# Novel Bcl-2 Inhibitors Selectively Disrupt the Autophagy-Specific Bcl-2−Beclin 1 Protein−Protein Interaction

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**ABSTRACT:** Autophagy plays essential roles in a wide variety of physiological processes, such as cellular homeostasis, metabolism, development, differentiation, and immunity. Selective pharmacological modulation of autophagy is considered a valuable potential therapeutic approach to treat diverse human diseases. However, development of such therapies has been greatly impeded by the lack of specific small molecule autophagy modulators. Here, we performed structure−activity relationship studies on a previously discovered weak Bcl-2 inhibitor SW076956, and developed a panel of small molecule compounds that selectively released Bcl-2-mediated inhibition of autophagy-related Beclin 1 compared to apoptosis-related Bax at nanomolar concentration. Our NMR analysis showed that compound **35** directly binds Bcl-2 and specifically inhibits the interaction between the Bcl-2 and Beclin 1 BH3 domains without disruption of the Bcl-2−Bax BH3 interaction. More broadly, this proof-of-concept study demonstrates that targeting protein−protein interactions of the intrinsic autophagy regulatory network can serve as a valuable strategy for the development of autophagy-based therapeutics.

**KEYWORDS:** Autophagy, apoptosis, Bcl-2 inhibitors, Beclin 1, Bax, protein−protein interaction, NMR binding studies
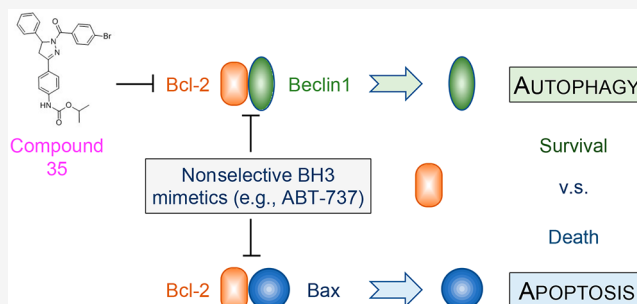
**M**acroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved catabolic pathway by which cells sequester unwanted cellular constituents (such as damaged organelles, protein aggregates ,and invading microbes) in double-membrane structures known as autophagosomes, and then target them for lysosomal degradation through formation of single-membrane structures called autolysosomes following the fusion between autophagosomes and lysosomes. Autophagy is conserved from plants to mammals and plays essential roles in a wide variety of physiological processes, such as cellular homeostasis, metabolism, development, differentiation, and immunity.[1,2] A large amount of evidence demonstrates that autophagy and certain autophagy genes are closely associated with many human diseases including diabetes, cancer, neurodegenerative diseases, and infection.[3,4] Whole-body or tissue-specific genetic disruption of autophagy in model animals leads to multiple pathologies, including tissue abnormalities, aberrant inflammation, impaired immunity, neurodegeneration, and susceptibility to tumorigenesis.[3,4] In contrast, genetic disruption of autophagy suppressors (such as Bcl-2 and Rubicon) enhances basal autophagy levels in vivo and improved the lifespan and health of different model animals.[5−8] Therefore, augmentation of autophagy through small molecule autophagy modulators is believed to be a promising novel candidate approach to treat diverse human diseases.[4,9,10]

To date, there are no autophagy-specific modulating drugs available in clinical use or trials. Although several drugs (e.g., mTOR inhibitors and calcium channel inhibitors) also activate autophagy, their mode of action is indirect and primarily operates through other mechanisms unrelated to autophagy modulation. Thus, there is considerable interest in identifying specific agents as selective autophagy modulators. We predicted Bcl-2 to be a valuable cellular target as it is known to inhibit autophagy through its interaction with the essential autophagy modulator Beclin 1.[11] Beclin 1 functions as a scaffold protein of the autophagy-specific class III phosphatidylinositol 3 kinase (PI3KC3 or PIK3C3) complexes that are required for autophagosomal biogenesis and maturation. Under basal conditions, Bcl-2 binds the BH3 domain of Beclin 1 and suppresses the assembly of PI3KC3 complexes. In response to diverse stress stimuli, Bcl-2-mediated inhibition is released through multiple mechanisms, including a multisite phosphorylation of the nonstructured loop of Bcl-2 and phosphorylation

