

Novel BIR Binding Ligands as XIAP Antagonists

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Abstract:

The Inhibitor of Apoptosis Protein (IAP) family of proteins plays a crucial role in regulating apoptosis, primarily by regulating caspase activity via the highly structured BIR (Baculovirus Inhibitory Repeat) motifs and by causing ubiquitination of binding partners through the E3 ligase motif. In the IAP family, XIAP is an important cancer target; XIAP antisense AEG35156, shows potent antitumor activity in various xenograft models and in multiple clinical trials. For a small molecule inhibitor approach, blocking interactions of XIAP with caspases through its BIR domains represent exceptional targets for modulation of anti-apoptotic properties. However, because of contrasting BIR activities within and between various IAPs, the impact of BIR selectivity for small molecule binders must be established. A series of novel BIR ligands with high binding affinity to IAP BIRs have been synthesized. In vitro characterization of a representative pre-clinical XIAP antagonist is reported.

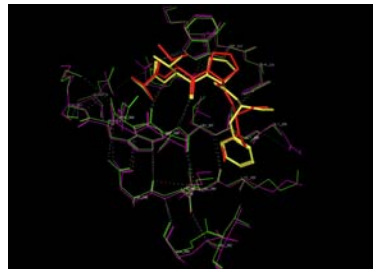


Figure 1: N-terminal tetrapeptides, (AVPI, AVPF) target XIAP Bir Domain

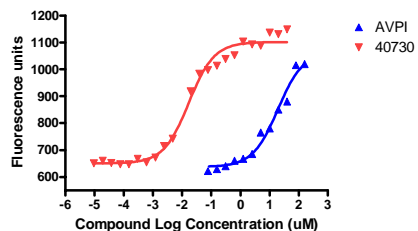


Figure 2: AEG40730 disrupts XIAP-Caspase-3 interaction. AEG40730 or AVPI peptide were incubated in the presence caspase-3 and an inhibitory amount of full-length XIAP. The ability of compound to displace caspase-3 from XIAP was evaluated by quantifying the amount of fluorescence released from Ac-DEVD-AMC by active caspase-3.

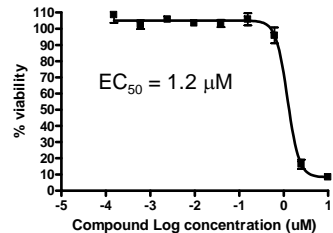


Figure 3: AEG40730 is relatively non-toxic to normal cells. Non-transformed WI38 cells were treated with various concentrations of AEG40730. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated.

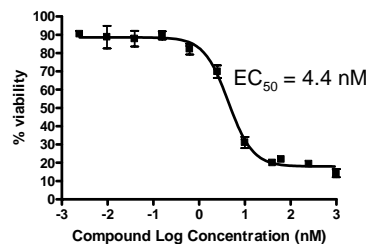


Figure 4: AEG40730 kills SK-OV-03 ovarian cancer cells. SK-OV-03 cells were treated with various concentrations of AEG40730. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated.

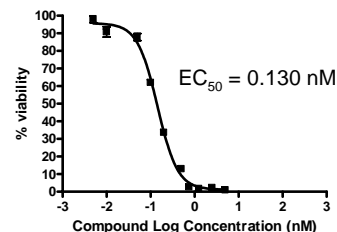


Figure 5: AEG40730 kills MDA-MB-231 breast cancer cells. MDA-MB-231 cells were treated with various concentrations of AEG40730. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated.

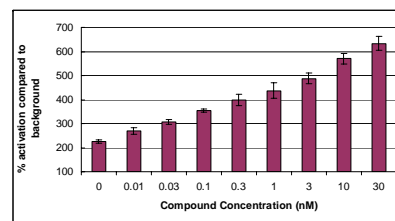


Figure 6: AEG40730 induces rapid activation of caspase-3. MDA-MB-231 cells were treated with various concentration of AEG40730. After 5 hours endogenous caspase-3 activity was evaluated by measuring the amount fluorescence released following cleavage of Ac-DEVD-AMC

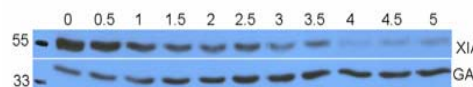


Figure 7: AEG40730 induces rapid loss of XIAP in cells. Human MDA-MB-231 breast cancer cells were treated with 0.5 nM of AEG40730. At the indicated time, cells were collected, the proteins extracted and the XIAP protein level evaluated by western blot analysis.

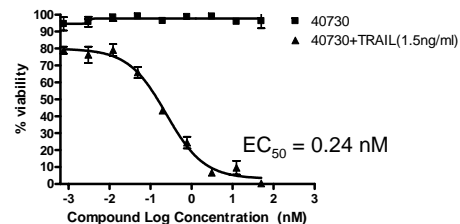


Figure 8: AEG40730 in combination with TRAIL induces apoptosis in colon cancer cells. HCT-116 cells were treated with a fix concentration of TRAIL (1.5 ng/mL) and various concentrations of AEG40730. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated.

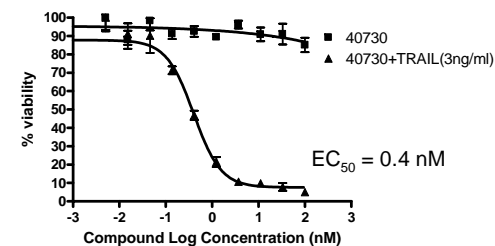


Figure 9: AEG40730 in combination with TRAIL induces apoptosis in PC3 prostate cancer cells. PC3 cells were treated with a fix concentration of TRAIL (3 ng/mL) and various concentrations of AEG40730. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated.

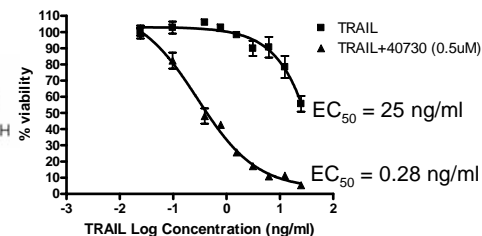


Figure 10: AEG40730 sensitizes PC3 cells to treatment with TRAIL. PC3 cells were incubated with a non-toxic dose of AEG40730 (0.5 μM) and increasing amounts of TRAIL. Cellular viability was determined after 72 hours by MTT staining and the EC_{50} calculated. Results indicate that AEG40730 increases TRAIL apoptotic activity by a factor of 2 logs.

Conclusions

A series of XIAP antagonist has been described which:

- Bind to XIAP and disrupt XIAP/caspase-3 interaction with nanomolar concentrations.
- Induce apoptosis and sensitize to TRAIL-induced apoptosis in various cancer cells at nanomolar concentrations.
- Exhibit therapeutic index over 1000 fold when comparing activity in cancer cells with normal cells