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Original Article

The catechol-*O*-methyltransferase inhibitory potential of *Z*-vallesiachotamine by *in silico* and *in vitro* approaches

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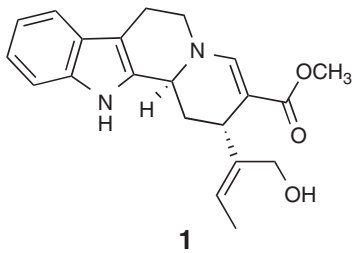
ABSTRACT

Z-Vallesiachotamine is a monoterpene indole alkaloid that has a β-*N*-acrylate group in its structure. This class of compounds has already been described in different *Psychotria* species. Our research group observed that *E*/*Z*-vallesiachotamine exhibits a multifunctional feature, being able to inhibit targets related to neurodegeneration, such as monoamine oxidase A, sirtuins 1 and 2, and butyrylcholinesterase enzymes. Aiming at better characterizing the multifunctional profile of this compound, its effect on cathecol-*O*-methyltransferase activity was investigated. The cathecol-*O*-methyltransferase activity was evaluated *in vitro* by a fluorescence-based method, using *S*-(5′-adenosyl)-L-methionine as methyl donor and aesculetin as substrate. The assay optimization was performed varying the concentrations of methyl donor (*S*-(5′-adenosyl)-L-methionine) and enzyme. It was observed that the highest concentrations of both factors (2.25 U of the enzyme and 100 μM of *S*-(5′-adenosyl)-L-methionine) afforded the more reproducible results. The *in vitro* assay demonstrated that *Z*-vallesiachotamine was able to inhibit the cathecol-*O*-methyltransferase activity with an IC_{50} close to 200 μM. Molecular docking studies indicated that *Z*-vallesiachotamine can bind the catechol pocket of catechol-*O*-methyltransferase enzyme. The present work demonstrated for the first time the inhibitory properties of *Z*-vallesiachotamine on cathecol-*O*-methyltransferase enzyme, affording additional evidence regarding its multifunctional effects in targets related to neurodegenerative diseases.

Introduction

Z-Vallesiachotamine (**1**) is a monoterpene indole alkaloid (MIA) firstly described for *Vallesia glabra* (Cav.) Link (= *V. dichotoma* Ruiz et Pav), Apocynaceae (Djerassi et al., 1966). It has a β-*N*-acrylate group, which confers a characteristic UV profile, with a high molar attenuation coefficient (ε) at 290 nm region. Therefore, vallesiachotamine-like alkaloids can be easily detected in alkaloid fractions by LC-DAD, based also on previous knowledge of the species or genus. This MIA had already been isolated from several different families, including Apocynaceae (Evans et al., 1968; Cheng et al., 2014), Loganiaceae (Zhong et al., 2014), and Rubiaceae (Heitzman et al., 2005). Specifically in Rubiaceae family, this alkaloid had already been described for several species of Palicoureeae Robbr. & Manen (Psychotrieae Cham. & Schltdl. s. lat.) tribe (Klein-Júnior et al., 2014), including *Psychotria dichroa* (Standl.) C.M. Taylor (=*Cephaelis dichroa* (Standl.) Standl.) (Solis et al., 1993), *Psychotria bahiensis* DC (Paul et al., 2003), *Psychotria cuspidata* Bredem. ex Schult. (=*Palicourea acuminata* (Benth.) Borhidi) (Berger et al., 2012), *Palicourea rigida* Kunth (Soares et al., 2012) and more recently, for *Psychotria suterella* Müll.Arg. and *Psychotria laciniata* Vell. (Passos et al., 2013a).