of the Beclin 1 BH3 domain to interrupt the Bcl-2−Beclin 1 protein−protein interaction, or the deployment of BH3-only proteins that competitively disrupt the Bcl-2−Beclin inter-action.[11] Recently, we and other investigators showed that genetically engineered mice carrying the point mutation F121A in Beclin 1 have decreased Bcl-2−Beclin 1 interaction and thereby increased autophagy in multiple tissues including brain, heart, muscle, liver, mammary gland, and kidney. These mice exhibited increased longevity and reduced neurodegeneration, and diminished aging-related renal and cardiac pathological changes and spontaneous tumorigenesis.[5−8] These findings highlight the therapeutic potential of specific small molecule modulation of the Bcl-2−Beclin 1 protein−protein interaction as a new approach to activate autophagy to treat human disease (Figure 1). The potential difficulty with this approach is the
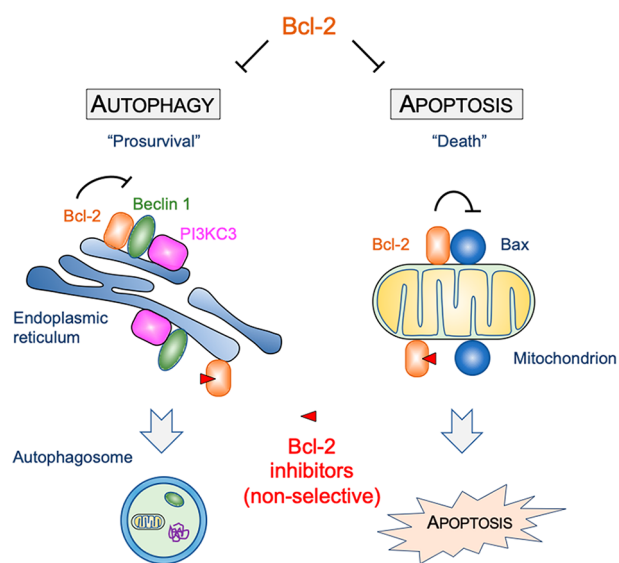


**Figure 1.** Bcl-2 is a key cellular regulator of both autophagy and apoptosis. Bcl-2 directly interacts with the essential autophagy modulator Beclin 1 to suppresses autophagy. Bcl-2 also suppresses apoptosis through binding to the proapoptotic protein Bax on the mitochondrion. Nonselective small molecules release Bcl-2-mediated inhibition of both autophagy and apoptosis. In contrast, small molecules that specifically target Bcl-2−Beclin 1 would be expected to activate autophagy without inducing apoptosis.

design of specific Bcl-2−Beclin 1 protein interaction inhibitors. Notably, Bcl-2 is also a cellular suppressor of apoptosis through its interaction with the BH3 domain of the proapoptotic protein Bax. To date, known Bcl-2 modulators such as ABT-737 that act as BH3 mimetics activate both autophagy and apoptosis.[12] Administration of such nonselective Bcl-2 modulators in vivo may thus raise substantial concern because of a potentially undesired induction of apoptosis. Therefore, an ideal Bcl-2-based autophagy modulator must have high selectivity for the suppression of the Bcl-2−Beclin 1 interaction while avoiding the disruption of the Bcl-2−Bax interaction (Figure 1). Here, we disclose a medicinal chemistry driven structure−activity relationship (SAR) study leading to a collection of small molecules that selectively suppress the Bcl-2−Beclin 1 protein−protein interaction at nanomolar concentration.

Previously, we reported a high-throughput screen (HTS) of the UTSW small molecule library and discovered compound SW076956 (1, Figure 2A) that suppressed the Bcl-2−Beclin 1 protein−protein interaction using an AlphaLISA protein−
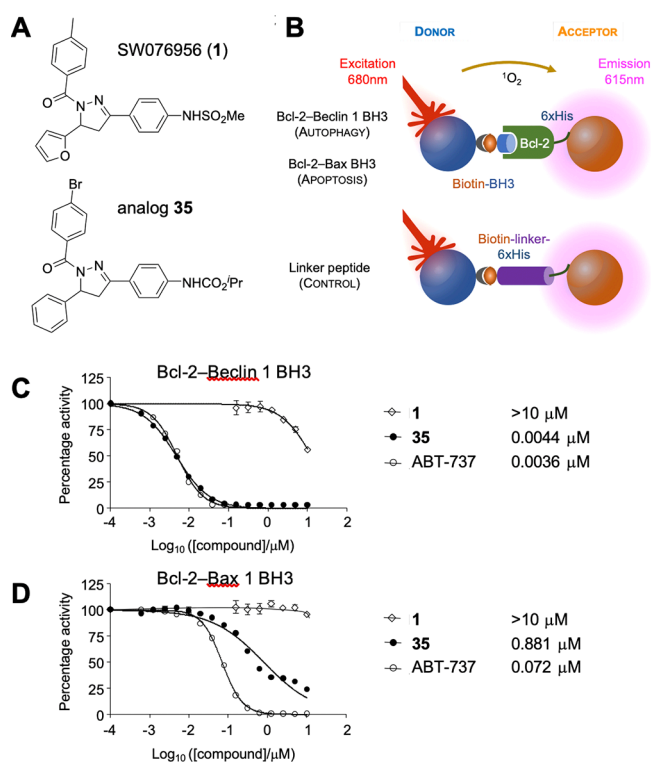


