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Laurent Hoffer, Manon Garcia, Raphael Leblanc, Mikael Feracci, Stéphane Betzi, et al.. Discovery of a PDZ Domain Inhibitor Targeting the Syndecan/Syntenin Protein–Protein Interaction: A Semi-Automated “Hit Identification-to-Optimization” Approach. *Journal of Medicinal Chemistry*, 2023, 10.1021/acs.jmedchem.2c01569 . hal-04048842

HAL Id: hal-04048842

<https://hal.science/hal-04048842v1>

Submitted on 28 Mar 2023

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Discovery of a PDZ Domain Inhibitor Targeting the Syndecan/Syntenin Protein-Protein Interaction: A Semi-Automated 'Hit Identification-to-Optimization' Approach

Laurent Hoffer,[†] Manon Garcia, Raphael Leblanc, Mikael Feracci,[†] Stéphane Betzi, Khaoula Ben Yaala, Avais M. Daulat, Pascale Zimmermann, Philippe Roche, Karine Barral,^{*,†,‡} and Xavier Morelli^{*,‡}

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KEYWORDS

Fragment-Based Drug Discovery (FBDD); Molecular Dynamics Simulation (MDS); Virtual Screening (VS); Hit-to-lead (H2L); PDZ domains; Protein-Protein Interaction (PPI)

ABSTRACT: The rapid identification of early hits by fragment-based approaches and subsequent hit-to-lead optimization represents a challenge for drug discovery. To address this challenge, we created a strategy called 'DOTS' that combines molecular dynamic simulations, computer-based library design (chemoDOTS) with encoded medicinal chemistry reactions, constrained docking, and automated compound evaluation. To validate its utility, we applied our DOTS strategy to the challenging target syntenin, a PDZ domain containing protein and oncology target. Herein, we describe the creation of a 'best-in-class' sub-micromolar small molecule inhibitor for the second PDZ domain of syntenin validated in cancer cell assays. Key to the success of our DOTS approach was the integration of protein conformational sampling during hit identification stage and the synthetic feasibility ranking of the designed compounds throughout the optimization process. This approach can be broadly applied to other protein targets with known 3D structures to rapidly identify and optimize compounds as chemical probes and therapeutic candidates.

INTRODUCTION

One of the major bottlenecks in the early phases of small molecule drug discovery is the 'hit-to-lead' optimization of compounds identified by experimental screening ¹. In recent years, we have developed an integrative strategy that combines virtual screening (VS) with automated synthesis in a platform called 'Diversity-Oriented Target-Focused Synthesis' (DOTS^{2,3}). This methodology was first validated by optimizing bromodomain inhibitors and enabled the design of several compounds with improved affinity by orders of magnitude. In DOTS, after the identification of a ligand and characterization of its binding mode using a structural biophysics method, such as X-ray crystallography, a virtual chemical library is generated by combining an appropriate activated chemical core analog with a collection of functionalized 'building blocks' (BB). This optimization step is based on the use of previously encoded *in silico* organic chemical reactions that are relevant to medicinal chemistry ⁴. The latter ensures that selected virtual compounds are feasibly synthesized in one or two steps and with high yields. Physicochemical filters are then automatically applied to extract a diverse subset of representative compounds that possess reasonable properties without any undesirable functions for medicinal chemists. The final chemical library is used for virtual screening with the 'S4MPLE' tool ⁵ to identify the best putative optimizations that create additional favorable contacts with the protein binding site while maintaining the original binding mode. The selected compounds are then synthesized using manual or robotic *de novo* synthesis. Finally, the synthesized compounds are evaluated using a variety of biochemical and biophysical assays on our automated screening platform in a rapid and cost-effective manner. Moreover, in a previous work, we published the 'McDSO' structure-based method which consists of identifying hits by coupling molecular dynamics simulation (MDS) with constrained docking and pharmacophore filters ⁶. We propose here to bring together 'McDSO' (for hit identification) and 'DOTS' (for hit-optimization) in an integrative and semiautomated 'fragment-based drug design (FBDD)' protocol for challenging targets.

Protein-protein interactions (PPIs), more particularly those of PDZ domains, which are protein modules that participate in many signaling pathways, represent an attractive but particularly challenging target for drug discovery ⁷. PDZ domains

recognize the C-termini of their protein partners and are critical for regulating several biological events such as scaffolding, trafficking and signaling cascades. More precisely, the last C-terminal residue of the PDZ partner is hydrophobic and its side chain is located within a hydrophobic pocket, while the terminal carboxylic group is involved in a canonical hydrogen-bond network. Thus, disrupting this PPI, for instance, by using small organic compounds, could have a major impact on the modulation of these biological processes.

PDZ2 Syntenin: Hit Identification & Hit-to-Lead Optimization

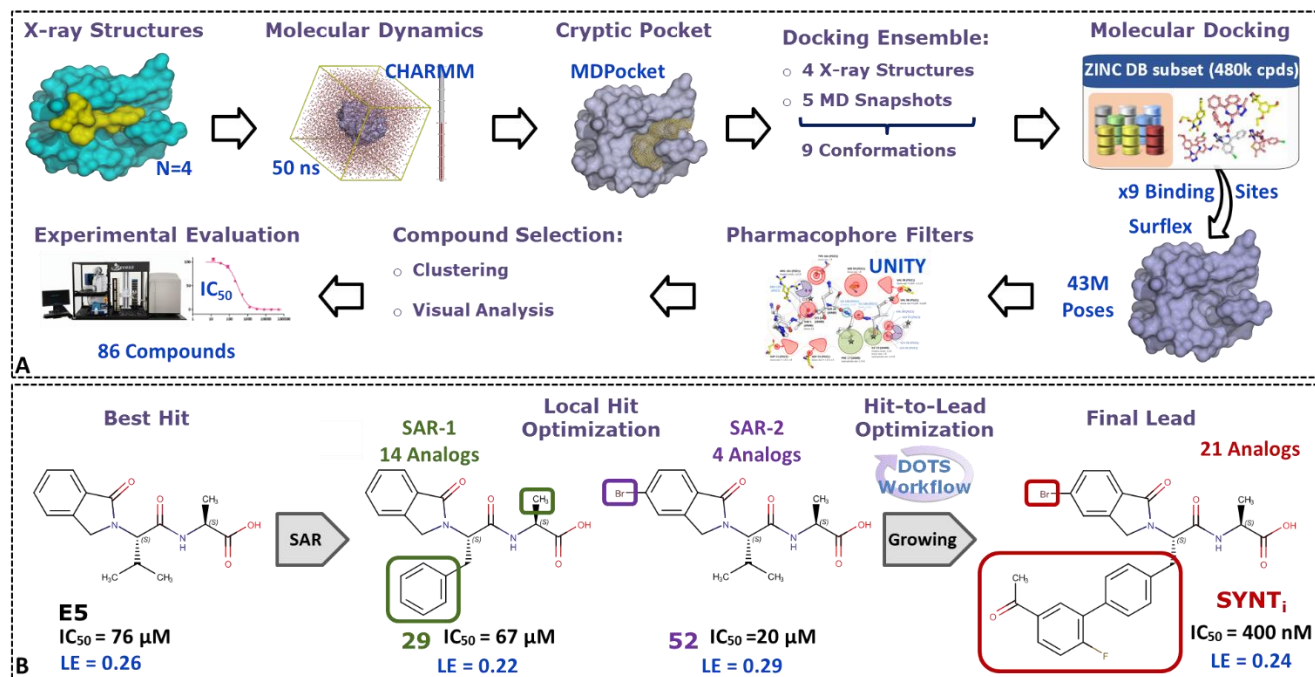


Figure 1. A. Detailed workflow of the hit identification stage. B. Main compounds from the hit-to-lead optimization stage. Both IC_{50} and ligand efficiency (LE) values are shown for each considered compound.

We previously reported the development of small molecule inhibitors targeting the second PDZ domain of syntenin (Synt-PDZ2) using an FBDD strategy⁸. A compound was first identified in an experimental screening of fragment-like compounds. Its binding mode was subsequently characterized using X-ray crystallography. Structure-activity relationship (SAR) and molecular modeling simulations were undertaken starting from this low-affinity fragment. However, none of the synthesized compounds resulting from these studies had the potential to be optimized as a potent lead.

In this study, we report a new series of potent Synt-PDZ2 inhibitors, from their initial identification to their hit-to-lead optimization, using an integrated workflow combining DOTS and McDSO methods.

RESULTS AND DISCUSSION

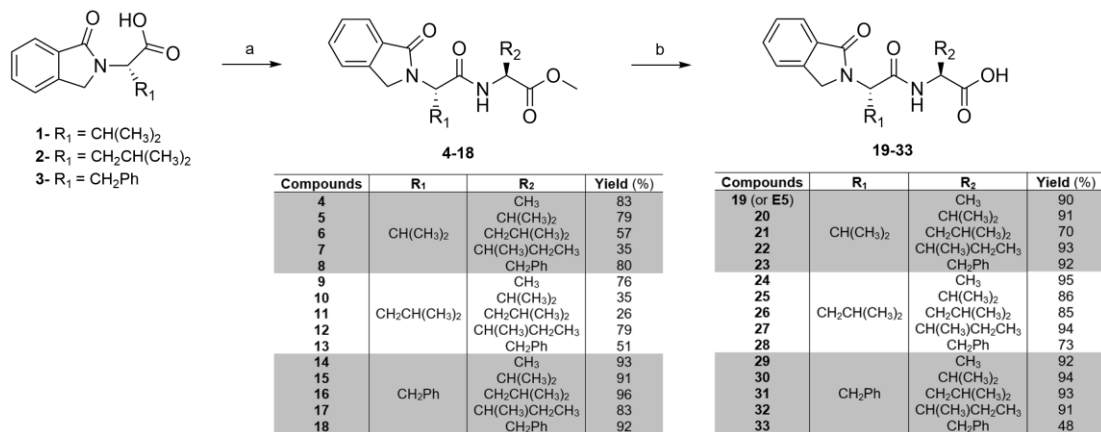
To identify putative cryptic pockets or deeper cavities to accommodate organic ligands mimicking the binding mode of the reference protein partners, we first used molecular dynamics simulations (MDS) starting from four reference Synt-PDZ2 X-ray structures. Interestingly, the induced-fit conformation around F213 from the 1W9E structure was reproduced within the trajectory of the 1N99 apo structure. This analysis finally led to the selection of 5 MDS snapshots that were further refined using a common minimization protocol. The final set of protein conformations for the subsequent ensemble docking stage consisted of the 4 original X-ray structures and 5 refined conformations from the MDS. The detailed workflow is depicted in Figure 1A. A subset of the ZINC database⁹ was then used to screen this ensemble of conformations for ligand binding using molecular docking. Forty-three million poses were generated and subsequently filtered out using a pharmacophore to extract compounds able to partially mimic the binding mode of syndecan, the endogenous protein partner of Synt-PDZ2. It should be noted that all four human syndecans (Syndecan-1/2/3/4) share the same C-terminal sequence (EFYA). This is peculiar because PDZ domains usually recognize larger terminal hydrophobic residues such as valine or leucine. In contrast, the PDZ domains from syntenin interacts with the smallest hydrophobic residue (alanine) due to its shallow hydrophobic pocket located near the carboxylate binding loop. Thus, as expected, large hydrophobic groups near the carboxylate group were not

tolerated by the shallow pocket from Syntenin PDZ domain (see discussion below about SAR-1). Therefore, some selectivity is expected in favour of Syntenin PDZ domains over alternative PDZ domains because of these specific structural constraints. However, while Synt-PDZ naturally recognizes protein partners with terminal small hydrophobic residues, previous work surprisingly reported the binding of peptides with larger C-terminal residues such as phenylalanine¹⁰. The associated structural data (PDB code 1W9E) highlighted an interesting induced-fit of Synt-PDZ to accommodate this larger sidechain. This structure was included in the protein conformation ensemble, and compounds targeting this larger pocket were selected for experimental evaluation. These compounds are highlighted in Table S1. The ability to trigger this known induced-fit with the prioritized small organic compounds will be discussed below.

Following the VS pipeline, 86 structurally diverse compounds, with molecular weights (MWs) ranging from 257 to 524 g.mol⁻¹ were selected, purchased and experimentally evaluated using an HTRF assay¹¹ as putative disruptors of the syntenin-syndecan PPI (Table S1). The 86 molecules were separated into fragment-like (17 compounds) and drug-like (69 compounds) based on their MW, and were tested in triplicate at 400 and 100 μ M, respectively. 17 compounds exhibiting more than 30% inhibition (5 fragments and 12 drug-like compounds) were re-evaluated at 200 μ M (Figure S1). Then, 8 compounds (1 fragment and 7 drug-like) exhibiting more than 60% inhibition at 200 μ M were reevaluated in the presence of NP-40 detergent (0.01%) to discard possible aggregators. In the end, one fragment hit that was not affected by NP-40 detergent, namely **E5**, was retained (Figure S1). These low affinities observed for these hits from the primary screening were expected because PDZ domains belong to protein-protein interaction interfaces, and are considered poorly druggable targets compared to enzymes. However, several small organic compounds have been reported in the literature as inhibitors of PDZ domains⁷ (Figure S2) but they mainly exhibited inhibition in the 10 micromolar range, even after intensive SAR and optimization studies⁷. Of particular note, all molecules that were expected to target the larger induced-fit subpocket (from PDB code 1W9E) showed no activity. One hypothesis was that the energy cost associated with the opening of the pocket was too high for these chemicals. Compound **E5** exhibited an IC₅₀ of 76 μ M and a good ligand efficiency (LE, value of 0.26, Table 1) for a PDZ domain hit. Moreover, additional selectivity experiments were achieved, **E5** appeared selective for syntenin, not affecting GRASP55-JAM-B complexes (Figure S3A) or Erbin-P0071 (Figure S3B) in HTRF.

