De-Novo Designed Library of Benzoylureas as Inhibitors of BCL-X_L: Synthesis, Structural and Biochemical Characterization

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Abstract.

The pro-survival BCL-2 proteins are attractive yet challenging targets for medicinal chemists. Their involvement in the initiation and progression of many, if not all, tumors makes them prime targets for developing new anti-cancer therapies. We present our approach based on *de novo* structure-based drug design. Using known structural information from complexes engaging opposing members of the BCL-2 family of proteins, we designed peptidomimetic compounds using a benzoylurea scaffold to reproduce key interactions between these proteins. A library stemming from the initial *de novo* designed scaffold led to the discovery of ligands with low micromolar potency (K_D = 4 μM) and selectivity for BCL-X_L. These compounds bind in the canonical BH3 binding groove in a binding mode distinct from previously known BCL-2 inhibitors. The results of our study provide insight into the design of a new class of antagonists targeting a challenging class of protein-protein interactions.

Introduction.

Programmed cell death (or apoptosis) is a critical biological function in multicellular organisms: the balance between cell death and cell survival, if not closely regulated, has implications in a number of disease states, most notably cancer.[1] Down-regulation of apoptosis allows cells carrying DNA defects to survive leading to cancer growth. The proteins of the BCL-2 family are central effectors of the apoptotic cascade.[2] These proteins can be divided into pro-apoptotic or pro-survival classes depending on whether they promote or repress cell death. The pro-survival clan comprises proteins such as BCL-2, BCL-X_L or MCL-1, and serves mainly to

repress cell death by inhibiting the pro-apoptotic proteins BAX and BAK. A further subgroup of pro-apoptotic proteins, known as the BH3-only proteins, acts upstream of BAX/BAK and the pro-survival proteins. The BH3-only proteins are activated in response to various apoptotic stimuli (such as DNA damage or cytokine withdrawal). By interacting with pro-survival proteins, the BH3-only proteins release the pro-apoptotic activity of BAX and/or BAK leading to release of cytochrome *c* from the mitochondrion, activation of the downstream apoptotic cascade and cell demolition.[3] In cancer, pro-survival proteins such as BCL-2, BCL-X_L or MCL-1 are often found over-expressed leading to suppression of the apoptotic cascade, an event that is now considered a hallmark of this disease.[4] Notably, amplification of *BCL2L1*, the gene encoding for BCL-X_L, is a common mechanism for cancers to evade apoptosis.[5] BCL-X_L has also been shown to drive resistance to many chemo-therapies.[6] It has been proposed that inhibiting the pro-survival proteins such as BCL-X_L with small molecules mimicking the structural and functional activity of the BH3-only proteins would offer a new avenue to treat cancer. A number of BCL-2 inhibitors have been reported but very few are presented with firm confirmation of their binding sites and fewer yet demonstrate mechanism-based activity.[7,8] The majority of the published molecules do not meet the most basic criteria defining the mechanism of action for true BH3-mimetics, which requires BAX/BAK-dependent cell killing activity.[9,10] Peptidomimetic scaffolds attempting to replicate the α-helical structure of BH3-peptides have been described previously by the Hamilton laboratory, who pioneered the terphenyl scaffold displaying sub-micromolar binding affinities for BCL-X_L.[11,12,13,14] The most successful approach to date remains the BCL-2 program at AbbVie where the 'SAR by NMR' strategy was used to initiate a program leading to the discovery of ABT-737 (**1**, Figure 1), a potent inhibitor of BCL-X_L, BCL-2 and BCL-W, which displays K_i values for these proteins below 1 nM.[15,16] Related compounds with improved

pharmacokinetic properties (navitoclax or ABT-263, **2**, Figure 1) or improved selectivity (ABT-199, **3**, Figure 1) have entered clinical trials.[17,18] Other potent BCL-2 inhibitors originating from a structure-based design approach have been reported more recently,[19-22] including WEHI-539 a potent and selective BCL-X_L inhibitor (**4**, Figure 1).[23,24] Despite these more recent advances, the overall number of BCL-2 inhibitors remains small and novel scaffolds are still needed.

Herein we present the use of a *de novo* interactive design strategy to build a library of small molecule α-helix mimetics based on a benzoylurea scaffold targeting the binding interface between BH3-only proteins and BCL-X_L. We describe the modular synthesis of this library and the structure activity relationship (SAR) as measured with a luminescence proximity assay (LPA) and further validated by direct binding affinity measurements (Surface Plasmon Resonance, SPR). These compounds bind preferentially to BCL-X_L over the other members of the pro-survival family (BCL-2, BCL-W, MCL-1 and A1). Critically, X-ray crystallography reveals a novel mode of binding within this series. This structurally validated proof-of-concept study opens promising opportunities. Because of their unique binding mode and their selectivity for BCL-X_L, the compounds describe herein represent ideal probes to investigate the conformational difference between BCL-2 and BCL-X_L, and to guide the future design of new selective BCL-X_L inhibitors or pan BCL-2/ BCL-X_L inhibitors. This library of benzoylurea analogues may also contribute to the discovery of starting points targeting other PPIs.

Results

***De novo* design of the initial peptidomimetic.** To find a suitable template for our library of small molecule BH3-mimetics, we used a computer-aided *de novo* interactive design approach.[25] This strategy involves iterative cycles of *in silico* structure-guided building and energy

minimization applied to molecular scaffolds that replicate the bond vector between the alpha and beta carbons (Cα-Cβ) of key amino acid side chains involved in the targeted binding interaction ("hot-spots").[26] By mimicking these Cα-Cβ bond vectors the functionalized side chains in the peptidomimetic should be able to sample the same conformational space as the parent peptide. This strategy has been successfully used within our group, for example in the design of conotoxin mimetics.[27-29] To support our *de novo* design strategy, we used information available at the inception of this work, specifically the NMR structural studies by Fesik and coworkers detailing the interface between the pro-survival BCL-X_L and the BH3 domains of BAK and BAD.[30,31] Confirmed by more recent structural studies,[32-34] these accounts provided a good understanding of the interaction interface between the BH3-only proteins and their pro-survival relatives.[35] The BH1, BH2 and BH3 domains of BCL-X_L form a hydrophobic groove on the surface of the protein. Upon binding, the naturally unstructured BH3 domain of pro-apoptotic proteins forms an amphipathic α-helix that inserts into the hydrophobic groove.[30] Binding in the groove occurs through key electrostatic and hydrophobic interactions, which were revealed by alanine scanning of mutant BAK peptides.[35] The results suggested that four hydrophobic residues, LEU78, ILE85, VAL74 and ILE81 project from one face of the α-helix of the BAK peptide into the hydrophobic groove of BCL-X_L. Analysis of other structures of BH3 domains in complex with pro-survival proteins revealed a common helix-in-groove interaction with the four key amino-acids located at position i, i+4, i+7 and i+11 along the helix projecting into four corresponding hydrophobic pockets (P1 to P4).

Having used the unsubstituted benzoylurea scaffold for other medicinal chemistry applications[36] together with information from the Cambridge Crystallographic Database, we found that this core served as a suitable α-helix mimetic, able to project side-chains along the

Cα-Cβ bond vectors of three hydrophobic amino-acids (Figure 2A-C).[37] This design strategy relied on the "closed" conformation of the benzoylurea core stabilized by an intramolecular hydrogen bond between the benzamide carbonyl group and the NH moiety. We have previously demonstrated that this intramolecular hydrogen bond is stable even in strongly polar and hydrogen bonding solvents, and that it can be used effectively to induce *cis*-amides and facilitate the formation of macrolactams.[38-40]

With a molecular scaffold identified, the *in silico* design of our mimetic involved the introduction of an aryl moiety at the *meta* position of the benzamide ring to overlay with the PHE Cα-Cβ bond vector (Figure 2A-C), projecting an aromatic ring into the P4 pocket. Owing to the variability of the hydrophobic residue binding in the P3 pocket, we opted to install a simple alkyl chain on the benzamide nitrogen atom to provide a reasonable surrogate at this position. Finally, *S*-benzylcysteine was incorporated to mimic the residue located in the P2 hydrophobic pocket. This choice was guided by several factors: (a) the side chain of commercially available *S*-benzylcysteine provides a reasonable match for the LEU151 (Figure 2A); (b) It was envisaged that the carboxylic acid group could participate in favourable electrostatic interactions during association, particularly with ARG139 that is in close proximity to the canonical binding groove;[41] (c) through its carboxylic acid moiety, this amino acid building block increases the polar surface area (PSA) of a fairly hydrophobic molecule. Altogether, the *de novo* design process provided a drug-like molecule that was amphiphilic in nature and that appeared to allow interaction with three important hydrophobic pockets. Importantly, we believed that this designed molecule was synthetically tractable and would be applicable as a platform for medicinal chemistry optimization.

Chemistry. We have previously described our novel strategy for the rapid synthesis of benzoylurea cores *via* a carbamoyl chloride intermediate, itself generated from a secondary amide and a carbonyl donor (Scheme 1).[42] Starting from amide **7a**, the formation of the carbamoyl chloride **8** is achieved *via* treatment of a silylated amide with a 20% solution of phosgene in toluene. In parallel, the carboxylic acid group of an amino acid is *in situ* protected as a trimethylsilyl ester in the presence of bistrimethylsilyl acetamide and reacted with carbamoyl chloride **8** in a mixture of acetonitrile and propylene oxide (acting as a "proton sponge" in this reaction), rapidly generating the benzoylurea in good yield. This synthesis avoids the use of protecting groups and proved extremely efficient, modular and highly appropriate for the preparation of diverse range of analogues. Indeed, the synthetic strategy enables the introduction of chemical diversity at key points in the synthesis scheme (Scheme 1): access to a wide range of amides (Scheme 1, diversification introduced during the formation of both the benzoic acid and of the amide), and convenient use of natural and non-natural amino-acids (step b, Scheme 1). Thus, although guided by our initial design, we also explored a wider chemical space made available by the chemistry of this scaffold.

The synthetic strategy was applied to the synthesis of initial designed mimetic **5** (Figure 2 and Scheme 1) as well as related analogues **9a-f** (Scheme 1). Here, 3-phenyl benzoic acid was used as a starting point to explore changes at the R^1 position, easily introduced *via* the synthesis of amides **7a**-**g**. The next set of analogues was designed to investigate SAR around the terminal *S*-benzylcysteine (Scheme 2). A series of amino acids as well as simple amines were used to probe the chemical space at this position furnishing final compounds **10a**-**j** (Scheme 2). A combination of palladium-mediated couplings and other approaches were used to explore SAR around the biaryl section of the molecule. Thus, compounds **13a**-**h** originated from biarylamides obtained

via Suzuki-Miyaura coupling reactions (Scheme 3). The benzylbenzoic acid **15** was obtained through the Huang-Minlon modification of the Wolff-Kishner reduction of the aromatic ketone **14** (Scheme 4). Preparation of amide **16** followed by formation of the benzoylurea led to analogue **17** (Scheme 4). The phenylacetylene derivative **21** was prepared from amide **20** *via* a solvent free Sonogashira coupling reaction (Scheme 5).[43] The *meta*-styryl derivative **26** was prepared from a Suzuki-Miyaura coupling using 1-styreneboronic acid pinacoyl ester (Scheme 6). Finally, this series of compounds exploring substituents on the aryl ring was completed with the synthesis of a phenyl ethyl analogue **30**, which was obtained using the efficient iron-mediated coupling reaction between the triflate derivative prepared from **27** and phenethylmagnesium bromide (Scheme 7).[44] Alkylation of cysteine according to Seko et *al*.[45] allowed easy access to derivatives **33a**-**g**, enabling SAR around the amino residues on a phenylacetylene template (Scheme 8). This series of phenylacetylenic derivatives was completed by using commercially available amino acids **34a**-**b**, affording the corresponding compounds **35a**-**b** (Scheme 8). Analogues of *iso*-butyl cysteine were easily prepared by reacting the advanced intermediate **36** with various aryl- and heteroaryl-acetylenic reagents using a Sonogashira coupling reaction (**37a**-**i**, Scheme 9). This strategy was not applicable to the two fluorinated analogues **39a-b**, which were prepared by first performing the Sonogashira coupling on amide **19** followed by formation of the benzoylureas **39a-b**. For crystallization purposes we also prepared the 4-bromo-*S*-benzyl analogue **41** (Scheme 10) *via* alkylation of cysteine **31**, followed by reaction with carbamoyl chloride derived from **38b** (Scheme 10). Altogether, the range of chemical transformations applied to the benzoylurea scaffold demonstrates its versatility in generating a library of potential α-helix mimetics.

Structure activity analysis. The ability of benzoylureas to mimic the BH3-domain and thus disrupt interaction with the pro-survival protein BCL-X_L was evaluated using a Luminescence Proximity Assay (LPA, see experimental section for details).[23,24] This bead-based competition assay provides a reliable and reproducible measure of ligand binding affinity especially in the case of PPIs.[46] The initial *de novo* designed benzoylurea **5** was found to have an IC_{50} of 128 μM for BCL-X_L. Whilst the binding affinity of the designed molecule was weak, we reasoned that, considering our structure-based approach, any appreciable binding was promising.[47] SAR around the P3 region (mimicking MET154, see Figure 2A) by varying the R^1 substituent (Table 1) showed that the *n*-propyl and *n*-butyl chains (**9a** and **9b**, IC_{50} = 109 and 138 μM, respectively, Table 1) maintained weak binding affinities while α-branched substituents such as *sec*-butyl (**9c**) and *iso*-propyl (**9d**) were effectively inactive (IC_{50} > 150 μM). We have previously shown that α-branched substituents cause benzoylureas to undergo a conformational change, adopting a "twisted" conformation.[38] We suggest that **9c** and **9d** cannot adopt the binding conformation without incurring an energy penalty that translates into loss of binding with the target. Arylmethylene groups at the same position (compounds **9e-f**) were also inactive.

SAR around the terminal amino-acid moiety of the molecule was explored (R^1, Table 2), while keeping the central *N*-alkyl constant (*n*-propyl). Leucine derivative **10b** was essentially inactive, suggesting an aryl ring might be important for binding. Phenylalanine and tyrosine derivatives (**10c**-**d**) were also effectively inactive as was homo-phenylalanine and 3-naphthyalanine (**10e-f**). These results suggested that shorter linkers between the terminal aromatic ring and the core molecule are not favorable. Removing the carboxylic acid moiety (**10h**) resulted in complete loss of activity (compound **10h**), as did masking the acid group through an ester (**10i**) or an amide (**10j**). These results suggest that the acid moiety is important

for binding. Replacing the entire amino acid with a methylamino group also led to loss of activity (**10a**, Table 2).

For the purposes of further SAR exploration, **9a** was retained as a template as we felt it represented the best chance of adopting most preferred binding modes while we explored alterations at other positions of the molecule focusing on the biphenyl section of the molecule (R, Table 3). These analogues ranged from substituted biphenyls to extended structures such as benzyl, phenylacetylene, phenylethyl and styryl groups. Although some increase in activity was observed with the more lipophilic 1- and 2-naphthyl groups (**13g**-**h**, IC_{50} = 62 and 63 μM, respectively), most of the analogues displayed only moderate activity. The exception in this series was the *meta*-phenylacetylenic derivative **21** which showed a promising IC_{50} value of 35 μM. The extended phenyl ring in **21** would sterically clash with the protein if it docked in the manner originally designed in Figure 2. We therefore inferred from this that **21** might adopt an alternate binding mode.

Having identified the triple bond extension as favorable for activity, we then decided to keep this feature constant while we reinvestigated the nature of the amino-acid residue. At this point we decided to follow the best biological activity regardless of the binding mode. A series of natural and non-natural amino acids, and various *S*-alkylated cysteine derivatives were tested (Scheme 8, Table 4). The *S*-Phenyl-, *S*-[4-MeO-benzyl and *S*-alkyl-substituted cysteine derivatives in general had increased potency compared to **21** except when the side chain was too bulky (e.g. **33f**) or too long (**33e**). Notably the best analogue in this series was the *iso*-butyl derivative **33c**, which displayed an IC_{50} value of 15 μM. This was a particularly exciting development as the moderate increase in activity seen in compound **33c** compared to the

designed compound **5** was achieved with minimal increase in molecular weight (462 to 466 g/mol). On this basis, we regarded **33c** as a breakthrough compound.

To complete the SAR within this sub-series, a set of analogues was prepared in which the terminal phenyl ring was substituted with various groups (Table 5). The best analogues from the series proved to be the fluorine-substituted phenyl compounds **37b** and **39b** (9 and 22 μM respectively). Compounds with bulkier side chains such as phenoxy or *tert*-butyl derivatives **37f**, **37g** or the more polar pyridyl groups (**37h** and **37i**) showed no binding affinity for BCL-X_L. We interpreted these results to suggest that the rod-like phenylacetylene moiety bound in a narrow and mostly hydrophobic pocket.

Measurement of direct binding to BCL-X_L. Validation of binding affinity is critical for any medicinal chemistry project but even more so in the context of the BCL-2 family of proteins. Indeed, the hydrophobic nature of the binding groove is conducive to non-selective hydrophobic binding, especially with hydrophobic compounds bearing a carboxylic acid. For example, it has been shown that the BCL-2 groove resembles human serum albumin domain III, a domain known to bind lipophilic acids.[15] We therefore wanted to validate the binding affinity of our compounds in an assay format distinct from that of the LPA. Our laboratory has developed a direct binding assay using the Surface Plasmon Resonance (SPR) technology that can discriminate between direct binding within the hydrophobic groove and non-selective background binding (see Supplementary Information Table S1).[48] To this end, we focused on two of the more potent compounds identified during our SAR study, **39b** and **41** (LPA BCL-X_L IC_{50} = 22 μM and 13 μM, respectively), and used a literature reference compound (compound **42**) belonging to the same class as compound **1** (Figures 1 and 3).[49] Compound **42** binds to BCL-X_L

with an IC_{50} of 2.2 μM in the LPA assay and displays steady state and kinetic K_D values of 0.9 and 1.4 μM, respectively in the SPR experiment (Figure 4A).[50] The sensorgrams for **39b** and **41** indicated a saturable binding interaction between protein and compounds (Figure 4B-C). The assay design confirmed that binding occurs within the hydrophobic groove of BCL-X_L as low compound binding interaction was observed in the presence of the BIM 26-mer BH3 peptide, which binds in the hydrophobic groove with high affinity. The K_D values measured for compound binding were determined from both steady state measurements and analysis of kinetic constants for molecular association and dissociation (4.9 and 2.1 μM, respectively for **39b** and 6.9 and 4.0 μM, respectively for **41**, Figure 4B-C) were in close agreement and in the same order of magnitude as the IC_{50} values provided by the LPA. Despite displaying similar levels of binding, compounds **39b**/**41** and **42** exhibited different binding kinetics (Supplementary Table S1). From the sensorgram of the phenylacetylenic derivatives **39b** and **42**, it was apparent that association of the former was significantly slower and the complex more stable (as indicated by the slower dissociation rate, Figure 4B-C and Supplementary Table S1). Although a number of factors can affect the association step, this result may suggest that, in the case of **39b**, significant protein conformational changes occur upon ligand binding. Once formed, the BCL-X_L•**39b** complex is more stable than that of BCL-X_L•**42**.

Selectivity profile. The selectivity profile of compound **39b** was assessed across a panel of 5 pro-survival proteins (Table 6) using a SPR competition assay.[51] As reference compound, we also included compound **42** (Figure 3).[49] While **1** binds potently to BCL-X_L, BCL-W and BCL-2, its precursor **42** exhibits good selectivity for BCL-X_L. Notably compound **42**'s binding profile reflects data reported previously and matches the relative selectivity profile reported by Bruncko

et al.[15] The binding affinity of benzoylurea compound **39b** for BCL-X_L was confirmed in this assay format (IC_{50} = 4.4 μM). Notably, it also displayed good selectivity for BCL-X_L with no observable binding for the remaining four pro-survival proteins tested.

