

Identification of novel Atg3-Atg8 inhibitors using virtual screening for autophagy modulation

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1. Introduction

Autophagy (self-eating) is a metabolic process for recycling damaged cellular material, which is captured by autophagosomes and directed to lysosomes for bulk degradation¹. Autophagy dysfunction is associated with many diseases, such as cancer, making the autophagy machinery a potential therapeutic target^{2,3}. A key step in this pathway is the complexation of the ubiquitin related Atg8 protein to the Atg3 polypeptide⁴. A small molecule non-cytotoxic autophagy inhibitor would open the door for adjunct therapies to enhance many established anticancer drugs, reducing their efficacious concentration. In addition, since many cancer types rely on the autophagy mechanism to survive a therapeutic regime, recurrence can potentially be reduced.

2. Methodology

9050 compounds from InterBioScreen Ltd natural product collection were screened against the binding pocket of human Atg8 (LC3). GOLD version 5.4.0 software suite was implemented. The selected compounds were verified using puncta formation of the LC3 protein and zebrafish model test.

3. Results

Forty-three compounds were identified using virtual screening. These compounds were tested using puncta formation of the LC3 protein. The results are shown in **Fig.1**. Compound **AT 95**, **AT102**, **AT109**, **AT110**, **AT111**, **AT112**, **AT115**, **AT130**, **AT136** were confirmed to be active. The structures of the active compounds are shown in **Fig. 2**.

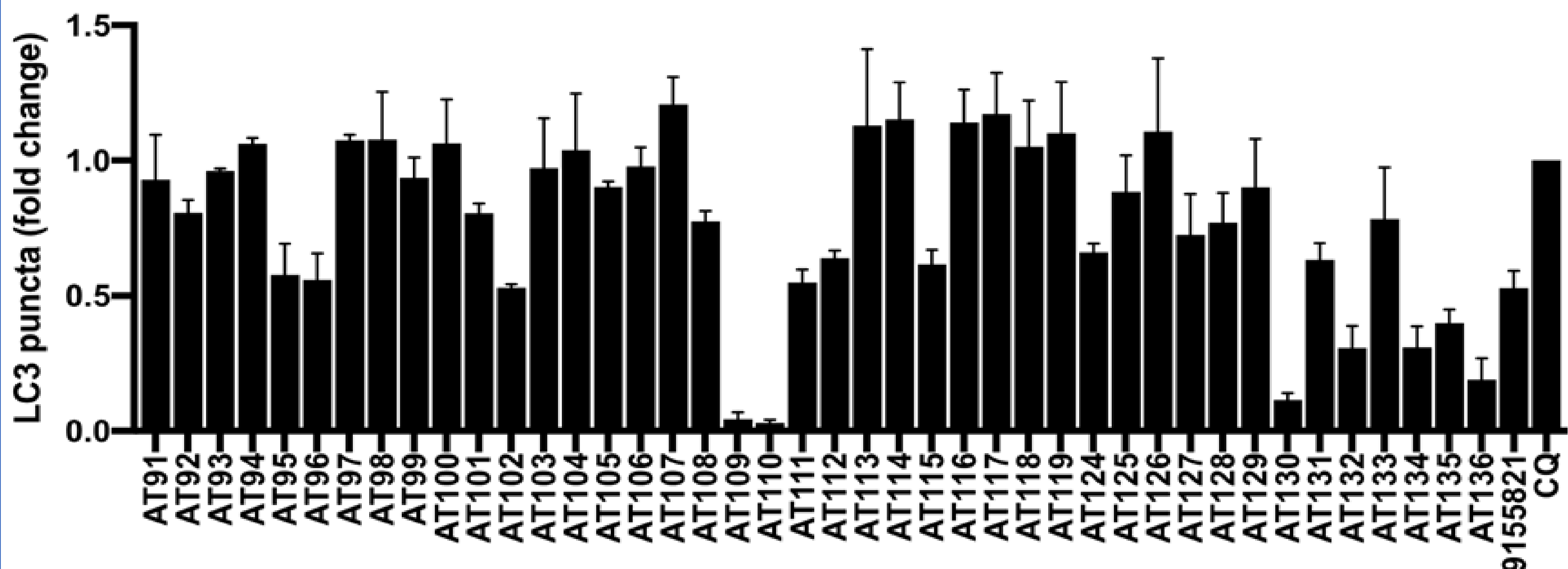


Fig. 1. The activity of the virtual hit matter (at 20 μ M). Compound 9155821 is a known Atg5- Atg16 inhibitor used as a reference compound.