Interestingly, this compound was identified using pharmacophore filters from 3 different protein conformations (2 X-ray + 1 MDS snapshot). This hit was further validated and its binding mode within Synt-PDZ2 resolved using X-ray crystallography (PDB code 8AAI) matching the one predicted by the docking algorithm (RMSD = 0.5 Å). This structure revealed that compound **E5** mimicked the binding mode of the C-terminus of syndecan (Figure 2A). This result was expected because compound **E5** can be described as a two residue peptidomimetic ('NH₂-Val-Ala-COOH'), in which the amine moiety of valine is substituted by an indolinone ring. The latter undergoes strong face to face π -stacking with Phe213 and its carbonyl moiety formed one hydrogen bond with the backbone from the same aromatic residue (Figure 2). In addition, the nitrogen from the **E5** central amide bond created a hydrogen bond with the backbone carbonyl of Phe211. The central hydrophobic isopropyl group creates strong Van der Waals (VdW) contacts with hydrophobic sidechains from His208 and Ile212. Finally, the terminal methyl group is located within the shallow hydrophobic pocket from syntenin, and the carboxylic acid functional group forms the expected canonical hydrogen-bonding interaction network with the backbone atoms of Val209, Gly210 and Phe211.

Based on these structural data, we then explored different modifications to improve the affinity of **E5**, while maintaining its canonical binding mode. The first SAR study (SAR-1, Figure 1B) of compound **E5** was performed, leading to the synthesis of 14 analogs exploring different *L*-amino acid residue moieties (**20-33**, Scheme 1 & Table 1). To proceed, we directly condensed the commercial iso-indolinone carboxylic acid derivatives (**1-3**) with the corresponding *L*-amino acid methyl ester hydrochloride (alanine, valine, leucine, iso-leucine or phenylalanine) in a mixture dichloromethane/trimethylamine at room temperature in the presence of coupling reagent EDCI¹². Methyl ester intermediates **4-18** obtained with good to excellent yields were then hydrolysed with LiOH to give derivatives **19-33**¹³. Fragment **E5** was synthesized, corresponding to compound **19**, to confirm the structure/affinity of our best commercial hit. Briefly, all modifications of terminal carboxylic acid function or nearby methyl groups were detrimental to the affinity. As with the compounds purchased after VS, the induced fit of syntenin was not reproduced using this new series (compounds **20 to 23**, mimicking terminal valine, leucine, isoleucine and phenylalanine residues, respectively).

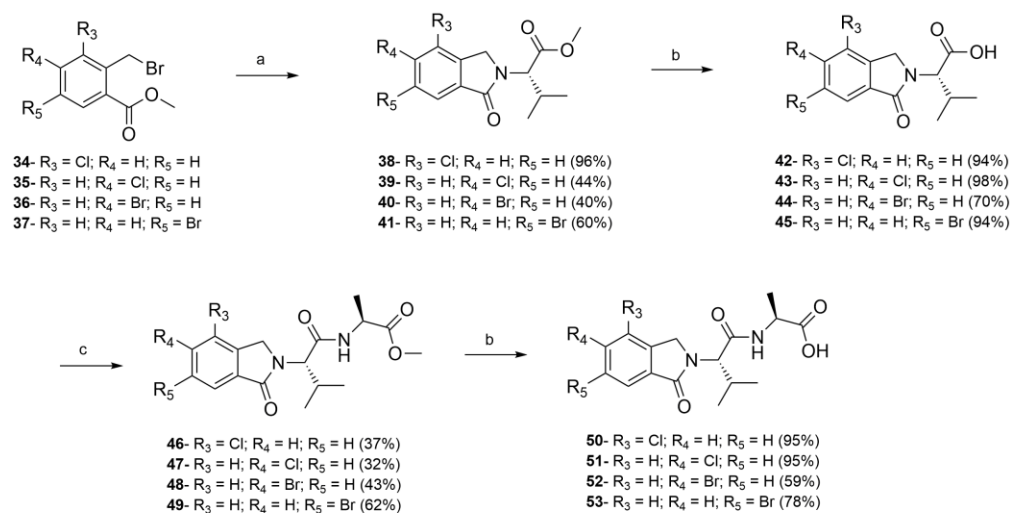


Reagents and conditions: (a) *L*-aminoacid methyl ester hydrochloride, EDCI, Et₃N, dry CH₂Cl₂, rt, 5-16h; (b) LiOH, THF/H₂O, 0°C-rt, 1h.

Scheme 1. Synthesis of iso-indolinone derivatives **19-33**.

Activity cliffs were obtained with subtle modifications of the original hit, highlighting an informative SAR in this first explored region of the ligand. In contrast, replacing the isopropyl group with a benzyl (Phe-like, compound **29**) slightly improved the IC₅₀ of the compound (IC₅₀ = 67 μM), but at the expense of the LE value (LE = 0.22). This last result was expected because endogenous syndecans contain an aromatic residue at the second to last position.

In parallel, a second SAR study (SAR-2, Figure 1B) around the indolinone core of **E5** (or **19**) was performed with a focus on small substitutions on the benzene ring (**50-53**, Scheme 2 & Table 1). Molecular modeling studies suggested that a single nonpolar heavy atom could be able to catch additional VdW interactions with the protein (e.g., carbon atoms from A251, S252 and A255). A cyclisation between the corresponding halogenated methyl (2-bromomethyl) benzoate derivatives **34-37** and (*L*)-valine methyl ester, in a mixture of DMF/DIPEA at 80°C, allowed the synthesis of halogenated indolinones intermediates **38-41** with moderate to good yields. Intermediates **38-41** were then hydrolysed with LiOH to give derivatives **42-45**¹³. As above, a peptide coupling with (*L*)-alanine methyl ester hydrochloride in the presence of EDCI yielded the methyl ester analogues **46-49**, that were then hydrolysed with LiOH to give the desired halogenated indolinone analogues **50-53** with good yields. Of the four synthesized compounds, the chloro analog **51** and the bromo analog **52** exhibited the most potent IC₅₀ in the 20 μM range. Thus, the addition of a single heavy atom increased the affinity by a factor of 7, highlighted by the impressive gain in LE (0.29 vs. 0.26 for compounds **51/52** and **E5**, respectively).



Reagents and conditions. (a) *L*-Valine methyl ester hydrochloride, DIPEA, dry DMF, 80°C, 5-8h; (b) LiOH, THF/H₂O, at 4°C-rt, 1h; (c) *L*-Alanine methyl ester hydrochloride, EDCI, NEt₃, dry DCM, rt, 8-18h;

Scheme 2. Synthesis of carboxylic acid intermediates **46-49** and derivatives **50-53**.

Then, the X-ray structure of compound **29** was resolved in complex with Synt-PDZ2 confirming the native binding mode of the series (PDB code 8AAK, Figure 2B). Interestingly, the benzyl group participated in a face-to-face π -stacking interaction with H208 and created strong VdW contacts with I212 and V222 (Figure S4). Moreover, analysis of this X-ray structure highlighted that a growing optimization around the terminal benzyl group could lead to more potent inhibitors. There is indeed a nearby subpocket that could be filled, and crystallographic water molecules could also be either displaced or involved in water-mediated hydrogen bonds between the protein and the optimized ligands.

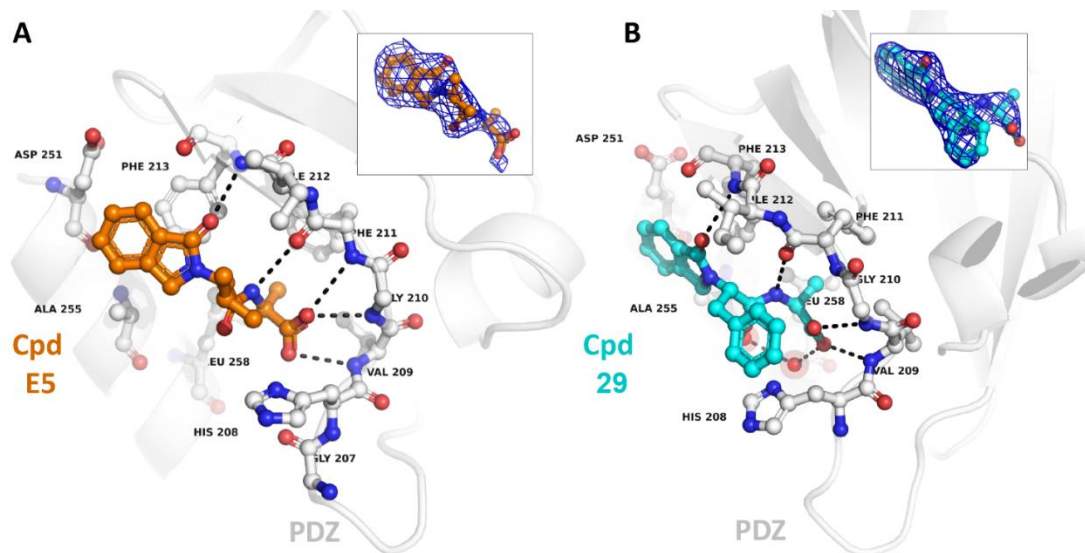
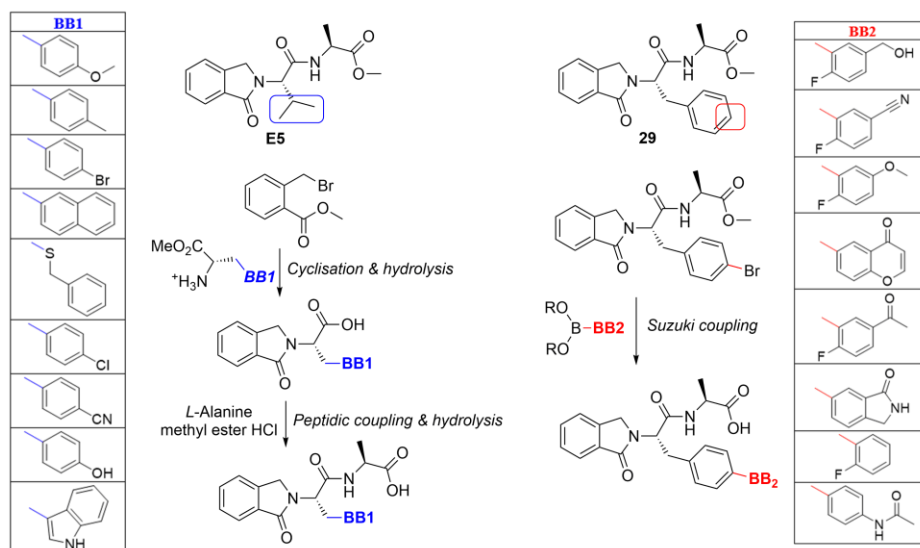


Figure 2. Molecular details of the syntenin/compound **E5** (**A**) and compound **29** (**B**) interactions showing the protein as white ball and sticks with its associated white cartoon, the small molecules as cyan (**E5**, PDB ID: 8AAO) or orange (**29**, PDB ID: 8AAP) balls and sticks, and the hydrogen bonds as black dotted lines. For each structure, the 2Fo-Fc electron density map of the compound contoured at 1σ is shown on the top right as a blue mesh surrounding the compound (1.6 carve distance).

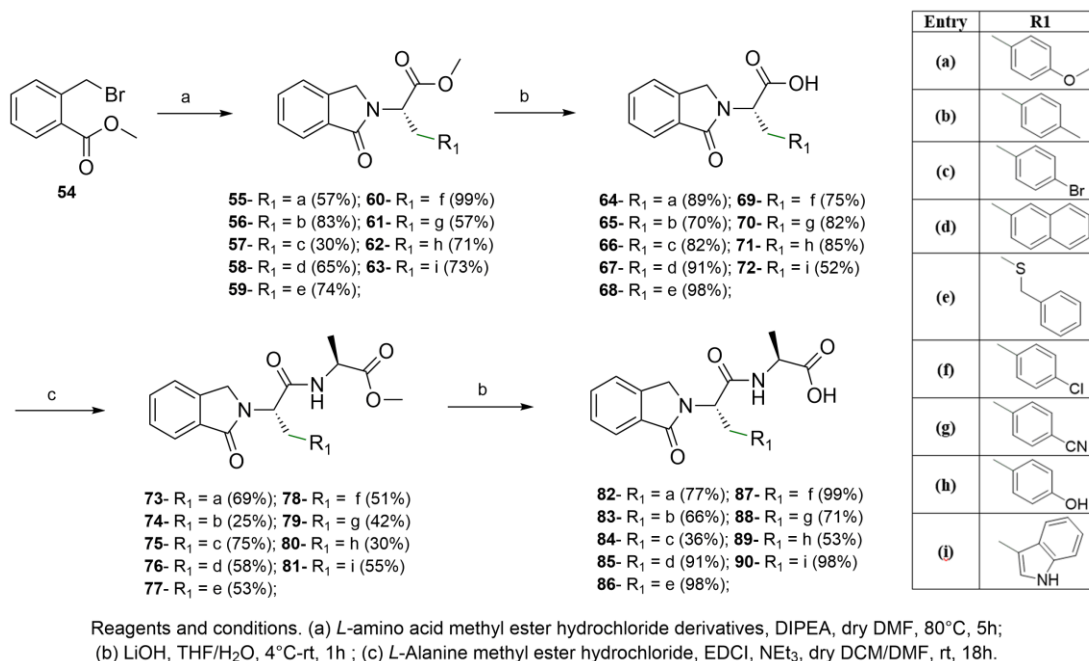
An *in silico*-based hit-to-lead (H2L) program, relying on DOTS², was then initiated. Two diverse chemical libraries of accessible compounds were first designed either to explore the chemical space around the original isopropyl moiety (**E5**) or to fill the previously described subpocket starting from **29** (Scheme 3).



