ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2019, **55**, 14765

Received 14th September 2019,
Accepted 12th November 2019

DOI: 10.1039/c9cc07217a

rsc.li/chemcomm

Utilizing PROTAC technology to address the on-target platelet toxicity associated with inhibition of BCL-X_L†

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BCL-X_L, an anti-apoptotic BCL-2 family protein, plays a key role in cancer cell survival. However, the potential of BCL-X_L as an anti-cancer target has been hampered by the on-target platelet toxicity because platelets depend on BCL-X_L to maintain their viability. Here we report the development of a PROTAC BCL-X_L degrader, XZ424, which has increased selectivity for BCL-X_L-dependent MOLT-4 cells over human platelets compared with conventional BCL-X_L inhibitors. This proof-of-concept study demonstrates the potential of utilizing a PROTAC approach to achieve tissue selectivity.

The B-cell lymphoma 2 (BCL-2) family proteins, consisting of pro- and anti-apoptotic members, play a critical role in determining cell life and death through regulation of the intrinsic apoptotic pathway.[1] The anti-apoptotic BCL-2 family proteins, including BCL-2, BCL-X_L, and MCL-1, are upregulated in many human cancers and associated with tumor initiation, progression, and resistance to chemotherapy and targeted therapies.[2,3] These proteins inhibit apoptosis by binding the α-helical BCL-2 homology-3 (BH3) domain of pro-apoptotic proteins Bax and Bak, thereby preventing their activation of the mitochondrial apoptotic pathway.[4] Thus, inhibiting the protein–protein interaction between anti- and pro-apoptotic BCL-2 proteins, and thus overcoming the apoptotic resistance of cancer cells, is a highly attractive cancer therapeutic strategy.[5–7]

Significant progress has been made in developing "BH3 mimetic" small-molecule inhibitors of the anti-apoptotic BCL-2 proteins.[8–11] Importantly, this therapeutic strategy has been validated by the FDA approval of venetoclax, a BCL-2 specific inhibitor, for chronic lymphocytic leukemia in 2016[12] and acute myeloid leukemia in 2018.[13] BCL-X_L is the most common BCL-2 family member overexpressed in solid tumors, as well as in a subset of leukemia and lymphoma cells.[14] In addition, it has been well established that BCL-X_L inhibition can sensitize cancer cells to chemotherapies.[15–17] More recently, we and others discovered that BCL-2/BCL-X_L dual inhibitors, navitoclax (also known as ABT-263) and ABT-737, and BCL-X_L specific inhibitors, A-1331852 and A-1155463, are able to selectively kill senescent cells (SnCs).[18–20] This is because BCL-X_L is a key anti-apoptotic protein in many types of SnCs. Accumulating evidence indicates that cellular senescence plays an important role in many age-related pathologies.[21] Studies on ABT-263 in mouse models have demonstrated that clearance of chemotherapy-induced SnCs reduces several short- and long-term adverse effects of the therapy, and inhibits cancer relapse and metastasis.[22] Thus, BCL-X_L has also been considered as a promising therapeutic target for the treatment of a range of age-related diseases and cancer therapy-induced adverse effects.

However, the clinical applications of BCL-X_L specific or BCL-2/BCL-X_L dual inhibitors currently in development are greatly limited by their on-target and dose-limiting thrombocytopenia toxicity. This is because platelets are solely dependent on BCL-X_L for survival.[23,24] Thus, traditional structural modifications of BCL-X_L inhibitors are unlikely to address this on-target toxicity. Here we report the utilization of proteolysis targeting chimera (PROTAC) as an approach to minimize the platelet toxicity associated with targeting BCL-X_L.

