations equal to or great than 100, 50 and 25 μM, respectively. The IC_{50} values and their 95 % confidence limits found were 78.42 (65.46 – 93.95) μM, 92.27 (85.10 – 100.50) μM, 44.98 (42.60 – 46.81) μM, for compounds **3**, **4** and **5**. Compounds **1** and **2** did not alter δ-ALA-D activity in the tested concentrations (Table 1).

Effect of DTT or $ZnCl_2$ as restoring agents for cerebral δ-ALA-D inhibition caused by 4-organoseleno-isoquinolines

DTT and $ZnCl_2$ were used to investigate whether the inhibition caused by isoquinoline derivatives was involved with the oxidation of sulfhydryl groups. The inhibitory effect of compounds **3**, **4** and **5** was completely restored by DTT, but not by $ZnCl_2$ (Table 2).

Effect of 4-organoseleno-isoquinolines on cerebral Na^+, K^+-ATPase activity *in vitro*

Similar to δ-ALA-D activity, compounds **1** and **2** did not inhibit cerebral Na^+, K^+-ATPase activity. By contrast, compounds **3**, **4** and **5**, which have halogens in the molecule, showed inhibitory effect on Na^+, K^+-ATPase activity at concentrations equal to or greater than 25, 100 and 50 μM, respectively (Table 3). The IC_{50} values and their 95 % confidence limits were 41.36 (27.80 – 61.44) μM, 89.43 (76.08 – 105.13) μM, 50.66 (32.07 – 80.05) μM, for compounds **3**, **4** and **5**.

Effect of DTT as restoring agent for Na^+, K^+-ATPase inhibition caused by 4-organoseleno-isoquinolines

DTT was able to restore Na^+, K^+-ATPase activity inhibited by compounds **3**, **4** and **5** to the control levels (Table 4).

Acute oral toxicity

A single intragastric administration of compound **1** at a dose of 300 mg/kg did not alter the body weight gain and the food intake. Nevertheless, the water intake was significantly reduced by treatment (See supplementary material, Table S1). During 14 days after the exposition, the female rats did not present any signs of toxicity (changes in skin and fur, eyes and mucous membranes, presence of tremors, convulsions, salivation, diarrhea and lethargy).

All markers of damage (LDH, AST and ALT activities as well as urea and creatinine levels) present unaltered by compound **1** (See supplementary material, Table S2). Treatment did not alter the hepatic, renal and cerebral δ-ALA-D activities. Cerebral Na^+, K^+-ATPase activity was not affected by isoquinoline derivative (See supplementary material, Table S3). Histological analyzes revealed normal aspect of brain structures and neuronal integrity demonstrated, by hematoxilin/eosin and cresyl violet staining (See supplementary material, Figure S1).

Discussion

The *in vitro* results obtained in this study clearly indicated that 4-organoseleno-isoquinolines substituted with electron-withdrawing groups to the selenium-bonded to the aromatic ring (fluoro, chloro and trifluoro, for compounds **3**, **4** and **5**, respectively) inhibited activities of both sulfhydryl enzymes: δ-ALA-D and Na^+, K^+-ATPase, whereas isoquinoline derivatives **1** and **2**, without substitution of electron-withdrawing group did not alter their enzymatic activities. Moreover, the enzyme inhibitions caused by compounds **3**, **4** and **5** appear to be involved with the oxidation of their sulfhydryl groups.

δ-ALA-D is responsible by the condensation of two δ-ALA molecules to form the monopyrrole PBG . Tetrapyrroles, such as heme, are formed from monopyrroles, being essential for aerobic metabolism and carbon fixation . Subsequently, toxic agents which interfere in this synthesis can affect the cell metabolism . Structurally, δ-ALA-D is an oligomeric enzyme that is fully active as an octamer, which have cysteinyl residues coordinate with Zn (II) ions. The reduction of its activity by 4-organoseleno-isoquinolines seems to be linked to the oxidation of sulfhydryl groups to disulfides, as demonstrated through of the reversion of inhibition by DTT. However, $ZnCl_2$ was not effective in restoring the δ-ALA-D inhibition, so the release of

essential zinc seems not to be related to the mechanism of enzymatic inhibition by compounds **3**, **4** and **5**.

Similarly δ-ALA-D, cerebral Na^+, K^+-ATPase was inhibited *in vitro* by 4-organoseleno-isoquinolines substituted with electron-withdrawing group to the selenium-bonded to the aromatic ring, and compounds without these groups did not inhibit its activity. Na^+, K^+-ATPase regulates the intracellular concentrations of Na^+ and K^+ ions, and consequently their gradients through of plasmatic membrane. The inactivation of Na^+, K^+-ATPase leads to partial membrane depolarization allowing excessive Ca^{2+} entry inside neurons with resultant toxic events like excitotoxicity . DTT was used as restoring agent, due to the presence of cysteinyl residues in Na^+, K^+-ATPase, which are essential for its activity. In the presence of DTT in the reaction medium, the cerebral Na^+, K^+-ATPase activity, previously inhibited by compounds **3**, **4** and **5,** was restored to the control levels, demonstrating that the enzymatic inhibition by isoquinoline derivatives seems to be involved to the oxidation of sulfhydryl groups.

Data of cerebral δ-ALA-D and Na^+, K^+-ATPase activities *in vitro* suggest that the inhibition of these enzymes may depend on electronic effects of the substituent in the selenium-bonded aromatic ring and on the involvement of sulfhydryl groups. In fact, the introduction of electron-withdrawing groups in the selenium-bonded aromatic ring could favor the bond cleavage between 4-phenylselenanyl and isoquinoline moieties. In agreement to our results, literature data demonstrated that organoselenium compounds substituted with electron-withdrawing groups showed higher inhibitory effect on these sulfhydryl enzymes than compounds non-substituted or substituted with an electron-donating group .

Based on data describe above, we investigated the *in vivo* effect of oral administration of compound **1** on markers of systemic toxicity in rats. In addition, this compound presents the lowest IC_{50} as MAO-B inhibitor among the 4-organoseleno-isoquinoline derivatives tested in our previous study. Compound **1** did not cause signs of toxicity, modification on body gain weight and food intake, however water intake was reduced. This result could be explained by the anorexigenic effect of organoselenium compounds, since at doses that do not cause toxic effects, organoselenium compounds can decrease food and water intakes and body weight gain . Moreover, the absence of cellular, hepatic, renal and cerebral damage, shown by results of the LDH, AST, ALT, δ-ALA-D and Na^+, K^+-ATPase activities, urea and

creatinine levels and histological analyzes, reinforce the probably low or absent toxicity of the compound **1**.

In conclusion, isoquinoline derivatives substituted with fluoro (**3**), chloro (**4**) and trifluoro (**5**) to the selenium-bonded to the aromatic ring, possibly due to electronic effects induced by these groups, inhibited cerebral δ-ALA-D and Na^+, K^+-ATPase activities *in vitro* by interacting with sulfhydryl residues of these enzymes. The compounds without electron-withdrawing group did not alter these enzymatic activities. In addition, compound **1** in female Wistar rats did not exhibit toxic signs *in vivo* and *ex vivo*. Although more studies are necessary about this specific compound, our findings suggest that 3-phenyl-4-(phenylselenyl) isoquinoline (compound **1**), that have pharmacological properties, is a safe compound.

Conflict of interest

The authors declare they have no conflicts of interest to disclose.

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References

[1] Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 2007;9:775-806.

[2] Nogueira CW, Rocha JBT. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Archives of Toxicology 2011;85:1313-59.

[3] Luchese C, Nogueira CW. Diphenyl diselenide in its selenol form has dehydroascorbate reductase and glutathione S-transferase-like activity dependent on the glutathione content. J Pharm Pharmacol 2010;62:1146-51.

[4] Chagas PM, Bortolatto CF, Wilhelm EA, Roehrs JA, Nogueira CW. Bis(phenylimidazoselenazolyl) diselenide: a compound with antinociceptive properties in mice. Behav Pharmacol 2013;24:37-44.

[5] da Rocha JT, Sampaio TB, Santos Neto JS, Nogueira CW, Zeni G. Cognitive effects of diphenyl diselenide and estradiol treatments in ovariectomized mice. Neurobiology of learning and memory 2013;99:17-24.