Scheme 3. Synthetic pathway of the two diverse chemical libraries designed to either explore the chemical space around original isopropyl moiety from **E5** (or **19**) or to fill the previously introduced subpocket starting from **29**.

Approximately 2,400 theoretically accessible compounds were virtually screened against the Synt-PDZ2/**29** X-ray structure using a constrained docking approach ² to identify the best putative modifications. Compounds from the top 20% based on the force field-based interaction energy were extracted. Both water and water-free binding site variations of the Synt-PDZ2/**29** structure were considered to either catch potential water-mediated hydrogen bonds or to virtually displace crystallographic waters. This subset of the best modifications was ultimately re-evaluated using SeeSAR software. The main goal was to rule out molecules with either poorly predicted affinity (mainly unfavorable desolvation penalties from either ligand or protein side according to the HYDE ¹⁴ scoring function) or geometry warnings (poor dihedral angles and/or slight intra/inter clashes). Following the rescoring workflow of the best compounds from VS, 17 compounds were prioritized, synthesized and evaluated as potential lead-like inhibitors of the syntenin-syndecan PPI (Scheme 3).

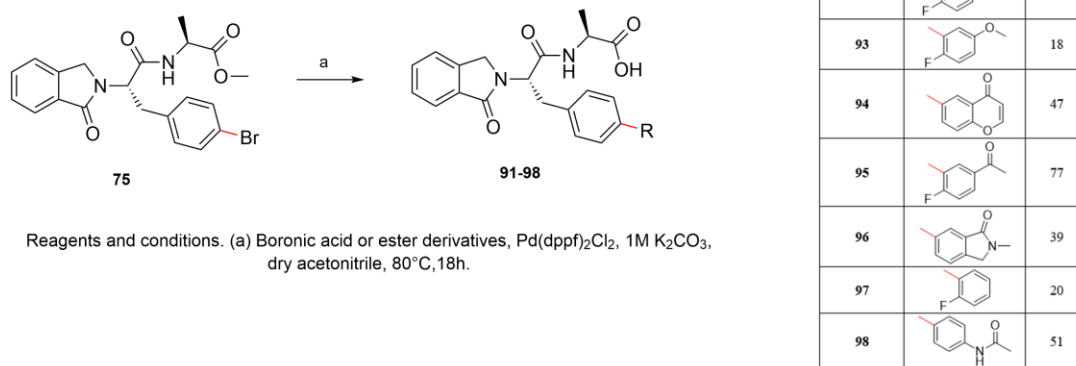
Nine compounds (**82-90**) were identified from this first DOTS-based VS around the isopropyl moiety (**E5**) and were synthesized according to Scheme 4 using the synthetic pathways previously described. A cyclisation between methyl (2-bromomethyl) benzoate **54** and nine selected commercial (L)-amino methyl ester derivatives, followed by an alkaline hydrolysis, a peptide coupling with (L)-alanine and a second alkaline hydrolysis led to the nine indolinone analogues **82-90** with good average yields (Scheme 4 & Table 1). Several minor structural modifications displayed a huge impact on potency. Thus, addition of methoxy (**82**), methyl (**83**), chlorine (**87**) and nitrile (**88**) groups on the *para*-position of the terminal phenyl ring exhibited lower potency than the reference analog, compound **29** (IC₅₀ value of 67 μM). By contrast, *p*-bromo (**84**) analog was slightly better with an IC₅₀ value of 35 μM. The phenol modification, mimicking the syndecan residue at the 2-position (EFYA), was also slightly better (**89**, IC₅₀ value of 44 μM). The inclusion of a larger flexible spacer between the core and the terminal phenyl ring had negligible impact on potency (**86**, IC₅₀ value of 63 μM). Finally, two bicycle variations were also explored for their predicted ability to add more contacts with close aromatic (H208) and aliphatic (V222) residues from the syntenin protein. The indole analog, used to mimic a 'Phe-to-Trp' mutation, displayed a slightly better potency (**90**, IC₅₀ value of 33 μM). The best molecule, compound **85** that contained a naphthyl group, exhibited an IC₅₀ value of 13 μM (LE=0.23, Table 1).



Scheme 4. Synthesis of carboxylic acid intermediates **73-81** and derivatives **82-90**.

Similarly, eight compounds (**91-98**) were prioritized from the second DOTS-based VS around the benzyl moiety (**29**). They were synthesized according to Scheme 5 from the *para*-bromobenzyl analogue **75** using the medicinal chemistry-relevant Suzuki-Miyaura reaction rule and a collection of selected BBs (Scheme 5 & Table 1). To improve the coupling, we set up a general procedure starting with the 4-bromophenyl intermediate **75** (Scheme 4) to generate the selected library. We turned to substitution of the bromo group at the C4-position under standard Suzuki-Miyaura conditions in the presence of Pd(PPh₃)₄ or Pd(dppf)Cl₂ under aqueous basic conditions ¹⁵. After few reaction optimizations such as performing the reaction with phenylboronic acid and **75** using different bases (sodium or potassium carbonate) in various solvents such toluene, acetonitrile or a mixture toluene/ethanol, we were able to conclude that use of Pd(dppf)Cl₂ and aqueous K₂CO₃ in acetonitrile at 80°C

achieved optimum yield (reactions monitored by LC/MS analysis). Furthermore, under these conditions, we noticed that the cross-coupled product is hydrolyzed to the corresponding carboxylic acid derivative, probably due to methyl ester sensitivity to basic hydrolysis. Therefore, intermediate **75** reacted readily with the eight selected boronic acids or boronic esters in the presence of Pd(dppf)Cl₂ and K₂CO₃ in acetonitrile at 80°C for 18h providing the corresponding carboxylic acid derivatives **91-98** with good to moderate yields (Scheme 5). These compounds displayed IC₅₀ values from 4.0 μM to 10.0 μM (LE values 0.19-0.21, Table 1). The simplest modification (biphenyl, **97**, IC₅₀=9.9 μM), within the Suzuki-Miyaura coupling context, already displayed a better potency than the reference analog, compound **29** (IC₅₀ value of 67 μM). Biphenyl analogs, with fluorine atom at the *o*-position between both aromatic rings, were prioritized when corresponding building blocks were commercially available. This was done because both rings were not coplanar according to the predicted binding modes from docking simulations. A Non-hydrogen atom at this position was introduced to optimize the torsion angle between both phenyl rings. Built-in dihedral angle analysis tool within SeeSAR also supported this move. The goal of the more elaborated modifications (compounds **91-98**, except **97**) was to either displace a crystallographic water molecule or create a water-mediated hydrogen-bond network with this solvent entity. All these subsequent modifications successfully improved the affinity for the syntenin protein. The *m*-methoxy variation exhibited the similar potency gain (**93**, IC₅₀=10 μM) as well as the *m*-cyano analog (**92**, IC₅₀=8.8 μM). Three additional modifications with single extra ring were considered for experimental testing. Thus, the *m*-hydroxymethyl analog (**91**) and the *m*-acetyl analog (**95**) exhibited a single digit micromolar potency, IC₅₀=5.3 μM and IC₅₀=4.2 μM, respectively. Similarly, the *p*-acetamide molecule (**98**) achieved an IC₅₀ value of 7.7 μM. Finally, two additional compounds with an extra bicycle moiety were also investigated. The N-methyl-dihydro-isoindolin-1-one analog (**96**) displayed an IC₅₀ value of 6.3 μM. Its chromone analog achieved the best potency from this sub-series (**94**, IC₅₀=4.0 μM). Interestingly, the carbonyl moiety from compounds **94**, **95** and **96** were superimposed in space according to the docking poses. This was the rationale behind the prioritization of these modifications despite their huge structural differences. It should be noted that these three carbonyl-containing compounds were also among the most potent ligands from the overall set of tested molecules so far.



Scheme 5. Synthesis of derivatives **91-98** via Suzuki coupling.

Several new compounds exhibiting improved affinities have been obtained starting from compounds **E5** (IC₅₀=76 μM, LE=0.26) and **29** (IC₅₀=67 μM, LE=0.22). The overall best compound, **94**, exhibited an IC₅₀ value of 4.0 μM, an almost 20-fold increase in potency after one optimization cycle. Similarly, the smallest compound within the most potent inhibitor subset, namely, compound **95**, displayed an LE value of 0.20 and an IC₅₀ value of 4.2 μM with the addition of a single decorated ring. The binding mode of **95** was subsequently resolved using X-ray crystallography (PDB code 8AAO, Figure 3A), and accurately matched that predicted by molecular modeling (RMSD=0.5 Å). In addition, the expected water-mediated hydrogen bond network with the backbone of both His208 and Ser226 was observed. Of note, the typical 10 μM range from small organic inhibitors of PDZ domains was already reached.

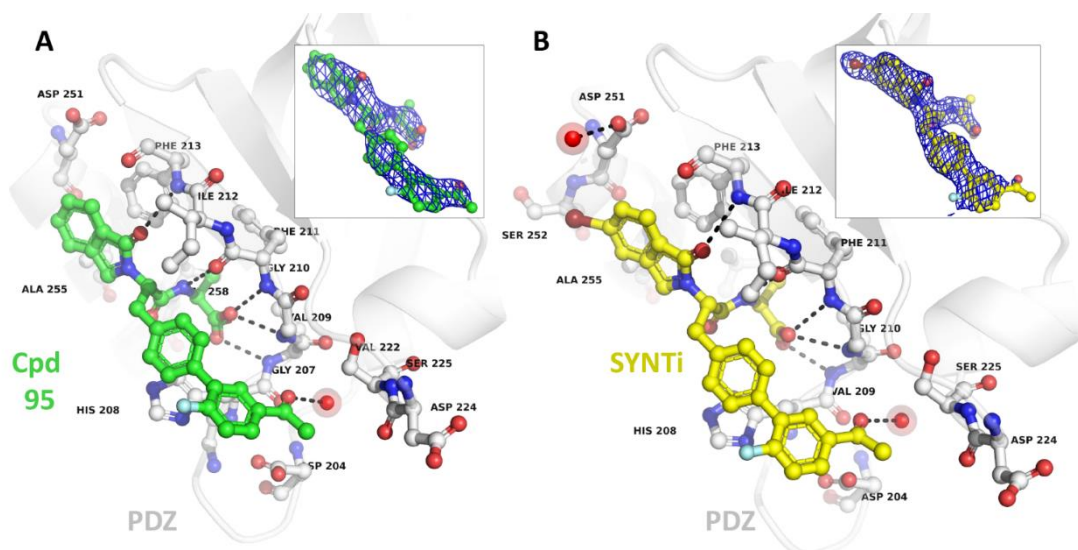
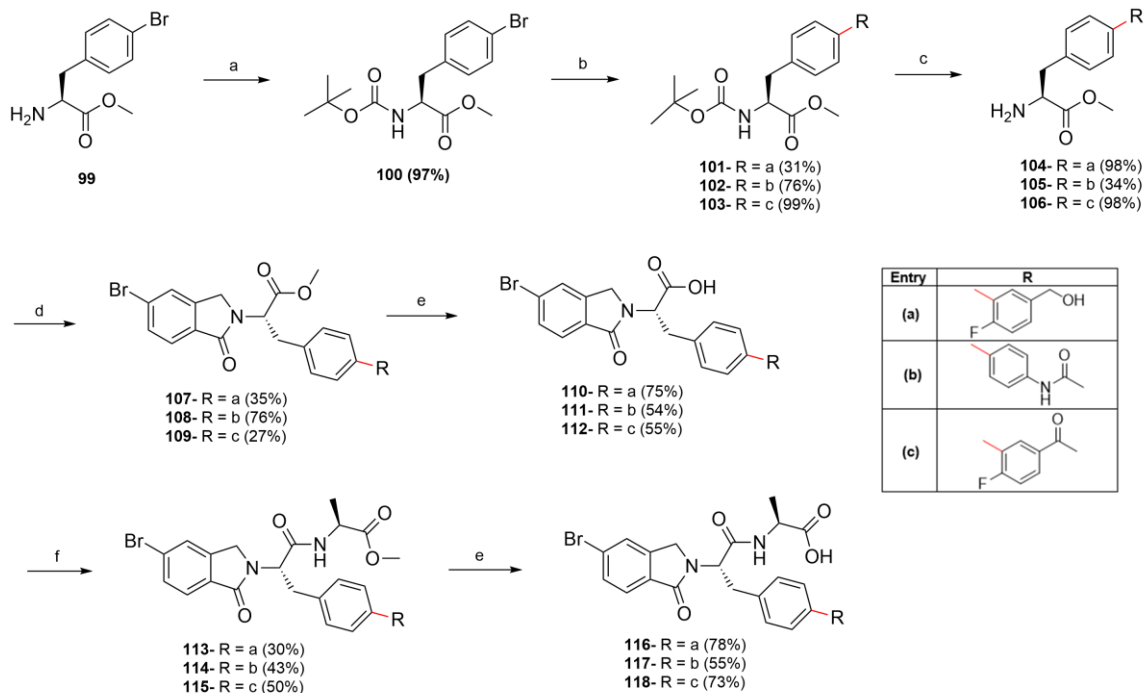


Figure 3. Molecular details of the syntenin/compound **95** (A) and **SYNTi** (B) interaction showing the protein as white balls and sticks with its associated white cartoon, the small molecules as cyan (**95**, PDB ID: 8AAO) or orange (**SYNTi**, PDB ID: 8AAP) balls and sticks, and the hydrogen bonds as black dotted lines. For each structure, the 2Fo-Fc electron density map of the compound contoured at 1σ is shown on the top right as a blue mesh surrounding the compound (1.6 carve distance).

