HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2024 November 25.

Published in final edited form as:

J Med Chem. 2023 December 28; 66(24): 16515–16545. doi:10.1021/acs.jmedchem.3c01130.

Small Molecule Inhibitors Targeting the "Undruggable" Survivin: The Past, Present, and Future from a Medicinal Chemist's Perspective

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Abstract

Survivin, a homodimeric protein and a member of the IAP family, plays a vital function in cell survival and cycle progression by interacting with various proteins and complexes. Its expression is upregulated in cancers but not detectable in normal tissues. Thus, it has been regarded and validated as an ideal cancer target. However, survivin is "undruggable" due to its lack of enzymatic activities or active sites for small molecules to bind/inhibit. Academic and industrial laboratories have explored different strategies to overcome this hurdle over the past two decades, with some compounds advanced into clinical testing. These strategies include inhibiting survivin expression, its interaction with binding partners and homodimerization. Here, we provide comprehensive analyses of these strategies and perspective on different small molecule survivin inhibitors to help drug discovery targeting "undruggable" proteins in general and survivin specifically with a true survivin inhibitor that will prevail in the foreseeable future.

Graphical Abstract

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.

1. INTRODUCTION

1.1. Inhibitor of Apoptosis Proteins.

Inhibitor of apoptosis proteins (IAPs) are major antiapoptotic regulators that inhibit cysteine proteases and caspases.[1,2] Humans have eight IAPs, including neuronal apoptosis inhibitory protein (NAIP), cellular IAP 1 (cIAP1), cellular IAP 2 (cIAP2), X-linked IAP (XIAP), survivin, Baculovirus IAP Repeat (BIR)-containing ubiquitin-conjugating enzyme (BRUCE), melanoma IAP (ML-IAP or Livin), and IAP-like Protein 2 (ILP2) (Figure 1A and Table 1).[3] The IAPs are characterized by the presence of one to three BIR domains and, hence, their corresponding genes are named BIRC (BIR containing) 1 through 8 (Figure 1A and Table 1). Five IAPs, including XIAP, cIAP1/2, Livin, and ILP2, contain a Really Interesting New Gene (RING) domain. While BRUCE has a ubiquitin-conjugating (UBC) domain, cIAP1/2, XIAP and ILP2 contain a ubiquitin-associated domain (UBA). In addition, NAIP has two leucine-rich repeat (LRR) domains, and one nucleotide-binding and oligomerization (NACHT) domain, and cIAP1/2 also contains a caspase recruitment domain (CARD).[4] Although the BIR domain was thought to mediate the binding of IAPs to caspases, XIAP is the only IAP that has been shown to interact with and inhibit caspase activity and ubiquitinate them to promote their degradation.[5,6]

Recent findings have shown that IAPs could serve as potential biomarkers and, more importantly, as therapeutic targets in cancer treatment due to their dysregulation and overexpression in cancer and association with poor prognosis and drug resistance.[22,23] Generally, IAPs exert their functions by interacting with vital players of both the intrinsic and extrinsic apoptosis pathways to thwart cell death, including (1) the initiators such as cytochrome *c*, and the apoptosome consisting of cytochrome *c*, apoptotic peptidase activating factor 1 (Apaf-1) and pro-caspase 9, and (2) the signal transducers/executioners such as caspase 3/8/9.[24,25] Because of their functions in antiapoptosis and association with cancer, small molecule inhibitors targeting IAPs have been identified, with some under clinical study as summarized in Table 1.

1.2. Survivin.

Human survivin, the smallest member of the IAP family with a molecular weight of 16.5 kDa, consists of 142 amino acid residues and exists as a homodimer with a single BIR domain in the N-terminus, a zinc-finger fold, and an extended C-terminal helical coiled coil (Figure 1B).[26] Unlike other IAPs, which are found mainly in the cytosol and to a lesser extent in the nucleus,[27] survivin is present in multiple subcellular locations, including the nucleus, the cytosol, mitochondria, and extracellular exosomes.[28–30] However, similar to other IAPs, survivin has been shown to inhibit both extrinsic and intrinsic apoptosis pathways in cell lines and animal models.[31,32]

Several mechanisms have been proposed to delineate how survivin inhibits apoptosis (Figure 1C). It may prevent apoptosis induction by interacting with pro-apoptotic proteins to inhibit caspase activation. For example, the second mitochondria-derived activator of caspases/direct IAP-binding protein with low pI (SMAC/DIABLO), a pro-apoptotic protein, promotes cytochrome *c*-dependent apoptosis by binding to and neutralizing IAPs following release from mitochondria and activating caspase 3.[33] Survivin binds directly to SMAC/DIABLO in mitochondria and prevents it from being released into the cytosol to activate apoptosis.[34] Indeed, disrupting the interaction between survivin and SMAC/DIABLO induces apoptosis.[35]

While survivin has been suggested to bind directly to and inhibit caspases 3 and 7 using recombinant proteins,[36] one study showed that the chemically synthesized survivin failed to inhibit caspase 3 activity even at 10 μM.[37] It is unclear if the chemically synthesized survivin is functional; however, another study using mouse and human survivin also failed to show caspase 3 inhibition.[38] Survivin has also been thought to bind to caspase 9 and inhibit its activation.[39] However, it was later shown that survivin alone does not bind to caspase 9 but rather works cooperatively with hepatitis B X-interacting protein (HBXIP) to bind to pro-caspase 9 and inhibits its activation.[40] Survivin may also form the survivin-XIAP complex that stabilizes XIAP against ubiquitination and proteasomal destruction to synergize the inhibition of caspase 9.[41] It appears that multiple BIR domains are required for caspase binding, evidenced by cIAP1/2[42] and XIAP.[43,44] Thus, it is more likely that survivin inhibits apoptosis by serving as a protein–protein interaction coordinator among IAPs. In addition, survivin also acts as a critical mitotic regulator in the nucleus, playing an essential role in proper mitosis and cytokinesis by interacting with the chromosomal passenger complex (CPC).[25,45–47]

Despite the progress in understanding the mechanism of survivin function, its detailed action modes at molecular and atomic levels remain largely unclear, including the functions of each domain and the critical residues in coordinating with or binding to its partners, and the signaling pathways orchestrated by survivin. While further studies are required in this aspect to assist drug discovery targeting survivin, inhibitors generated thus far may also be used as chemical probes to dissect the biology of survivin function at the molecular and atomic levels.

1.3. Survivin as a Therapeutic Target.

Survivin is overexpressed in almost all human cancers,[48,49] but undetectable in most normal adult tissues.[50] The high survivin expression level is predictive of poor clinical outcome and is associated with tumor relapse.[51,52] Survivin has been consistently demonstrated to be a pivotal contributor to radiotherapy and chemotherapy resistance due to its role in antiapoptosis.[53–55] It has been shown that survivin knockdown in glioma and breast cancer cells decreased angiogenesis.[56,57] Silencing or downregulating survivin expression using ribozyme or siRNAs also led to increased cell death and chemosensitivity.[58,59] These findings together indicate that survivin is clinically relevant and perhaps an ideal target for anticancer drug discovery.[25]

2. MEDICINAL CHEMISTRY OF SMALL MOLECULE SURVIVIN INHIBITORS

It has been very challenging to target survivin, which has been historically considered "undruggable" due to the lack of known enzymatic activities or active sites to be targeted by small molecule compounds.[60] To bypass this obstacle, several strategies have been tested. The first one was to inhibit transcription of the survivin gene. The small molecule inhibitors identified to do so include compounds **1** (YM155), **2** (EM-1421), and **3** (FL118) (Figure 2A). As discussed above, survivin exerts most of its functions by interacting with other proteins. Thus, inhibiting survivin interaction with these proteins using small molecules has been attempted, with representative inhibitors including compound **4** (UC-112) and its analogs **5** (MX106) and **6** (12b). Finally, survivin exists and functions as a homodimer and, thus, inhibiting its homodimerization to induce its degradation in the proteasome has also exhibited some success, as exemplified by compounds **7** (LQZ-7F), **8** (7F1), **9** (LQZ-7I), **10** (LLP3), and **11** (4a′). These inhibitors (Figure 2A) are at different stages of development, with most in preclinical studies for their efficacies, safety, pharmacodynamics (PD), and pharmacokinetics (PK) profiles, and only **1** and **2** advanced into clinical testing (Table 2).[61,62]

However, because **1** and **2** do not act directly on the survivin protein but inhibit its upstream transcription factors (see below), they are not survivin inhibitors per se and readers are advised to be cautious when citing these compounds as survivin inhibitors. Nevertheless, because these compounds inhibit survivin expression, selectively target cancer cells, and have been studied in detail and are being tested in clinical trials without major toxicity, critically examining these compounds may provide valuable perspectives on developing inhibitors to selectively inhibit survivin expression.

In this perspective, we will focus on the medicinal chemistry of the well-characterized small molecule compounds by examining their chemical structures, pharmacological properties, and provide insights and perspectives on their future improvements or developments. Additionally, we will critically discuss the different strategies in targeting survivin and place these in perspective for future investigations. Biologics, including dominant negative survivin and antisense oligonucleotides as well as vaccines, are out of scope and, thus, are excluded from discussion. In addition to the small molecule inhibitors shown in Figure 2A that have been the subject of extensive medicinal chemistry studies and analyses, a few others that are nonselective and for which there is a limited understanding of mechanism of action and lack of associated pharmacological or medicinal chemistry studies, are excluded. These inhibitors include compounds **12** (indinavir),[63] **13** (nelfinavir),[64] **14** (GDP366),[65] **15** (withanone),[66] **16** (S12),[67] **17** (SF002–96–1),[68] **18** (ZINC2243688), and **19** (ZINC057885)[69] (Figure 2B).

Here, we attempt to interpret the drug-likeness of the prominent inhibitors using the well-defined Lipinski's Rule of Five (RO5) as a guide,[86] and by analyzing their drug design, structure–activity relationship (SAR), key medicinal chemistry and pharmacological properties from published work. Note that RO5 is used with caution as these rules were established based on the orally administered drugs approved prior to 1997[86] and the drug-

like properties are constantly changing with a growing number of approved drugs that do not strictly follow these rules.[87]

2.1. Compound 1 (YM155).

Compound **1** (sepantronium bromide) is an ammonium salt with a positively charged imidazolium ring (Figure 3A), termed an onium compound. It was first identified in 2007 by Astellas Pharma using a high throughput screening (HTS) assay of survivin promoter-driven luciferase reporter expression aiming for small molecule inhibitors that may bind to the promoter sequence of the survivin gene and inhibit its expression.[74] Compound **1** indeed suppressed survivin expression at both mRNA and protein levels in various cancer cells with nanomolar potency after a 6-h treatment.[88,89] It had favorable selective profiles over other IAPs including cIAP2 and XIAP, and had no effect on the expression of antiapoptotic Bcl-2-related genes such as Bcl-2, Bcl-xL, and Bad.[74] More importantly, **1** preferably accumulates in tumors, with ~20-fold higher concentration than that in plasma.[74]

2.1.1. Biological Properties.—Compound 1 inhibited cancer cell proliferation with an average IC_{50} of ~15 nM in 119 human cancer cell lines, and an IC_{50} range of 0.49 nM to 248 nM in a series of drug-resistant neuroblastoma and pancreatic cancer cell lines, suggesting that it is a highly potent anticancer agent.[74,90,91] Although **1** is toxic to normal cells such as primary human astrocytes, WA09 human ES cell-derived neural progenitor cells, and primary human lung fibroblasts cells,[70] with IC_{50} values ranging from 5 to 90 nM, it requires much lower concentrations (~1–100 fold) to kill glioblastoma (GBM) cells, with IC_{50} values ranging from 0.78 to 4.5 nM, suggesting the potential for a safety margin.[70] In orthotopic xenograft PC-3 prostate tumor and ectopic xenograft tumors of breast, lung, and bladder as well as melanoma, administration of **1** via a continuous 3- or 7-day infusion significantly suppressed the tumor growth, without significant toxicity as indicated by low body weight loss in the animals.[74,91]

Importantly, **1** is well tolerated by humans, with a maximum tolerated dose (MTD) of 4.8 mg/m^2.[92] However, limited or modest efficacy at best was observed in clinical trials against various solid tumors. No improvement in response rate was observed for nonsmall cell lung cancer (NSCLC) patients in two phase II trials of **1**, either as a single agent or in combination with carboplatin or paclitaxel.[15,93] In another phase II clinical trial, modest efficacy was observed with 25% of castration-resistant prostate cancer (CRPC) patients displaying prolonged stable disease.[94] In a study testing **1** in combination with erlotinib in patients with advanced NSCLC that are refractory to EGFR inhibitors, a favorable safety profile with moderate clinical efficacy and little significant drug–drug interactions were observed.[61] Currently, there are no ongoing or active clinical trials on **1** in the U.S. but it is still active in China and Japan.

One of the major issues with compound **1** is its poor PK profile due to rapid elimination from plasma at a rate of 34–48 L/h (Table 3),[92,96] requiring it to be continuously infused intravenously (IV) 24 h a day in a 7-day dosing cycle (168 h).[92,96] Another issue is the lack of understanding in the precise mechanism of its action. As previously mentioned, although **1** was identified using a HTS assay for inhibitors that bind to and inhibit the

survivin promoter and it indeed inhibits survivin transcription,[74] it was later shown that **1** does not bind to the survivin promoter as anticipated but rather inhibits the transcription factor Sp1[97] and disrupts the interaction between interleukin enhancer-binding protein factor 3 (ILF3) and $p54^{nrb}$, a critical complex for survivin transcription.[72] These findings are not surprising because the cell-based HTS assay used did not eliminate the potential inhibition of transcription factors that may activate the survivin promoter. Furthermore, there is compelling evidence suggesting that **1** may be a DNA damage-inducing agent and its inhibition of survivin expression is secondary to this event.[98] However, a later study showed that **1**-induced DNA damage in neuroblastoma UKF-NB-3 cells may be secondary to survivin down-regulation since survivin knockdown using siRNA induced DNA damage without **1**.[75] The latter observation is consistent with previous studies that provided indirect evidence of survivin-depletion leading to DNA damage.[99,100] These findings indicate that survivin may play a role in protecting against DNA damage.

Most recently, it was shown that **1** inhibits the receptor-interacting protein kinase 2 (RIPK2), which also mediates its effect on survivin expression.[70,101] Considering that Sp1 is a ubiquitous transcription factor with multiple downstream target genes, RIPK2 also has multiple target proteins, and compound **1** does not bind to survivin, designating **1** as a survivin inhibitor is inappropriate and misleading. Nevertheless, as discussed above the fact that it continues to be actively investigated calls for perspective insight and discussion on future directions of its study, and we believe that there is some value in thoroughly examining its medicinal chemistry and pharmacology. Hence, we provide our thoughts on its medicinal chemistry and pharmacology for readers with interest in **1** and perhaps also in targeting gene transcription in general and survivin transcription specifically.

2.1.2. Medicinal Chemistry.—While hundreds of **1** analogs have been disclosed in the patent application,[102] the SARs for the benzene ring (I) and imidazolium ring (II) of the dioxonaphthoimidazolium scaffold and the relationship to anticancer activity have not been clearly defined.[102] There are two recent reports on SAR studies of **1**, including a study showing DNA intercalation and cytotoxicity using the clear cell renal cell carcinoma (ccRCC) cell lines RCC786–0 and RCC4/VA, NSCLC cell lines H1299 and H1666, and nonmalignant human lung fibroblast IMR-90,[103] and another study showing anticancer activity of **1** derivatives using human pluripotent stem cells (hPSCs).[104] In these two studies, all its derivatives have the intact dioxonaphthoimidazolium scaffold, the essential pharmacophore of **1**, but with various substituents on N^1 (Figure 3B) and N^3 (Figure 3C) of ring II. The key findings associated with the SARs of **1** are summarized in Table 4.[103,104]

Currently, about three hundred analogs of compound **1** have been synthesized and tested. However, only several derivatives, including **20** (AB7), **21**, and **22** (Figure 3D), exhibited favorable in vitro and in vivo tumor-inhibiting activities.[103,104] Unfortunately, none of these derivatives showed superior activity compared to **1** in either inhibiting survivin expression or suppressing cancer cell proliferation. Compound **23** (UFSHR, Figure 3D) also exhibited lower potency than **1** in suppressing survivin expression in the pancreatic cancer cell lines PPCL-46 and PPCL-LM1, which were generated from patient-derived xenograft tumors of stage III or IV pancreatic ductal adenocarcinoma (PDAC). However, 0.1 mg/kg **23** via

intraperitoneal (IP) injection achieved similar and better activity than 0.01 mg/kg **1** (IP) in inhibiting PPCL-LM1 and PPCL-46 xenograft tumors, respectively, but at a 10-fold higher dose.[48]

In addition, compound **1** was confirmed to be a substrate of the ATP-binding cassette (ABC) transporter subfamily B member 1 (ABCB1, also known as P-glycoprotein) of which overexpression facilitated its efflux from cancer cells and thereby conferred its resistance.[105,106] Thus, it cannot be utilized for cancer patients overexpressing ABCB1.

2.1.3. Key Structural Properties.—Compound **1** is an ionized and positively charged molecule. Ionized molecules account for only ~4% of all approved drugs, and most of these drugs are for external use to treat infectious diseases.[107,108] While in most cases, cationic molecules are more potent cytotoxins toward cancer cells than the anionic molecules,[109,110] ionized molecules when used in vivo usually have absorption issues due to lower membrane permeability than neutral molecules.[111] However, positively charged molecules may enter cancer cells using the organic cation transporters, as has been suggested for **1**.[74,112] Solute carrier family 35 member F2 (SLC35F2) has also been suggested to contribute to **1**'s uptake and its expression may predict the in vitro and in vivo efficacy of **1**.[73,75]

Intriguingly, many tool compounds are cationic, including the commercially available fluorescent tracking/imaging MitoTracker probes.[113,114] These compounds typically possess a chemical formula of $(R_3)\ N^+(\text{halogen})^-$ or $(Ph_3)\ P^+(\text{halogen})^-$, which may favorably target and accumulate in the negatively charged inner mitochondrial membranes.[113] Since **1** also contains the $(R_3)\ N^+(\text{halogen})^-$ motif, it may also accumulate in mitochondria. Indeed, a recent study showed that it accumulates in mitochondria and causes mitochondrial dysfunction, suggesting another potential mechanism of action for compound **1**.[115]

Compound **1** has a 1,4-benzoquinone containing dual carbonyl groups which is prevalent in both biological molecules and approved drugs.[116] The two carbonyl groups together with the vinyl group can form a conjugated system that appears to be stable. However, the 1,4-benzoquinone motif is oxidatively active under physiological conditions. It can undergo specific redox reactions with reductive biomolecules or by metabolic enzymes, which may contribute to its poor PK profile.[116] Indeed, it was shown recently that the carbonyl groups on dioxonaphthoimidazoliums analogs with the same pharmacophore as **1** underwent redox reaction catalyzed by respiratory enzyme type II NADH dehydrogenase (NDH2), leading to increased production of reactive oxygen species (ROS).[117,118] Future studies to modify the two carbonyl groups may help reduce their chemical reactivity and improve the PK profile.