Originally described by Crews and Deshaies in 2001,[25] PROTAC has emerged as a powerful drug discovery technology.[26,27] PROTACs are bivalent small-molecules containing a pharmacophoric unit that recognizes the target protein linked to a second pharmacophoric unit that binds to a specific E3 ubiquitin ligase. They can recruit the target protein to an E3 ligase, promote proximity-induced ubiquitination of the target protein, and lead to its degradation through the ubiquitin proteasome system (UPS).[28] Because PROTACs rely on E3 ligases to induce protein degradation, it is possible for them to achieve cell/tissue selectivity even when their target proteins are ubiquitously expressed, if they target an E3 ligase that is differentially expressed in different cells or tissues. To our delight, by analysing published human

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9cc07217a

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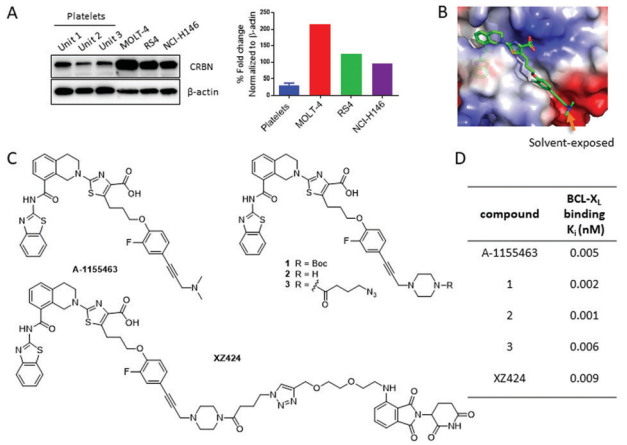
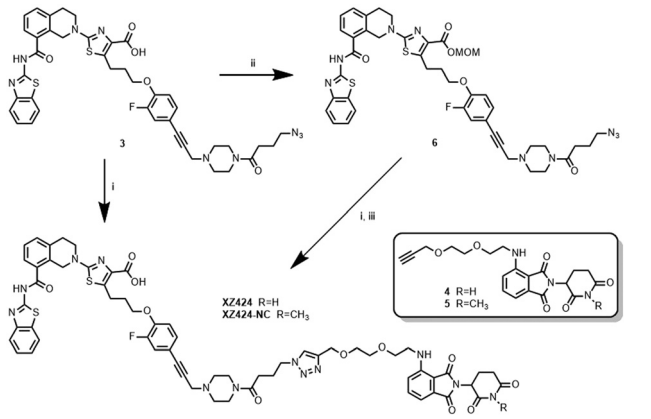


compound	BCL-X_L binding K_i (nM)
A-1155463	0.005
1	0.002
2	0.001
3	0.006
XZ424	0.009

Fig. 1 (A) Left panel: representative immunoblot analyses of CRBN expression in three human cancer cell lines and human platelets from three individuals indicated by units 1–3; right panel: densitometric analyses of CRBN expression. (B) X-ray crystal structure of A-1155463 (green) bound to BCL-X_L (PDB code: 4QVX). (C) Structures of A-1155463, compounds **1–3** and XZ424. (D) Binding affinities of various compounds to BCL-X_L; $n \geq 2$.



Scheme 1 (i) **4** or **5**, $CuSO_4{\cdot}5H_2O$, sodium L-ascorbate, ^{t}BuOH, THF, and H_2O, at 55–65 °C. (ii) Chloromethyl methyl ether, Na_2CO_3, and DMF. (iii) HCl in 1,4-dioxane, DCM, and MeOH.

platelet RNA-seq data,[29,30] we found that cereblon (CRBN), one of the two most popular E3 ligases being recruited by PROTACs to induce targeted protein degradation, is modestly expressed in human platelets. This finding was confirmed by Western blot, which indicates a significantly lower CRBN level in human platelets compared to a number of cancer cell lines (Fig. 1A).

Through the analysis of the co-crystal structure of A-1155463, a potent and selective BCL-X_L inhibitor (Fig. 1C), in complex with BCL-X_L (Fig. 1B),[31] we found that the *N*,*N*-dimethylamino moiety on A-1155463 is solvent-exposed, thus representing a potential linker tethering position. To confirm that this position is amenable to linker attachment without a major loss of BCL-X_L binding affinity, we synthesized compounds **1** and **2**, in which the dimethylamino group was replaced with a piperazine ring, and compound **3**, an azide derivative of **2** (Fig. 1C and Scheme S1, ESI†). All three compounds exhibited BCL-X_L binding affinities, measured by a bead-based AlphaScreen competition binding assay,[32] that are comparable to A-1155463 (Fig. 1D). We therefore generated a focused series of PROTACs by conjugating **3** with the CRBN ligand pomalidomide (POM). The most potent PROTAC in inducing BCL-X_L degradation, XZ424 (Fig. 1C), was selected for the proof-of-concept studies described below.