Structural studies. Our α-helix mimetic program stemmed from a *de novo* structure-based design. Even though it is possible that early compounds such as **5** bind to BCL-X_L as designed, the SAR associated with more optimized compounds such as **39b** suggested that they may adopt binding modes that do not match the original design. To clarify the mode of binding of these compounds to BCL-X_L we obtained X-ray crystal structures of selected compounds. Gaining access to good quality crystals of BCL-2•inhibitor complexes suitable for X-ray crystallography has been difficult especially with weakly binding small molecules. We have made important breakthroughs in this field thanks to our ability to identify protein constructs suitable for crystallization.[52] Here again we focused our structural biology study on compound **39b**. Additionally, to increase our chances of obtaining good quality structural data, we prepared **41**, an analogue of **39b** bearing a bromophenyl group on the amino-acid moiety of the molecule. Pleasingly, both compounds led to good quality crystals, which resulted in high-resolution electron density map (2.1 Å for **39b**, and 2.3 Å for **41**).

Key observations can be made from the X-ray structures (Figure 5). Both compounds **39b** and **41** adopt a binding mode different from the original design, as we had surmised based on the SAR. In the complexes with BCL-X_L, both compounds adopt a conformation maintained by an intramolecular hydrogen bond, as predicted by our earlier conformational studies: this pseudo-6-membered ring conformation of the benzoylurea is likely to be the preferred in the hydrophobic environment of the BCL-X_L groove.[38] The phenylacetylene group of these two compounds

projects into a tight pocket created between P1 and P2. This snug pocket is reminiscent of the complex with previously described BCL-X_L ligands (e.g. **1** or **4**, Figure 1).[21,24,52] However, closer inspection reveals a novel binding mode, distinct from that of existing BCL-X_L ligands: for example overlay of compound **39b** and **1** (Figure 5B) indicates that the phenylacetylene moiety and the biphenyl groups point in different orientations when bound to BCL-X_L. In the X-ray structure of compound **1**, the biphenyl group projects toward P2. Extensive remodeling of the P1/P2 region to accommodate **39b** and **41** perhaps explains the slow dissociation rate observed by SPR (Figure 4B-C). Intriguingly, the unique orientation of the phenylacetylene in the P1/P2 pockets may contribute to the selectivity of this series of compounds for BCL-X_L. Our results, as well as those reported for other chemical series, demonstrate the considerable plasticity within this region of the pro-survival proteins, which has provided opportunities for the discovery of potent and, in some cases, selective ligands of these proteins.[16,18,24,53]

The benzoylurea core of these compounds lies along the area around P2 and P3, while the *S-iso*-propyl (**39b**) or *S*-bromobenzyl (**41**) groups point towards P4 without reaching it (Figure 5C-D). For these compounds, the carboxylic acid moiety does not appear to engage in any active interactions with the protein in the bound complexes. Since removal of the carboxylic acid or modification to an ester or an amide (**10a** and **10i**-**j**, Table 2) abrogate activity, it is likely that the carboxylic acid is important for the association phase of the complex, possibly as a recognition pattern for the conserved ARG139 on BCL-X_L.

Our contention is that the early, weaker compounds such as **9a** may adopt the binding mode as designed but that during optimization, induced fit occurs. It is also possible that these weaker early compounds actually bind in the same mode as observed for the optimized analogues **39b** and **41**. However, this remains to be determined and current SAR allows for both possibilities.

Ultimately, our results demonstrate that our library of *de novo* structure-based designed benzoylureas represents a powerful tool to identify PPI inhibitors. Indeed, in a field where only a small number of BCL-X_L ligand are known and where even fewer compounds have been structurally characterized, our benzoylurea library has led to the discovery of a ligand with a validated binding mode offering a significant number of possibilities to improve potency.

Conclusion

PPIs are difficult to target with small molecules and have proven to be challenging for medicinal chemistry programs. This is exemplified by the small number of biologically validated small molecules targeting the pro-survival members of the BCL-2 family of proteins. Here we have shown that a library of compounds originating from a *de novo* structure-based design enabled us to discover hits validated in three separate binding assays (LPA, direct binding assay SPR and competition assay SPR). Our lead compound, **39b,** demonstrates selectivity for BCL-X_L over four other pro-survival proteins (Table 6). Critically, we were able to obtain details of the binding mode at the molecular level from a high-resolution crystal structure (Figure 5). These X-ray structures highlight the unique features of the binding interaction between compounds **39b/41** and BCL-X_L: indeed these compounds are the first examples of small molecules engaging the protein in the P1/P2 region of the hydrophobic groove. Although the actual binding modes depart from the initial design, the strategy employed in this work is still highly significant. The benzoylurea library we have assembled has obviously shown its utility in targeting the pro-survival protein BCL-X_L. We have now the opportunity to build on this series of compounds to improve their binding affinities, a task that should be greatly facilitated by the structural information we have been able to gather. Our work described herein also allows for the

formulation of broader design principles. In particular, that 1st generation type-III peptidomimetics may serve as tractable "hooks" to identify more potent compounds through activity-driven optimization, that may adopt different binding modes and that may induce conformational changes in the host protein. An implication is that once a designed hit is found, it may be inappropriate to use a putative binding mode hypothesis to further guide what analogues to make, or indeed what analogues not to make. Rather, this should be driven by SAR elucidation until firm structural information is obtained, especially for proteins characterized by conformational plasticity.

Our work will have an impact on future studies targeting pro-survival BCL-2 proteins: we disclose new leads in this field with a distinct chemical class compared to known inhibitors (e.g. **1** and its derivatives). The facile chemistry to construct these compounds will enable rapid progression towards more potent BCL-X_L inhibitors. In addition, strategies such as "scaffold-hopping" are particularly suited to our molecules. We also believe that our approach has wider applications including for the targeting of other members of the BCL-2 family of proteins (e.g. MCL-1) and other therapeutically relevant PPIs. In each of these cases, we are confident that the modular chemistry utilized to assemble the benzoylurea scaffold will prove amenable to a broad exploration of chemical space.

Experimental Section

Chemistry

General Chemistry Methods. All non-aqueous reactions were performed in oven-dried glassware under an atmosphere of dry nitrogen, unless otherwise specified. Tetrahydrofuran was

freshly distilled from sodium/benzophenone ketyl under nitrogen. Dichloromethane was freshly distilled from CaH_2 under N_2. All other solvents were reagent grade. Petroleum ether describes a mixture of hexanes in the bp range 40-60 °C. Analytical thin-layer chromatography was performed on Merck silica gel $60F_{254}$ aluminium-backed plates and were visualized by fluorescence quenching under UV light. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.063 mm). NMR spectra were recorded on a Bruker Avance DRX 300 with the solvents indicated (^{1}H NMR at 300 MHz, ^{13}C at 75 MHz). Chemical shifts are reported in ppm on the δ scale and referenced to the appropriate solvent peak. HRMS were recorded at the Australian National University Mass Spectrometry Facility using a Waters LCT Premier XE (ESI TOF Mass Spectrometer). The purity of all final compounds was assessed using high performance liquid chromatography (Gemini C18 column, water/acetonitrile), UV-Vis (100-600 nm) and photodiode array detections coupled to an ESI ion trap Mass Spectrometer (Finnigan LCQ advantage MAX). Unless otherwise noted, all tested compounds were found to be >95% pure as determined by HPLC analysis. Reference compound **42** was prepared according to literature procedures (see Supplementary Information for NMR data of synthesized sample).[49]

General procedure 1 – Amide formation. An appropriately substituted benzoic acid was refluxed in neat $SOCl_2$ (150 μL for 1 mmol of acid). The reaction was concentrated and the residue treated with toluene. The mixture was concentrated and the toluene addition was performed two more times. The resulting acid chloride was dissolved in dichloromethane and treated at 0 °C successively with triethylamine (1.2 equivalents) and an amine substituted with R^1 (1.2 equivalents). The reaction was stirred at room temperature for 16 h. The resulting amide was

washed with HCl (2N), followed by saturated $NaHCO_3$, then brine. The resulting solution was then dried over $MgSO_4$.

General procedure 2 – Biaryl compounds prepared by Suzuki coupling. Biphenyl compounds may be introduced using Suzuki coupling between *meta*-halogenated phenyl derivatives and substituted phenyl boronic acids. The two starting materials (1.1 equivalent of phenyl boronic acid) are dissolved in toluene (2.2 mL for 1 mmol of halogenated phenyl derivative). Ethanol (530 μL for 1 mmol of halogenated phenyl derivative), 2 N Na_2CO_3 (1 mL for 1 mmol of halogenated phenyl derivative) and 5 mol% $Pd(PPh_3)_4$ were added successively to the reaction. The reaction was stirred at 80 °C until the palladium precipitates. The resulting reaction mixture was dissolved with ethyl acetate and poured onto water. The aqueous phase was extracted three times with ethyl acetate. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated under vacuum.

General procedure 3 – Preparation of carbamoyl chloride. To a stirred solution or suspension of amide from General Procedure 1 in ether (2 mL for 0.5 mmol) was added dry triethylamine (1.1 equivalent/amide). Trimethylsilyltriflate was then added (1.1 equivalent/amide). The reaction was stirred under nitrogen atmosphere at room temperature for 16 h. After that time, an orange oil formed, which was then removed using a syringe. The remaining clear solution was cooled at 0 °C and treated with a solution of phosgene in toluene (CAUTION!! 20% in toluene, 100 mL for 0.1 mmol). The reaction was then slowly warmed up to room temperature and stirred over a 4 h period. After this time the reaction flask was connected to a vacuum line and the reaction was concentrated leaving the carbamoyl chloride as

a thick oily residue. The compound was then used directly in the next step without further purification.

General procedure 4: General method for *in situ* protection of amino acids

To a suspension of amino acid in acetonitrile (4 mL per 1 mmol amino acid) was added successively propylene oxide (2 mL per 1 mmol amino acid) and *N*,*O*-bistrimethylsilylacetamide (1.5 equivalent per 1 mmol amino acid). The reaction mixture was stirred at room temperature under nitrogen for 30 min after which time it was used directly in the following step.

General procedure 5 – Formation of benzoylurea. To a solution of carbamoyl chloride from General Procedure 3 in acetonitrile (1 mL for 0.5 mmol) was added a mixture of *in situ* protected amino-acid from General Procedure 4 (1.2 equivalent of amino-acid per equivalent of carbamoyl chloride) in acetonitrile at 0 °C. The ice bath was then removed and the reaction was stirred at room temperature for 1 h. After completion of the reaction as shown by TLC (CH_2Cl_2), the reaction mixture was diluted with ethyl acetate and poured onto 2 N HCl (5 mL for 1 mmol carbamoyl chloride). The aqueous phase was extracted three times with ethyl acetate and the combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The compounds were purified using silica gel (SiO_2) and CH_2Cl_2/MeOH/AcOH 99:0.5:0.5. The purified product was dissolved in toluene, and then concentrated three times before drying under high vacuum.

General procedure 6 – Synthesis of Boc-protected cysteine derivatives. According to Seko *et al*.,[45] cysteine was reacted in EtOH (1 mL per mmol) with 2 equivalents of 2N NaOH, 3% of

tetrabutylammonium iodide and an alkyl halide (in case of alkyl iodide, tetrabutylammonium iodide was omitted) for three days at room temperature. After this time Boc_2O was added (250 mL per mmol) and the reaction was stirred at room temperature for a further 24 h. The reaction mixture was then concentrated. Cold 1 N HCl was added (1.65 mL per mmol) and followed by EtOAc and water. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine and dried over $MgSO_4$. Concentration of the organic phase afforded compounds, which were pure enough to be used in the following step without further purification.

General procedure 7 – Synthesis of benzoylurea from Boc-protected cysteine derivatives. The Boc-protected cysteine derivatives were dissolved in 1,4-dioxane (1.43 mL per mmol) and 4N HCl in dioxane (1.43 mL per mmol of amino-acid) was added. The deprotection reaction was followed by TLC and upon completion of the reaction, the mixtures was concentrated. The residue was dried under vacuum and redissolved in EtOH (1 mL per mmol) and treated with 1 equivalent of NaOH 2 M. To this reaction mixture was added 1.1 equivalent of a CH_3CN solution of carbamoyl chloride prepared according to General Procedure 3. The mixtures were concentrated. At this stage compounds can be redissolved in MeOH and purified HPLC semi-preparative (see detail for each compound). Alternatively, the residue was dissolved in dichloromethane and treated two times with HCl (2N). The organic solution was purified directly by flash chromatography (see detail for each compound).

General procedure 8 – Synthesis of benzoylurea from unprotected amino-acids. The amino acid is suspended in EtOH (1 mL per mmol) and treated with 1 equivalent of NaOH 2 M

(in the case of an amino acid hydrochloride salt, 2 equivalents of NaOH 2 M are used). To this reaction mixture was added 1.1 equivalent of a CH_3CN solution of carbamoyl chloride prepared according to preparation method 3. The mixtures were concentrated down and purified either by flash chromatography of HPLC semi-preparative.

General procedure 9 – Parallel synthesis of S-substituted cysteine derived benzoylureas. Cysteine was reacted in EtOH (1 mL per mmol) with 2 equivalents of NaOH 2 M, 3% of tetrabutylammonium iodide and 1 equivalent of an alkyl halide (in case of alkyl iodide, tetrabutylammonium iodide was omitted) for three days at room temperature. To this reaction mixture was added 1 equivalent of carbamoyl chloride prepared according to General Procedure 3 in CH_3CN (4 mL per mmol). After stirring for 30 min, the mixture was concentrated. The residue was then dissolved in EtOAc and treated two times with 2 N HCl. The organic solution can be purified by flash chromatography (see detail for each compound). Alternatively, the desired compound may be isolated by passing through a SAX Acetate solid phase extraction column.

General procedure 10 – Synthesis of ethynylbenzoylureas. (3-Iodobenzoyl)urea was dissolved in DMF/Et_3N 80:20 (5 mL per mmol) along with CuI (0.2 equivalent) and a substituted ethyne (1.5 equivalent). $Pd(PPh_3)_2Cl_2$ (0.1 equivalent) was added and stirring at room temperature was applied for 16 h. The reaction mixture was diluted with EtOAc then filtered. The solution was washed twice with HCl 2 N, then once with water, then once with brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The residue was passed through

a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 to obtain the desired compound.

N-Ethyl-biphenyl-3-carboxamide (**7a**). Using general procedure 1, 3-phenylbenzoic acid (150 mg, 0.76 mmol) was reacted with ethylamine (70% solution in water) in presence of 2M $NaHCO_3$. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2 (100%) to CH_2Cl_2/MeOH 99:1 to give **7a** a pale yellow oil (139 mg, 81%). ^{1}H (ppm, $CDCl_3$): δ 8.0 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.61 (d, *J* = 5.4 Hz, 2H), 7.52-7.35 (m, 4H), 6.14 (br s, 1H), 3.58-3.49 (m, 2H), 1.28 (t, *J* = 7.5 Hz, 3H); MS (ES+), *m/z* 226.1, (M + H).

N-Propyl-biphenyl-3-carboxamide (**7b**). Using general procedure 1, 3-phenyl benzoic acid (200 mg, 1 mmol) was reacted with *n*-propylamine. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2 (100%) to CH_2Cl_2/MeOH 99:1 to give **7b** as an off-white solid (173 mg, 72%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.97 (t, *J* = 1.5 Hz, 1H), 7.69 (dd, *J* = 7.9 and 1.8 Hz, 2H), 7.61-7.58 (m, 2H), 7.51-7.24 (m, 4H), 6.17 (br s, 1H), 3.47-3.40 (m, 2H), 1.65 (sext, *J* = 7.3 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 240.1, (M + H).

N-Butyl-biphenyl-3-carboxamide (**7c**). Using general procedure 1, 3-phenylbenzoic acid (200 mg, 1 mmol) was reacted with *n*-butylamine (247 μL, 2.5 mmol) to afford **7c** as a white solid (88 mg, 70%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.51-7.34 (m, 4H), 6.11 (br s, 1H), 3.51-3.44 (m, 2H), 1.64 (sext, *J* = 7.6 Hz, 2H), 1.43 (quint, *J* = 8.0 Hz , 2H), 0.96 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 254.1, (M + H).

N-sec-Butyl-biphenyl-3-carboxamide (**7d**). Using general procedure 1, 3-phenylbenzoic acid (200 mg, 1 mmol) was reacted with s*ec*-butylamine (253 μL, 2.5 mmol) affording **7d** as a white solid (89 mg, 70%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.95 (s, 1H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.6 Hz, 2H), 7.50-7.33 (m, 4H), 5.97 (br s, 1H), 4.30 (hept, *J* = 6.9 Hz, 1H), 1.58 (quint, *J* = 7.3 Hz, 1H), 1.23 (d, *J* = 6.6 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 254.1, (M + H).

N-iso-propyl-biphenyl-3-carboxamide (**7e**). Using general procedure 1, 3-phenylbenzoic acid (200 mg, 1 mmol) was reacted with *iso*-propylamine (511 μL, 6 mmol). The residue was purified by flash chromatography: SiO_2, CH_2Cl_2/EtOAc 95:5 to afford **7e** as a white solid (167 mg, 58%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 2H), 7.59 (d, *J* = 7.1 Hz, 2H), 7.50-7.33 (m, 4H), 5.93 (br s, 1H), 4.14 (oct, *J* = 6.7 Hz, 1H), 1.27 (d, *J* = 6.4 Hz, 6H); MS (ES+), *m/z* 240.3, (M + H).

N-Benzyl-biphenyl-3-carboxamide (**7f**). Using general procedure 1, 3-phenylbenzoic acid (200 mg, 1 mmol) was reacted with benzylamine (268 μL, 2.5 mmol) affording **7f** as a white solid (105 mg, 73%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.01 (s, 1H), 7.72 (t, *J* = 7.9 Hz, 2H), 7.58 (d, *J* = 7.1 Hz, 2H), 7.49-7.24 (m, 9H), 6.57 (br s, 1H), 4.64 (d, *J* = 5.3 Hz, 2H); MS (ES+), *m/z* 288.1, (M + H).

N-para-Methylbenzyl-biphenyl-3-carboxamide (**7g**). Using general procedure 1, 3-phenylbenzoic acid (125 mg, 0.63 mmol) was reacted with 4-methylbenzylamine (88 μL, 0.69 mmol) affording **7f** as a white solid (89 mg, 47%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.99 (s, 1H), 7.73-7.69 (m, 2H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.50-7.33 (m, 4H), 7.26 (d, *J* = 7.4 Hz, 2H), 7.15 (d, *J* =

7.8 Hz, 2H), 6.36 (br s, 1H), 4.62 (d, *J* = 5.5 Hz, 2H), 2.33 (s, 3H); MS (ES+), *m/z* 302.1, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-ethylureido)propanoic acid (**5**). Amide **7a** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **5** as a colorless glassy solid (72 mg, 65%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.63 (d, *J* = 6.9 Hz, 1H), 7.63-7.54 (m, 2H), 7.51-7.45 (m, 2H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.38-7.11 (m, 9H), 4.65 (dd, *J* = 12.0 and 6.7 Hz, 1H), 3.74-3.62 (m, 4H), 2.85 (qd, *J* = 14.0 and 5.9 Hz, 2H), 1.03 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (ppm, $CDCl_3$): δ 175.1, 175.0, 155.0, 142.0, 139.9, 137.7, 136.8, 129.3, 129.2, 129.1, 128.8, 128.1, 127.4, 127.3, 124.9, 53.6, 42.6 , 36.8, 32.9, 15.2. RP-HPLC: t_R 5.81 min, >96%. MS (ES+); *m/z* 462.9, (M + H). HRMS (ES+) Calculated for $C_{26}H_{26}N_2O_4S$ (M + H): 463.1615; found 463.1689.

