**Results:** Ginger treatment resulted in dose-dependent sequences of events marked by apoptosis, as shown by loss of cell viability, chromatin condensation, DNA fragmentation, activation of caspase 3, and cleavage of poly (ADP-ribose) polymerase. At the molecular level, the apoptotic cell death mediated by ginger could be attributed in part to up-regulation of Bax and down-regulation of Bcl-2 proteins. Ginger treatment modulated expression of proteins involved in cell cycle regulation; it decreased expression of cyclin D1, cyclin-dependent kinase-4 (CDK-4), but increased expression of CDK inhibitor, p21. It also inhibited the expression of the two prominent molecular targets of cancer, c-Myc and the human telomerase reverse transcriptase (hTERT).

**Conclusion:** These findings suggest that the ginger may be a promising candidate for the treatment of breast carcinomas.

*Keywords*: *Zingiber officinale*, medicinal plants, apoptosis, gene expression, breast cancer, cell line
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## 12. Structural Interpretation for the Subnanomolar Affinity of Muscarinic Toxin 7 for Human Muscarinic Acetylcholine Receptor 1 by Modeling Protein-Protein Interaction

Jianrong Xu [1], Jun Xu [2], Hongzhuan Chen [1]
[1] *Department of Pharmacology, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China*
[2] *Research Center for Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, P. R. China*
*E-mail address:* janker.xu@gmail.com (J. Xu).

**Background:** Human muscarinic acetylcholine receptor 1 (hM1) is closely related to several diseases, such as Alzheimer's disease, schizophrenia, and peptic ulcer. MT7, a muscarinic toxin isolated from the snake venom of *Dendroaspis angusticeps*, is the only natural selective hM1 allosteric binder with subnanomolar affinity ($K_d$ = 14pM). MT7 is a peptide with 66 residues, possessing a three-finger structure; and hM1 is a GPCR membrane protein, sharing the conserved structure of seven transmembrane regions. Understanding the binding mode of hM1- MT7 will give insights to discover small molecular selective ligand for hM1.

**Methods:** The structure of hM1 was constructed by homology modeling, and the bilayer membrane was created by the VMD program. The initial interaction coordinate of hM1-MT7 was generated by protein-protein docking in PatchDock program. Explicit membrane molecular dynamics (MD) simulations with Amber program were utilized to produce the dynamic trajectories of hM1-MT7. Binding energy between hM1 and MT7 was calculated with MM/PBSA method and decomposed into every residue to reveal the binding mode of hM1-MT7.

**Results and Discussion:** The hM1-MT7 binding mode is discovered to consist of five residue interaction clusters, which are separated in three interaction regions. By analyzing the cluster representative structures, the cluster residues form an interaction network, which shows a multiple-point-to-site binding mode. It is revealed in hydrogen binding statistical analysis that Glu170 (hM1) and Arg34 (MT7) are both locked in electrostatic cages with counter charges, respectively. It makes hM1-MT7 hard to dissociate, leading to the high binding affinity of MT7. This is confirmed by the dynamic distances calculation between these residues, and it is consistent with biological mutant experiments.

**Conclusions:** MT7 is discovered to bind to hM1 in a multiple-point-to-site mode. By forming a core in the interaction network, Glu170 (hM1) and Arg34 (MT7) are responsible for the subnanomolar affinity, which is consistent with the biological experimental data.

*Keywords*: muscarinic toxin, muscarinic acetylcholine receptor, molecular dynamics simulations, protein-protein docking
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## 13. Crotamine Pharmacology Revisited: Novel Insights Based on the Inhibition of Kv Channels

Steve Peigneur [1], Diego Orts [2], Alvaro Prieto da Silva [3], Nancy Oguiura [4], Malvina Boni-Mitake [5], Eduardo Brandt de Oliveira [6], André Junqueira Zaharenko [3], J.C. de Freitas [2], J. Tytgat [1]
[1] *Laboratory of Toxicology, University of Leuven (K.U. Leuven), Campus Gasthuisberg O&N2, Herestraat 49, P.O. Box 922, B-3000 Leuven, Belgium*
[2] *Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil*
[3] *Laboratório de Genética, Instituto Butantan, São Paulo, SP, Brazil*
[4] *Laboratório de Ecologia e Evolução, Instituto Butantan, São Paulo, SP, Brazil*
[5] *Gerência de Radioproteção, IPEN, CNEN, São Paulo, SP, Brazil*
[6] *Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
*E-mail address:* steve.peigneur@pharm.kuleuven.be (S. Peigneur).