XZ424 was initially synthesized *via* Huisgen cycloaddition of azide **3** with alkyne **4** derived from POM (Scheme 1). The reaction suffered from low yields and the product was difficult to purify, due to the presence of the carboxylic acid group which is attributed to the low solubility of XZ424 in organic solvents. Azide **3** was then converted to the corresponding benzyl and ethyl esters. The cycloaddition of both esters with **4** and the subsequent purification of the products were straightforward. However, removal of the benzyl and ethyl groups from the corresponding product appeared to be difficult due to the presence of a triple bond, which prevents the use of hydrogenolysis for the cleavage of benzyl groups, and an imide moiety that is unstable under alkaline hydrolysis conditions. Methoxymethyl (MOM) ester **6**, which was converted from **3** in an 84% yield, was then employed. The click reaction between **6** and **4**, as well as the following cleavage of the MOM group under mild acidic conditions, underwent smoothly to afford XZ424 (84%, 2 steps).

As expected, XZ424 had similar BCL-X_L binding affinity compared with A-1155463 (Fig. 1D). The BCL-X_L degradation ability of XZ424 was examined in MOLT-4, a human T-cell acute lymphoblastic leukemia cell line primarily dependent on BCL-X_L for survival.[33] XZ424 dose-dependently induced BCL-X_L degradation in MOLT-4 cells, with a DC_{50} value (the concentration for 50% protein degradation) of 50 nM under 16 h treatment (Fig. 2A). In contrast, no significant changes in BCL-X_L protein levels were observed in human platelets treated with up to 1.0 μM of XZ424 for 16 h (Fig. 2B). In addition, the BCL-X_L degradation induced by XZ424 in MOLT-4 was time-dependent, starting within 2 h, and after drug treatment for 16 h, more than 85% protein was degraded with 100 nM of XZ424 (Fig. 2C). The effects of XZ424 on BCL-X_L protein levels in MOLT-4 were long-lasting and also reversible, as indicated in the "washout" assay (Fig. 2D). Furthermore, pre-incubation of MOLT-4 cells with an excess of the CRBN ligand pomalidomide (POM) or a proteasome inhibitor MG132 blocked XZ424-induced BCL-X_L degradation (Fig. 2E), indicating that the degradation depends on both CRBN E3 ligase and the UPS. To further confirm that the CRBN E3 ligase is involved in XZ424-induced BCL-X_L degradation, we synthesized a negative control compound XZ424-NC (Scheme 1), in which a methyl group is installed on the amino group in the POM moiety of XZ424. It has been shown that adding the methyl to thalidomide analogues abolishes their binding to CRBN.[28f,34] Not surprisingly, XZ424-NC did not induce BCL-X_L degradation in MOLT-4 cells (Fig. 2F).

We next evaluated the effects of XZ424 on the viability of MOLT-4 and human platelets, along with A-1155463 and ABT-263. As expected, A-1155463 and ABT-263 exhibited no selective cytotoxicity for MOLT-4 over platelets (Fig. 3A), confirming the on-target platelet toxicity of BCL-X_L inhibitors. In contrast, XZ424 showed potent cytotoxicity against MOLT-4 cells with an IC_{50} value

