[1-8-NαC]-Zanriorb A1, a Proapoptotic Orbitide from Leaves of *Zanthoxylum riedelianum*

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Figure S10. ZVAD-FMK inhibits cell death induced by **1**. Cells were incubated initially for 1 h with ZVAD-FMK, a caspase inhibitor, and Nec-1, a necroptosis inhibitor and then the cells ($1x10^5$/mL) were treated with **1** (218 nM) for 24 h. Cell viability was determined using annexin-V and PI staining. Results represent means ± SEM of three independent experiments performed in triplicate. ANOVA followed by Dunnett's multiple comparison test (** p <0.01 and *** p <0.001 vs. **1**).

Figure S11. [1-8-NαC]-Zanriorb A1 (**1**) induces caspase-3 activation. Cells ($1x10^5$/mL) were incubated with **1 (**218 nM) or 1 µM staurosporine for 12 h. Caspase 3 activation was accessed by flow cytometry using a cleaved caspase 3 antibody conjugated with Alexa Fluor 488. (a) Representative flow cytometry histograms. (b) Mean of intensity of cleaved caspase 3 using geometric mean. Results represent the means ± SEM of three independent experiments performed in triplicate, with ANOVA followed by Dunnett's multiple comparison test (** p <0.01 and *** p <0.001 vs. untreated control).

Figure S12. [1-8-NαC]-Zanriorb A1 (**1**) elicited a decrease of Ψ_{mit} in Jurkat cells. Cells ($1x10^5$/mL) were incubated with the compound **1** (218 nM) or CCCP 50 µM for 24 h, then, JC-1 dye was added and samples were analyzed by flow cytometry. (a) Representative flow cytometry density plots. (b) Mean of ratio of green fluorescence/red fluorescence. Each column represents the mean of three independent experiments performed in triplicate. Results represent the means ± SEM of three independent experiments performed in triplicate, with ANOVA followed by Dunnett's multiple comparison test (*** p <0.001 vs untreated control).

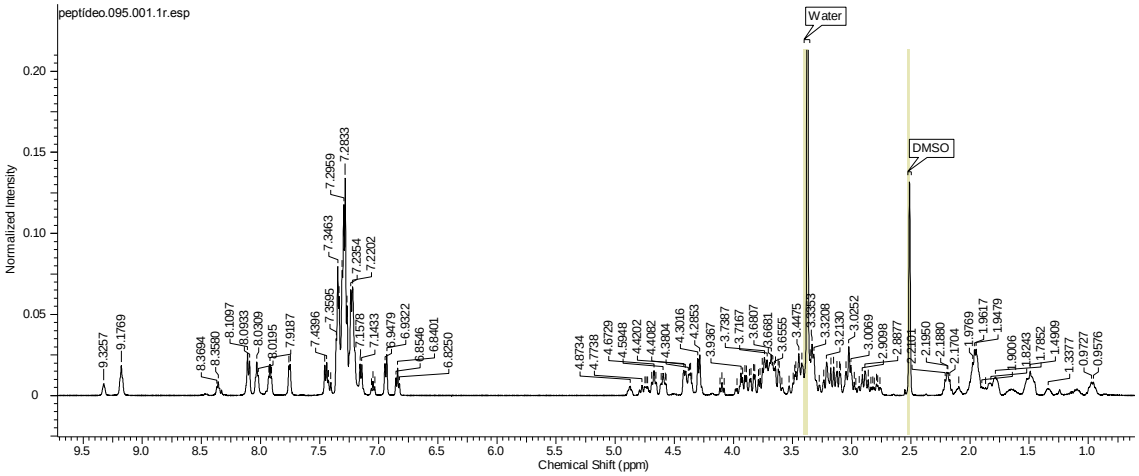


Figure S1. ^{1}H-NMR spectrum of [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).

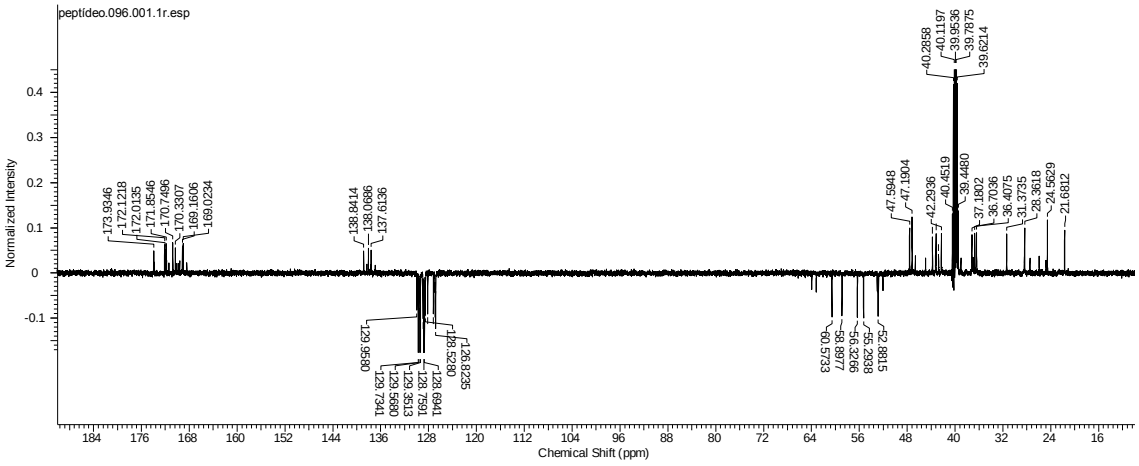
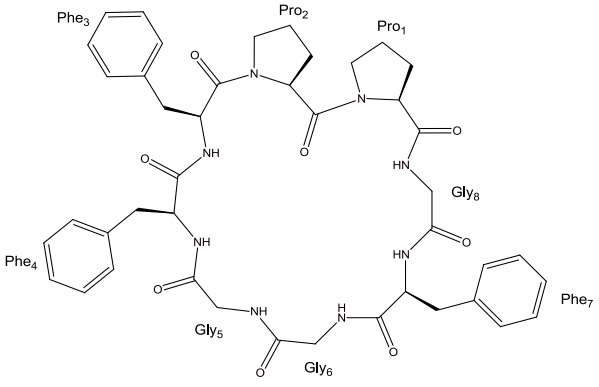


Figure S2. DEPTQ NMR spectrum of [1-8-NαC]-zanriorb A1 (**1**) (125 MHz in DMSO-d_6).