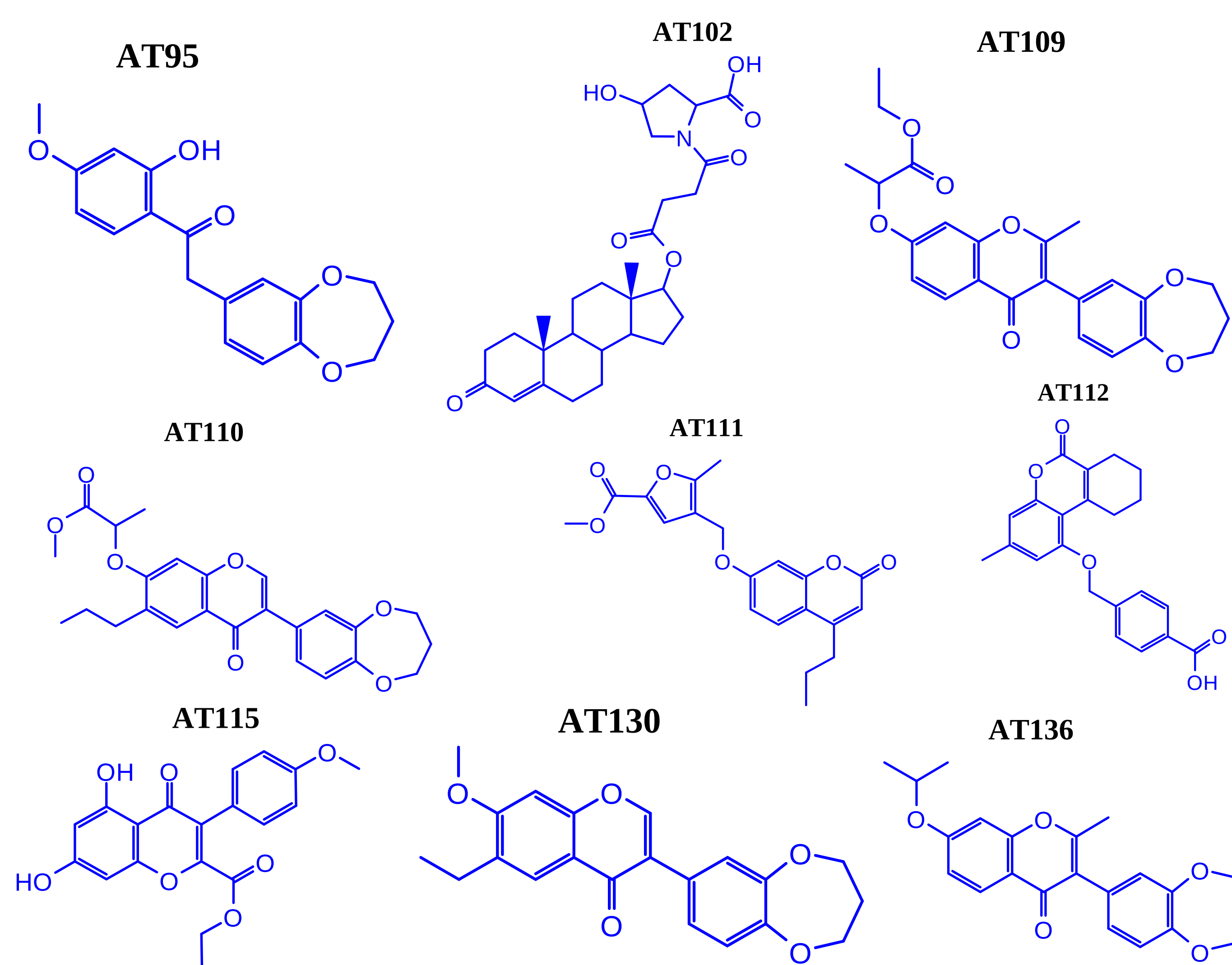


Fig. 2. Structures of the active compounds.