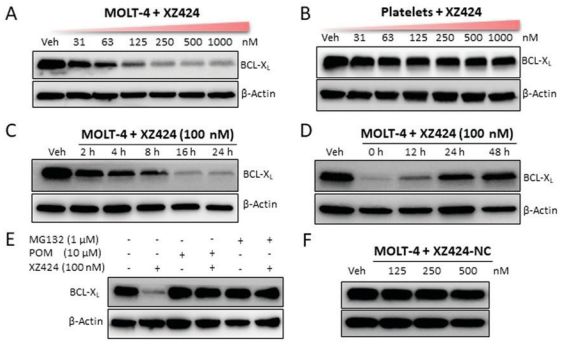


Fig. 2 XZ424-induces BCL-X_L degradation. (A) Western blot showing the BCL-X_L protein levels in MOLT-4 cells treated with the indicated concentration of XZ424 for 16 h. (B) Western blot analysis of BCL-X_L levels after treatment of human platelets with the indicated concentration of XZ424 for 16 h. (C) Time-dependent experiments in MOLT-4 cells after treatment with 100 nM XZ424 at the indicated time points. (D) MOLT-4 cells were incubated with 100 nM of XZ424 for 16 h followed by drug washout, resuspension and incubation of the cells for an additional time as indicated in drug-free medium. (E) Pretreatment with 10 μM pomalidomide (POM) or 1 μM MG132 for 2 h blocked the degradation of BCL-X_L by XZ424. Data are presented as representative figures of two independent experiments. (F) Western blot analysis of BCL-X_L in MOLT-4 cells treated with XZ424-NC at the indicated concentrations for 16 h.

A

compound	Platelets 24 h (EC_{50}, nM)	MOLT-4 72 h (EC_{50}, nM)	EC_{50} ratio (Platelets/MOLT-4)
A-1155463	7.1 ± 2.6	6.2 ± 4.3	1.1
ABT-263	591 ± 126	220 ± 26	2.7
XZ424	1136 ± 27	51 ± 23	22.3
XZ424+POM	1167 ± 20	561 ± 181	2.1

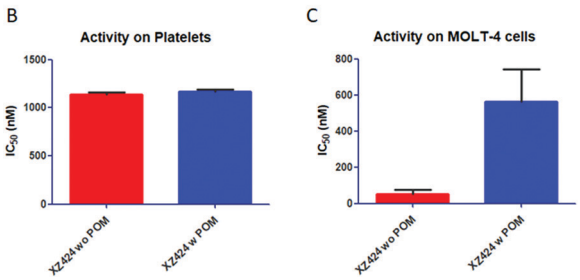


Fig. 3 Human platelet toxicity studies. (A) Platelets and MOLT-4 cells were cultured with BCL-X_L inhibitors or XZ424 for 24 h and 72 h, respectively. The selectivity for MOLT-4 over platelets was calculated. (B and C) The competitive cytotoxicity assay with 10 μM POM co-treatment in platelets and MOLT-4 cells. Data are presented as mean ± SD (n = 3 replicates) of two independent experiments.

of 51 nM and a 22-fold selectivity over platelets (Fig. 3A). The improved selectivity of XZ424 in comparison to A-1155463 is likely due to the different BCL-X_L degradation efficiency in MOLT-4 and platelets. The cytotoxicity of XZ424 to platelets most likely derived from BCL-X_L inhibition rather than degradation as pre-incubation of platelets with POM did not affect the cytotoxicity of XZ424 to platelets (Fig. 3B). Since XZ424 and A-1155463 had similar binding affinity to BCL-X_L, the largely reduced toxicity of XZ424 to platelets is likely due to a decrease in cell permeability compared to A-1155463. On the other hand,

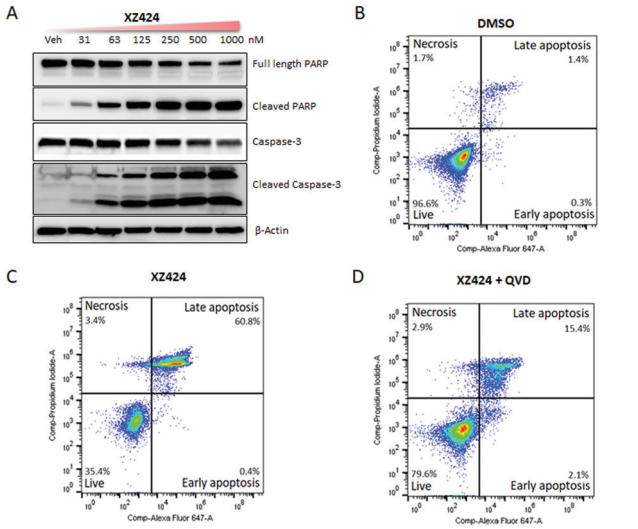


Fig. 4 Characterization of XZ424 mediated apoptosis in MOLT-4 cells. (A) Western blot analysis of PARP and caspase-3 after XZ424 treatment for 16 h. (B–D) Flow cytometry analysis of apoptosis using Annexin-V and PI staining. Cells were treated with DMSO and XZ424 (100 nM) for 48 h, and XZ424 (100 nM) significantly increased the percentage of apoptotic cells and QVD (10 μM) pre-treatment for 2 h inhibited the apoptosis induced by XZ424. Data are representative figures of two independent experiments.