**Figure 2.** Compound structures, AlphaLISA assay, and representative dose−response quantification of Bcl-2−Beclin 1 BH3 and Bcl-2−Bax BH3 protein−protein interactions. (A) Structures of SW076956 (1) and analogue 35. (B) Design of the AlphaLISA assay. Details can be found in ref 13. (C and D) Quantification of the Bcl-2−Beclin 1 (C) and the Bcl-2−Bax interaction (D). Dose−response curves for compounds 1, 35, and the nonselective Bcl-2 inhibitor ABT-737 for the indicated interactions. Dose−response curves were analyzed using the nonlinear regression function in Prism 7.0 (GraphPad).

protein interaction assay (Figure 2B).[13] Although SW076956 was not very potent at inhibiting the Bcl-2−Beclin 1 BH3 interaction ($IC_{50} \sim 16 \ \mu M$), we were encouraged by the complete lack of inhibition of the corresponding Bcl-2−Bax BH3 interaction over a similar concentration range (Figure 2C, D). For comparison, the known Bcl-2 modulator ABT-737 was significantly more potent ($IC_{50} = 3.6$ nM, Figure 2C), but unfortunately also significantly inhibited the Bcl-2−Bax BH3 interaction ($IC_{50} = 72$ nM, Figure 2D). To obtain potent and selective autophagy inducers with sufficient drug-like properties for proof-of-concept studies, our initial studies around HTS hit 1 aimed at exploring SAR around the benzamide, furyl, and phenylmethanesulfonamide rings. All new analogues were evaluated in the AlphaLISA assay for both the Bcl-2−Beclin 1 and Bcl-2−Bax interaction.

As shown in Table 1, removing the 4-Me substituent ($Ar^1$) killed activity (9) as did replacement with a 4-MeSO_2, 4-MeC(O), 4-CF_3C(O), or 4-$^t$Bu substituent (analogues 26−29). Other *para*-substitutions either had little effect (4-F, 4-CN, and 4-Me; analogues 2, 7, and 8) or modestly improved activity 5.5−8-fold (4-Cl, 4-Br, 4-CF_3, 4-NO_2, analogues 3−6). *Meta*-substituted benzamides 10 and 11 were inactive, as were furyl (12) and pyridinyl analogues 30−32. Linking $Ar^1$ via a sulfonamide or methylene instead of an amide, or replacement of $Ar^1$ with a cyclohexyl resulted in inactive compounds (Table S1), as did substituting the methylsulfonamide in the right-hand ring with a trifluoromethylsulfonamide (13), acetamide (14), or

