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Rationally Designed Covalent BCL6 Inhibitor That Targets a Tyrosine Residue in the Homodimer Interface

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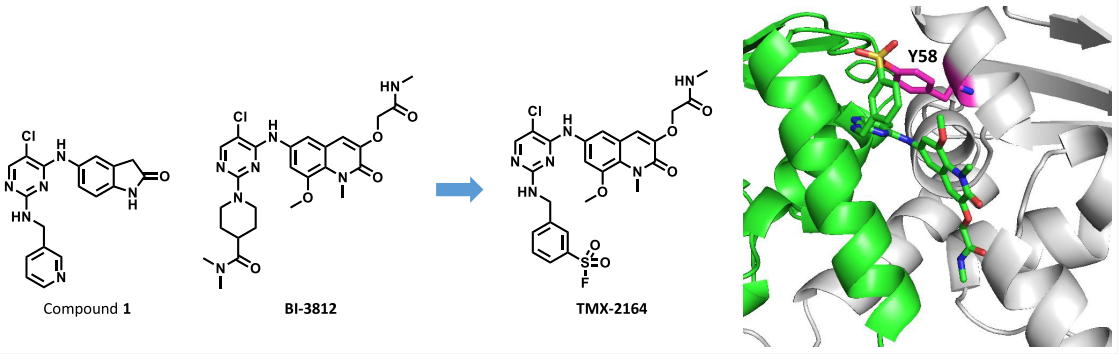

ABSTRACT: B-cell lymphoma 6 (BCL6) is a transcriptional repressor frequently deregulated in lymphoid malignancies. BCL6 engages with number of corepressors, and these protein−protein interactions are being explored as a strategy for drug development. Here, we report the development of an irreversible BCL6 inhibitor **TMX-2164** that uses a sulfonyl fluoride to covalently react with the hydroxyl group of Tyrosine 58 located in the lateral groove. **TMX-2164** exhibits significantly improved inhibitory activity compared to that of its reversible parental compound and displays sustained target engagement and antiproliferative activity in cells. **TMX-2164** therefore represents an example of a tyrosine-directed covalent inhibitor of BCL6 which demonstrates advantages relative to reversible targeting.

KEYWORDS: *BCL6, Protein−protein interactions, Covalent inhibitor, Sulfonyl fluoride*

B-cell lymphoma 6 (BCL6) is an essential protein for the formation and maintenance of the germinal center (GC) during the humoral immune response[1−3] and is therefore important for B-cell development. GCs are transient and dynamic substructures of lymph nodes that are dedicated to the selection of B-cells expressing high-affinity antibodies in response to T-cell-dependent antigen stimulation.[4,5] Within GCs, BCL6 represses the expression of genes that are required to sustain mutagenic activity without activating the DNA damage response or apoptosis,[6−8] ensuring that GC B-cells undergo immunoglobulin affinity maturation.[6,9] BCL6 also represses genes required for exit from the GC cycle, ensuring that GC B-cells have sufficient time to acquire somatic hypermutation of their immunoglobulin genes.[6] The activity of BCL6 must be switched off once GC B-cells acquire appropriate affinity for the inciting antigen, which allows their differentiation into memory B-cells and plasma cells.[10] However, deregulation of BCL6 results in a highly proliferative GC phenotype with accumulating DNA damage, eventually leading to malignant transformation of B-cells.[11,12] Overexpression of BCL6 has been found in diffuse large B-cell lymphoma (DLBCL), nodular lymphocyte predominant Hodgkin lymphomas (NLPHL), and follicular lymphoma (FL). Transgenic IμHA-BCL6 mice developed DLBCL-like tumors,[11] and genetic knockdown of BCL6 with shRNA induced lethality in DLBCL cell lines,[13] suggesting that BCL6 represents a therapeutic target for cancer treatment.