pre-incubation of MOLT-4 with POM resulted in 11-fold reduction of the cytotoxicity of XZ424 (Fig. 3C), suggesting that the effect of XZ424 on MOLT-4 viability is largely derived from BCL-X_L degradation.

Western blot analysis showed that XZ424 dose-dependently increased the poly (ADP-ribose) polymerase (PARP) cleavage and caspase-3 cleavage in MOLT-4 cells (Fig. 4A), suggesting the apoptotic cell-death mechanism. Furthermore, to determine that XZ424 induces cell death through caspase mediated apoptosis, we did flow cytometry analysis of apoptosis using Annexin V and propidium iodide (PI) staining. We found that 100 nM of XZ424 treatment for 48 h significantly increased the percentage of Annexin-V-positive cells in MOLT-4 cells compared to the vehicle group (Fig. 4B and C), whereas pretreatment with 10 μM of the pan-caspase inhibitor Q-VD-OPh (QVD) for 2 h inhibited the XZ424 induced apoptosis, which confirms that XZ424 induces cell death through caspase-dependent apoptosis (Fig. 4D).

Taken together, we demonstrate the development of novel BCL-X_L-PROTACs that can degrade BCL-X_L selectively in MOLT-4 cells but not in platelets. Western blot analyses confirmed that the PROTACs induced BCL-X_L protein degradation in a dose- and time-dependent manner, and mediated by E3 ligase and UPS. Compared with conventional BCL-X_L inhibitors, XZ424 possesses a unique selectivity for MOLT-4 cells over platelets, suggesting that an improved therapeutic window can be achieved by the conversion of an inhibitor into a PROTAC. This study demonstrated an additional utility of the PROTAC technology. A similar strategy could be used to reduce on-target toxicities of other antitumor agents by taking advantage of the tissue-specific expression of E3 ligases. In addition, because XZ424 is a potent and selective BCL-X_L degrader, it might be a useful toolkit to

chemically dissect the functions of BCL-2 family proteins in multiple biological processes.

This work was supported in part by NIH grants R01CA211963, R01CA219836, R21CA223371, and S10OD021758.

Conflicts of interest

X. Z., D. T., X. L., P. Z., D. Z. and G. Z. are inventors of pending patent application(s) for the use of BCL-X_L PROTACs as anticancer agents. D. Z. and G. Z. are co-founders of and have equity of Dialectic Therapeutics, which develops BCL-X_L PROTACs to treat cancer.