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The pharmacological potential of vallesiachotamine was also reported. Shang et al. (2010) evaluated the *in vitro*

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anti-inflammatory activity of several alkaloids, however no significant effect was observed for vallesiachotamine, which displayed only 41.9% of cyclooxygenase-2 inhibition at 100 μM. The cytotoxicity in human melanoma cells was analyzed by MTT assay, giving an IC_{50} value of 14.7 μM. By flow cytometry analysis, it was observed that this MIA induced G0/G1 arrest, increasing the sub-G1 hypodiploid cells number. The authors concluded that the cytotoxicity observed for this compound is mainly due to apoptosis and necrosis processes (Soares et al., 2012). In addition to the reported activities, different central effects were also described for *Z*-vallesiachotamine, including butyrylcholinesterase (BChE) and monoamine oxidase-A (MAO-A) inhibitory activity (Passos et al., 2013b) and sirtuins (SIRT1 and SIRT2) modulation (Sacconnay et al., 2015).

Another important target related to neurodegenerative disorders is the cathecol-*O*-methyltransferase (COMT) enzyme (Mannisto and Kaakkola, 1999). COMT is a methyltransferase S-adenosylmethionine-dependent responsible for catalyzing the methylation of catecholamines, such as dopamine, leading to their inactivation. Two isoforms are described: a soluble (S-COMT) and a membrane bounded (MB-COMT). S-COMT and MB-COMT can be differentiated by their affinity for substrates and their tissue distribution. The MB-COMT has a high affinity for catecholamine neurotransmitters, being found in neurons and glial cells of rat brains (Shirakawa et al., 2004). However, little is known regarding the regional distribution and cellular localization of COMT in the human brain. Kastner et al. (2006) found imunoreactivity for COMT into neurons and glial cells in the striatum in *postmortem* studies of human brains. Additionally, the presence of COMT mRNA was demonstrated in neurons of striatum and prefrontal cortex (Matsumoto et al., 2003).

COMT is the major enzyme responsible for dopamine degradation in the prefrontal cortex. In the striatum, in addition to COMT, MAO-B enzyme and the dopamine transporter (DAT) are involved in the inactivation of this neurotransmitter (Rybakowski et al., 2006). The role of COMT in the dopaminergic transmission makes this enzyme an interesting target for the discovery of new substances for the treatment of diseases such as schizophrenia, obsessive-compulsive disorder, bipolar disorder, anxiety, and panic disorder (Brisch et al., 2009). Furthermore, the COMT inhibitor entacapone is clinically used in association with levodopa in the treatment of Parkinson's disease (PD) (Hamaue et al., 2010).

Taking into account the already described BChE, MAO-A, and sirtuin inhibitory effects described for vallesiachotamine alkaloids, the present study aimed at developing and implementing a protocol for the evaluation of COMT activity suitable for the screening of plant extracts and isolated natural products. Further, we used the optimized protocol to evaluate the potential effects of vallesiachotamine on COMT activity, and an *in silico* approach to elucidate the molecular mechanisms involved in the interactions between vallesiachotamine and COMT.

Materials and methods

Chemicals

Potassium phosphate monobasic, potassium phosphate dibasic, L-cysteine, magnesium chloride hexahydrate, esculetin, scopoletin, DMSO, S-(5′-adenosyl)-L-methionine iodide (SAM), 3,5-dinitrocatechol (DNC), and porcine COMT were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *Z*-Vallesiachotamine was isolated from *Psychotria laciniata* Vell. collected from the Atlantic Forest of Cocal do Sul (Santa Catarina, Brazil S26°80'; W48°95'; ICN 182552, Herbarium of the Federal University of Rio Grande do Sul), according to previously described (Passos et al., 2013b). Briefly, the dried leaves were extracted with EtOH at room temperature. The solvent was removed under vacuum and the residual extract dissolved in 1 N HCl and exhaustively extracted with CH_2Cl_2 in order to remove non-polar constituents. Subsequently, the acid extracts were alkalinized with 25% NH_4OH (pH 9–10) and partitioned with CH_2Cl_2, resulting in the *P. laciniata* alkaloid-enriched extract. This extract was fractionated by reversed-phase medium pressure chromatography (RP-MPLC) with a H_2O-CH_3CN gradient step. Fraction containing *Z*-vallesiachotamine was submitted to preparative HPLC with isocratic elution (H_2O:CH_3CN, 40:60, v/v), rate 5 ml/min, and detection at 280 nm, affording the isolated compound (purity > 95%).