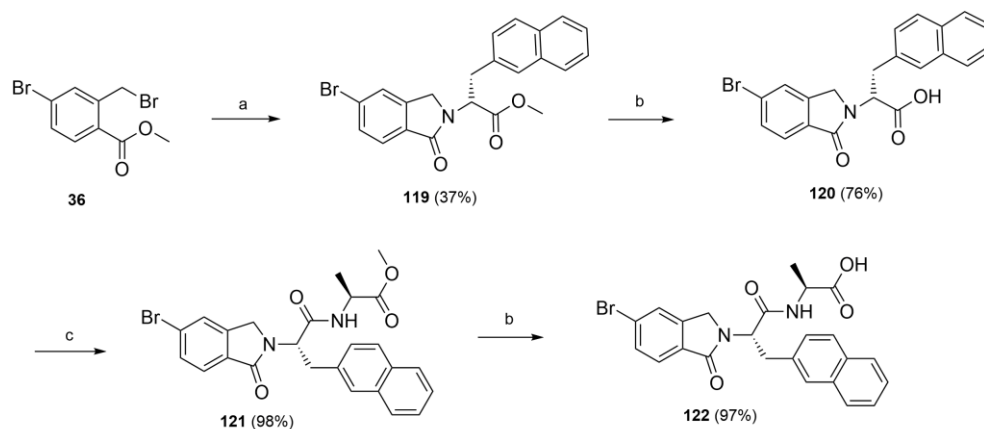
However, the chimera compounds between the most potent inhibitors synthesized from the *in silico* growing stage and those from the second SAR study were expected to be even more potent, while maintaining the overall original binding mode. Bromo analog **52**, available in higher amount ($IC_{50}=20\ \mu\text{M}$) was selected for this stage. The previous synthetic pathways were combined and four brominated chimera compounds **116-118** and **122** were synthesized and evaluated (Figure S5, Schemes 6 & 7, Table 1). For this purpose, the primary amine group of the commercial methyl (2*S*)-2-amino-3-(4-bromophenyl)propanoate **99** was protected with di-*tert*-butyl dicarbonate in a mixture of DCM/TEA, leading to the intermediate **100** in 97% yield. Once again, we performed few Suzuki-Miyaura reaction optimizations with phenylboronic acid and (2*S*)-3-(4-bromophenyl)-2-[[*tert*-butoxy]carbonyl]amino}propanoate **100** in the presence of Pd(PPh₃)₄ or Pd(dppf)Cl₂ using different bases (sodium or potassium carbonate) in various solvents such toluene, THF, acetonitrile or a mixture toluene/ethanol^{15,16}. We were able to conclude that use of Pd(PPh₃)₄ and aqueous Na₂CO₃ in THF at 70°C achieved optimum yield (reactions monitored by LC/MS analysis). Therefore, intermediate **100** reacted readily under these conditions with the three selected boronic acids or boronic esters for 1h providing the corresponding derivatives **101-103** with moderate to good yields (Scheme 6). Deprotection of Boc-protected amines **101-103** was rapidly accomplished using TFA in DCM (4°C to rt) for 4h leading to *L*-aminoacid methyl ester derivatives **104-106**. As previously, a cyclisation between the methyl 4-bromo-2-(bromomethyl) benzoate **36** and derivatives **104-106**, in a mixture DMF/DIPEA at 80°C, allowed the synthesis of halogenated indolinones intermediates **107-109** with moderate to good yields. Intermediates **107-109** were hydrolysed with LiOH to give the halogenated indolinone analogues **110-112** with good yields. At the end, a peptide coupling with (L)-Alanine methyl ester hydrochloride in the presence of EDCI yielded the methyl ester analogues **113-115**, that were then hydrolysed with LiOH to give the desired halogenated indolinone analogues **116-118** with good yields (Scheme 6).



Reagents and conditions. (a) Di-*tert*-butyl dicarbonate, NEt₃, dry DCM, 4°C-rt, 2h; (b) boronic acid or ester derivative, PdCl₂(PPh₃)₂, Na₂CO₃, dry THF, 70°C, 1h; (c) TFA, DCM, 4°C-rt, 4h; (d) methyl 4-bromo-2-(bromomethyl)benzoate **36**, DIPEA, dry DMF, 80°C, 3h; (e) LiOH, THF/H₂O, 4°C-rt, 1h; (f) *L*-Alanine methyl ester hydrochloride, EDCI, NEt₃, dry DCM/DMF, rt, 18h.

Scheme 6. Synthesis of "chimera" derivatives **116-118**.

Similar syntheses were achieved starting with intermediate **100** and the *L*-aminoacid methyl ester derivative related to the most potent chromone derivative **94**. The Suzuki coupling, followed by the acidic deprotection step and the cyclisation with methyl 4-bromo-2-(bromomethyl) benzoate **36** worked properly. However, the alkaline hydrolysis did not lead to the desired indolinone analogue. Instead, we isolated the 'opened' chromone form. Despite various attempts under milder alkaline hydrolysis, we could not readily synthesize the desired chromone chimera compound. The last chimera compound was obtained starting with the cyclisation between the methyl 4-bromo-2-(bromomethyl) benzoate **36** and the methyl (2*S*)-2-amino-3-(naphthalen-2-yl)propanoate in a mixture of DMF/DIPEA at 80°C for 5h (Scheme 7). This cyclisation step allowed the synthesis of the brominated indolinone intermediate **119** in 37% yield and was followed by a hydrolysis with LiOH to give the brominated indolinone **120** in 76% yield. A peptide coupling with (*L*)-Alanine methyl ester hydrochloride in the presence of EDCI yielded the methyl ester analogue **121**, that were then hydrolysed with LiOH to give the desired halogenated indolinone analogue **122** with an excellent yield (Scheme 7).



Reagents and conditions. (a) methyl (2*S*)-2-amino-3-(naphthalen-2-yl)propanoate hydrochloride, DIPEA, dry DMF, 80°C, 5h; (b) LiOH, THF/H₂O, 4°C-rt, 1h; (c) *L*-Alanine methyl ester hydrochloride, EDCI, NEt₃, dry DCM/DMF, rt, 18h.

Scheme 7. Synthesis of "chimera" derivative **122**.

Compound **118** was the most potent inhibitor out of the 4 tested chimera compounds (**116-118** and **122**). More precisely, the final lead (compound **118**, **SYNTi**) exhibited an IC_{50} value of 400 nM and LE value of 0.24 (Table 1). Despite the undruggable status of Synt-PDZ2, the LE value was almost maintained between the original hit and the final lead (0.26 vs. 0.24 for compounds **E5** and **SYNTi**, respectively), while the affinity was increased by almost 200-fold. Noteworthy, compound **SYNTi** had no effect on other PDZ complexes such as GRASP55-JAM-B and Erbin-P0071 complexes in HTRF (Figure S3).

The structure of the Synt-PDZ2/**SYNTi** complex was resolved using X-ray crystallography (PDB code 8AAP, Figure 3B and Figure S4), confirming the shared binding mode within the full series (**E5**, **29** and **SYNTi**). In addition, the observed binding mode was again consistent with the binding mode predicted by molecular modeling (RMSD=0.7 Å).

An orthogonal biophysical validation was also included after the HTRF campaign to measure **SYNTi** direct interaction to syntenin using Isothermal Titration Calorimetry (ITC). The experiment confirmed a 1:1 syntenin:**SYNTi** binding stoichiometry (one site) in agreement with the proposed model (Figure 4). The measured energetic forces confirmed that the optimization campaign did not result in an unfavourable entropy-enthalpy compensation and an entropy driven compound. Indeed, **SYNTi** displays a balanced thermodynamic binding profile with contributions from both favourable enthalpic and entropic components as shown in Figure 4 associated histogram. The enthalpic component confirms the expected net hydrogen bonds formation as shown in the X-ray structures (Figure 3), while the favourable entropic component validates potential release of structured water molecules and increased level of freedom. Analysis of three independent experiments (Supp Table S2 and Figure S6) allowed to calculate an average K_D value of $1.8 \pm 0.7 \mu M$, although slightly lower than the measured HTRF IC_{50} under these experimental conditions.

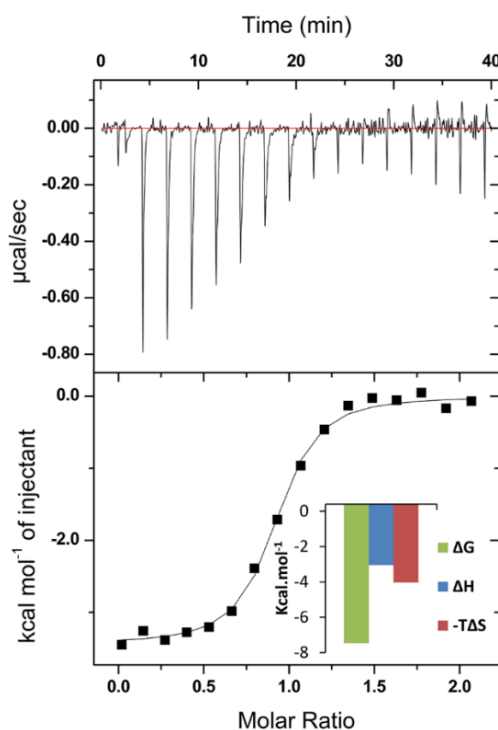


Figure 4. Representative ITC experiment of the Syntenin/**SYNTi** interaction at 25°C. For this titration experiment, Syntenin (cell) and **SYNTi** (syringe) were used at 100 μM and 1000 μM respectively. The thermodynamics parameters (ΔH (blue), $-T\Delta S$ (red) and ΔG (green)) measured/deduced from this experiment are shown at the bottom right of the figure. The full details of this experiment are listed Table S2 along with the two other performed experiments used to calculate the interaction K_D (Figure S6).

Entry	Structure	IC_{50} (μM) LE	Entry	Structure	IC_{50} (μM) LE	Entry	Structure	IC_{50} (μM) LE

E5 (19)		76 ± 7.98 0.26	20		> 500 ND	21		> 500 ND
22		> 500 ND	23		> 500 ND	24		213 ± 15.19 0.22
25		> 500 ND	26		> 500 ND	27		> 500 ND
28		> 500 ND	29		67 ± 1.92 0.22	30		> 500 ND
31		> 500 ND	32		> 500 ND	33		> 200 ND
50		> 200 ND	51		14 ± 1.22 0.29	52		20 ± 6.19 0.29
53		> 200 ND	82		128 ± 21.27 0.19	83		> 200 ND
84		35 ± 3.13 0.23	85		13 ± 2.28 0.23	86		63 ± 9.07 0.21
87		78 ± 7.33 0.21	88		180 ± 5.19 0.18	89		44 ± 2.33 0.22
90		33 ± 1.01 0.21	91		5.3 ± 0.84 0.20	92		8.8 ± 0.06 0.20

93		10 ± 0.07 0.20	94		4.0 ± 0.31 0.21	95		4.2 ± 0.39 0.20
96		6.3 ± 0.57 0.19	97		9.9 ± 0.91 0.21	98		7.7 ± 0.89 0.19
116		1.5 ± 0.05 0.22	117		0.82 ± 0.08 0.23	SYNTi (118)		0.40 ± 0.02 0.24
122		5.2 ± 0.2 0.23						

Table 1. Structure, IC₅₀ and LE values of analogues **19-33**, **50-53**, **82-98**, **116-118(SYNTi)** and **122** on syntenin-syndecan2 complex HTRF assay.

Finally, both the original hit **E5** and final lead **SYNTi** were tested using cellular assays. In wild - type MDA-MB-231 breast carcinoma cells and in MDA-MB-231 cells with syntenin - KO (Synt KO; CRISPR/Cas9 - engineered), compounds **E5** and **SYNTi** were not toxic at concentrations up to 100 μM (Figure S7). This concentration of compounds **E5** and **SYNTi** significantly affected the migration speed and Euclidean distance of the wild-type MDA-MB-231 cells to the same extent as the knock-out of syntenin (Figure S8). Moreover, no such effect was observed in MDA-MB-231 cells with syntenin knockout (MDA-MB-231 Synt KO), confirming the biological specificity of the effects of **E5** and **SYNTi** on syntenin. We next compared the efficiency of compounds **E5** and **SYNTi** by performing dose–response assays on wild-type MDA-MB-231 single cell migration (Figure 5). Compound **30** was used as an inactive control compound. First, we noticed that only the highest concentration (100 μM) of compound **E5** markedly reduced the migration speed and the Euclidean distance. A treatment with 100μM of compound **30** significantly affected the Euclidean distance and the migration speed of wild-type MDA-MB-231 cells (Figure S9).

Treatment with 1 μM **SYNTi** reduced the migration speed and affect the Euclidean distance of MDA-MB-231 cells. Interestingly, a single dose of 10 μM **SYNTi** significantly decreased both the migration speed and the Euclidean distance of MDA-MB-231 cells to the same extent as treatment at 100 μM **SYNTi** (Figure 5). Taken together, our data indicated that **SYNTi** inhibits MDA-MB-231 cell migration more potently than compound **E5**.