The active compounds were further tested *in vivo* using Zebra fish embryos (**Fig.3**).

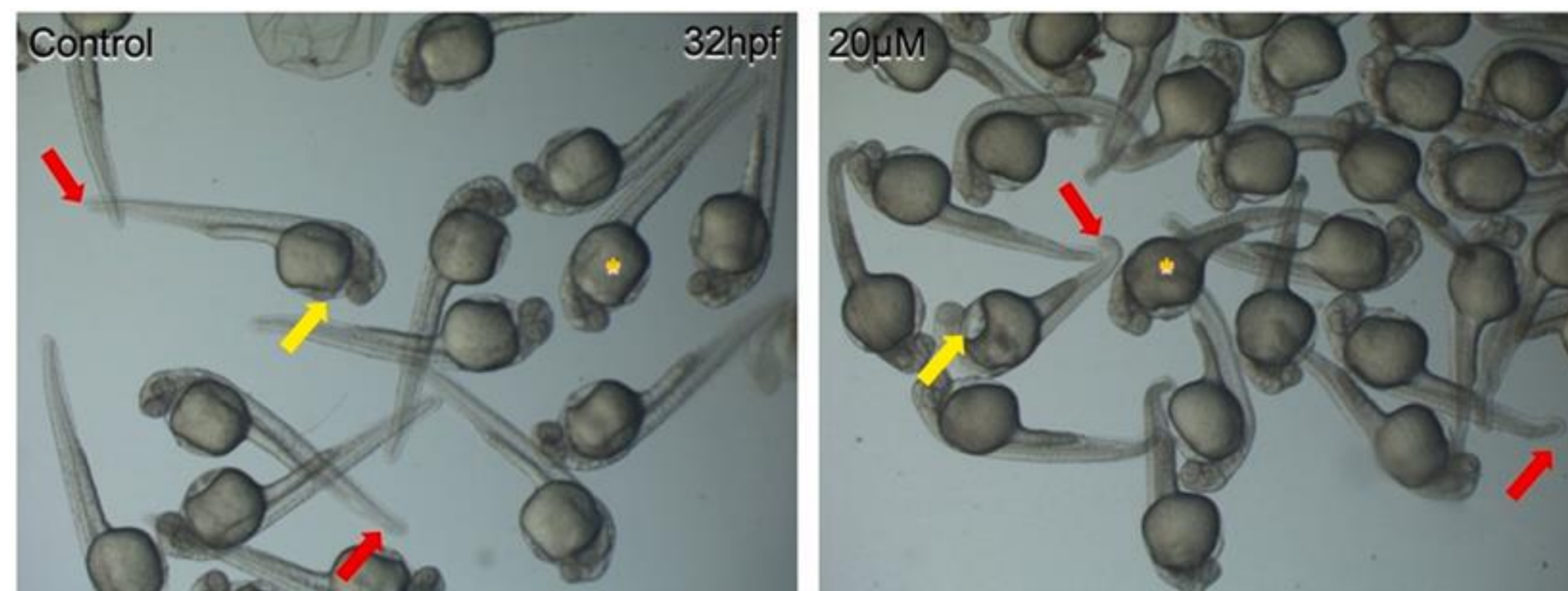


Fig. 3. AT110 is toxic at 20 μ M in zebrafish. Embryos exposed to the drug show evidences of toxicity such as grey yolk (orange asterisk), bent tail (red arrow) and pericardial oedema (yellow arrow).