COMT enzymatic assay optimization

The COMT inhibitory activity was assessed based on methods previously described in the literature (Kurkela et al., 2004; Yalcin and Bayraktar, 2010), using aesculetin as COMT substrate and SAM as the methyl donor. The *O*-methylation of aesculetin catalyzed by COMT to afford scopoletin was monitored by fluorescence measurements (λ_{ex} = 355 nm and λ_{em} = 460 nm). The general reaction is presented below:

$$ES + SAM \xrightarrow{COMT} SAH + Scopoletin$$

where ES is aesculetin, SAM is the methyl donor S-(5′-adenosyl)-L-methionine iodide, COMT is catechol-*O*-methyltransferase, and SAH is S-adenosyl-homocystein.

For setting up the experimental conditions, different concentrations of the porcine COMT and of the methyl donor substrate, SAM, were tested. COMT was evaluated in concentrations of 0.75, 1.50 and 2.25 U/well while SAM was tested in concentrations of 20, 60, and 100 μM. The concentration of the catechol substrate aesculetin was kept constant at 0.6 μM. The control for COMT inhibition, DNC, was tested in concentration corresponding to its IC_{50} (35 nM) (Kurkela et al., 2004). Calibration curves of aesculetin and scopoletin (0.1–2 μM) were constructed in triplicate in order to calculate the rates of the *O*-methylation of aesculetin into scopoletin.

Evaluation of vallesiachotamine in COMT enzymatic assay

For the assay, 190 μl potassium buffer (pH 7.4) containing 20 mM L-cysteine, 5 mM magnesium chloride, 15 μl of 100 μM aesculetin (final concentration 6 μM), 15 μl of 150 U/ml porcine COMT (final concentration 2.25 U/well), and 5 μl of sample (final concentration varying from 15.62 to 500 μM) solubilized in 100% DMSO were used. In the blank wells, the enzyme was replaced by the corresponding amount of buffer. The plates were preincubated for 5 min at 37 °C, followed by the addition of 25 μl of 100 mM SAM (final concentration 100 μM). For the controls, samples were replaced by the corresponding amount of DNC solution (final concentration corresponding to the IC_{50} value, 35 nM) or DMSO. Readings were performed at temperature of 37 °C, for 60 min, in 4 min intervals. A microplate reader (SpectraMax® Molecular Devices, CA, USA) was used for fluorescence measurements. The excitation and emission wavelengths were adjusted to 355 and 460 nm, respectively. Calculations were performed using Excel and were based on the following equation:

$$\%\,\text{inhibition} = 100 - \frac{\Delta_{test} \times 100}{\Delta_{NC}}$$

where Δ_{test} is the difference between the test fluorescence reading in time 60 and 0 min; and Δ_{NC} is the difference between the negative control (DMSO) fluorescence readings in time 60 and 0 min.

Molecular modeling

The crystallographic structure of human soluble COMT (human S-COMT) (PDB ID: 3BWM) (Rutherford et al., 2008) was retrieved from the protein data bank (http://www.wwpdb.org/). Protein was prepared for structural inspection and docking using MOE 2014.09. The crystallized water molecules and the co-crystallized ligand DNC were removed, and the missing hydrogen atoms were added. The molecular docking protocol was developed using GOLD, version 5.2 (CCDC). COMT pocket was centered in the space occupied by the co-crystallized ligand DNC and delimited by an 8 Å radius. For each ligand, 100 docking solutions were generated by using 100,000 GOLD Genetic Algorithm interactions (Preset option). Docking poses were evaluated and ranked according to the ChemPLP scoring function. Docking protocol was validated by re-docking the co-crystallized ligand DNC in the COMT active site. RMSD values between the best ranked docking solution and the co-crystallized ligand lower than 2.0 Å were accepted as satisfactory.