**Table 1. SAR of Trisubstituted 4,5-Dihydropyrazoles 1−44**
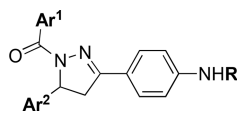


| compd[a] | Ar$^1$ | Ar$^2$ | R | IC$_{50}$ ($\mu$M)[b] |
|---|---|---|---|---|
| 1 | 4-MePh | 2-furyl | SO$_2$Me | 15.9 ± 0.8 |
| 2 | 4-FPh | 2-furyl | SO$_2$Me | 12.66 ± 0.22 |
| 3 | 4-ClPh | 2-furyl | SO$_2$Me | 2.72 ± 0.11 |
| 4 | 4-BrPh | 2-furyl | SO$_2$Me | 2.47 ± 0.36 |
| 5 | 4-CF$_3$Ph | 2-furyl | SO$_2$Me | 2.88 ± 0.06 |
| 6 | 4-NO$_2$Ph | 2-furyl | SO$_2$Me | 1.97 ± 0.12 |
| 7 | 4-CNPh | 2-furyl | SO$_2$Me | 7.44 ± 0.21 |
| 8 | 4-MeOPh | 2-furyl | SO$_2$Me | 10.11 ± 0.30 |
| 9 | Ph | 2-furyl | SO$_2$Me | inactive |
| 10 | 3-MePh | 2-furyl | SO$_2$Me | inactive |
| 11 | 3-FPh | 2-furyl | SO$_2$Me | inactive |
| 12 | 2-furyl | 2-furyl | SO$_2$Me | inactive |
| 13 | 4-MePh | 2-furyl | SO$_2$CF$_3$ | inactive |
| 14 | 4-MePh | 2-furyl | C(O)Me | inactive |
| 15 | 4-MePh | 2-furyl | H | inactive |
| 16 | 4-MePh | 2-furyl | CO$_2^t$Bu | 3.29 ± 0.16 |
| 17 | 4-BrPh | 2-furyl | CO$_2^t$Bu | 1.26 ± 0.03 |
| 18 | 4-BrPh | 3-furyl | CO$_2^t$Bu | 3.82 ± 0.09 |
| 19 | 4-BrPh | thiazol-2-yl | CO$_2^t$Bu | 1.61 ± 0.01 |
| 20 | 4-BrPh | thiazol-5-yl | CO$_2^t$Bu | 4.36 ± 0.08 |
| 21 | 4-BrPh | 2-pyridinyl | CO$_2^t$Bu | 0.65 ± 0.04 |
| 22 | 4-BrPh | 3-pyridinyl | CO$_2^t$Bu | 1.63 ± 0.11 |
| 23 | 4-BrPh | 4-pyridinyl | CO$_2^t$Bu | 2.78 ± 0.13 |
| 24 | 4-BrPh | Ph | CO$_2^t$Bu | 0.262 ± 0.039 |
| 25 | 4-NO$_2$Ph | Ph | CO$_2^t$Bu | 0.439 ± 0.016 |
| 26 | 4-MeSO$_2$Ph | Ph | CO$_2^t$Bu | inactive |
| 27 | 4-MeC(O)Ph | Ph | CO$_2^t$Bu | inactive |
| 28 | 4-$^t$BuPh | Ph | CO$_2^t$Bu | inactive |
| 29 | 4-CF$_3$C(O)Ph | Ph | CO$_2^t$Bu | inactive |
| 30 | 2-pyridinyl | Ph | CO$_2^t$Bu | inactive |
| 31 | 3-pyridinyl | Ph | CO$_2^t$Bu | 14.5 ± 3.7 |
| 32 | 4-pyridinyl | Ph | CO$_2^t$Bu | inactive |
| 33 | 4-BrPh | Ph | CO$_2$Me | 2.10 ± 0.07 |
| 34 | 4-BrPh | Ph | CO$_2$Et | 0.0328 ± 0.0006 |
| 35 | 4-BrPh | Ph | CO$_2^i$Pr | 0.0044 ± 0.0002 |
| 36 | 4-BrPh | Ph | CO$_2^c$Pr | 0.0441 ± 0.0028 |
| 37 | 4-BrPh | Ph | CO$_2^t$Bu | 0.164 ± 0.033 |
| 38 | 4-BrPh | Ph | CO$_2$allyl | 0.0318 ± 0.0028 |
| 39 | 4-BrPh | Ph | CO$_2$(CH$_2$)$_2$C$_2$H | 0.065 ± 0.022 |
| 40 | 4-BrPh | Ph | C(O)NHMe | 5.53 ± 0.12 |
| 41 | 4-BrPh | Ph | C(O)NMe$_2$ | 2.41 ± 0.03 |
| 42 | 4-BrPh | Ph | C(O)pyrrolidine | inactive |
| 43 | 4-BrPh | Ph | C(O)NH$^i$Pr | 0.350 ± 0.009 |
| 44 | 4-BrPh | Ph | C(O)NH$^t$Bu | 9.22 ± 0.51 |

[a]All compounds are racemates. [b]IC$_{50}$ values represent the half maximal (50%) inhibitory concentration for the Bcl-2−Beclin 1 BH3 interaction as determined in the AlphaLISA assay. Error represents SD ($n$ = 3). All compounds were inactive (at 10 $\mu$M) against the Bcl-2−Bax BH3 interaction except compounds **34** (IC$_{50}$ = 6.62 ± 0.27 $\mu$M), **35** (IC$_{50}$ = 0.88 $\mu$M ± 0.04 $\mu$M), **36** (IC$_{50}$ = 15.1 ± 0.04 $\mu$M), and **38** (IC$_{50}$ = 8.83 ± 0.39 $\mu$M).

