# New Analogs of [1-8-NαC]-zanriorb A1 as nitric oxide inhibitors

**Anila Bashir**[1], Nadeem-ul-Haque[1], Humira Karim[1], Sadiq Noor Khan[1], Zafar Ali Shah[2], Almas Jabeen[3], Shaista Qayyum[3], A. Ganesan[4], M. Iqbal Choudhary[1,3,5] and Farzana Shaheen [1]

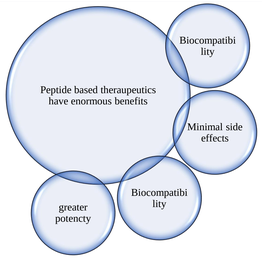
[1]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
*Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*
*Email Address: anila.bano58@ymail.com

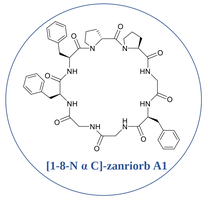
## ABSTRACT

**The [1-8-NαC]-zanriorb A1 (1) was reported as natural orbitide constituent of traditional medicinal plant *Zanthoxylum riedelianum.* In the current study, new cyclic analogues of zanriorb A1 were designed and synthesized by replacing glycine and proline residues of this peptide. These peptides were synthesized through solid-phase peptide synthetic strategy and cyclized *via* two ways, solution-phase macrocyclization and on-resin solid-phase macrocyclization. These peptide analogues were fully characterized by using mass, 1D, and 2D-NMR techniques. The natural orbitide was reported cytotoxic to Jurket leukemia T-cells ($IC_{50}$ = 218.0 nM), while our synthetic analogs were inactive against Jurkat cell line but many of them inhibited the production of nitric oxide in LPS stimulated macrophages. The most active synthetic analogue showed an $IC_{50}$ = 22 µM and it was more potent as compared to the standard L-NMMA ($IC_{50}$ = 98 µM) indicating its strong anti-inflammatory potential.**

## INTRODUCTION

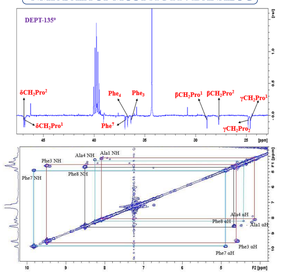
**[1-8-NαC]-zanriorb A1 is a cyclic peptide belongs to class orbitide. It's Isolated from the leaves of *Zanthoxylum reidelianum* plant which belongs to Rutaceae family. The plants of this genus are good source of bioactive compounds which includes alkaloids, aromatic and aliphatic amides, Cumarines and lignans. The plant *Zanthoxylionum riedelianum* Engl. has been used as an analgesic for toothache and reported folk uses of this species has been confirmed by the *in-vitro* anti-inflammatory and antinociceptive activities of the leaves and stem bark. Our research group already synthesized the natural product through sulfonamide safety catch linker and performed on-resin cyclization which was inactive toward Jurket leukemia T-cells . In the current study we have synthesized glycine and proline substituted 16 analogs of zanriorb A1 and evaluate them for anticancer activity and nitric oxide inhibition.**



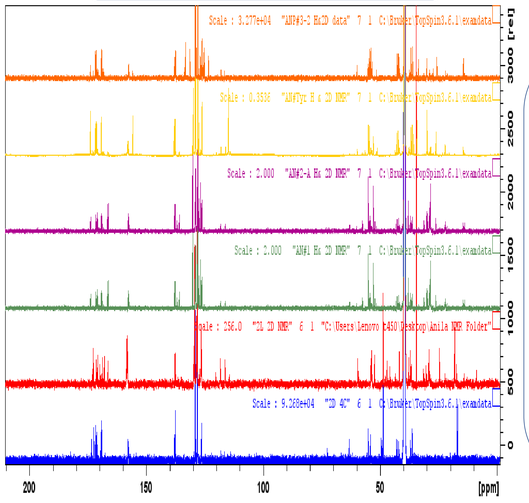



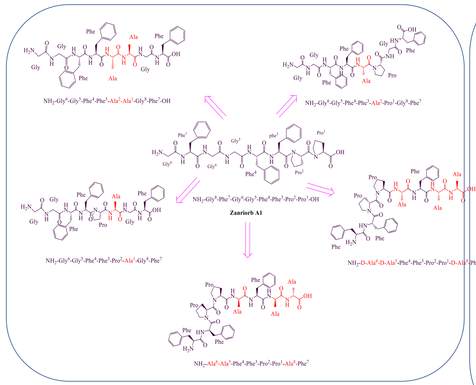

- A cyclic Peptide (Orbitide)
- Show cytotoxic activity against Jurka leukemia T cells
- $IC_{50}$ value is 218 nM

