Supplementary Materials: Novel Carbazolepiperazine Hybrid Small Molecule Induces Apoptosis by Targeting BCL-2 and Inhibits Tumor Progression in Lung Adenocarcinoma *In vitro* and Xenograft Mice Model

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Figure S1. RMSF plot of Bcl2 (Black) and Bcl2-ligand complex (Red).



Figure S2. Secondary structure of Bcl2.



Figure S3. Secondary structure of Bcl2-ligand.





Figure S4. Western blot images with molecular weight for CDK6, CyclinD1, BCL-2, Bcl-XL, Mcl-1, XIAP, Bax, Bid and respective β -actin control bands shown in Figure 4G and Figure 5A. The red dotted rectangle indicates the area shown in Figure 4G as well as Figure 5A.



Figure S5. Western blot images with molecular weight for Cytochrome-C, VDAC1, Caspase-9, Caspase-3, APAF1 and the respective β -actin control bands shown in Figure 5D and 5E. The red dotted rectangle indicates the area shown in Figure 5D and 5E.



Figure S6. Western blot images with molecular weight for PARP1, c-Myc, p53, IL-4, IL-6, NF-kB, IKK β and the respective β -actin control bands shown in Figure 5F. The red dotted rectangle indicates the area shown in Figure 5F.



Figure S7. Inhibition of Bcl-2 protein in vivo and immunohistomistry study. IHC study showed ECPU-0001 significantly inhibits protein expression of Bcl2 in A549 induced xenograft tumor, similarly, Bcl-2 protein inhibited in A549 cells in-vitro. Scale bar: 400X.

Antigens	Species Antibodies	Dilution	Supplier
	Raised in	(WB)	
Bax	Mouse, monoclonal	1:500	Santa Cruz Biotechnology Cat. #sc-7480
Bak	Rabbit, Polyclonal	1:500	Santa Cruz Biotechnology, Cat. #SC-832
VDAC1	Mouse, monoclonal	1:1000	Santa Cruz Biotechnology, Cat. #SC-390996
PARP-1	Rabbit, polyclonal	1:1000	Cell Signaling Technology, USA #9542
CDK6	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-7961
Cyclin D1	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-450
c-Myc	Mouse, monoclonal	1:1000	Santa Cruz Biotechnology, Cat. #sc-56634
p53	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-47698
IL-4	Rabbit, monoclonal	1:1000	Cell Signaling Technology, #12227
IL-6	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-28343
Bid	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc 56025
Bcl2	Mouse, monoclonal	1:1000	Cell Signaling Technology, #15071
XIAP	Rabbit, monoclonal	1:1000	Cell Signaling Technology, #14334
Bcl-XL	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-56021
Cyto-C	Rabbit, monoclonal	1:1000	Cell Signaling Technology, #4280
Apaf-1	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-135836
NF-κB	Mouse, monoclonal	1:1000	Cell Signaling Technology, #6956
Ικκβ	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-56918
Caspase-3	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-56053
MCL-1	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-53951
Caspase-9	Mouse, monoclonal	1:500	Santa Cruz Biotechnology, Cat. #sc-70505

Table S1. List of antibodies.



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