SUPPLEMENTAL INFORMATION

The Dynamic Character of the *BCL2* Promoter i-Motif Provides a Mechanism for Modulation of Gene Expression by Compounds That Bind Selectively to the Alternative DNA Hairpin Structure

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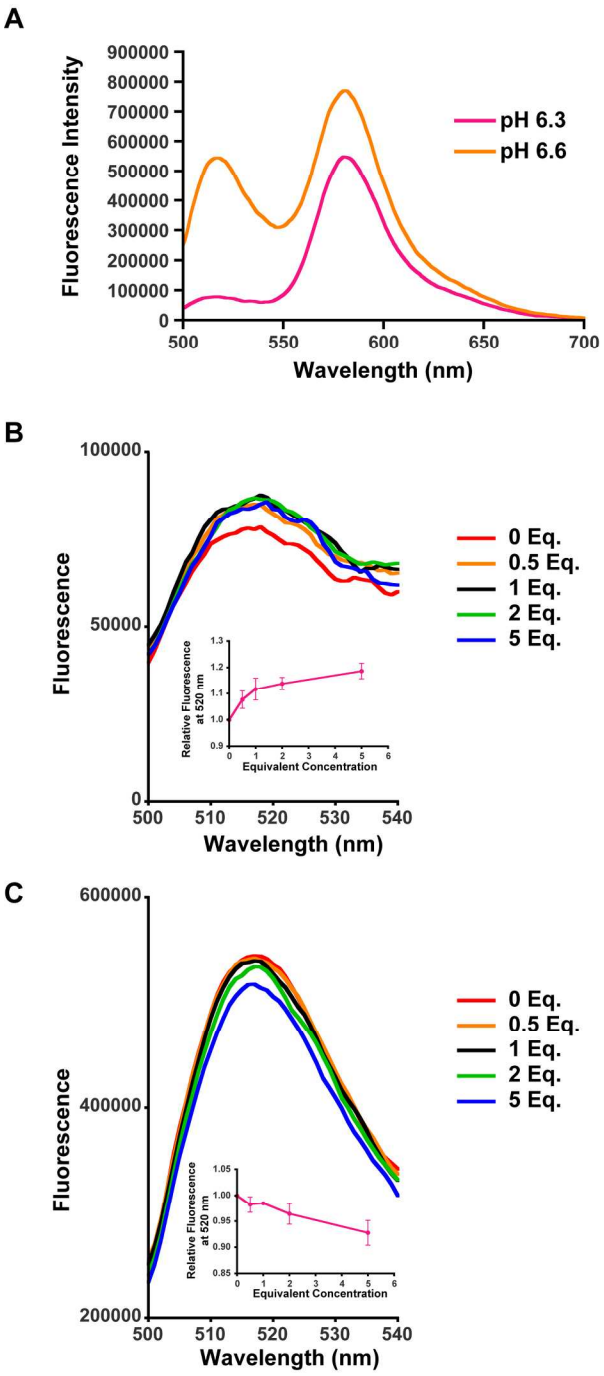
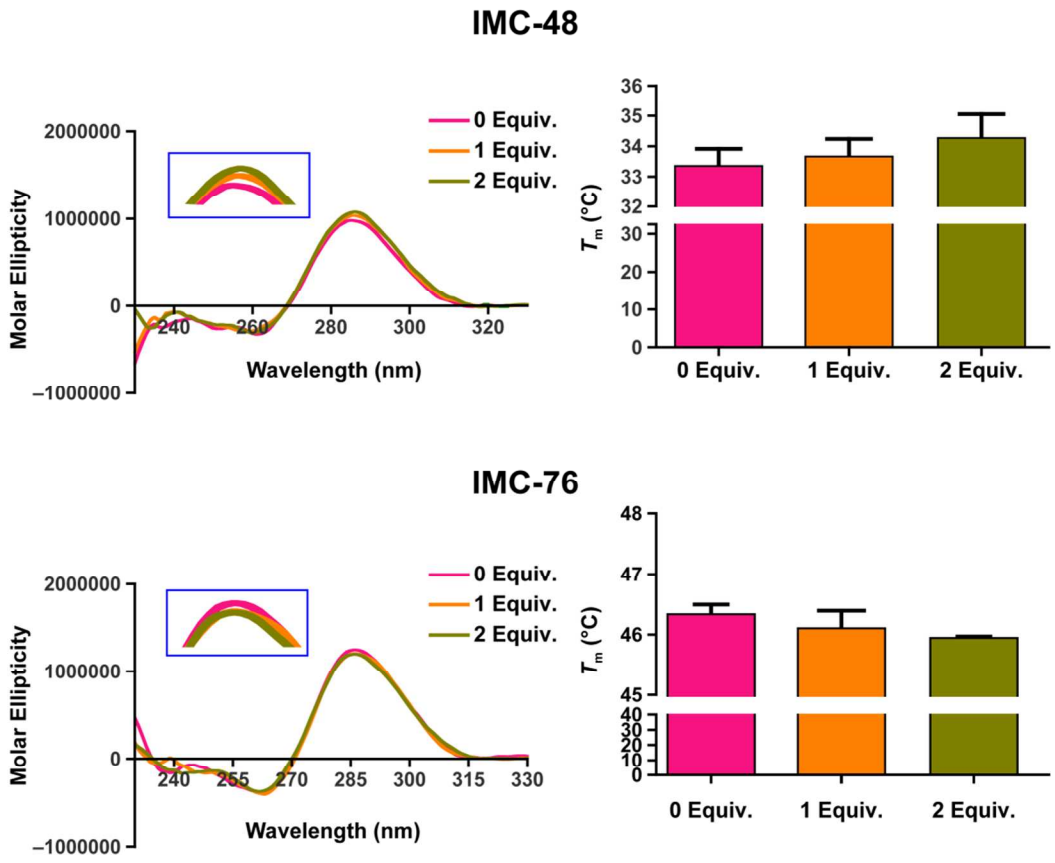
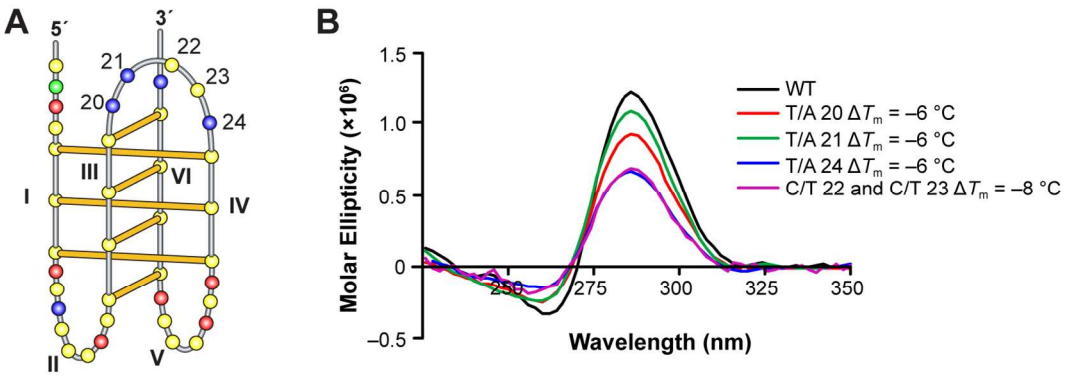