(*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-propylureido)propanoic acid (**9a**). Amide **7b** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9a** as a colorless glassy solid (51 mg, 44%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.86 (s, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.19-7.13 (m, 1H), 7.02 (br s, 1H), 3.33-3.27 (m, 2H), 1.54 (sext, *J* = 7.3 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 476.9, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-butylureido)propanoic acid (**9b**). Amide **7c** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9b** as a colorless glassy oil (61 mg, 44%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.51-7.34 (m, 4H), 6.11 (br s, 1H), 3.51-3.44 (m,

2H), 1.64 (sext, *J* = 7.6 Hz, 2H), 1.43 (quint, *J* = 8.0 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 490.9, (M + H).

(2*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-*sec*-butylureido)propanoic acid (**9c**). Amide **7d** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9c** as a colorless glassy solid (118 mg, 86%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.95 (s, 1H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.6 Hz, 2H), 7.50-7.33 (m, 4H), 5.97 (br s, 1H), 4.30 (hept, *J* = 6.9 Hz, 1H), 1.58 (quint, *J* = 7.3 Hz, 1H), 1.23 (d, *J* = 6.6 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 490.9, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-*iso*-propylureido)propanoic acid (**9d**). Amide **7e** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9d** as a colorless glassy oil (151 mg, 63%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.73 (br s, 1H), 8.52 (d, *J* = 7.0 Hz, 1H), 7.73 (s, 1H), 7.71-7.63 (m, 1H), 7.59-7.56 (m, 5H), 7.28-7.09 (m, 5H), 4.67-4.60 (m, 1H), 4.37 (hept, *J* = 6.8 Hz, 1H), 3.68 (s, 2H), 2.80 (d, *J* = 5.8 Hz, 2H), 1.48-1.44 (m, 6H); MS (ES+), *m/z* 476.9, (M + H).

(*R*)-2-(3-Benzyl-3-(biphenylcarbonyl)ureido)-3-(benzylthio)propanoic acid (**9e**). Amide **7f** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9e** as a colorless glassy oil (85 mg, 66%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.01 (s, 1H), 7.72 (t, *J* = 7.9 Hz, 2H), 7.58 (d, *J* = 7.1 Hz, 2H), 7.49-7.24 (m, 9H), 6.57 (br s, 1H), 4.64 (d, *J* = 5.3 Hz, 2H); MS (ES+), *m/z* 524.9, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(biphenylcarbonyl)-3-(4-methylbenzyl)ureido)propanoic acid (**9f**). Amide **7g** and TMS protected *S*-benzyl-(L)-cysteine were reacted according to general procedures 3 to 5, affording **9f** as a colorless oil (60 mg, 43%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.99 (s, 1H), 7.73-7.69 (m, 2H), 7.58 (dd, *J* = 7.0 and 1.4 Hz, 2H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.35 (tt, *J* = 7.2 and 2.4 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.36 (br s, 1H), 4.59 (d, *J* = 5.5 Hz, 2H), 3.79 (s, 3H); MS (ES+), *m/z* 539.1, (M + H).

N-(Methylcarbamoyl)-*N*-propylbiphenyl-3-carboxamide (**10a**). 600 μL of a 0.5M solution of carbamoylchloride in CH_3CN prepared from **7b** according to general procedure 3 was reacted with methylamine (0.5 mL, 33% solution in ethanol) at 0 ^{o}C. The crude material was obtained and purified following the same work-up and purification procedures as those described in general procedure 5. A colorless oil was obtained (75 mg, 84 %). ^{1}H NMR (ppm, $CDCl_3$): δ 8.93 (br d, 1H), 7.67 (dq, *J* = 7.8, 1.8 and 1.1 Hz, 2H), 7.61-7.55 (m, 3H), 7.52-7.41 (m, 3H), 7.39-7.33 (m, 2H), 3.70-3.65 (m, 2H), 2.91 (d, *J* = 4.7 Hz, 3H), 1.55 (s, *J* = 7.5 Hz, 2H), 0.72 (t, *J* = 7.4 Hz, 3H). MS (ES+), *m/z* 297.3, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-4-methylpentanoic acid (**10b**). Amide **7b** and TMS protected (L)-Leucine were reacted according to general procedures 3 to 5, affording benzoylurea **10b** as a colorless glassy oil (106 mg, 89%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.35 (d, *J* = 6.8 Hz, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.65 (s, 1H), 7.58 (d, *J* = 7.3 Hz, 2H), 7.53-7.34 (m, 5H), 4.56-4.52 (m, 1H), 3.69 (m, 2H), 1.80-1.68 (m, 2H), 1.58 (sext, *J* = 7.5 Hz, 2H), 0.99-0.96 (m, 6H), 0.72 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 396.9, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-3-phenylpropanoic acid (**10c**). Amide **7b** and TMS protected (L)-phenylalanine were reacted according to general procedures 3 to 5, affording benzoylurea **10c** as a colorless glassy oil (110 mg, 85%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.45 (d, *J* = 6.8 Hz, 1H), 9.44 (br s, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.62-7.57 (m, 3H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.33-7.26 (m, 5H), 4.87-4.80 (m, 1H), 3.66 (m, 2H), 3.34-3.11 (m, 2H), 1.51 (sext, *J* = 7.3 Hz, 2H), 0.70 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 430.9, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-3-(4-hydroxyphenyl)propanoic acid (**10d)**. Amide **7b** and TMS protected (L)-tyrosine were reacted according to general procedures 3 to 5, affording benzoylurea **10d** as a colorless glassy oil (114 mg, 85%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.43 (d, *J* = 6.7 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.61 (s, 1H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.51-7.34 (m, 5H), 7.06 (d, *J* = 8.3 Hz, 2H), 6.71 (d, *J* = 8.3 Hz, 2H), 4.80-4.73 (m, 1H), 3.65 (m, 2H), 3.18-3.03 (m, 2H), 1.51 (sext, *J* = 7.4 Hz, 2H), 0.69 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 446.9, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-4-phenylbutanoic acid (**10e**). Amide **7b** and TMS protected (L)-homophenylalanine were reacted according to general procedures 3 to 5, affording benzoylurea **10e** as a colorless glassy oil (122 mg, 92%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.28 (br s, 1H), 9.56 (d, *J* = 7.0 Hz, 1H), 7.73-7.69 (m, 2H), 7.62-7.60 (m, 2H), 7.56-7.37 (m, 6H), 7.32-7.21 (m, 3H), 4.65-4.59 (m, 1H), 3.73 (m, 2H), 2.80 (t, *J* = 8.0 Hz, 2H), 2.39-2.08 (m, 2H), 1.59 (sext, *J* = 7.5 Hz, 2H), 0.76 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 445.0, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-3-(naphthalen-2-yl)propanoic acid (**10f**). Amide **7b** and TMS protected (L)-3-naphthylalanine were reacted according to general procedures 3 to 5, affording benzoylurea **10f** as a colorless glassy oil (128 mg, 90%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.9 (br s, 1H), 9.56 (d, *J* = 6.5 Hz, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 7.87 (dd, *J* = 8.0 and 1.1 Hz, 1H), 7.79 (dd, *J* = 6.81 and 2.33 Hz, 1H), 7.67 (ddd, *J* = 7.7, 2.8 and 1.0 Hz, 1H), 7.62-7.56 (m, 4H), 7.53-7.37 (m, 8H), 5.00-4.97 (m, 1H), 3.94-3.3.87 (m, 1H), 3.63-3.58 (m, 2H), 3.52-3.45 (m, 1H), 1.46 (sext, *J* = 7.6 Hz, 2H), 0.68 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 481.0, (M + H).

(*S*)-2-(3-(Biphenylcarbonyl)-3-propylureido)-3-(1H-indol-3-yl)propanoic acid (**10g**). Amide **7b** and TMS protected (L)-tryptophane were reacted according to general procedures 3 to 5, affording benzoylurea **10g** as a colorless glassy oil (113 mg, 80%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.95 (br s, 1H), 9.49 (d, *J* = 6.3 Hz, 1H), 8.31 (s, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.3 Hz, 4H), 7.50-7.36 (m, 4H), 7.32-7.25 (m, 2H), 7.16-7.08 (m, 2H), 4.91-4.85 (m, 1H), 3.65 (m, 2H), 3.49-3.30 (m, 2H), 1.52 (sext, *J* = 7.4 Hz, 2H), 0.69 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 470.0, (M + H).

N-(2-(Benzylthio)ethylcarbamoyl)-*N*-propylbiphenyl-3-carboxamide (**10h**). Amide **7b** (42 mg, 0.18 mmol) was reacted according to general procedure 3 to prepare the corresponding carbamoylchloride. The crude carbamoylchloride was then redissolved in 1 mL of CH_3CN. Triethylamine (33 μL, 0.22 mmol) and (2-benzylthio)-ethylamine (56 mg, 0.21 mmol) were added to this solution at 0 ^{o}C. The reaction was stirred at room temperature for 16 hours. It was then diluted with AcOEt and 2M HCl solution (50 mL each). The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with water and brine and

then dried over $MgSO_4$. The crude material was purified by flash chromatography on SiO_2 using dichoromethane. A yellow oil was obtained (55 mg, 73%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.18 (m), 7.68 (d, *J* = 7.8 Hz, 2H), 7.63-7.57 (m, 3H), 7.53-7.43 (m, 3H), 7.40-7.23 (m, 7H), 3.75 (s, 2H), 3.71-3.66 (m, 2H), 2.63 (t, *J* = 6.8 Hz, 2H), 1.56 (sext, *J* = 7.5, 2H), 0.73 (t, *J* = 7.4, 3H). MS (ES+), *m/z* 433.3, (M + H).

(*R*)-Methyl 3-(benzylthio)-2-(3-(biphenylcarbonyl)-3-propylureido)propanoate (**10i**). Amide **7b** and TMS protected (L)-*S*-benzylcysteine methyl ester were reacted according to general procedures 3 to 5, affording benzoylurea **10i** as a colorless oil (89 mg, 51%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.65 (d, *J* = 7.2 Hz, 1H), 7.71-7.20 (m, 14H), 4.80-4.74 (m, 1H), 3.77 (s, 2H), 3.76 (s, 3H), 3.70 (t, *J* = 7.5 Hz, 2H), 2.99-2.84 (m, 2H), 1.57 (sext, *J* = 7.5 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 491.0, (M + H).

(*R*)-*N*-(1-Amino-3-(benzylthio)-1-oxopropan-2-ylcarbamoyl)-*N*-propylbiphenyl-3-carboxamide (**10j**). Amide **7b** and TMS protected (L)-*S*-benzylcysteine amide were reacted according to general procedures 3 to 5, affording benzoylurea **10j** as a colorless oil (72 mg, 42%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.44 (d, *J* = 7.2 Hz, 1H), 7.66-7.19 (m, 14H), 6.44 (br s, 1H), 6.03 (br s, 1H), 4.60-4.53 (m, 1H), 3.79 (s, 2H), 3.69 (t, *J* = 7.5 Hz, 2H), 2.96-2.80 (m, 2H), 1.56 (sext, *J* = 7.5 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 475.9, (M + H).

3-Bromo-*N*-propylbenzamide (**11**). Using general procedure 1, 3-bromobenzoic acid was reacted with *n*-propylamine. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2 (100%) to CH_2Cl_2/MeOH 99:1 to give 3-bromo-*N*-propylbenzamide as an off-white solid (3.8 g,

79%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.86 (s, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.19-7.13 (m, 1H), 7.02 (br s, 1H), 3.33-3.27 (m, 2H), 1.54 (sext, *J* = 7.3 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 242.1, (M[^{79}Br] + H, 100%), 244.0, (M[^{81}Br] + H, 95%).

4'-Fluoro-*N*-propylbiphenyl-3-carboxamide (**12a**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 4-fluorophenylboronic acid. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2/petroleum ether 80:20 to CH_2Cl_2/EtOAc 80:20 to give a white solid (179 mg, 70%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.94 (t, *J* = 2.1 Hz, 1H), 7.66 (tt, *J* = 7.8 Hz, *J* = 0.9 Hz, 2H), 7.58-7.53 (m, 2H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 9 Hz, 2H), 6.12 (br s, 1H), 3.47-3.40 (m, 2H), 1.65 (sext, *J* = 7.5 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 258.1, (M + H).

4'-Methyl-*N*-propylbiphenyl-3-carboxamide (**12b**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 4-tolylboronic acid. The resulting reaction mixture was purified using SiO_2 with EtOAc/CH_2Cl_2 10:90. A white crystalline solid was obtained (197 mg, 78%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.99 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.74 (br s, 1H), 3.42-3.35 (m, 2H), 2.36 (s, 3H), 1.61 (sext, *J* = 7.2 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 254.1, (M + H).

2'-Chloro-*N*-propylbiphenyl-3-carboxamide (**12c**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 2-chloro-phenyl boronic acid. The resulting reaction mixture was purified using SiO_2 with petroleum ether/CH_2Cl_2 5:95 to CH_2Cl_2 100% to

EtOAc/CH_2Cl_2 5:95. A white crystalline solid was obtained (216 mg, 79%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.82 (d, *J* = 8.5 Hz, 2H), 7.46-7.43 (m, 3H), 7.28-7.26 (m, 3H), 6.59 (br s, 1H), 3.44-3.37 (m, 2H), 1.63 (sext, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 274.1, (M + H).

4'-Methoxy-*N*-propylbiphenyl-3-carboxamide (**12d**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 4-methoxyphenylboronic acid. The resulting reaction mixture was purified using SiO_2 with EtOAc/CH_2Cl_2 10:90. A yellow crystalline solid was obtained (141 mg, 52%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.93 (s, 1H), 7.75-7.62 (m, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.45 (t, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.15 (br s, 1H), 3.84 (s, 3H), 3.47-3.40 (m, 2H), 1.65 (sext, *J* = 7.3 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 270.1, (M + H).

4'-Chloro-*N*-propylbiphenyl-3-carboxamide (**12e**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 4-chlorophenylboronic acid. The resulting reaction mixture was purified using SiO_2 with EtOAc/CH_2Cl_2 10:90. A pale yellow solid was obtained (282 mg, 79%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.95 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.43 (dt, *J* = 8.6 Hz, *J* = 2.2 Hz, 2H), 7.35 (t, *J* = 7.7 Hz, 1H), 7.31 (dt, *J* = 8.6 Hz, *J* = 2,2 Hz, 2H), 6.95 (br s, 1H), 3.39-3.32 (m, 2H), 1.59 (sext, *J* = 7.3 Hz, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). MS (ES+), *m/z* 274.3, (M + H).

3',4'-Dichloro-*N*-propylbiphenyl-3-carboxamide (**12f**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 3,4-dichlorophenylboronic acid. The resulting reaction

mixture was purified using SiO_2 with EtOAc/CH_2Cl_2 10:90. A yellow solid was obtained (255 mg, 92%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.83 (d, *J* = 8.5 Hz, 2H), 7.67 (d, *J* = 2.0 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.41 (dd, *J* = 8.3 Hz, *J* = 2.1 Hz, 1H), 6.17 (br s, 1H), 3.47-3.40 (m, 2H), 1.65 (sext, *J* = 7.3 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 308.1, (M + H).

3-(Naphthalen-1-yl)-*N*-propylbenzamide (**12g**). Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 1-naphthylboronic acid. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2/ petroleum ether 80:20 to CH_2Cl_2/AcOH 80:20 to give a thick yellow oil (216 mg, 75%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.92-7.78 (m, 5H), 7.67-7.40 (m, 6H), 6.16 (br s, 1H), 3.50-3.36 (m, 2H), 1.64 (sext, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 290.1, (M + H).

3-(Naphthalen-2-yl)-*N*-propylbenzamide (**12h**)**.** Using general procedure 2, 3-bromo-*N*-propylbenzamide **11** was reacted with 2-naphthylboronic acid. The resulting reaction mixture was purified using SiO_2 with CH_2Cl_2/ petroleum ether 80:20 to CH_2Cl_2/AcOH 80:20 to give a white crystalline solid (164 mg, 57%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.12 (t, *J* = 1.7 Hz, 1H), 8.06 (s, 1H), 7.91 (t, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.73 (td, *J* = 8.5 Hz, *J* = 1.79 Hz, 2H), 7.55-7.46 (m, 3H), 6.18 (br s, 1H), 3.49-3.43 (m, 2H), 1.67 (sext, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 290.1, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(4'-fluorobiphenylcarbonyl)-3-propylureido)propanoic acid (**13a**)**.** Amide **12a** and TMS protected (L)-*S*-benzylcysteine were reacted according to general

procedures 3 to 5, affording benzoylurea **13a** as a colorless glassy oil (128 mg, 86%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.93 (br s, 1H), 9.67 (d, *J* = 6.9 Hz, 1H), 7.67-7.63 (m, 2H), 7.57-7.48 (m, 3H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.32-7.11 (m, 7H), 4.80-4.74 (m, 1H), 3.79 (s, 2H), 3.71 (m, 2H), 3.04-2.88 (m, 2H), 1.58 (sext, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 494.9, (M + H).