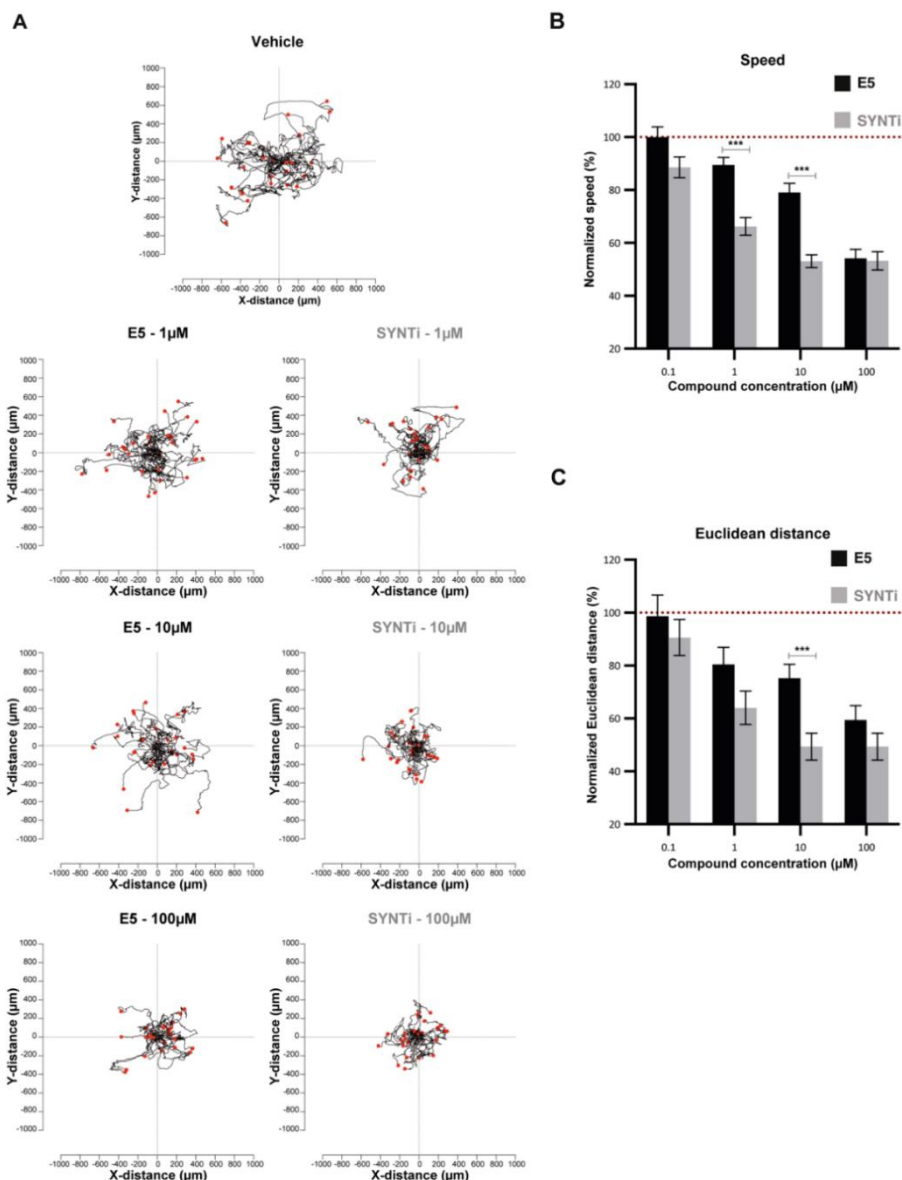


Figure 5. SYNTi is more efficient than E5 *in vitro*. **A.** Cell migration evaluation by 2D phase contrast video microscopy and single-cell tracking analysis (24h video recorded; 1-min intervals) of Wild-type MDA-MB-231 treated with increasing concentration (0.1 to 100 μM) of compounds E5 or SYNTi. Wind-rose plots show cell tracks of 25 cells for each condition. **B & C.** The bar graph indicates the percentage of migration velocity (**B**) and euclidean distance (**C**) of the tracked cells relative to Wild-type MDA-MB-231 treated with vehicle. Values correspond to the mean ± SEM of 25 cells taken from 10 different fields from 3 independent experiments. Statistical analysis was performed using the one-way analysis of variance (ANOVA) with a Bonferroni post-test (**P < 0.01, ***P < 0.001).

CONCLUSION

Here, we report an integrated strategy combining molecular dynamics simulations and virtual screening for hit-identification, and focused virtual library design using medicinal-chemistry rules followed by constrained structure-based VS for hit optimization. Due to the inclusion of chemistry knowledge during the virtual design stage, the selected compounds can be easily synthesized in one or two steps for a quick evaluation of suggested modifications, after purchasing the relevant building blocks. Then, more complex chemistry can be considered on the most promising compounds, as highlighted by the chimera derivatives synthesis. Besides, reassessing the FF-based top compounds from VS using an independent method can improve the prioritization process.

As a conclusion, the approach was successfully applied to the development of potent small organic inhibitors that can disrupt the PPI interface between the second PDZ domain of syntenin and its partner syndecan. To our knowledge, the most potent small organic compounds from this series are the best syntenin PDZ domain inhibitors described thus far in the literature,

highlighting the success of this integrated academic approach combining chemoinformatics, molecular modeling, organic synthesis, structural biology and biological experimental assays. Finally, this whole strategy can be easily applied to any protein with a known 3D structure.

EXPERIMENTAL SECTION

Synthetic procedures

All compounds are $\geq 95\%$ pure by HPLC analysis. Representative HPLC traces are shown in the Supporting Information. All commercial reagents and solvents were purchased from Sigma Aldrich, Fisher Scientific, Fluorochem, Enamine or Molport companies.

General information

All dry solvents were obtained via Sigma Aldrich with Sure/Seal™ system and regular solvents were obtained via Sigma Aldrich at technical grade. Analytical thin layer chromatography (TLC) of the reactions was performed on silica gel 60F 254 aluminium plates (Merck) of 0.2 mm thickness with appropriate solvents. The spots were examined with UV light ($\lambda = 254$ nm) and Ninhydrin Spray. Preparative flash column chromatographies were performed using silica gel (Merck) G60 230-240 under compressed air. The ^1H NMR and ^{13}C NMR spectra were determined with a BRUCKER AMX 250 MHz or BRUKER Avance III nanobay 400 MHz. The chemical shifts are reported in ppm and coupling constants (J) are reported in hertz. Reaction monitoring and purity of compounds were recorded by using analytical Agilent Infinity high performance liquid chromatography (Column Zorbax SB-C18 1.8 μM (2.1x50 mm); Mobile phase (A: 0.1% FA H_2O , B: 0.1% FA CH_3CN , Time/%B: 0/10, 4/90, 7/90, 9/10, 10/10); Flow rate 0.3 mL/min with DAD at 254 nm. Low-resolution mass spectra were obtained with Agilent SQ G6120B mass spectrometer in positive and/or negative electrospray modes. Resolution Mass Spectra (HRMS) were obtained on a SYNAPT G2-S WATERS mass spectrometer.

Chemistry

General procedure for the synthesis of isoindolinone methyl ester intermediates (**4-18**)

To a stirred solution of the corresponding isoindolinone propionic acid derivative (**1-3**) (1 eq, 0.5 mmol) and *L*-aminoacid methyl ester hydrochloride (Alanine, Valine, Leucine, Isoleucine or Phenylalanine) (1 eq, 1.0 mmol) in dry dichloromethane, *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (2 eq, 1.0 mmol) and triethylamine (2 eq, 1 mmol) were added. The mixture was stirred under argon atmosphere during 5-16 h at room temperature. After completion, the resulting mixture was washed twice with 1N HCl solution, once with water, once with brine, dried over Na_2SO_4 , and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 95:5).

methyl (2S)-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]propanoate (4)

^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.81-7.74$ (m, 1H), 7.53-7.44 (m, 1H), 7.43-7.35 (m, 2H), 6.63 (bd, $J = 6.7$ Hz, 1H), 4.60 (d, $J = 17.6$ Hz, 1H), 4.41 (q, $J = 7.2$ Hz, 1H), 4.35 (d, $J = 17.6$ Hz, 1H), 4.32 (d, $J = 11.2$ Hz, 1H), 3.65 (s, 3H), 2.47-2.28 (m, 1H), 1.28 (d, $J = 7.2$ Hz, 3H), 1.00 (d, $J = 6.5$ Hz, 3H), 0.80 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (CDCl_3 , 101 MHz): $\delta = 172.85, 169.44, 169.16, 141.87, 131.87, 131.75, 128.04, 123.88, 122.92, 61.95, 52.39, 48.13, 47.76, 27.91, 19.35, 18.96, 17.77$. LC/MS (ESI): 316.7 [M-H] $^-$; 318.7 [M+H] $^+$; yellow solid. Yield = 83%.

methyl (2S)-3-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]butanoate (5)

^1H NMR ($\text{DMSO}-d_6$, 250 MHz): $\delta = 7.79$ (bd, $J = 8.2$ Hz, 1H), 7.54-7.35 (m, 3H), 6.66 (bd, $J = 7.7$ Hz, 1H), 4.56 (d, $J = 17.2$ Hz, 1H), 4.46-4.29 (m, 3H), 3.65 (s, 3H), 2.51-2.28 (m, 1H), 2.22-1.97 (m, 1H), 0.99 (d, $J = 6.6$ Hz, 3H), 0.82 (d, $J = 6.6$ Hz, 3H), 0.75 (d, $J = 7.0$ Hz, 3H), 0.71 (d, $J = 6.9$ Hz, 3H). LC/MS (ESI): 344.7 [M-H] $^-$; 346.7 [M+H] $^+$; white foam. Yield = 79%.

methyl (2S)-4-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]pentanoate (6)

^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.82-7.75$ (m, 1H), 7.53-7.45 (m, 1H), 7.44-7.36 (m, 2H), 6.56 (bd, $J = 7.6$ Hz, 1H), 4.56 (d, $J = 17.6$ Hz, 1H), 4.40-4.32 (m, 1H), 4.35 (d, $J = 17.6$ Hz, 1H), 4.32 (d, $J = 11.2$ Hz, 1H), 4.46-4.29 (m, 3H), 3.64 (s, 3H), 2.49-2.29 (m, 1H), 1.68-1.31 (m, 3H), 1.00 (d, $J = 6.5$ Hz, 3H), 0.82 (d, $J = 6.7$ Hz, 3H), 0.71 (d, $J = 6.0$ Hz, 3H), 0.69 (d, $J = 6.1$ Hz, 3H). LC/MS (ESI): 359.5 [M-H] $^-$; 361.3 [M+H] $^+$; yellow solid. Yield = 57%.

methyl (2S)-3-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]pentanoate (7)

^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.83-7.74$ (m, 1H), 7.54-7.43 (m, 1H), 7.44-7.34 (m, 2H), 6.67 (bd, $J = 8.2$ Hz, 1H), 4.54 (d, $J = 17.5$ Hz, 1H), 4.42 (dd, $J = 8.4$ Hz and 5.0 Hz, 1H), 4.35 (d, $J = 17.4$ Hz, 1H), 4.33 (d, $J = 11.2$ Hz, 1H), 3.65 (s, 3H), 2.48-2.31 (m, 1H), 1.82-1.61 (m, 1H), 1.28-1.02 (m, 2H), 0.97 (d, $J = 6.5$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H), 0.74 (d, $J = 7.4$ Hz, 3H), 0.70 (d, $J = 7.0$ Hz, 3H). LC/MS (ESI): 359.5 [M-H] $^-$; 361.3 [M+H] $^+$; white solid. Yield = 35%.

methyl (2S)-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]-3-phenylpropanoate (8)

¹H NMR (CDCl₃, 250 MHz): δ = 7.82 (bd, *J* = 7.4 Hz, 1H), 7.52 (td, *J* = 7.4 Hz and 1.4 Hz, 1H), 7.44 (td *J* = 7.4 Hz and 1.1 Hz, 1H), 7.35 (bd, *J* = 7.4 Hz, 1H), 6.90-6.84 (m, 2H), 6.83-6.73 (m, 2H), 6.58 (bd, *J* = 8.4 Hz, 1H), 4.82 (dd, *J* = 8.6 Hz and 4.9 Hz, 1H), 4.26 (d, *J* = 11.2 Hz, 1H), 4.18 (d, *J* = 17.4 Hz, 1H), 4.00 (d, *J* = 17.5 Hz, 1H), 3.66 (s, 3H), 3.07 (dd, *J* = 13.8 Hz and 4.9 Hz, 1H), 2.76 (dd, *J* = 13.8 Hz and 8.6 Hz, 1H), 2.35-2.20 (m, 1H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H). LC/MS (ESI): 395.2 [M+H]⁺; white foam = 80%.

methyl (2S)-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]propanoate (9)

¹H NMR (CDCl₃, 250 MHz): δ = 7.89-7.83 (m, 1H), 7.60-7.53 (m, 1H), 7.50-7.44 (m, 2H), 6.64 (bd, *J* = 7.2 Hz, 1H), 4.95 (t, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 17.2 Hz, 1H), 4.48 (q, *J* = 7.2 Hz, 1H), 4.40 (d, *J* = 17.4 Hz, 1H), 3.19 (s, 3H), 1.85 (t, *J* = 7.2 Hz, 2H), 1.56-1.48 (m, 1H), 1.35 (d, *J* = 7.2 Hz, 3H), 0.97 (d, *J* = 6.5 Hz, 6H). NMR (101 MHz, CDCl₃) δ = 172.98, 170.23, 169.06, 141.81, 131.93, 131.74, 128.06, 123.88, 122.93, 53.07, 52.42, 48.15, 47.40, 38.00, 24.78, 22.89, 22.01, 17.83. LC/MS (ESI): 330.6 [M-H]⁻, 332.8 [M+H]⁺; yellow solid. Yield = 76%.

methyl (2S)-3-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]butanoate (10)

¹H NMR (CDCl₃, 250 MHz): δ = 7.90-7.84 (m, 1H), 7.58-7.52 (m, 1H), 7.51-7.42 (m, 2H), 6.70 (bd, *J* = 8.7 Hz, 1H), 4.95 (t, *J* = 7.9 Hz, 1H), 4.54 (d, *J* = 17.2 Hz, 1H), 4.48-4.43 (m, 1H), 4.40 (d, *J* = 17.0 Hz, 1H), 3.73 (s, 3H), 2.18-1.96 (m, 1H), 1.86 (t, *J* = 7.5 Hz, 2H), 1.63-1.40 (m, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.80 (d, *J* = 6.9 Hz, 3H), 0.77 (d, *J* = 6.9 Hz, 3H). LC/MS (ESI): 358.7 [M-H]⁻, 360.8 [M+H]⁺; yellow solid. Yield = 35%.