1

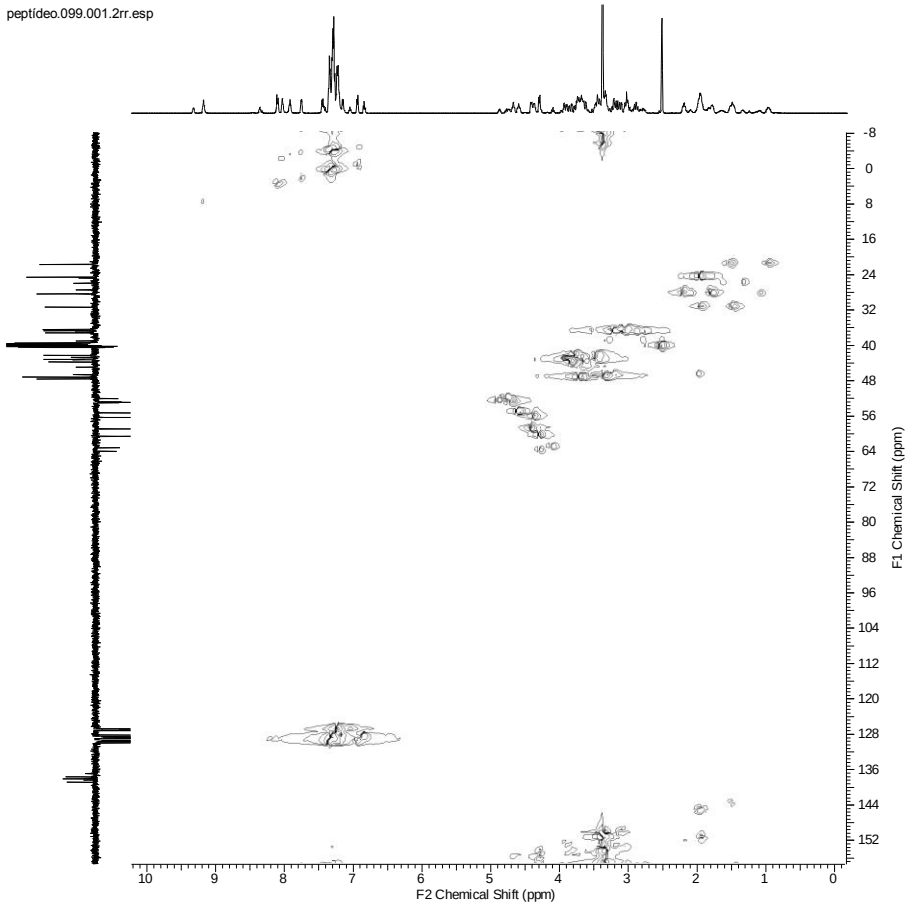


Figure S3. HSQC spectrum of [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).