(*R*)-3-(Benzylthio)-2-(3-(4'-methylbiphenylcarbonyl)-3-propylureido)propanoic acid (**13b**). Amide **12b** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13b** as a colorless solid (104 mg, 71%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.73 (d, *J* = 6.9 Hz, 1H), 9.64 (br s, 1H), 7.70-7.68 (m, 2H), 7.52-7.47 (m, 3H), 7.41-7.23 (m, 8H), 4.82-4.76 (m, 1H), 3.79 (s, 2H), 3.72 (t, *J* = 7.5 Hz, 2H), 3.05-2.89 (m, 2H), 2.40 (s, 3H), 1.58 (sext, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 491.0 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(2'-chlorobiphenylcarbonyl)-3-propylureido)propanoic acid (**13c**). Amide **12c** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13c** was as a pale yellow oil (141 mg, 63%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.63 (d, *J* =6.9 Hz, 1H), 7.52-7.23 (m, 13H), 4.76-4.69 (m, 1H), 3.79 (s, 2H), 3.72 (t, *J* = 7.5 Hz, 2H), 3.02-2.87 (m, 2H), 1.57 (sext, *J* = 7.4 Hz, 2H), 0.76 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 510.9 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(4'-methoxybiphenylcarbonyl)-3-propylureido)propanoic acid (**13d**). Amide **12d** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13d** as a colorless oil (49 mg, 32%). ^{1}H NMR (ppm,

$CDCl_3$): δ 9.70 (d, *J* = 7.0 Hz, 1H), 9.19 (br s, 1H), 7.66-7.20 (m, 11H), 6.98 (d, *J* = 8.7 Hz, 2H), 4.79-4.73 (m, 1H), 3.84 (s, 3H), 3.78 (s, 2H), 3.70 (t, *J* = 7.5 Hz, 2H), 3.03-2.87 (m, 2H), 1.56 (sext, *J* = 7.4 Hz, 2H), 0.73 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 506.9 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(4'-chlorobiphenylcarbonyl)-3-propylureido)propanoic acid (**13e**). Amide **12e** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13e** as a colorless oil (123 mg, 80%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.0 (br s, 1H), 9.65 (d, *J* = 7.0 Hz, 1H), 7.66-7.23 (m, 13H), 4.81-4.75 (m, 1H), 3.78 (s, 2H), 3.69 (t, *J* = 7.4 Hz, 2H), 3.04-2.87 (m, 2H), 1.57 (sext, *J* = 7.3 Hz, 2H), 0.73 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 510.9 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(3',4'-dichlorobiphenylcarbonyl)-3-propylureido)propanoic acid (**13f**). Amide **12f** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13f** as a colorless oil (134 mg, 82%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.65 (br s, 1H), 9.63 (d, *J* = 7.1 Hz, 1H), 7.66-7.14 (m, 12H), 4.81-4.75 (m, 1H), 3.78 (s, 2H), 3.69 (t, *J* = 7.5 Hz, 2H), 3.04-2.88 (m, 2H), 1.57 (sext, *J* = 7.5 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 544.9 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(3-(naphthalen-1-yl)benzoyl)-3-propylureido)propanoic acid (**13g).** Amide **12g** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13g** as a colorless oil (235 mg, 87%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.76 (br s, 1H), 9.72 (d, *J* = 6.99 Hz, 1H), 7.91 (t, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.64-7.48 (m, 5H), 7.43 (t, *J* = 6.7 Hz, 2H), 7.34-7.21 (m, 4H), 7.17 (t, *J* = 7.6 Hz, 2H),

4.82-4.76 (m, 1H), 3.78 (s, 2H), 3.77 (m, 2H), 3.04-2.88 (m, 2H), 1.61 (sext, *J* = 7.5 Hz, 2H), 0.79 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 527.0 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(3-(naphthalen-2-yl)benzoyl)-3-propylureido)propanoic acid (**13h**). Amide **12h** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **13h** as a colorless oil (167 mg, 88%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.76 (d, *J* = 7.0 Hz, 1H), 9.67 (br s, 1H), 8.07 (s, 1H), 7.94 (t, *J* = 8.5 Hz, 2H), 7.85 (d, *J* = 7.3 Hz, 3H), 7.74 (d, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H), 7.60-7.46 (m, 4H), 7.37-7.25 (m, 5H), 4.85-4.79 (m, 1H), 3.82 (s, 2H), 3.77 (m, 2H), 3.08-2.92 (m, 2H), 1.63 (sext, *J* = 7.4 Hz, 2H), 0.78 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 527.0 (M + H).

3-Benzylbenzoic acid (**15**)**.** 3-Phenylmethylbenzoic acid was prepared according to the method of Van Herwijnen *et. al.*[54] 3-Benzoylbenzoic acid (904 mg, 4 mmol), powdered NaOH (700 mg), hydrazine hydrate (0.7 mL), and triethylene glycol (20 mL) were stirred at 190 °C for 4 h. Upon cooling, the reaction mixture was washed twice with diethyl ether. Concentrated HCl was added dropwise to the aqueous phase until an acidic solution was obtained. The resulting precipitate was filtered off, washed with water and dried to give pale yellow flakes (780 mg, 92%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96-7.93 (m, 2H), 7.43-7.17 (m, 7H), 4.04 (s, 2H).

3-Benzyl-*N*-propylbenzamide (**16**)**.** Using general procedure 1, 3-phenylmethylbenzoic acid **15** was reacted with propylamine. The resulting reaction mixture was purified using SiO_2, CH_2Cl_2/petroleum ether 80:20 to CH_2Cl_2/EtOAc 80:20 to give a white solid (422 mg, 53%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.61 (s, 1H), 7.56 (dt , *J* = 6.8 and 1.9 Hz, 1H), 7.32-7.26 (m, 4H), 7.21-

7.15 (m, 3H), 6.10 (br s, 1H), 4.00 (s, 2H), 3.44-3.36 (m, 2H), 1.61 (sext, *J* = 7.2 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 254.1 (M + H).

(*R*)-2-(3-(3-Benzylbenzoyl)-3-propylureido)-3-(benzylthio)propanoic acid (**17**). Amide **16** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **17** as a colorless glassy oil (2.3 g, 72%)**.** ^{1}H NMR (ppm, $CDCl_3$): δ 9.68 (br s, 1H), 9.66 (d, *J* = 6.9 Hz, 1H), 7.38-7.20 (m, 14H), 4.75-4.69 (m, 1H), 4.01 (s, 2H), 3.76 (s, 2H), 3.58 (m, 2H), 3.00-2.84 (m, 2H), 1.47 (sext, *J* = 7.5 Hz, 2H), 0.66 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 491.0 (M + H).

3-Iodo-*N*-propylbenzamide (**19).** Using general procedure 1, 3-iodobenzoic acid was reacted with *n*-propylamine. The resulting reaction mixture was purified *via* column chromatography eluting with CH_2Cl_2/petroleum ether 95:5 to CH_2Cl_2/EtOAc 95:5 to give **19** as an off-white solid (474 mg, 82%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.07 (t, *J* = 1.6 Hz, 1H), 7.80 (dt, *J* = 7.9 and 1.1 Hz, 1H), 7.69 (dt, *J* = 7.8 and 1.1 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.06 (br s, 1H), 3.43-3.36 (m, 2H), 1.62 (sext, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).

3-(Phenylethynyl)-*N*-propylbenzamide (**20).** A mixture of 3-iodo-*N*-propylbenzamide **19** (m = 289, 1 mmol), phenylacetylene (165 μL, 1.5 mmol) and $Pd(PPh_3)_2Cl_2$ (35 mg, 5 mol%) in piperidine (297 μL, 3 mmol) was heated at 70 °C for 30 min in a sealed tube. The solidified residue was dissolved with CH_2Cl_2 and water and poured onto 2N HCl. The acidic phase was extracted three times with CH_2Cl_2. The combined organic layers were washed once with 2N HCl, twice with saturated $NaHCO_3$, once with water and once with brine. The organic layer was then

dried over $MgSO_4$ and concentrated. The resulting residue was purified using SiO_2 with CH_2Cl_2/petroleum ether 80:20 to CH_2Cl_2 100% to give 3-(phenylethynyl)-*N*-propylbenzamide **20** as a yellow solid (237 mg, 90%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.88 (s, 1H), 7.74-7.68 (m, 1H), 7.63-7.60 (m, 1H), 7.53-7.50 (m, 2H), 7.42-7.33 (m, 4H), 6.22 (br s, 1H), 3.44-3.38 (m, 2H), 1.64 (sext, *J* = 7.3 Hz, 2H), 0.98 (t, *J* = 7.3 Hz, 3H); MS (ES^+) *m/z* 264.1 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**21**). Amide **20** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5, affording benzoylurea **21** as a colorless oil (102 mg, 68%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 6.9 Hz, 1H), 7.64-7.21 (m, 14H), 4.75-4.69 (m, 1H), 3.78 (s, 2H), 3.67 (t, *J* = 7.5 Hz, 2H), 3.02-2.87 (m, 2H), 1.54 (sext, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES^+) *m/z* 500.9 (M + H).

(*E*)-Methyl 3-styrylbenzoate (**23**). Methyl 3-bromobenzoate **22** (458 mg, 2 mmol), 1-styreneboronic acid pinacoyl ester (484 mg, 2.1 mmol), Na_2CO_3 (222 mg, 2.1 mmol), tetrakis(triphenylphosphino)palladium (116 mg, 0.1 mmol), water (4 mL) and 1,2-dimethoxyethane (6 mL) were stirred under reflux conditions for 1 h, under nitrogen. The reaction mixture was diluted with water and was extracted three times with EtOAc. The organic layers were combined, washed with water and then brine, dried over $MgSO_4$ and concentrated. The product was purified by trituration with Et_2O. White crystals were obtained (393 mg, 82%). ^{1}H NMR (ppm, $CDCl_3$): δ 8.19 (t, *J* = 1.6 Hz, 1H), 7.91 (dt, *J* = 7.7 and 1.3 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.30-7.26 (m, 1H), 7.19 (d, *J* = 16.3 Hz, 1H), 7.11 (d, *J* = 16.4 Hz, 1H), 3.06 (s, 3H).

(*E*)-*N*-Propyl-3-styrylbenzamide (**25**). (*E*)-Methyl 3-styrylbenzoate **23** (357 mg, 1.5 mmol) was suspended in EtOH (2 mL) and aqueous NaOH (1 M, 6 mL) and was heated to 80 °C for 30 min. Upon cooling to room temperature, the solution was acidified with concentrated HCl, was cooled to 0 °C, and the white solid isolated by filtration. Using General Procedure 1, the resultant carboxylic acid was (224 mg, 1 mmol) was reacted with *n*-propylamine (90 μL, 65 mg, 1.1 mmol). The product was purified by flash chromatography on SiO_2 using CH_2Cl_2/hexanes 40:60 to CH_2Cl_2/hexanes 60:40. White crystals were obtained (154 mg, 58%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.92 (s, 1H), 7.60 (t, *J* = 7.7 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.29-7.26 (m, 1H), 7.18 (d, *J* = 16.4 Hz, 1H), 7.10 (d, *J* = 16.3 Hz, 1H), 6.12 (br s, 1H), 3.47-3.40 (m, 2H), 1.65 (sext, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); MS (ES^+) *m/z* 266.1 (M + H).

(*R*,*E*)-3-(Benzylthio)-2-(3-propyl-3-(3-styrylbenzoyl)ureido)propanoic acid (**26**). (E)-*N*-Propyl-3-styrylbenzamide **25** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5. Purification of the resulting crude product using CH_2Cl_2 100%, then CH_2Cl_2/AcOH 99.5:0.5, then CH_2Cl_2/AcOH/MeOH 99:0.5:0.5 gave compound **26** as a colorless oil (68 mg, 54%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.69 (d, *J* = 7.0 Hz, 1H), 7.61-7.24 (m, 14H), 7.16 (d, *J* = 18.3 Hz, 1H), 7.08 (d, *J* = 16.2 Hz, 1H), 4.80-4.74 (m, 1H), 3.79 (s, 2H), 3.70 (t, *J* = 7.5 Hz, 2H), 3.04-2.87 (m, 2H), 1.57 (sext, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES^+) *m/z* 503.0 (M + H).

3-Phenethyl-benzoic acid methyl ester (**28**). Methyl 3-hydroxybenzoate **27** (1.52 g, 10 mmol) was dissolved in dry CH_2Cl_2 (25 mL) along with 4-dimethylaminopyridine (244 mg, 2 mmol) and 2,4,6-collidine (1.6 mL, 1.45 g, 12 mmol). The solution was immersed in a dry ice/CH_3CN bath, and stirred under nitrogen. Triflic anhydride (2 mL, 3.36 g, 12 mmol) was added by dropping funnel. The reaction mixture was permitted to reach room temperature and stirring was maintained for 30 min. The reaction mixture was quenched with citric acid (10 %) and was extracted three times with CH_2Cl_2. The combined organic layers were washed with water and then brine, were dried over $MgSO_4$ and were concentrated. The product was purified by flash chromatography on SiO_2 using EtOAc/hexanes 3:97. A colorless oil was obtained (2.50 g, 88 %). The resulting triflate (1.14 g, 4 mmol), *N*-methylpyrrolidinone (2.2 mL), $Fe(acac)_3$ (70 mg, 0.2 mmol) and dry THF (25 mL) were stirred under nitrogen at room temperature. Phenethylmagnesium bromide (1.0 M in THF, 5 mL) was added by syringe. After stirring for fifteen minutes, HCl (1 M, 10 mL) was slowly added. The mixture was diluted with water and extracted three times with EtOAc. The organic layers were combined, washed with water and then brine, were dried over $MgSO_4$ and were concentrated. Purification was achieved using flash chromatography on SiO_2 with hexanes/toluene 50:50. A colorless oil was obtained (660 mg, 69 %). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96-7.85 (m, 2H), 7.33-7.15 (m, 7H), 3.91 (s, 3H), 2.97-2.93 (m, 4H); MS (ES^+) *m/z* 241.3 (M + H).

3-Phenethyl-*N*-propylbenzamide (**29**). Compound **28** (660 mg, 2.75 mmol) was suspended in EtOH (2 mL) and aqueous NaOH (1 M, 6 mL) and was heated to 80 °C for 30 min. Upon cooling to room temperature, the solution was acidified with HCl (concentrated), was cooled to 0°C, and was filtered. The white crystalline precipitate was dried under vacuum (356 mg, 57 %).

Using General Procedure 1, the carboxylic acid (226 mg, 1 mmol) was reacted with *n*-propylamine (90 μL, 65 mg, 1.1 mmol). The crude amide was clean and used in the next step without purification. White solids were obtained (107 mg, 40%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.96-7.89 (m, 2H), 7.56-7.12 (m, 7H), 6.03 (br s, 1H), 3.42-3.38 (m, 2H), 3.04-2.87 (m, 4H), 1.63 (sext, *J* = 7.3 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H); MS (ES^+) *m/z* 268.1 (M + H).

(*R*)-3-(Benzylthio)-2-(3-(3-phenethylbenzoyl)-3-propylureido)propanoic acid (**30**). 3-Phenethyl-*N*-propylbenzamide **29** and TMS protected (L)-*S*-benzylcysteine were reacted according to general procedures 3 to 5 affording benzoylurea **30** as a colorless oil (45 mg, 36%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.69 (d, *J* = 6.9 Hz, 1H), 7.41-7.11 (m, 14H), 4.76-4.79 (m, 1H), 3.78 (s, 2H), 3.58 (t, *J* = 7.5 Hz, 2H), 3.02-2.86 (m, 2H), 1.50 (sext, *J* = 7.5 Hz, 2H), 0.70 (t, *J* = 7.4 Hz, 3H); MS (ES^+) *m/z* 505.0 (M + H).

Boc-*S*-propyl-L-cysteine (**32a**). Using general procedure 6, cysteine (242 mg, 2 mmol) was reacted with propyl iodide (219 μL, 2.2 mmol) to provide Boc-(S)-propyl-L-cysteine as thick pale yellow oil (474 mg, 90%). ^{1}H NMR (ppm, DMSO-d_6): δ 4.27 (br t, *J* = 7.0 Hz, 1H), 3.00-2.77 (m, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 1.59 (sext, *J* = 7.3 Hz, 2H), 1.45 (s, 9H), 0.98 (t, *J* = 7.31 Hz, 3H).

Boc-*S*-*iso*-propyl-L-cysteine (**32b**). Using general procedure 6, cysteine (242 mg, 2 mmol) was reacted with 2-bromopropane (207 μL, 2.2 mmol) to provide Boc-*S*-*iso*-propyl-L-cysteine as thick pale yellow oil (480 mg, 91%). ^{1}H NMR (ppm, DMSO-d_6): δ 4.28 (br t, *J* = 7.0 Hz, 1H), 3.03-2.80 (m, 3H), 1.44 (s, 9H), 1.24 (d, *J* = 6.8 Hz, 6H).

Boc-*S*-*iso*-butyl-L-cysteine (**32c**). Using general procedure 6, cysteine (242 mg, 2 mmol) was reacted with 2-methyl-1-bromopropane (239 μL, 2.2 mmol) to provide Boc-(S)-*iso*-butyl-L-cysteine as thick pale yellow oil (510 mg, 92%). ^{1}H NMR (ppm, DMSO-d_6): δ 4.27 (br t, J = 7.1 Hz, 1H), 2.99-2.77 (m, 2H), 2.44 (d, J = 6.8 Hz, 2H), 1.75 (hept, J = 6.7 Hz, 1H), 1.44 (s, 9H), 0.99 (d, J = 6.6 Hz, 6H).

Boc-*S*-phenylpropyl-L-cysteine (**32e**). Using preparation method 6, cysteine (242 mg, 2 mmol) was reacted with 1-bromo-3-phenylpropane (334 μL, 2.2 mmol) to provide Boc-(S)-phenylpropyl-L-cysteine as thick pale yellow oil (638 mg, 94%). ^{1}H NMR (ppm, DMSO-d_6): δ 7.26-7.10 (m, 5H), 4.27-4.24 (m, 1H), 3.02-2.78 (m, 2H), 2.69 (t, J = 7.3, 2H), 2.55 (t, J = 7.3 Hz, 2H), 1.84 (q, J = 7.4 Hz, 2H), 1.41 (s, 9H).

Boc-*S*-biphenyl-2-ylmethyl-L-cysteine (**32f**). Using preparation method 6, cysteine (35.5 mg, 0.25 mmol) was reacted with 2- biphenyl-2-ylmethylbromide (50.5 μL, 0.28 mmol) to provide Boc-(S)-biphenyl-2-ylmethyl-L-cysteine as thick pale yellow oil (252 mg, 65%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.86 (br s, 1H), 7.42-7.18 (m, 9H), 5.20 (d, J = 7.1 Hz, 1H), 4.44 (br s, 1H), 3.77-3.67 (m, 2H), 2.96 (m, 2H), 1.43 (s, 9H).

Boc-*S*-phenethyl-L-cysteine (**32g**). Using preparation method 6, cysteine (242 mg, 2 mmol) was reacted with phenylethylbromide (207 μL, 2.2 mmol) to provide Boc-(S)-phenethyl-L-cysteine as thick pale yellow oil (586 mg, 90%). ^{1}H NMR (ppm, DMSO-d_6): δ 7.24-7.13 (m, 5H), 4.30-4.26 (m, 1H), 3.00-2.76 (m, 6H), 1.41 (s, 9H).

(*R*)-2-(3-(3-(Phenylethynyl)benzoyl)-3-propylureido)-3-(propylthio)propanoic acid (**33a**). Using general procedure 3, amide **20** (445 mg, 1.7 mmol) was reacted with phosgene to provide the corresponding carbamoyl chloride, which was dissolved in acetonitrile to obtain a 0.4 M solution. Using General Procedure 7, compound **33a** (58 mg, 0.22 mmol) was deprotected and reacted with 660 μL of the carbamoyl chloride solution. The reaction was then stirred at room temperature for 10 min. Purification by flash chromatography using CH_2Cl_2/petroleum ether 80:20 then CH_2Cl_2 100% then CH_2Cl_2/MeOH/AcOH 99:0.5:0.5 to give compound **33a** as a pale yellow oil (25 mg, 25%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 6.7 Hz, 1H), 7.63-7.60 (m, 2H), 7.54-7.51 (m, 2H), 7.43-7.40 (m, 2H), 7.36-7.34 (m, 3H), 4.78-4.71 (m, 1H), 3.70-3.62 (m, 2H), 3.13-3.00 (m, 2H), 2.57 (t, *J* = 7.24 Hz, 2H), 1.68-1.48 (m, 4H), 0.97 (t, *J* = 7.30 Hz, 3H), 0.74 (t, *J* = 7.35 Hz, 3H); MS (ES-), *m/z* 451.1 (M - H).

(*R*)-3-(*iso*-Propylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**33b**). Using general procedure 7, compound **32b** (56 mg, 0.21 mmol) was deprotected and reacted with 640 μL of the carbamoyl chloride solution from amide **20**. The reaction was then stirred at room temperature for 10 min. Purification by HPLC semi-preparative gave **33b** as a colorless yellow oil (25 mg, 24%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.60 (d, *J* = 6.8 Hz, 1H), 7.63-7.60 (m, 2H), 7.54-7.50 (m, 2H), 7.46-7.40 (m, 2H), 7.36-7.32 (m, 3H), 4.78-4.72 (m, 1H), 3.70-3.65 (m, 2H), 3.14-2.94 (m, 3H), 1.54 (sext, *J* = 7.5 Hz, 2H), 1.27 (d, *J* = 6.7 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 452.9 (M - H).