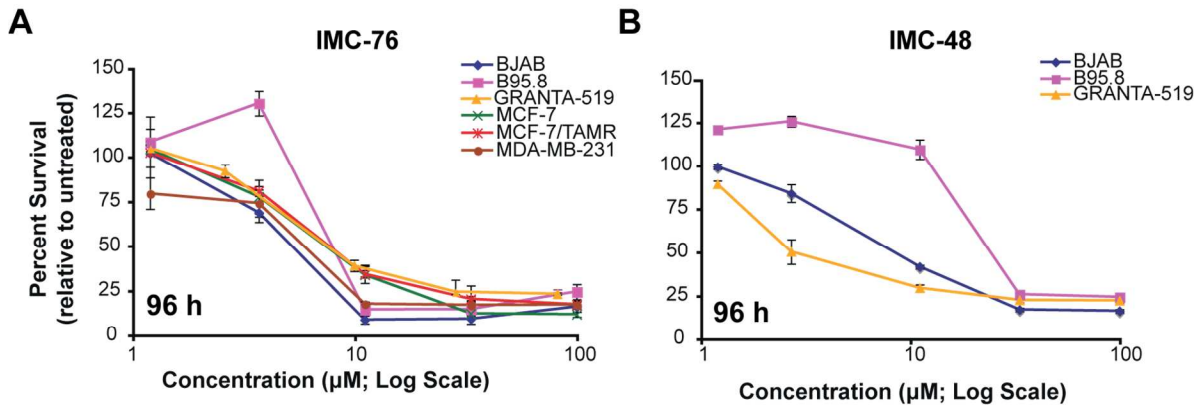
Supplemental Figure 1. Fluorescence spectra analysis to confirm the conformational change of the *BCL2* i-motif produced by IMC-76 and IMC48. (A) pH-dependent FRET changes in the BCL2 i-motif labeled with FAM at the 5´-end and TAMRA at the 3´-end of the oligomer. At low pH (pH 6.3), the I_{580}/I_{520} ratio is 7.45, showing a closer distance between two dyes by folding the i-motif, while at high pH (pH 6.6) the I_{580}/I_{520} ratio is 1.45, showing a longer distance between two dyes by unfolding the i-motif. (B) Dose-dependent spectra change by IMC-76 at pH 6.3, I_{520}. (C) Dose-dependent spectra change by IMC-48 at pH6.6, I_{580}. Insets in (B) and (C) show a graph of the dose response from the spectral analysis at I_{580}. A PTI fluorometer was used to measure the spectra.



