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# Effect of scopoletin on monoamine oxidases and brain amines

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ABSTRACT

Naturally, occurring compounds with MAO inhibitory property may provide promising lead molecules against neurodegenerative disorders. We report MAO inhibitory activity of a naturally occurring coumarin (validated chemical scaffold as MAO inhibitors), scopoletin. It selectively (and reversibly) inhibits human (Ki = 20.7 μM) and mouse (Ki = 22 μM) MAO-B, ~3.5 times more selective towards MAO-B than MAO-A. Docking studies revealed its molecular recognition and explained the selectivity mechanism towards MAO isoforms. Scopoletin occupied the hydrophobic aromatic pockets showing favorable interactions for MAO-B; experimental $K_i$ agreed with the predicted $K_i$. *In vivo*, scopoletin (80 mg/kg, i.p.) treatment significantly increases dopamine level and decreases its metabolite DOPAC in striatum. Overall, scopoletin is a partially selective MAO-B inhibitor that increases brain dopamine level.

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## 1. Introduction

Monoamine oxidase (MAO) is a mitochondrial bound flavoenzyme that catalyzes oxidative deamination of xenobiotics and biogenic amines. Based on their substrate and inhibitor specificities they are classified into two isoforms: MAO-A and MAO-B. MAO-A preferentially metabolizes bulky endogenous amines (serotonin, noradrenaline, and adrenaline); MAO-B metabolizes small exogenous amines such as benzylamine and phenethylamine. DA and p-tyramine are common substrates for both isoforms. MAO (A and B) are validated drug targets for treating neurological disorders. Selective MAO A inhibitors are used as antidepressants and MAO B inhibitors are used in Parkinson's and Alzheimer's disease (Carradori and Petzer, 2015).

Recently, coumarins (2H-1-benzopyran-2-one) have been reported as MAO inhibitors (Carradori et al., 2014). We explored MAO inhibitory property of scopoletin (1, 6-methoxy-7-hydroxy coumarin), which is a naturally occurring coumarin. It is found in the roots of plant genus Scopolia (Sethna and Shah, 1945), in many edible plants, and fruits such as *Avena sativa*, *Allium ampeloprasum*, *Apium graveolens*, *Capsicum annuum*, *Capsicum frutescens*, *Daucus carota*, *Cichorium intybus*, *Citrus limon*, *and Citrus paradise*. It's occurrence in these plants indicate-safety for consumption (Carpinella et al., 2005). Scopoletin is reported as anti-inflammatory (Ding et al., 2008), anti-depressant (Capra et al., 2010), antioxidant (Panda and Kar, 2006; Shaw et al., 2003), choline acetyltransferase, acetylcholinesterase inhibitor (Hornick et al., 2011; Rollinger et al., 2004), aldose reductase inhibitor (Lee et al., 2010), and gama-aminotransferase inhibitor (Mishra et al., 2010). It has also been mentioned as MAO inhibitor but requires detailed exploration (Yun et al., 2001).

Here, we present detailed study on scopoletin: a) exploring its selectivity towards MAO-A/B using both rat and human enzymes; b) molecular docking to understand its binding mode; and c) demonstrated the effect of scopoletin on metabolism of brain amines.

## 2. Material and methods

### 2.1. Animals

All animal studies were carried out using male Swiss albino mice, weighing 25–30 g. They were housed in a controlled environment (12 h light/dark cycles, 21 ± 2 °C temperature) with food, and water ad libitum. This study protocol has been approved by Ethical Committee for Laboratory Animals of Birla Institute of Technology, Mesra, Ranchi, India. All experiments were carried out

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in accordance with CPSCE guidelines. The scopoletin was suspended in 1% tween 80 (vehicle), injected intra-peritoneally (80 mg/kg, i.p.) and during all the experiments vehicle control was used for comparison.

### 2.2. Monoamine amine oxidase assay

MAO-A and B inhibitory activity of scopoletin was estimated using both rat and human enzymes. Scopoletin and recombinant human MAO-A and B was purchased from Sigma–Aldrich Chemical Co. Scopoletin stock solution was prepared in DMSO, and tested in the range of 1–100 μM concentration. DMSO concentration was maintained 4% in the final assay reaction mixture. Rat MAO-A/B enzymes were purified from its liver. Rat was sacrificed, liver was removed, and homogenized (1:10 w/v) in ice-cold potassium phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 1000 g, 4 °C for 15 min and the supernatant was used as a source of MAO enzymes.

The MAO inhibitory activity of scopoletin was estimated as previously reported (Holt et al., 1997). Enzyme solution 125 μl (with or without scopoletin), assay mixture 125 μl (vanillinic acid, 4-aminoantipyrine and peroxidase type II in potassium phosphate buffer (pH 7.6)), and substrate 500 μl (p-tyramine, 500 mM for MAO-A and 2.5 mM for MAO-B) were mixed, and increase in absorbance was monitored at 498 nm at 37 °C for 60 min. Lineweaver burk plot was used to estimate the inhibition constant (Ki) of the scopoletin. It is a plot of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor, versus inhibitor [I] concentration. For selective MAO-A and MAO-B activity measurement, mice liver homogenate was pre-incubated with selective MAO inhibitors (Chlorgyline or Pargyline) 50 mM, 1:100v/v. Human MAO-A/B were pure commercial enzymes thus no pre-incubation was required.