[6] Barbosa NB, Rocha JB, Wondracek DC, Perottoni J, Zeni G, Nogueira CW. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. Chem Biol Interact 2006;163:230-8.

[7] Pesarico AP, Sartori G, dos Santos CF, Neto JS, Bortolotto V, Santos RC, et al. 2,2'-Dithienyl diselenide pro-oxidant activity accounts for antibacterial and antifungal activities. Microbiol Res 2013;168:563-8.
[8] Bortolatto CF, Jesse CR, Wilhelm EA, Ribeiro LR, Rambo LM, Royes LF, et al. Protective effect of 2,2'-dithienyl diselenide on kainic acid-induced neurotoxicity in rat hippocampus. Neuroscience 2011;193:300-9.
[9] Pinton S, Souza AC, Sari MH, Ramalho RM, Rodrigues CM, Nogueira CW. p,p'-Methoxyl-diphenyl diselenide protects against amyloid-beta induced cytotoxicity in vitro and improves memory deficits in vivo. Behav Brain Res 2013;247:241-7.
[10] Stranges S, Sieri S, Vinceti M, Grioni S, Guallar E, Laclaustra M, et al. A prospective study of dietary selenium intake and risk of type 2 diabetes. BMC Public Health 2010;10:564.
[11] Vinceti M, Bonvicini F, Bergomi M, Malagoli C. Possible involvement of overexposure to environmental selenium in the etiology of amyotrophic lateral sclerosis: a short review. Ann Ist Super Sanita 2010;46:279-83.
[12] Vinceti M, Maraldi T, Bergomi M, Malagoli C. Risk of chronic low-dose selenium overexposure in humans: insights from epidemiology and biochemistry. Rev Environ Health 2009;24:231-48.
[13] Clark RF, Strukle E, Williams SR, Manoguerra AS. Selenium poisoning from a nutritional supplement. JAMA 1996;275:1087-8.
[14] Chasteen TG, Bentley R. Biomethylation of selenium and tellurium: microorganisms and plants. Chem Rev 2003;103:1-25.
[15] Paulmier C. Selenoorganic functional groups. In: Paulmier C, editor. Selenium Reagents and Intermediates in Organic Synthesis. Oxford: Pergamon press, 1986. p. 25-51.
[16] Plano D, Baquedano Y, Ibanez E, Jimenez I, Palop JA, Spallholz JE, et al. Antioxidant-prooxidant properties of a new organoselenium compound library. Molecules 2010;15:7292-312.
[17] Stewart MS, Spallholz JE, Neldner KH, Pence BC. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radic Biol Med 1999;26:42-8.
[18] Chen JJ, Boylan LM, Wu CK, Spallholz JE. Oxidation of glutathione and superoxide generation by inorganic and organic selenium compounds. Biofactors 2007;31:55-66.
[19] Nogueira CW, Borges VC, Zeni G, Rocha JBT. Organochalcogens effects on delta-aminolevulinate dehydratase activity from human erythrocytic cells in vitro. Toxicology 2003;191:169-78.
[20] Farina M, Folmer V, Bolzan RC, Andrade LH, Zeni G, Braga AL, et al. Selenoxides inhibit delta-aminolevulinic acid dehydratase. Toxicology Letters 2001;119:27-37.
[21] Goeger DE, Ganther HE. Oxidation of Dimethylselenide to Dimethylselenoxide by Microsomes from Rat-Liver and Lung and by Flavin-Containing Monooxygenase from Pig-Liver. Archives of Biochemistry and Biophysics 1994;310:448-51.
[22] McNaught KS, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, et al. Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease. Biochemical Pharmacology 1998;56:921-33.
[23] Niwa T, Takeda N, Sasaoka T, Kaneda N, Hashizume Y, Yoshizumi H, et al. Detection of Tetrahydroisoquinoline in Parkinsonian Brain as an Endogenous

Amine by Use of Gas-Chromatography Mass-Spectrometry. J Chromatogr-Biomed 1989;491:397-403.
[24] Antkiewicz-Michaluk L. Endogenous risk factors in Parkinson's disease: dopamine and tetrahydroisoquinolines. Pol J Pharmacol 2002;54:567-72.
[25] Dipasquale B, Marini AM, Youle RJ. Apoptosis and DNA degradation induced by 1-methyl-4-phenylpyridinium in neurons. Biochem Biophys Res Commun 1991;181:1442-8.
[26] Rocha JBT, Saraiva RA, Garcia SC, Gravina FS, Nogueira CW. Aminolevulinate dehydratase (delta-ALA-D) as marker protein of intoxication with metals and other pro-oxidant situations. Toxicol Res-Uk 2012;1:85-102.
[27] Markham GD, Myers CB, Harris KA, Jr., Volin M, Jaffe EK. Spatial proximity and sequence localization of the reactive sulfhydryls of porphobilinogen synthase. Protein Sci 1993;2:71-9.
[28] Goncalves TL, Erthal F, Corte CL, Muller LG, Piovezan CM, Nogueira CW, et al. Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women. Clin Biochem 2005;38:1071-5.
[29] Fernandez-Cuartero B, Rebollar JL, Batlle A, Enriquez de Salamanca R. Delta aminolevulinate dehydratase (ALA-D) activity in human and experimental diabetes mellitus. Int J Biochem Cell Biol 1999;31:479-88.
[30] Bonfanti G, Ceolin RB, Valcorte T, De Bona KS, de Lucca L, Goncalves TL, et al. delta-Aminolevulinate dehydratase activity in type 2 diabetic patients and its association with lipid profile and oxidative stress. Clin Biochem 2011;44:1105-9.
[31] Kaplan JH. Biochemistry of Na,K-ATPase. Annu Rev Biochem 2002;71:511-35.
[32] Stein AL, Bilheri FN, da Rocha JT, Back DF, Zeni G. Application of Copper(I) Iodide/Diorganoyl Dichalcogenides to the Synthesis of 4-Organochalcogen Isoquinolines by Regioselective C?N and C?Chalcogen Bond Formation. Chem-Eur J 2012;18:10602-8.
[33] Sassa S. Delta-Aminolevulinic-Acid Dehydratase Assay. Enzyme 1982;28:133-43.
[34] Barbosa NB, Rocha JB, Zeni G, Emanuelli T, Beque MC, Braga AL. Effect of organic forms of selenium on delta-aminolevulinate dehydratase from liver, kidney, and brain of adult rats. Toxicol Appl Pharmacol 1998;149:243-53.
[35] Emanuelli T, Rocha JB, Pereira ME, Nascimento PC, Souza DO, Beber FA. delta-Aminolevulinate dehydratase inhibition by 2,3-dimercaptopropanol is mediated by chelation of zinc from a site involved in maintaining cysteinyl residues in a reduced state. Pharmacol Toxicol 1998;83:95-103.
[36] Wyse ATD, Streck EL, Worm P, Wajner A, Ritter F, Netto CA. Preconditioning prevents the inhibition of Na+,K+-ATPase activity after brain ischemia. Neurochemical Research 2000;25:971-5.
[37] Fiske CH, Subbarow Y. Phosphorus Compounds of Muscle and Liver. Science 1929;70:381-2.
[38] Borges VC, Rocha JB, Nogueira CW. Effect of diphenyl diselenide, diphenyl ditelluride and ebselen on cerebral Na(+), K(+)-ATPase activity in rats. Toxicology 2005;215:191-7.
[39] OECD. Guideline 423 for testing of chemicals. Acute Oral Toxicity - Acute Toxic Class Method. 2001.