primary amine (**15**). The corresponding *tert*-butylcarbamates improved activity but not dramatically (2−5-fold, e.g., **16**, **17** vs **1** and **4**). Comparing compounds **18−23** to analogue **17** indicates that exchanging the 2-furanyl ring (Ar$^2$) with other heterocyclic rings did not improve the activity significantly, whereas eliminating this ring altogether resulted in loss of activity (Table S1). However, a phenyl replacement enhanced activity 5-fold (**24** vs **17**). Selected other 2-, 3-, and 4-substituted

phenyls (Br, Me, NO$_2$) significantly impaired activity (Table S1). Thus far, a combination of 4-BrPh (Ar$^1$), Ph (Ar$^2$) and a *tert*-butylcarbamate (R) provided the most potent Bcl-2−Beclin 1 antagonist (analogue **24**) with an in vitro AlphaLISA IC$_{50}$ of 262 nM and no activity against the corresponding Bcl-2−Bax BH3 interaction.

As we advanced analogues for improved potency, we also evaluated a select subset for metabolic stability (Table S2). All

**Table 2. Additional Miscellaneous SAR**
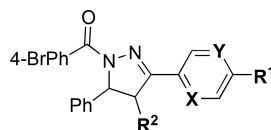
| compd[a] | $R^1$ | $R^2$ | X | Y | $IC_{50}$ ($\mu$M)[b] |
|---|---|---|---|---|---|
| 24 | $NHCO_2{}^tBu$ | H | CH | CH | $0.262 \pm 0.039$ |
| 45 | H | H | CH | $CNHCO_2{}^tBu$ | $11.06 \pm 0.33$ |
| 46 | $NHCO_2{}^tBu$ | H | CH | N | $0.309 \pm 0.010$ |
| 47 | $NHCO_2{}^tBu$ | H | N | CH | $0.372 \pm 0.038$ |
| 48 | $NHCO_2{}^tBu$ | Me[c] | CH | CH | $0.0274 \pm 0.0012$ |
| 49 | $NHCO_2{}^tBu$ | Phe[c] | CH | CH | $0.172 \pm 0.075$ |
| 50 | $NHCO_2{}^iPr$ | Me[c] | CH | N | $0.002 \pm 0.001$ |
| 51 | oxazolidinone | H | CH | CH | $5.16 \pm 0.07$ |
| 52 | $CO_2H$ | H | CH | CH | inactive |
| 53 | $CO_2Me$ | H | CH | CH | $1.64 \pm 0.27$ |
| 54 | $CO_2{}^iPr$ | H | CH | CH | $0.110 \pm 0.049$ |
| 55 | $OCO^iPr$ | H | CH | CH | $5.45 \pm 0.23$ |
| 56 | $CONMe_2$ | H | CH | CH | $5.91 \pm 0.27$ |
| 57 | $CONH^tBu$ | H | CH | CH | inactive |