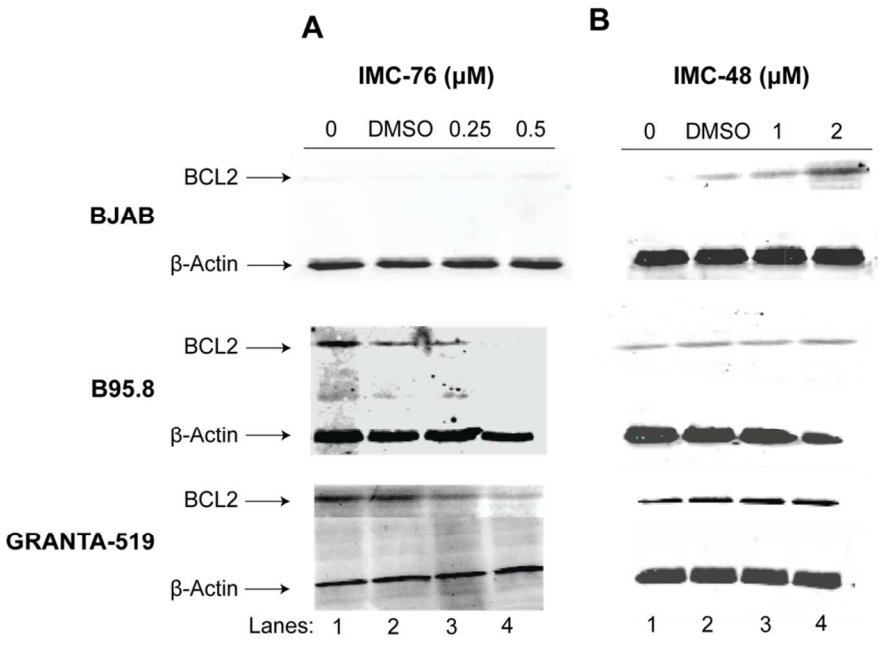
Supplemental Figure 2. CD spectra of the BCL2 i-motif with IMC-48 and IMC-76. IMC-48 and IMC-76 were incubated with the DNA at 50 mM Na cacodylate buffer (pH 6.6 and pH 6.3, respectively). The ellipticity at 286 nm was monitored with 1 °C/min of temperature increase for melting analysis, and then sigmoidal curve fitting was used to calculate T_m. DMSO was treated as a control.