(*R*)-3-(*iso*-Butylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**33c**). Using general procedure 7, compound **32c** (62 mg, 0.22 mmol) was deprotected and reacted with 670 μL of the carbamoyl chloride solution from amide **20**. The reaction was then stirred at room temperature for 10 min. Purification by HPLC semi-preparative to give compound **33c** as a colorless oil (25 mg, 24%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 6.8 Hz, 1H), 7.64-7.61 (m, 2H), 7.54-7.50 (m, 2H), 7.43-7.40 (m, 2H), 7.36-7.33 (m, 3H), 4.78-4.72 (m, 1H), 3.70-3.65 (m, 2H), 3.10-2.99 (m, 2H), 2.47 (d, *J* = 6.9 Hz, 2H), 1.80 (hept, *J* = 6.7 Hz, 1H), 1.54 (sext, *J* = 7.5 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 467.1 (M + H).

(*R*)-3-(Cyclohexylmethylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido) propanoic acid (**33d**). Using general procedure 9, (L)-cysteine (12 mg, 0.1 mmol) was treated with (bromomethyl)cyclohexane (14 μL, 18 mg, 0.1 mmol), and then treated with the carbamoyl chloride of amide **20**. Purification using a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 provided compound **33d** as a colorless oil (11 mg, 22%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.55 (d, *J* = 6.0 Hz, 1H), 7.62-7.59 (m, 2H), 7.53-7.50 (m, 2H), 7.44-7.34 (m, 5H), 5.17 (br s, 1H), 4.71-4.65 (m, 1H), 3.68-3.63 (m, 2H), 3.16-3.00 (m, 2H), 2.49 (d, *J* = 9.5 Hz, 2H), 1.83-1.36 (m, 8H), 1.23-1.12 (m, 3H), 0.96-0.86 (m, 4H), 0.72 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 507.0 (M - H).

(*R*)-2-(3-(3-(Phenylethynyl)benzoyl)-3-propylureido)-3-(3-phenylpropylthio)propanoic acid (**33e**). Using general procedure 7, compound **32e** (48 mg, 0.14 mmol) was deprotected and reacted with 423 μL of the carbamoyl chloride solution from amide **20**. The reaction was then stirred at room temperature for 10 min. Purification by semi-preparative HPLC to give

compound **33e** as colorless oil (29 mg, 39%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.66 (d, *J* = 6.8 Hz, 1H), 7.64-7.59 (m, 2H), 7.54-7.50 (m, 2H), 7.46-7.33 (m, 5H), 7.28-7.23 (m, 2H), 7.18-7.14 (m, 3H), 4.78-4.72 (m, 1H), 3.70-3.64 (m, 2H), 3.13-3.00 (m, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 1.91 (q, *J* = 7.6 Hz, 2H), 1.53 (sext, *J* = 7.5 Hz, 2H), 0.73 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 528.9 (M - H).

(*R*)-3-(Biphenyl-2-ylmethylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**33f**). Using general procedure 7, compound **32f** (47 mg, 0.12 mmol) was deprotected and reacted with 360 μL of the carbamoyl chloride solution from amide **20**. The reaction was then stirred at room temperature for 10 min. Purification by semi-preparative HPLC to give compound **33f** as colorless oil (6 mg, 9%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.55 (d, *J* = 6.7 Hz, 1H), 7.64-7.58 (m, 2H), 7.54-7.50 (m, 2H), 7.46-7.23 (m, 14H), 4.59-4.53 (m, 1H), 3.76 (s, 2H), 3.67-3.62 (m, 2H), 3.07-3.85 (m, 2H), 1.52 (sext, *J* = 7.5 Hz, 2H), 0.73 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 576.9 (M + H).

(*R*)-3-(Phenethylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**33g**). Using general procedure 7, compound **32g** (78 mg, 0.24 mmol) was deprotected and reacted with 528 μL of the carbamoyl chloride solution from amide **20**. The reaction was then stirred at room temperature for 10 min. Purification by flash chromatography using CH_2Cl_2/petroleum ether 80:20, then CH_2Cl_2 100% then CH_2Cl_2/MeOH/AcOH 99:0.5:0.5 gave compound **33g** as a pale yellow oil (63 mg, 51%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.67 (d, *J* = 6.9 Hz, 1H), 7.63-7.58 (m, 2H), 7.54-7.51 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.36-7.34 (m, 3H), 7.26-7.7.24 (m, 1H), 7.21-7.17

(m, 3H), 4.80-4.74 (m, 1H), 3.69-3.64 (m, 2H), 3.15-3.00 (m, 2H), 2.92-2.80 (m, 4H), 1.49 (sext, *J* = 7.4 Hz, 2H), 0.72 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 514.9 (M - H).

(*R*)-2-(3-(3-(Phenylethynyl)benzoyl)-3-propylureido)-3-(phenylthio) propanoic acid (**35a**). Amide **20** and TMS protected (L)-*S*-phenylcysteine were reacted according to general procedures 3 to 5 affording benzoylurea **35a** as a colorless oil (mass, 61%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 7.0 Hz, 1H), 9.24 (br s, 1H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.54-7.50 (m, 4H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.33-7.14 (m, 5H), 4.80-4.74 (m, 1H), 3.78 (s, 2H), 3.71 (t, *J* = 7.4 Hz, 2H), 3.03-2.87 (m, 2H), 1.57 (sext, *J* = 7.4 Hz, 2H), 0.75 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 486.9 (M + H).

(R)-3-(4-Methoxybenzylthio)-2-(3-(3-(phenylethynyl)benzoyl)-3-propylureido)propanoic acid (**35b**). Amide **20** and TMS protected (L)-*S*-4-methoxybenzylcysteine were reacted according to general procedures 3 to 5 affording benzoylurea **35b** as a colorless oil (16 mg, 34%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.61 (d, *J* = 6.9 Hz, 1H), 7.63-7.33 (m, 9H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.80 (br s), 4.76-4.70 (m, 1H), 3.77 (s, 3H), 3.74 (s, 2H), 3.70-3.65 (m, 2H), 3.01-2.86 (m, 2H), 1.54 (sext, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 530.9 (M - H).

(*R*)-2-(3-(3-Iodobenzoyl)-3-propylureido)-3-(*iso*-butylthio)propanoic acid (**36**). Using general procedure 3, *N*-*n*-propyl-3-iodobenzamide **19** (820 mg, 2.84 mmol) was reacted with phosgene to provide the corresponding carbamoyl chloride. Using general procedure 4, *S*-*iso*-butyl-(L)-cysteine hydrochloride was protected *in situ*. The carbamoyl chloride and TMS protected amino acid were reacted using preparation method 5. Purification by flash chromatography using SiO_2

with CH_2Cl_2/MeOH 95:5 gave **36** as a pale amber oil (1.2 g, 85%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.53 (d, *J* = 6.3 Hz, 1H), 7.85-7.79 (m, 2H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.18 (t, *J* = 7.7 Hz, 1H), 4.75-4.69 (m, 1H), 3.60-3.55 (m, 2H), 3.06-2.96 (m, 2H), 2.47 (d, *J* = 6.7 Hz, 2H), 1.84-1.75 (m, 1H), 1.54 (sext, *J* = 7.6 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.5 Hz, 3H). MS (ES+), *m/z* 493.1 (M + H).

(*R*)-2-(3-(3-((3-Fluorophenyl)ethynyl)benzoyl)-3-propylureido)-3-(*iso*-butylthio)propanoic acid (**37a**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 3-fluorophenylacetylene (18 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37a** as a golden solid (37 mg, 76%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.60-9.51 (m, 1H), 8.05-7.04 (m, 8H), 4.78-4.71 (m, 1H), 3.70-3.60 (m, 2H), 3.09-2.99 (m, 2H), 2.48-2.43 (m, 2H), 1.81-1.75 (m, 1H), 1.54 (sext, *J* = 7.0 Hz, 2H), 0.97 (d, *J* = 6.5 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 485.1 (M + H).

(*R*)-2-(3-(3-((4-Fluorophenyl)ethynyl)benzoyl)-3-propylureido)-3-(*iso*-butylthio)propanoic acid (**37b**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 4-fluorophenylacetylene (18 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37b** as a golden oil (15 mg, 31%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 6.9 Hz, 1H), 7.64-7.32 (m, 8H), 4.77-4.71 (m, 1H), 3.70-3.65 (m, 2H), 3.12-2.99 (m, 2H), 2.48 (d, *J* = 6.9 Hz, 2H), 1.87-1.73 (m,

1H), 1.54 (sext, *J* = 7.4 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 485.3 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-propyl-3-(3-(*o*-tolylethynyl)benzoyl)ureido)propanoic acid (**37c**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 2-ethynyltoluene (17.4 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37c** as an off-white solid (20 mg, 42%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.63 (d, *J* = 6.8 Hz, 1H), 8.40 (br s, 1H), 7.63-7.60 (m, 2H), 7.49-7.37 (m, 3H), 7.27-7.13 (m, 3H), 4.79-4.73 (m, 1H), 3.70-3.65 (m, 2H), 3.13-2.99 (m, 2H), 2.51-2.47 (m, 5H), 1.84-1.74 (m, 1H), 1.55 (sext, *J* = 7.4 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 481.3 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-propyl-3-(3-(*m*-tolylethynyl)benzoyl)ureido)propanoic acid (**37d**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 3-ethynyltoluene (17.4 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37d** as an amber solid (29 mg, 60%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.61 (d, *J* = 6.9 Hz, 1H), 8.41 (br s, 1H), 7.61-7.59 (m, 2H), 7.45-7.31 (m, 4H), 7.26-7.14 (m, 2H), 4.79-4.73 (m, 1H), 3.70-3.65 (m, 2H), 3.12-2.99 (m, 2H), 2.47 (d, *J* = 7.0 Hz, 2H), 2.34 (s, 3H), 1.84-1.75 (m, 1H), 1.54 (sext, *J* = 7.4 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 481.3 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-propyl-3-(3-(*p*-tolylethynyl)benzoyl)ureido)propanoic acid (**37e**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 4-ethynyltoluene (17.4 mg,

0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37e** as an amber solid (12 mg, 25%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 5.9 Hz, 1H), 7.61-7.10 (m, 8H), 5.85 (br s, 1H), 4.77-4.71 (m, 1H), 3.70-3.65 (m, 2H), 2.36-2.34 (m, 5H), 1.82-1.77 (m, 1H), 1.54 (sext, *J* = 7.4 Hz, 2H), 0.97 (d, *J* = 6.5 Hz, 6H), 0.73 (t, *J* = 7.4 Hz, 3H); MS (ES+), *m/z* 481.3 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-(3-((4-phenoxyphenyl)ethynyl)benzoyl)-3-propylureido) propanoic acid (**37f**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 4-phenoxyphenylacetylene (29.1 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37f** as an amber oil (33 mg, 59%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.61 (d, *J* = 6.8 Hz, 1H), 8.37 (br s, 1H), 7.60-7.33 (m, 8H), 7.14 (t, *J* = 8.1 Hz, 1H), 7.04-6.90 (m, 4H), 4.79-4.73 (m, 1H), 3.70-3.65 (m, 2H), 3.12-2.99 (m, 2H), 2.47 (d, *J* = 6.9 Hz, 2H), 1.84-1.75 (m, 1H), 1.54 (sext, *J* = 7.3 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.73 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 559.4 (M + H).

(*R*)-2-(3-(3-((4-*tert*-Butylphenyl)ethynyl)benzoyl)-3-propylureido)-3-(*iso*-butylthio) propanoic acid (**37g**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 4-*tert*-butylphenylacetylene (25 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37g** as a colorless oil (3 mg, 6%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.62 (d, *J* = 6.8 Hz, 1H), 7.62-7.35 (m, 8H), 4.75-4.71 (m, 1H), 3.70-3.65 (m, 2H), 3.12-2.99 (m, 2H), 2.48 (d, *J* = 6.7 Hz, 2H), 1.84-1.75 (m,

1H), 1.54 (sext, *J* = 7.3 Hz, 2H), 1.31 (s, 9H), 0.97 (d, *J* = 6.4 Hz, 6H), 0.74 (t, *J* = 7.2 Hz, 3H); MS (ES+), *m/z* 523.7 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-propyl-3-(3-(pyridin-4-ylethynyl)benzoyl)ureido)propanoic acid (**37h**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 4-ethynylpyridine hydrochloride (21 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37h** as a red oil (10 mg, 21%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.50-9.35 (m, 1H), 7.81-7.14 (m, 8H), 4.76-4.70 (m, 1H), 3.70-3.65 (m, 2H), 3.12-2.99 (m, 2H), 2.46 (d, *J* = 6.9 Hz, 2H), 1.82-1.73 (m, 1H), 1.54 (sext, *J* = 7.2 Hz, 2H), 0.95 (d, *J* = 6.6 Hz, 6H), 0.74 (m, 3H); MS (ES+), *m/z* 468.2 (M + H).

(*R*)-3-(*iso*-Butylthio)-2-(3-propyl-3-(3-(pyridin-3-ylethynyl)benzoyl)ureido)propanoic acid (**37i**). Using general procedure 10, **36** (49 mg, 0.1 mmol) was reacted with 3-ethynylpyridine (15 mg, 0.15 mmol). Purification by passing through a SAX Acetate solid phase extraction column with MeOH 100% then MeOH/AcOH 85:15 gave compound **37i** as an amber oil (32 mg, 69%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.34 (d, *J* = 6.8 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.65-7.43 (m, 7H), 4.78-4.72 (m, 1H), 3.69-3.64 (m, 2H), 3.12-2.99 (m, 2H), 2.46 (d, *J* = 6.6 Hz, 2H), 1.85-1.72 (m, 1H), 1.55 (sext, *J* = 7.3 Hz, 2H), 0.95 (d, *J* = 6.5 Hz, 6H), 0.75 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 468.3 (M + H).

3-((2-Fluorophenyl)ethynyl)-*N*-propylbenzamide (**38a**): The procedure used to prepare compound **20** was followed using compound **19** (145 mg, 0.5 mmol) and 1-ethynyl-2-fluorobenzene (90 mg, 0.75 mmol). Purification of the crude mixture by flash chromatography

on SiO_2 using CH_2Cl_2/AcOEt (98:2) afforded compound **38a** as an orange solid (121 mg, 86%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.89 (s, 1H), 7.34 (dd, *J* = 7.8 and 1.1 Hz, 1H), 7.61 (dd, *J* = 7.7 and 1.2 Hz, 1H), 7.48 (td, *J* = 8.0 and 1.6 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.34-7.24 (m, 1H), 7.13-7.05 (m, 2H), 3.39 (q, *J* = 6.2 Hz, 2H), 1.62 (sext., *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.6 Hz, 3H); MS (ES+), *m/z* 282.3 (M + H).

3-((2,4-Difluorophenyl)ethynyl)-*N*-propylbenzamide (**38b**): The procedure used to prepare compound **20** was followed using compound **19** (289 mg, 1 mmol) and 1-ethynyl-2,4-difluorobenzene (207 mg, 1.5 mmol). Purification of the crude mixture by flash chromatography on SiO_2 using CH_2Cl_2/AcOEt (95:5) afforded compound **38b** as an orange solid (252 mg, 84%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.89 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 7.7 Hz, 1H), 7.51-7.37 (m, 2H), 6.89-6.83 (m, 2H), 6.27 (br s, 1H), 3.40 (q, *J* = 6.5 Hz, 2H), 1.62 (sext., *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H); MS (ES+), *m/z* 300.3 (M + H).

(*R*)-2-(3-(3-((2-Fluoropenyl)ethynyl)benzoyl)-3-propylureido)-3-(*iso*-butylthio) propanoic acid (**39a**). Using general procedure 3, **38a** (84 mg, 0.3 mmol) was reacted with phosgene to provide the corresponding carbamoyl chloride. According to general procedure 5, this intermediate was reacted at 0 ^{o}C with a solution of *in situ* protected *S*-*iso*-butyl-(L)-cysteine hydrochloride (0.31 mmol in CH_3CN, prepared according to general procedure 4). Purification by flash chromatography using SiO_2 with CH_2Cl_2/MeOH/AcOH (100:0:0 then 99.5:0.5:0 then 99:0.5:0.5) gave **39a** as a pale amber glassy residue (90 mg, 62%). ^{1}H NMR (ppm, $CDCl_3$): δ 10.4 (br s, 1H), 9.60 (d, *J* = 6.9 Hz, 1H), 7.66-7.62 (m, 2H), 7.53-7.47 (m, 1H), 7.44-7.39 (m, 2H), 7.36-7.28 (m, 1H), 7.16-7.07 (m, 2H), 4.75 (q, *J* = 6.4 Hz, 1H), 3.70-3.65 (m, 2H), 3.12-2.98 (m, 2H), 2.47 (d,

J = 6.6 Hz, 2H), 1.79 (sept., *J* = 6.7 Hz, 1H), 1.53 (sext, *J* = 7.5 Hz, 2H), 0.96 (d, *J* = 6.6 Hz, 6H), 0.74 (t, *J* = 7.4 Hz, 3H); MS (ES^+), *m/z*, 485.1 (M + H).

(*R*)-2-(3-(3-((2,4-Difluoropenyl)ethynyl)benzoyl)-3-propylureido)-3-(*iso*-butylthio) propanoic acid (**39b**). Using general procedure 3, **38b** (90 mg, 0.3 mmol) was reacted with phosgene to provide the corresponding carbamoyl chloride. According to general procedure 5, this intermediate was reacted at 0 ^oC with a solution of *in situ* protected *S*-*iso*-butyl-(L)-cysteine hydrochloride (0.31 mmol in CH_3CN, prepared according to general procedure 4). Purification by flash chromatography using SiO_2 with CH_2Cl_2/MeOH/AcOH (100:0:0 then 99.5:0.5:0 then 99:0.5:0.5) gave **39b** as a pale amber glassy residue (85 mg, 56%). ^1H NMR (ppm, $CDCl_3$): δ 9.59 (d, *J* = 6.9 Hz, 1H), 8.35 (br s, 1H), 7.64-7.61 (m, 2H), 7.52-7.40 (m, 3H), 6.91-6.87 (m, 1H), 4.75 (q, *J* = 5.6 Hz, 1H), 3.69-3.64 (m, 2H), 3.11-2.97 (m, 2H), 2.46 (d, *J* = 7.1 Hz, 2H), 1.79 (sept, *J* = 6.7 Hz, 1H), 1.53 (sext, *J* = 7.5 Hz, 2H), 0.96 (d, *J* = 6.7 Hz, 6H), 0.73 (t, *J* = 7.5 Hz, 3H); MS (ES^+), *m/z*, 503.1 (M + H).

(*R*)-2-Amino-3-(4-bromobenzylthio)propanoic acid hydrochloride salt (**40**). Using general procedure 6, cysteine (200 mg, 1.65 mmol) was reacted with 1-bromo-4-(bromomethyl)benzene (433 mg, 1.73 mmol) with a slight modification: After the reaction mixture was concentrated the residue on ice was acidified to pH 3 by slowly adding 1N HCl. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were concentrated and redissolved in MeOH then dried over $MgSO_4$. The white precipitate was triturated with diethyl ether before yielding the pure compound (*R*)-2-amino-3-(4-bromobenzylthio)propanoic acid as a hydrochloride salt (393 mg, 73%) used in the following step without further purification. ^1H NMR (ppm, DMSO-

d_6): δ 7.55 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 3.88 – 3.70 (m, 3H), 2.96 (dd, *J* = 14.2, 4.5 Hz, 2H), 2.83 (dd, *J* = 14.2, 7.2 Hz, 2H); MS (ES+), *m/z* 291.8, (M + H).