Notes and references

1 P. E. Czabotar, G. Lessene, A. Strasser and J. M. Adams, *Nat. Rev. Mol. Cell Biol.*, 2014, **15**, 49–63.
2 F. H. Igney and P. H. Krammer, *Nat. Rev. Cancer*, 2002, **2**, 277–288.
3 J. M. Adams and S. Cory, *Oncogene*, 2007, **26**, 1324–1337.
4 J. Kale, E. J. Osterlund and D. W. Andrews, *Cell Death Differ.*, 2018, **25**, 65–80.
5 S. Thomas, B. A. Quinn, S. K. Das, R. Dash, L. Emdad, S. Dasgupta, X. Y. Wang, P. Dent, J. C. Reed, M. Pellecchia, D. Sarkar and P. B. Fisher, *Expert Opin. Ther. Targets*, 2013, **17**, 61–75.
6 A. R. Delbridge and A. Strasser, *Cell Death Differ.*, 2015, **22**, 1071–1080.
7 J. T. Opfermann, *FEBS J.*, 2016, **283**, 2661–2675.
8 A. R. Delbridge, S. Grabow, A. Strasser and D. L. Vaux, Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies, *Nat. Rev. Cancer*, 2016, **16**, 99–109.
9 J. L. Yap, L. Chen, M. E. Lanning and S. Fletcher, *J. Med. Chem.*, 2017, **60**, 821–838.
10 T. P. Garner, A. Lopez, D. E. Reyna, A. Z. Spitz and E. Gavathiotis, *Curr. Opin. Chem. Biol.*, 2017, **39**, 133–142.
11 W. Xiang, C.-Y. Yang and L. Bai, *OncoTargets Ther.*, 2018, **11**, 7301–7314.
12 E. D. Deeks, *Drugs*, 2016, **76**, 979–987.
13 C. D. DiNardo, K. Pratz, V. Pullarkat, B. A. Jonas, M. Arellano, P. S. Becker, O. Frankfurt, M. Konopleva, A. H. Wei, H. M. Kantarjian, T. Xu, W.-J. Hong, B. Chyla, J. Potluri, D. A. Pollyea and A. Letai, *Blood*, 2019, **133**, 7–17.
14 M. Vogler, *Adv. Med.*, 2014, 943648.
15 M. Wong, N. Tan, J. Zha, F. V. Peale, P. Yue, W. J. Fairbrother and L. D. Belmont, *Mol. Cancer Ther.*, 2012, **11**, 1026–1035.
16 J. D. Leverson, D. C. Phillips, M. J. Mitten, E. R. Boghaert, D. Diaz, S. K. Tahir, L. D. Belmont, P. Nimmer, Y. Xiao, X. M. Ma, K. N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T. J. Magoc, K. S. Vaidya, D. H. Albert, J. M. Tarrant, N. La, L. Wang, Z. F. Tao, M. D. Wendt, D. Sampath, S. H. Rosenberg, C. Tse, D. C. S. Huang, W. J. Fairbrother, S. W. Elmore and A. J. Souers, *Sci. Transl. Med.*, 2015, **7**, 279ra40.
17 C. Wang, S.-B. Huang, M.-C. Yang, Y.-T. Lin, I.-H. Chu, Y.-N. Shen, Y.-H. Chiu, S.-H. Hung, L. Kang, Y.-R. Hong and C.-H. Chen, *PLoS One*, 2016, **10**, e0120913.
18 J. Chang, Y. Wang, L. Shao, R. M. Laberge, M. Demaria, J. Campisi, K. Janakiraman, N. E. Sharpless, S. Ding, W. Feng, Y. Luo, X. Wang, N. Aykin-Burns, K. Krager, U. Ponnappan, M. Hauer-Jensen, A. Meng and D. Zhou, *Nat. Med.*, 2016, **22**, 78–83.
19 R. Yosef, N. Pilpel, R. Tokarsky-Amiel, A. Biran, Y. Ovadya, S. Cohen, E. Vadai, L. Dassa, E. Shahar, R. Condiotti, I. Ben-Porath and V. Krizhanovsky, *Nat. Commun.*, 2016, **7**, 11190.
20 Y. Zhu, E. J. Doornebal, T. Pirtskhalava, N. Giorgadze, M. Wentworth, H. Fuhrmann-Stroissnigg, L. J. Niedernhofer, P. D. Robbins, T. Tchkonia and J. L. Kirkland, *Aging*, 2017, **9**, 955–963.