Mechanistically, BCL6 regulates gene transcription through recruiting corepressor proteins such as BCL6 corepressor (BCOR). BCL6 functions as an obligatory dimer, and this dimerization, which is mediated by the BTB domain, creates two identical lateral grooves that engage binding partners.[14,15] The lateral groove also is a hotspot for small molecule inhibitor binding, such as compound **79-6** which was the first reported small molecule inhibitor of BCL6.[16] Further optimization efforts resulted in inhibitors with improved activity, including

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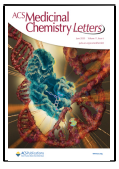

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compound **1**,[17] **BI-3812**,[18] and macrocyclic compound **2**.[19] All of these compounds employ a reversible mode of binding. Recently, Sameshima et al. reported an irreversible BCL6 inhibitor **BCL-i** that targets cysteine 53 (Cys53) located within a cavity found in the BTB domain.[20] In addition to these compounds that bind BCL6 and inhibit protein−protein interactions, more recently, several compounds that induce BCL6 degradation have also been reported. For example, **BI-3802** was serendipitously identified as a BCL6 degrader,[18] whereas heterobifunctional molecule **3** that hijacks E3 ligase cereblon (CRBN) to induce proteasome-mediated degradation of BCL6 was rationally designed[21] (Figure 1). Here, we report

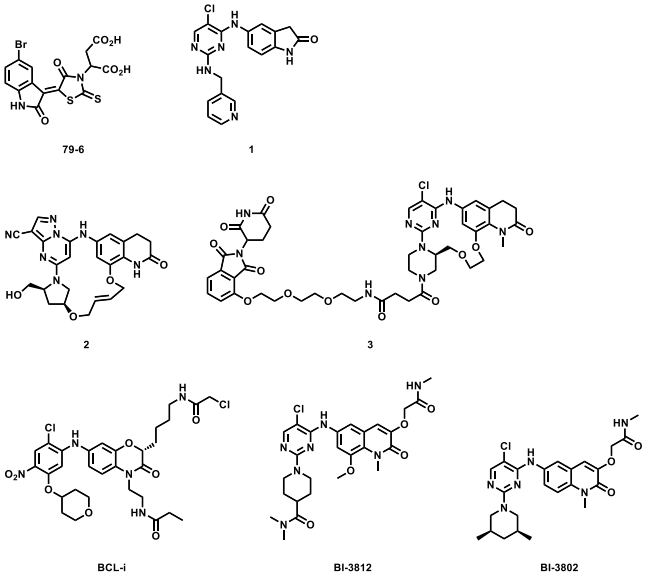

Figure 1. Chemical structures of published BCL6 inhibitors and BCL6 degraders.

the rational design of **TMX-2164**, a covalent inhibitor that targets tyrosine 58 (Tyr58) located in the lateral groove of BCL6. We validate that **TMX-2164** covalently binds Tyr58 using a range of *in vitro* assays and confirm that it engages BCL6 in cells. **TMX-2164** exhibits single digit micromolar antiproliferative activity in DLBCL cells and outperforms the reversible parental compound.

The cocrystal structure of compound **1** with BCL6 (PDB: 5X4Q) (Figure 2) provided the basis for the design of a covalent inhibitor. Tyr58 of BCL6 is favorably located within 4.2 Å as measured by the distance between Tyr58 −OH group

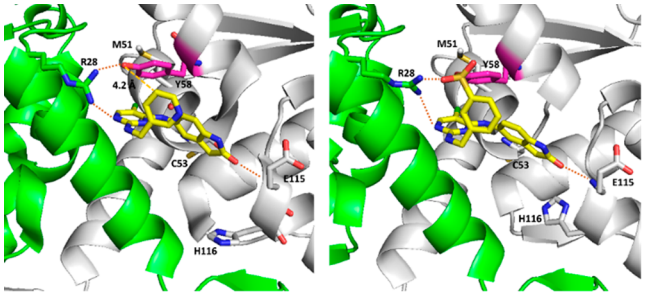

Figure 2. (Left) Co-crystal structure of compound **1** in complex with BCL6BTB (PDB code: 5X4Q). The side chain of Tyr58 (colored pink) is located near the 5-position of the pyridine ring (4.2 Å). (Right) Docking-based structure of **TMX-1120** bound to BCL6^{BTB}.

and meta-carbon of pyridine ring. We also noted that the guanidinium group of Arg28 is within 2.4 Å of the Tyr58 −OH and likely to facilitate deprotonation of the Tyr58 −OH, as was previously observed in reports that describe covalent targeting of tyrosines.[22−24] Additionally, we observed that the pyridine moiety was solvent-exposed and disordered, suggesting that there should be no steric hindrance to reach the phenol group. Taken together, we reasoned that introducing sulfonyl fluoride, a previously used electrophilic warhead for targeting tyrosines[22,25−27] to compound **1**, would potentially yield a covalent inhibitor for BCL6. Thus, compound **TMX-1120** (Figure 3) was designed and docked into the BCL6 crystal