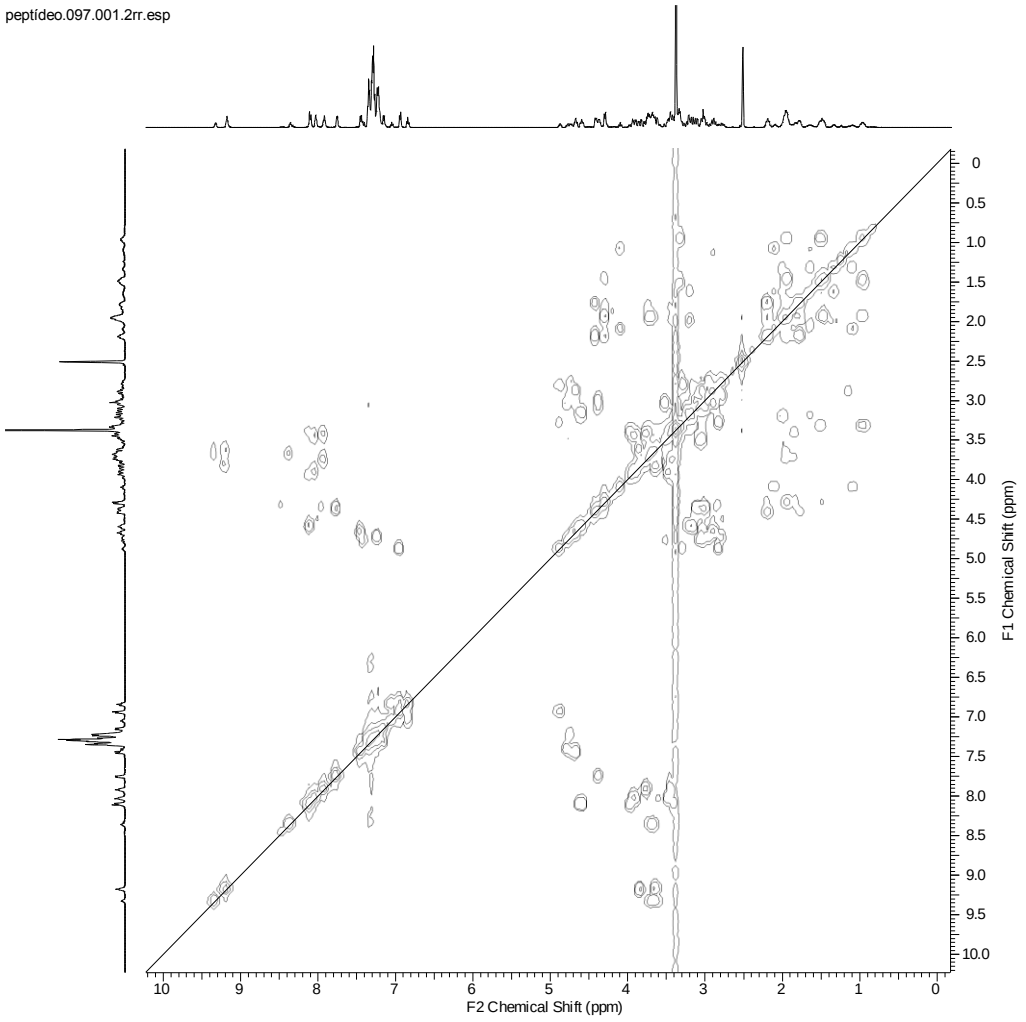
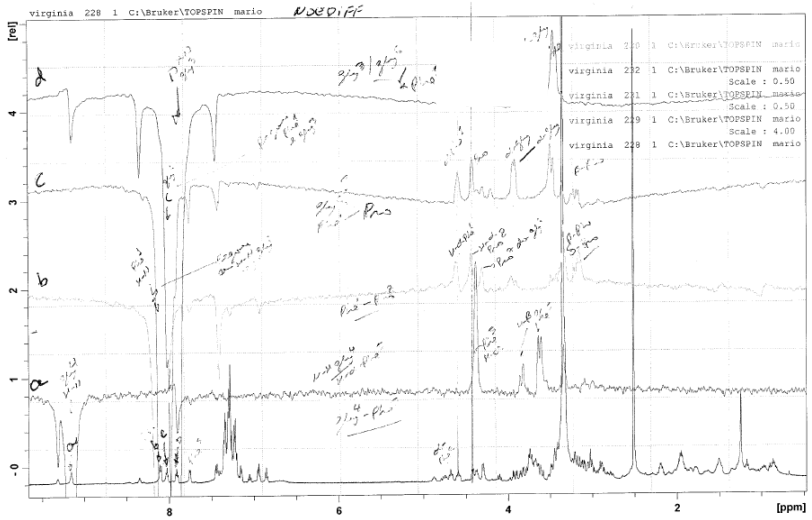


Figure S4. COSY spectrum of [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).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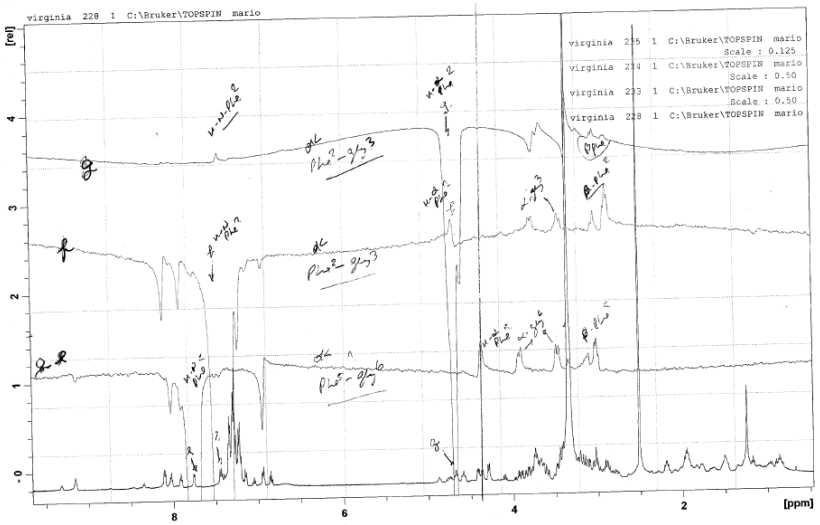
[ppm]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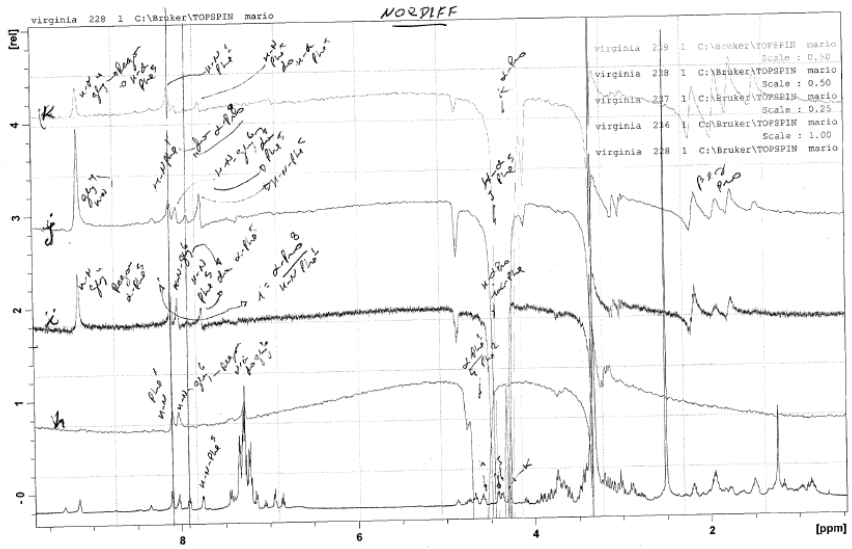
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[ppm]

Figure S5. NOEDIFF experiments spectra for [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).