**Background:** Crotamine, a 5KDa peptide possesses a unique biological versatility. Not only its cell-penetrating activity has become of clinical interest but moreover, its potential selective anti-tumor activity is of great pharmacological importance. Before, several studies have attempted to elucidate the exact molecular target responsible for the crotamine-induced skeletal muscle spasm. The aim of this study was to investigate whether crotamine affects voltage-gated potassium ($K_V$) channels in an effort to explain its *in vivo* effects.

**Methodology/Principal findings:** Crotamine was studied on ion channel function using the two-electrode voltage clamp technique on 16 cloned ion channels (12 $K_v$ channels and 4 $Na_v$ channels), expressed in *Xenopus laevis* oocytes. Crotamine selectively inhibits $K_v$1.1, $K_v$1.2 and $K_v$1.3 channels with an $IC_{50}$ of ~300 nM and the key amino acids responsible for this molecular interaction are highlighted. Our results demonstrate for the first time that the symptoms which are observed in the typical crotamine syndrome may result from the inhibition of $K_v$ channels.

**Conclusions/significance:** The ability of crotamine to inhibit the potassium current through $K_v$ channels unravels it as the first snake peptide with the unique multifunctionality such as cell penetrating, antitumoral activity and $K_v$ channel inhibiting properties. The potent and selective $K_v$ channel inhibiting properties, as demonstrated in this work, can be an advantage for the use of crotamine or its derivatives as anti-tumor drug. This new property of crotamine might explain

some experimental observations and opens new perspectives of pharmacological uses.

*Keywords*: Crotamine, voltage-gated potassium channel, snake toxin, *Crotalus durissus terrificus*, anti-tumor drug
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## 14. Evaluation of the Cytotoxic Activity of *Rhinella schneideri* Toad Poison on Tumor Cells and on Healthy Mononuclear Cells

Elisa C. Fornari Baldo [1], Cássio P. da Silva [2], Suely V. Sampaio [2], Eliane C. Arantes [1]
[1] *Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[2] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
*E-mail address:* lifornari@yahoo.com.br (E.C. Fornari Baldo).

**Introduction:** Amphibian poisons, especially from Anura order, contain a variety of biologically active compounds such as biogenic amines, cardiotonic steroids, alkaloids and peptides. Cardiotonic steroids have been shown to induce apoptosis in a wide spectrum of tumor cell. However, the detailed molecular mechanisms of inducing apoptosis are still unclear. The aim of this study was the comparative evaluation of the cytotoxicity of *Rhinella schneideri* poison (Rsp) on tumor cell lines and on healthy mononuclear cells.

**Material and Methods:** A MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay was used to detect cell viability using tumor cell lines B16F10 (Murine Melanoma Cells), HL-60 (Murine Acute Promyelocytic Leukemia Cells), HepG2 (Hepatocellular Carcinoma Human Cells), PC-12 (Murine Pheochromocytoma Cells) and PBMC (Human Peripheral Blood Mononuclear Cells). The cells (5 x 104 per well) were plated in 96-well plates and incubated with Rsp (5, 10, 20, 50 and 100 μg/mL) for 24 h. Then, the MTT was added (10 μL) and after 3 h of incubation at 37C°, in 5% of CO2, it was observed the production of formazan crystals by viable cells. The solubilization of crystals was performed by addition of DMSO (100 μL) and the absorbance (DO) at 570 nm was measured. The cell viability was calculated by the equation: cell viability (%) = (DO treated group/DO control group) x 100%.

