

Waking the Executioner: Procaspase-3 Activating Compounds (PACs) that Selectively Induce Apoptosis in Cancer Cells

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Most modern anticancer regimens utilize drugs that are general cytotoxins in order to target malignancies due to their intensified proliferation relative to non-cancerous tissue. However, the lack of selectivity results in side-effects and dose-limiting toxicity. Because cancer typically avoids apoptosis via mutation and aberrant expression of upstream pro- and anti-apoptotic proteins, reestablishment of apoptosis could prove to be a more efficient therapy. Caspase-3 is an “executioner” cysteine protease which facilitates apoptosis via the degradation of a wide variety of cellular targets but is kept in check by its *in vivo* expression as an inactive zymogen, procaspase-3. Procaspase-3 is paradoxically upregulated in many neoplasms, making activation of the zymogen an attractive anticancer strategy. Screening over 20,000 structurally diverse compounds led to the discovery of PAC-1, the first procaspase-3 activating compound. PAC-1 has been shown to induce apoptotic death in cell culture, in mouse models, and in primary isolates from human patients treated at Carle Hospital, all with a level of efficacy that correlates with the relative amount of procaspase-3 expressed within each tissue.

Extensive structural manipulations have been performed on PAC-1 via individual and combinatorial synthesis. SAR confirmed the critical nature of the benzyl and phenol moieties, showed that the tertiary amines in the piperazine core were dispensable, demonstrated the tolerance for N-alkylation of the amide, and led us to conclude that conformational restrictions within the N-acylhydrazone are relevant for biological activity. Mindful of the critical functional groups, an array constitutive of 11 hydrazides and 15 aldehydes were coupled in parallel to afford 165 PAC-1 derivatives containing varied functionality on each aromatic ring. Seven members of this library were 10- to 20-fold more potent in cell culture relative to PAC-1.

Additionally, PAC derivatives are being utilized to elucidate the mechanism by which these molecules activate procaspase-3. Crystallographic structural determinations as well as measurement of binding affinity and thermodynamics are underway with PAC-1. Photo-labile azido-PAC derivatives are being utilized for crosslinking with procaspase-3 to determine which residues constitute the PAC binding site. Further structural optimizations of PACs will be guided by data garnered from these mechanistic studies.