2.1.4. Perspective.—Compound 1 has a favorable molecular weight of 443, zero H-bond donors (HBDs), and seven H-bond acceptors (HBAs), but an unfavorable cLog *P* of −3.58. The negative cLog *P* value indicates a strong hydrophilicity that may cause absorption issues following oral administration. We used a Radar Chart to visualize the overall properties of **1** (Figure 4A), which shows that it is an extremely unbalanced candidate without an optimal cLog *P* value. The low cLog *P* value renders it a highly polar and soluble compound that can only be administered in clinical trials via infusion. The high polarity along with the fact that its uptake may rely on transporters may exclude it from

being developed for tumors in the central nervous system due to the need to traverse the blood-brain barrier (BBB).[70,119]

Given that **1** is a highly potent candidate and has been tested in clinical trials, future studies should be focused on correcting its PK profiles by modifying its structure or developing pharmaceutical formulations that may eliminate the need for a 3- or 7-day continuous infusion.[15,94] With the above medicinal chemistry and key structural characteristics of **1**, we envision a few studies to improve it based on several candidates, including compounds **20** and **23**. It has been shown that minor structural modifications on lead compounds can have meaningful impacts on pharmacological profiles due to significant effects on molecular and physicochemical properties and intra- and intermolecular interactions.[120] Thus, minor structural modifications to eliminate the positive charge may help improve the PK profile of **1**. For example, a minor modification by reducing the C=N in ring II to CH–N or replacing the nitrogen atom (N) with a carbon atom (C), as shown in compounds **24–29** (Figure 4B), respectively, is expected to lead to an electrically neutral molecule that retains all of the essential pharmacophore of **1** and may possess comparable anticancer potencies with possible better PK/PD profiles than **1**.

Next, structural modifications to increase the in vivo stability should be considered. For example, compound **30** with specific halogen substitution on the benzene ring, including fluoro (−F), chloro (−Cl), bromo (−Br), iodo (−I), or trifluoromethyl ($-CF_3$) (Figure 4B) may help to stabilize the benzene ring, leading to enhanced resistance to metabolism.[121,122]

Also, protection of the carbonyl group(s) by ethylene glycol (Figure 4B) may generate **31** with a ketal group for development of a new chemical entity or a potential prodrug although such a moiety is stable in neutral or even acidic conditions and, thus, cannot convert back into **1**.[123] The ketal moiety is used as a common chemical strategy to protect a carbonyl group in organic chemistry[124] and also as a motif in drug structures, as in the compound **32** (G-5555), a p21activated kinases (PAKs) inhibitor,[125] and **33**, the approved drug doxofylline (Figure 4C).[126] A quinone to ketal molecular edit has been successfully exemplified in the synthesis of **34** (lactonamycin),[127] an antimicrobial drug that has also been shown to be a potent anticancer agent with nanomolar IC_{50} values (Figure 4D)[128]. However, a ketal derivative of a quinone-based molecule has yet to be developed or approved. Nevertheless, a compound with a ketal derived from the quinone may be worthy of consideration for testing whether it can function as a new chemical entity.

The successful enforcement of these proposed modifications may help further develop **1** into an effective anticancer drug with an improved PK/PD profile.

2.2. Compound 2 (EM-1421).

Compound **2** (Figure 5A), tetra-*O*-methyl-nordihydroguaiaretic acid, also known as terameprocol or M4N, is a semisynthetic compound derived from **35**, the natural product nordihydroguaiaretic acid (NDGA, Figure 5A) from *Larrea tridentata*.[129] Compound **35**, a poly phenolic antioxidative compound categorized as a lignan, was discovered over one century ago and has been used in food chemistry as a preservative for more than 70 years.[130] As the main active component, **35**, developed by the University of Arizona Cancer Center

under the trade name Actinex, was approved by the FDA in 1992 for skin-related damage/cancer due to sun exposure. However, it was withdrawn in 1996 due to unexpected side effects and a small market. Compound **2** is generated directly by the methylation of the four phenolic hydroxy groups of **35**.[77] Similar to **1, 2** is also a transcriptional repressor of the survivin gene by inhibiting the ubiquitous transcription factor Sp1.[76,131] As such, it also inhibits the expression of cyclin dependent kinase 1 (CDK1), a downstream gene of Sp1,[132] and the expression of vascular endothelial growth factor (VEGF).[133] It has also been shown to inhibit AKT phosphorylation and activation.[134,135] Similar also to **1**, new targets may continue to be identified with continued study. Thus, designating **2** as a specific survivin inhibitor is also inappropriate and misleading since it does not bind to survivin. Nevertheless, **2** is now under evaluation in multiple phase I/II clinical studies sponsored by Erimos Pharmaceuticals.

2.2.1. Biological Properties.—Compound **2** has IC_{50} values ranging from 16 to 85 μM in various cancer cell lines, including A375, HT-29, MCF-7, HaCat, and HepG2 cells, and suppresses the growth of xenografts derived from breast, prostate, colorectal, and liver tumors in mouse models.[77,95,136] In addition, it was able to sensitize radiotherapy in NSCLC cells.[137] The bioavailability of orally administered **2** in mice is very good, reaching 88% (Table 3).[95] While **2** shares similarity with **1** in that it requires IV administration in clinical trials, the infusion time is shorter and dosing is of a lower frequency than for **1**, with a cycle of 1500 mg dosing over 6 h, three times/week for 2 weeks followed by a week of rest.[134] The first clinical trial with **2** was conducted in 2003 for refractory malignant head and neck tumors (NCT00057512). However, the results of this trial have not been posted. In 2007 and 2008, the safety profile of **2** was reported as a vaginal ointment ultimately intended as treatment for cervical intraepithelial neoplasia caused by human papilloma virus (HPV) in a phase I/II trial in healthy adult women.[138,139] The ointment formulated with 1–2% compound **2** showed a promising safety profile with no severe adverse effects in healthy subjects.[138,139] Among advanced leukemia patients recruited, compound **2** at 1000, 1500, or 2200 mg, three times/week via IV, demonstrated a safe profile with partial responses in a few patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) in a phase I clinical study conducted in 2015.[140] In another phase I clinical study in 2012 for high-grade gliomas, **2** did not display any significant response, although 32% of patients showed stable disease.[16] In 2019, a phase I/II clinical trial (NCT00404248) to study the side effects and to optimize the dose of **2** in patients with recurrent high-grade glioma was completed, with the results showing that 1700 mg/day for five consecutive days was safe. Another phase I clinical trial (NCT02575794) to test MTD in patients with high-grade glioma has been scheduled but is inactive in recruiting patients. As disclosed in the pipeline of Erimos Pharmaceuticals, **2** is proposed for multiple phase I clinical trials for solid tumors, advanced leukemia, glioma, and head and neck cancer.[16,134]

2.2.2. Medicinal Chemistry.—Compound **2** is a relatively simple compound with two major chemical groups, the linker and the two methyl groups to protect the two hydroxy groups (−OH) of each catechol (Figure 5A). Most of the known derivatives and analogs have the two benzene rings but vary in the identity of substituents or protective groups on the catechol moieties, and the linker between the two catechols (Figure 5B–F). These derivatives

include substitutions of four methyl groups on the two catechols using other functional groups via the ether bond (Figure 5B), ester bond (Figure 5C), or end-ring (Figure 5D), and modification of the linear carbon chain linker using different length and ring substitution motifs (Figure 5E). Although hundreds of **2** derivatives and analogs have been synthesized and characterized, no derivative with better drug-likeness than the parent drug **2** has been identified.[141,142] While one study showed that the introduction of chlorine on each benzene ring increased anticancer potency as compared to **2,** the compound **36** (NDGA-Cl_2, Figure 5F) was eventually abandoned due to its high toxicity in vivo.[77] Table 5 shows a summary of key SAR information associated with **2**.

2.2.3. Key Structural Properties.—There are several potential key/interesting modification strategies of the natural product **35**, the parent compound of **2**. First, phenolic hydroxy groups (PHGs) have long been regarded as free radical traps that can capture various forms of free radicals, including ROS and reactive nitrogen species (RNS), leading to reduced oxidative stress and acting as antioxidants that can be applied as cytoprotective agents.[145] However, PHGs, especially the poly phenolic groups, are unstable and easily oxidized into quinones.[146,147] Therefore, PHGs in small molecules are usually protected by other functional groups through an ester or ether bond and these simple modifications may lead to a more potent drug candidates.[148,149] Ester bond modification of PHGs often results in prodrugs that can be hydrolyzed into its active form in vivo, mediated by mild base/acidic conditions or carboxylesterases enriched in the liver and blood.[150,151] In contrast, ethers are a much more stable form and the product usually acts as an intact molecule to bind to its target or exert its therapeutic function.[149] In the case of compound **2**, methylation of PHGs in NDGA resulted in a promising product that is currently being studied in clinical trials. However, the phenol ether could be metabolized to the free phenol by cytochrome P450 (CYP) as shown previously[152] although no such study has been conducted for **2**. Future PK studies are necessary to test this possibility.

Second, the length of the carbon chain in ether or ester derivatives of PHGs will affect lipophilicity.[153,154] While generally the longer the side chain, the more lipophilic the molecule, the correlation with bioactivity is usually more complex and unpredictable.[153,154] In addition, as the side chains grow longer and bigger, the molecules tend to be more stable against hydrolysis due to steric hindrance.[155,156] Given that the essential pharmacophore of **2** is actually the NDGA, bulkier side chains may reduce potency and should be avoided.[77]

Third, **2** does not contain a nitrogen atom while many of its ester or ether derivatives do (Figure 5B/C).[141] Such nitrogen-containing groups (NCGs) are one class of solubilizing moieties commonly adopted in medicinal chemistry to help address solubility issues of lead compounds.[157] NCGs in biologically active compounds usually exist in different forms including amine salt, neutral amide, or heterocyclic compounds.[158] It is estimated that nearly 75% of FDA-approved small molecule drugs contain at least one nitrogen atom, and ~60% have a nitrogen-containing heterocyclic, suggesting that NCGs are prevalent in approved drugs.[158,159] Thus, the addition of NCGs to NDGA was speculated to increase its drug-likeness by protecting PHGs, forming a hydrogen bond with its target, and improving water solubility. Unfortunately, no better derivative than **2** using this approach has been

identified (Figure 5B). More such compounds may need to be synthesized to further test this possibility.

2.2.4. Perspective.—Structurally, **2** is not an ideal drug since it offers extremely limited space for further improvement and possesses a high cLog *P* value, as shown in the Radar Chart (Figure 6A). Since all four polar hydroxy groups are protected as methyl ethers, compound **2** is hydrophobic with poor solubility. However, this hydrophobicity may help it penetrate the BBB, as suggested by its trials for glioma.[16] Similar to **1**, we offer below some perspective on future development since **2** is being actively investigated in clinical trials. In this regard, modifying **37** with four free PHGs (Figure 5G) and an IC_{50} value of 0.3 μM against a small cell lung cancer cell line H69 may provide a better option for development.[143]

First, given that the four free PHGs in **37** are unstable, they likely need to remain protected similar to that in **2**. Esterification or etherification with short side chains as proposed in **42** or **43** (Figure 6B) is preferred based on previous findings.

Second, the liner linker appears to be more favorable for cytotoxicity although the data for this conclusion are limited. However, several other types of linkers such as butanediols in secoisolariciresinol,[160] and tetrahydrofuran in taxiresinol[161] have been used and shown to have promising activities. Thus, further studies testing additional linkers such as those in the proposed **44** and **45** may result in better derivatives.

Finally, considering that poly phenolic compounds may possess cytoprotective activity, **37**, the hydrolysis product of **42/43** (Figure 6B), could be explored for its ability to alleviate adverse effects induced by toxic chemotherapeutics in combination therapy.

Similar to **1, 2** also inhibits the transcription factor Sp1, which indirectly inhibits survivin expression. Interestingly, these two compounds are safe for use in animal models and in clinical trials despite the fact that they inhibit a ubiquitous transcription factor Sp1 with multiple downstream target genes,[25,60] indicating that Sp1 may be a potential target without major toxicity. However, their limited clinical efficacy suggests that targeting Sp1 to suppress survivin expression along with other Sp1 downstream genes may not be feasible and represents an inappropriate strategy for therapeutic development. Nevertheless, the information accumulated from more than ten years of preclinical study and clinical trials may be useful to transform **1** and **2** into potent candidates that are not only safe but also effective.

2.3. Compound 3 (FL118).

Compound **3** (Figure 7A) was identified via HTS in 2012 using a reporter assay of the survivin gene promoter in an attempt to identify novel inhibitors that disrupt the promoter activity, similar to the strategy used in the discovery of **1**.[78,162] However, **3** also inhibits the expression of Mcl-1, and two other IAPs including XIAP, and cIAP2.[163] Thus, **3** is also a nonselective inhibitor of survivin expression. Interestingly, **3** structurally resembles the topoisomerase I inhibitors, **46** (camptothecin), **47** (irinotecan), and **48** (topotecan), which have been approved by FDA for cancer treatments (Figure 7A/B).

2.3.1. Biological Properties.—Compound 3 displayed nanomolar IC_{50} values in a series of cancer cell lines with strong antitumor activity in xenografts of squamous cell carcinoma, colon cancer, multiple myeloma cell lines, as well as a patient-derived head and neck cancer xenograft when combined with the first-line therapeutic gemcitabine.[17,164,165] It was also found that SW620 and HCT-8 cancer cells are more sensitive to **3** than normal adult human dermal fibroblast and human gingival fibroblast cells, suggesting the existence of a therapeutic window.[78] However, **3** appears to have a short half-life in mice, with a $t_{1/2}$ of only 1.8 h following IV administration (Table 3). A recent study on its mechanism of action showed that **3** may work as a molecular glue to directly target and bind to DEAD-box helicase 5 (DDX5), an ATP-dependent RNA helicase with transcription coactivator function, with a K_d of 34.4 nM, leading to dephosphorylation and degradation of DDX5 in the proteasome.[79] DDX5 promotes cancer progression by interacting with transcription factors including signal transducer and activator of transcription 3 (STAT3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), cyclin D1, c-Myc, and β-catenin.[166] Compound **3**-induced degradation of DDX5 is expected to down-regulate the expression of many downstream target genes of these transcription factors, including survivin.[79] Indeed, in addition to inhibiting XIAP and cIAP2 expression,[163] **3** has been shown to inhibit the expression of c-Myc, a known target gene of DDX5-β-catenin coactivation.[79] Furthermore, **3** with oral dosing at 2.5 mg/kg (four times a week for five to 7 weeks) is more effective on PDAC patient-derived xenograft (PDX) tumors with high DDX5 level than those with low-level.[79] DDX knockdown in colon cancer and PDAC cells also caused insensitivity to **3**. These results suggest that DDX5 is indeed a target of **3**. Currently, **3** is the basis of IND application although clinical studies do not appear to have been scheduled.

Many **3** analogs have been reported, including nine new derivatives (**50–58**) recently synthesized with different R7 substituents on ring II (Figure 7C),[82] two compounds with different R9 substituents on ring I (Figure 7D), and five compounds with modifications on the hydroxy group (Figure 7E).[167] Compounds **51** and **52** (Figure 7C) have a similar IC_{50} values of 11 and 10 nM in A2008 cells, respectively, and 0.17 and 0.11 nM in SW620 cells, respectively, and both are ~3- to 6-fold more potent than **3**. **52** was selected for further evaluation over **51** due to convenience of synthesis and purification and because it consistently showed higher potency than **3** in a series of cancer cell lines with IC_{50} values ranging from 0.78 to 19 nM.[82] Compound **52** (Figure 7C) was also more potent than **3** in inducing apoptosis of NCI-H446 lung cancer cells and in drug-resistant H69AR cells. It also appears to be more efficient in reducing both mRNA and protein levels of survivin in H69AR cells than in NCI-H445 cells.[82] However, similar to **3**, **52** also reduced the levels of XIAP in both cell lines, suggesting that it too is nonselective in inhibiting survivin expression. In addition, the irinotecan-resistant NCI-H446/Iri and etoposide/cisplatin combination (EP)-resistant NCI-H446/EP cells are more sensitive to **52** than their respective parental cells, suggesting that the drug-resistant cancer cells may have gained collateral sensitivity to inhibition of survivin expression. More importantly, intragastric (IG) dosing of **52** at 0.75, 1.5, and 3 mg/kg (once/week for 4 weeks) dose-dependently inhibited NCI-H446 xenograft growth, and at 3 mg/kg it was more potent than **3**, consistent with the in vitro results.[82] At 3 mg/kg, **52** also inhibited the growth of xenograft tumors derived from drug-resistant cancer cells NCI-H446/Iri and NCI-H446/EP.

However, significant weight loss was observed in test animals, indicating that **52** may have toxicity.[82]

2.3.2. Medicinal Chemistry.—Preliminary SAR studies of the R7 and R9 substituents and the hydroxy group on ring V have been reported (Figure 7).[82,164,167,168] Compounds **59** (9-Q6) and **60** (9-Q20) as shown in Figure 7D were well-absorbed across 2D and 3D CaCo-2 cell models. The IC_{50} values for **60** in HCT116, MCF-7, HepG2, HeLa, and A549 cells range from 0.78 to 3.58 μM, indicating that it is 16 to 30-fold less potent than **3**.[164] While the activity of **59** has not been reported, it is reasonable to predict that it may possess cytotoxicity comparable to **60** considering their structural similarity. The cytotoxicity of **61** (Val-FL118) in Figure 7E is weaker but comparable to **3** in HepG2 and HCT116 cells.[169] However, **61** may be a good candidate with improved water solubility due to the hydrophilic valine motif. Four other derivatives, **62–65**, with modifications of the hydroxy group (Figure 7E) all are much less potent than 3 in FaDu, SW620, and HCT-8 cells, suggesting that the free hydroxy group is important.[167] Recently, it was found that a nitrogen at the para site of pyridine ring (**50**) or a bis-trifluoromethyl phenyl group at R7 (**58**) in Figure 7C were less favorable than the other rings.[82] On the basis of these limited medicinal chemistry studies and the patent published in 2015,[170] we performed a preliminary SAR analysis of **3** (Table 6).

2.3.3. Key Structural Properties.—Although **3** is structurally related to topoisomerase 1 inhibitors including **46** (camptothecin), **47** (irinotecan), **48** (topotecan), and **49** (SN-38, the active metabolite of irinotecan), it has been shown that **3** works differently[78] and that its anticancer activity is unrelated to topoisomerase 1 inhibition.[171] Thus, it would be interesting to analyze the structural differences between **3** and these topoisomerase 1 inhibitors, which may confer different biological events in cancer cells.