## NMR DATA OF MOST ACTIVE ANALOG



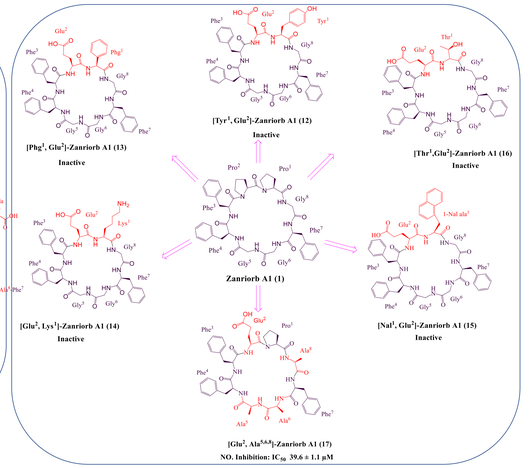

## $^{13}$C NMR SPECTRA

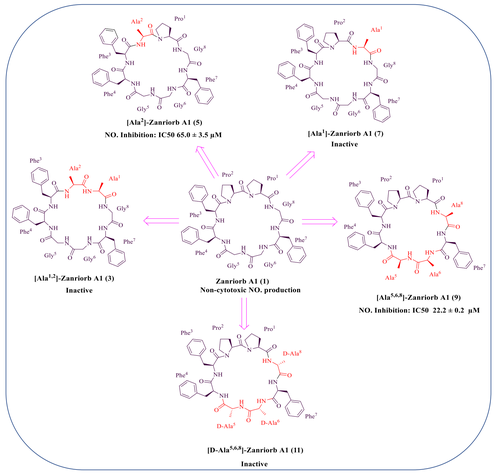


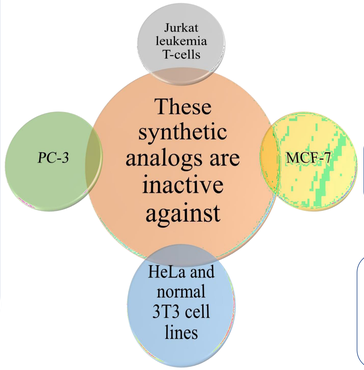

## RESULTS AND DISCUSSIONS

**All synthetic analogs were synthesized *via* Solis Phase Peptide Synthesis (SPPS) standard procedure by using Fmoc protected amino acids on Wang resin (1.2 mmol/g). DMAP used as a catalyst, ethyl hydroxyiminocyanoacetate (oxymapure) used to prevent racemization while di-isopropylcarbodiimide (DIC) used as a coupling agent. TFA cocktails used to cleave the cyclic peptide from resin. Crude peptides further purified on RP-HPLC by using ACN: $H_2O$: TFA (60:40:0.08) solvent system and the purified compounds were further analyzed through mass, and NMR spectroscopies.**




## CYTOTOXIC EVALUATION




## CONCLUSION

- SPPS was utilized to obtain sixteen analogues of Zanriorb A1, and were further cyclized by both solution-phase and on-resin macrocyclization, but solution-phase macrocyclization yielded better results.
- The synthetic analogues were found inactive against Jurkat and MCF-7 cell lines, in addition non-toxic against normal fibroblast NIH-3T3 cells.
- The cyclic [Ala5,6,8]-zanriorb A1 (9) with all three Glycine residues replaced with Alanine was identified as a potent inhibitor of nitric oxide production in LPS activated J774.2 macrophage.
- This cyclic [Ala5,6,8]-zanriorb A1 analog possesses a great potential as nitric oxide inhibitor, hence can act as a lead anti-inflammatory molecule.

## ACKNOWLEDGEMENT

**This work was supported by a grant 5738/Sindh/NRPU/R&D/HEC/2016 from Higher Education Commission Pakistan, and a grant PSF/NSLP/S-HEJ (290) from the Pakistan Science Foundation.**

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- dos S. Beirigo, P.J., Torquato, H.F., Dos Santos, C.H., Carvalho, M.G.D., Castro, R.N., Paredes-Gamero, E.J., de Sousa Jr, P.T., Jacinto, M.J. and da Silva, V.C., 2016. [1-8-NαC]-Zanriorb A1, a proapoptotic orbitide from leaves of Zanthoxylum riedelianum. Journal of Natural Products, 79(5), pp.1454-1458.