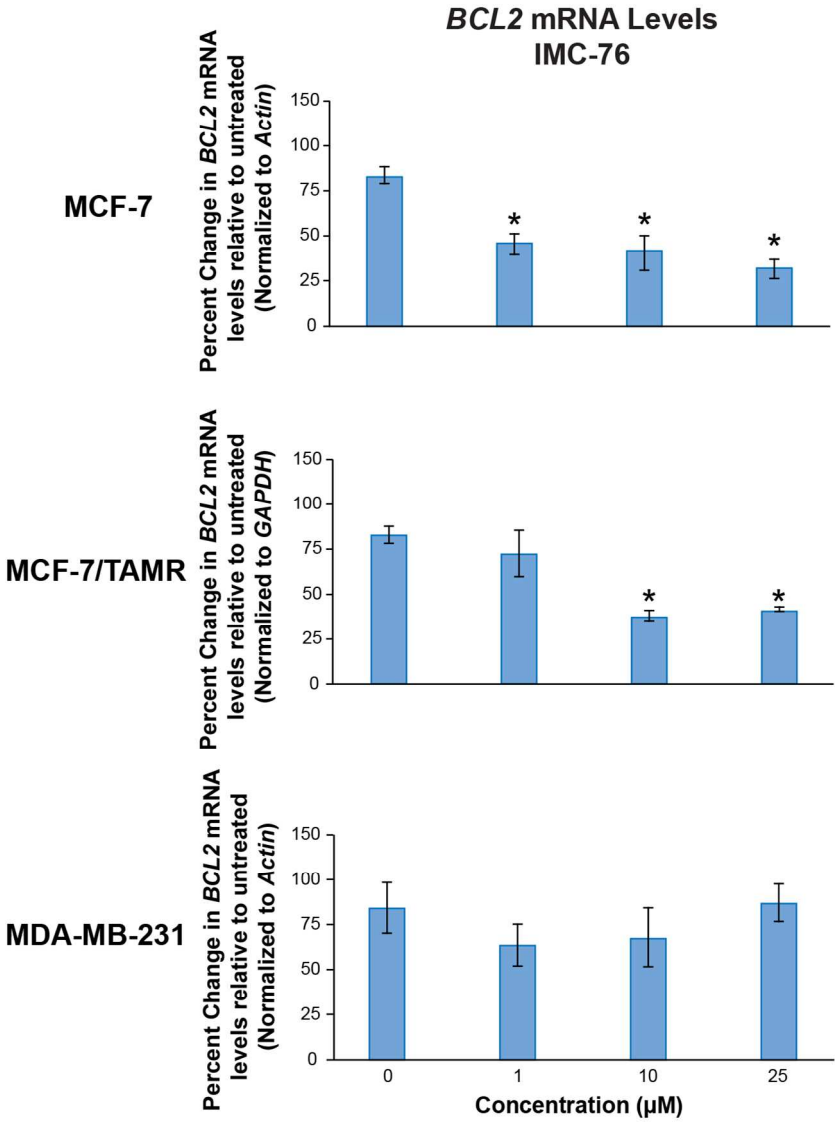
Supplemental Figure 3. CD spectral and thermal stability analyses of capping mutants. (A) Folding pattern of the *BCL-2* i-motif with the capping structure nucleotides labeled. (B) CD spectra of the *BCL-2* i-motif capping mutants C22, C23, T20, T21, and T24 in comparison to the wild-type sequence.



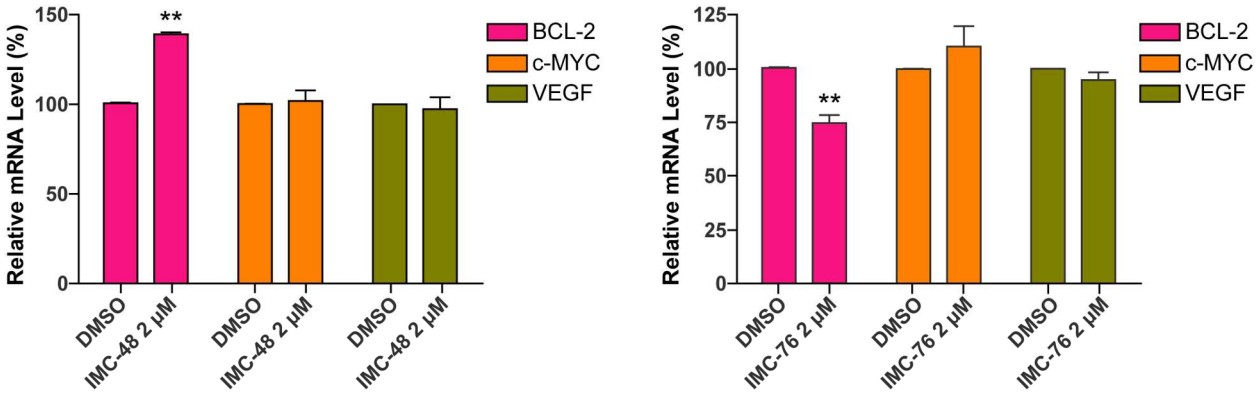
Supplemental Figure 4. Effect of IMC-76 and IMC-48 on cell toxicity in lymphoma and breast cancer cell lines. Percent survival determined by the MTS cytotoxicity assay in response to treatment with IMC-76 (A) for BJAB, B95.8 ,and GRANTA-519 lymphoma and MCF-7, MCF-7/TAMR, and MDA-MB-231 breast cancer cell lines or IMC-48 (B) for BJAB and B95.8 lymphoma cell lines at 96 h. Percent survival was calculated relative to untreated controls from three independent experiments.