Molecular Modelling

The modelling of hit molecules showed plausible binding modes for all the scoring function. AT110 occupies the binding pocket. The ester containing chain is anchored by hydrogen bonding in a cleft. The propyl chain sits in the lipophilic Atg3 Leu343 pocket. Finally the two membered ring system 3,4-dihydro-2H-benzo-1,5-benzodioxepine is embedded in the pocket occupied by the indole moiety of Trp340 in the Atg3 polypeptide. The ester moiety forms hydrogen bond with the guanidino group of arginine. Also, the carbonyl group in the isoflavone system forms hydrogen bond with the backbone amine of leucine. Finally, a π - π stacking is formed between the isoflavone system and phenylalanine as shown in **Fig. 4**.

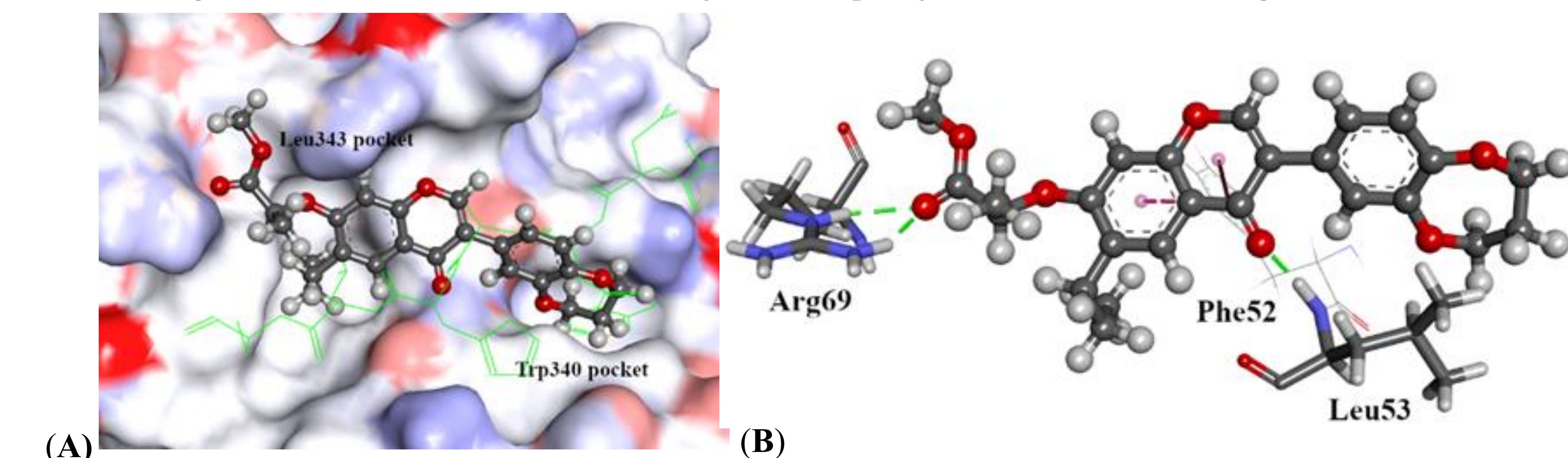


Fig 4. The docked pose of AT110 in the complexation site of Atg3-Atg8 **(A)** The Atg8 protein surface is rendered; blue depicts regions with a partial positive charge on the surface; red depicts regions with a partial negative charge and grey shows neutral areas. The co-crystallised Atg3 is green in line. **(B)** The predicted configuration is shown in the ball-and-stick format and the adjacent amino acid residues are depicted in the stick format except the Phe52 amino acid is shown in the line format for clarity. Hydrogen bonds are shown as green lines whiles π - π stacking is shown in pink.

4. Conclusion

Three hits showed single digit μ M IC₅₀ values with AT110, an isoflavone derivative, being the most active at 1.2 ± 0.6 μ M. The discovery of AT110 is an important step in establishing an adjunct therapy.

5. References

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