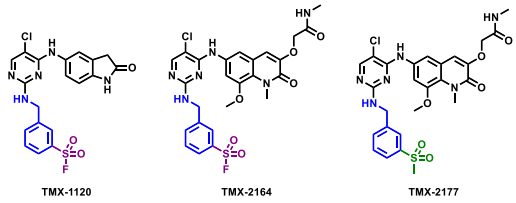

Figure 3. Chemical structures of covalent BCL6 inhibitors and nonreactive methylsulfonyl-bearing control compound used in this study.

structure. As shown in Figure 2, **TMX-1120** is predicted to bind to BCL6^{BTB} in a binding mode similar to that of compound **1**. The carbonyl oxygen of the cyclic amide moiety was observed to interact with Glu115; the linker nitrogen formed a hydrogen bond with the main-chain oxygen of Met51, and one of the pyrimidine nitrogen atoms interacted with Arg28. Importantly, the original interaction of Tyr58 −OH with Arg28 was replaced by the oxygen of the sulfonyl moiety, strongly suggesting the potential for covalent bond formation.

To validate that **TMX-1120** binds and inhibits BCL6, we performed a series of *in vitro* experiments. To assess the binding, we used a TR-FRET-based biochemical assay where a BodipyFL-labeled BCOR peptide was displaced from BCL6 upon titration with increasing concentrations of **TMX-1120**. At a fixed time point of 30 min, **TMX-1120** exhibited an IC_{50} of 251 nM and, in contrast, the reversible compound **1** demonstrated an IC_{50} of only 2699 nM (Figure 4). To examine

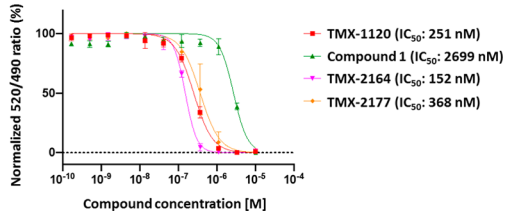

Figure 4. BCL-6 corepressor peptide displacement assay.

whether the improved activity was due to the covalent bond formation with Tyr58, we analyzed recombinant expressed BCL6 protein by LC-MS after incubation with a 10-fold molar excess of **TMX-1120** for 2 h at room temperature. We observed a mass shift consistent with the stoichiometric modification of the protein by **TMX-1120** (accompanied by the loss of HF as expected for the reaction between Tyr −OH with the sulfonyl fluoride warhead, Figure 5A). To determine the site of modification, the labeled protein was digested with trypsin, and peptides were analyzed by capillary electro-

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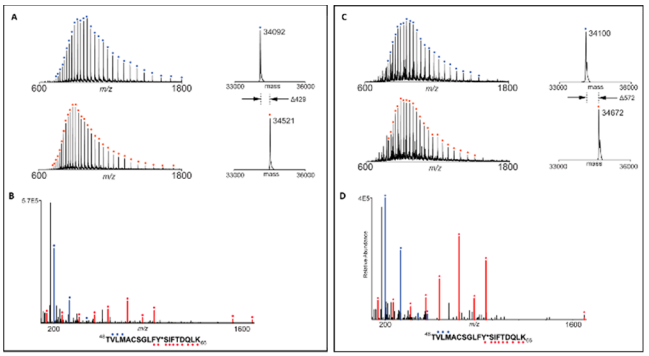


Figure 5. Mass spectrometry analysis reveals that **TMX-1120** and **TMX-2164** react with BCL6 protein at Tyr58. (A) Mass spectra (left) and zero-charge mass spectra (right) of BCL6 protein treated with DMSO (top) or 10-fold molar excess of **TMX-1120** (bottom) for 2 h at room temperature. The observed mass shift of 429 Da is consistent with covalent addition of a single molecule of **TMX-1120** (with loss of HF). (B) CE-MS/MS spectrum of tryptic BCL6 peptide (residues 48−66) with Y58 modified by **TMX-1120**. Ions of type b and y are indicated with blue and red glyphs, respectively. Y*, **TMX-1120**-modified tyrosine. Panels C and D are the same as panels A and B except with **TMX-2164** (mass shift of 572 Da is consistent with **TMX-2164** with loss of HF).

