Study aims at boosting antitumoral activity of compound extracted from an Amazon plant

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Uncaria guianensis, in bloom, Cacuri, Venezuela. CC3.0

Researchers in Brazil have prepared modified forms of the alkaloids produced by *Uncaria guianensis*, a woody vine native to the Amazon Rainforest.

U. guianensis is a medicinal plant commonly known as cat's claw. The natural versions of alkaloids are widely used to combat tumors and inflammation, and they can help modulate the immune system. The scientists sought to design therapeutically more potent chemicals.

The study was supported by São Paulo Research Foundation—FAPESP and conducted by researchers affiliated with the University of Ribeirão Preto (UNAERP) and the Federal University of São Carlos (UFSCar), both in São Paulo State. The results are published in *Scientific Reports*.

Alkaloids, which are organic compounds produced by plants or microorganisms, have long been used in medicine. One example is morphine, which is extracted from the opium poppy (*Papaver somniferum*).

Recent studies have shown that minor modifications in the chemical structure of certain alkaloids can enhance their therapeutic effects. Fluorovinblastine, for example, results from the addition of fluorine to the chemical structure of vinblastine, a natural alkaloid produced by the Rosy periwinkle (*Catharanthus roseus*).

US researchers have demonstrated that the anti-tumor activity of fluorovinblastine is 30 times more potent than that of the natural compound.

"When we compared the structure of vinblastine and of the alkaloids from *U. guianensis*, we found highly similar biosynthetic pathways. One of the modifications we made was therefore similar: we substituted a fluorine atom for a hydrogen in the aromatic ring [a small part of the

structure of the molecule]," said Adriana Aparecida Lopes, a professor in UNAERP's Biotechnology Unit and first author of the article.

The outcome of this small modification was a new alkaloid called 6-fluoro-isomitraphylline, which has three aromatic hydrogens and one fluorine instead of the natural alkaloid's four aromatic hydrogens. The researchers also produced an analogue called 7-methyl-isomitraphylline by substituting a methyl group for an aromatic hydrogen.

The research was supported by FAPESP via a Young Investigator Grant and a regular research grant under the São Paulo Researchers in International Collaboration program.

A project continuing the research has just been selected in a call for proposals issued jointly by FAPESP; the National Council of State Funding Agencies (CONFAP); the National Council for Scientific and Technological Development (CNPq, an agency of the Brazilian government); and the European Research Council (ERC).

The new project will be conducted in collaboration with Sarah O;Connor, Director of the Max Planck Institute for Chemical Ecology in Germany.

Laboratory plants

Modifications were made to the alkaloids using *U. guianensis*'s own metabolic pathways. Plantlets (young plants) not more than 15 cm tall were grown in the laboratory and were fed with water and nutrients. Precursors of the natural alkaloids with small modifications to their structures were added to this liquid medium.

"This protocol is called precursor-directed biosynthesis," Lopes explained. "The synthesis is done by the plant. I provide it with an

analogous key intermediate [precursor], which is captured and inserted into its metabolic route, forming a new alkaloid. This is 'green chemistry," totally free of solvents or reagents, and uses an in vitro plantlet system."

The plants were grown for 30 days and then extracted. The extracts were subjected to different types of liquid chromatography and mass spectrometry to identify the substances present in the extract based on their corresponding ions. Following this characterization, chemical (chromatographic) processes were used to isolate the alkaloid analogues.

The two novel alkaloid analogues modified with fluorine and methyl groups were analyzed by nuclear magnetic resonance (NMR) spectroscopy to confirm their chemical structure.

Using this method, approximately one to two milligrams of the new alkaloids were obtained. The next steps will entail increasing the output. To do so, it will be necessary to silence the production of the natural alkaloids by the plant, as the researchers want the plant to produce only the fluorinated version in their laboratory.

"We'll have to silence the enzyme TDC, which is present in the plant's metabolism and converts the amino acid tryptophan into tryptamine. The plant will cease producing natural tryptamine and will produce only the modified version," Lopes said.

The team expects the therapeutic efficacy of the novel compounds obtained from *U. guianensis* using precursor-directed biosynthesis to be more potent than those produced naturally by the plant.

More information: Adriana A. Lopes et al, Unnatural spirocyclic oxindole alkaloids biosynthesis in Uncaria guianensis, *Scientific Reports* (2019). DOI: 10.1038/s41598-019-47706-3

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