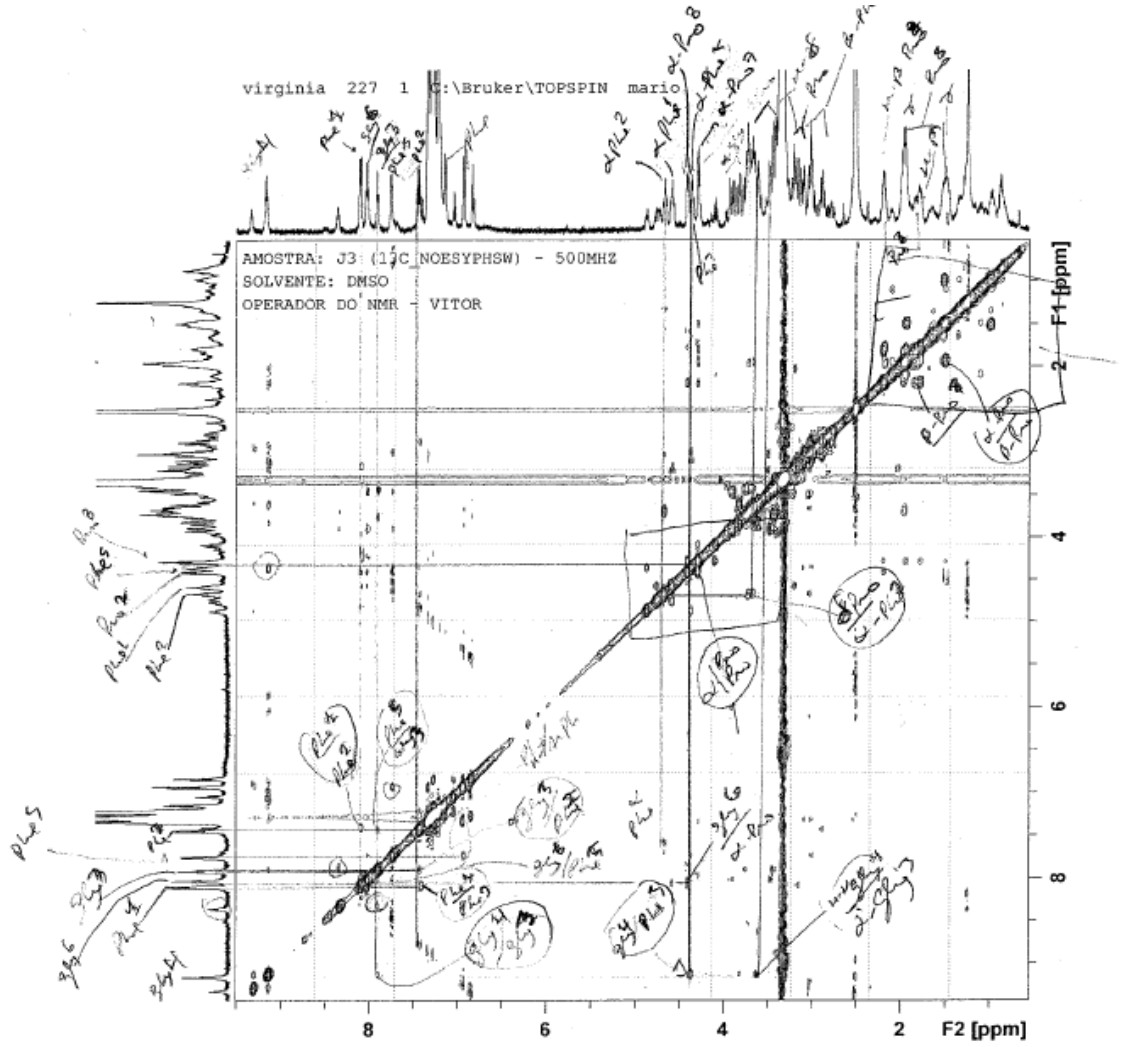


Figure S6. NOESY spectrum of [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).