Results and discussion

One of the aims of this study was to optimize the parameters for the evaluation of alkaloid fractions and MIAs on COMT. This enzyme is responsible for the methylation of dopamine and other endogenous and xenobiotic catechol substrates. Therefore, its inhibition can increase dopamine levels and lead to an improvement of the motor symptoms associated with PD (Dickinson and Elvevág, 2009). In fact, COMT inhibitors have been used clinically for some years in combination with levodopa. The first generation of these inhibitors, as tropolone, demonstrated low *in vivo* efficacy with high toxic effects. The second generation, represented by tolcapone and entacapone, replaced first generation COMT inhibitors, however they still have several dopaminergic and gastrointestinal side-effects. Nowadays, opicapone is under clinical trials, starting the third generation of this class, with higher potency and lower toxicity (Jatana et al., 2013; Bonifácio et al., 2014).

Different methods have already been reported for the *in vitro* evaluation of COMT inhibition, including spectrophotometric, fluorimetric and radioactive methods. Most of these methods describe the quantification of the product and of the remaining substrate by using chromatographic techniques; however, they are not well applicable for screening assays. Yalcin and Bayraktar (2010) developed a fluorescence-based method for the COMT evaluation, using aesculetin as the catechol substrate which is methylated to afford the fluorescent product scopoletin.

Aiming at optimizing a screening method based on Yalcin and Bayraktar (2010) study, the linearity of both esculetin and scopoletin were checked in five different concentrations, by triplicate experiments. It was possible to observe that both compounds demonstrated a linear behavior in the concentrations evaluated, with coefficient of determination higher than 0.99, which is in accordance to the literature for enzymatic assays (Tiwari and Tiwari, 2010).

The activity of COMT was evaluated using the enzyme obtained from porcine liver. Incubation temperature (37 °C), incubation time (60 min) and substrate concentration (0.6 μM) were kept constant in all experiments. The parameters evaluated were the concentration of the methyl donor substrate (SAM) and the amount of enzyme in the incubation medium. The combined variation of these parameters allows us to observe that higher concentrations of the enzyme and of the donor led to more reproducible results. With 0.75 U of the enzyme, and 20 μM of the substrate (lowest levels), the relative standard deviation (RSD) was 59.9%, while with 2.25 U of the enzyme and 100 μM of SAM (highest levels), the RSD was just 6.6%, which is in accordance to bioanalytical methods validation (Tiwari and Tiwari, 2010). Taking this into account, both enzyme and methyl donor higher levels were chosen to evaluate vallesiachotamine effect.

Yalcin and Bayraktar (2010) evaluated phenolic and alkaloids enriched extracts obtained from *Cistus parviflorus* Lam., *Vitex agnus-castus* L. and *Peganum harmala* L. The authors observed that an alkaloid fraction from *P. harmala* demonstrated better inhibition results than the phenolic extracts, with an IC_{50} value of 1.09 μg/ml. Taking into account that harmaline and harmalol were the main compounds detected for *P. harmala*, the effect of both β-carboline (βC) alkaloids were also evaluated, exhibiting IC_{50} values of 0.98 and 3.59 μM, respectively. Kinetic experiments revealed that harmaline showed a mixed-type COMT inhibition regarding the methyl donor substrate SAM while harmalol displayed an uncompetitive mode of inhibition.

In the present study, the COMT inhibitory effect of *Z*-vallesiachotamine was investigated. Just a few is known regarding alkaloids effects on COMT; however, considering the COMT inhibition displayed by plant-derived βC, it was expected that *Psychotria* MIA, as *E*/*Z*-vallesiachotamine, might also inhibit this enzyme. When evaluating the compound in a range from 15 to 500 μM, it was possible to determine an IC_{50} value of 196 μM (92% maximum inhibition). Compared to planar β-carbolines, the lower activity observed may be due to the differentiated geometry given by the monoterpenoid unit that is not present in *P. harmala* alkaloids. In addition, the saturation of the *N*-containing ring affords flexibility to the ligand, and this feature can decrease the potency of THβC alkaloids as vallesiachotamine for COMT inhibition.

