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STIMULATION OF APOPTOSIS BY COMPUTATIONALLY DERIVED SMALL MOLECULES THAT BIND TO BCL-2

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INTRODUCTION. Bcl-2 and its family members are key regulators of apoptosis or programmed cell death, implicated in human diseases including cancer (1). The family has both anti-apoptotic members such as Bcl-2 and Bcl-x_L and also death-promoting members such as Bax, Bak, Bid and Bad (1,2). The interactions between these two groups of proteins have been shown to modulate the sensitivity of a cell to apoptotic stimuli (3,4). Most cancers typically show high levels of Bcl-2 expression, and this overexpression is believed to contribute to the resistance of cancers to a wide variety of chemotherapeutic drugs and radiation therapy (5). In our studies, we used structure-based computer screening technology to identify small molecule Bcl-2 antagonists. These compounds were assayed for binding to Bcl-2 and then in cell-based functional assays. Positive compounds can potentially be developed as anti-cancer therapies.

METHODS. The NMR structure of Bcl-x_L complexed with a portion of the BH3 region of its ligand Bak (PDB accession number 1BXL) (6) was used to generate a computational model of Bcl-2 complexed with a corresponding portion of its intracellular ligand Bax. The model was used in a 3-D computational search for non-peptide small molecules that disrupt Bcl-2/Bax interaction. Selected compounds were tested for their ability to inhibit binding of Eu^{3+}-labelled Bax to Bcl-2 as measured by time-resolved fluorometry. Compounds with Ki values less than 50 μM were tested for their ability to induce apoptosis in Jurkat cells that overexpress Bcl-2 (Jurkat/Bcl-2 cells). The published Bcl-2 binding compound HA14-1 (7), was used as a control in the biochemical and cellular assays.

RESULTS. Two compounds with Ki values of 6 μM and 25 μM in the binding assay showed anti-Bcl-2 activities in cell-based assays. Jurkat/Bcl-2 cells are resistant to apoptosis induced by 1 μM staurosporine, as measured by cleavage of caspase 3 and PARP. However, caspase 3 and PARP cleavage were observed when these cells were incubated with 1 μM staurosporine in addition to either of these two compounds at 30 and 100 μM. Furthermore, the compounds induced apoptosis in a breast cancer cell line, MCF-7, which overexpresses Bcl-2.

CONCLUSION. Two chemically distinct compounds were identified as Bcl-2 antagonists, leading to induction of apoptosis in Bcl-2 overexpressing cells. These results suggest that targeting Bcl-2 may lead to the development of new anti-cancer agents.

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