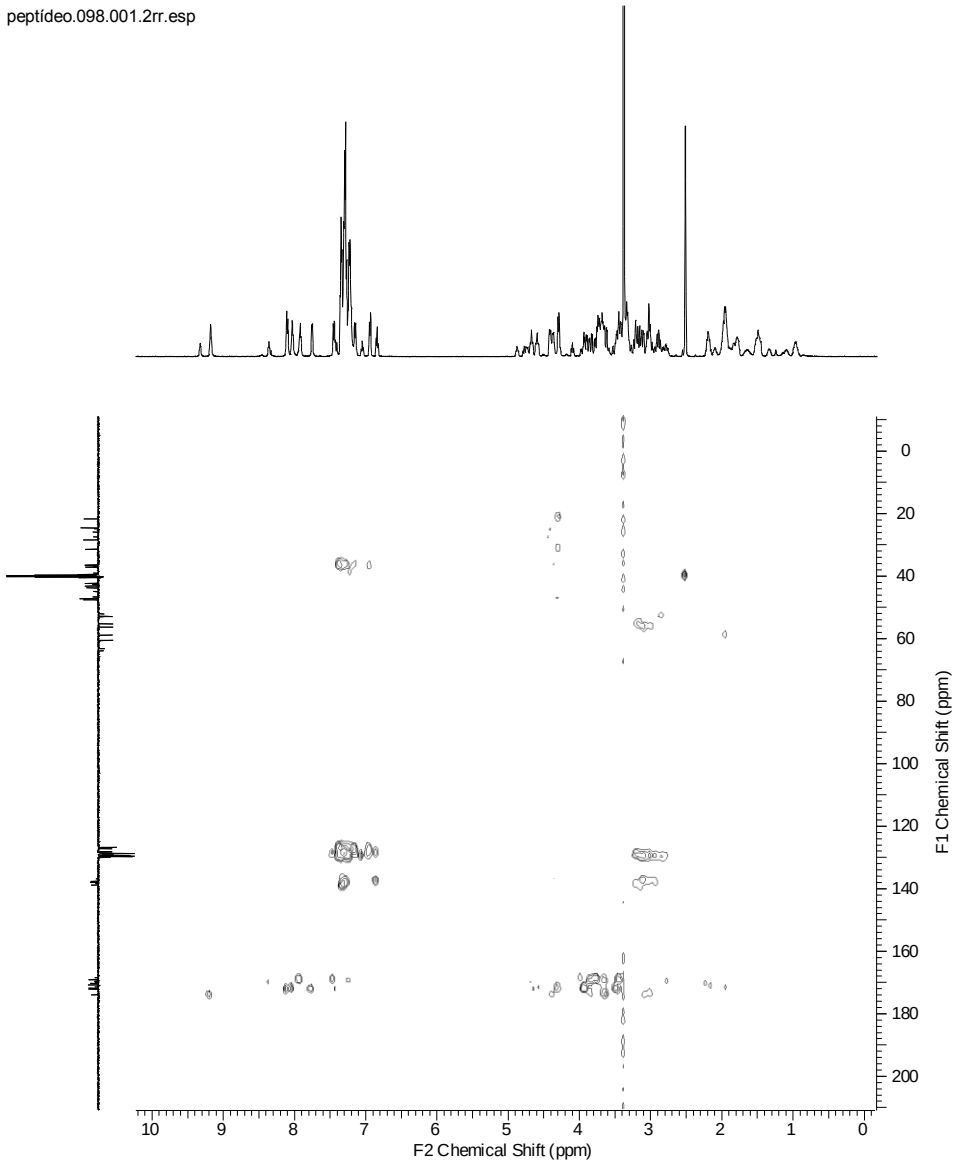
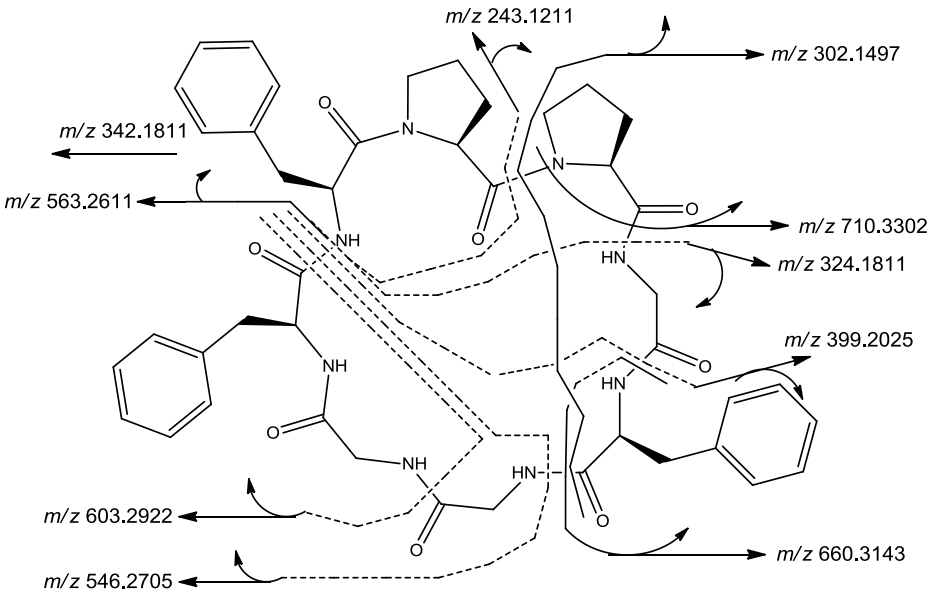


Figure S5. HMBC spectrum of [1-8-NαC]-zanriorb A1 (**1**) (500 MHz in DMSO-d_6).