methyl (2S)-4-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]pentanoate (11)

¹H NMR (CDCl₃, 250 MHz): δ = 7.82-7.76 (m, 1H), 7.54-7.45 (m, 1H), 7.45-7.36 (m, 2H), 6.52 (bd, *J* = 7.8 Hz, 1H), 4.89 (d, *J* = 7.9 Hz, 1H), 4.47 (d, *J* = 17.2 Hz, 1H), 4.51-4.38 (m, 1H), 4.33 (d, *J* = 17.2 Hz, 1H), 3.65 (s, 3H), 1.81 (d, *J* = 7.1 Hz, 1H), 1.78 (d, *J* = 7.1 Hz, 1H), 1.59-1.28 (m, 4H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.69 (d, *J* = 6.4 Hz, 3H), 0.67 (d, *J* = 6.4 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.92, 170.26, 169.14, 141.68, 132.02, 131.70, 128.07, 123.80, 122.88, 53.20, 52.22, 50.81, 47.27, 41.05, 37.51, 24.79, 24.78, 22.88, 22.54, 22.05, 21.59. LC/MS (ESI): 372.8 [M-H]⁻, 374.8 [M+H]⁺; yellow solid. Yield = 26%.

methyl (2S)-4-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]pentanoate (12)

¹H NMR (CDCl₃, 250 MHz): δ = 7.68-7.63 (m, 1H), 7.38-7.31 (m, 1H), 7.29-7.22 (m, 2H), 6.54 (bd, *J* = 8.5 Hz, 1H), 4.76 (t, *J* = 7.9 Hz, 1H), 4.35 (d, *J* = 17.3 Hz, 1H), 4.27 (dd, *J* = 8.6 Hz and 5.2 Hz), 4.19 (d, *J* = 17.3 Hz, 1H), 3.52 (s, 3H), 1.68-1.56 (m, 3H), 1.39-1.24 (m, 1H), 1.15-1.06 (m, 2H), 0.76 (d, *J* = 6.7 Hz, 3H), 0.75 (d, *J* = 6.5 Hz, 3H), 0.56 (t, *J* = 7.4 Hz, 3H), 0.54 (d, *J* = 6.9 Hz, 3H). LC/MS (ESI): 375.3 [M-H]⁻, 377.2 [M+H]⁺; yellow foam. Yield = 79%.

methyl 2-[4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]-3-phenylpropanoate (13)

¹H NMR (CDCl₃, 250 MHz): δ = 7.87 (d, *J* = 7.3 Hz, 1H), 7.58 (td, *J* = 7.4 Hz and *J* = 1.3 Hz, 1H), 7.50 (bd, *J* = 7.1 Hz, 1H), 7.39 (d, *J* = 7.4 Hz, 1H), 6.93-6.85 (m, 3H), 6.85-6.76 (m, 2H), 6.60 (bd, *J* = 8.6 Hz, 1H), 4.96-4.79 (m, 2H), 4.19 (d, *J* = 17.1 Hz, 1H), 3.89 (d, *J* = 17.1 Hz, 1H), 3.73 (s, 3H), 3.15 (dd, *J* = 13.9 Hz and *J* = 4.9 Hz, 1H), 2.81 (dd, *J* = 13.9 Hz and *J* = 8.9 Hz, 1H), 1.83-1.65 (m, 2H), 1.52-1.34 (m, 1H), 0.91 (d, *J* = 6.6 Hz, 6H). ¹³C NMR (CDCl₃, 75 MHz) δ = 171.65, 169.69, 168.88, 141.71, 135.74, 132.02, 131.62, 128.83, 128.16, 128.06, 126.63, 123.89, 123.00, 52.80, 52.65, 52.43, 46.65, 38.12, 36.98, 24.64, 22.93, 21.95. LC/MS (ESI): 407.2 [M-H]⁻, 409.2 [M+H]⁺; yellow solid. Yield = 51%.

methyl (2S)-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]propanoate (14)

¹H NMR (CDCl₃, 250 MHz): δ = 7.76 (bd, *J* = 7.1 Hz, 1H), 7.47 (td, *J* = 7.4 Hz and 1.3 Hz, 1H), 7.43-7.36 (m, 2H), 7.21 (s, 4H), 7.17-7.09 (m, 1H), 6.73 (bd, *J* = 7.4 Hz, 1H), 5.07 (dd, *J* = 9.0 Hz and 7.0 Hz, 1H), 4.45-4.33 (m, 3H), 3.62 (s, 3H), 3.37 (dd, *J* = 14.3 Hz and 7.0 Hz, 1H), 3.17 (dd, *J* = 14.5 Hz and 9.1 Hz, 1H), 1.97 (d, *J* = 7.2 Hz, 1H). LC/MS (ESI): 364.8 [M-H]⁻, 366.7 [M+H]⁺; yellow solid. Yield = 93%.

methyl (2S)-3-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]butanoate (15)

¹H NMR (CDCl₃, 250 MHz): δ = 7.76 (bd, *J* = 6.9 Hz, 1H), 7.45 (td, *J* = 7.3 Hz and 1.3 Hz, 1H), 7.41-7.30 (m, 2H), 7.18 (s, 4H), 7.14-7.05 (m, 1H), 6.75 (bd, *J* = 8.8 Hz, 1H), 5.07 (dd, *J* = 9.0 Hz and 7.1 Hz, 1H), 4.40-4.27 (m, 3H), 3.60 (s, 3H), 3.35 (dd, *J* = 14.5 Hz and 7.0 Hz, 1H), 3.18 (dd, *J* = 14.4 Hz and 9.1 Hz, 1H), 2.09-1.92 (m, 1H), 0.71 (d, *J* = 6.9 Hz, 3H), 0.69 (d, *J* = 6.8 Hz, 3H). LC/MS (ESI): 392.6 [M-H]⁻, 394.7 [M+H]⁺; yellow solid. Yield = 91%.

methyl (2S)-4-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]pentanoate (16)

¹H NMR (CDCl₃, 250 MHz): δ = 7.75 (bd, *J* = 7.0 Hz, 1H), 7.46 (td, *J* = 7.4 Hz and 1.3 Hz, 1H), 7.42-7.30 (m, 2H), 7.19 (s, 4H), 7.15-7.07 (m, 1H), 6.66 (bd, *J* = 8.0 Hz, 1H), 5.08 (dd, *J* = 9.0 Hz and 6.8 Hz, 1H), 4.47-4.34 (m, 3H), 3.60 (s, 3H), 3.36 (dd, *J* = 14.5 Hz and 6.9 Hz, 1H), 3.17 (dd, *J* = 14.4 Hz and 9.1 Hz, 1H), 1.54-1.26 (m, 3H), 0.68 (d, *J* = 6.4 Hz, 6H). LC/MS (ESI): 406.8 [M-H]⁻, 408.7 [M+H]⁺; white foam. Yield = 96%.

methyl (2S)-3-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]pentanoate (17)

¹H NMR (CDCl₃, 250 MHz): δ = 7.77 (bd, *J* = 7.6 Hz, 1H), 7.47 (td, *J* = 7.4 Hz and 1.4 Hz, 1H), 7.42-7.32 (m, 2H), 7.19 (s, 4H), 7.16-7.07 (m, 1H), 6.79 (bd, *J* = 8.5 Hz, 1H), 5.09 (dd, *J* = 9.0 Hz and 7.0 Hz, 1H), 4.45-4.34 (m, 3H), 3.61 (s, 3H), 3.35 (dd, *J* =

14.5 Hz and 7.0 Hz, 1H), 3.19 (dd, $J = 14.5$ Hz and 9.1 Hz, 1H), 1.29-1.13 (m, 2H), 1.07-0.89 (m, 1H), 0.71 (t, $J = 7.4$ Hz, 3H), 0.68 (d, $J = 6.9$ Hz, 3H). LC/MS (ESI): 407.4 [M-H]⁻, 409.2 [M+H]⁺; yellow solid. Yield = 83%.

methyl (2S)-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]-3-phenylpropanoate (18)

¹H NMR (CDCl₃, 250 MHz): $\delta = 7.76$ (dd, $J = 7.1$ Hz and 1.0 Hz, 1H), 7.49 (td, $J = 7.5$ Hz and 1.5 Hz, 1H), 7.40 (bt, $J = 7.4$ Hz, 1H), 7.29 (bd, $J = 7.4$ Hz, 1H), 7.21-7.01 (m, 5H), 6.86-6.69 (m, 5H), 6.65 (bd, $J = 7.8$ Hz, 1H), 5.08 (dd, $J = 9.1$ Hz and 6.9 Hz, 1H), 4.77 (td, $J = 8.8$ Hz and 4.9 Hz, 1H), 4.15 (d, $J = 16.9$ Hz, 1H), 3.84 (d, $J = 17.1$ Hz, 1H), 3.62 (s, 3H), 3.28 (dd, $J = 14.7$ Hz and 6.9 Hz, 1H), 3.14-2.95 (m, 2H), 2.73 (dd, $J = 13.9$ Hz and 8.9 Hz, 1H). LC/MS (ESI): 440.5 [M-H]⁻, 442.7 [M+H]⁺; yellow foam. Yield = 92%.

General procedure for the synthesis of isoindolinone amino acid derivatives (19-33)

To a stirred solution of ester intermediate derivative (**4-18**) (1eq, 0.5 mmol) in THF at 0°C, aqueous solution of LiOH (3 eq, 1.5 mmol) was added dropwise. The resulting mixture was stirred 2 h at room temperature. After completion, THF was removed in vacuo. Then, 1N HCl solution was added dropwise until pH 2-3 and the aqueous layer was extracted with ethyl acetate. The organic layer was washed once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo to afford a crude residue which does not require further purification.

(2S)-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]propanoic acid (19 or E5)

¹H NMR (DMSO-*d*₆, 250 MHz): $\delta = 8.76$ (bd, $J = 6.3$ Hz, 1H), 7.74 (d, $J = 7.4$ Hz, 1H), 7.69-7.60 (m, 2H), 7.58-7.47 (m, 1H), 4.82 (d, $J = 18.2$ Hz, 1H), 4.59 (d, $J = 11.1$ Hz, 1H), 4.52 (d, $J = 18.4$ Hz, 1H), 4.22-3.98 (m, 1H), 2.37-2.15 (m, 1H), 1.30 (d, $J = 7.3$ Hz, 3H), 1.07 (d, $J = 6.4$ Hz, 3H), 0.79 (d, $J = 6.5$ Hz, 3H). ¹³C NMR (DMSO-*d*₆, 63 MHz): $\delta = 173.78, 169.59, 167.52, 142.21, 131.53, 131.45, 127.78, 123.45, 122.86, 59.78, 47.66, 47.21, 28.34, 19.12, 18.94, 16.63$; LC/MS (ESI): 302.7 [M-H]⁻, 304.8 [M+H]⁺; white solid. Yield = 90%.

(2S)-3-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]butanoic acid (20)

¹H NMR (DMSO-*d*₆, 250 MHz): $\delta = 8.46$ (bd, $J = 7.8$ Hz, 1H), 7.71 (d, $J = 7.4$ Hz, 1H), 7.65-7.53 (m, 2H), 7.54-7.42 (m, 1H), 4.80 (d, $J = 18.2$ Hz, 1H), 4.70 (d, $J = 10.6$ Hz, 1H), 4.48 (d, $J = 18.4$ Hz, 1H), 4.10-3.92 (m, 1H), 2.33-2.16 (m, 1H), 2.13-1.97 (m, 1H), 1.01 (d, $J = 6.3$ Hz, 3H), 0.87 (d, $J = 7.0$ Hz, 3H), 0.83 (d, $J = 7.0$ Hz, 3H), 0.77 (d, $J = 6.4$ Hz, 3H). ¹³C NMR (DMSO-*d*₆, 63 MHz): $\delta = 172.56, 170.31, 167.58, 142.26, 131.59, 131.42, 127.76, 123.43, 122.86, 59.75, 57.49, 47.32, 29.26, 28.28, 19.21, 18.94, 18.89, 18.15$; LC/MS (ESI): 330.8 [M-H]⁻, 332.8 [M+H]⁺; white solid. Yield = 91%.

(2S)-4-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]pentanoic acid (21)

¹H NMR (DMSO-*d*₆, 250 MHz): $\delta = 8.62$ (bd, $J = 7.3$ Hz, 1H), 7.71 (bd, $J = 7.4$ Hz, 1H), 7.66-7.61 (m, 2H), 7.54-7.46 (m, 1H), 4.79 (d, $J = 18.3$ Hz, 1H), 4.57 (d, $J = 10.8$ Hz, 1H), 4.50 (d, $J = 18.4$ Hz, 1H), 4.23-4.06 (m, 1H), 2.35-2.17 (m, 1H), 1.62-1.47 (m, 3H), 1.03 (d, $J = 6.6$ Hz, 3H), 0.86 (d, $J = 6.0$ Hz, 3H), 0.78 (d, $J = 6.5$ Hz, 3H), 0.77 (d, $J = 5.8$ Hz, 3H). ¹³C NMR (DMSO-*d*₆, 63 MHz): $\delta = 173.65, 169.86, 167.48, 142.21, 131.51, 131.45, 127.78, 123.46, 122.87, 59.87, 50.37, 47.22, 39.32, 28.25, 24.29, 22.70, 21.17, 19.15, 18.96$; LC/MS (ESI): 345.4 [M-H]⁻, 347.3 [M+H]⁺; white solid. Yield = 70%.