21 Y. Ovadya and V. Krizhanovsky, *J. Clin. Invest.*, 2018, **128**, 1247–1254.
22 M. Demaria, M. N. O'Leary, J. Chang, L. Shao, S. Liu, F. Alimirah, K. Koenig, C. Le, N. Mitin, A. M. Deal, S. Alston, E. C. Academia, S. Kilmarx, A. Valdovinos, B. Wang, A. de Bruin, B. K. Kennedy, S. Melov, D. Zhou, N. E. Sharpless, H. Muss and J. Campisi, *Cancer Discovery*, 2017, **7**, 165–176.
23 K. D. Mason, M. R. Carpinelli, J. I. Fletcher, J. E. Collinge, A. A. Hilton, S. Ellis, P. N. Kelly, P. G. Ekert, D. Metcalf, A. W. Roberts, D. C. S. Huang and B. T. Kile, *Cell*, 2007, **128**, 1173–1186.
24 H. Zhang, P. M. Nimmer, S. K. Tahir, J. Chen, R. M. Fryer, K. R. Hahn, L. A. Iciek, S. J. Morgan, M. C. Nasarre, R. Nelson, L. C. Preusser, G. A. Reinhart, M. L. Smith, S. H. Rosenberg, S. W. Elmore and C. Tse, *Cell Death Differ.*, 2007, **14**, 943–951.
25 K. M. Sakamoto, K. B. Kim, A. Kumagai, F. Mercurio, C. M. Crews and R. J. Deshaies, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 8554–8559.
26 A. C. Lai and C. M. Crews, *Nat. Rev. Drug Discovery*, 2017, **16**, 101–114.
27 I. Churcher, *J. Med. Chem.*, 2018, **61**, 444–452.
28 Examples of published PROTACs: (*a*) G. E. Winter, D. L. Buckley, J. Paulk, J. M. Roberts, A. Souza, S. Dhe-Paganon and J. E. Bradner, *Science*, 2015, **348**, 1376–1381; (*b*) A. C. Lai, M. Toure, D. Hellerschmied, J. Salami, S. Jaime-Figueroa, E. Ko, J. Hines and C. M. Crews, *Angew. Chem., Int. Ed.*, 2016, **55**, 807–810; (*c*) D. Remillard, D. L. Buckley, J. Paulk, G. L. Brien, M. Sonnett, H. S. Seo, S. Dastjerdi, M. Wuhr, S. Dhe-Paganon, S. A. Armstrong and J. E. Bradner, *Angew. Chem., Int. Ed.*, 2017, **56**, 5738–5743; (*d*) C. M. Robb, J. I. Contreras, S. Kour, M. A. Taylor, M. Abid, Y. A. Sonawane, M. Zahid, D. J. Murry, A. Natarajan and S. Rana, *Chem. Commun.*, 2017, **53**, 7577–7580; (*e*) P. M. Cromm, K. T. G. Samarasinghe, J. Hines and C. M. Crews, *J. Am. Chem. Soc.*, 2018, **140**, 17019–17026; (*f*) B. Zhou, J. Hu, F. Xu, Z. Chen, L. Bai, E. Fernandez-Salas, M. Lin, L. Liu, C.-Y. Yang, Y. Zhao, D. McEachern, S. Przybranowski, B. Wen, D. Sun and S. Wang, *J. Med. Chem.*, 2018, **61**, 462–481; (*g*) M. Schiedel, D. Herp, S. Hammelmann, S. Swyter, A. Lehotzky, D. Robaa, J. Olah, J. Ovadi, W. Sippl and M. Jung, *J. Med. Chem.*, 2018, **61**, 482–491; (*h*) Q. Zhao, T. Lan, S. Su and Y. Rao, *Chem. Commun.*, 2019, **55**, 369–372; (*i*) B. Zhang and K. Burgess, *Chem. Commun.*, 2019, **55**, 2704–2707; (*j*) Y. Li, J. Yang, A. Aguilar, D. McEachern, S. Przybranowski, L. Liu, C.-Y. Yang, M. Wang, X. Han and S. Wang, *J. Med. Chem.*, 2019, **62**, 448–466; (*k*) X. Han, C. Wang, C. Qin, W. Xiang, E. Fernandez-Salas, C.-Y. Yang, M. Wang, L. Zhao, T. Xu, K. Chinnaswamy, J. Delproposto, J. Stuckey and S. Wang, *J. Med. Chem.