Compound **3** is 100- to 1000-fold more active in inhibiting survivin expression/transcription than inhibiting topoisomerase 1 activity.[78] However, **48** and **49** exhibited 10 to 100-fold lower activity than **3** in inhibiting the expression of Mcl-1, XIAP, and cIAP2.[81] Furthermore, **3** is not a substrate of ABC transporters such as ABCB1 or ABCG2,[81] which differentiates it from **48** and **49**.[172] These findings suggest that although structurally similar, **3** is more selective than topoisomerase 1 inhibitors in suppressing survivin expression and less potent toward topoisomerase 1 inhibition with an ability to bypass ABC transporter-mediated drug efflux as a source of resistance.

The cocrystal structure and computer-assisted docking analysis of **46** and **48** with topoisomerase 1 showed that the active groups for topoisomerase 1 binding were the lactone (ring V), the hydroxy adjacent to the lactone, the hydroxy on ring I, and the carbonyl group on the pyridine ring (IV), while no direct binding interactions were found for the quinolone motif (I and II).[173,174] Considering that the only structural differences among these topoisomerase 1 inhibitors are the substituents on rings I and II (Figure 7B) and that **3** differs from them also on ring I, it would be of interest to examine these differences in relationship to their target selectivity. While the hydroxy group on ring I of topotecan contributes to binding to topoisomerase 1, as discussed above, and hydrolysis of **47** results

in the free hydroxy group in **49**, **3** cannot be hydrolyzed to give the free hydroxy group on ring I with both oxygen atoms protected by the intervening methylene group, which is much more stable than the ester bond in **47**. This particular entity maybe the major factor that prevents **3** from inhibiting topoisomerase 1 and may also be responsible for selective binding to and inhibition of DDX5.

2.3.4. Perspective.—Both compounds **3** and **52** are drug-like molecules given that several topoisomerase 1 inhibitors with similar structures have been approved by the FDA. The Radar Charts presented in Figure 8A/B suggest that both compounds follow the RO5. However, **3** has very low water solubility of less than 0.001 mg/mL. Due to its hydrophobicity, FL118 can permeate cells, as determined using 2D and 3D CaCo-2 cell models.[164]

Considering that rings I/II are tolerant of structural modifications and that the lack of a hydrophilic motif as compared with **47** and **48** may cause poor water solubility, we envision a few minor modifications on rings I or II of **3** to improve its solubility and/or potency without undermining its drug-likeness (Figure 8C).

First, the nitrogen atom in the pyridine ring may form a salt with either hydrochloric acid (HCl) or methanesulfonic acid (MSA), two commonly used acids among FDA-approved drugs,[175] that should possess improved water solubility (**66**).

Second, introducing a hydrophilic dimethylaminomethyl group on ring I (**67**), similar to **48**, may improve water solubility significantly. The resulting compound **67** could also be converted into a salt by exposing to HCl or MSA to further improve solubility.

Third, the protective methylene group could be replaced by two separate methyl groups (**68**) or a crown ether (**69**). The two methyl groups linked via ether bonds are also resistant to hydrolysis (see discussion above for **2**) and may resemble the effects of **3** on survivin expression. The crown ether-modified **3** may not only represent a new chemical entity, but also possess better bioavailability from modified membrane permeability.[176] A successful example of such a molecule is **74** (icotinib) compared with its predecessor **75** (erlotinib) as shown in Figure 8D.[177]

Fourthly, it is possible to combine the modifications in **67**–**69** to create **70** and **71** with advantages from **67**–**69** (Figure 8C). While **70** has a suitable molecular weight, **71** may be too big with a molecular weight 551.6. In addition, the synthetic method to create **70** and **71** may be technically challenging.

Finally, with higher molecular weight (Figure 8B) and better potency than **3**,[82] **52** can be further developed by introducing a hydrophilic tail, such as a dimethylaminomethyl group, at the R9 position on ring I, generating **72** (Figure 8C), which may increase its drug-likeness. Additionally, a crown ether can also be used to replace the mathelene on ring I of **52**, generating **73**. However, caution needs to be exercised due to the toxicity observed with **52** in mice.

2.4. Compound 4 (UC-112) and Its Analogs.

Survivin functions by binding to and interacting with many other essential cellular proteins. Thus, there have been various efforts in targeting survivin interaction with its binding partners using small molecule inhibitors. In this regard, a series of compounds containing a 7-(pyrrolidin-1-ylmethyl)quinolin-8-ol motif constructed with rings I–III (Figure 9A) were identified in a shape-based structural screen for SMAC mimetics that inhibit the interaction between SMAC and IAPs inspired by the SMAC-XIAP complex structure.[83] One of these compounds, **4** (Figure 9B), inhibited the expression of XIAP, cIAP1, cIAP2, and survivin. It possessed IC_{50} values ranging from 0.7 to 3.4 μM in suppressing melanoma A375 and M14 cell proliferation as well as prostate cancer PC-3 and DU145 cell lines by activating caspases.[83] Varying the linker between the 7-(pyrrolidin-1-ylmethyl)quinolin-8-ol motif and the benzene ring IV (Figure 9B–D) and substituents on the benzene ring IV or its replacement by other aromatic rings (Figure 9E–G) led to several second generation analogs including **5** (Figure 9F),[18] **76**, and **77** (Figure 9D), as well as **78–80** (Figure 9E) with structural extension by varying substituents on the indole moiety.[84,178] Compound **6** is a third generation of **4** analog discovered recently (Figure 9G).[179]

2.4.1. Biological Properties.—Compound **4** is ~2.5-fold more effective against cancer cell lines than normal human keratinocyte Hacat cells or dermal fibroblast adult cells.[83] Thus, there may be a small therapeutic window. Nevertheless, treatment using 20 and 40 mg/kg of **4** (IP, 5 days/week for 3 weeks) significantly inhibited the growth of A375 melanoma xenografts, with little reduction in the body weight of the mice,[83] suggesting that it may be tolerable at the dose used, at least in mice. Although the initial intention was to inhibit SMAC binding to all IAPs, **4** dose-dependently inhibited the expression of survivin the most in a proteasome-dependent manner and its effect on other IAPs, including cIAP1/2 and XIAP, is relatively weaker.[83] A docking analysis suggested that **4** might bind to the BIR domain of survivin,[84] a proposal that needs experimental validation. Since cIAP1/2 and XIAP all have multiple BIR domains, it is intriguing that **4** is less effective to these proteins than survivin, which has only one BIR domain. It is also unclear how **4** binding to the BIR domain leads to proteasome-dependent survivin degradation.

Compound **5** (Figure 9F), a second generation of **4** analog which has an isopropyl group on benzene ring IV, showed an average of 4-fold increase in activity in inhibiting cancer cell proliferation and an increase in selectivity to survivin when compared to **4**.[18] Compound **5** at 20 and 40 mg/kg via IP injection (5 days/week for 3 weeks) was effective at inhibiting the A375 melanoma xenograft growth in mice.[18] The third generation compound **6** (Figure 9G) had an average IC_{50} value of 1.4 μM in a variety of cancer cell lines and showed antitumor effects in an A375 melanoma xenograft model and orthotopic ovarian cancer model at 20 and 40 mg/kg via IP administration (3 days/week for 15 days).[179]

2.4.2. Medicinal Chemistry.—7-(Pyrrolidin-1-ylmethyl)-quinolin-8-ol has been shown to be the essential pharmacophore that is critical for anticancer effects, and any other structural modifications/changes on this scaffold led to reduced or lost bioactivity.[178] The major structural modifications of **4** were on the ring IV and the linker between the ring IV and II (Figure 9) with key findings summarized in Table 7.

2.4.3. Key Structural Properties.—Compound **4** has two key structural properties. First, the quinoline ring is a prevalent scaffold among synthetic drug candidates and approved drugs,[180] and from this perspective, **4** and its analogs are drug-like. Furthermore, **4** and its analogs appear to have higher cytotoxicity toward drug-resistant cancer cells that overexpress specific ABC transporters, including ABCB1, ABCC1, and ABCG2, which are known to cause resistance to anticancer drugs by actively exporting them from cells.[181,182] Interestingly, **4**, **5**, **76**, and **79** were all found to inhibit survival of ABCB1-overexpressing cancer cells ~3- to 10-fold more efficiently than cells without ABCB1 overexpression.[84,178,179] However, no interactions between these compounds and ABCB1 have been demonstrated and the mechanism for this potential collateral sensitivity is unclear.

The second structural property that is worthy of discussion is the relatively low metabolic stability of **4** and **5**, as compared to compound **6**. The free and exposed benzene ring in **4** or the iso-propyl benzene in **5** usually undergoes oxidative reaction quickly in phase-I metabolism in vivo.[183] Indeed, a recent stability study using human liver microsomes showed that **6** was metabolically more stable with a $t_{1/2}$ of 1.5 h and clearance rate of 15.7 μL/min/mg than **5** with a $t_{1/2}$ of 0.85 h and clearance rate of 136.6 μL/min/mg.[179] It is noteworthy that both **4** and **5** require daily administration while **6** could be administered every other day (all three dosed at 20 and 40 mg/kg) for in vivo studies using melanoma A375 xenograft models, consistent with the notion that **6** is metabolically more stable and slower in clearance than **5**, as discussed above. It is tempting to speculate that the trifluoromethyl group on the benzene ring as well as the triazole linker in **6** may contribute to its improved stability.

Furthermore, the core pharmacophore 7-(pyrrolidin-1-ylmethyl)quinolin-8-ol was synthesized via a Mannich reaction.[179] It is possible that this moiety may undergo a retro-Mannich reaction, generating a reactive Michael acceptor that may cause stability issue or off-target effects. This speculation needs to be tested and, if proven true, medicinal chemistry work is necessary to stabilize it.

2.4.4. Perspectives.—Compound **4** appears to have all critical drug-like properties (Figure 10A) and no predicted major pitfalls including solubility and bioavailability were identified except the possible retro-Mannich reaction as discussed above. Structural modifications on the quinolone moiety and its substitutions without changing the R_1 (a–f) are possible to further improve **5**, **6**, **76**, **77**, **79**, and **80** (Figure 10B).

First, quinolone may be replaced with either quinoxaline (UC-P1 series) or quinazoline (UC-P2 series), leaving R_1 (a–f) of each compound unchanged (Figure 10B). Quinoxaline and quinazoline represent another two common moieties among approved drugs especially tyrosine kinase inhibitors (TKIs), including the epidermal growth factor receptor (EGFR) inhibitors, lapatinib, afatinib, gefitinib, dacomitinib, **74** (icotinib), and **75** (erlotinib).[184,185] While quinoxaline and quinazoline possess similar physical and chemical properties, they each have one additional nitrogen atom than the quinoline in **4**, which may lead to higher polarity and increase the solubility or drug-likeness.[186] The replacement of quinoline with quinoxaline or quinazoline may also introduce additional activity in inhibiting ABC transporters since growing evidence supports that TKIs containing quinoxaline or

quinazoline are capable of inhibiting ABCB1 or ABCG2, leading to sensitization to anticancer drugs that are substrates of these transporters.[187,188]

Second, the hydroxy at R_2 may be replaced by bioisosteres such as HBD groups like NH_2, SH, or an HBA group like CN (Figure 10B). Bioisosteres are a class of atoms or chemical groups with different structures but similar physical or chemical properties conferring similar biological activities.[189,190] Generally, bioisosteric replacement is to enhance target-binding affinity, reduce toxicity and correct poor PK, or generate new chemical entities. Further, CN anchored here may also help prevent possible retro-Mannich reaction of the 7-(pyrrolidin-1-ylmethyl)quinolin-8-ol.

2.5. Compound 7 (LQZ-7F) and Its Analogs.

As discussed above, inhibiting survivin expression by targeting its upstream regulators such as transcription factor Sp1 or coactivator DDX5 do not selectively inhibit survivin expression. Inhibiting survivin interaction with its binding partners may also inhibit other IAPs as discussed above. Selective inhibition may limit to one-binding partner of survivin while spare other partners which bind to different sites on survivin. Thus, a specific strategy to eliminate survivin selectively is necessary to target this protein. In this regard, it was hypothesized that effective inhibition of homodimerization of survivin would expose its hydrophobic interface, similar to the exposure of the hydrophobic core in a globular protein during denaturation or misfolding, leading to recognition of the monomeric survivin as a misfolded protein by the cell quality control system and target it for destruction by the proteasome (Figure 11) and, thus, effectively eliminate survivin.[20] However, unlike targeting heterodimerization of two different proteins, targeting homodimerization is more challenging since HTS assays such as the use of amplified luminescent proximity homogeneous assay (Alpha) are not readily applicable.

To overcome this challenge, a computer-based screen was used to target the homodimerization of survivin. Briefly, critical residues responsible for hydrophobic interactions between the two identical survivin subunits were studied using a computational analysis that identified Leu6, Pro7, Pro8, Ala9, Trp10, Phe93, Glu94, Glu95, Leu96, Thr97, Leu98, Gly99, Phe101, and Leu102 as potentially important residues.[20] In silico screening of small molecules targeting these critical residues at the dimerization interface of survivin was performed with the top-scoring candidates assessed with a nondenaturing polyacrylamide gel electrophoresis (PAGE) assay of purified survivin.[20] These studies led to the identification of a hit compound, **81** (LQZ-7, Figure 12A) that induced dissociation of the recombinant homodimeric survivin and inhibited homodimerization of nascent survivin newly synthesized in a cell-free system.[20] It also induced proteasome-dependent survivin degradation, as expected. As shown in Figure 12B, several **81** analogs, **82** (LQZ-7A), **83** (LQZ-7B), **84** (LQZ-7C), **85** (LQZ-7D), **86** (LQZ-7E), and **7** (LQZ-7F), were also identified from the chemical library that had different activities in targeting survivin and inducing spontaneous apoptosis in prostate cancer cell lines (Table 8).[20] Further medicinal chemistry study to optimize these inhibitors has also been attempted, leading to the identification of the two novel survivin inhibitors **8** (7F1, Figure 12B) and **9** (LQZ-7I, Figure 12C) that

are very active in inducing survivin degradation, suppressing proliferation and inducing the spontaneous apoptosis of CRPC cells.[19–21]

2.5.1. Biological Properties.—Compound **81** and its active derivatives inhibited homodimerization of survivin as determined using a nondenaturing PAGE analysis of purified recombinant proteins or using a mammalian two-hybrid assay.[19–21] These inhibitors induced survivin degradation in proteasome and reduced survivin half-life from ~2–3 h to 25–50 min.[19,20] Interestingly, the other members of the IAP family such as cIAP1/2 and XIAP were not significantly affected, suggesting that this class of inhibitors is selective for survivin. Furthermore, **7** with an amino group in its tail was immobilized using CNBr-activated Sepharose to pull down purified recombinant survivin while **81** was analyzed for its interaction with purified survivin using a fluorogenic assay, which demonstrated their direct binding to survivin.[20] The affinity of **81** binding to survivin was estimated to be 0.24 μM (K_d) using the fluorogenic assay.[20] The IC_{50} value for **81** toward the proliferation of prostate cancer cell lines DU145 and PC-3 was significantly improved from 20 to 25 μM to 1.9–4.3 μM for **7** (Table 8). The low potency of **81** compared with its active analogs was thought to be due to the presence of the carboxylic acid which may negatively impact cellular permeability.

The third-generation derivative **8**, which expresses an IC_{50} value of ~160 nM toward CRPC cell lines, is much more potent than its predecessor **7** (Table 8).[21] These inhibitors have also been shown to induce spontaneous apoptosis of prostate and other cancer cell lines and synergize with docetaxel in prostate cancer cells (Table 8).[19–21] However, only **7** and **9** have been tested in vivo where, at 25 mg/kg (every 3 days for eight treatments, IP) and 100 mg/kg (every other day for ten treatments, oral), respectively, and they both significantly inhibited the growth of PC-3 xenograft tumors without any obvious toxicity (Table 8).[19,20] However, the pharmacokinetics of these compounds have not been studied.

2.5.2. Medicinal Chemistry.—Among analogs **82** to **86**, all have a similar range of IC_{50} values with the exception of **86**, which has a much higher IC_{50} value than that of the parent **81** (Table 8). This observation suggests that a ring structure fused with benzene ring a as in **86** (Figure 12B) is unfavorable, and that the hydrophilic hydroxy group on benzene ring b of 81 is also unfavorable for cytotoxicity possibly due to its poor membrane permeability or low affinity toward the hydrophobic dimeric interface of survivin. It appears that nonpolar groups, including methyl, nitro, and halogens such as −Cl and −Br, on both benzene rings a/b are favorable (Figure 12A/B), which may direct further structural modification for optimization.

Molecular dynamic simulation analysis of **81** docked in the survivin dimerization interface suggested that benzene ring a and the secondary amine bond are two significant motifs that bind to the survivin dimeric interface engaging residues Glu94, Phe93, Phe101, and Leu98, while the furazanopyrazine ring I and II (Figure 12A) did not contribute significantly to survivin binding.[20] Therefore, analogs were synthesized using a benzene ring to replace the furazan ring and using an amide (**87** and **88**) or secondary amine linker (**9**, **89**, and **90**) to replace the labile hydrazone linker (Figure 12C). Compound **9**, a quinoxaline linked with

two *para*-F benzyl rings via two secondary amine bonds, showed the most potent anticancer effect among the derivatives without the hydrazone linker.[19]

Because the hydrazone linker is labile and may undergo hydrolysis, **7** was thought to be a prodrug with its hydrolysis under acidic conditions mimicking that in the tumor microenvironment that may lead to the production of **8** (Figure 12B), which was tested for inhibition of survivin homodimerization. Interestingly, **8** was ~20-fold more potent than **7** in inhibiting PC-3 and C4–2 cells (Table 8).[21] It also effectively inhibited survivin homodimerization, induced its degradation via the proteasome, and induced spontaneous apoptosis as expected.[21] Furthermore, compounds **82** to **86** may be subject to redox activation due to the iminoquinone moiety. Thus, cautions should be practiced when further evaluating these compounds. The key SAR analyses of **81** and **9** are summarized in Table 9.

2.5.3. Key structural properties.—The survivin homodimerization inhibitors discussed above can be categorized into two groups of structurally different scaffolds, with **8** representative of one group and **9** the other. Compound **8** has a rigid structure with a locked tetracyclic ring system consisting of two 5-membered rings (I and III) and two 6-membered rings (II and IV in Figure 12B). While rigid structures are not rare among approved drugs, the absence of the flexibility provided by rotatable bonds and the deficiency of hydrophilic groups is usually problematic for solubility.[191,192] Interestingly, the FDA-approved topoisomerase inhibitors **46** and doxorubicin both have rigid backbone structures but with hydrophilic tails to adjust their polarity and aqueous solubility. Indeed, **7** with a hydrophilic tail is more soluble than **8**, and it was successfully administered via IP injection and validated to be effective in the prostate cancer cell PC-3 xenograft model.[20] However, **8** was not studied in vivo due to low solubility.[21] Thus, structural modifications of **8** to increase its solubility while maintaining its potency is desirable.