$C_{43}H_{51}N_8O_8$ *m/z* 807.382986 Obs: 807.3832 $[M + H]^+$

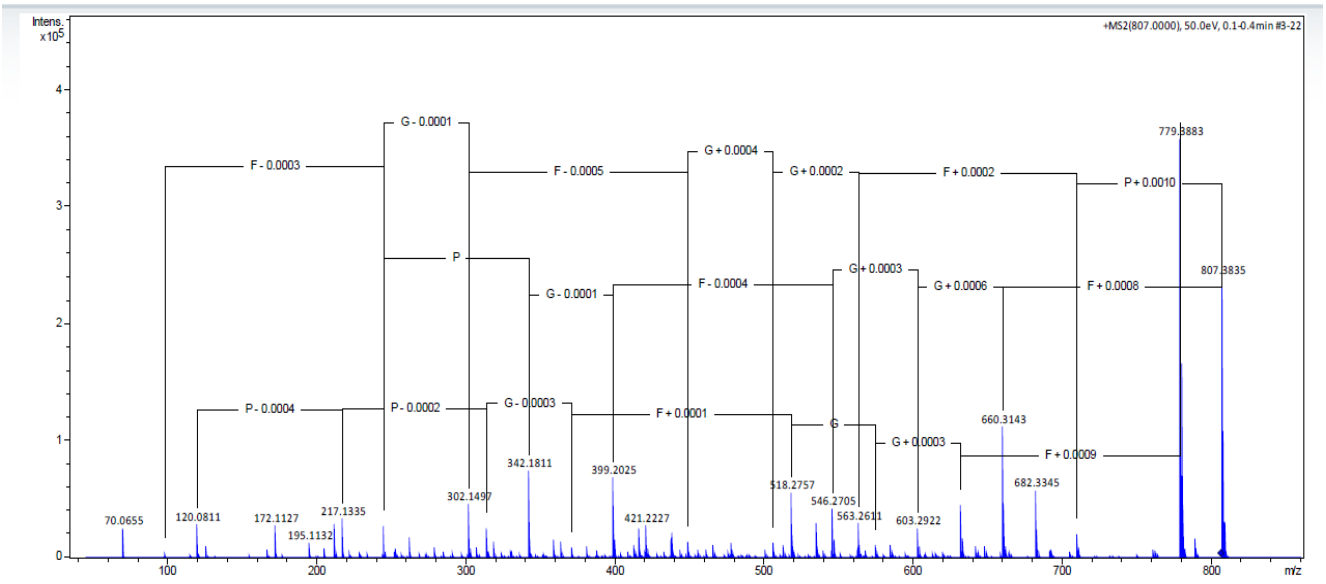
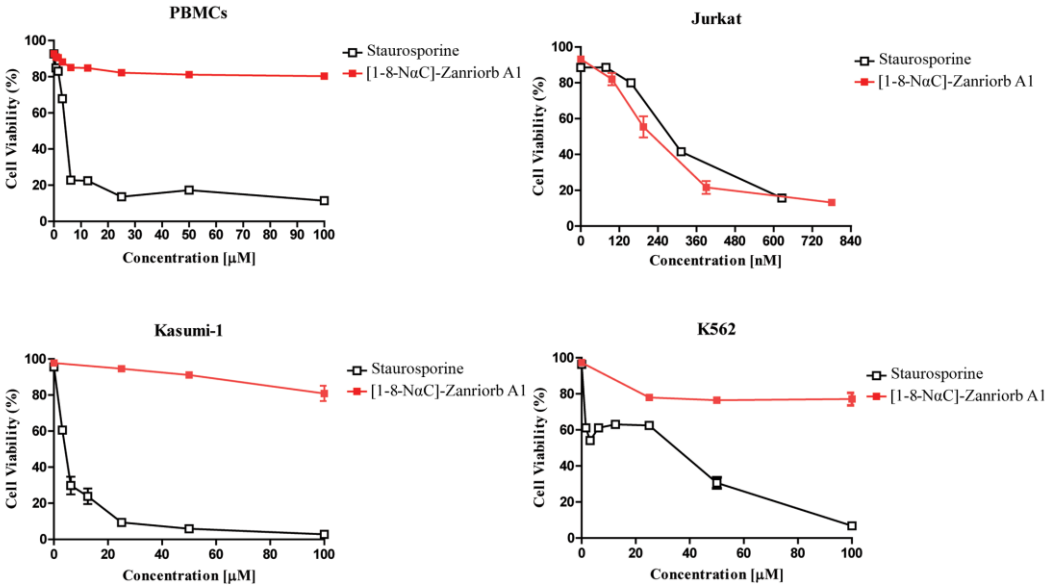
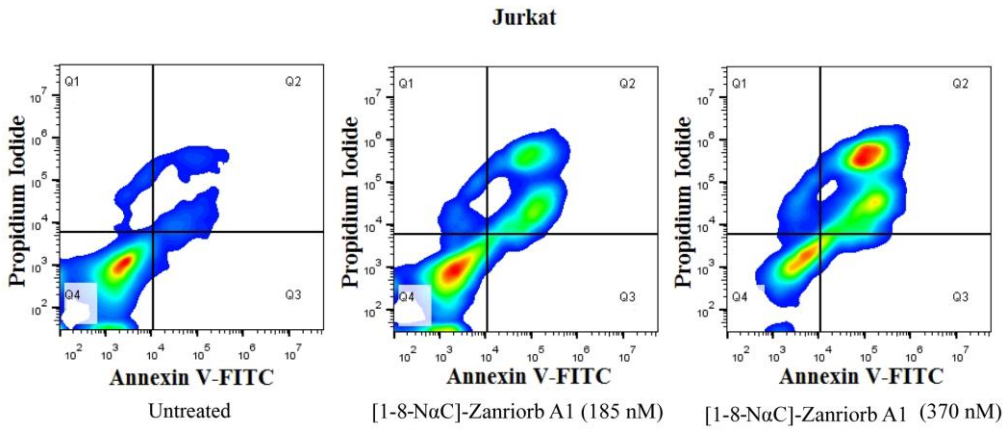


Figure S8. MS/MS mass spectrum of [1-8-NαC]-zanriorb A1 (**1**).

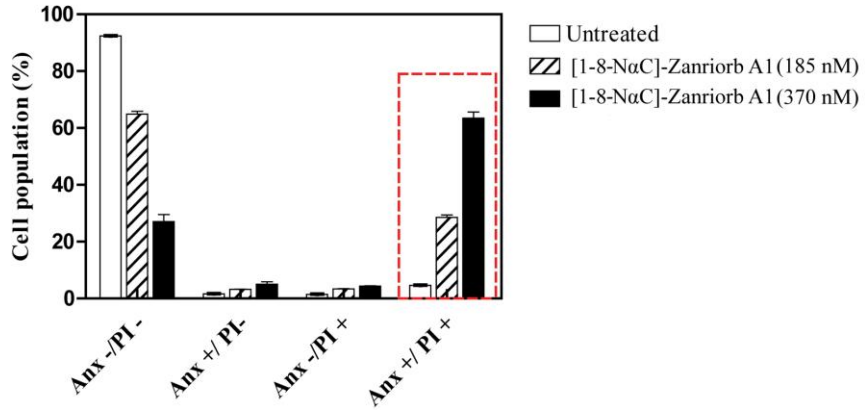
a


PBMCs
Staurosporine
[1-8-NαC]-Zanriorb A1
Cell Viability (%)
Concentration [μM]
Jurkat
Staurosporine
[1-8-NαC]-Zanriorb A1
Cell Viability (%)
Concentration [nM]
Kasumi-1
Staurosporine
[1-8-NαC]-Zanriorb A1
Cell Viability (%)
Concentration [μM]
K562
Staurosporine
[1-8-NαC]-Zanriorb A1
Cell Viability (%)
Concentration [μM]

b


Jurkat
Propidium Iodide
Annexin V-FITC
Q1
Q2
Q3
Q4
Untreated
[1-8-NαC]-Zanriorb A1 (185 nM)
[1-8-NαC]-Zanriorb A1 (370 nM)

c


Cell population (%)
Untreated
[1-8-NαC]-Zanriorb A1 (185 nM)
[1-8-NαC]-Zanriorb A1 (370 nM)
Anx -/PI -
Anx +/ PI-
Anx -/PI +
Anx +/ PI +