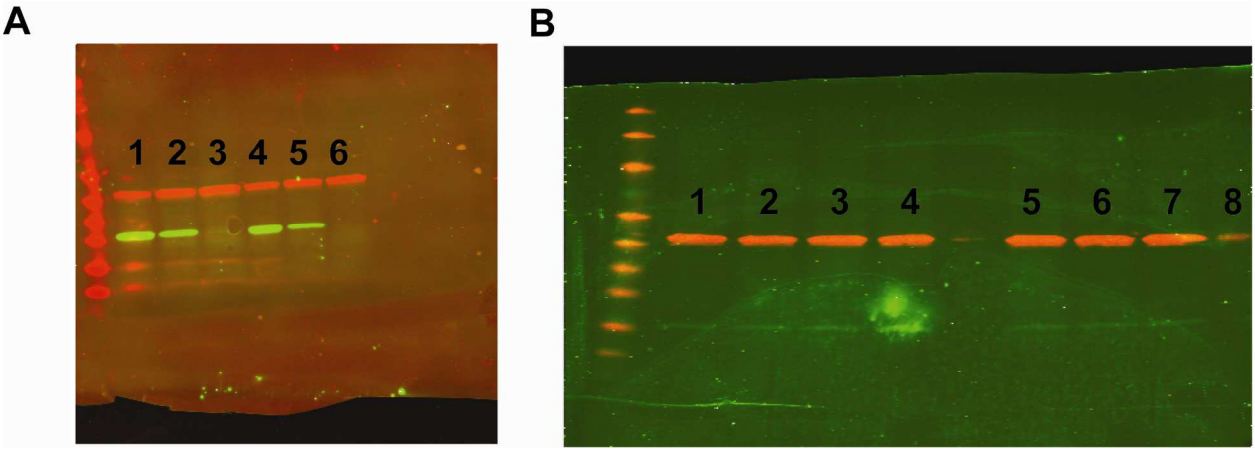
Supplemental Figure 5. Effect of IMC-76 and IMC-48 on BCL-2 protein levels in lymphoma cell lines. Western blot analysis of BCL-2 protein levels in BJAB, B95.8, and GRANTA-519 cell lines following 24 h treatment with IMC-76 (A) or IMC-48 (B). In both (A) and (B), lanes 1 and 2, represent untreated and DMSO vehicle cell lysates, respectively. In (A), lanes 3 and 4 contain lysates from cells treated with 0.25 and 0.5 μM IMC-76, respectively. In (B), lanes 3 and 4 contain lysates from cells treated with 1 and 2 μM IMC-48, respectively. The western blots are representative of three independent experiments with β-actin as a loading control.



Supplemental Figure 6. Effect of IMC-76 on *BCL2* mRNA levels in breast carcinoma cell lines. The *BCL2* mRNA levels as determined by qPCR for MCF-7, MCF-7/TAMR and MDA-MB-231 breast carcinoma cell lines following 24 h treatment with IMC-76. Percent change in mRNA levels were calculated relative to untreated controls. MCF-7 and MDA-MB-231 *BCL-2* mRNA levels were normalized to β-actin and MCF-7/TAMR levels were normalized to *GAPDH*. **P* ≤0.02.



Supplemental Figure 7. Effect of IMC-48 and IMC-76 on the mRNA levels of BCL2, c-MYC, and VEGF. A BJAB cell line with a low basal level of BCL2 mRNA (left panel) and an MCF-7 cell line with a high basal level of BCL2 mRNA (right panel) were used for IMC-48 and IMC-76, respectively. Cells were treated for 24 h with compounds and DMSO served as a control. Relative mRNA level was determined by normalization of Ct values to GAPDH and the DMSO by qPCR assay.