[40] Bradford MM. Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. Anal Biochem 1976;72:248-54.
[41] Jaffe EK, Lawrence SH. Allostery and the dynamic oligomerization of porphobilinogen synthase. Arch Biochem Biophys 2012;519:144-53.
[42] Heinemann IU, Jahn M, Jahn D. The biochemistry of heme biosynthesis. Arch Biochem Biophys 2008;474:238-51.
[43] Veldhuis WB, van der Stelt M, Delmas F, Gillet B, Veldink GA, Vliegenthart JF, et al. In vivo excitotoxicity induced by ouabain, a Na+/K+-ATPase inhibitor. J Cereb Blood Flow Metab 2003;23:62-74.
[44] Bruning CA, Prigol M, Barancelli DA, Nogueira CW, Zeni G. Disubstituted diaryl diselenides inhibit delta-ALA-D and Na+, K+-ATPase activities in rat brain homogenates in vitro. Mol Cell Biochem 2009;332:17-24.
[45] Chagas PM, Bortolatto CF, Wilhelm EA, Nogueira CW. High doses of 2,2'-dithienyl diselenide cause systemic toxicity in rats: an in vitro and in vivo study. J Appl Toxicol 2013;33:480-7.
[46] Meotti FC, Borges VC, Perottoni J, Nogueira CW. Toxicological evaluation of subchronic exposure to diphenyl diselenide in rats. J Appl Toxicol 2008;28:638-44.
[47] Savegnago L, Jesse CR, Nogueira CW. Structural modifications into diphenyl diselenide molecule do not cause toxicity in mice. Environ Toxicol Pharmacol 2009;27:271-6.

Figure Captions

Fig. 1: Chemical structures of 4-organoseleno-isoquinoline derivatives tested: 3-phenyl-4-(phenylselenyl) isoquinoline **1**; 4-(4-methylphenylselenyl)-3-phenylisoquinoline **2**; 4-(4-fluorophenylselenyl)-3-phenylisoquinoline **3**; 4-(4-chlorophenylselenyl)-3-phenylisoquinoline **4**, and 4-[(3-trifluoromethyl)phenylselenyl]-3-phenylisoquinoline **5**.

Tables

Table 1: Effect of isoquinoline derivatives on cerebral δ-ALA-D activity *in vitro*.

Isoquinoline	0	1 µM	5 µM	10 µM	25 µM	50 µM	100 µM
1	34.24 ± 1.51	34.22 ± 1.61	33.88 ± 1.90	35.66 ± 2.29	34.56 ± 2.89	37.09 ± 2.98	33.06 ± 1.26
2	35.77 ± 1.25	34.71 ± 0.47	33.69 ± 2.09	33.92 ± 0.64	33.88 ± 0.58	33.57 ± 0.83	32.24 ± 0.73
3	36.24 ± 2.30	37.32 ± 1.67	37.17 ± 1.93	35.31 ± 2.50	32.10 ± 3.54	26.99 ± 5.49	16.70 ± 4.37**
4	34.88 ± 1.69	33.70 ± 2.12	36.16 ± 2.89	34.24 ± 1.69	33.33 ± 2.33	28.78 ± 1.95*	17.76 ± 1.40***
5	32.12 ± 1.78	32.92 ± 2.64	31.90 ± 2.07	29.39 ± 1.44	20.26 ± 2.40***	12.60 ± 2.64***	4.89 ± 0.30***

Data are expressed as means ± S.E.M. of 3-4 individual experiments. δ-ALA-D activity is expressed as nmol of porphobilinogen /mg protein /hour. The values found were considered statistically significant when $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ (one-way ANOVA/Newman-Keuls test). **1**, 3-Phenyl-4-(phenylseleno) isoquinoline; **2**, 4-(4-methylphenylseleno)-3-phenylisoquinoline; **3**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline; **4**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline; **5**, 4-(4-trifluorophenylseleno)-3-phenylisoquinoline.

Table 2: Reversibility of δ-ALA-D inhibition *in vitro* by DTT and $ZnCl_2$.

Isoquinoline	Control	DTT	$ZnCl_2$	IC_{50}	IC_{50} + DTT	IC_{50} + $ZnCl_2$
3	35.06 ± 1.61	36.34 ± 1.14	33.33 ± 2.05	11.75 ± 1.19**	34.61 ± 0.90##	13.66 ± 0.72**
4	35.98 ± 0.90	36.03 ± 1.42	33.96 ± 2.13	28.34 ± 0.93*	36.25 ± 1.54#	29.71 ± 1.54*
5	35.06 ± 1.61	36.34 ± 1.14	33.33 ± 2.05	12.66 ± 1.56**	33.88 ± 0.42##	14.30 ± 1.20**

Results are expressed as means ± S.E.M. for 3–4 independent experiments. δ-ALA-D activity is expressed as nmol of porphobilinogen /mg protein /hour. The values found were considered statistically significant when **P* < 0.01 and ***P* < 0.001 compared to the control, and #*P* < 0.01 and ##*P* < 0.001 compared to the IC_{50} of isoquinoline derivatives (two-way ANOVA/ Bonferroni test). **3**, 4-(4-Fluorophenylseleno)-3-phenylisoquinoline; **4**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline; **5**, 4-(4-trifluorophenylseleno)-3-phenylisoquinoline.

Table 3: Effect of isoquinoline derivatives on cerebral Na^+, K^+ - ATPase activity *in vitro*.

Isoquinoline	0	1 µM	5 µM	10 µM	25 µM	50 µM	100 µM
1	37.43 ± 1.72	38.64 ± 1.39	34.57 ± 1.94	35.82 ± 0.63	33.96 ± 2.09	35.12 ± 2.99	33.29 ± 1.85
2	40.56 ± 3.17	44.41 ± 3.02	42.09 ± 3.43	44.01 ± 0.96	36.76 ± 3.09	38.10 ± 3.21	36.19 ± 4.11
3	42.97 ± 0.51	42.42 ± 2.23	36.66 ± 1.58	37.84 ± 2.74	34.66 ± 3.47*	34.37 ± 2.23***	24.00 ± 3.39***
4	44.45 ± 1.94	46.57 ± 5.22	37.68 ± 1.92	39.29 ± 2.58	38.35 ± 5.51	31.58 ± 3.49	28.08 ± 4.90**
5	40.28 ± 3.47	45.79 ± 3.92	39.75 ± 3.92	38.91 ± 2.55	31.03 ± 3.74	29.35 ± 1.40***	19.26 ± 5.25***

Data are reported as means ± S.E.M. for 3-4 individual experiments. Cerebral Na^+, K^+ - ATPase activity is expressed as nmol Pi /mg protein /minute. The values found were considered statistically significant when **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 compared with the control (one-way ANOVA/Newman–Keuls test). **1**, 3-Phenyl-4-(phenylseleno) isoquinoline; **2**, 4-(4-methylphenylseleno)-3-phenylisoquinoline; **3**, 4-(4-fluorophenylseleno)-3-phenylisoquinoline; **4**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline; **5**, 4-(4-trifluorophenylseleno)-3-phenylisoquinoline.

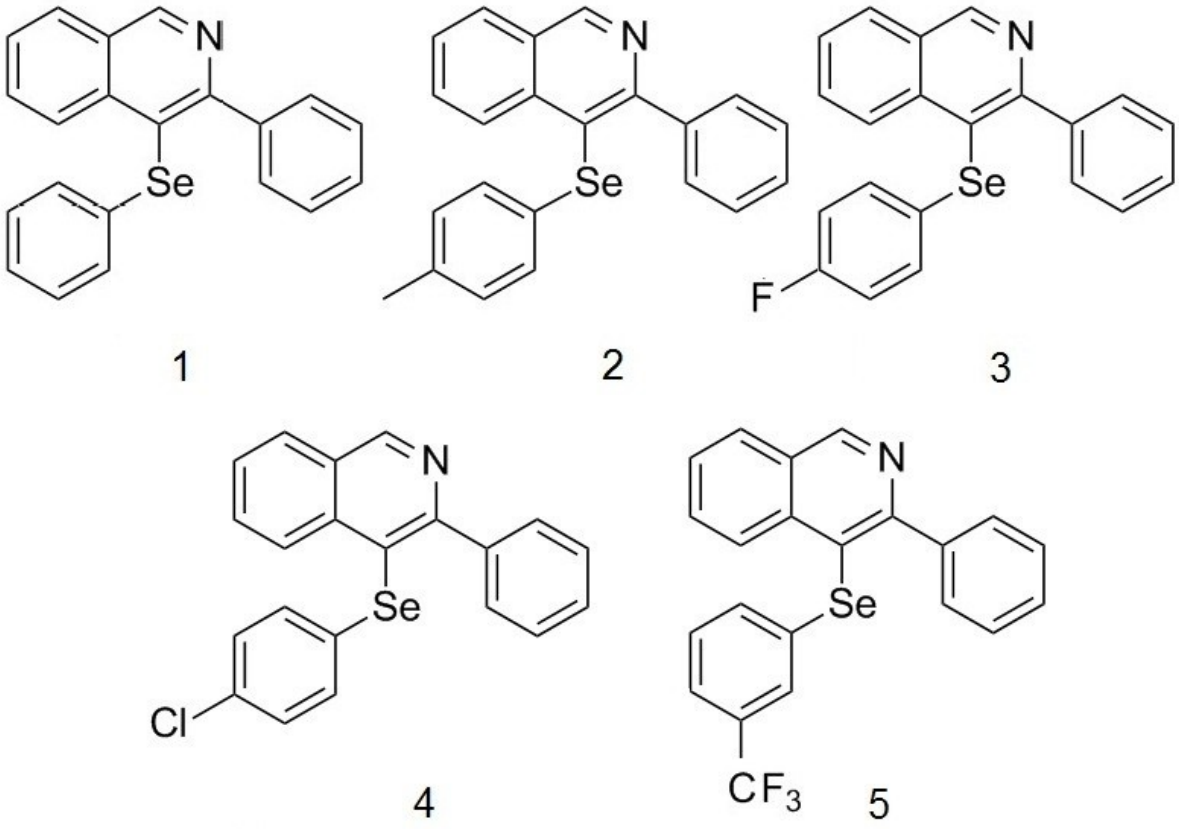
Table 4: Reversibility Na^+, K^+ - ATPase inhibition *in vitro* by DTT.