(2S,3S)-3-methyl-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]pentanoic acid (22)

¹H NMR (DMSO-*d*₆, 250 MHz): $\delta = 8.52$ (bd, $J = 7.7$ Hz, 1H), 7.75 (d, $J = 7.4$ Hz, 1H), 7.70-7.61 (m, 2H), 7.58-7.48 (m, 1H), 4.84 (d, $J = 18.3$ Hz, 1H), 4.72 (d, $J = 10.8$ Hz, 1H), 4.53 (d, $J = 18.3$ Hz, 1H), 4.04-4.11 (m, 1H), 2.36-2.15 (m, 1H), 1.86-1.40 (m, 1H), 1.32-1.10 (m, 2H), 1.04 (d, $J = 6.6$ Hz, 3H), 0.85 (d, $J = 7.1$ Hz, 6H), 0.81 (d, $J = 6.6$ Hz, 3H). ¹³C NMR (DMSO-*d*₆, 63 MHz): $\delta = 173.68, 171.33, 168.68, 143.39, 132.69, 132.57, 128.90, 124.58, 123.99, 60.82, 57.70, 48.42, 36.82, 29.43, 25.92, 20.36, 20.00, 16.54, 12.33$; LC/MS (ESI): 345.4 [M-H]⁻, 347.3 [M+H]⁺; yellow solid. Yield = 93%.

(2S)-2-[(2S)-3-methyl-2-(1-oxo-3H-isoindol-2-yl)butanamido]-3-phenylpropanoic acid (23)

¹H NMR (CDCl₃, 250 MHz): $\delta = 7.84$ (d, $J = 7.3$ Hz, 1H), 7.56 (td, $J = 7.3$ Hz and 1.0 Hz, 1H), 7.47 (bt, $J = 7.3$ Hz, 1H), 7.37 (d, $J = 7.6$ Hz, 1H), 7.33 (d, $J = 8.4$ Hz, 1H), 6.86-6.79 (m, 2H), 6.76-6.57 (m, 3H), 4.88 (td, $J = 8.4$ Hz and 5.0 Hz, 1H), 4.50 (d, $J = 11.1$ Hz, 1H), 4.19 (d, $J = 18.2$ Hz, 1H), 3.99 (d, $J = 18.1$ Hz, 1H), 3.13 (dd, $J = 13.7$ Hz and 4.9 Hz, 1H), 2.59 (dd, $J = 13.7$ Hz and 8.5 Hz, 1H), 2.36-2.15 (m, 1H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.71 (d, $J = 6.7$ Hz, 3H). ¹³C NMR (CDCl₃, 63 MHz): $\delta = 172.46, 168.24, 167.35, 140.96, 135.04, 130.98, 130.51, 128.23, 127.12, 126.84, 125.35, 122.94, 121.99, 60.48, 51.74, 46.47, 37.16, 26.47, 18.31, 17.40$; LC/MS (ESI): 379.4 [M-H]⁻, 381.2 [M+H]⁺; white solid. Yield = 92%.

(2S)-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]propanoic acid (24)

¹H NMR (CDCl₃, 250 MHz): $\delta = 7.83-7.74$ (m, 1H), 7.55-7.35 (m, 4H), 5.12 (bt, $J = 7.8$ Hz, 1H), 4.61 (d, $J = 17.9$ Hz, 1H), 4.55-4.44 (m, 1H), 4.39 (d, $J = 17.9$ Hz, 1H), 1.77 (t, $J = 7.4$ Hz, 2H), 1.50-1.34 (m, 1H), 1.31 (d, $J = 7.1$ Hz, 3H), 0.89 (d, $J = 6.5$ Hz, 6H). ¹³C NMR (CDCl₃, 63 MHz): $\delta = 174.07, 168.78, 168.35, 140.82, 130.99, 130.63, 127.15, 122.93, 121.93, 52.15, 47.39, 46.58, 37.31, 23.69, 21.83, 21.05, 17.06$. LC/MS (ESI): 316.7 [M-H]⁻, 318.8 [M+H]⁺; white solid. Yield = 95%.

(2S)-3-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]butanoic acid (25)

¹H NMR (CDCl₃, 250 MHz): $\delta = 7.83-7.77$ (m, 1H), 7.57-7.30 (m, 3H), 7.34 (bd, $J = 8.5$ Hz, 1H), 5.10 (bt, $J = 7.8$ Hz, 1H), 4.60 (d, $J = 17.7$ Hz, 1H), 4.49 (dd, $J = 8.6$ Hz and 4.8 Hz, 1H), 4.39 (d, $J = 17.8$ Hz, 1H), 2.22-1.02 (m, 1H), 1.86-1.68 (m, 2H), 1.50-1.36

(m, 1H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.5$ Hz, 3H), 0.78 (d, $J = 7.0$ Hz, 3H), 0.74 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (CDCl_3 , 63 MHz): $\delta = 174.03, 170.18, 169.31, 141.76, 131.98, 131.70, 128.20, 123.98, 122.94, 57.20, 53.40, 47.65, 38.07, 31.08, 24.77, 22.82, 22.16, 18.84, 17.69$; LC/MS (ESI): 344.7 [M-H] $^-$; 346.8 [M+H] $^+$; white solid. Yield = 86%.

(2S)-4-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]pentanoic acid (26)

^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.83\text{-}7.75$ (m, 1H), 7.55-7.47 (m, 1H), 7.46-7.34 (m, 2H), 7.34 (bd, $J = 8.0$ Hz, 1H), 5.05 (bt, $J = 7.8$ Hz, 1H), 4.56 (d, $J = 17.7$ Hz, 1H), 4.52 (m, 1H), 4.38 (d, $J = 17.8$ Hz, 1H), 1.84-1.71 (m, 2H), 1.66-1.37 (m, 4H), 0.89 (d, $J = 6.4$ Hz, 6H), 0.72 (d, $J = 6.0$ Hz, 3H), 0.70 (d, $J = 6.0$ Hz, 3H). ^{13}C NMR (CDCl_3 , 63 MHz): $\delta = 175.39, 170.12, 169.40, 141.77, 132.00, 131.69, 128.19, 123.93, 122.93, 53.25, 50.95, 47.61, 41.13, 37.97, 24.87, 24.74, 22.85, 22.60, 22.12, 21.88$; LC/MS (ESI): 358.8 [M-H] $^-$; 360.8 [M+H] $^+$; yellow solid. Yield = 85%.

(2S,3S)-3-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]pentanoic acid (27)

^1H NMR (CD_3OD , 250 MHz): $\delta = 7.79$ (dd, $J = 7.6$ Hz and 0.8 Hz, 1H), 7.64-7.55 (m, 2H), 7.53-7.46 (m, 1H), 5.12 (dd, $J = 9.5$ Hz and 6.4 Hz, 1H), 4.79 (d, $J = 17.9$ Hz, 1H), 4.52 (d, $J = 17.9$ Hz, 1H), 4.34 (d, $J = 5.8$ Hz, 1H), 1.96-1.76 (m, 3H), 1.58-1.46 (m, 1H), 1.29-1.14 (m, 2H), 0.98 (d, $J = 6.7$ Hz, 3H), 0.97 (d, $J = 6.5$ Hz, 3H), 0.8 (d, $J = 6.9$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (CD_3OD , 63 MHz): $\delta = 173.16, 171.72, 169.60, 142.39, 131.80, 131.36, 127.78, 122.97, 122.90, 56.79, 52.90, 47.53, 38.25, 36.74, 26.57, 24.79, 21.86, 20.75, 14.62, 10.36$; LC/MS (ESI): 359.2 [M-H] $^-$; 361.3 [M+H] $^+$; yellow solid. Yield = 94%.

(2S)-2-methyl-2-[(2S)-4-methyl-2-(1-oxo-3H-isoindol-2-yl)pentanamido]-3-phenylpropanoic acid (28)

^1H NMR (CD_3OD , 250 MHz): $\delta = 7.76$ (d, $J = 7.5$ Hz, 1H), 7.59 (td, $J = 7.5$ Hz and $J = 1.2$ Hz, 1H), 7.49 (bd, $J = 7.5$ Hz, 1H), 7.45 (d, $J = 7.4$ Hz, 1H), 6.99 (dd, $J = 7.9$ Hz and $J = 1.5$ Hz, 2H), 6.84-6.69 (m, 3H), 4.94 (dd, $J = 8.6$ Hz and $J = 7.1$ Hz, 1H), 4.61 (dd, $J = 10.5$ Hz and $J = 4.5$ Hz, 1H), 4.24 (d, $J = 17.8$ Hz, 1H), 3.94 (d, $J = 17.8$ Hz, 1H), 3.18 (dd, $J = 13.8$ Hz and $J = 4.5$ Hz, 1H), 2.79 (dd, $J = 13.8$ Hz and $J = 10.3$ Hz, 1H), 1.72-1.63 (m, 2H), 1.41-1.30 (m, 1H), 0.89 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR (CD_3OD , 63 MHz): $\delta = 172.90, 170.33, 169.18, 142.28, 136.83, 131.71, 131.40, 128.69, 127.74, 127.69, 126.04, 123.07, 122.95, 53.07, 52.67, 46.82, 37.80, 37.11, 24.56, 21.75, 20.92$; LC/MS (ESI): 393.2 [M-H] $^-$; 395.2 [M+H] $^+$; yellow solid. Yield = 73%.

(2S)-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]propanoic acid (29)

^1H NMR ($\text{DMSO-}d_6$, 250 MHz): $\delta = 8.73$ (d, $J = 7.2$ Hz, 1H), 7.68-7.57 (m, 3H), 7.53-7.42 (m, 1H), 7.36 (d, $J = 7.5$ Hz, 2H), 7.27 (bt, $J = 7.0$ Hz, 2H), 7.19 (bd, $J = 7.0$ Hz, 1H), 5.34 (dd, $J = 11.4$ Hz and 4.2 Hz, 1H), 4.85 (d, $J = 17.6$ Hz, 1H), 4.52 (d, $J = 17.8$ Hz, 1H), 4.36-4.18 (m, 1H), 3.21 (d, $J = 11.8$ Hz, 1H), 3.15 (d, $J = 11.9$ Hz, 1H), 1.36 (d, $J = 7.3$ Hz, 3H); ^{13}C NMR ($\text{DMSO-}d_6$, 63 MHz): $\delta = 173.84, 170.24, 167.68, 142.11, 137.45, 131.63, 131.41, 128.49, 128.26, 127.70, 126.39, 123.32, 122.77, 54.34, 47.61, 47.24, 35.43, 16.92$; LC/MS (ESI): 350.8 [M-H] $^-$; 352.7 [M+H] $^+$; yellow solid. Yield = 92%.

(2S)-3-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]butanoic acid (30)

^1H NMR ($\text{DMSO-}d_6$, 250 MHz): $\delta = 8.48$ (d, $J = 8.4$ Hz, 1H), 7.67-7.57 (m, 3H), 7.51-7.42 (m, 1H), 7.36 (bd, $J = 7.2$ Hz, 2H), 7.26 (bt, $J = 7.3$ Hz, 2H), 7.16 (bd, $J = 7.2$ Hz, 1H), 5.44 (dd, $J = 11.5$ Hz and 4.3 Hz, 1H), 4.86 (d, $J = 17.8$ Hz, 1H), 4.45 (d, $J = 17.7$ Hz, 1H), 4.18 (dd, $J = 8.3$ Hz and 5.9 Hz, 1H), 3.24-3.05 (m, 2H), 2.19-2.04 (m, 1H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR ($\text{DMSO-}d_6$, 63 MHz): $\delta = 170.45, 168.72, 165.48, 139.85, 135.14, 129.39, 129.16, 126.22, 125.99, 125.46, 124.15, 121.04, 120.50, 55.01, 51.90, 45.12, 33.17, 27.38, 16.80, 15.73$; LC/MS (ESI): 378.7 [M-H] $^-$; 380.7 [M+H] $^+$; yellow solid. Yield = 94%.

(2S)-4-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]pentanoic acid (31)

^1H NMR ($\text{DMSO-}d_6$, 250 MHz): $\delta = 8.63$ (d, $J = 8.0$ Hz, 1H), 7.70-7.58 (m, 3H), 7.55-7.44 (m, 1H), 7.36 (bd, $J = 7.0$ Hz, 2H), 7.27 (bt, $J = 7.3$ Hz, 2H), 7.19 (bd, $J = 7.0$ Hz, 1H), 5.35 (dd, $J = 11.3$ Hz and 4.3 Hz, 1H), 4.84 (d, $J = 17.7$ Hz, 1H), 4.51 (d, $J = 17.9$ Hz, 1H), 4.34-4.21 (m, 1H), 3.29-3.10 (m, 2H), 1.70-1.50 (m, 3H), 0.93 (d, $J = 5.9$ Hz, 3H), 0.86 (d, $J = 5.9$ Hz, 3H); ^{13}C NMR ($\text{DMSO-}d_6$, 63 MHz): $\delta = 173.78, 170.56, 168.67, 167.66, 142.10, 137.43, 131.63, 131.43, 128.48, 128.27, 127.72, 126.40, 123.32, 122.78, 54.34, 50.32, 47.25, 35.40, 24.31, 22.82, 21.16, 21.11$; LC/MS (ESI): 392.8 [M-H] $^-$; 394.8 [M+H] $^+$; white solid. Yield = 93%.

(2S)-3-methyl-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]pentanoic acid (32)

^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.77\text{-}7.70$ (m, 1H), 7.52-7.44 (m, 1H), 7.41-7.31 (m, 3H), 7.18-7.10 (m, 4H), 7.09-7.00 (m, 1H), 5.28 (dd, $J = 8.9$ Hz and 7.0 Hz, 1H), 4.57-4.39 (m, 3H), 3.31 (dd, $J = 14.4$ Hz and 6.9 Hz, 1H), 3.17-3.04 (dd, $J = 14.3$ Hz and 9.0 Hz, 1H), 1.87-1.73 (m, 1H), 1.34-1.25 (m, 1H), 1.14-0.91 (m, 1H), 0.76 (d, $J = 7.2$ Hz, 3H), 0.70 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (CDCl_3 , 63 MHz): $\delta = 173.90, 169.46, 169.32, 141.66, 136.37, 132.02, 131.54, 128.82, 128.66, 128.17, 126.89, 123.92, 122.91, 56.68, 56.33, 48.13, 37.64, 35.45, 25.11, 15.21, 11.59$; LC/MS (ESI): 392.6 [M-H] $^-$; 394.7 [M+H] $^+$; white solid. Yield = 91%.