Compound **9** contains a quinoxaline ring, which is a prominent scaffold to inhibit ABC transporters as discussed above for the quinoline in **4** (Section 2.4.4). Compound **9** and its analogs may also possess similar biological activities to overcome MDR mediated by ABCB1 or ABCG2, warranting further evaluation. Interestingly, **9** was also predicted to be a substrate of ABCB1 following an analysis using an online tool that predicts drug-likeness and ADME parameters (http://www.swissadme.ch/). Further studies are necessary to determine if **9** and its analogs are substrates or inhibitors of ABC transporters and caution needs to be exercised during its development for potential ABC transporter-mediated resistance.

2.5.4. Perspectives.—Because **7** has an appropriate cLog *P* value of 3.92 (Figure 13A) and it has been successfully administered in an animal model, it has potential for further preclinical evaluation. On the other hand, **8** is more lipophilic (Figure 13B) with possible solubility issues due to its rigid structure as discussed above. Interestingly, **8** has a lower cLog *P* compared with **7**, suggesting that its solubility could be easily improved. For example, the two nitrogen atoms in the pyridine with a pK_a values of 1.4 and 1.1 for N^1 and N^2,[193] respectively, could be used to convert **8** into organic salts using HCl or MSA (**91** or **92**, Figure 13D) without structural modification to increase its solubility. It is, however,

noteworthy that both nitrogen atoms are weak bases and this approach may be difficult. Nevertheless, given its high potency and low molecular weight, **8** may serve as a lead compound for structural modification designed to increase its solubility as discussed below.

Linking 8 with solubilizing moieties such as dimethylaminomethyl group (R_1) may increase its polarity and allow facile transformation into organic salts using HCl or MSA (**93**, Figure 13E) with improved aqueous solubility.[194] Additionally, introducing hydrophilic groups on the benzene ring, such as a carboxyl group (**94**) and its sodium salt (**95**) (Figure 13E) may also increase its solubility although such modifications may compromise membrane permeability.

It was shown previously that **7** can work as the prodrug of **8**,[21] thus, versatile solubilizing moieties can also be linked with **8** via either imine or hydrazone bond at the carbonyl group.

Although **9** is a lipophilic compound and has a cLog *P* of nearly 6 (Figure 13C), it was successfully solubilized in corn oil containing 10% DMSO and was successfully administered into mice via oral gavarge.[19] Clearly, an optimized drug delivery system is required for this lipophilic compound. In addition, **9** has IC_{50} values ranging from 3 to 5 μM in prostate cancer cells. Thus, its potency needs to be improved. Considering that **9** has two strong electronegative F groups and that the other analogs of lower potency possess either one F or electro-positive groups (e.g., methylenedioxy in **88**, methoxy in **89**, or *iso*-butyl in **90**), it remains to be determined if the two unsubstituted benzene rings or with other electronegative substituents could improve potency. A thorough and systematic SAR study is needed to evaluate optimal substituents on the two benzene rings. The potential modifications include substituents of electronegative groups, the types, position, and number of electronegative groups.

2.6. Compound 10 (LLP3) and Its Analogs.

In 2007, Abbott Laboratories conducted a nuclear magnetic resonance (NMR)- and affinity-based screening of their libraries for compounds that can bind to survivin and identified a hit compound 96 (Abbott 1), composed of three linked rings (I–III, Figure 14A).[195] Compound **96** is insoluble and, thus, follow-up structural modifications to rings I and III were conducted, leading to the identification of **97** (Abbott 8, Figure 14B/C) which was shown to bind to the dimeric interface of survivin using NMR while a K_d of 75 μM was determined using an enzymatic assay.[195]

A series of **97** analogs modified with NCGs as solubilizing moieties were subsequently synthesized and studied (Figure 14D), leading to the identification of several compounds with adequate water solubility and cell permeability.[195] While some of these compounds possessed a high affinity for survivin with a K_d of ~40–90 nM in in vitro assays, cell-based anticancer activity and effect on survivin stability or in vivo efficacy have yet to be published.[195] Using **96** and **97** as the starting scaffold, additional analogs linking various functional groups on the two benzene rings (I and II) were also synthesized and tested in cell-based assays, leading to the identification of two active compounds, including **10** (LLP3) and **98** (LLP9) (Figure 14E) with enhanced affinity toward the dimeric interface of survivin due to extended benzyloxy moieties.[196,197] A series of new **97** analogs with a

fused three-membered ring (Figure 14F) has also been synthesized and tested.[198] However, no derivative from this round showed better binding affinity to survivin as determined by the ability to form a compound–survivin complex using recombinant L54 M mutant survivin. Recently, six series including **99–113** (4a-e, 5a-e, and 6a-e) and **11**, **114–124**, (4a′–d′, 5a′–d′ and 6a′–d′) of **97** analogs were reported in which the carbonyl group (C=O) was edited to either an amino (NH_2) or thiocarbonyl (C=S) on ring II, as well as different modifications on ring I/III (Figure 14G/H).[199,200] Of these compounds, **108** and **11** were found to be the most potent in suppressing cancer cell proliferation (see below).

2.6.1. Biological Properties.—Compounds **10** and **98** are potent in suppressing the mitosis and proliferation of prostate cancer PC-3 cells.[196,197] Compounds **10** could also synergize with irinotecan in XIAP-associated factor 1 (XAF1)-positive but not p53-mutated cancer cells.[201] However, no further details were provided on whether this synergism was due to targeting survivin.

Compound **108** was found to be the most effective among the 4a-6e series (Figure 14G) in suppressing cancer cell proliferation with IC_{50} values of 4.46 μM, 3.59 μM, and 6.01 μM for PC-3, MDA-MB-231, and HepG2 cells, respectively.[199] In addition, **108** was about 17- to 29-fold more effective against these cancer cells than normal human fetal lung fibroblast WI-8 cells, suggesting a good therapeutic window.[199] Compounds **99–103** possess different ring II elements when compared to **104–113**, and they did not show obvious cytotoxicity at ~50 μM. With IC_{50} values of >100 μM for **99–101** and **103** and IC_{50} values ranging from 30 to 66 μM for **102** in PC-3, MDA-MB-231, and HepG2 cell lines, the anticancer potency of these compounds is poor. The IC_{50} values of **104–107** range from 14.4 to 52 μM and are generally more cytotoxic than **109–113** at 23 to 67 μM, suggesting that the carbonyl group on ring III is necessary for cytotoxicity. Compound **108**, with IC_{50} values ranging from 3.59 to 6 μM, is most potent among all of the tested compounds in these three series and may be more potent than the approved drug 5-FU in these three cancer cell lines.[199] Compound **108** reduced survivin expression, consistent with its disruption of survivin homodimerization as discussed above. However, no data have been provided on inhibition of survivin homodimerization or if the reduced survivin expression is due to proteasome-dependent degradation as discussed above for **7–9**. Furthermore, unlike the compounds **7–9** of homodimerization inhibitors, **108** at 1–10 μM also downregulated the expression of XIAP, cIAP, and Livin, suggesting that it is unlikely a selective survivin homodimerization inhibitor.[199]

Interestingly, after removal of the fluorine substituent on the benzene ring, the resulting compounds **11**, **114–116** (Figure 14H) showed 100-fold higher potency than **99–101**, as revealed in another study.[200] The most potent compound **11** possessed IC_{50} values of 0.42 μM, 0.66 μM and 1.22 μM, in PC-3, MDA-MB-231, and HepG2 cells, respectively, ~5- to 10-fold better than that of **108**, and almost 100-fold more potent than **99**.[200] It is noteworthy that **11** has a much higher IC_{50} value of 193 μM for inhibiting normal WI-38 fibroblast cells, suggesting a good therapeutic window. Compound **11** also induced apoptosis, arrested cell cycle at G2/M and pre-G1 phases of PC-3 cells. Furthermore, **11** at 1, 5, and 10 μM downregulated survivin and Bcl-2 protein levels and induced cleavage of PARP and caspase

7, suggesting induction of apoptosis possibly via both survivin and Bcl-2.[200] However, it is unclear if **11** has any effects on the expression of other IAPs and if reduction of Bcl-2 expression is indirect or an off-target effect. It also remains to be determined if **11** inhibits survivin homodimerization and whether that may contribute to **11**-induced survivin loss. Furthermore, its antitumor effects remain to be validated using in vivo models.

Using NMR, **97** was found to interact with the dimeric interface of both subunits of survivin primarily via hydrophobic interaction between the ring III and Phe101 and Leu6 in one subunit and residue Leu102 and Leu98 in the other, and the ring I contacting the side chains of residues Leu96, Phe93, Val89, Leu104, and Phe86 in the same subunit which donates Phe101 and Leu6 to interaction with the ring III.[195] If this prediction is true, then **97** will unlikely dissociate the survivin dimer due to its binding to both subunits; however, this binding may cause a conformational change in survivin that leads to its degradation.

The three rings on **10**, including the core scaffold 3-cyano-2-pyridone and two benzyloxy groups, are predicted to form three strong π-stacking interactions with Phe86, Phe93, and Phe101 of survivin as determined by Autodock-Vina, with a docking score of −11.3 kcal/mol which is slightly lower than **98** with a score of −10.6 kcal/mol.[197]

2.6.2. Medicinal Chemistry.—Compound **97** derivatives constructed with various three-membered rings (Figure 14F) have been synthesized and tested.[198] However, no derivative showed better binding affinity to survivin as determined by their ability in forming compound-survivin complexes using recombinant L54 M mutant survivin.[198] Additional **97** analogs have been reported, but biological data have been described only for **108** and **11**.[199,200] Thus, we can only extract useful information from **10**, **11**, **99**, and **108** for a critical SAR analysis of **97** as shown in Table 10.

2.6.3. Key Structural Properties.—The relationship between the structures and related pharmacological study of the **97** series compounds are largely unavailable due to lack of consistent cell-based assays or direct evidence of on-target validation. However, **97** presented solubility issues[195] and, thus, effort was made to introduce hydrophilic moieties into the molecule, as shown in Figure 14D.[195] While compounds with higher binding affinity to survivin were found, no detailed physicochemical properties and biological activities of these derivatives, e.g., cytotoxicity and their effects on survivin, were reported.

The finding of significant improvement in potency from **99** to **11** by removal of the fluorine substituent is very intriguing. Usually in the structural modification of a hit compound, the addition of a fluorine substituent improves affinity and thereby bioactivity,[202] as represented by **9**,[19] although there are also many examples of erosion due to the addition of a fluorine substituent.[203] However, **11** with fluorine has a predicted binding energy of −8.5 kcal/mol,[199,200] compared with −4.9 kcal/mol for **99** that has a fluorine[199] as both determined using Molecular Operating Environment (MOE-Dock 2014.09). While this difference in binding energy requires practical validation, it suggests that **11** has a higher affinity toward survivin than **99**. Upon examination of the docking results from these studies,[199,200] we found that neither the fluorobenzyloxy in **99** nor the benzyloxy in **11** contributed significantly to survivin binding. Clearly, how the removal of fluorine generates

such a major difference in cytotoxicity and whether this difference is due to off-target effect or a change in binding to survivin remains to be determined. Future studies to investigate these issues are likely important for further improving the selectivity and affinity to survivin.

It is also noteworthy that the predicted binding mode of **108** and **11** to the survivin dimeric interface differs from that of **10**. While the interaction of **10** with survivin was predicted to involve mostly π-stacking,[197] **108** can engage in hydrogen bonding interactions with Thr97 and Leu98,[199] and **11** with Glu40, Lys15, and Arg18.[200] It has been predicted that four residues, Phe93, Glu94, Leu98, and Phe101, in the dimeric interface are essential for hydrophobic interactions between survivin and **108** and **11**.[199,200] These findings are consistent with that of the compounds **7–9** of dimerization inhibitors discussed above.[20] However, structural studies of the interaction between these inhibitors and survivin is required to delineate the detailed binding mode at an atomic level.

2.6.4. Perspectives.—On the basis of the SAR information associated with **97**, **108**, and **11**, it is reasonable to conclude that ring II is necessary for the activity and that the other two rings are tolerant of modification. Figure 15A–C shows that **97** could serve as a lead for further modification, including the addition of hydrophilic motif/solubilizing moieties to improve its solubility, and **11** appears to be a good candidate for further evaluation. However, **10**, due to its high molecular weight (535.00) and unfavorable cLog *P* value (6.94), may have issues with solubility and PK profiles, making it a less favorable candidate for further study.

Therefore, we propose the synthesis of molecular hybrids of **10** with **108** and **11** using **11** as the core structure as described below. Molecular hybridization is a feasible and prevalent method in medicinal chemistry and drug design, which fuses two pharmacophores or key structure features of two different drugs into one molecule that usually possesses all merits of its parent drugs.[204,205]

First, **108** with a methoxy group is more potently cytotoxic than **104–107** with different substituent patterns on ring III. Ring III in **11**, and **114–116** does not incorporate a methoxy group. Thus, we propose to add a methoxy group to ring III of **11** at different positions including *para*-(**125**), *meta*- (**126**) or *ortho*- (**127**) (Figure 15D). Similarly, other common electron-donating groups that have been used among approved drugs, such as $-C(CH_3)_3$ (**128–130**), or $-N(CH_3)_2$ (**131–133**), could also be considered.

Second, it is known that **10** with two benzyloxy groups on ring III is more potently cytotoxic than with either one or no benzyloxy group. Thus, adding two benzyloxy groups into ring III, generating **134**, may increase the potency of **11**.

It has been shown that Cl and OH substituents on ring I of **10** show higher cytotoxicity than those without these two substituent.[195] However, in **11**, no such substituents were linked on ring I. Thus, fusing the scaffold on ring I of **10** with **11** via introducing both Cl and OH to ring I, generating **135** or **136**, may help further enhance the potency of **11**.

Finally, because **11** has a high cLog *P* value, indicative of poor water solubility, transforming it into a salt with HCl (**137**) or MSA (**138**) may improve its solubility as discussed above.

3. SUMMARY AND FUTURE PERSPECTIVE

In this perspective, we have analyzed the medicinal chemistry of **1** to **11**. Although **1** and **2** have been tested in phase I/II clinical studies, defining them as survivin inhibitors may be inappropriate and misleading because their true targets are not survivin nor do they selectively inhibit survivin expression. However, survivin may be used as a biomarker to determine their biological effects. Because of the unsuccessful outcome of the clinical trials with **1** or **2**, it is tempting to speculate that targeting the ubiquitous transcription factors such as Sp1 may not be feasible in suppressing survivin expression for clinical therapy. One possible caveat is the simultaneous inhibition of other downstream target genes that may counteract the reduction of survivin expression and rescue cancer cells from survivin downregulation-induced apoptosis. Similarly, **3** is also a multitargeting compound that can downregulate survivin expression but does not bind directly to and inhibit survivin protein.

Compound **4**, however, was identified using the crystal structure of SMAC-XIAP BIR3 domain (PDB 1G73),[83] and it is reasonable to predict that **4** may directly bind to the survivin protein. However, it may also bind to the BIR domains of other IAP members. Indeed, it affects survivin expression and the expression of other IAPs although to a lesser extent compared to survivin. Compounds **7–9**, however, were designed to target the hydrophobic dimeric interface of survivin to induce survivin degradation in the proteasome. They have been shown to bind directly to survivin and are selective for survivin without any effect on other IAPs. Therefore, these compounds are considered to be selective survivin inhibitors. Compounds **10** and **11** also fall into this category although it is unclear if their binding to the dimeric interface inhibits survivin dimerization and/or causes survivin degradation. Nevertheless, these inhibitors that target the dimeric interface of survivin identified using two different approaches deserve to be considered survivin inhibitor leads for more in-depth studies and development. Future studies focusing on the binding modes of these inhibitors to survivin at atomic level in structural studies in combination with medicinal chemistry optimization will likely lead to break-through findings that can assist further design and discovery of selective survivin inhibitors for development.

From the perspective of a medicinal chemist, **1** may be of little value for further laborious modification, but rather for minor changes as proposed above to improve its PK profile. One critical pitfall for **2** and its analog **37**, other than promiscuity, is the poor quality as a lead compound. The simple structure, the paucity of active and functional groups, as well as a lack of feasible/available synthetic methodology for further modification render it a challenging candidate. Compound **3**, a later-confirmed DDR, but not survivin, inhibitor, can be further improved in potency and solubility via medicinal chemistry optimization, as discussed above. Compound **6**, a third generation **4** analog, appears to be an optimized lead for preclinical studies due to its improved potency and metabolic stability. Modifications to simplify the linker may help decrease the complexity and improve its potency. Finally, given that **8**, **9** and **11** are direct survivin inhibitors, as discussed above, more medicinal chemistry

effort to improve their potency, solubility, and drug-likeness along with a thorough and systematic SAR analysis of each functional group are necessary.

While targeting survivin transcription was initially thought to be appropriate if its promoter DNA sequence could be selectively targeted since survivin was considered “undruggable”, recent successes in targeting survivin dimerization or its interaction with its binding partners suggest that it is possible to develop inhibitors targeting the survivin protein directly. The approach in inhibiting survivin homodimerization leading to its degradation is interesting considering that the strategy in targeted protein degradation (TPD) including the technology of proteolysis-targeting chimeras (PROTAC)[206,207] and molecular-glues[208] are gaining rapid traction with nearly 50 TPD agents currently undergoing clinical evaluation.[206,207] It is noteworthy that the advantage of the compounds **7–9** in targeting the homodimerization interface to induce survivin degradation is that it does not require any moiety to recruit E3 ligase to initiate proteasome-dependent degradation of survivin. Future studies in this direction are warranted to selectively induce degradation of homodimeric target proteins in general and inhibit survivin protein specifically for further development.

On the basis of the above critical evaluation of targeting survivin over the past two decades, we envision that the future discovery and development of survivin inhibitors may need to focus on in-depth systematic SAR study and structural optimization of the existing true survivin inhibitors, as described above. Other foci are envisioned to include deciphering detailed interactions between inhibitor(s) and survivin at atomic level and more biological and pharmacological studies of the roles and modes of action of survivin in cancer cells to assist drug discovery targeting survivin. Development of PROTACs that selectively induce survivin degradation is thought to be achievable and may open a new area of study. Finally, the successes in targeting survivin homodimerization may inspire similar studies using the same approach targeting other “undruggable” proteins that exist and function as homodimers.