Passos et al. (2013b) evaluated *E* and *Z*-vallesiachotamine regarding its monoamine oxidases (MAOs) and cholinesterases (ChEs) inhibitory activities. The alkaloids were able to inhibit butyrylcholinesterase (BChE) activity with IC_{50} of 7.08 and 9.77 μM, respectively. By docking studies, it was observed that the indole ring of *E*/*Z*-vallesiachotamine can establish hydrogen bonds with Ser-198 and His-438 residues of BChE, while the residues Trp-82, Trp-231, Leu-286, Phe-329, and Ile-442 can stabilize the compound through van der Waals contacts with the carbon atoms of the ring system. In addition, both compounds were able to inhibit MAO-A activity, with IC_{50} of 2.14 and 0.85 μM. Molecular docking on MAO-A revealed that the binding of *E*/*Z*-vallesiachotamine in MAO-A is stabilized by hydrophobic interactions between the ligand and the non-polar side chains of amino acids residues in the MAO-A pocket. Polar contacts between the ligand and two conserved water molecules, and Gln-215 side chain also contribute to the complex stabilization. Finally, the carboxymethyl moieties of the compounds are close to FAD cofactor, allowing a nucleophilic attack by the N5 of FAD, creating a covalent bond and reinforcing the irreversible binding.

Recently, the potential of *E*/*Z*-vallesiachotamine on sirtuins (SIRT1 and SIRT2) modulation was also evaluated. SIRT are class III histone deacetylases, responsible for the deacetylation of the *N*-acetyl-lysine tails of histones and of non-histone substrates, such as α-tubulin. Sirtuin inhibition, especially SIRT2, has been proposed as a new target to treat neurodegenerative diseases (Dillin and Kelly, 2007; Maxwell et al., 2011; Donmez and Outeiro, 2013). The nonselective inhibition of SIRT1 and SIRT2 enzymes by nicotinamide has shown to promote the restoration of cognitive deficits in a mice model of Alzheimer's disease (Green et al., 2008). By docking studies, Sacconnay et al. (2015) demonstrated that the vallesiachotamine ring structure of *E*/*Z*-vallesiachotamine and vallesiachotamine lactone is able to interact with Ala-262, Phe-273, Phe-297, Ile-347, and Val-445 residues of SIRT1. In addition, the acetyl group of these MIAs was able to make a hydrogen bond with Ser-442 backbone. For SIRT2, Ala-85, Phe-96, Ile-69, Phe-119, and Val-266 were involved in the stabilization of the complex between protein and natural product. By *in vitro* assays, this nonselective