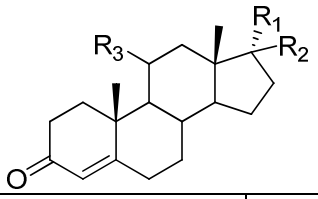


Supplemental Figure 8. Uncut western blot gels. (A) Western blot gel of basal BCL2 protein levels from GRANTA-519 (lanes 1 and 4), B95.8 (lanes 2 and 5), and BJAB (lanes 3 and 6) cell line lysates shown in Figure 5B. (B) Western blot gel of BJAB cells following 24 h treatment with IMC-76 from Supplemental Figure 5. Lanes 1 and 5 and lanes 2 and 6 represent untreated and DMSO vehicle cell lysates, respectively. Lanes 3 and 7 and lanes 4 and 8 contain lysates from cells treated with 0.25 and 0.5 μM IMC-76, respectively. The lysates represent two of three independent experiments with β-actin as a loading control.

Supplemental Table 1. Sequences of *BCL2* FRET and NdU probes.

DNA Probe	Sequence (5′ → 3′)
BCL2 i-motif for HTS	FAM-CAG**CCCC**GCT**CCC**G**CCCCC**TTCCT**CCC**GCG**CCC**G**CCCC**T-BHQ
BCL2 i-motif	FAM-CAG**CCCC**GCT**CCC**G**CCCCC**TTCCT**CCC**GCG**CCC**G**CCCC**T-TAMRA
BCL2 i-motif mutant	FAM -CAG***TTTT***GCT**CCC**G**C*TTT*C**TTCCT***TTT***GCG**CCC**G**CCCC**T-TAMRA
BCL2 G-quadruplex	FAM-A**GGGG**C**GGG**CGC**GGG**AGGAA**GGGGG**C**GGG**AGC**GGGG**CTG-TAMRA
c-MYC i-motif	FAM- TCCCCACCTTCCCCACCCTCCCCACCCTCCCCA-TAMRA
VEGF i-motif	FAM-CTCCG**CCCC**GCCGGGA**CCCCC**G**CCCCC**GG**CCC**G**CCCC**-TAMRA
20T	CAG**CCCC**GCT**CCC**G**CCCCC***NdU*TCCT**CCC**GCG**CCC**G**CCCC**T
21T	CAG**CCCC**GCT**CCC**G**CCCCC**T*NdU*CCT**CCC**GCG**CCC**G**CCCC**T
24T	CAG**CCCC**GCT**CCC**G**CCCCC**TTCC*NdU***CCC**GCG**CCC**G**CCCC**T
39T	CAG**CCCC**GCT**CCC**G**CCCCC**TTCCT**CCC**GCG**CCC**G**CCCC***NdU*

Supplemental Table 2. Structures and FRET values for steroidal compounds from the NCI Diversity Set and ChemDiv library.



Compound #	R_1	R_2	R_3	% Increase in FRET value compared to probe (set to 100 %) (10 equivalent compared to DNA)
IMC-76	–	–	–	270
IMC-48	–	–	–	52
5829-8948	-OH	–H	–H	200.3
N050-0008	$-OCOCH_3$	–H	–H	163.0
N050-0013	–OH	$-COCH_3$	–H	213.0
N050-0014	–OH	$-COCH_2OH$	–H	177.3
5776-0002	$-OCOCH_3$	$-COCH_2OCOCH_3$	–H	234.6
N050-0017	–OH	$-COCH_2OH$	=O	190.4
N056-0002	$-OCOCH_3$	$-COCH_2OH$	–OH	183.0
N056-0003	$-OCOCH_3$	$-COCH_2OCOCH_3$	–OH	181.0

Analysis of structure and FRET values: IMC-76 was identified from the NCI Diversity Set and shows a FRET value of 270. It is a steroidal molecule predominantly substituted on ring D at C17. Screening of similar steroidal molecules from the ChemDiv collection resulted in identification of compounds that exert a similar effect on FRET: an increase indicating destabilization of the BCL2 i-motif. Although definite conclusions cannot be drawn about structure–activity relationships (SAR), activity trend was observed for steroidal compounds with C17 substitution. A detailed SAR would require design, synthesis, and testing of analogs; hence this would be a part of future studies on these compounds.

Supplemental Table 3. IMC-76 and IMC-48 IC_{50} values at 96 h for breast carcinoma and lymphoma cell lines as determined by the MTS cytotoxicity assay.

	IC_{50} (μM)	
Cell Line	**IMC-76 (96 h)**	**IMC-48 (96 h)**
MCF-7	9.4	ND
MCF-7/TAMR	9.4	ND
MDA-MB-231	5.8	ND
BJAB	5.2	8.4
B95.8	7.9	24.2
GRANTA-519	8.4	2.7

ND: not determined