As discussed above, while a few inhibitors targeting the survivin protein rather than its upstream transcription factors have been tested for their cytotoxicity and effect on survivin, only **7**, **81**, and **97** and its analogs have been shown to directly bind to survivin. However, no study has been conducted to investigate the detailed interaction between survivin and the inhibitors with the exception of **97**. Thus, one of the future challenges in the discovery and development of survivin inhibitors is to generate atomic structures of survivin bound with these inhibitors using X-ray crystallography, Cryo-EM, and/or NMR. The structural information generated from these studies will likely help propel further optimization and guide optimization to more potent and selective survivin inhibitors.

There are also many open questions regarding the biology of survivin in its mechanism of action and specific residues involved in binding to its partners. The new information generated from these studies will likely assist design of appropriate assays to screen for survivin inhibitors and approaches for selectively targeting survivin. In addition, the survivin inhibitors discussed here may serve as chemical probes to facilitate decoding the biology of survivin function and its biochemistry, including its dimerization, stability, and interaction with other partners.

The finding that disrupting survivin homodimerization led to survivin destruction, as successfully shown using **7–9**,[20] suggests that it is reasonable to develop PROTAC or molecular glues that may eliminate the survivin protein more effectively. PROTAC or molecular glues act by hijacking a ubiquitin E3 ligase to mediate targeted protein degradation.[209] These molecules have considerable potential to eliminate "undruggable" protein targets such as survivin.[210,211] Many promising molecules generated using the PROTAC technology have been developed with about 50 currently in clinical trials.[206,207,212–214] Given the availability of survivin-targeting small molecules, including **4** and **7** to **9**, such a PROTAC targeting survivin is not out of reach in the near future.

The successful identification of **8**, **9**, and **4** may inspire studies on targeting survivin homodimerization and its interaction with binding partners. It may also inspire studies on other "undruggable" proteins that exist as homodimers and/or function by interacting with other proteins similarly to survivin. Because these "undruggable" proteins have no known enzymatic activity and lack active centers/pockets that could easily be targeted, inhibiting homodimerization to destabilize them or inhibiting their interaction with binding partners may represent a superior strategy to selectively eliminate them or disrupt their interaction with their partners. However, in many cases such as survivin, we lack an understanding of how these target proteins interact with their binding partners and whether they bind to different partners at different domains of the target protein. The compound that inhibits target protein binding to one partner may not inhibit its interaction with another. However, eliminating these target proteins by disrupting their homodimerization, as demonstrated here for survivin, will affect all downstream effects. It is also noteworthy that eliminating a target protein may result in compensatory changes in survival mechanisms of cancer cells, leading to resistance to such inhibitors. Considering that survivin is a member of the IAP family, eliminating survivin following long-term treatment with homodimerization inhibitors may result in resistance due to possible compensatory upregulation of other IAP family members.

HTS helps to facilitate the identification of hit compounds and may be used for targeting homodimerization or interaction of a target protein such as survivin with its binding partners. While such a HTS assay may be developed by adopting the Alpha assay which has been successful for inhibiting heterodimerization, it is very challenging to develop such a functional assay for HTS targeting homodimerization. Virtual screening to identify top-scoring homodimerization inhibitory compounds, which can then be used for testing using low throughput assays such as nondenaturing PAGE and mammalian two-hybrid assays, should be able to help overcome the lack of functional HTS assays as demonstrated for the discovery of **81**.[20]

In conclusion, one of the major highlights among many drug-discovery lessons learned over the past half century is the difficulty in translating new targets into new drugs which are plagued with a high failure rate in every step including (1) identification of hit compounds, (2) structural modification and optimization, (3) cell- and animal-based PK/PD and therapeutic evaluation, (4) further structural modification for optimization, and (5) clinical trials.[215] These challenges are further compounded when targeting an "undruggable" protein such as survivin. It is noteworthy that ~80% of the human proteome are "undruggable" despite the fact that many of them have been shown to be ideal targets

using molecular approaches.[216,217] Developing novel strategies targeting these proteins will likely result in breakthroughs for drug discovery and development. Different strategies attempted in targeting survivin protein or its expression regulators as discussed here provide valuable information and lessons learned on targeting “undruggable” proteins in general, which will help future drug discovery targeting other “undruggable” proteins with a true survivin inhibitor that will prevail in benefiting cancer patients in the foreseeable future.

ACKNOWLEDGMENTS

This work was supported in part by the NIH grant R01 GM127656 (JYL).

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Jing-Yuan Liu received her Ph.D. in Structure Biology from Indiana University School of Medicine in 2004. Dr. Liu worked as a postdoctoral fellow studying Computational Biology and Bioinformatics in the Department of Medical & Molecular Genetics followed by appointment as Assistant Professor of Computer and Information Science at Indiana University Purdue University of Indianapolis and of Pharmacology and Toxicology at Indiana University School of Medicine. She is currently Associate Professor of Medicine at the University of Toledo College of Medicine and Life Sciences. Her research mainly focuses on understanding the interactions between protein and protein or between protein and small molecules for drug discovery using bioinformatics and computational approaches and then validate those findings using biophysical and biochemical methods and/or patient data mining.

Jian-Ting Zhang obtained his Ph.D. in Molecular and Cell Biology from the State University of New York at Buffalo in 1989 followed by a postdoctoral training in Experimental Therapeutics at Ontario Cancer Institute/Princess Margret Hospital affiliated with the University of Toronto. He was appointed Assistant Professor of Physiology and Biophysics in 1993 at University of Texas Medical Branch and Associate Professor of Pharmacology and Toxicology in 1998 at Indiana University School of Medicine, and later promoted to Professor in 2003 and Andrew and Peggy Thompson Chair in Hematology/Oncology in 2007. Currently, Dr. Zhang is Professor and the Chair of the Department of Cell and Cancer Biology and the McMaster Endowed Chair in Biochemistry at the University

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ABBREVIATIONS USED

AKT	protein kinase B
Alpha	amplified luminescent proximity homogeneous assay
AML	acute myeloid leukemia
Apaf-1	activating factor 1
BBB	blood-brain barrier
BIR	Baculovirus IAP Repeat
BIRC	BIR containing
BRUCE	BIR repeat-containing ubiquitin conjugating enzyme
C	carbon atom
CARD	caspase recruitment domain
ccRCC	clear cell renal cell carcinoma
cIAP	cellular IAP
CDK1	cyclin-dependent kinase-1
CIN	cervical intraepithelial neoplasia
CML	chronic myeloid leukemia
CPC	chromosomal passenger complex
CRPC	castration-resistant prostate cancer
DDX5	DEAD-box helicase 5
DLBCL	diffuse large B-cell lymphoma
EGFR	epidermal growth factor receptor
GBM	glioblastoma
γH2AX	γ-histone H2A
HBA	H-bond acceptor
HBD	H-bond donor
HBXIP	hepatitis B X-interacting protein

HCl	hydrochloric acid
HPV	human papilloma virus
hPSCs	human pluripotent stem cells
IAPs	inhibitor of apoptosis proteins
ILP2	IAP-like Protein 2
ILF3	Interleukin enhancer-binding factor 3
IND	Investigational New Drug
IG	intragastric
IP	intraperitoneal
IV	intravenous
LRR	leucine-rich repeat
ML-IAP	melanoma IAP
MDR	multidrug resistance
MSA	methanesulfonic acid
MTD	maximum tolerated dose
N	nitrogen atom
NACHT	nucleotide-binding and oligomerization
NAIP	neuronal apoptosis inhibitory protein
NCGs	nitrogen-containing groups
NDGA	nordihydroguaiaretic acid
NDH2	Type II NADH dehydrogenase
NHL	non-Hodgkin’s lymphoma
NMR	nuclear magnetic resonance
NSCLC	nonsmall-cell lung cancer
PAGE	polyacrylamide gel electrophoresis
PAKs	p21-activated kinases
PD	pharmacodynamics
PDAC	pancreatic ductal adenocarcinoma
PDX	patient-derived xenograft

PHGs	phenolic NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells
PK	pharmacokinetics
PROTAC	proteolysis targeting chimera
RING	Really Interesting New Gene
RIPK2	receptor-interacting protein kinase 2
rHGG	recurrent high-grade glioma
RNS	reactive nitrogen species
RO5	Rule of Five
ROS	reactive oxygen species
RRMM	relapsed/refractory multiple myeloma
SAR	structure–activity relationship
SLC35F2	solute carrier family 35 member F2
SMAC/DIABLO	the second mitochondria-derived activator of caspases/ direct IAP-binding protein with low pI
STAT3	signal transducer and activator of transcription 3
TKIs	tyrosine kinase inhibitors
TPD	targeted protein degradation
UBA	ubiquitin-associated domain
UBC	ubiquitin-conjugating
VEGF	vascular endothelial growth factors
$t_{1/2}$	half-life
XAF1	XIAP-associated factor 1
XIAP	X-linked IAP

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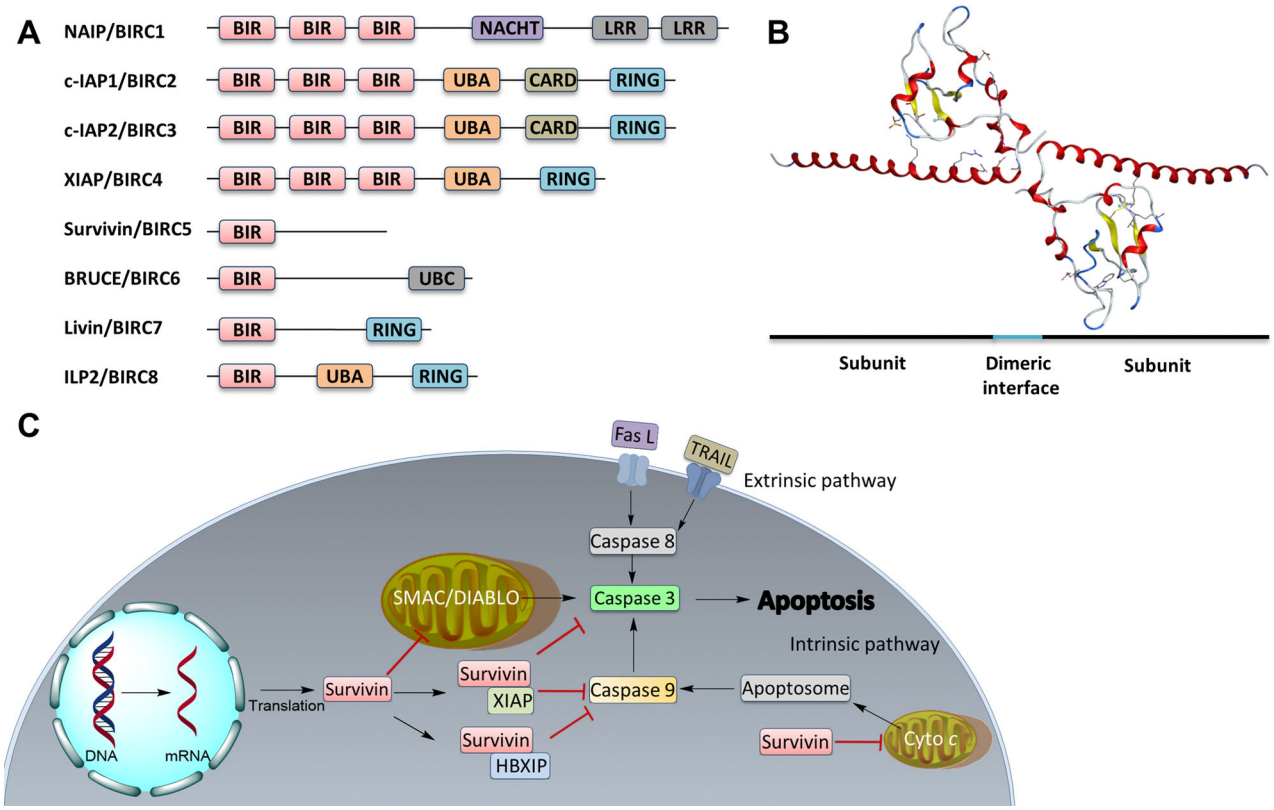


Figure 1.

IAP family members and survivin. (A) Linear domain structures of all eight IAP family members. (B) Crystal structure of survivin as a homodimer (PDB code: 1F3H). (C) Survivin function in extrinsic and intrinsic apoptosis. Fas L, Fas ligand; TRAIL, TNF-related apoptosis-inducing ligand; Cyto *c*, cytochrome *c*.

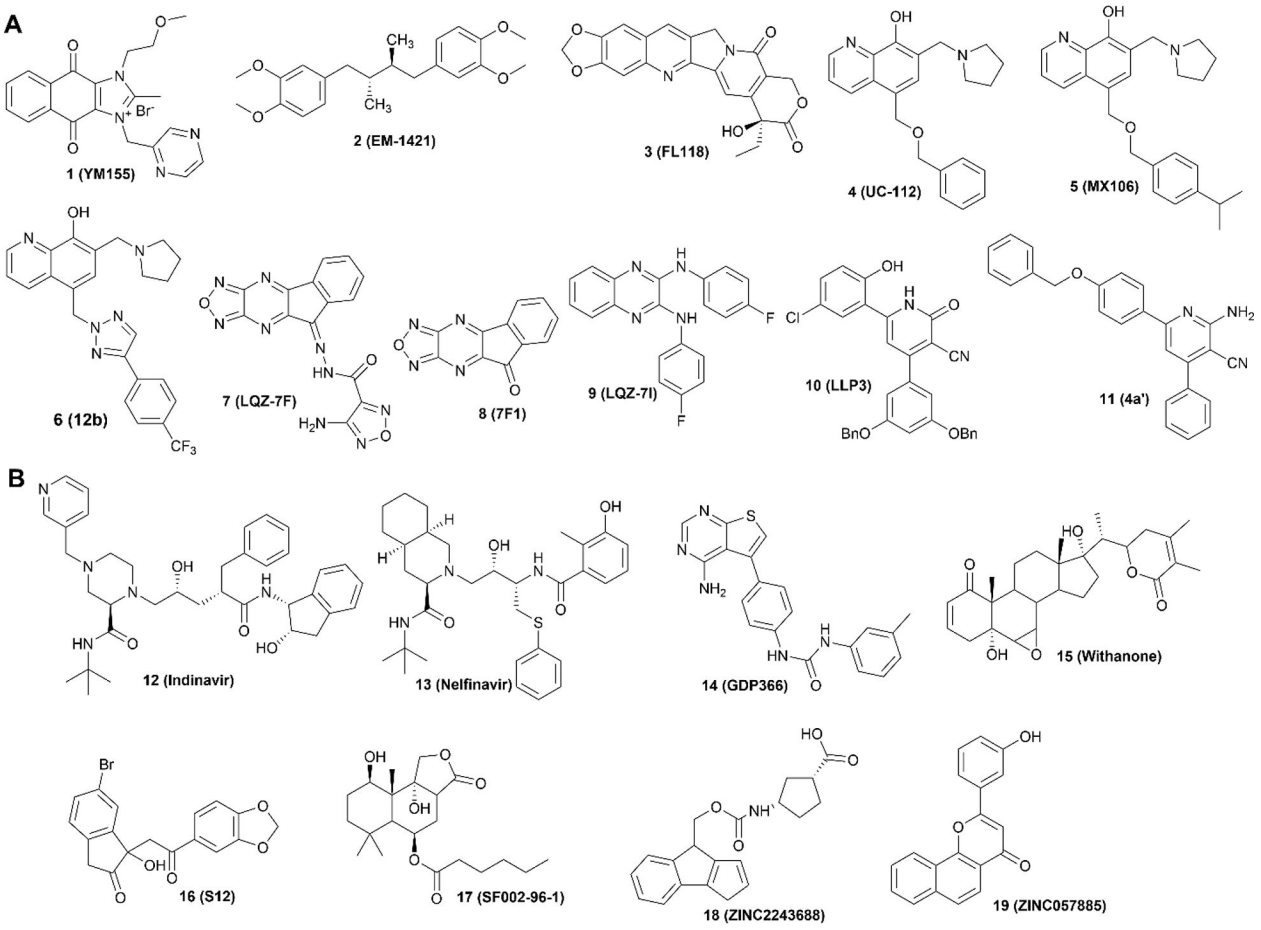


Figure 2.

Structures of small molecule inhibitors that are included (A) in or excluded (B) from discussion in this work.

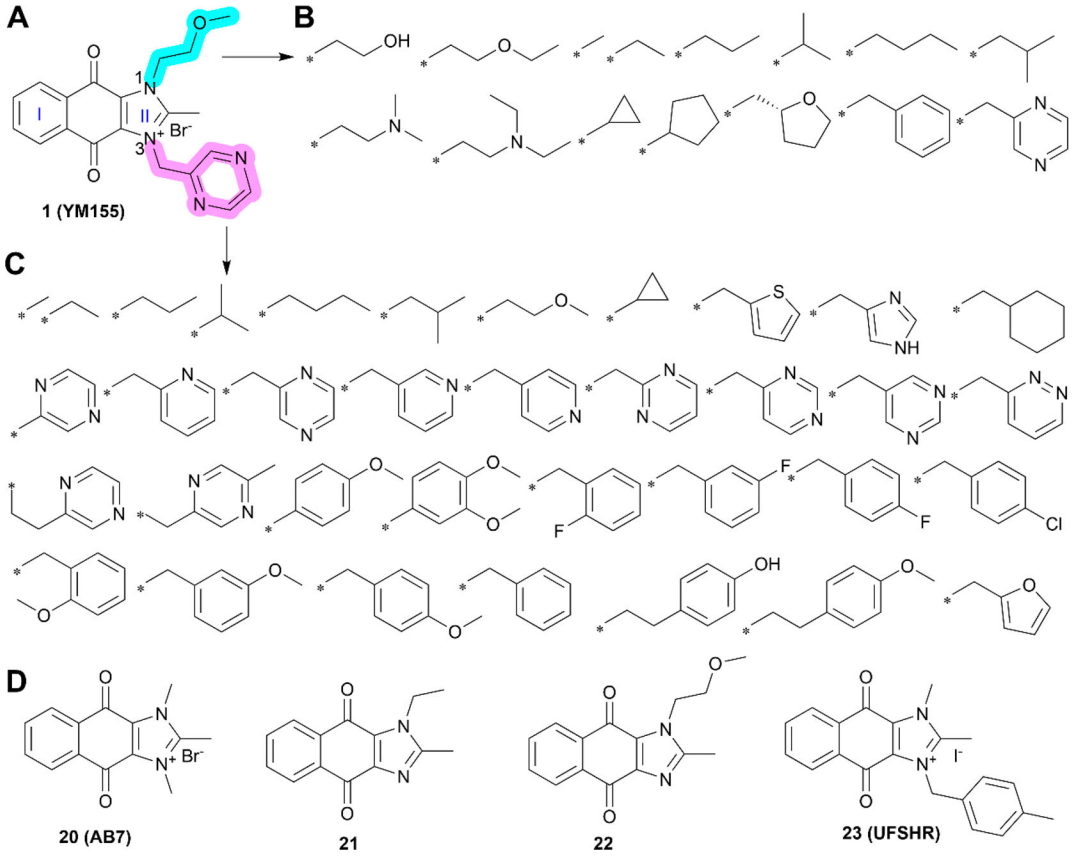


Figure 3.