(*R*)-3-(4-Bromobenzylthio)-2-(3-(3-((2,4-difluorophenyl)ethynyl)benzoyl)-3-propylureido)propanoic acid (**41**). Amide **38b** and TMS protected (L)-*S*-4-bromobenzylcysteine **40** were reacted according to general procedures 3 to 5, affording benzoylurea **41** as a colorless oil (15 mg, 33%). ^{1}H NMR (ppm, $CDCl_3$): δ 9.64 (d, *J* = 7.1 Hz, 1H), 7.66-7.63 (m, 2H), 7.53-7.40 (m, 4H), 7.24-7.17 (m, 4H), 6.92-6.84 (m, 2H), 4.83-4.7 (m, 1H), 3.73 (s, 2H), 3.71-3.66 (m, 1H), 3.02-2.88 (m, 2H), 1.59-1.51 (m, 2H), 0.75 (t, *J* = 7.5 Hz, 3H); MS (ES^+), *m/z* 616.9, (M + H).

***De novo* design of ligand 5.**

The modeling was performed using the BCL-X_L•BAK structure published by Petros *et al.*[30,31]. Ligands were built using the SYBYL6.8 (Tripos Associates; http://www.tripos.com) software. For all minimizations and force field calculations, the Tripos force field was used with Gasteiger–Huckel charges as implemented in SYBYL6.8. Default settings were used for the minimization process, including the use of a distance dependent dielectric of 1 and using a non-bonded cut-off of 8 Å. Full coordinates of the resulting modeling for compound **5** are included as Supplementary Material.

Luminescence Polarization Assay: AlphaScreen™

The aim of the BH3 proteins AlphaScreen™ assay is to identify active small molecules inhibiting the protein-protein interaction between BCL-X_L and a BH3-peptide derived from BIM.

Reagents and Materials

GST-BCL-X_L proteins were prepared and provided by established procedures.[34,51] Biotinylated-GST (for the counter-screen) were purchased (Perkin-Elmer). These constructs were stored as stock solutions at -80°C. The AlphaScreen GST (Glutathione-S-Transferase) detection kits were obtained from Perkin Elmer Lifesciences. White, 384, low-volume assay plates and polypropylene, 384, compound storage plates were obtained from Greiner Bio One and Matrical, respectively. Optical-grade, adhesive plate seals for assay plates were obtained from Applied Biosystems whilst adhesive aluminium foil seals used for compound plate storage were obtained from Beckman-Coulter. DMSO was purchased from AnalaR. Assay Buffer (25 mM HEPES/25 mM Tris-HCl/50 mM NaCl, pH 7.5) stock was warmed to room temperature from 4°C, followed by the addition of 5 mM DTT (aliquots stored at -20°C), 0.1 mg/mL casein (sodium salt; aliquots stored at -20 ºC) and 0.03% v/v Tween 20 (all final concentrations) fresh on a daily basis.

Methods

Screening of the test compounds was performed using the AlphaScreen™ GST (glutathione s-transferase) detection kit system (Perkin Elmer Lifesciences). Briefly, test compounds were titrated into the assay, which consisted of GST tagged BCL-X_L ΔC25 protein (0.6 nM final concentration) and biotinylated BIM BH3-26mer peptide, Biotin-DLRPEIRIAQELRRIGDEFNETYTRR (5.0 nM final concentration), anti-GST coated acceptor beads and streptavidin coated donor beads (both bead types at a final concentration of 15 μg/mL) and a room temperature incubation time of 4h before reading. More specifically: (i) a 384-well plate was prepared with 4.75 μL of buffer and 0.25 μL of compound stock (20 mM in DMSO) per well; (ii) binding partners were mixed – in one tube BCL-X_L was added with the acceptor beads, while in the second tube the biotinylated BH3 peptide was added with the donor beads;

(iii) these two pairs of binding partners were pre-incubated for 30 minutes; (iv) 10 μL of the acceptor beads/BCL-X_L protein complex was then added to each of the 384 wells; (v) plates were sealed and incubated at room temperature for a further 30 minutes; (vi) 10 μL of the donor bead/BH3 peptide complex was then added to each of the 384 wells; (vii) plates were sealed, covered with foil and incubated for a further 4 hours and then read. Assay buffer contained 50 mM Hepes pH 7.4, 10 mM DTT, 100 mM NaCl, 0.05% Tween20 and 0.1 mg/mL casein. Bead dilution buffer contained 50 mM Tris-HCl, pH 7.5, 0.01% Tween20 and 0.1 mg/mL casein. The final DMSO concentration in the assay was 1.0% (v/v). Assays were performed in 384-well white Optiplates (Perkin Elmer Lifesciences) and analysed on the PerkinElmer Fusion Alpha plate reader (Ex680, Em520-620 nM).

Determination of binding constant by Surface Plasmon Resonance direct binding assay (Biacore S51).

Immobilization of anti-GST antibody: Anti-GST antibody surfaces were prepared by using standard amine-coupling procedures as instructed by the GST Capture Kit from Biacore. The running buffer was 10 mM $NaH_2PO_4.H_2O$, 40 mM $Na_2HPO_4 \bullet 2H_2O$, 150 mM NaCl, 1.0 mM EDTA, 0.03% Tween 20, 5% DMSO, pH 7.4. Flow cells (both spot 1 and spot 2) were activated by injecting 200 μL of (11.5 mg/mL) NHS and EDC (75 mg/mL). Fifty microliters of anti-GST antibody (30 μg/mL) in 10 mM sodium acetate pH 5.0 was injected for 10 min at 5 μL/min, followed by the injection of 175 μL Ethanolamine (1 M). Inject 36 seconds of Biadesorb1 solution (0.5% SDS) and 40 seconds of Biadesorb2 solution (50 mM glycine pH 9.5) at 30 μL/min at the end of the run. These resulted in about 10,000 RU anti-GST antibody immobilized.

Capture of GST-BCL-X_L: The running buffer utilized was the same as for immobilization of anti-GST antibody. GST-BCL-X_L (0.1 mg/mL to 0.2 mg/mL) in the running buffer was injected at 10 μL/min for 3 min across spot 2 only, resulting in the capture of about 1200 RU protein.

Kinetic Analysis: 30 nM of 26-mer-wt-BIM (10 μl/min x3 minutes) was loaded into spot 1 only at the beginning of each cycle using hydronamic addressing resulting in BCL-X_L BH3 binding groove "pre-blocked" with wt-BIM 26mer. A concentration series of each compound was injected at a flow rate of 90 µL/min over three spots (spot 1: coated with BCL-X_L bound to BIM peptide, spot 2: coated with GST-BCL-X_L, spot r: free) at 25 °C. The association and dissociation measurement times were 60 and 120 seconds, respectively. The buffer blanks were also injected periodically for double referencing. The buffer samples containing 4% to 6% DMSO were injected for solvent correction. The sensorgrams of (spot 1 – spot r) reflect the non-specific binding to the GST-BCL-X_L protein (prior to DMSO solvent correction). The sensorgrams of (spot 2 – spot 1) reflect the specific binding to the GST-BCL-X_L protein's binding groove (prior to DMSO solvent correction).

Data Analysis: All sensorgrams were processed by using double referencing: 1. The response from the reference spot (spot 1) was subtracted from the binding response (spot 2), followed by solvent correction. 2. The response from an average of buffer injection was subtracted. To obtain kinetic rate constants (k_a and k_d), corrected response data were then fitted to a one to one binding site model, which includes correction for mass transport limitations. The equilibrium dissociation constant (K_d) was determined by k_d/k_a. For more details about the experimental design, refer to previously published studies by our group.[23,24]

Determination of Selectivity Profile by Surface Plasmon Resonance analysis (Biacore 3000). *Recombinant proteins*: Expression and purification of loop-deleted human BCL-2 ΔC22 and human BCL-W ΔC29 (C29S/A128E) as well as human BCL-X_L ΔC24, human/ mouse chimeric MCL-1 (ΔN170,ΔC23) and mouse A1(P104K) was performed as described previously.[34,51,55] Synthetic peptides were synthesized by Mimotopes and purified by reverse-phase HPLC to >90% purity.

Binding Affinity Measurements – Solution Competition Assays. Solution competition assays were performed using a Biacore 3000 instrument as described previously.[21,56] Briefly, pro-survival proteins (10 nM) were incubated with varying concentrations of compounds (25 μM – 0.024 μM) for 2 h in running buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, and 0.005% (v/v) Tween 20, pH 7.4) prior to injection onto a CM5 sensor chip onto which either a wild-type mBIMBH3 (DLRPEIRIAQELRRIGDEFNETYTRR) peptide or an inert mBIMBH3 (DLRPEIR**E**AQE**E**RR**E**GDE**E**NETYTRR) mutant peptide (mutations in bold) were immobilized (via N-hydroxysuccinimide coupling). Specific binding of the prosurvival protein to the surface in the presence and absence of compounds was quantified by subtracting the signal from the BIM mutant channel from that obtained on the wild-type BIM channel, following 'global analysis' baseline correction for each using the BIAevaluation Software (version 4.1, Biacore). IC_{50} values were calculated by nonlinear curve fitting of the data with Prism Software (version 6.0b for Macintosh OS X, GraphPad Software).

X-ray Structural Data – Crystallization, Data Collection and Structure Refinement

Crystallization of both the BCL-X_L•**39b** and BCL-X_L•**41** complexes was performed as previously described.[24,52] Briefly, BCL-X_L, a C-terminally truncated, "loop-deleted" form of

human BCL-X_L (Δ27-82 ΔC24)[57] was combined with a 1.5 molar excess of either compound in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, supplemented with 5 % DMSO. The complex was then concentrated to 10 mg/mL. Crystals of the BCL-X_L•**39b** complex were grown in hanging drops at 295 K with a reservoir solution consisting of 1.4 M ammonium sulphate, 0.1 M MES pH 6.5. Crystals of the BCL-X_L•**41** complex were grown similarly, but instead with a reservoir solution consisting of 1.1 M ammonium sulphate, 0.1 M MES pH 6.5. Both sets of crystals were soaked for 30 seconds in cryo-protectant (consisting of the mother liquor, supplemented with 25 % ethylene glycol) and flash frozen in liquid nitrogen. X-ray data for the BCL-X_L•**39b** complex were collected at 100 K using an in-house RAXIS-IV++ detector with a micro-max007 rotating anode x-ray source, then integrated and scaled with HKL2000.[58] Data for the BCL-X_L•**41** complex were collected at 100 K at the Australian Synchrotron (MX-1), then integrated and scaled with XDS.[59] Each structure was solved by molecular replacement with PHASER.[60-62] In the case of the BCL-X_L•**39b** complex the BCL-X_L coordinates from the crystal structure of human BCL-X_L (1MAZ),[63] were used as the search model and in the case of the BCL-X_L•**41** complex, the isolated co-ordinates of BCL-X_L taken from the structure of the BCL-X_L•**39b** complex. Several rounds of building in COOT[64] and refinement with REFMAC5[65] and PHENIX[66] incorporating in each case an initial round of simulated annealing, led to the models described in Supplementary Table 2 and Figure 5. Structural alignments were performed, and figures constructed, using PyMOL (PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC). Structures were deposited in the PDB with the following access codes: 4C52 (**39b**) and 4C5D (**41**).

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37. Our work based on the benzoylurea scaffold has been presented in a number of fora: posters (1st Conforth conference, Sydney 2002; 19th RACI conference, Lorne 2003; 20th RACI conference, Cairns 2004) and oral presentations (Connect 2005, 12th RACI Convention, Sydney 2005; 5th and 7th ESH conference on Mechanisms of Cell Death and Disease: Advances in Therapeutic Intervention and Drug Development, 2004 and 2008). Inspired by our results (note 11 and 13 in Rodriguez, J. M.; Ross, N. T.; Katt, W. P.; Dhar, D.; Lee, G.-i.; Hamilton, A. D. Structure and Function of Benzoylurea-Derived α-Helix Mimetics Targeting the Bcl-xL/Bak Binding Interface. *ChemMedChem* **2009**, *4*, 649–656), the Hamilton group reported a similar use of this core for the preparation of extended α-helical mimetics.

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41. While the possibility of a long range interaction between the carboxylic acid and ARG139 might still be possible in the bound complex, we hypothesized that introducing this moiety may also be beneficial to increase the rate of productive encounters between the small molecule and the canonical binding groove through charged interactions between a highly conserved element of the pro-survival protein binding groove (arginine) and a complementary interacting group on our inhibitor (carboxylic acid).

42. It is also likely that the excess trimethylsilylating agent also leads to the formation of trimethylsilylamine, which effectively activates the amino group towards nucleophilic addition ("neutral alkyl amide").

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46. Other methods such as Fluorescence Polarization (especially used without confirmation by separate screening methods) are prone to give false positive results in particular in the context of protein-protein interactions.

47. Around 130 analogues were prepared during this work. We present here the most important results.

48. See Supplementary Data section

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50. We attribute the difference between the AlphasScreen IC_{50} value and the reported K_i value (see Ref 49) to the method used to obtain K_i. The original report does not present the IC_{50} obtained by fluorescence polarization for this compound from which the K_i was determined. We judge that, because the IC_{50} value obtained by AlphaScreen and the K_D are in close agreement, they are good representative of the real level of binding affinity for **42**.

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ASSOCIATED CONTENT

Supporting Information: Coordinates for structure based design of compound **5**, steady state curves, sensorgrams and parameters from SPR experiments for compounds **39b**, **40** and **41**, and crystallographic information for compounds **39b** and **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVATIONS USED

BAK: BCL-2 homologous antagonist/killer; BAX: BCL-2 associated X protein; BCL-2: B-Cell Lymphoma 2; BCL-X_L: B-Cell Lymphoma Extra Large; BCL-W: BCL-2-like protein 2; BIM: BCL-2 interacting mediator of cell death; BH: BCL-2 homology domain; MCL-1: Myeloid

cell leukemia sequence 1; LPA: Luminescence Proximity Assay; PPI: Protein-Protein Interactions; SPR: Surface Plasmon Resonance.

FIGURES

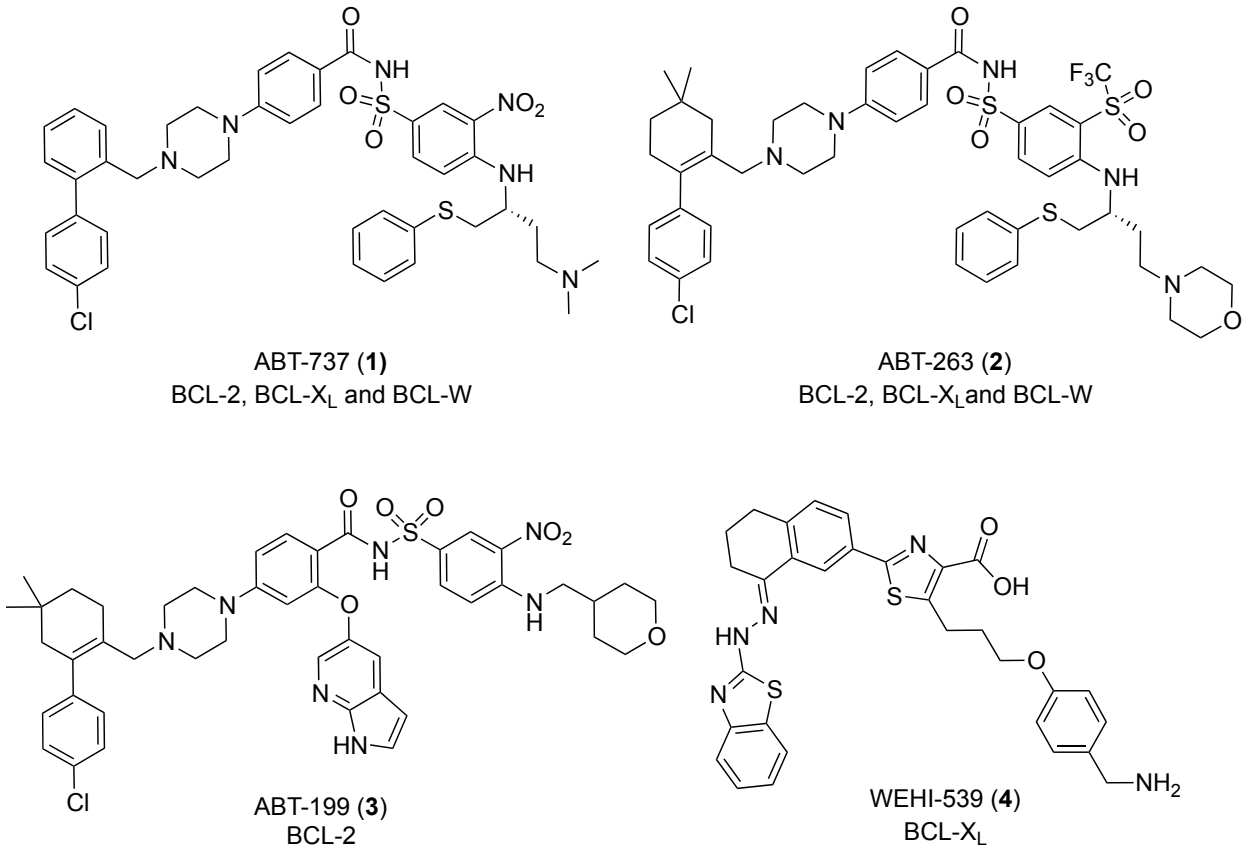


Figure 1. Structure of ABT-737, navitoclax (ABT-263), ABT-199 and WEHI-539. The selectivity profile for each compound is indicated.

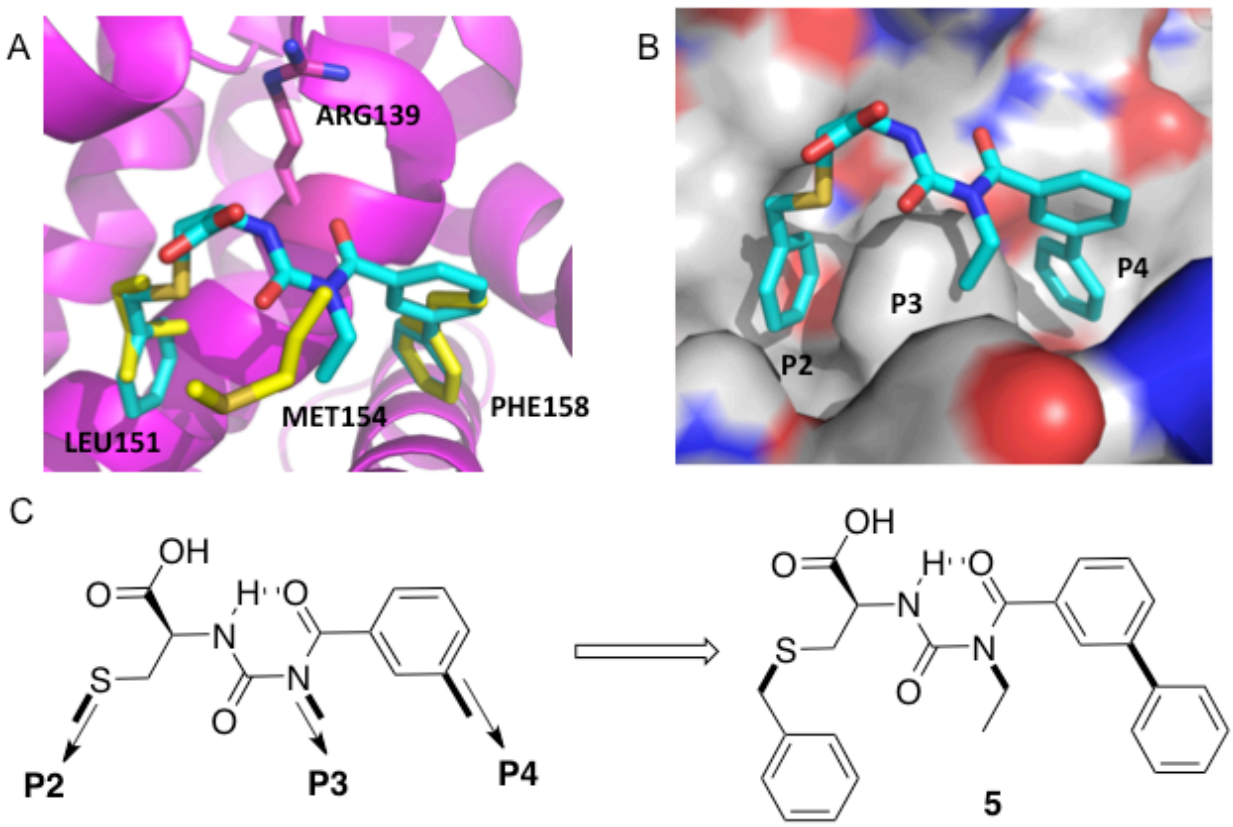


Figure 2. *De novo* designed benzoylurea. (A) Modeling and *de novo* design using the BAD•BCL-X_L NMR solution structure (PDB ID: 1G5J) by Petros *et al*. (Ref 31). In yellow: three hydrophobic residues of BAD (LEU151, MET154 and PHE158 which equate to i+4, i+7 and i+11 respectively) in complex with BCL-X_L (main chain of the BH3 peptide has been removed for clarity). In cyan: *de novo* designed benzoylurea. Ribbon in magenta: BCL-X_L. (B) Overall docking of *de novo* designed compound within the hydrophobic pocket of BCL-X_L. (C) Designed molecule **5**.