Isoquinoline	Control	DTT	IC_{50}	IC_{50} + DTT
3	32.73 ± 0.83	30.33 ± 1.28	14.81 ± 1.06*	30.45 ± 1.50$^{\#\#}$
4	32.69 ± 0.58	31.36 ± 1.37	17.47 ± 0.60*	29.30 ± 3.19$^{\#}$
5	33.09 ± 0.60	32.24 ± 1.49	15.55 ± 1.94*	34.71 ± 1.75$^{\#\#}$

Results are demonstrated as means ± S.E.M. for 3-4 individual experiments. Cerebral Na^+, K^+ - ATPase activity is expressed as nmol Pi /mg protein /minute. The values found were considered statistically significant when $^{*}P < 0.001$ compared with the control and $^{\#}P < 0.01$ and $^{\#\#}P < 0.001$ (two-way ANOVA/Bonferroni test). **3**, 4-(4-Fluorophenylseleno)-3-phenylisoquinoline; **4**, 4-(4-chlorophenylseleno)-3-phenylisoquinoline; **5**, 4-(4-trifluorophenylseleno)-3-phenylisoquinoline.

Figures

Figure 1:



SUPPORTING INFORMATION

4-Organoseleno-isoquinolines effects *in vitro* on cerebral δ-aminolevulinate dehydratase and Na^+, K^+-ATPase activities

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Table S1: Effect of intragastric administration of compound **1** on the body weight gain, water and food intakes in rats.

	Control	**1**
Weight gain	23.88 ± 5.75	22.25 ± 4.39
Food intake	23.53 ± 1.01	23.36 ± 1.02
Water intake	37.93 ± 1.58	32.07 ± 1.56 *

Data are reported as means ± S.E.M. of 8 animals. Weight gain and food intake are expressed as g per animal, and water intake as ml per animal. Data were considered statistically significant when *P < 0.05 compared to the control group (Student's *t* test). **1**, 3-phenyl-4-(phenylseleno) isoquinoline.

Table S2: Effect of a single acute dose of compound 1 administered to female rats on plasmatic markers of toxicity.

	LDH[a]	AST[a]	ALT[a]	Creatinine[b]	Urea[b]
Control	615.3 ± 132.3	165.5 ± 33.98	98.86 ± 10.59	0.68 ± 0.06	46.9 ± 3.21
1	561.3 ± 176.0	166.8 ± 29.9	102.4 ±12.0	0.61 ± 0.07	51.11 ± 2.4

Data are reported as the mean ± S.E.M. of 6-8 animals. Statistical analysis was performed by Student's *t* test. [a] Expressed as U/l and [b] expressed as mg/dl.

Table S3: δ-ALA-D activity of liver, kidney and brain and cerebral Na^+, K^+-ATPase activity of female rats treated with isoquinoline derivative **1.**

	δ-ALA-D[a]			Na^+, K^+-ATPase[b]
	Brain	Kidney	Liver	
Control	0.74 ± 0.10	1.26 ± 0.10	5.61 ± 0.54	4.28 ± 0.68
1	0.74 ± 0.03	1.25 ± 0.09	4.23 ± 0.47	5.52 ± 1.07

The results are demonstrated as means ± S.E.M. of 6-8 animals per group (Student's *t* test). [a] Expressed as nmol of PBG/mg protein/hour and [b] expressed as nmol of Pi/mg protein/minute.

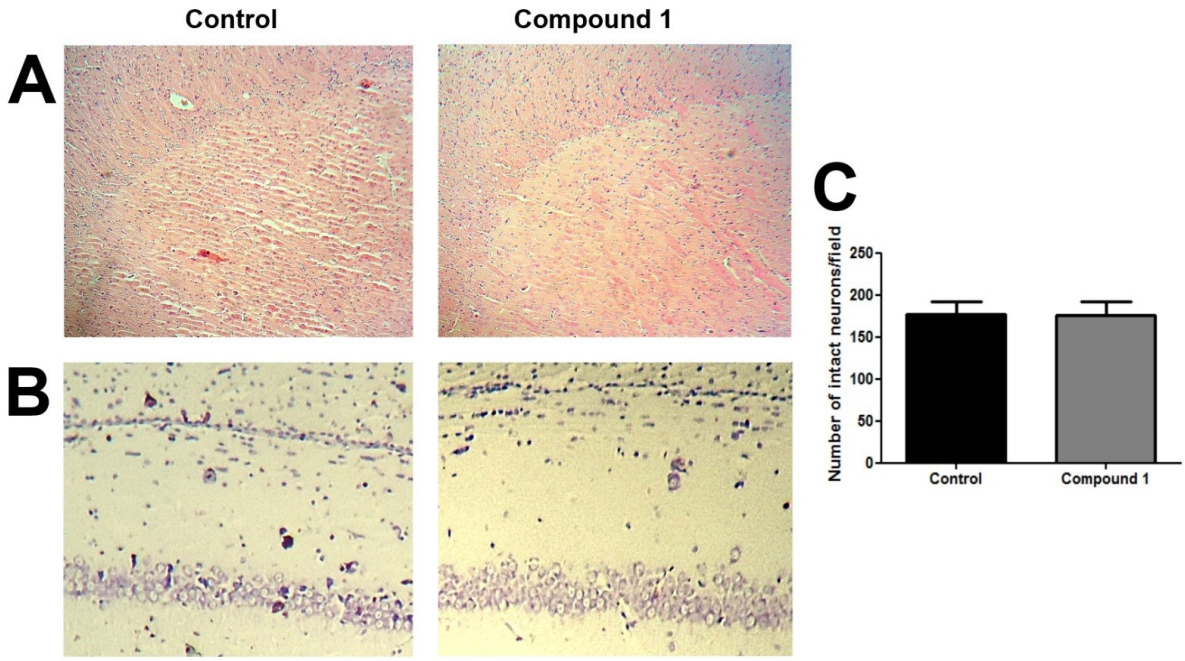

Figure S1: Photomicrography of rat brain 14 days after the treatment with compound **1**. (A) H/E staining; (B) Cresyl violet staining; (C) Count of intact neurons by cresyl violet staining did not demonstrate significant difference between the groups (Student's *t* test). Data are reported as means ± S.E.M. of 3 animals per group. **1**, 3-phenyl-4-(phenylselenyl) isoquinoline.

4 DISCUSSÃO

Os inibidores da MAO são divididos em três gerações, as quais apresentam propriedades cinéticas distintas. Na década de 50, a iproniazida foi o primeiro inibidor da MAO introduzido na clínica. A partir da sua descoberta, desenvolveu-se derivados hidrazínicos como inibidores da MAO de 1ª geração, os quais possuíam inibição não seletiva, usados para o tratamento da depressão. Entretanto, estes compostos apresentaram toxicidade hepática, crises hipertensivas, hemorragia e, em alguns casos, de morte, resultando na sua retirada do mercado (COSTA et al., 2012).