Medicinal chemistry of compound **1** and its derivatives. (A) Chemical structure of compound **1**. (B–C) Analogs of compound **1** with substitutions on N^1 (B) and N^3 (C) as highlighted in cyan and pink, respectively. (D) Proposed lead derivatives **20–23**.

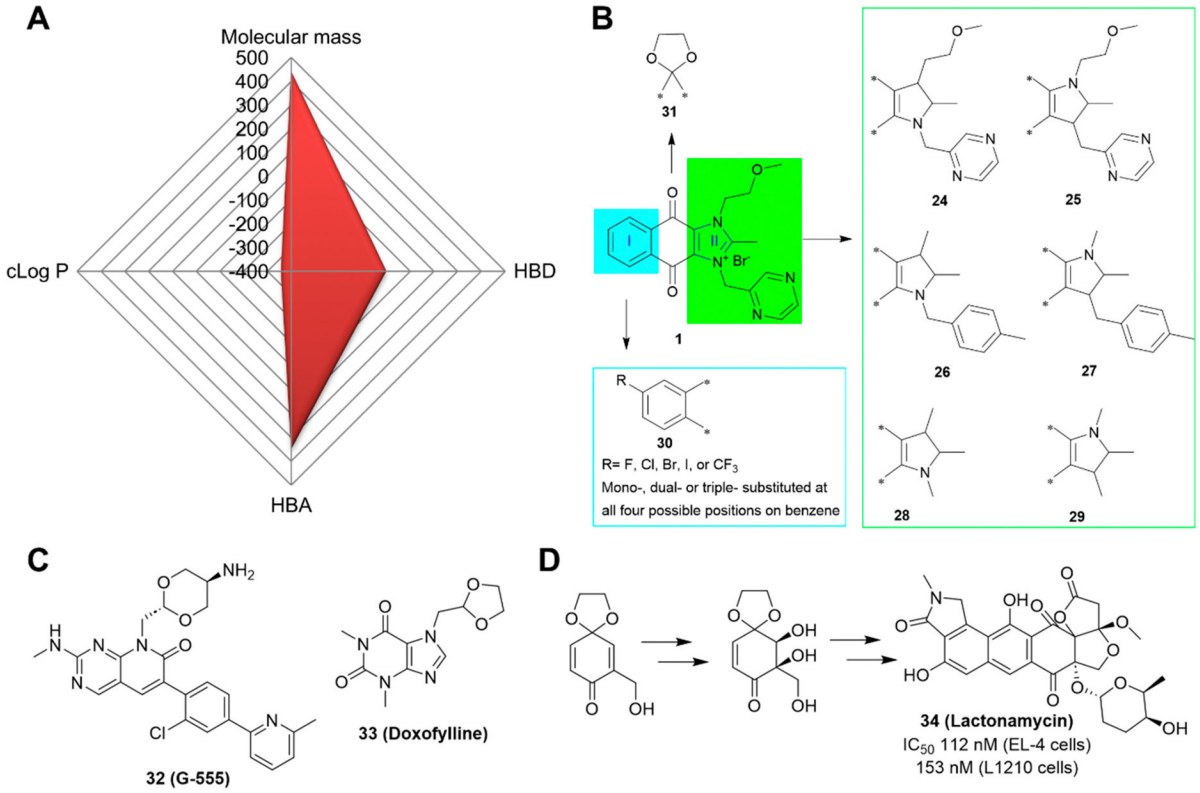


Figure 4.

Perspectives of compound **1**. (A) Radar Chart analysis of **1**. The scale of cLog *P* (calculated by ChemDraw 20.0) and HBD is equivalent to the original value multiplied by 100 and that of HBA multiplied by 50. (B) Proposed modifications of **1**. (C) The structures of compounds **32** (G-555) and **33** (doxofylline). (D) Ketal moiety is used in the synthesis of **34** (lactonamycin).

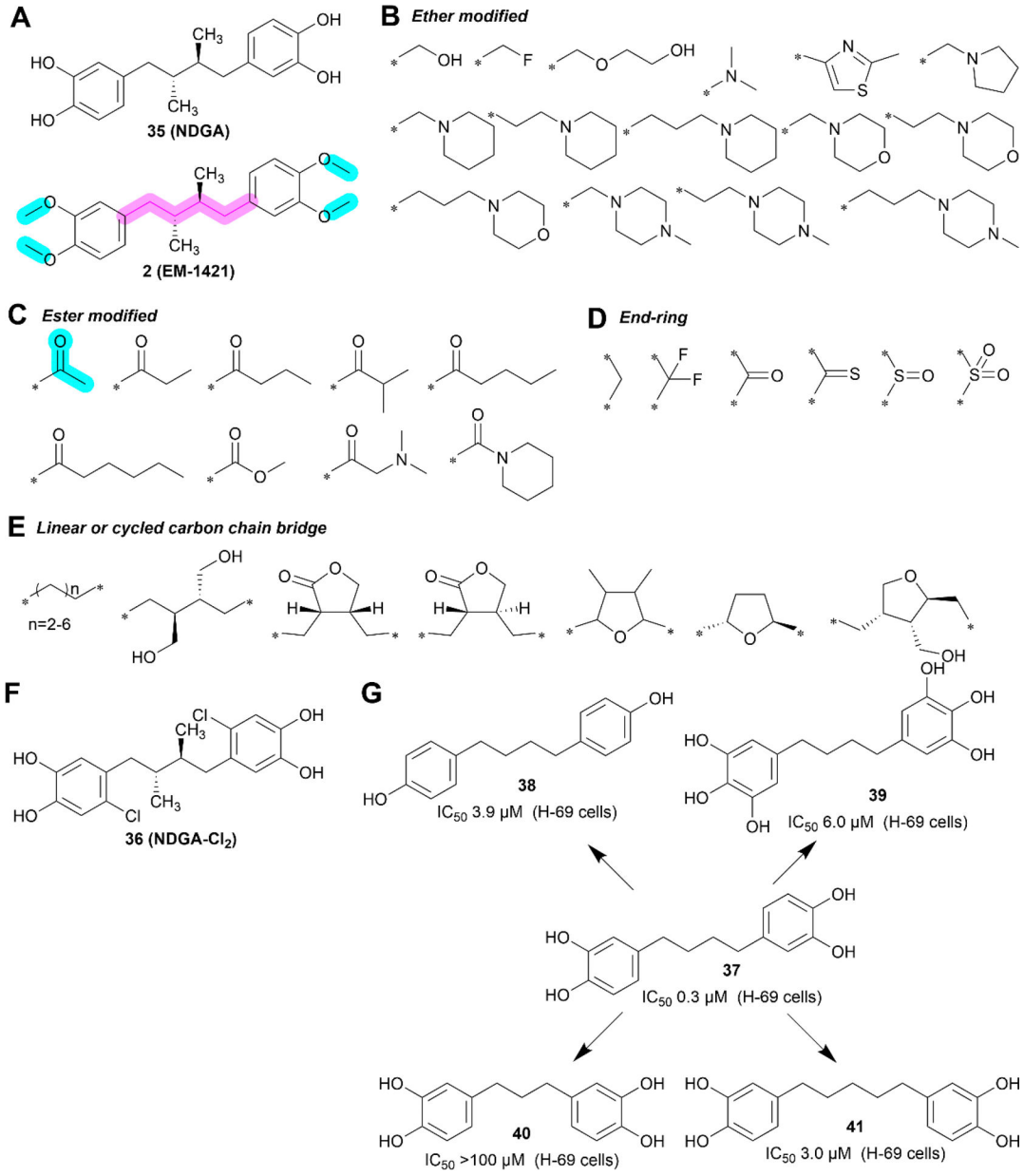


Figure 5.

Medicinal chemistry of **2**. (A) Chemical structures of **35** (NDGA) and **2**. (B–E) Ether bond (B), ester bond (C), end-ring (D), and linear or cycled carbon chain bridge (E) modifications on compound **2**. (F) Chemical structure of **36** (NDGA-Cl_2). (G) Compound **2** analogs with different length of the carbon-bridge or number of hydroxyl groups on benzene rings.

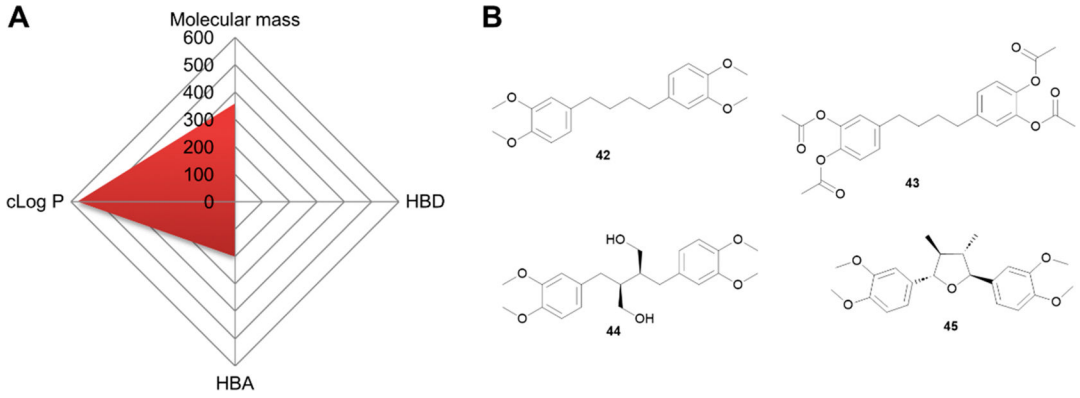


Figure 6.

Perspective of **2**. (A) Radar Chart analysis of **2**. The scale of cLog *P* and HBDs is equivalent to the original value multiplied by 100 and that of HBAs multiplied by 50. (B) Chemical structure of proposed derivatives **42–45**.

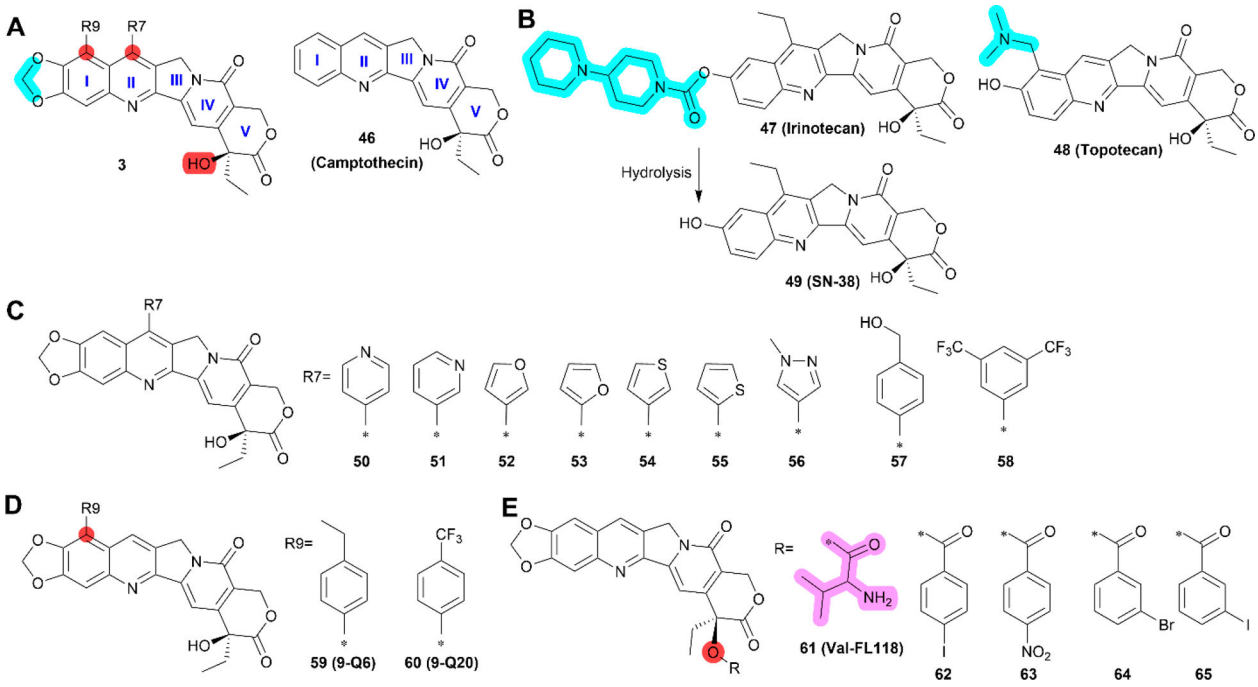


Figure 7.

Medicinal chemistry of **3**. (A) Chemical structures of **3** and **46** (camptothecin). (B) Chemical structures of camptothecin-based topoisomerase 1 inhibitors **47** (irinotecan), **48** (topotecan), and irinotecan metabolite **49** (SN-38). (C) Derivatives **50–58** with modified R7. (D) Derivatives **59** (9-Q6) and **60** (9-Q20) with modified R9. (E) Derivative **61** (Val-FL118) with addition of valine and **62–65** with other modifications at the hydroxyl group. The positions that are tolerable to modification are highlighted as R7 or R9 in claret.

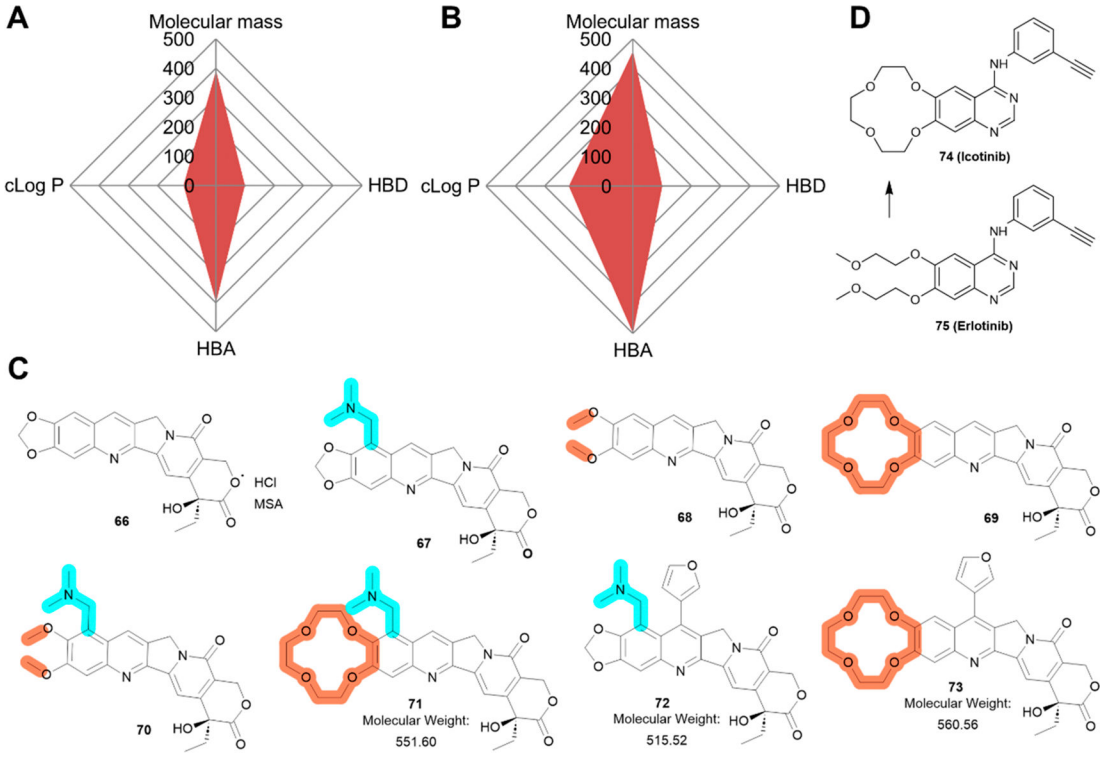


Figure 8.

Perspective of **3**. (A-B) Radar Chart analysis of compounds **3** (A) and **52** (B). The scale of cLog *P* and HBDs is equivalent to the original value multiplied by 100 and that of HBAs multiplied by 50. (C) Proposed modifications/optimizations of **3**. Compound **67** is derived from topotecan; **68** and **69** are inspired by compound **2**, and the discovery of **74** (icotinib), respectively. (D) **74** (icotinib) and **75** (erlotinib).

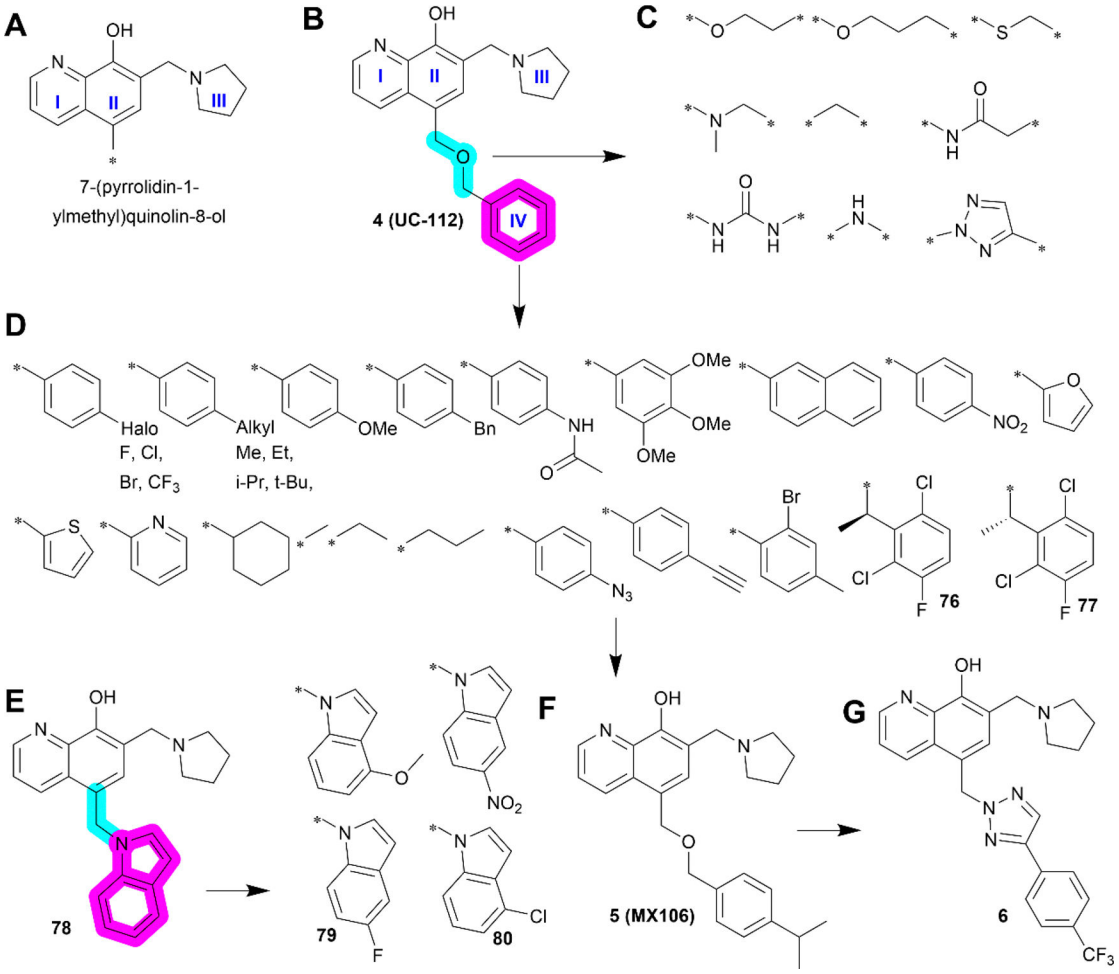


Figure 9.