phoresis-mass spectrometry (CE-MS). Database search revealed exclusive modification of BCL6 Tyr58 (Figure 5B). Taken together, our mass spectrometry-based analysis confirmed that **TMX-1120** forms a covalent adduct with BCL6 by reacting with Tyr58. Next, we took advantage of the well-established structure−activity relationship (SAR) for this scaffold to hybridize the sulfonyl fluoride warhead with **BI-3812**, which is a more potent reversible BCL6 inhibitor compared to compound **1**. This resulted in the design of compound **TMX-2164** and its reversible counterpart compound **TMX-2177** (Figure 3). After confirming the covalent binding of **TMX-2164** with BCL6 by mass spectrometry (Figure 5C and D), we measured the affinity of **TMX-2164** using the TR-FRET-based displacement assay described above. As shown in Figure 4, **TMX-2164** displayed an IC_{50} of 152 nM. The reversible counterpart, **TMX-2177**, showed a comparable activity, in agreement with a stronger reversible binding with BCL6 compared to compound **1**.

To determine whether our compounds indeed function as covalent inhibitors in cells, we developed a fluorescence-activated cell sorting (FACS)-based reporter assay in HEK293T-cells, which allows quantification of BCL6 levels by monitoring the eGFP to mCherry ratio (Figure 6A).[28] Cells were treated with either covalent inhibitors **TMX-1120** and **TMX-2164** or reversible compounds **1** and **TMX-2177** at a fixed concentration of 5 μM for 30 h, and excess compound was then washed away. The cells were then exposed to BCL6 degrader **BI-3802** at various concentrations, and BCL6 protein levels were monitored using the ratio of eGFP over mCherry. As shown in Figure 6B, while **TMX-1120** and **TMX-2164** rescued BCL6 from **BI-3802**-induced degradation, compound **1** and **TMX-2177** did not, demonstrating a prolonged occupancy on BCL6 protein by a covalent inhibitor. Furthermore, we evaluated the antiproliferative activity of these covalent inhibitors in SU-DHL-4 cells (Figure S1), a DLBCL model system. With 5-day treatment, **TMX-2164** showed the most effective cell growth inhibition with single digit micromolar GI_{50} (Figure 6C). However, all the reversible

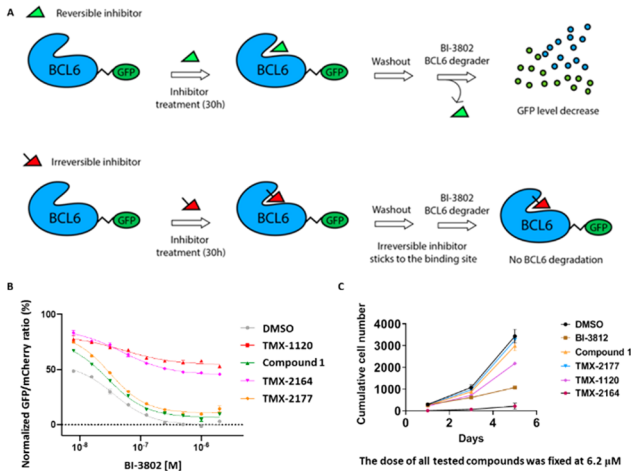


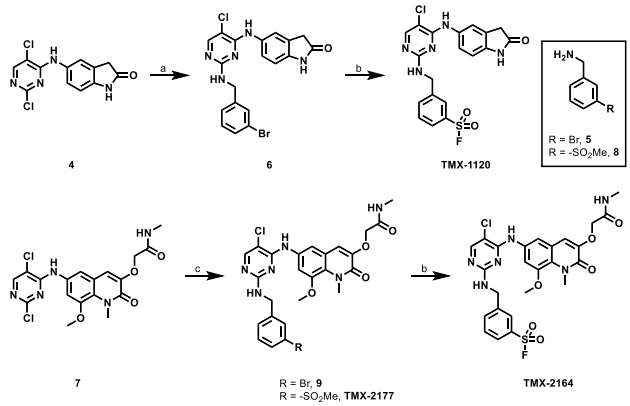
Figure 6. (A) A schematic cartoon describing the washout assay in BCL6-eGFP reporter cells. (B) The BCL6 target engagement for the covalent (irreversible) and reversible BCL6 inhibitors in HEK293T-cells. (C) Antiproliferative effects of the covalent and reversible BCL6 inhibitors after 1-day, 3-day, and 5-day treatment in SU-DHL-4 cells at a fixed dose of 6.2 μM. See also Figure S1 for additional information.

compounds, including **TMX-2177** that had a biochemical activity comparable to that of **TMX-2164**, significantly lost ability to protect BCL6 from degradation as well as antiproliferative activity, suggesting that a covalent inhibitor may be superior to a reversible one under the conditions tested.