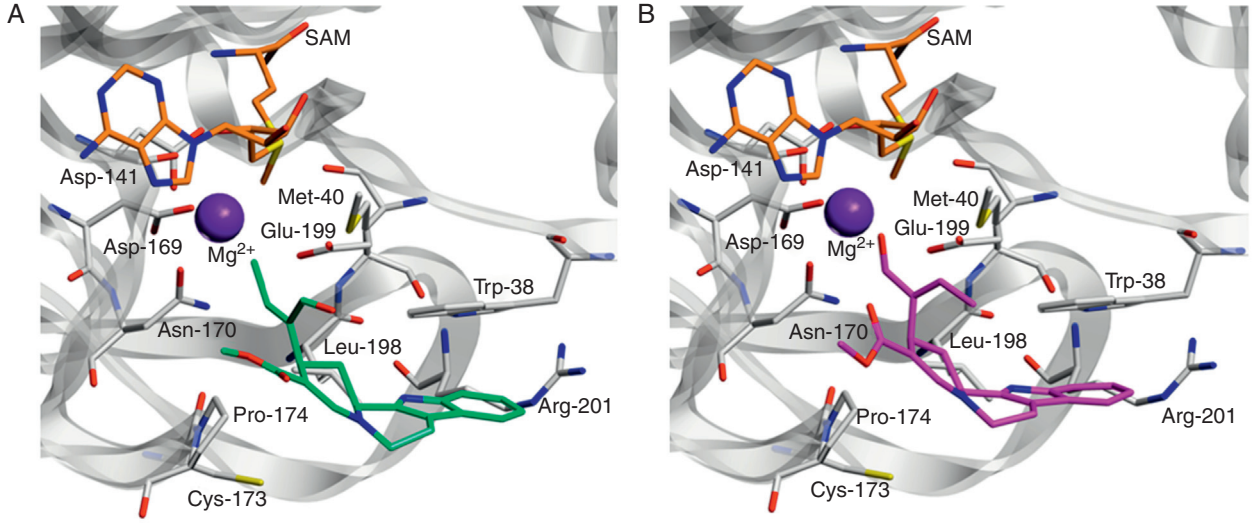

Fig. 1. Molecular docking results of *Z*-vallesiachotamine (A) and *E*-vallesiachotamine (B) in the cathecol-*O*-methyltransferase (COMT) catechol binding pocket (PDB ID: 3BWM; the carbon atoms of the protein are shown in gray, the carbon atoms of SAM are shown in orange, and Mg^{2+} ion is shown as a purple sphere). (A) The best ranked solution obtained for *Z*-vallesiachotamine (green) docking in COMT. (B) The best ranked solution obtained for *E*-vallesiachotamine (magenta) docking in COMT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

effect of vallesiachotamine was confirmed in a dose-dependent way.

Aiming at better understanding the interactions between *Z*-vallesiachotamine and COMT, molecular docking calculations were performed. As demonstrated in Fig. 1A, *Z*-vallesiachotamine was able to bind human COMT in the same pocket occupied by the co-crystallized DNC. Nonpolar contacts, namely π–π stacking interactions with Trp-38, and van der Waals interactions with Met-40, Pro-174, and Leu-198, seem to stabilize the protein-ligand complex. On the contrary to what is observed for COMT inhibitors containing cathecol groups, no coordination with the Mg^{2+} ion was verified for *Z*-vallesiachotamine, which can explain the IC_{50} value around 200 μM. In order to get additional clues regarding to the binding mode of vallesiachotamine-like alkaloids on COMT enzyme, the same docking protocol was performed to evaluate the potential interactions between *E*-vallesiachotamine and COMT. *E*-vallesiachotamine was able to bind the DNC pocket of human COMT, with the THβC nucleus in the same orientation observed for its *Z* isomer. In addition to the nonpolar contacts with Trp-38, Met-40, Pro-174, and Leu-198, the aldehyde oxygen of *E*-vallesiachotamine can coordinate with Mg^{2+}. This feature could suggest that these two structure-related MIA might be able to inhibit COMT showing different potencies and modes of inhibition.

Taking into account the obtained results, different MIA may be evaluated using the proposed screening assay in order to confirm or not their potential as COMT inhibitors, and to determine their structure–activity relationship. These secondary metabolites represent a source of chemical diversity and, based on this, they might be used as scaffolds for the development of new chemical entities acting on targets related to neurodegeneration, and possessing favorable pharmacokinetic and toxicological features.

Author contributions

CSP (Postdoctoral Researcher) performed the *in silico* experiments and part of the *in vitro* method optimization. LCKJ (PhD Student) performed the *in vitro* experiments and wrote the paper. JMMA (PhD Student) performed part of the *in vitro* experiments. Both CSP and JMMA contributed to write the manuscript. CM and ATH supervised the experimental work and contributed to the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Berger, A., Fasshuber, H., Schinnerl, J., Brecker, L., Greger, H., 2012. Various types of tryptamine-iridoid alkaloids from *Palicourea acuminata* (=*Psychotria acuminata*, Rubiaceae). Phytochem. Lett. 5, 558–562.

Bonifácio, M.J., Sutcliffe, J.S., Torrão, L., Wright, L.C., Soares-da-Silva, P., 2014. Brain and peripheral pharmacokinetics of levodopa in the cynomolgus monkey following administration of opicapone, a third generation nitrocatechol COMT inhibitor. Neuropharmacology 77, 334–341.