[a]All compounds are racemates. [b]$IC_{50}$ values represent the half maximal (50%) inhibitory concentration for the Bcl-2−Beclin 1 BH3 interaction as determined in the AlphaLISA assay. Error represents SD ($n$ = 3). All compounds were inactive (at 10 $\mu$M) against the Bcl-2−Bax BH3 interaction except compounds 50 ($IC_{50}$ = 0.157 $\pm$ 0.048 $\mu$M) and 54 ($IC_{50}$ = 4.36 $\pm$ 0.03 $\mu$M). [c]Single diastereomer; configuration not assigned.
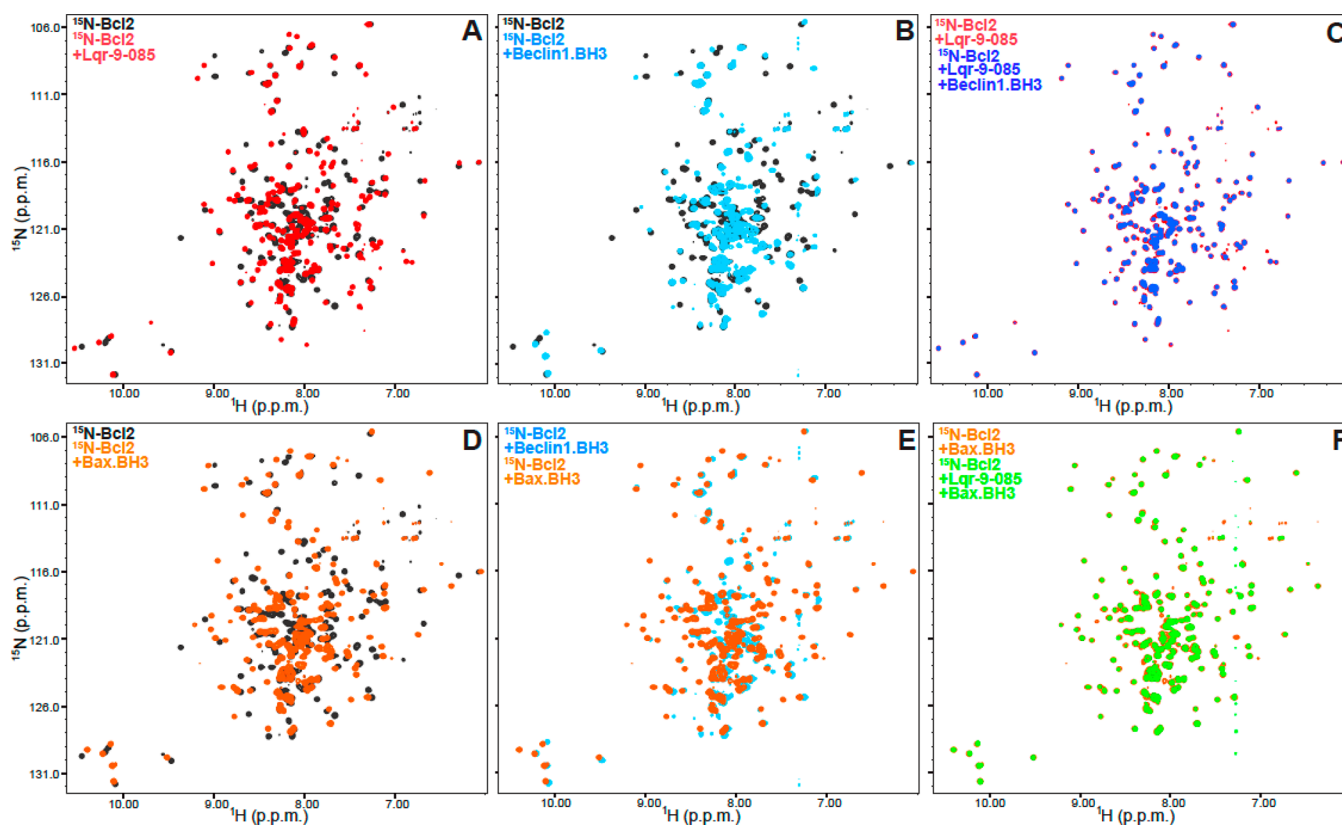


**Figure 3.** Compound 35 displaces the Beclin 1 BH3 domain from Bcl-2, but not the Bax BH3 domain. (A−F) Superpositions of $^1H−^{15}N$ TROSY-HSQC spectra of (A) Bcl-2 alone (black contours) or in the presence of compound 35 (red contours); (B) Bcl-2 alone (black contours) or in the presence of the Beclin 1 BH3 peptide (light blue contours); (C) Bcl-2 in the presence of compound 35 and the absence (red contours) or presence (blue contours) of the Beclin 1 BH3 peptide; (D) Bcl-2 alone (black contours) or in the presence of the Bax BH3 peptide (orange contours); (E) Bcl-2 in the presence of the Beclin 1 BH3 peptide (light blue contours) or the Bax BH3 peptide (orange contours); (F) Bcl-2 in the presence of the Bax BH3 peptide and the absence (orange contours) or presence (gray contours) of compound 35.