A toxicidade hepática foi evitada pelo desenvolvimento de inibidores não hidrazínicos, como tranicilpromina e pargilina (ROBINSON, 2002), no entanto as crises hipertensivas não foram prevenidas. Este efeito colateral, chamado de reação do queijo, ocorre quando aminas simpatomiméticas, metabolizadas pela MAO-A presente no intestino e no fígado, potencializam a atividade cardiovascular simpática pela liberação de noradrenalina (YOUDIM, M. B. H., 1995).

Devido a estes efeitos colaterais, desenvolveu-se a segunda geração de inibidores da MAO, os quais possuem como característica em comum a inibição seletiva de uma das isoformas. Como exemplos dessa classe, podemos citar a clorgilina e a selegilina, inibidores seletivos da MAO-A e MAO-B (JOHNSTON, 1968), respectivamente. A clorgilina foi muito utilizada como antidepressivo e ansiolítico (COSTA et al., 2012), enquanto que a selegilina atua como adjuvante no tratamento da doença de Parkinson (GERLACH et al., 1996).

Todos os inibidores da primeira e segunda geração possuem inibição irreversível, sendo assim a atividade enzimática só poderá ser restabelecida após a síntese de novas moléculas de MAO. Somado a isto, estes inibidores tendem a perder a seletividade inicial com doses maiores ou tratamento crônico (YAMADA e YASUHARA, 2004). Dessa forma, as ações produzidas pelo uso destes compostos podem ter limitações importantes na terapia, como efeitos nervosos centrais, disfunções cardiovasculares, reações hipertensivas graves e distúrbios sexuais (BENEDETTI e DOSTERT, 1985; COSTA et al., 2012).

Com base nas primeiras gerações, uma terceira geração de inibidores da MAO foi desenvolvida: os inibidores seletivos reversíveis. Estes novos inibidores da MAO propiciaram a perda de muitos efeitos colaterais, de nível central e periférico. No entanto, estes inibidores ainda desenvolvem outros tipos de reações adversas, como distúrbios do sono, aumento da ansiedade, agitação e dor de cabeça (YAMADA e YASUHARA, 2004). Desta forma, a busca por novas moléculas capazes de inibir as isoformas da MAO de maneira seletiva, potente, reversível, e ausentes de efeitos adversos é desejada.

As isoquinolinas e os compostos orgânicos contendo calcogênios em suas estruturas despertam o interesse em pesquisar suas atividades farmacológicas, visto o histórico dessas classes de compostos. Compostos orgânicos de calcogênios possuem atividade antioxidante bem estabelecida, assim como propriedades bioquímicas e farmacológicas que modulam os efeitos benéficos destes compostos em modelos animais de diversas doenças (NOGUEIRA et al., 2004). Da mesma forma as isoquinolinas apresentam muitos compostos de origem natural com uso clínico, como a morfina, ementina e colchicina (FACCHINI, 2001).

Com base no exposto, novas 4-organocalcogeno-isoquinolinas foram sintetizadas e testadas em relação ao seu potencial inibitório das isoformas da MAO *in vitro*, bem como seu perfil cinético em relação a estas enzimas.

O primeiro manuscrito desta dissertação demonstra que todos os derivados de isoquinolina testados apresentam inibição seletiva da MAO-B. Entre os compostos não substituídos com diferentes calcogênios em suas estruturas, o derivado de isoquinolina contendo Se (3-fenil-4-(selenofenil) isoquinolina) apresentou o menor valor de IC_{50}. Desta forma, substituintes (Me, Cl, F e CF_3) foram inseridos no anel aromático ligado ao átomo de Se do composto 3-fenil-4-(selenofenil) isoquinolina, sendo que entre os compostos substituídos o maior potencial inibitório foi apresentado pelo derivado de isoquinolina substituído com F (4-(4-fluorofenilseleno)-3-fenilisoquinolina). Entretanto, independente do substituinte inserido o potencial inibitório do composto 3-fenil-4-(selenofenil) isoquinolina foi reduzido. Devido a estes resultados, os compostos 3-fenil-4-(selenofenil) isoquinolina e 4-(4-fluorofenilseleno)-3-fenilisoquinolina foram utilizados para que a interação composto x MAO-B fosse melhor compreendida. Estes derivados apresentaram perfil cinético coerente aos inibidores de 3ª geração da MAO, seletivo e reversível. O *docking* molecular *in silico* demonstrou-se coerente à seletividade

exibida nos testes *in vitro*, demonstrando a interação dos compostos com o resíduo de isoleucina 199, o qual está envolvido na inibição seletiva da MAO-B (TSUGENO e ITO, 1997).

As isoformas da MAO possuem três domínios bem definidos: o domínio do substrato, o domínio do FAD e o domínio de ligação dos inibidores clássicos (BINDA et al., 2002). A porção 4-fenilcalcogenanil do composto interage com o resíduo de tirosina 398, demonstrando que este é o sítio de ligação do cofator FAD, o qual se liga no resíduo de cisteína 397, sendo assim a inibição mista da MAO-B exibida pelos compostos está de acordo com o observado nos estudos de modelagem molecular, no qual foi demonstrado que o composto se liga à enzima em uma região diferente ao sítio de ligação do substrato.

A MAO-B participa na biotransformação de neurotoxinas como MPTP, uma neurotoxina que mimetiza a doença de Parkinson em modelos animais. Estudos mostram que a inibição seletiva desta isoforma pode conferir neuroproteção por aumentar os níveis de dopamina no estriado e também por causar efeitos antioxidantes através da redução da formação de H_2O_2 (LANGSTON et al., 1999). Dessa forma justifica-se a utilização destes inibidores como adjuvantes no tratamento da doença de Parkinson (FERNANDEZ e CHEN, 2007). Alguns outros estudos também ressaltam a importância da MAO-B no desenvolvimento da doença de Alzheimer (RIEDERER et al., 2004).

Devido aos resultados positivos deste primeiro trabalho, houve a necessidade de investigar a toxicologia dos 4-organoseleno-isoquinolinas, pois tanto os compostos orgânicos de Se quanto as isoquinolinas, também, apresentam-se relacionadas com situações pró-oxidantes. Deste modo, investigou-se, no segundo manuscrito, o efeito *in vitro* das 4-organoseleno-isoquinolinas frente à atividade das enzimas δ-ALA-D e Na^+, K^+-ATPase em homogeneizado de cérebro de ratos. A enzima δ-ALA-D é utilizada como um biomarcador de estresse oxidativo, devido a sua alta sensibilidade a situações pró-oxidantes. Esta sensibilidade está relacionada a resíduos essenciais de cisteína presentes no seu sítio ativo, os quais são facilmente oxidados, levando a redução da sua atividade enzimática (ROCHA et al., 2012). De maneira similar, a Na^+, K^+-ATPase, enzima responsável por regular as concentração dos íons Na^+ e K^+ no interior da célula, também apresenta resíduos de cisteína essências para sua função catalítica (KAPLAN, 2002).

Sendo assim, no segundo manuscrito desta dissertação, demonstrou-se que as diferenças estruturais dos 4-organoseleno-isoquinolina levam a diferentes efeitos *in vitro* na atividade das enzimas δ-ALA-D e Na^+, K^+-ATPase. O composto 3-fenil-4-(selenofenil) isoquinolina e seu derivado substituído com Me não alteraram a atividade de ambas as enzimas até a concentração testada de 100 μM. Entretanto, o oposto foi observado pelos compostos substituídos com Cl, F e CF_3 no anel aromático ligado ao átomo de Se do composto 3-fenil-4-(selenofenil) isoquinolina, ou seja, esses derivados de isoquinolina inibiram ambas as atividades cerebrais das enzimas sulfidrílicas testadas.

Os dados reportados no manuscrito 2, demonstram claramente que a inserção de substituintes retiradores de elétrons (Cl, F e CF_3) no anel aromático ligado ao átomo de Se do composto 3-fenil-4-(selenofenil) isoquinolina, aumentam a ação pró-oxidante deste derivados de isoquinolina, o que não foi observado no composto substituído com um grupamento neutro (metila), nem no composto sem substituinte. Além disso, através da reversibilidade da inibição pelo DTT, pode-se predizer que a inibição das enzimas sulfidrílicas δ-ALA-D e Na^+, K^+-ATPase está relacionada à oxidação dos resíduos de cisteína, essenciais para que ambas as enzimas realizem a sua função.