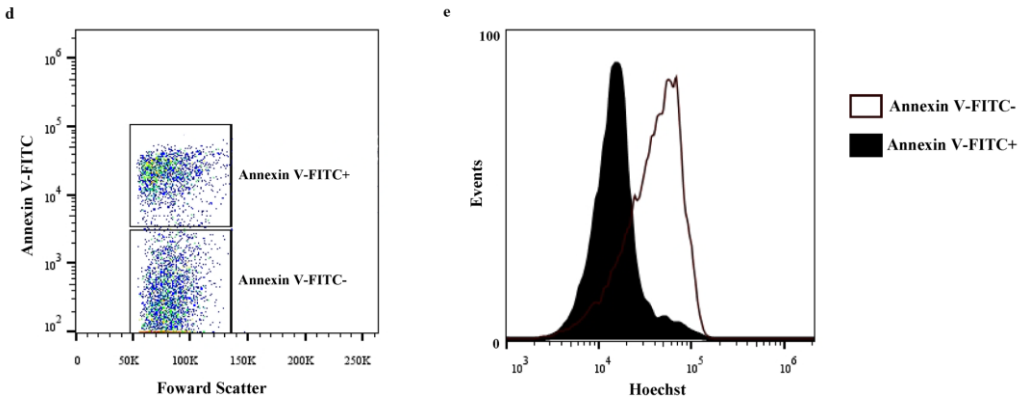
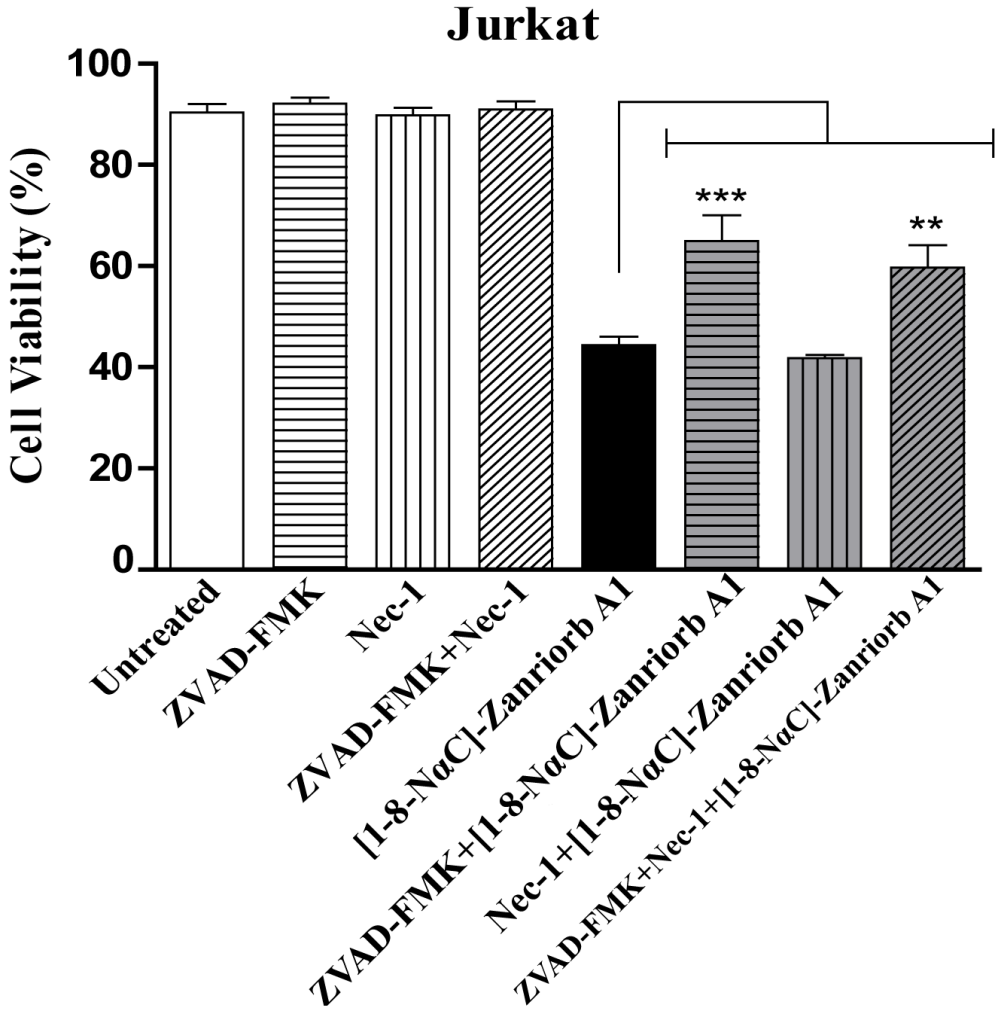


Figure S9. [1-8-NαC]-Zanriorb A1 (**1**) selectively induces cell death in the human T-cell leukemia cell line Jurkat. Cells were treated with **1** for 24 h, and then evaluated. Cell viability was determined using annexin-V and PI staining. (a) Cytotoxicity curves were determined for the Jurkat, Kasumi-1 and K562 cells lines and for PBMCs ($1x10^5$/mL). Cells were incubated with different test compound concentrations. (b) Representative flow cytometry density plots of double labeling with annexin-V-FITC and PI. (c) Mean of populations by staining with annexin V-FITC and PI. (d/e) DNA content quantified using Hoechst 33342 in annexin-V^- and annexin-V^+ populations after treatment with **1**. Results represent the means ± SEM of three independent experiments performed in triplicate.