**Results:** In the presence of Rsp (5, 10, 20, 50 and 100 μg/mL) the cell viability (%) to HL-60 and B16F10 were 19.1, 19.1, 19.0, 17.5, 20.4, and 40.3, 37.1, 38.2, 32.4, 34.8, respectively. The values obtained to HepG2 and PC-12 were 82.4, 76.7, 79.2, 83.1, 84.2 and 72.8, 85.6, 93.4, 92.7, 76.9, respectively. The cell viability to PBMC was also high (87.4, 77.2, 81.3, 95.6, 99.7).

**Discussion:** The MTT assays showed that the tumor cell lines HL-60 and B16F10 are more sensitive to Rsp, which induced a marked inhibition of cell proliferation. The cell lines HepG2 and PC-12 showed high cell viability, meaning that Rsp slightly interfered with the replication process of these cells. Rsp showed no cytotoxicity to PBMC cells.

**Conclusion:** These results show that Rsp has selective cytotoxic activity for the different tumor cells evaluated, indicating the potential application of its components as therapeutic agents in oncology.

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*Keywords*: *Rhinella schneideri*, cardiotonic steroids, cell viability, anti-cancer activity
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## 15. New Perspective for Therapy Against Seizures Using Molecules From *Rhinella schneideri* Toad Poison

Mateus Amaral Baldo [1], José Luiz Liberato [2], Lívea Dornela Godoy [2], Wagner Ferreira dos Santos [2], Eliane Candiani Arantes [1]
[1] *Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[2] *Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
*E-mail address:* mateuseus@yahoo.com.br (M.A. Baldo).

**Background:** The number and diversity of compounds produced by amphibians in their glands is surprisingly high. Parotoid gland secretions from toads are useful source of chemical compounds with potential medical-pharmaceutical applications, among them biogenic amines, cardiotonic steroids, alkaloids and peptides. The aim of this study was to isolate and characterize components from *Rhinella schneideri* (Rs) poison and evaluate its potential use for inhibiting CNS seizures.

**Material and Methods:** The soluble poison was submitted to chromatography in HPLC system using C2C18 column. Five main fractions were obtained and Rs5 showed neuroprotective action. Male Wistar rats (250 g) were cannulated in the right lateral ventricle, following stereotactic coordinates. Rats were divided into groups (n=6) and the control animals received injections of saline (0.9%; i.c.v.) followed by the convulsants PTZ (80 mg/kg,i.p.) or NMDA (20 μg/μL, i.c.v.). Different concentrations of Rs5 (0.05, 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0 μg/μL; i.c.v.) were analyzed. Additionally, the lower concentration (0.05 μg/μL) was used concomitant with carbamazepine (CBZ) (20 μg/μL – protected 50% of rat against seizures) for evaluation of synergism between the two drugs.

**Results:** Pretreatment with Rs5 at concentrations of 0.5, 1.0, 1.5 and 2.0 μg/μL protected 40, 60, 84 and 100% of rats against tonic-clonic seizures induced by PTZ, respectively. Additionally, 50, 62, 62, 83 e 100% of rats treated with Rs5 at concentrations of 0.05, 0.1, 0.5, 1.0 and 2.0 μg/μL, respectively, were protected of seizures induced by NMDA. To evaluate the Rs5 toxicity rats were submitted to the rotarod assay after injection of Rs5 (2 μg/μL e 6 μg/μL μg/μL) and ataxia was not observed.

**Discussion:** Rs5 concentration able to inhibit seizures is lower than the concentration of CBZ which promotes the same effect, showing that it is a molecule with high neuroprotective activity. Additionally, was demonstrated synergism between CBZ (20 μg/μL) and Rs5 (0.05 μg/μL) showing 100% protection against seizures. Furthermore, at the rotaroad assay no animal fell from the apparatus indicating that Rs5 did not cause motor impairment.