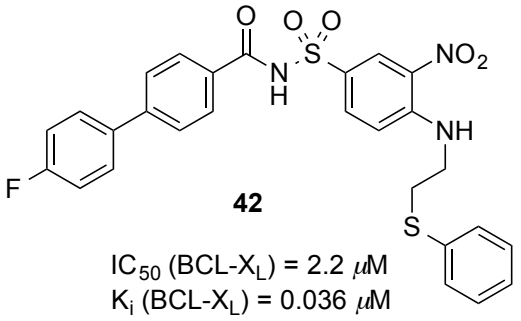


Figure 3. Benchmark compound from Ref 49.

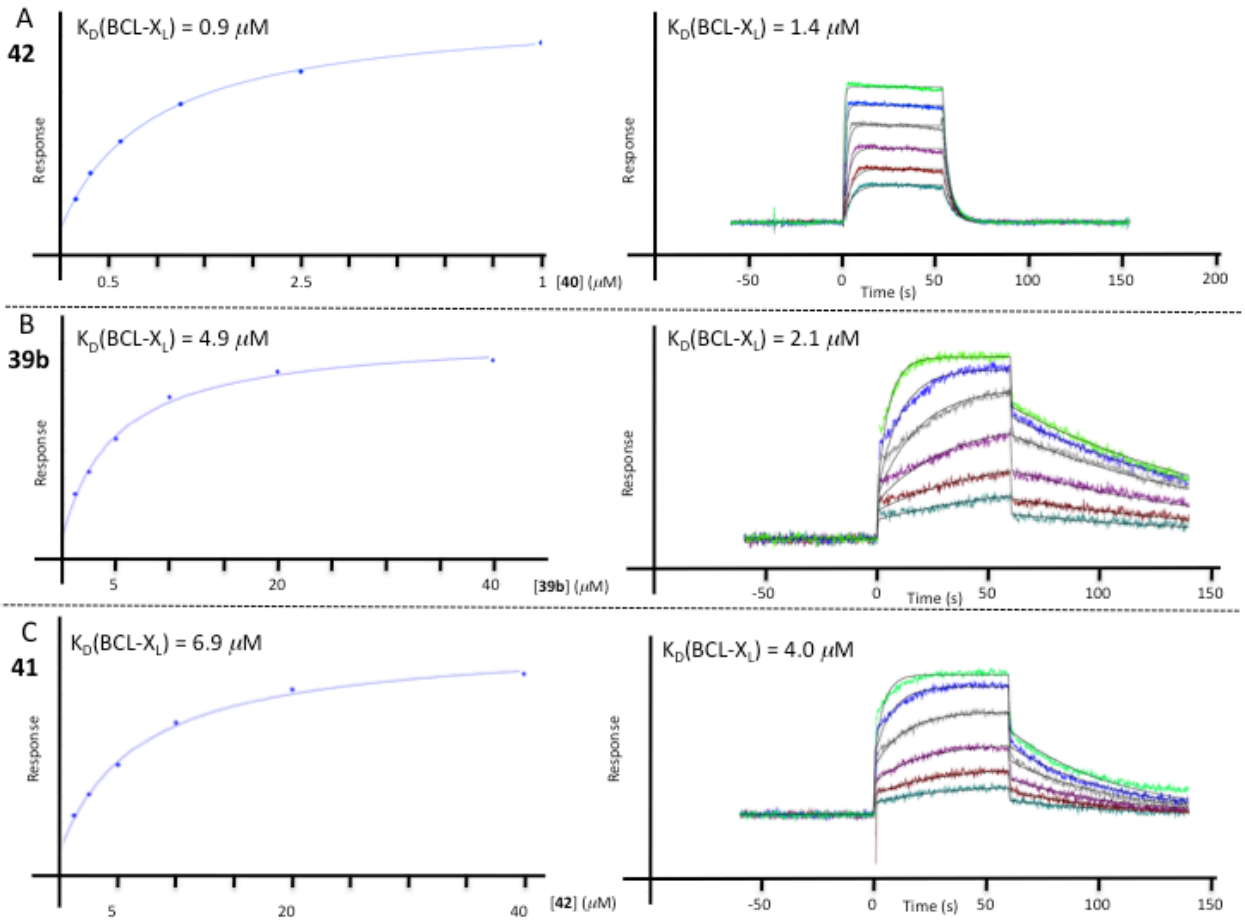


Figure 4. Compounds from the benzoylurea series bind directly to BCL-X_L by engaging the hydrophobic cleft. Graphs show direct binding affinity between compounds and BCL-X_L as measured by Surface Plasmon Resonance (direct binding, Biacore S51). On the left: Steady State

curves show Response (RU: Resonance Unit, Y axis) as a function of compound concentration (X axis, in μM). On the right: sensorgrams showing association and dissociation rates. Response (RU; Resonance Unit, Y axis) and Time (X-axis, s). Steady State and Kinetic K_D values are indicated on each graph. (A) Control compound **42**; (B) Compound **39b**; (C) Compound **41**.

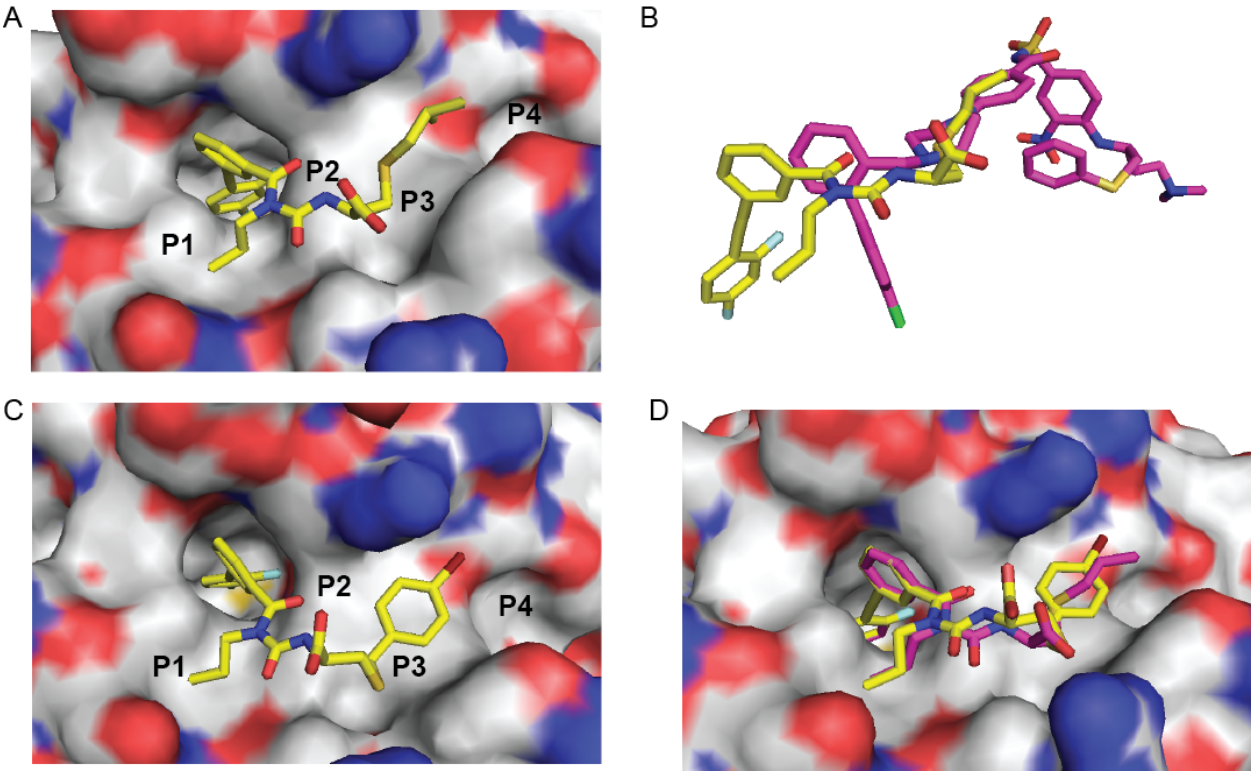
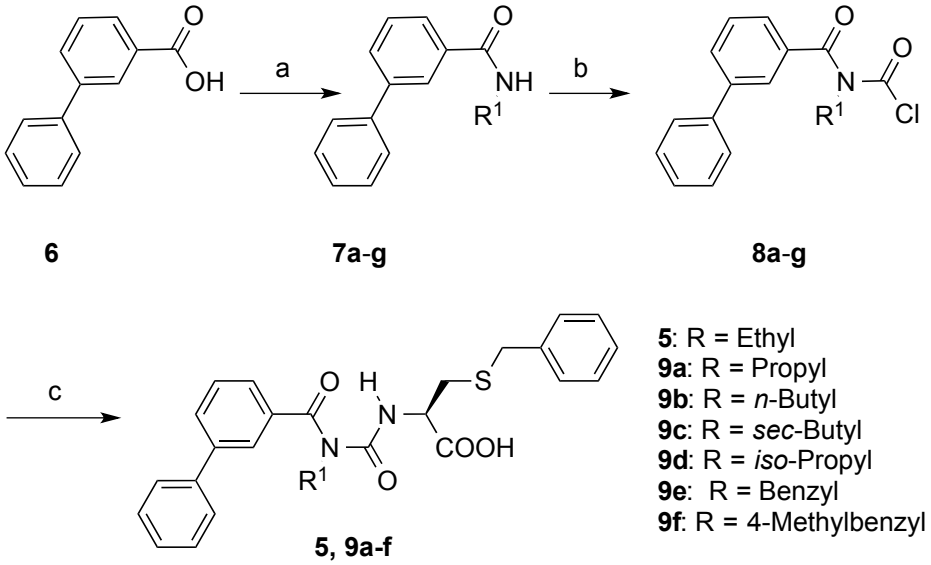


Figure 5. X-ray crystallography shows that compound **39b** (PDB ID: 4C52) and **41** (PDB ID: 4C5D) bind into the hydrophobic groove of BCL-X_L by creating a deep hole around the P1/P2 region. (A) Overall binding mode of compound **39b** within the hydrophobic groove of BCL-X_L. The regions in which the canonical hydrophobic binding pockets P1 to P4 are located are indicated. (B) Overlay of compound **39b** and **1** bound to BCL-X_L (PDB ID: 2YXJ) with the protein omitted for clarity. (C) Overall binding mode of compound **41** within the hydrophobic

groove of BCL-X_L. Canonical hydrophobic binding pockets P1 to P4 are indicated. (D) Overlay of compound **39b** and **41** bound to BCL-X_L. The protein is shown as a surface representation colored according to the nature of the atoms (carbon and hydrogen in grey, oxygen in red and nitrogen in blue). Compounds are shown as a stick representation with carbon atoms in yellow (5A-D) or magenta (5B and 5D), oxygen atoms in red, nitrogen atoms in blue and sulfur atoms in yellow.

SCHEMES

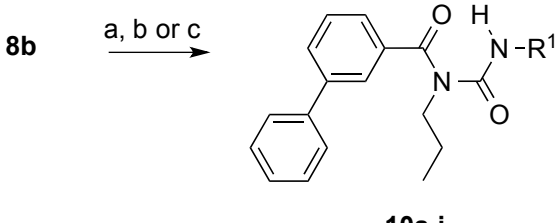
Scheme 1. Synthesis of the metabiphenyl derived benzoylureas.[a]



5: R = Ethyl
9a: R = Propyl
9b: R = *n*-Butyl
9c: R = *sec*-Butyl
9d: R = *iso*-Propyl
9e: R = Benzyl
9f: R = 4-Methylbenzyl

[a]Reagents and conditions: (a) EDCI, HOBt, DCM, 12 h, R^1NH_2 (b) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (c), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

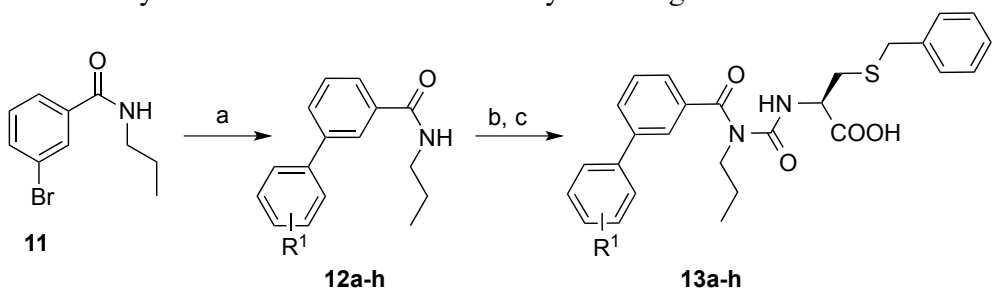
Scheme 2. Synthesis of benzoylureas varying the terminal amino acid substituent.[a]



10a: $R^1 = CH_3$
10b: R^1 = Leucine
10c: R^1 = Phenylalanine
10d: R^1 = Tyrosine
10e: R^1 = Homo-phenylanine
10f: R^1 = 3-Napthylalanine
10g: R^1 = Tryptophan
10h: $R^1 = CH_2CH_2SCH_2Ph$
10i: R^1 = *S*-Benzyl-cysteine-OMe
10j: R^1 = *S*-Benzyl-cysteine-NH_2

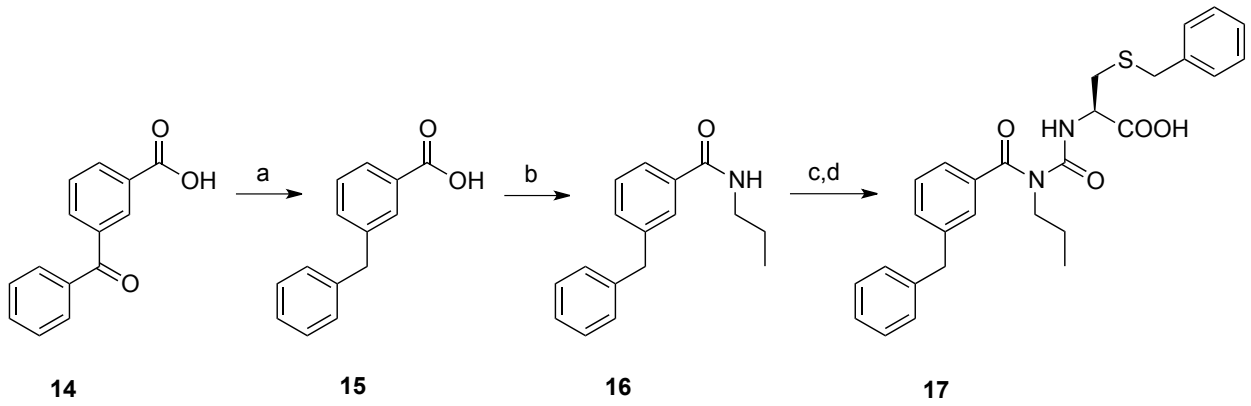
[a]Reagents and conditions: (a) for **10a**: Propylene oxide, $MeNH_2$ 33% in EtOH, CH_3CN (b) for **10b-g** and **10i-j**: Propylene oxide, BTSA, amino acid or amine R^1NH_2, CH_3CN (c) for **10h**: 2-(benzylthio)ethanamine, NEt_3, CH_3CN.