sulfonamides tested (1, 4−6, 61) displayed poor stability with T1/2 of 2.9−18.1 min upon incubation with mouse microsomes, whereas the corresponding *tert*-butylcarbamates improved stability 5−7-fold. The two most potent analogues (24 and 25) coincidentally were also metabolically the most stable with an excellent T1/2 of 120 min (Table S2). Because a

nitrophenyl represent a known toxicophore (**25**), we focused our next round of SAR optimization on exploring various carbamates. Using compound **24** as the reference ($IC_{50}$ = 262 nM), we found that sterically less encumbered alkylcarbamates **34-39** improved activity significantly, up to 60-fold for isopropylcarbamate **35** ($IC_{50}$ = 4.4 nM). The smallest carbamate (R = $NHCO_2Me$, **33**) was an exception and was 8-fold less active than **24**. Replacing carbamate functionality with ureas (**40-44**) was unfruitful and resulted in significant loss of activity. Although we started to observe some inhibition of the undesired Bcl-2−Bax protein−protein interaction with some of the more potent carbamates **34-36**, and **38** (Table 1 footnotes), a significant selectivity for disruption of the Bcl-2−Beclin 1 interaction was maintained (>200-fold). Dose−response curves and selectivity for the most potent analogue **35** are included in Figure 1.

In Table 2, we explore some additional miscellaneous SAR related to the right-hand aromatic ring, or additional substitution in the central dihydropyrazole ring. First, transposing the carbamate from the para to the meta-position was detrimental for activity (**45** vs **24**). In an attempt to improve polarity (compound **24** has a Clog P of 6.2), we prepared more polar pyridine analogues **46** and **47** with an acceptable ClogP of 4.7 below the Lipinski Rule-of-Five recommended threshold. Fortunately, this polarity improvement did not come at a cost of potency. Introduction of an additional methyl substituent on the central dihydropyrazole ring creates a new stereogenic center and resulted in a 10-fold improvement of activity for compound **48** versus comparator **24**, and was also beneficial for the more polar pyridine analogue **50** ($IC_{50}$ = 2 nM; ClogP 4.8). However, the selectivity of compound **50** for the Bcl-2−Beclin 1 interaction dropped to 71-fold (Bcl-2−Bax, $IC_{50}$ 157 nM; vs 200-fold selectivity for **35**). Finally, other functionality including acids, esters, carbonates, and amides (**51-57**) worsened activity and did not lead to new avenues for improved analogue design.

Compound **35** was selected for further follow-up. It provided the best balance of potency, selectivity, and in vitro metabolic stability. Compound **35** had a 3600-fold improved activity in suppressing autophagy-related Bcl-2−Beclin 1 BH3 interaction by the AlphaLISA assay ($IC_{50}$ = 4.4 nM) compared to HTS hit **1** ($IC_{50}$ ∼ 16 $\mu$M), which is comparable to the potency of the commercial Bcl-2 inhibitor ABT-737 ($IC_{50}$ = 3.6 nM; Figure 2C). Notably, ABT-737 also disrupted the apoptosis-related Bcl-2−Bax BH3 interaction with an $IC_{50}$ of 72.2 nM (Figure 2D), thus providing only ∼20-fold selectivity. In contrast, compound **35** was 200-fold more selective at suppressing the Bcl-2−Beclin 1 BH3 interaction. Gratifyingly, compound **35** was also one of the most stable analogues with a T1/2 of 120 min in murine microsomal fractions (Table S2). Compound **35** also showed favorable in vivo pharmacokinetics after intraperitoneal delivery, with marginally better exposure than the corresponding tert-butylcarbamate **24** and excellent oral bioavailability (Table S3).

To determine whether compound **35** directly binds to Bcl-2, we acquired $^1H-^{15}N$ transverse relaxation optimized (TROSY) heteronuclear single quantum coherence (HSQC) spectra of a $^{15}N$-labeled fragment corresponding to the cytoplasmic domain of Bcl-2 (Bcl-2 aa1−218) in the absence and presence of compound **35**. We found that compound **35** induced dramatic changes in the spectrum (Figure 3A), demonstrating that compound **35** directly interacts with Bcl-2. As expected, a peptide corresponding to the BH3 domain of Beclin 1 also resulted in cross-peak changes in the $^1H-^{15}N$ TROSY-HSQC spectrum of Bcl-2 (Figure 3B) which is consistent with the prior

structure biology studies indicating a direct interaction between the Beclin 1 BH3 domain and Bcl-2 family members.[14−16] Notably, the shifts of the Bcl-2 spectrum caused by the Beclin 1 BH3 peptide were clearly distinct from those caused by compound **35** (when comparing red contours in Figure 3A with light blue contours in Figure 3B). This indicates that Beclin 1 BH3 peptide and compound **35** likely bind to somewhat different confirmations of Bcl-2. Furthermore, incubation with Beclin 1 BH3 peptide failed to alter the $^1H-^{15}N$ TROSY-HSQC spectrum of compound **35**-bound Bcl-2 (Figure 3C), showing that compound **35** prevents binding of the Beclin 1 BH3 peptide to Bcl-2.