EXPERIMENTAL SECTION

Synthetic methods for compounds **TMX-1120**, **TMX-2164**, and **TMX-2177** are shown in Scheme 1. 5-((2,5-Dichloropyrimidin-4-

Scheme 1. Synthesis of Compounds TMX-1120, TMX-2164, and TMX-2177[a]



[a]Reagents and conditions: (a) **5**, TEA, DMSO, 95 °C, 67%; (b) DABSO, $PdCl_2$(AmPhos)$_2$, TEA, *i*-PrOH, 75 °C; then NFSI, rt, 6% (for **TMX-1120**) or 7% (for **TMX-2164**); (c) **5** or **8**, TEA, DMF, MeOH, 70 °C, 59% (for **9**) or 9% (for **TMX-2177**).

yl)amino)indolin-2-one (**4**) and 2-[(6-((2,5-dichloropyrimidin-4-yl)-amino)-8-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)oxy]-*N*-methylacetamide (**7**) were used as staring materials. *N*-(3-Bromobenzyl)pyrimidin-2-amines **6** and **9** were then generated through aromatic nucleophilic substitution of 2-chloropyrimidine **4** or **7**, respectively, by using benzylamine as the nucleophile. Next, a one-pot palladium-catalyzed synthesis of sulfonyl fluoride from aryl

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bromide, an elegant methodology recently developed by the Willis's group,[29] was applied to successfully generate both **TMX-1120** and **TMX-2164**, albeit in low yields.

In summary, here we report the development of a covalent BCL6 inhibitor, **TMX-1120**. We used a structure-guided rational design strategy that introduced a tyrosine-directed covalent warhead, sulfonyl fluoride, to a reversible BCL6 inhibitor **1**. We validated that **TMX-1120** forms a covalent bond with Tyr58 on BCL6 and sustainably inhibits BCL6 in cellular context after washout, whereas the reversible inhibitors required continuous exposure. Further optimization resulted in the development of **TMX-2164**, which showed an improved antiproliferation activity in SU-DHL-4 cells, a DLBCL model system. Thus, the covalent inhibitor **TMX-2164** represents a new example of tyrosine-directed covalent targeting strategy applied to BCL6 with advantages over reversible targeting.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00111.

Details of biological assays, synthetic procedures, and analytical data (PDF)

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Author Contributions

M.T., T.Z., and N.S.G. generated the concept. M.T. designed and synthesized compounds, analyzed and interpreted data, and prepared the manuscript. S.B.F. and J.A.M. carried out mass spectrum labeling and trypsin digestion. H.Y. and E.S.F. expressed and purified protein constructs and performed TR-FRET-based biochemical assay and washout assay. J.C. built the docking model. J.Z. performed cell proliferation assay. T.Z. and N.S.G. provided strategic direction and coordinated manuscript preparation. All authors discussed the results and commented on the manuscript. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): Eric S. Fischer is a founder, science advisory board (SAB) member, and equity holder in Civetta Therapeutics and an equity holder and SAB member in C4 Therapeutics. The Fischer lab receives or has received research funding from Astellas, Novartis, Voronoi, and Deerfield. Jarrod A. Marto serves on the SAB of 908 Devices and has received sponsored research support from AstraZeneca and Vertex. Nathanael S. Gray is a founder, SAB member, and equity holder in Gatekeeper, Syros, Petra, C4, B2S, Aduro, and Soltego. The Gray lab receives or has received research funding from Novartis, Takeda, Astellas, Taiho, Janssen, Kinogen, Voronoi, Her2llc, Deerfield, and Sanofi.

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■ ABBREVIATIONS

BCL6 B-cell lymphoma 6; TEA triethylamine; DMSO dimethyl sulfoxide; DABSO 1,4-diazabicyclo[2.2.2]octane bis(sulfur dioxide) adduct; AmPhos di*tert*-butyl(4-dimethylaminophenyl)phosphine; NFSI *N*-fluorobenzenesulfonimide; *i*-PrOH isopropanol; rt room temperature; DMF dimethylformamide

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