Reversibility of the MAO inhibition by scopoletin was assessed by recovering the MAO-A/B activity from scopoletin and enzyme mixture by dialysis. For reversibility study, we have used human recombinant MAO-A and MAO-B enzymes in dialysis tubing cellulose membrane (Sigma–Aldrich Chemical Co.), with a molecular weight cut-off of 12,000 Da. Scopoletin at higher concentrations were added to MAO-A and MAO-B enzymes, and pre-incubated for 30 min at 37 °C. The controls include, similarly pre-incubated MAO-A and MAO-B enzymes in the presence of the irreversible inhibitors clorgyline, pargyline, and no inhibitor. These enzyme–inhibitor mixtures were subsequently dialyzed at 4 °C in dialysis buffer (0.1 mM potassium phosphate, pH 7.4). The dialysis buffer was replaced with fresh buffer every 3 h and 7 h after commencement of dialysis. After 24 h the recovered enzyme was solubilized in potassium phosphate buffer (0.1 M, pH 7.4) from different dialysis reactions and residual enzyme activity was assayed as described above.

### 2.3. Docking

All docking studies were performed as reported earlier (Jagrat et al., 2011; Karuppasamy et al., 2010; Sahoo et al., 2010) described as follows.

#### 2.3.1. Protein preparation

Crystallographic models 2BXR (hMAO-A) and 2BYB (hMAO-B) were downloaded from www.rcsb.org. Hydrogens were added and bond order was adjusted. Water molecules within 5 Å were kept and rest were deleted. The covalently linked ligand was also deleted, and bond order was corrected for FAD. Thereafter, partial charges (Gasteiger-Huckel) were assigned, protonation state for charged aminoacid side chains were finally energy minimization (1000 iteration and convergence threshold 0.1) was performed using OPLS2005 force field. The protein files written by Maestro for prepared proteins were re-written by Open Babel for AutoDock compatible atom type.

#### 2.3.2. Ligand preparation

Structure of scopoletin were sketched using built panel of Maestro and were energy minimized using OPLS2005 (1000 iterations, convergence threshold 0.05) force field of Macro Model (MacroModel, version 9.6, Schrödinger, LLC, New York, NY, 2005) and saved in PDB format.

#### 2.3.3. Simulations

All the docking experiments were performed with AutoDock 4.0. Lamarckian Genetic Algorithm was employed as the docking algorithm (Mishra and Sasmal, 2011). Grid parameter file (.gpf) and Docking parameter files (.dpf) were written using MGLTools-1.4.6. Receptor grids were generated using 60 × 60 × 60 grid points in xyz with grid spacing of 0.375 Å. Grid box was centered on N5 atom of FAD. Map types were generated using autogrid4. Docking parameters: Number of Genetic Algorithm (GA) runs: 10, Population size: 150, Maximum number of evaluation: 2,500,000, Maximum number of generation: 27,000. Best scoring docked poses from the largest cluster were used for further structural and interaction studies.

The docking protocol for MAO enzymes has been standardized as reported earlier. This docking protocol provided significant results. We used the protocol as is without any modifications (Badavath et al., 2015; Jagrat et al., 2011; Jayaprakash et al., 2008; Karuppasamy et al., 2010; Sahoo et al., 2010).

### 2.4. Determination of levels of biogenic amines in mouse brain

We studied the chronic (21 days) effect of scopoletin (8 mg/kg, i.p.) on mice brain amines and compared with vehicle control. Mice were sacrificed after 1 h of last administered dose. Striatum was rapidly dissected (over ice bath). Levels of DA, NA, 5-HT, and their metabolites DOPAC and HIAA were determined by HPLC (Waters HPLC systems, Milford, MA, USA), equipped with electrochemical detector. Striatum was homogenized in mobile phase (600 μl), and centrifuged at 2000 *g*. The mobile phase was made up of methanol: buffer (7.5: 92.5, v/v). The buffer composition was: 0.07 M sodium acetate, 0.04 M citric acid, 130 μM EDTA and 230 μM sodium octane sulfonate. HPLC analysis was performed at 35 °C, pH 4.5 and flow rate 1 ml/min. For protein estimation the tissue pellets were neutralized with 1 N NaOH (Bradford, 1976).

### 2.5. Statistical analysis

Mean data are shown together with standard error; mean values ± S.E.M. Significance analysis was done by t test and tukey post hoc test.

## 3. Results and discussion

### 3.1. MAO inhibitory activity in vitro

MAO activity was quantified by spectrophotometric method. We measured the metabolism of tryramine (common substrate) that releases hydrogen peroxide. In this reaction, 4-aminoantipyrine (proton donor) is condensed with vanillic acid to form a red quinonemine dye, which absorbs maximally at 498 nm. Metabolism of tyramine (with or without scopoletin) by MAO-A/B was observed

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