De acordo com os resultados obtidos em ambos os trabalhos, o composto 3-fenil-4-(selenofenil) isoquinolina foi escolhido para avaliação de seus efeitos toxicológicos *in vivo* em fêmeas de ratos. Esta escolha foi baseada nos resultados do primeiro manuscrito, no qual o composto 3-fenil-4-(selenofenil) isoquinolina apresentou o maior potencial inibitório da MAO-B *in vitro* e um perfil cinético dos inibidores de 3ª geração da MAO, sendo relevante o estudo de seus efeitos em modelos animais de doenças que possuam a MAO-B como alvo farmacológico; somado a isto no segundo manuscrito este composto não apresentou inibição *in vitro* de duas importantes enzimas sulfidrílicas, a δ-ALA-D e Na^+, K^+-ATPase, aumentando a possibilidade de este composto ser seguro para que mais estudos referentes a sua farmacologia sejam desenvolvidos.

A fim de determinar os efeitos tóxicos do 3-fenil-4-(selenofenil) isoquinolina, fêmeas de ratos foram tratadas com uma alta dose do composto (300 mg/kg, i.g, 1ml/kg) e os sinais de toxicidade foram observados por 14 dias. No último dia os animais foram mortos e as amostras teciduais e plasmáticas foram recolhidas para posteriores análises. Os sinais de toxicidade aguda, como letargia, alteração nos

pelos e salivação foram observados, assim como o consumo de água, comida e ganho de peso. Exceto pela redução da ingesta de água, nenhum outro sinal de toxicidade foi observado. Entretanto sabe-se que compostos orgânicos que contem Se tendem a possuir um efeito anorexígeno sem causar toxicidade (CHAGAS et al., 2013a; MEOTTI et al., 2008). Nos parâmetros toxicológicos avaliados, marcadores de dano celular, hepático, renal e cerebral, nenhuma alteração foi observada.

Em relação ao descrito anteriormente, pode-se concluir que os derivados de isoquinolina são inibidores de 3ª geração – seletivos e reversíveis - da MAO-B *in vitro*, sendo o composto 3-fenil-4-(selenofenil) isoquinolina o que apresentou maior potencial inibitório entre eles. Além disso, conclui-se que a inserção de grupos retiradores de elétrons a estrutura deste composto não somente reduz o seu potencial inibitório em relação a MAO-B, como também aumenta o seu poder pró-oxidante *in vitro*, evidenciado através da inibição das enzimas δ-ALA-D e Na^+, K^+-ATPase. Por fim, o derivado de isoquinolina 3-fenil-4-(selenofenil) isoquinolina apresentou-se como um composto seguro *in vitro*, *in vivo* e *ex vivo.*

Considerando a necessidade do desenvolvimento de novos inibidores da MAO, o composto 3-fenil-4-(selenofenil) isoquinolina torna-se um candidato interessante a estudos em modelos animais que possuam a atividade da MAO-B como alvo terapêutico, visto que este composto demonstrou inibição seletiva e reversível desta enzima somado a um baixo potencial toxicológico.

5 CONCLUSÃO

Os resultados apresentados nesta dissertação nos permitem concluir que:

- Todos os 4-organocalcogeno-isoquinolinas testados inibem seletivamente a atividade cerebral da MAO-B *in vitro;*
- O *docking* molecular *in silico* demonstrou que a seletividade dos compostos pela isoforma B da MAO está relacionada com a interação dos derivados de isoquinolina com os resíduos de isoleucina 199 e tirosina 398;
- Os compostos 3-fenil-4-(selenofenil) isoquinolina e 4-(4-fluorofenilseleno)-3-fenilisoquinolina apresentaram os melhores potenciais inibitórios, em relação aos derivados de isoquinolina não substituídos e substituídos no anel aromático ligado ao átomo de Se do composto 3-fenil-4-(selenofenil) isoquinolina, respectivamente. Determinou-se que estes compostos possuem inibição do tipo mista e reversível da atividade da MAO-B;
- 4-Organoseleno-isoquinolinas substituídas com grupos retiradores de elétrons (F, Cl e CF_3) inibiram, *in vitro,* a atividade das enzimas δ-ALA-D e Na^+, K^+-ATPase em homogeneizado de cérebro de ratos;
- O possível mecanismo pelo qual os 4-organoseleno-isoquinolinas inibem as enzimas sulfidrílicas está relacionado com a oxidação dos resíduos de cisteína presentes no sítio ativo destas enzimas.

6 REFERÊNCIAS

ABDEL AZIZ, M. M. Memory switching of germanium tellurium amorphous semiconductor. **Appl. Surf. Sci.,** v. 253, p. 2059-2065, 2006.

ANDERSSON, C. M. et al. Glutathione Peroxidase-Like Activity of Diaryl Tellurides. **Bioorganic & Medicinal Chemistry Letters,** v. 3, n. 12, p. 2553-2558, Dec 1993.

AVILA, D. S. et al. An organotellurium compound with antioxidant activity against excitotoxic agents without neurotoxic effects in brain of rats. **Brain Res Bull,** v. 76, n. 1-2, p. 114-23, May 15 2008.

BARBOSA, N. B. et al. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. **Chem Biol Interact,** v. 163, n. 3, p. 230-8, Nov 7 2006.

BEBER, F. A. et al. delta-Aminolevulinate dehydratase inhibition by ascorbic acid is mediated by an oxidation system existing in the hepatic supernatant. **Int J Vitam Nutr Res,** v. 68, n. 3, p. 181-8, 1998.

BENEDETTI, M. S.; DOSTERT, P. Stereochemical Aspects of Mao Interactions - Reversible and Selective Inhibitors of Monoamine-Oxidase. **Trends in Pharmacological Sciences,** v. 6, n. 6, p. 246-251, 1985.

BERTORELLO, A. M. K., A. I. Regulation of Na+-K+ Pump Activity: Pathways Between Receptors and Effectors. **Physiology,** v. 10, n. 6, p. 253-259, 1995.

BINDA, C. et al. Structural properties of human monoamine oxidases A and B. **Int Rev Neurobiol,** v. 100, p. 1-11, 2011.

BINDA, C. et al. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. **Nature Structural Biology,** v. 9, n. 1, p. 22-26, Jan 2002.

BORTOLATTO, C. F. et al. 2,2'-dithienyl diselenide, an organoselenium compound, elicits antioxidant action and inhibits monoamine oxidase activity in vitro. **J Enzyme Inhib Med Chem,** v. 28, n. 4, p. 677-84, Aug 2013.

BORTOLATTO, C. F. et al. Protective effect of 2,2'-dithienyl diselenide on kainic acid-induced neurotoxicity in rat hippocampus. **Neuroscience,** v. 193, p. 300-9, Oct 13 2011.

BRAGA, A. L. et al. Synthesis of telluroamino acid derivatives with remarkable GPx like activity. **Org Biomol Chem,** v. 7, n. 1, p. 43-5, Jan 7 2009.

BRAGA, A. L. Z., G.; ANDRADE, L. H.; SILVEIRA, C. C. Stereoconservative formation and reativity of alpha-chalcogen-functionalized vinylithium compounds from bromo-vinylic chalcogens. **Synlett,** v. 5, p. 595-596, 1997.

BRANDAO, R. et al. Diphenyl diselenide protects against glycerol-induced renal damage in rats. **J Appl Toxicol,** v. 29, n. 7, p. 612-8, Oct 2009.

BRANDAO, R. et al. Diphenyl diselenide protects against hematological and immunological alterations induced by mercury in mice. **J Biochem Mol Toxicol,** v. 22, n. 5, p. 311-9, Sep-Oct 2008.

BRUNING, C. A. et al. Disubstituted diaryl diselenides inhibit delta-ALA-D and Na+, K+-ATPase activities in rat brain homogenates in vitro. **Mol Cell Biochem,** v. 332, n. 1-2, p. 17-24, Dec 2009a.

BRUNING, C. A. et al. Involvement of the serotonergic system in the anxiolytic-like effect caused by m-trifluoromethyl-diphenyl diselenide in mice. **Behav Brain Res,** v. 205, n. 2, p. 511-7, Dec 28 2009b.