Brisch, R., Bernstein, H.G., Krell, D., Dobrowonly, H., Bielau, H., Steiner, J., Gos, T., Funke, S., Stauch, R., Knüppel, S., Bogerts, B., 2009. Dopamine–glutamate abnormalities in the frontal cortex associated with the catechol-*O*-methyltransferase (COMT) in schizophrenia. Brain Res. 1269, 166–175.

Cheng, G.-G., Zhao, Y.-L., Zhang, Y., Lunga, P.-K., Hu, D.-B., Li, Y., Gu, J., Song, C.-W., Sun, W.-B., Liu, Y.-B., Luo, X.-D., 2014. Indole alkaloids from cultivated *Vinca major*. Tetrahedron 70, 8723–8729.

Dickinson, D., Elvevág, B., 2009. Genes, cognition and brain through a COMT lens. Neuroscience 164, 72–87.

Dillin, A., Kelly, J.W., 2007. The yin-yang of sirtuins. Science 317, 461–462.

Djerassi, C., Monteiro, H.J., Walser, A., Durham, L.J., 1966. Alkaloids studies. LVI. The constitution of vallesiachotamine. J. Am. Chem. Soc. 88, 1792–1798.

Donmez, G., Outeiro, T.F., 2013. SIRT1 and SIRT2: emerging targets in neurodegeneration. EMBO Mol. Med. 5, 344–352.

Evans, D.A., Joule, J.A., Smith, G.F., 1968. The alkaloids of *Rhazya orientalis*. Phytochemistry 7, 1429–1431.

Green, K.N., Steffan, J.S., Martinez-Coria, H., Sun, X., Schreiber, S.S., Thompson, L.M., Laferla, F.M., 2008. Nicotinamide restores cognition in Alzheimer's disease transgenic mice via a mechanism involving sirtuin inhibition and selective reduction of Thr231-Phosphotau. J. Neurosci. 28, 11500–11510.

Hamaue, N., Ogata, A., Terado, M., Tsuchida, S., Yabe, I., Sasaki, H., Hirafuji, M., Togashi, H., Aoki, T., 2010. Entacapone, a catechol-*O*-methyltransferase inhibitor, improves the motor activity and dopamine content of basal ganglia in a rat model of Parkinson's disease induced by Japanese encephalitis virus. Brain Res. 1309, 110–115.

Heitzman, M.E., Neto, C.C., Winiarz, E., Vaisberg, A.J., Hammond, G.B., 2005. Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae). Phytochemistry 66, 5–29.

Jatana, N., Sharma, A., Latha, N., 2013. Pharmacophore modeling and virtual screening studies to design potential COMT inhibitors as new leads. J. Mol. Graph. Model. 39, 145–164.

Kastner, R.S., Van Os, J., Steinbusch, H., Schimtz, C., 2006. Gene regulation by hypoxia and the neurodevelopmental schizophrenia. Schizophr. Res. 84, 253–271.

Klein-Júnior, L.C., Passos, C.S., Moraes, A.P., Wakui, V.G., Konrath, E.L., Nurisso, A., Carrupt, P.-A., Oliveira, C.M.A., Kato, L., Henriques, A.T., 2014. Indole alkaloids and semisynthetic indole derivatives as multifunctional scaffolds aiming the inhibition of enzymes related to neurodegenerative diseases – a focus on *Psychotria* L. genus. Curr. Top. Med. Chem. 14, 1056–1075.

Kurkela, M., Siiskonen, A., Finel, M., Tammela, P., Taskinen, J., Vuorela, P., 2004. Microplate screening assay to identify inhibitors of human catechol-*O*-methyltransferase. Anal. Biochem. 331, 198–200.

Mannisto, P.T., Kaakkola, S., 1999. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol. Rev. 51, 593–628.

Matsumoto, M., Weickert, C.S., Beltaifa, S., Kolachana, B., Chen, J., Hyde, T.M., Herman, M.M., Weinberger, D.R., Kleinman, J.E., 2003. Catechol-*O*-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. Neuropsychopharmacology 28, 1521–1530.