Medicinal chemistry of **4**. (A–B) Chemical structures of 7-(pyrrolidin-1-ylmethyl)quinolin-8-ol motif (A) and **4** (B). Replacement of the linker and benzene ring IV in **4** with different groups led to **76** and **77** (C and D). (E) Using methyl as a linker and replacing benzene with indole motif led to the discovery of **78** and its analogs **79** and **80**. Further study revealed another lead **5** (F) and **6** (G).

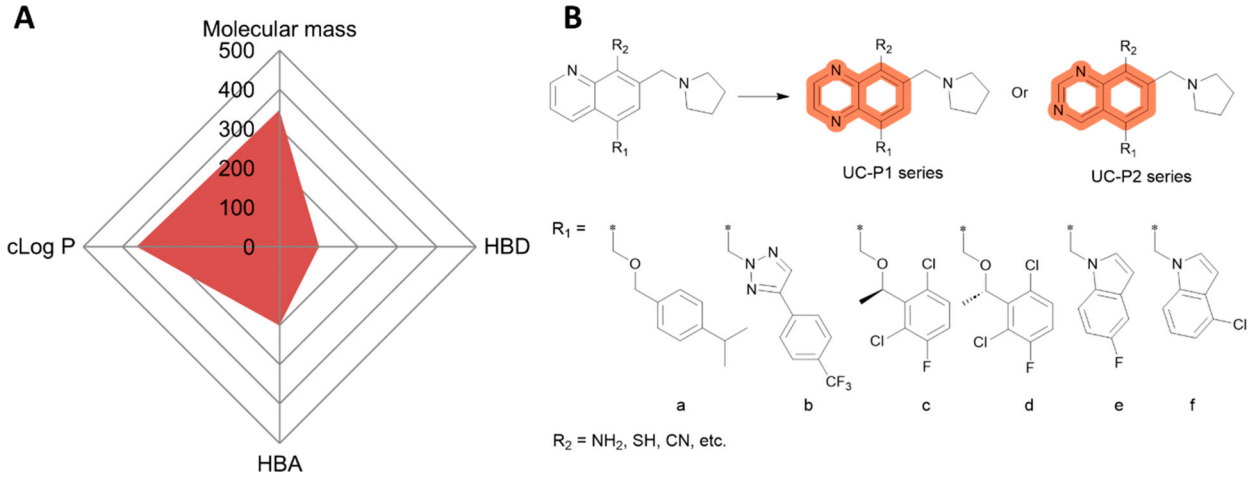


Figure 10.

Perspective of **4** and its analogs. (A) Radar Chart analysis of **4**. The scale of cLog *P* and HBDs is equivalent to the original value multiplied by 100 and that of HBAs multiplied by 50. (B) Proposed modification/optimization of **4**. Quinoxaline (UC-P1 series) or quinazoline (UC-P2 series) are used to replace quinolone and −NH_2, −SH, or CN are used to replace −OH in R_2. Residues in R_1 (a–f) are adopted from **5**, **6**, **76**, **77**, **79**, and **80**.

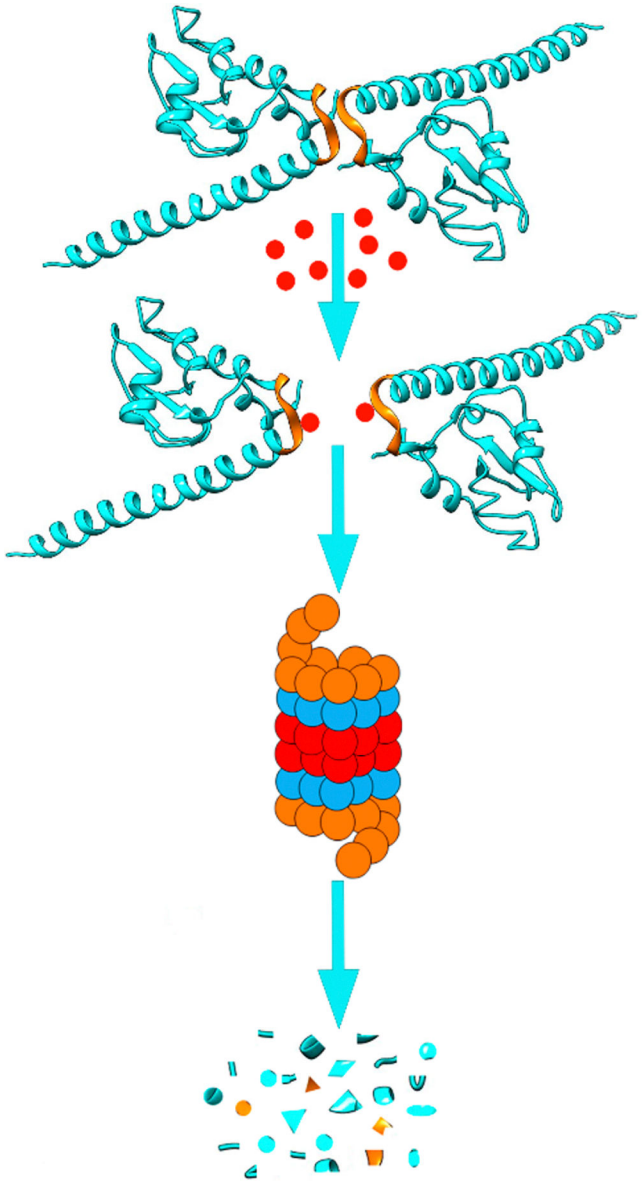


Figure 11.

Scheme of survivin dissociation and degradation in the proteasome. The two identical survivin subunits in a homodimer are shown in blue with the hydrophobic patch in the dimerization interface indicated in gold. The red dot represents inhibitors that bind to the hydrophobic dimerization interface, inhibiting dimerization, leading to destruction of survivin in proteasome.

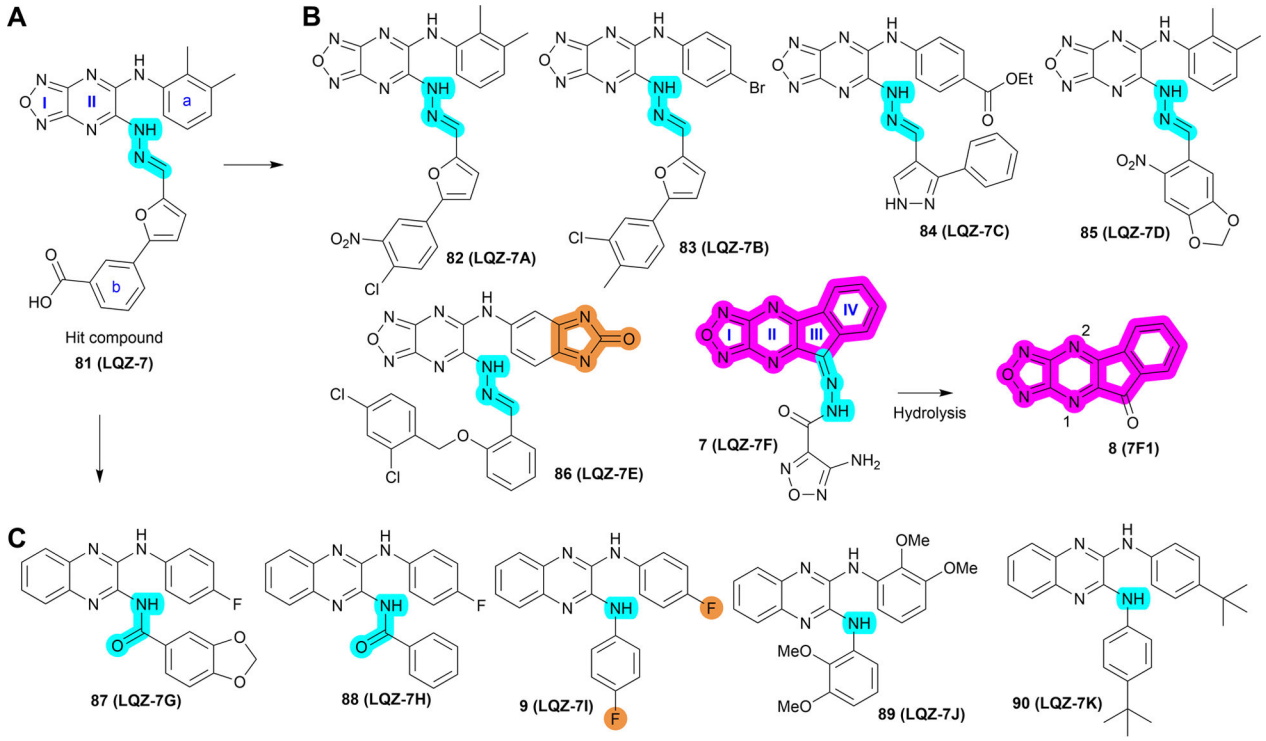


Figure 12.

Medicinal chemistry associated with **81** (LQZ-7). (A, B) Chemical structures of the hit compound **81** and its six analogs including **7** and its hydrolysis product **8**. (C) Chemical structures of **87** (LQZ-7G), **88** (LQZ-7H), **9** (LQZ-7I), **89** (LQZ-7J), and **90** (LQZ-7K) originated from **81**. Labile hydrazone linker and other linkers are highlighted in cyan. **7** has a fused tetracyclic ring. **9** differs from other analogs **87–90** mainly by the substitution patterns on the two benzene rings.

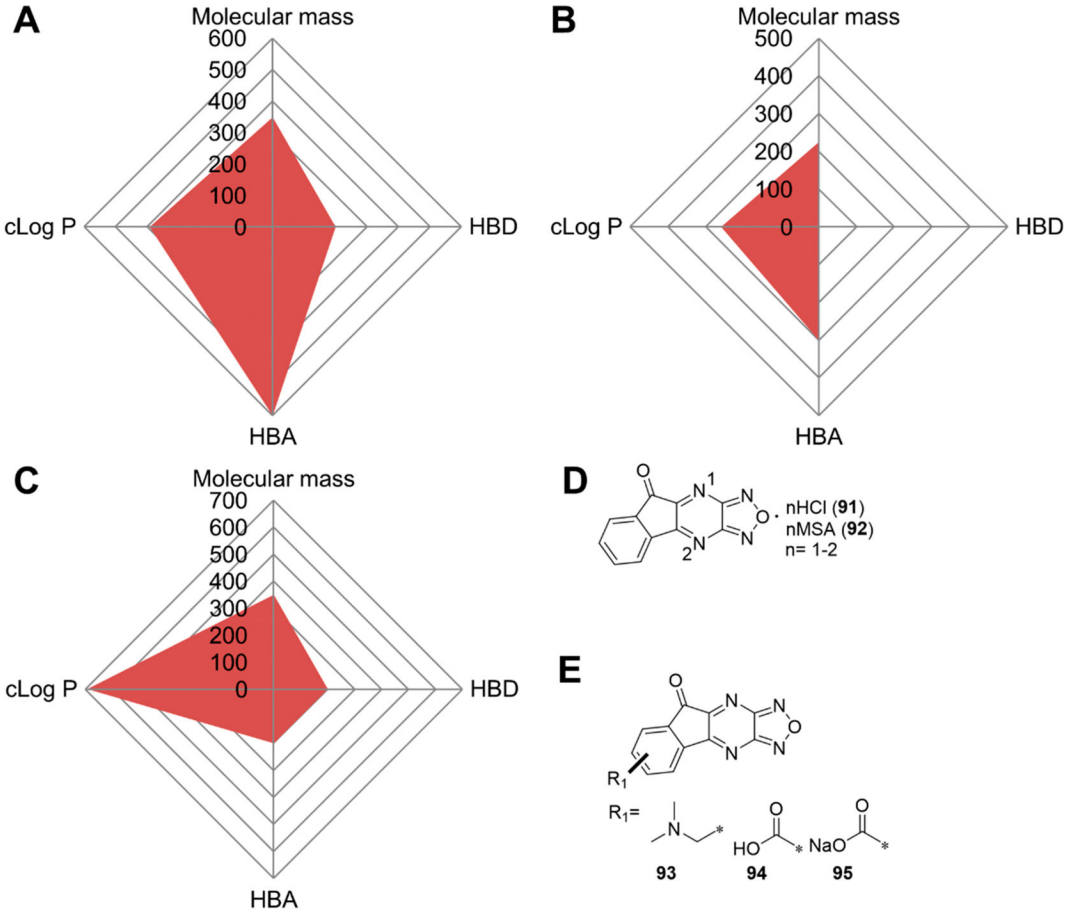


Figure 13.

Perspectives of compounds **7–9**. (A–C) The radar charts of **7** (A), 8 (B) and **9** (C). (D–E) Proposed further structural modifications of 8.

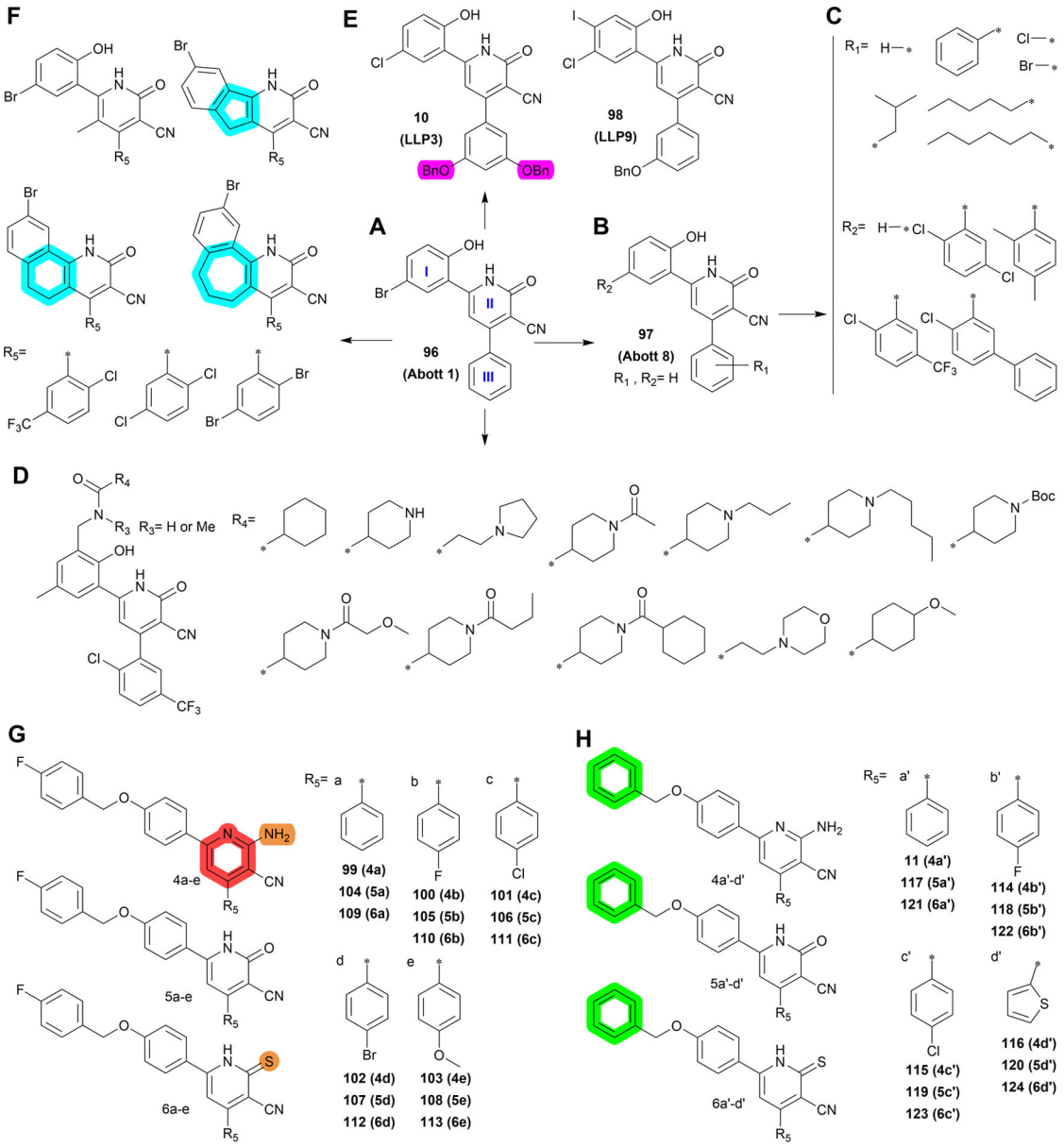


Figure 14.

Medicinal chemistry associated with **97** (Abbott 8). (A-B) Chemical structures of the hit compound **96** (Abbott 1), (A) and its analog **97** (B). (C) Functional groups linked to the two benzene rings on **96**. (D) Hydrophilic moieties that are used to modify ring I. (E) The leading compound **10** (LLP3) and its analog **98** (LLP9). (F) A series of novel fused tricyclic ring analogs. (G–H) Six series of **97** analogs with different substitutions. Bn, benzyl. Key structural differences were highlighted.