BRUNING, C. A. et al. Evidence for the involvement of mu-opioid and delta-opioid receptors in the antinociceptive effect caused by oral administration of m-trifluoromethyl-diphenyl diselenide in mice. **Behav Pharmacol,** v. 21, n. 7, p. 621-6, Oct 2010.

CHAGAS, P. M. et al. High doses of 2,2'-dithienyl diselenide cause systemic toxicity in rats: an in vitro and in vivo study. **J Appl Toxicol,** v. 33, n. 6, p. 480-7, Jun 2013a.

CHAGAS, P. M. et al. Bis(phenylimidazoselenazolyl) diselenide: a compound with antinociceptive properties in mice. **Behav Pharmacol,** v. 24, n. 1, p. 37-44, Feb 2013b.

CHANDER, P. N. et al. Nephropathy in Zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. **J Am Soc Nephrol,** v. 15, n. 9, p. 2391-403, Sep 2004.

CLARK, R. F. et al. Selenium poisoning from a nutritional supplement. **JAMA,** v. 275, n. 14, p. 1087-8, Apr 10 1996.

COSTA, M. J. J. et al. Monoamine Oxidase inhibitors: An important but underutilized treatment Part I: Efficacy. **Psychopharm Rev,** v. 47, n. 10, p. 73-80, 2012.

DA ROCHA, J. T. et al. Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice. **J Pharm Pharmacol,** v. 61, n. 12, p. 1673-9, Dec 2009.

DUMONT, E. et al. Selenium speciation from food source to metabolites: a critical review. **Analytical and Bioanalytical Chemistry,** v. 385, n. 7, p. 1304-23, Aug 2006.

EMANUELLI, T. et al. delta-Aminolevulinate dehydratase inhibition by 2,3-dimercaptopropanol is mediated by chelation of zinc from a site involved in maintaining cysteinyl residues in a reduced state. **Pharmacol Toxicol,** v. 83, n. 3, p. 95-103, Sep 1998.

FACCHINI, P. J. Alkaloid biosynthesis in plants: Biochemistry, cell biology, molecular regulation, and metabolic engineering applications. **Annual Review of Plant Physiology and Plant Molecular Biology,** v. 52, p. 29-66, 2001.

FAO/OMS. Report 7ª Joint FAO/OMS Expert Consultation. Bangkok. Thailand. **Human Vitamin and Mineral Requirements**, p. p. xxii + 286, 2002.

FARINA, M. et al. Selenoxides inhibit delta-aminolevulinic acid dehydratase. **Toxicology Letters,** v. 119, n. 1, p. 27-37, Feb 3 2001.

FERNANDEZ, H. H.; CHEN, J. J. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. **Pharmacotherapy,** v. 27, n. 12 Pt 2, p. 174S-185S, Dec 2007.

FREI, G. M. et al. Antitumour effects in mycosis fungoides of the immunomodulatory, tellurium-based compound, AS101. **Br J Dermatol,** v. 158, n. 3, p. 578-86, Mar 2008.

FRIEDMAN, M. et al. Topical treatment for human papillomavirus-associated genital warts in humans with the novel tellurium immunomodulator AS101: assessment of its safety and efficacy. **Br J Dermatol,** v. 160, n. 2, p. 403-8, Feb 2009.

GAWESKA, H.; FITZPATRICK, P. F. Structures and Mechanism of the Monoamine Oxidase Family. **Biomol Concepts,** v. 2, n. 5, p. 365-377, Oct 1 2011.

GAY, B. M. et al. Antidepressant-like pharmacological profile of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene: Involvement of serotonergic system. **Neuropharmacology,** v. 59, n. 3, p. 172-9, Sep 2010.

GERLACH, M. et al. Pharmacology of selegiline. **Neurology,** v. 47, n. 6 Suppl 3, p. S137-45, Dec 1996.

GOEGER, D. E.; GANTHER, H. E. Oxidation of Dimethylselenide to Dimethylselenoxide by Microsomes from Rat-Liver and Lung and by Flavin-Containing Monooxygenase from Pig-Liver. **Archives of Biochemistry and Biophysics,** v. 310, n. 2, p. 448-451, May 1 1994.

GRINBERG, L. et al. N-acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. **Free Radical Biology and Medicine,** v. 38, n. 1, p. 136-145, Jan 1 2005.

INEU, R. P. et al. Diphenyl diselenide reverses gastric lesions in rats: Involvement of oxidative stress. **Food Chem Toxicol,** v. 46, n. 9, p. 3023-9, Sep 2008.

INGENBLEEK, Y.; KIMURA, H. Nutritional essentiality of sulfur in health and disease. **Nutrition Reviews,** v. 71, n. 7, p. 413-432, Jul 2013.

JACOB, C. et al. Water-soluble organotellurium compounds: catalytic protection against peroxynitrite and release of zinc from metallothionein. **Chem Res Toxicol,** v. 13, n. 1, p. 3-9, Jan 2000.

JAFFE, E. K.; LAWRENCE, S. H. Allostery and the dynamic oligomerization of porphobilinogen synthase. **Arch Biochem Biophys,** v. 519, n. 2, p. 144-53, Mar 15 2012.

JOHNSTON, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. **Biochem Pharmacol,** v. 17, n. 7, p. 1285-97, Jul 1968.

KAPLAN, J. H. Biochemistry of Na,K-ATPase. **Annu Rev Biochem,** v. 71, p. 511-35, 2002.

KONDOH, S. et al. Effects of ebselen on cerebral ischemia and reperfusion evaluated by microdialysis. **Neurol Res,** v. 21, n. 7, p. 682-6, Oct 1999.

KOTAKE, Y. et al. A Novel Endogenous Amine, 1-Benzyl-1,2,3,4-Tetrahydroisoquinoline in the Mouse-Brain and Parkinsonian Csf - Relation to Parkinsons-Disease. **Journal of Neurochemistry,** v. 65, p. S46-S46, 1995.

KRYUKOV, G. V. et al. Characterization of mammalian selenoproteomes. **Science,** v. 300, n. 5624, p. 1439-43, May 30 2003.

LANGSTON, J. W. et al. Chronic Parkinsonism in Humans Due to a Product of Meperidine-Analog Synthesis. **Science,** v. 219, n. 4587, p. 979-980, 1983.

LANGSTON, J. W. et al. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. **Annals of Neurology,** v. 46, n. 4, p. 598-605, Oct 1999.

LI, G. S. et al. Keshan disease: an endemic cardiomyopathy in China. **Hum Pathol,** v. 16, n. 6, p. 602-9, Jun 1985.

LUCHESE, C.; NOGUEIRA, C. W. Diphenyl diselenide in its selenol form has dehydroascorbate reductase and glutathione S-transferase-like activity dependent on the glutathione content. **J Pharm Pharmacol,** v. 62, n. 9, p. 1146-51, Sep 2010.

MCNAUGHT, K. S. et al. Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease. **Biochemical Pharmacology,** v. 56, n. 8, p. 921-933, Oct 15 1998.

MEDVEDEV, A. E. et al. QSAR analysis of indole analogues as monoamine oxidase inhibitors. **Journal of Chemical Information and Computer Sciences,** v. 36, n. 4, p. 664-671, Jul-Aug 1996.

MEOTTI, F. C. et al. Toxicological evaluation of subchronic exposure to diphenyl diselenide in rats. **J Appl Toxicol,** v. 28, n. 5, p. 638-44, Jul 2008.

MEOTTI, F. C. et al. Thiophenes and furans derivatives: a new class of potential pharmacological agents. **Environmental Toxicology and Pharmacology,** v. 15, n. 1, p. 37-44, Dec 2003.

MULLER, R. et al. Tellurium-intoxication. **Klin Wochenschr,** v. 67, n. 22, p. 1152-5, Nov 17 1989.

NIWA, T. et al. Detection of Tetrahydroisoquinoline in Parkinsonian Brain as an Endogenous Amine by Use of Gas-Chromatography Mass-Spectrometry. **Journal of Chromatography-Biomedical Applications,** v. 491, n. 2, p. 397-403, Jul 21 1989.

NOGUEIRA, C. W. et al. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. **Inflamm Res,** v. 52, n. 2, p. 56-63, Feb 2003.

NOGUEIRA, C. W.; ROCHA, J. B. T. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. **Archives of Toxicology,** v. 85, n. 11, p. 1313-1359, Nov 2011.