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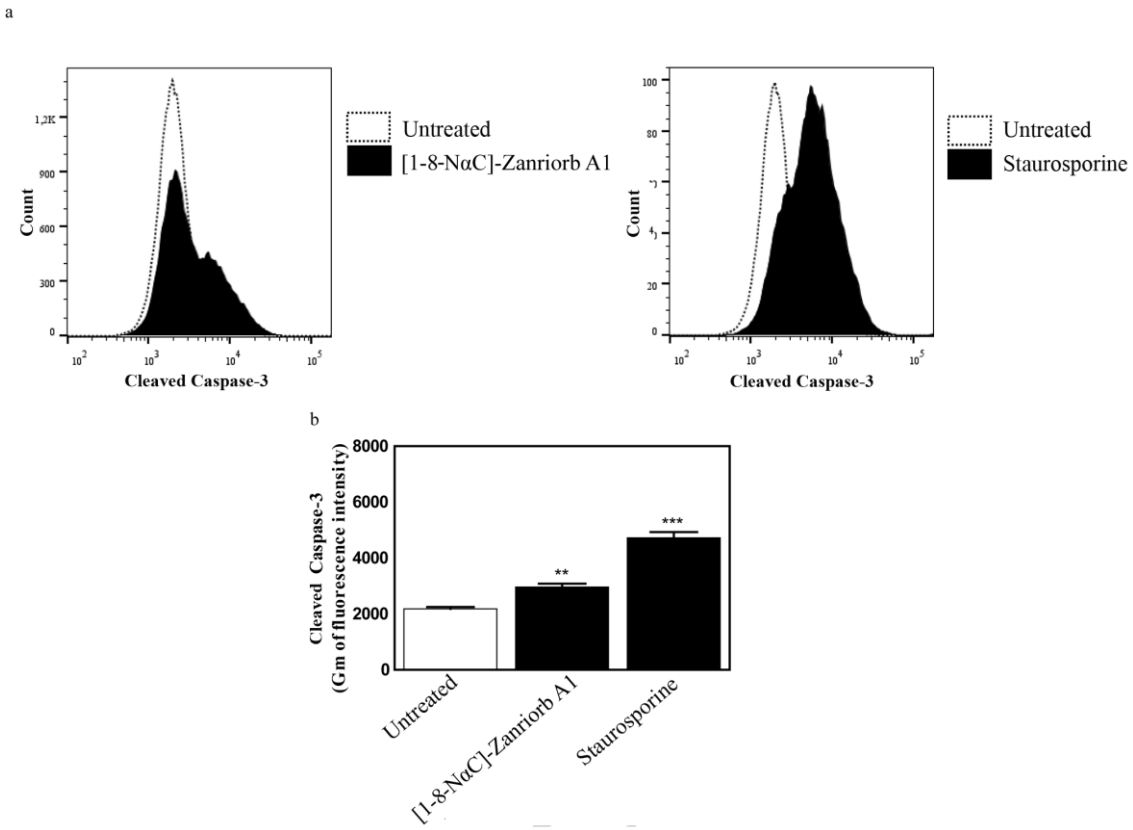

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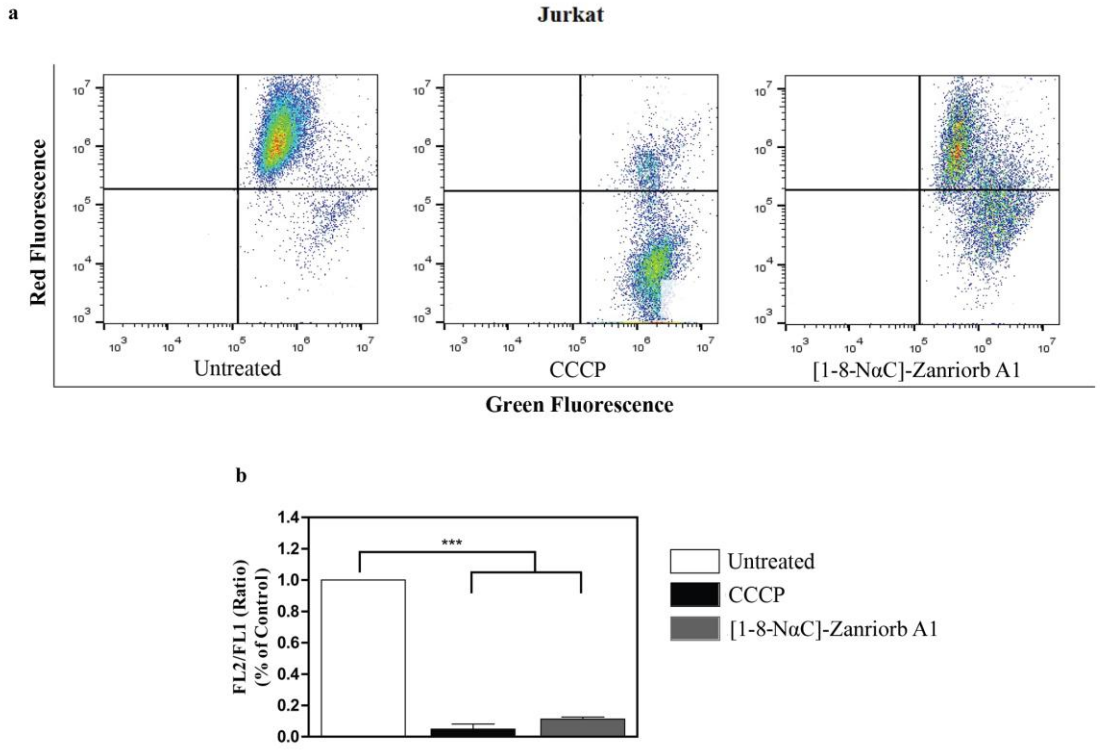

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