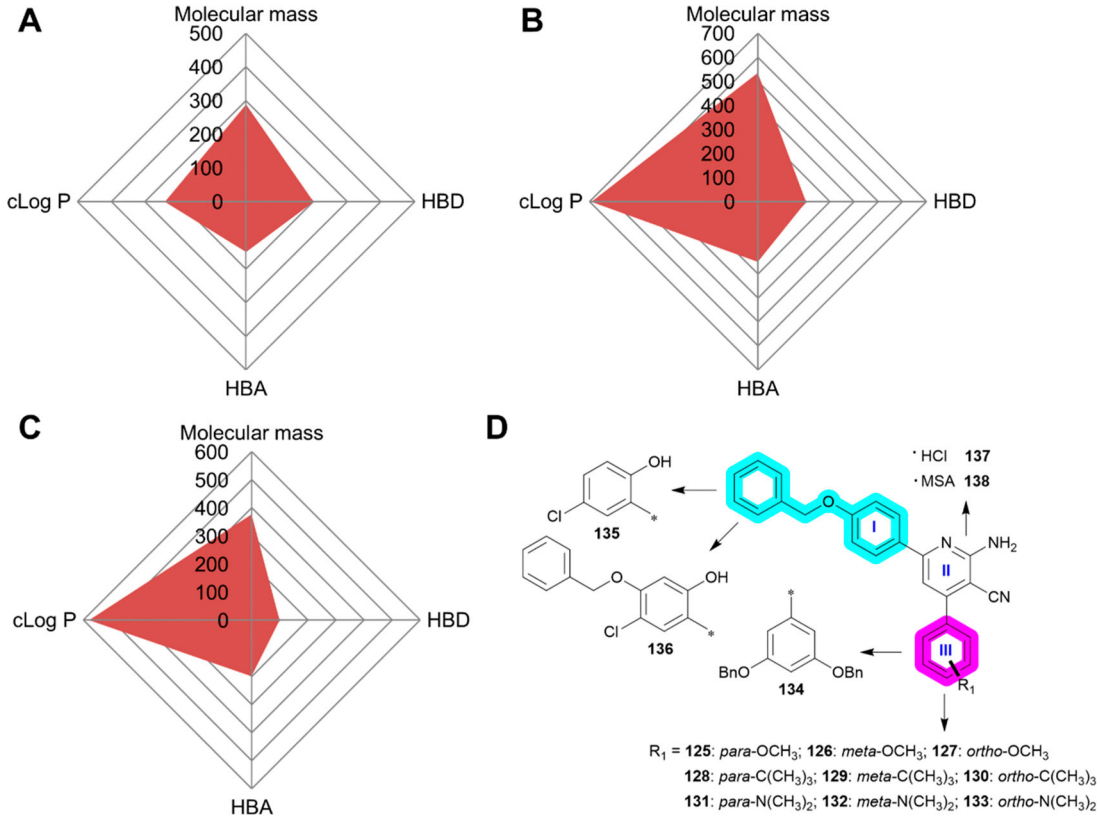


Figure 15.

Perspective of **97** and its analogs. (A–C) The Radar Charts for **97** (A), 10 (B), and **11** (C). (D) The proposed future structural optimization based on leading compounds **10**, **11**, and **108**.

Table 1.

Members of the IAPs, and the Status of Reported Inhibitors

gene name	protein name	year identified	molecular mass (kDa)	inhibitors[a]	development stage
BIRC1	NAIP	1995	160	none reported	
BIRC2	cIAP1	1995	69	SM-1295	Preclinical[7]
				AZD5582	Preclinical[8]
				AT-406	Phase I[9]
				ASTX660	Phase I/II[10]
				Birinapant	Phase I/II[11]
BIRC3	cIAP2	1995	68	SM-1295	Preclinical[7]
				AZD5582	Preclinical[8]
				AT-406	Phase I[9]
				LCL161	Phase I[12]
BIRC4	XIAP	1996	55	MX69	Preclinical[13]
				AZD5582	Preclinical[8]
				GDC-0152	Phase I[14]
				LCL161	Phase I[12]
				ASTX660	Phase I/II[10]
BIRC5	Survivin	1997	16.5	YM155	Phase II[15]
				EM-1421	Phase I[16]
				FL118	IND[b],[17]
				UC-112/MX106	Preclinical[18]
				LQZ-7*F*7F1/7I	Preclinical[19–21]
BIRC6	BRUCE	1998	528	None reported	
BIRC7	ML-IAP or Livin	2000	33	None reported	
BIRC8	ILP2	2001	27	None reported	

[a] Small molecule agents.

[b] IND, investigational new drug.

J Med Chem. Author manuscript; available in PMC 2024 November 25.

Table 2.

Representative Compounds

compounds	mechanism of action	in vitro IC_{50} (μM)	application/clinical trial (year initiated)	SAR[a] status
1 (YM155)	Inhibiting Sp1 and RIPK2[70,71]	0.008 (PC-3), 0.002 (PPC-1), 0.004 (DU145)	Phase I for advanced cancer (2009) and NSCLC (2010)	Limited SAR without better compound identified
	Disrupting ILF3-p54^{nrb} complex[72]	0.008 (TSU-Pr1), 0.011 (22Rv1), 0.004 (SK-MEL-5), 0.006 (A375)[74]	Phase II for CRPC (2007), DLBCL (2007), NHL (2009), melanoma (2009), metastatic breast cancer (2009), high-grade B-cell lymphoma (2022)	
	Inducing DNA damage[73]	0.0005 (NKF-NB-3), 0.0007 (NKF-NB-3)[75]		
2 (EM-1421)	Inhibiting Sp1[76]	16.6 (A375), 61.5 (HT-29), 42.4 (MCF-7) 85.0 (HaCat), 44.5 (HepG2)[77]	Phase I or II for head and neck tumors (2003), CIN (2005), leukemia (2008), refractory solid tumors (2008), lymphoma and rHGG (2015), and high-grade glioma (NCT02575794)	Systematic SAR[b] with a more potent compound **37** identified
3 (FL118)	Inhibiting expression of survivin, Mcl-1, XLAP, cIAP2[78]	0.0003 (H460), 0.0006 (EKVX), 0.0009 (A549)	Preclinical for cancers of head and neck, colon, and pancreas, as well as metastatic lung cancer and RRMM	Limited SAR with a more potent compound **52** (9c) identified
	Inhibiting	0.0004 (HCT116)[80]	Under IND	
	DDX5[79]	0.0005 (HCT-8)[81] 0.037 (A2008), 0.009 (SW620)[82]		
4 (UC-112)	Disrupting SMAC and survivin interaction[83]	1.6 (A375), 2.5 (M14)	Preclinical for P-glycoprotein- overexpressing colorectal and cervical cancers	Systematic SAR with several leads identified
	Inhibiting expression of other IAPs[83]	3.2 M14/LCC6MDR1), 0.7 (PC-3), 3.4 (DU145)[84]		
7 (LQZ-7F)	Inhibiting survivin dimerization and inducing survivin degradation[20]	4.3 (PC-3), 1.9 (C4–2)[20]	Preclinical for CRPC	Limited SAR with **7–9** as candidates
10 (FFP3)	Binding to the dimerization domain[85]	31.2 (GBM528)[c,85]	Preclinical for glioma	Systematic SAR with **10** and **11** as leads

[a] Abbreviations use: CIN, cervical intraepithelial neoplasia. CRPC, castration-resistant prostate cancer. DLBCL, diffuse large B-cell lymphoma. ILF3, interleukin enhancer-binding factor 3. NHL, non-Hodgkin's lymphoma. NSCLC, nonsmall-cell lung cancer. rHGG, recurrent high-grade glioma. RIPK2, receptor-interacting protein kinase 2. RRMM, relapsed/refractory multiple myeloma. SAR, structure–activity relationship. Sp1, specific protein 1.

[b] Systematic SAR is defined as available SAR for each functional group.

[c] Determined by sphere formation assay.

Table 3.

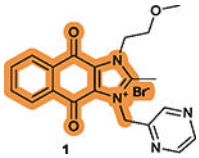
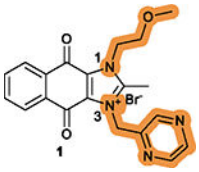
Pharmacokinetic Profiles of Compounds 1, 2 and 3

compounds	species	route	T_{max} [a](h)	$T_{1/2}$(h)	C_{max}(ng/mL)	Cl(mL/h/kg)	AUC (ng × h/mL)	*F* (%)
1 (YM155)	human[92]	IV	ND	26.3	10.7	47 700	1439	ND
	human[61]	IV	45.98	17.18	11.95	34 030	1832	ND
2 (EM-1421)	mouse[95]	IV	0.08	ND	1064.60	ND	2984.54	ND
	mouse[95]	Oral	8.0	ND	441.51	ND	2635.47	88.29
	human[16]	IV	ND	17.1	ND	54 000	ND	ND
3 (FL118)	mouse[81]	IV	0.17	1.8	43	14 287	82	ND

[a]Abbreviations used: T_{max}, the time it takes for a drug to reach the maximum concentration. $T_{1/2}$, the time required for plasma concentration of a drug to decrease by 50%. C_{max}, the maximum concentration in plasma. Cl, clearance rate. AUC, the aera under the curve. F, fraction absorbed (bioavailability). ND, not determined. IV, intravenously.

Table 4.

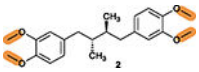
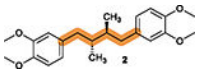
SARs Associated with Compound 1[a]

position/scaffold	SAR
	The intact dioxonaphthoimidazolium scaffold is required; The critical motif on the tricyclic scaffold has an order of quinone > imidazolium > benzene.[104]
	Substitutions at the N^1 and N^3 positions are tolerable; Short, unbranched, and cyclized alkyl substitutions at both N^1 and N^3 are favorable; A pyrazine moiety at N^3 is necessary and usually optimal; Substituting pyrazine with benzene decreases the potency; The combination of substitutions at N^1 and N^3 is complex, resulting in no compounds with higher potency.[103,104]
	Substitutions with electron-withdrawing groups on the benzene ring are optimal, without differences for electron-withdrawing groups at different positions.[103,104]

[a]The highlighted elements are the points of discussion.

Table 5.

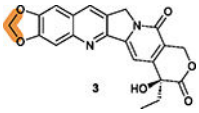
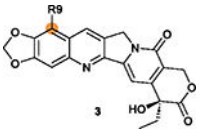
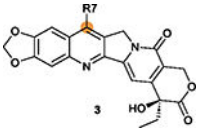
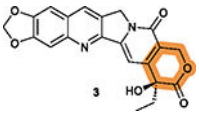
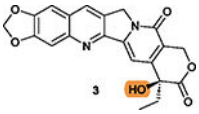
SARs Associated with Compound 2[a]

position/scaffold	SAR
	End-ring modification (ester or ether bond) decreases activity;[77,141] Short chain phenol esters show comparable activity to **2**;[77] The effect of longer chain or other ether bond modifications on activity is unknown; Two phenolic groups on each benzene ring (e.g., **37**) are better than either one (**38**) or three (**39**) such groups.[141]
	A linear carbon chain linker is better than a cyclized linker;[142] The two methyl groups are dispensable;[141] The optimum length of the carbon linker is 4 (**37**) and shorter (**40**) or longer (**41**) one reduces activity.[143,144]

[a]The highlighted elements are the points of discussion.[144]

Table 6.

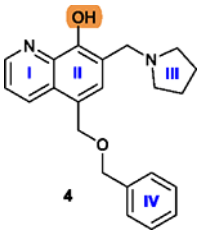
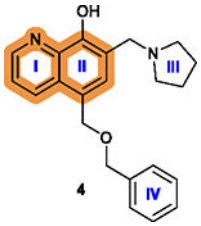
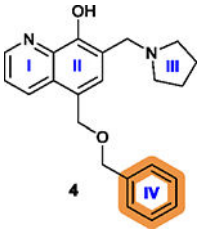
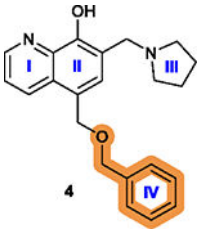
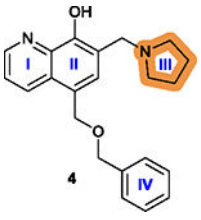
SARs Associated with Compound 3[a]

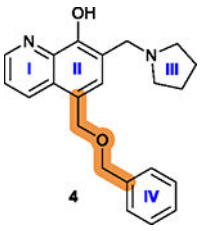
position/scaffold	SAR
	Methylene group is essential for inhibiting DDX5, down-regulating survivin expression and dispensing of topoisomerase 1 inhibition.[78]
	R9 position on ring I is tolerant of modifications with the substituted benzene (R-Ph) ring decreasing cytotoxicity (e.g., **59** and **60**).[164,168]
	R7 on ring II is tolerant of modifications with different rings; Five-membered heterocyclic rings are generally better than a six-member pyridine or benzene ring.[82]
	The lactone ring V may be unstable under acidic or basic conditions that may undermine **3**'s activity and stability.[170]
	The free hydroxy group on ring V is critical for cytotoxicity;[167] Protection by hydrophilic groups via ester bond such as that in **61** and compounds **62–65** reduces cytotoxicity.[167,169]

[a]The highlighted elements are the points of discussion.

Table 7.

SARs Associated with Compound 4[a]

position/scaffold	SAR
	The free hydroxy group is essential and its removal leads to a loss of cytotoxicity.[178]
	The quinolone ring is essential; Adding a halogen atom, replacing ring I with a benzene ring, or removing ring I significantly decreases cytotoxicity.[178]
	Introduction of an electron-withdrawing group increases activity and the increase in the size of substituent from –F to –Br generally increases the activity, although bulky substitutions are unfavorable; Introduction of a hydrophobic group to the para-position is favorable;[18] Replacing the isopropyl group with an azide group or an ethynyl group on the *para*-position is less favorable while a more lipophilic group is slightly more favorable;[178] Replacing the phenyl ring IV with heterocyclic rings is less favorable.[18]
	Replacing the benzyloxy with substituted indole is favorable, with monosubstitution better than di-substitution on the indole moiety and analogs substituted at the 5- or 6-position offering improved anticancer potency.[84]
	Pyrrolidine is the best and most favorable among many substitutions.[83]

position/scaffold	SAR
	Increasing the length between the oxygen atom and ring IV is not favorable;[18] Replacing the oxygen atom of the linker with sulfur is favorable, while a methylamine linker is unfavorable;[18,178] A simple methylene as a linker is favorable;[84] Limited structural modifications to the linker are tolerated in maintaining the efficiency of disrupting protein-protein interactions.[178]

[a]The highlighted elements are the points of discussion.

Table 8.

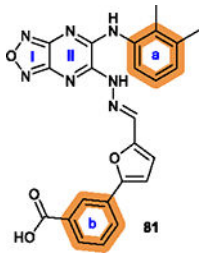
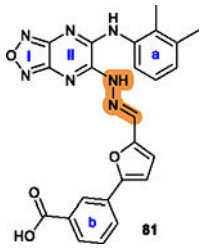
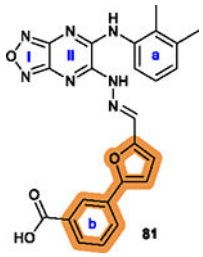
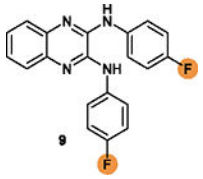
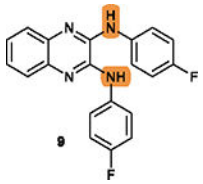
Survivin Homodimerization Inhibitors

			interaction with survivin				
compd	**IC_{50} (μM)**	**apoptosis induction**	**binding**	**dimerization inhibition**	**degradation**	**synergism with docetaxel**	**in vivo activity**
81 (LQZ-7)	20 (PC-3) 25 (DU145)	ND[a]	yes	yes	yes	ND	ND
82 (LQZ-7A)	4.8 (PC-3) 7.2 (DU145)	ND	ND	ND	no	ND	ND
83 (LQZ-7B)	3.5 (PC-3) 3.3 (DU145	ND	ND	ND	yes	ND	ND
84 (LQZ-7C)	3.5 (PC-3) 3.5 (DU145)	ND	ND	ND	yes	ND	ND
85 (LQZ-7D)	2.1 (PC-3) 2.6 (DU145)	ND	ND	ND	no	ND	ND
86 (LQZ-7E)	49.8 (PC-3) 60.3 (DU145)	ND	ND	ND	no	ND	ND
7 (LQZ-7F)	4.3 (PC-3) 1.9 (C4–2)	yes	yes	yes	yes	yes	yes
87 (LQZ-7G)	20.7 (PC-3) 14.3 (C4–2)	ND	ND	no	ND	ND	ND
88 (LQZ-7H)	19.7 (PC-3) 12.7 (C4–2)	ND	ND	no	ND	ND	ND
9 (LQZ-7I)	4.8 (PC-3) 3.1 (C4–2)	yes	ND	yes	yes	yes	yes
89 (LQZ-7J)	24.8 (PC-3) 17.6 (C4–2)	ND	ND	no	ND	ND	ND
90 (LQZ-7K)	18.2 (PC-3) 18.6 (C4–2)	ND	ND	no	ND	ND	ND
8 (7F1)	0.16 (PC-3) 0.17 (C4–2)	yes	ND	yes	yes	yes	ND

[a]ND, not determined.

Table 9.

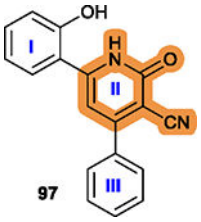
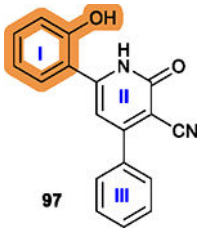
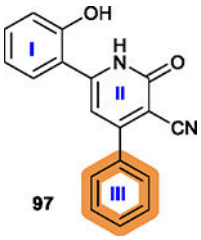
SARs Associated with Compounds 81 and 9[a]

position/scaffold	SAR
	Benzene rings a and b are tolerant of modification with hydrophobic substituents, such as methyl, nitro, halogen or carboxylate ester; Other fused ring structures in a are less favorable.[20]
	The hydrazone linker is unstable and may undergo hydrolysis under acidic conditions;[21] Its replacement with a secondary amine bond is generally favorable.[19]
	The motif of phenylfuran is tolerable to be replaced.[20]
	Two fluorine substituents are favorable for cytotoxicity; Their replacement with electropositive substituent may decrease cytotoxicity;[19]
	The NHs is necessary to form hydrogen bonds with residues in the survivin dimeric interface; Its replacement with OH is less favorable.[19]

[a]The highlighted elements are the points of discussion.

Table 10.

SARs Associated with Compound 97[a]

position/scaffold	SAR
	The intact pyridone ring II and its carbonyl group are necessary for anticancer activity; Replacement of the carbonyl with $–NH_2$ or C=S is generally less favorable.[199]
	Ring I is tolerant of modification; The position *para*- to the OH is more favorable to target survivin; Saturated 5- or 6-membered rings or benzene, benzyloxy ring substituents are favorable for binding affinity.[195,199]
	Hydrophobic groups are preferred; Electron-donating groups like methyl, methoxy groups are favorable;[195,199] Benzyloxy (–OBn) on ring III is favorable.[195,197]

[a]The highlighted elements are the points of discussion.