NOGUEIRA, C. W. et al. Organoselenium and organotellurium compounds: Toxicology and pharmacology. **Chemical Reviews,** v. 104, n. 12, p. 6255-6285, Dec 2004.

PAPP, L. V. et al. From selenium to selenoproteins: synthesis, identity, and their role in human health. **Antioxid Redox Signal,** v. 9, n. 7, p. 775-806, Jul 2007.

PESARICO, A. P. et al. 2,2'-Dithienyl diselenide pro-oxidant activity accounts for antibacterial and antifungal activities. **Microbiol Res,** v. 168, n. 9, p. 563-8, Nov 7 2013.

PIENAAR, I. S. et al. Closing the gap between clinic and cage: sensori-motor and cognitive behavioural testing regimens in neurotoxin-induced animal models of Parkinson's disease. **Neurosci Biobehav Rev,** v. 36, n. 10, p. 2305-24, Nov 2012.

PINTON, S. et al. Comparison of the antioxidant properties and the toxicity of p,p'-dichlorodiphenyl ditelluride with the parent compound, diphenyl ditelluride. **Biol Trace Elem Res,** v. 139, n. 2, p. 204-16, Feb 2011.

PINTON, S. et al. p,p'-Methoxyl-diphenyl diselenide prevents neurodegeneration and glial cell activation induced by streptozotocin in rats. **J Alzheimers Dis,** v. 33, n. 1, p. 133-44, 2013.

POSSER, T. et al. Antidepressant-like effect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system. **Eur J Pharmacol,** v. 602, n. 1, p. 85-91, Jan 5 2009.

PRIGOL, M. et al. Protective effect of unsymmetrical dichalcogenide, a novel antioxidant agent, in vitro and an in vivo model of brain oxidative damage. **Chem Biol Interact,** v. 176, n. 2-3, p. 129-36, Nov 25 2008.

RIEDERER, P. et al. Monoamine oxidase-B inhibition in Alzheimer's disease. **Neurotoxicology,** v. 25, n. 1-2, p. 271-7, Jan 2004.

ROBINSON, D. S. Monoamine oxidase inhibitors: a new generation. **Psychopharmacol Bull,** v. 36, n. 3, p. 124-38, Summer 2002.

ROCHA, J. B. T. et al. Ebselen and diphenyl diselenide change biochemical hepatic responses to overdosage with paracetamol. **Environ Toxicol Pharmacol,** v. 19, n. 2, p. 255-61, Feb 2005.

ROCHA, J. B. T. et al. Aminolevulinate dehydratase (delta-ALA-D) as marker protein of intoxication with metals and other pro-oxidant situations. **Toxicology Research,** v. 1, n. 2, p. 85-102, 2012.

SAAD, S. Y. et al. Cardioprotective effects of subcutaneous ebselen against daunorubicin-induced cardiomyopathy in rats. **Basic Clin Pharmacol Toxicol,** v. 99, n. 6, p. 412-7, Dec 2006.

SAITO, I. et al. Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. **Neurosurgery,** v. 42, n. 2, p. 269-77; discussion 277-8, Feb 1998.

SATO, F.; KUMAGAI, H. Microbial production of isoquinoline alkaloids as plant secondary metabolites based on metabolic engineering research. **Proc Jpn Acad Ser B Phys Biol Sci,** v. 89, n. 5, p. 165-82, 2013.

SAUSEN DE FREITAS, A. et al. Reduction of diphenyl diselenide and analogs by mammalian thioredoxin reductase is independent of their gluthathione peroxidase-like activity: a possible novel pathway for their antioxidant activity. **Molecules,** v. 15, n. 11, p. 7699-714, Nov 2010.

SAVEGNAGO, L. et al. Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats. **Progress in Neuro-Psychopharmacology & Biological Psychiatry,** v. 31, n. 6, p. 1261-1269, Aug 15 2007.

SCANSETTI, G. Exposure to metals that have recently come into use. **Sci Total Environ,** v. 120, n. 1-2, p. 85-91, Jun 9 1992.

SOUZA, A. C. et al. 2-phenylethynyl-butyltellurium improves memory in mice. **Neurochem Int,** v. 60, n. 4, p. 409-14, Mar 2012.

SOUZA, A. C. et al. 2-Phenylethynyl-butyltellurium enhances learning and memory impaired by scopolamine in mice. **Behav Pharmacol,** v. 24, n. 4, p. 249-54, Aug 2013a.

SOUZA, A. C. et al. Antioxidant effect of a novel class of telluroacetilene compounds: studies in vitro and in vivo. **Life Sci,** v. 84, n. 11-12, p. 351-7, Mar 13 2009.

SOUZA, A. C. et al. 2-Phenylethynyl-butyltellurium attenuates amyloid-beta peptide(25-35)-induced learning and memory impairments in mice. **J Neurosci Res,** v. 91, n. 6, p. 848-53, Jun 2013b.

SREDNI, B. et al. A new immunomodulating compound (AS-101) with potential therapeutic application. **Nature,** v. 330, n. 6144, p. 173-6, Nov 12-18 1987.

TABUCHI, Y. et al. Ebselen, a seleno-organic compound, protects against ethanol-induced murine gastric mucosal injury in both in vivo and in vitro systems. **Eur J Pharmacol,** v. 272, n. 2-3, p. 195-201, Jan 16 1995.

TASAKI, Y. et al. 1-Methyl-1,2,3,4-Tetrahydroisoquinoline, Decreasing in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Treated Mouse, Prevents Parkinsonism-Like Behavior Abnormalities. **Journal of Neurochemistry,** v. 57, n. 6, p. 1940-1943, Dec 1991.

TAYLOR, A. Biochemistry of tellurium. **Biol Trace Elem Res,** v. 55, n. 3, p. 231-9, Dec 1996.

TSUGENO, Y.; ITO, A. A key amino acid responsible for substrate selectivity of monoamine oxidase A and B. **J Biol Chem,** v. 272, n. 22, p. 14033-6, May 30 1997.

VELDHUIS, W. B. et al. In vivo excitotoxicity induced by ouabain, a Na+/K+-ATPase inhibitor. **J Cereb Blood Flow Metab,** v. 23, n. 1, p. 62-74, Jan 2003.

VINCETI, M. et al. Risk of chronic low-dose selenium overexposure in humans: insights from epidemiology and biochemistry. **Rev Environ Health,** v. 24, n. 3, p. 231-48, Jul-Sep 2009.

WIESER, M. E. C., T. B. Atomic weights of the elements 2009 (IUPAC Technical Report). **Pure and Applied Chemistry,** v. 83, n. 2, p. 359 - 396, 2011.

WOUTERS, J. Structural aspects of monoamine oxidase and its reversible inhibition. **Curr Med Chem,** v. 5, n. 2, p. 137-62, Apr 1998.

YAMADA, M.; YASUHARA, H. Clinical pharmacology of MAO inhibitors: Safety and future. **Neurotoxicology,** v. 25, n. 1-2, p. 215-221, Jan 2004.

YAMAKAWA, T.; OHTA, S. Isolation of 1-methyl-1,2,3,4-tetrahydroisoquinoline-synthesizing enzyme from rat brain: A possible Parkinson's disease-preventing enzyme. **Biochemical and Biophysical Research Communications,** v. 236, n. 3, p. 676-681, Jul 30 1997.

YAREMA, M. C.; CURRY, S. C. Acute tellurium toxicity from ingestion of metal-oxidizing solutions. **Pediatrics,** v. 116, n. 2, p. e319-21, Aug 2005.

YOUDIM, M. B.; BAKHLE, Y. S. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. **Br J Pharmacol,** v. 147 Suppl 1, p. S287-96, Jan 2006.

YOUDIM, M. B. H. The Advent of Selective Monoamine-Oxidase-a Inhibitor Antidepressants Devoid of the Cheese Reaction. **Acta Psychiatrica Scandinavica,** v. 91, p. 5-7, 1995.

YOUNG, V. R. et al. Selenium bioavailability with reference to human nutrition. **Am J Clin Nutr,** v. 35, n. 5, p. 1076-88, May 1982.

ZENI, G. et al. Synthesis and anti-inflammatory activity of acetylenic thiophenes. **Tetrahedron Letters,** v. 42, n. 45, p. 7921-7923, Nov 5 2001.