*, 2019, **62**, 941–964; (*l*) J. Hu, B. Hu, M. Wang, F. Xu, B. Miao, C.-Y. Yang, M. Wang, Z. Liu, D. F. Hayes, K. Chinnaswamy, J. Delproposto, J. Stuckey and S. Wang, *J. Med. Chem.*, 2019, **62**, 1420–1442; (*m*) J. W. Papatzimas, E. Gorobets, R. Maity, M. I. Muniyat, J. L. MacCallum, P. Neri, N. J. Bahlis and D. J. Derksen, *J. Med. Chem.*, 2019, **62**, 5522–5540; (*n*) Z. Wang, N. He, Z. Guo, C. Niu, T. Song, Y. Guo, K. Cao, A. Wang, J. Zhu, X. Zhang and Z. Zhang, *J. Med. Chem.*, 2019, **62**, 8152–8163; (*o*) V. Zoppi, S. J. Hughes, C. Maniaci, A. Testa, T. Gmaschitz, C. Wieshofer, M. Koegl, K. M. Riching, D. L. Daniels, A. Spallarossa and A. Ciulli, *J. Med. Chem.*, 2019, **62**, 699–726; (*p*) W. Farnaby, M. Koegl, M. J. Roy, C. Whitworth, E. Diers, N. Trainor, D. Zollman, S. Steurer, J. Karolyi-Oezguer, C. Riedmueller, T. Gmaschitz, J. Wachter, C. Dank, M. Galant, B. Sharps, K. Rumpel, E. Traxler, T. Gerstberger, R. Schnitzer, O. Petermann, P. Greb, H. Weinstabl, G. Bader, A. Zoephel, A. Weiss-Puxbaum, K. Ehrenhöfer-Wölfer, S. Wöhrle, G. Boehmelt, J. Rinnenthal, H. Arnhof, N. Wiechens, M.-Y. Wu, T. Owen-Hughes, P. Ettmayer, M. Pearson, D. B. McConnell and A. Ciulli, *Nat. Chem. Biol.*, 2019, **15**, 672–680; (*q*) C. P. Tinworth, H. Lithgow, L. Dittus, Z. I. Bassi, S. E. Hughes, M. Muelbaier, H. Dai, I. E. D. Smith, W. J. Kerr, G. A. Burley, M. Bantscheff and J. D. Harling, *ACS Chem. Biol.*, 2019, **14**, 342–347.
29 P. F. Bray, S. E. McKenzie, L. C. Edelstein, S. Nagalla, K. Delgrosso, A. Ertel, J. Kupper, Y. Jing, E. Londin, P. Loher, H. W. Chen, P. Fortina and I. Rigoutsos, *BMC Genomics*, 2013, **14**, 1.
30 A. Kissopoulou, J. Jonasson, T. L. Lindahl and A. Osman, *PLoS One*, 2013, **8**, e81809.
31 Z.-F. Tao, L. Hasvold, L. Wang, X. Wang, A. M. Petros, C. H. Park, E. R. Boghaert, N. D. Catron, J. Chen and P. M. Colman, *ACS Med. Chem. Lett.*, 2014, **5**, 1088–1093.
32 G. Lessene, P. E. Czabotar, B. E. Sleebs, K. Zobel, K. N. Lowes, J. M. Adams, J. B. Baell, P. M. Colman, K. Deshayes, W. J. Fairbrother, J. A. Flygare, P. Gibbons, W. J. A. Kersten, S. Kulasegaram, R. Moss, J. P. Parisot, B. J. Smith, I. P. Street, H. Yang, D. C. S. Huang and K. G. Watson, *Nat. Chem. Biol.*, 2013, **9**, 390–397.
33 J. D. Leverson, D. C. Phillips, M. J. Mitten, E. R. Boghaert, D. Diaz, S. K. Tahir, L. D. Belmont, P. Nimmer, Y. Xiao, X. M. Ma, K. N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T. J. Magoc, K. S. Vaidya, D. H. Albert, J. M. Tarrant, N. La, L. Wang, Z. F. Tao, M. D. Wendt, D. Sampath, S. H. Rosenberg, C. Tse, D. C. S. Huang, W. J. Fairbrother, S. W. Elmore and A. J. Souers, *Sci. Transl. Med.*, 2015, **7**, 279ra40.
34 L. Lu, Y. Qian, M. Altieri, H. Dong, J. Wang, K. Raina, J. Hines, J. D. Winkler, A. P. Crew, K. Coleman and C. M. Crews, *Chem. Biol.*, 2015, **22**, 755–763.