Scheme 3. Synthesis of meta-substituted alkyne analogues.[a]



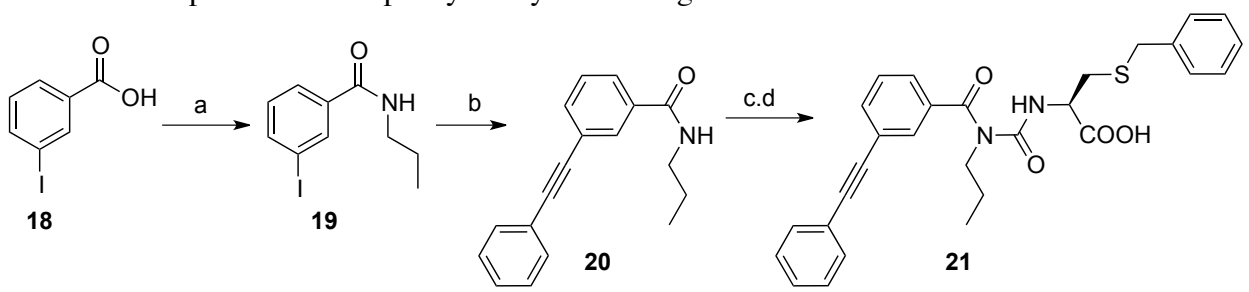
[a]Reagents and conditions: (a) Boronic acids, $Pd(PPh_3)_4$, Na_2CO_3, EtOH; (b) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (c), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

Scheme 4. Synthesis of the methylene linked biphenyl analogue **17**.[a]



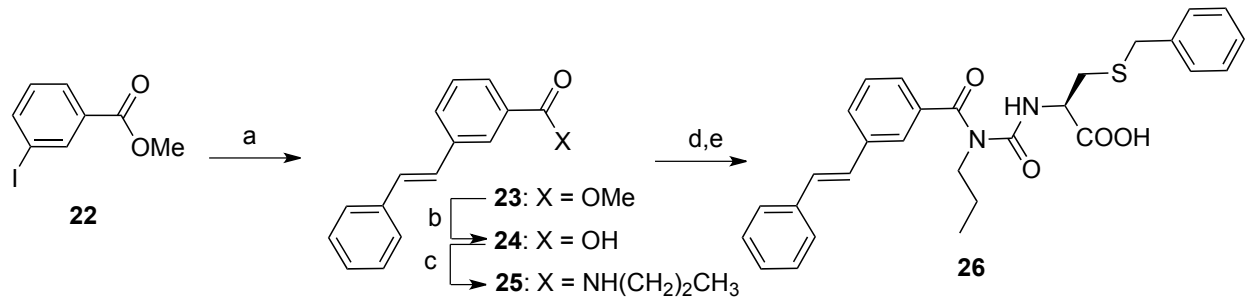
[a]Reagents and conditions: (a) Hydrazine hydrate, NaOH, triethyleneglycol, 190ºC. (b) (i) $SOCl_2$, reflux; (ii) propylamine, NEt_3, CH_2Cl_2. ; (c) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (d), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

Scheme 5. Preparation of the phenyl acetylene analogue.[a]



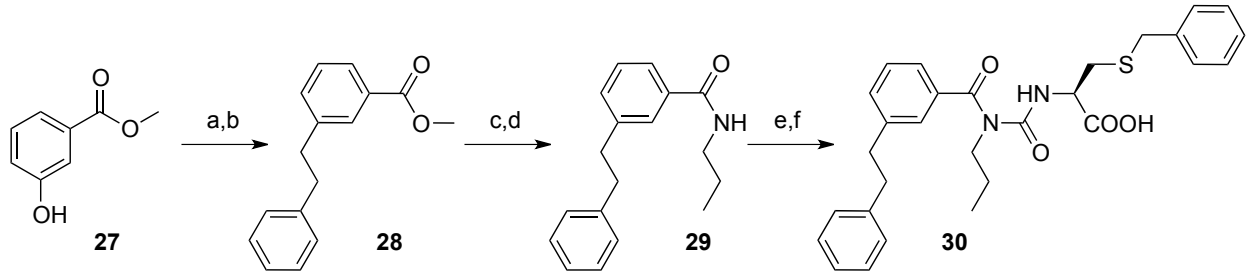
[a]Reagents and conditions: (a) (i) $SOCl_2$, reflux; (ii) propylamine, NEt_3, CH_2Cl_2. (b) Phenylacetylene, $Pd(PPh_3)_4$, Piperidine, 70ºC. (c) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (d), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

Scheme 6. Synthesis of the phenyl alkene analogue **26**.[a]



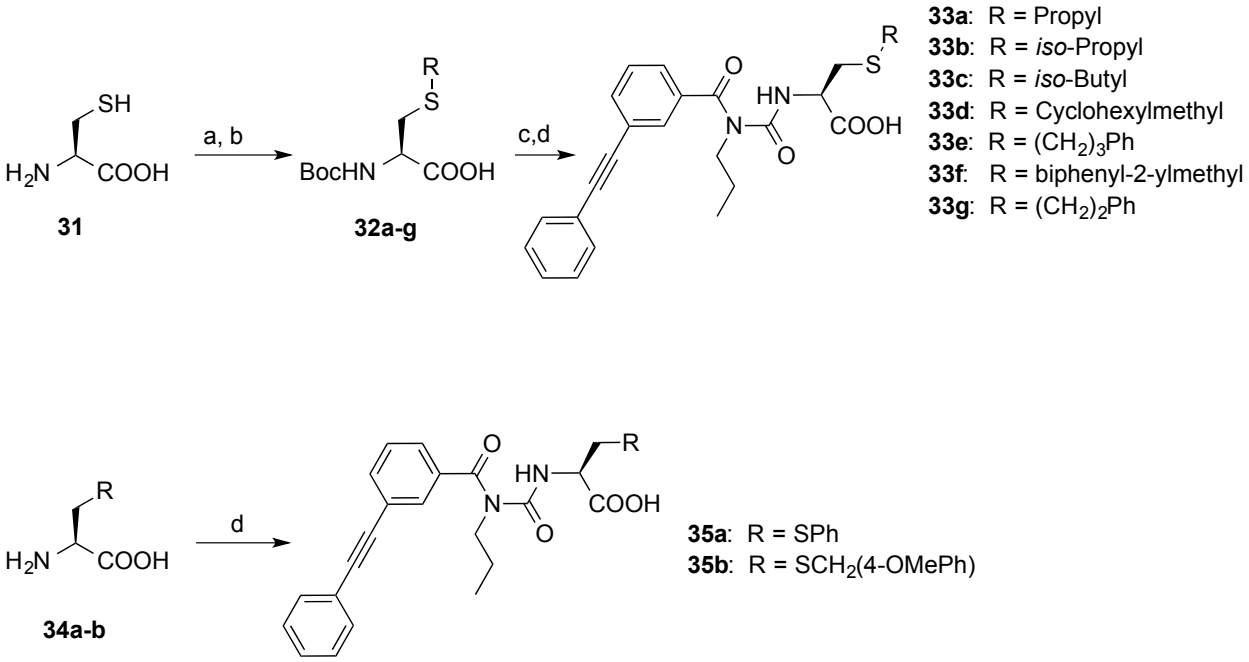
[a]Reagents and conditions: (a) 1-Styreneboronic acid pinacoyl ester, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, reflux. (b) NaOH, EtOH, 80ºC. (c) (i) $SOCl_2$, reflux; (ii) propylamine, NEt_3, CH_2Cl_2. (d) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (e), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

Scheme 7. Synthesis of the phenyl ethylene analogue.[a]



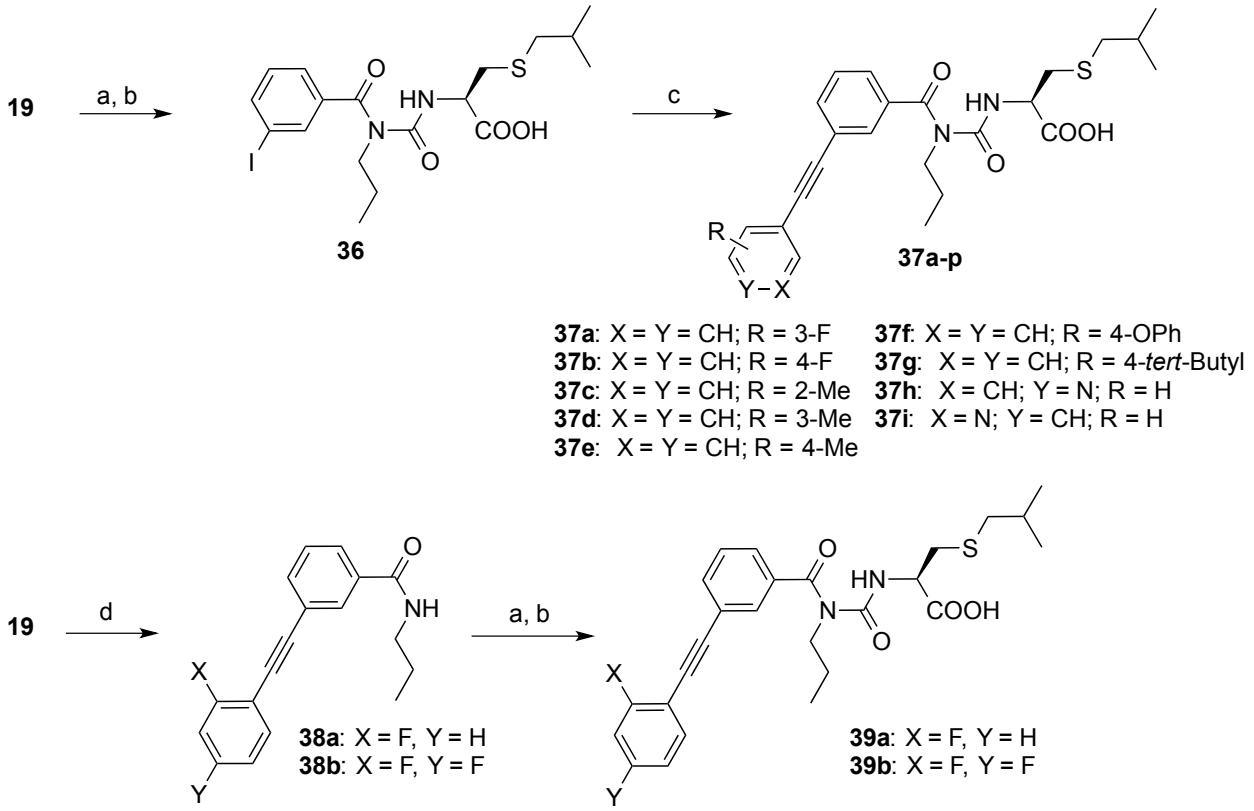
[a]Reagents and conditions: (a) Tf_2O, DMAP, 2,4,6-collidine, CH_2Cl_2. (b) Phenethylmagnesium bromide, $Fe(acac)_3$, NMP. (c) NaOH, EtOH, 80°C. (d) (i) $SOCl_2$, reflux; (ii) propylamine, NEt_3, CH_2Cl_2. (e) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (f), Propylene oxide, BTSA, *S*-benzyl-L-cysteine, CH_3CN.

Scheme 8. Preparation of phenylacetylenic derivatives.[a]



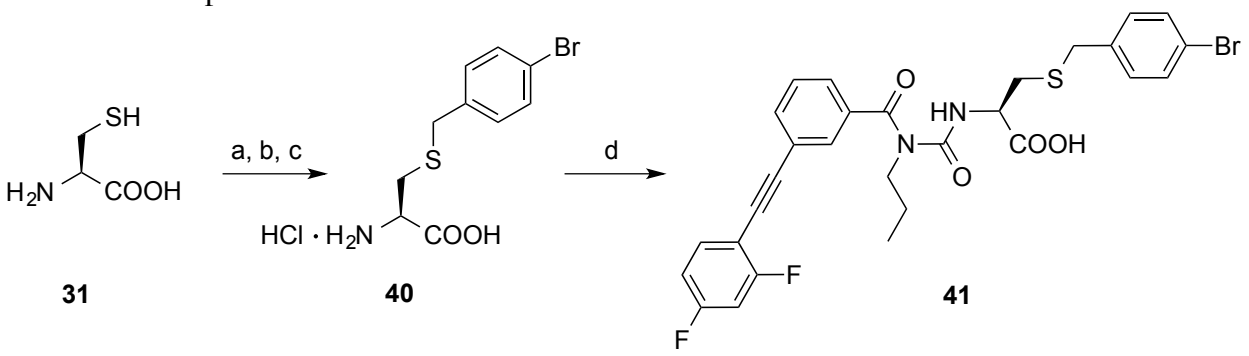
[a]Reagents and conditions: (a) Alkyl iodide, NaOH, EtOH or alkyl bromide, tetrabutyl ammonium iodide, NaOH EtOH. (b) Boc_2O, EtOH. (c) HCl 4N, dioxane. (d) 3-(Phenylethynyl)benzoyl(propyl)carbamoyl chloride, propylene oxide, BTSA, CH_3CN.

Scheme 9. Preparation of acetylenic derivatives.[a]



[a]Reagents and conditions: (a) (i) TMSOTf, Et_3N, Et_2O, 1 h (ii) 20% $COCl_2$ in toluene, 1 h (c), Propylene oxide, BTSA, *S-iso*-propyl-L-cysteine, CH_3CN. (c) Arylalkynyl, CuI, $Pd(PPh_3)_2Cl_2$, NEt_3, DMF. (d) 1-ethynyl-2-fluorobenzene or 1-ethynyl-2,4-difluorobenzene, $Pd(PPh_3)_2Cl_2$, pipridine, 70ºC.

Scheme 10. Preparation of derivative **41**.[a]

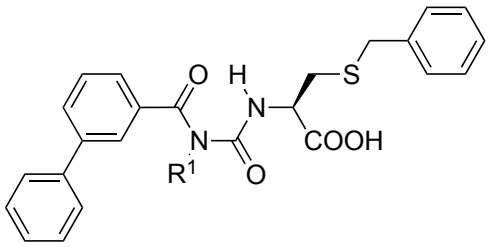


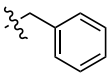
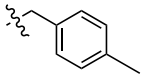
31 **40** **41**

[a]Reagents and conditions: (a) 4-bromobenzylbromide, NaOH, EtOH, tetrabutyl ammonium iodide, NaOH, EtOH. (b) Boc_2O, EtOH. (c) HCl 4N, dioxane. (d) Propylene oxide, BTSA, carbamoylchloride of **38b**, CH_3CN.

TABLES

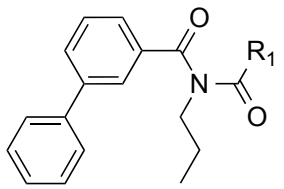
Table 1. Analogues at amide 1 position

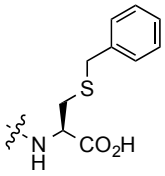
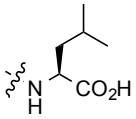
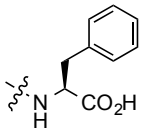
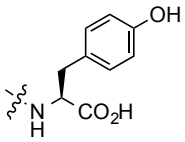
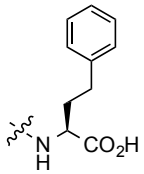
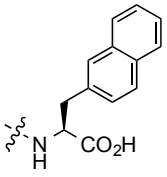


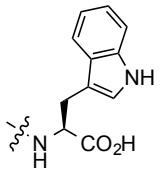
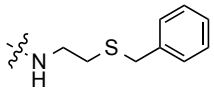
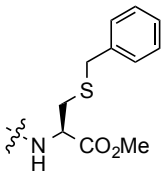
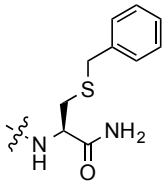
Compounds	R^1	IC_{50} (BCL-X_L, μM) [a,b]
5		128 (±11)
9a		109 (±13)
9b		138 (±15)
9c		> 150
9d		> 150
9e		> 150
9f		> 150

[a] All IC_{50} values measured by LPA are an average of at least two separate experiments with standard deviation in brackets. [b] All compounds were tested by serial dilution starting from 150 μM. Upper bound values (i.e. IC_{50} > 150 μM) reflect compounds for which IC_{50} values are above this threshold.

Table 2. Variations of the amino-acid moiety

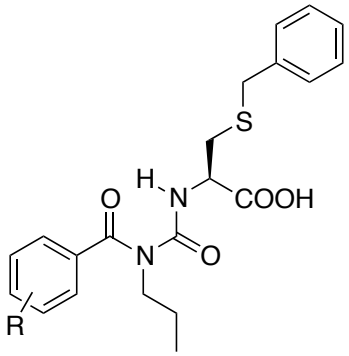


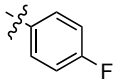
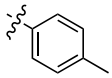
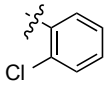
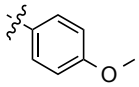
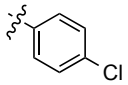
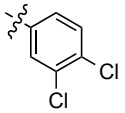
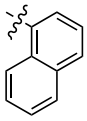
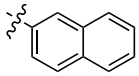
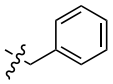
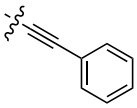
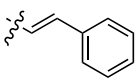
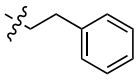
Compounds	R^1	IC_{50} (BCL-X_L, μM) [a,b]
9a		109 (±13)
10a		> 150
10b		> 150
10c		> 150
10d		> 150
10e		> 150
10f		>150

10g		108 (±2)
10h		> 150
10i		> 150
10j		> 150

[a] All IC_{50} values measured by LPA are an average of at least two separate experiments with standard deviation in brackets. [b] All compounds were tested by serial dilution starting from 150 μM. Upper bound values (i.e. IC_{50} > 150 μM) reflect compounds for which IC_{50} values are above this threshold.

Table 3. SAR exploration of phenyl substitution

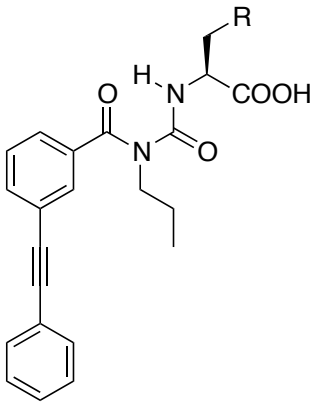


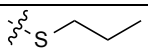
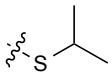
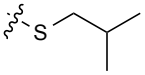
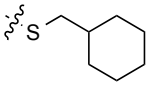
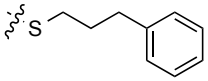
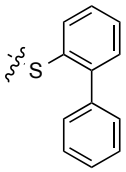
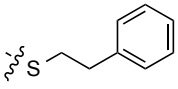
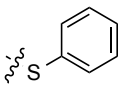
Compounds	R	IC_{50}(BCL-X_L, μM)[a,b]
9a		109 (±13)
13a		125 (±55)
13b		77 (±23)
13c		102 (±29)
13d		>150
13e		86 (±4)
13f		78 (±1)
13g		62 (±8)
13h		63 (±1)
17		> 150
21		35 (±4)
26		141 (±15)
30		55 (±6)

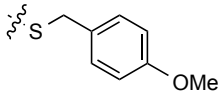
[a] All IC_{50} values measured by LPA are an average of at least two separate experiments with standard deviation in brackets. [b] All compounds were tested by serial dilution starting from 150

μM. Upper bound values (i.e. IC_{50} > 150 μM) reflect compounds for which IC_{50} values are above this threshold.

Table 4. SAR exploration of the amino-acid residue on the phenyl-acetylene derivative

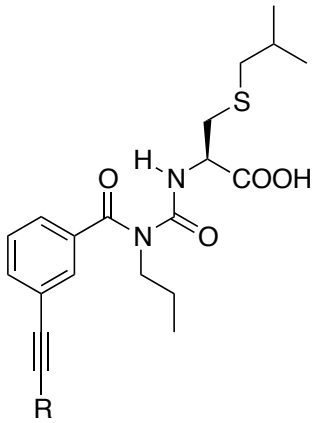


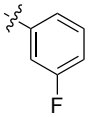
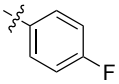
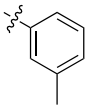
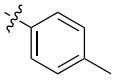
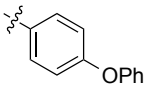
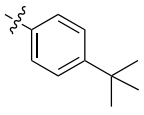
Compounds	R	IC_{50}(BCL-X_L)[a]
33a		21 (±0.3)
33b		33 (±2)
33c		15 (±1)
33d		42 (±3)
33e		73 (±1)
33f		146 (±10)
33g		21 (±2)
35a		25 (±0.1)

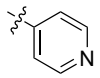
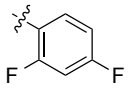
35b		21 (±6)

[a] All IC_{50} values measured by LPA are an average of at least two separate experiments with standard deviation in brackets.

Table 5. Phenyacetylene analogues



Compounds	R	$IC_{50}(BCL\text{-}X_L)$ [a,b]
33c		15 (±0.5)
37a		69 (±35)
37b		9 (±1)
37c		57 (±38)
37d		51 (±17)
37e		50 (±3)
37f		> 150
37g		> 150

37h		> 150
37i		> 150
39a		39 (±6.3)
39b		22 (±3.3)

[a] All IC_{50} values measured by LPA are an average of at least two separate experiments with standard deviation in brackets. [b] All compounds were tested by serial dilution starting from 150 μM. Upper bound values (i.e. IC_{50} > 150 μM) reflect compounds for which IC_{50} values are above this threshold.

Table 6. Selectivity profile for compounds **39b** and **42** obtained by SPR competition experiments.

Compounds	**BCL-X_L** **(IC_{50}, μM)**[a]	**BCL-2** **(IC_{50}, μM)**[a]	**BCL-W** **(IC_{50}, μM)**[a]	**MCL-1** **(IC_{50}, μM)**[a]	**A1** **(IC_{50}, μM)**[a]
39b	4.4 (±0.2)	> 25	> 25	> 25	> 25
42	0.196 (±0.007)	14.7 (±0.1)	> 25	22 (±3)	14.2 (±0.4)

[a] IC_{50} values are shown as average of three independent SPR experiments with standard error of the mean in brackets. [b] All compounds were tested by serial dilution starting from 25 μM. Upper bound values (i.e. IC_{50} > 25 μM) reflect compounds for which IC_{50} values are above this threshold.

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