Next, we studied the effect of compound **35** on the Bcl-2−Bax BH3 interaction, which is essential for the apoptotic regulation. Incubation of a peptide corresponding to the BH3 domain of Bax with Bcl-2 led to dramatic shifts in the $^1H-^{15}N$ TROSY-HSQC cross-peaks of Bcl-2 (Figure 3D), which is consistent with prior findings regarding the direct interaction between Bcl-2 and the Bax BH3 domain.[17] Notably, the resulting spectrum of Bcl-2 bound to the Bax BH3 peptide was almost identical to that observed for Bcl-2 bound to the Beclin 1 BH3 peptide (Figure 3E). This finding is consistent with the fact that Beclin 1 BH3 domain and Bax BH3 domain both bind to the same evolutionarily conserved hydrophobic groove on Bcl-2 family members.[18] Since the spectrum of Bcl-2 bound to the Beclin 1 BH3 peptide had fewer cross-peaks, we speculated that the moderate affinity of the Beclin 1 BH3 peptide with Bcl-2 likely causes exchange broadening, while the Bax BH3 peptide binds to Bcl-2 with higher affinity that does not lead to exchange broadening. Importantly, compound **35** did not cause marked changes in the $^1H-^{15}N$ TROSY-HSQC spectrum of Bcl-2 bound to the Bax BH3 peptide (Figure 3F). These findings show that compound **35** did not displace the Bax BH3 peptide from Bcl-2 under the conditions of these experiments, even though compound **35** completely displaced the Becling 1 BH3 peptide at the same concentrations. These differences are consistent with the AlphaLisa results (Figure 2C,D) and can be attributed to the much higher affinity of the Bax BH3 peptide for Bcl-2 than the Beclin 1 BH3 peptide.

In summary, we identified a set of new Bcl-2 inhibitors that can potently suppress the desired Bcl-2−Beclin 1 BH3 interaction while avoiding to disrupt the Bcl-2−Bax BH3 interaction that could lead to off-target induction of apoptosis. Compound **35** provided for a reasonable balance of high potency ($IC_{50}$ = 4.4 nM), selectivity (200-fold), metabolic stability and in vivo pharmacokinetic properties. Mechanistically, the selectivity of the compound in inhibiting the Bcl-2-Beclin 1 interaction can be explained by the differences in affinity of the Bax and Beclin 1 BH3 domains for Bcl-2. Hence, this proof-of-concept study demonstrates Bcl-2, an essential cellular regulator of both autophagy and apoptosis, as an excellent cellular target for the development of agents to specifically augment autophagy without induction of apoptosis. More broadly, our study highlighted the importance in thorough characterization of protein−protein interaction networks and the development of selective small molecule compounds that can specifically modulate one protein−protein interaction among distinct protein complexes that share a common partner protein.[19]

## ■ ASSOCIATED CONTENT

### ⓈⒾ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00309.

> Data for additional compounds; in vitro metabolic stability; pharmacokinetic data for compounds **24** and **35**; materials and methods; procedures and characterization of synthetic compounds; copies of $^1$H and $^{13}$C NMR spectra (PDF)

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### Author Contributions

‡X.D. and Q.L. contributed equally. X.D., B.L., and J.K.D.B. conceived and designed the study; Q.L. synthesized and characterized all the synthetic compounds in the lab of J.K.D.B.; N.S.W. and X.W. performed all the in vitro and in vivo pharmacology; X.D. and W.-C.C. performed all the biological assays in the lab of B.L.; Y.-Z.P., Y.-C.K., X.Z., and J.R. performed the NMR studies and analyzed the corresponding data; X.D., J.R., B.L., and J.K.D.B. analyzed the data; X.D., N.S.W., J.R., and J.K.D.B. wrote and edited the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

Bax, Bcl-2 associated X; Bcl-2, B-cell lymphoma 2; Beclin 1, Coiled-coil moesin-like Bcl-2-interacting protein 1; BH3, Bcl-2 homology domain 3; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; PI3KC3, class III phosphatidylinositol 3 kinase; TROSY, transverse relaxation optimized spectroscopy

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