Maxwell, M.M., Tomkinson, E.M., Nobles, J., Wizeman, J.W., Amore, A.M., Quinti, L., Chopra, V., Hersch, S.M., Kazantsev, A.G., 2011. The sirtuin 2 microtubule deacetylase is an abundant neuronal protein that accumulates in the aging CNS. Hum. Mol. Genet. 20, 3986–3996.

Passos, C.S., Soldi, T.C., Abib, R.T., Apel, M.A., Simões-Pires, C., Marcourt, L., Gottfried, C., Henriques, A.T., 2013a. Monoamine oxidase inhibition by monoterpene indole alkaloids and fractions from *Psychotria suterella* and *Psychotria laciniata*. J. Enzyme Inhib. Med. Chem. 28, 611–618.

Passos, C.S., Simões-Pires, C., Nurisso, A., Soldi, T.C., Kato, L., Oliveira, C.M.A., Faria, E.O., Marcourt, L., Gottfried, C., Carrupt, P.-A., Henriques, A.T., 2013b. Indole alkaloids of *Psychotria* as multifunctional cholinesterases and monoamine oxidases inhibitors. Phytochemistry 86, 8–20.

Paul, J.H.A., Maxwell, A.R., Reynolds, W.F., 2003. Novel Bis(monoterpenoid) indole alkaloids from *Psychotria bahiensis*. J. Nat. Prod. 66, 752–754.

Rutherford, K., Le Trong, I., Stenkamp, R.E., Parson, W.W., 2008. Crystal structures of human 108 V and 108 M catechol-*O*-methyltransferase. J. Mol. Biol. 380, 120–130.

Rybakowski, J.K., Borkowska, A., Czerski, P.M., Dmitrzak-Weglarz, M., Skibinska, M., Kapelski, P., Hauser, P., 2006. Performance on the Wisconsin Card Sorting Test in schizophrenia and genes of dopaminergic inactivation (COMT, DAT, NET). Psychiat. Res. 143, 13–19.

Sacconnay, L., Ryckewaert, L., Passos, C.S., Guerra, M.C., Kato, L., Oliveira, C.M.A., Henriques, A.T., Carrupt, P.-A., Simões-Pires, C., Nurisso, A., 2015. Alkaloids from *Psychotria* target sirtuins: *in silico* and *in vitro* interaction studies. Planta Med. 81, 517–524.

Shang, J.-H., Cai, X.-H., Feng, T., Zhao, Y.-L., Wang, J.-K., Zhang, L.-Y., Yan, M., Luo, X.-D., 2010. Pharmacological evaluation of *Alstonia scholaris*: anti-inflammatory and analgesic effects. J. Ethnopharmacol. 129, 174–181.

Shirakawa, T., Abe, M., Oshima, S., Mitome, M., Oguchi, H., 2004. Neuronal expression of the catechol-*O*-methyltransferase nRNA in neonatal rat suprachiasmatic nucleus. Neuroreport 15, 239–1243.

Soares, P.R.O., Oliveira, P.L., Oliveira, C.M.A., Kato, L., Guillo, L.A., 2012. In vitro antiproliferative effects of the indole alkaloid vallesiachotamine on human melanoma cells. Arch. Pharm. Res. 35, 565–571.

Solis, P.N., Wright, C.W., Gupta, M.P., Phillipson, J.D., 1993. Alkaloids from *Cephaelis dichroa*. Phytochemistry 33, 1117–1119.

Tiwari, G., Tiwari, R., 2010. Bioanalytical method validation: an update review. Pharm. Methods 1, 25–38.

Yalcin, D., Bayraktar, O., 2010. Inhibition of catechol-*O*-methyltransferase (COMT) by some plant-derived alkaloids and phenolics. J. Mol. Catal. B: Enzym. 64, 162–166.

Zhong, X.-H., Xiao, L., Wang, Q., Zhang, B.-J., Bao, M.-F., Cai, X.-H., Peng, L., 2014. Cytotoxic 7S-oxindole alkaloids from *Gardneria multiflora*. Phytochem. Lett. 10, 55–59.