(2S)-2-[(2S)-2-(1-oxo-3H-isoindol-2-yl)-3-phenylpropanamido]-3-phenylpropanoic acid (33)

^1H NMR ($\text{DMSO-}d_6$, 250 MHz): $\delta = 8.51$ (bd, $J = 8.4$ Hz, 1H), 7.64-7.39 (m, 4H), 7.28-7.05 (m, 9H), 5.21 (dd, $J = 10.5$ Hz and 4.9 Hz, 1H), 4.54-4.42 (m, 1H), 4.31 (bd, $J = 5.0$ Hz, 1H), 4.03 (dd, $J = 14.0$ Hz and 6.8 Hz, 1H), 3.13-2.77 (m, 4H). ^{13}C NMR ($\text{DMSO-}d_6$, 63 MHz): $\delta = 172.64, 169.88, 167.46, 141.96, 137.53, 137.42, 131.60, 131.37, 128.97, 128.58, 128.21, 127.99, 127.69,$

126.34, 126.26, 123.33, 122.79, 54.40, 53.44, 46.78, 36.30, 35.15; LC/MS (ESI): 426.6 [M-H]⁻, 428.7 [M+H]⁺; white foam. Yield = 48%.

General procedure for the synthesis of intermediates (38-41)

To a stirred solution of *L*-valine methyl ester hydrochloride (1.2 eq) and the corresponding methyl 2-(bromomethyl)benzoate derivative (**34-37**) (200 mg, 1 eq) in dry DMF (4 mL), *N,N*-diisopropylethylamine (3 eq) was added. The mixture was stirred under argon atmosphere during 5h-8h at 80°C. After completion, the resulting mixture was washed twice with 1N HCl solution, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with cyclohexane-ethyl acetate (9:1 to 8:2).

methyl (2S)-2-(4-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoate (38)

¹H NMR (CDCl₃, 400 MHz): δ = 7.71 (dd, *J* = 7.5 Hz and 1.0 Hz, 1H), 7.46 (dd, *J* = 7.9 Hz and 1.0 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 4.74 (d, *J* = 10.3 Hz, 1H), 4.66 (d, *J* = 17.7 Hz, 1H), 4.33 (d, *J* = 17.7 Hz, 1H), 3.67 (s, 3H), 2.37 – 2.25 (m, 1H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (DMSO-*d*₆, 63 MHz): δ = 171.37, 168.08, 139.89, 133.87, 131.68, 129.75, 129.30, 122.47, 59.92, 52.05, 46.57, 29.07, 19.42, 19.20; LC/MS (ESI): 282.1 [M+H]⁺; yellow oil. Yield = 96%.

methyl (2S)-2-(5-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoate (39)

¹H NMR (CDCl₃, 400 MHz): δ = 7.72 (dd, *J* = 7.9 Hz and 0.8 Hz, 1H), 7.41 – 7.35 (m, 2H), 4.72 (d, *J* = 10.3 Hz, 1H), 4.66 (d, *J* = 17.4 Hz, 1H), 4.33 (d, *J* = 17.3 Hz, 1H), 3.65 (s, 3H), 2.34 – 2.17 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.45, 167.95, 143.26, 138.10, 130.33, 128.71, 125.24, 123.29, 59.84, 52.01, 46.91, 29.08, 19.39, 19.14; LC/MS (ESI): 282.1 [M+H]⁺; beige solid. Yield = 44%.

methyl (2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoate (40)

¹H NMR (CDCl₃, 400 MHz): δ = 7.66 (d, *J* = 7.5 Hz, 1H), 7.57 – 7.52 (m, 2H), 4.72 (d, *J* = 10.3 Hz, 1H), 4.65 (d, *J* = 17.3 Hz, 1H), 4.32 (d, *J* = 17.3 Hz, 1H), 3.65 (s, 3H), 2.32 – 2.18 (m, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.42, 168.04, 143.49, 131.56, 130.79, 126.45, 126.25, 125.45, 59.83, 52.02, 46.84, 29.09, 19.40, 19.14; LC/MS (ESI): 326.1/328.1 [M+H]⁺; white solid. Yield = 40%.

methyl (2S)-2-(6-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoate (41)

¹H NMR (CDCl₃, 250 MHz): δ = 8.01 (d, *J* = 1.6 Hz, 1H), 7.69 (dd, *J* = 8.0 Hz and 1.7 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 4.81 (d, *J* = 10.3 Hz, 1H), 4.71 (d, *J* = 17.3 Hz, 1H), 4.39 (d, *J* = 17.3 Hz, 1H), 3.74 (s, 3H), 2.41-2.28 (m, 1H), 1.07 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CDCl₃, 63 MHz): δ = 171.39, 167.50, 140.30, 134.64, 133.87, 127.20, 124.42, 122.10, 59.88, 51.99, 47.06, 29.11, 19.38, 19.12; LC/MS (ESI): 325.8/327.9 [M+H]⁺; yellow solid. Yield = 60%.

General procedure for the synthesis of carboxylic acid intermediates (42-45) and derivatives (50-53)

To a stirred solution of ester intermediate derivative (**38-41** and **46-49**) (1eq) in THF at 0°C, aqueous solution of LiOH (4 eq, 0.75 ml) was added dropwise. The resulting mixture was stirred 1 h at room temperature. After completion, THF was removed in vacuo. Then, 1N HCl solution was added dropwise until pH 2-3 and the aqueous layer was extracted with ethyl acetate. The organic layer was washed once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo to afford a crude residue which does not requires further purification.

(2S)-2-(4-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoic acid (42)

¹H NMR (CD₃OD, 400 MHz): δ = 7.76 (dd, *J* = 7.5 Hz and 1.0 Hz, 1H), 7.66 (dd, *J* = 7.9 Hz and 1.0 Hz, 1H), 7.56 (t, *J* = 7.7 Hz, 1H), 4.79 (d, *J* = 18.0 Hz, 1H), 4.69 (d, *J* = 9.9 Hz, 1H), 4.56 (d, *J* = 18.0 Hz, 1H), 2.48 – 2.35 (m, 1H), 1.14 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 171.90, 168.78, 140.07, 133.32, 131.80, 129.89, 129.06, 121.80, 60.47, 46.76, 28.70, 18.51, 18.29; LC/MS (ESI): 266.1 [M-H]⁻, 268.1 [M+H]⁺; yellow oil. Yield = 94%.

(2S)-2-(5-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoic acid (43)

¹H NMR (CD₃OD, 400 MHz): δ = 7.65 (d, *J* = 8.2 Hz, 1H), 7.55 (bd, *J* = 1.0 Hz, 1H), 7.43 (dd, *J* = 8.2 Hz and 1.8 Hz, 1H), 4.71 (d, *J* = 18.1 Hz, 1H), 4.53 (d, *J* = 9.8 Hz, 1H), 4.46 (d, *J* = 18.1 Hz, 1H), 2.32 – 2.20 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 172.09, 168.77, 144.18, 138.06, 129.87, 128.36, 124.41, 123.42, 60.58, 47.88, 28.68, 18.51, 18.22; LC/MS (ESI): 266.1 [M-H]⁻, 268.1 [M+H]⁺; white solid. Yield = 98%.

(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoic acid (44)

¹H NMR (CD₃OD, 400 MHz): δ = 7.73 – 7.71 (m, 1H), 7.59 (d, *J* = 1.0 Hz, 2H), 4.70 (d, *J* = 18.1 Hz, 1H), 4.53 (d, *J* = 9.8 Hz, 1H), 4.46 (d, *J* = 18.1 Hz, 1H), 2.35 – 2.19 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 171.94, 168.89, 144.35, 131.28, 130.26, 126.46, 126.35, 124.58, 60.45, 47.30, 28.67, 18.50, 18.23; LC/MS (ESI): 310.0/312.0 [M-H]⁻, 312.0/314.0 [M+H]⁺; white solid. Yield = 70%.

(2S)-2-(6-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanoic acid (45)

¹H NMR (CD₃OD, 400 MHz): δ = 7.80 (d, *J* = 1.7 Hz, 1H), 7.66 (dd, *J* = 8.1 Hz and 1.8 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 4.65 (d, *J* = 18.0 Hz, 1H), 4.55 (d, *J* = 9.8 Hz, 1H), 4.44 (d, *J* = 18.0 Hz, 1H), 2.33-2.18 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.83 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (CD₃OD, 101 MHz): δ = 171.85, 168.33, 141.23, 134.75, 133.31, 125.96, 124.95, 121.55, 60.46, 47.50, 28.68, 18.49, 18.23; LC/MS (ESI): 310.9/312.9 [M-H]⁻, 311.8/313.9 [M-H]⁺; pale yellow solid. Yield = 94%.

(2S)-2-[(2S)-2-(4-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoic acid (50)

¹H NMR (CD₃OD, 400 MHz): δ = 7.64 (dd, *J* = 7.5 Hz and 0.7 Hz, 1H), 7.53 (dd, *J* = 7.9 Hz and 0.8 Hz, 1H), 7.43 (bt, *J* = 7.7 Hz, 1H), 4.71 (d, *J* = 18.5 Hz, 1H), 4.48 (d, *J* = 11.0 Hz, 1H), 4.43 (d, *J* = 18.5 Hz, 1H), 4.24 (q, *J* = 7.3 Hz, 1H), 2.37 – 2.23 (m, 1H), 1.29 (d, *J* = 7.3 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.77 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.16, 170.23, 168.46, 140.13, 133.43, 131.71, 129.84, 129.06, 121.71, 61.14, 47.88, 47.67, 28.44, 18.16, 18.05, 15.92; LC/MS (ESI): 337.1 [M-H]⁻, 339.1 [M+H]⁺; HRMS (TOF, ESI⁺) calcd for C₁₆H₂₀ClN₂O₄ [M+H]⁺ 339.1106, found 339.1117; white solid. Yield = 95%.

(2S)-2-[(2S)-2-(5-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoic acid (51)

¹H NMR (CD₃OD, 400 MHz): δ = 7.65 (d, *J* = 8.2 Hz, 1H), 7.54 (bd, *J* = 0.9 Hz, 1H), 7.42 (dd, *J* = 8.2 Hz and 1.7 Hz, 1H), 4.70 (d, *J* = 18.5 Hz, 1H), 4.48 – 4.42 (m, 2H), 4.23 (q, *J* = 7.3 Hz, 1H), 2.32 – 2.20 (m, 1H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.25, 170.25, 168.46, 144.22, 138.02, 129.95, 128.33, 124.34, 123.42, 61.14, 48.10, 47.30, 28.39, 18.16, 18.01, 15.95; LC/MS (ESI): 337.1 [M-H]⁻, 339.1 [M+H]⁺; HRMS (TOF, ESI⁺) calcd for C₁₆H₂₀ClN₂O₄ [M+H]⁺ 339.1106, found 339.1120; white solid. Yield = 95%.

(2S)-2-[(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoic acid (52)

¹H NMR (CD₃OD, 400 MHz): δ = 7.76 – 7.65 (m, 1H), 7.58 (d, *J* = 0.9 Hz, 2H), 4.69 (d, *J* = 18.5 Hz, 1H), 4.48 – 4.42 (m, 2H), 4.22 (q, *J* = 7.3 Hz, 1H), 2.32 – 2.19 (m, 1H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.19, 170.24, 168.56, 144.39, 131.23, 130.36, 126.44, 126.28, 124.50, 61.11, 48.03, 47.25, 28.40, 18.17, 18.03, 15.93; LC/MS (ESI): 381.0/383.1 [M-H]⁻, 383.1/385.1 [M+H]⁺; HRMS (TOF, ESI⁺) calcd for C₁₆H₂₀BrN₂O₄ [M+H]⁺ 383.0601, found 383.0583; white solid. Yield = 59%.

(2S)-2-[(2S)-2-(6-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoic acid (53)

¹H NMR (CD₃OD, 400 MHz): δ = 7.81 (bd, *J* = 1.7 Hz, 1H), 7.67 (dd, *J* = 8.1 Hz and 1.8 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 4.67 (d, *J* = 18.4 Hz, 1H), 4.45 (d, *J* = 11.0 Hz, 1H), 4.42 (d, *J* = 18.4 Hz, 1H), 4.22 (q, *J* = 7.3 Hz, 1H), 2.33 – 2.20 (m, 1H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.32, 170.19, 168.01, 141.29, 134.68, 133.42, 125.87, 124.96, 121.50, 61.19, 47.87, 47.66, 28.43, 18.16, 18.01, 15.98; LC/MS (ESI): 381.1/383.1 [M-H]⁻, 383.1/385.1 [M+H]⁺; HRMS (TOF, ESI⁺) calcd for C₁₆H₂₀BrN₂O₄ [M+H]⁺ 383.0601, found 383.0611; pale yellow solid. Yield = 78%.

General procedure for the synthesis of methyl ester intermediates (46-49)

To a stirred solution of *L*-alanine methyl ester hydrochloride (1 eq) and the corresponding carboxylic acid derivative (42-45) (1 eq) in dry dichloromethane (5 mL), *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (2 eq) and triethylamine (2 eq, 1 mmol) were added. The mixture was stirred under argon atmosphere during 8-18 h at room temperature. After completion, the resulting mixture was washed twice with 1N HCl solution, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 98:2).

methyl (2S)-2-[(2S)-2-(4-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoate (46)

¹H NMR (CDCl₃, 400 MHz): δ = 7.69 (dd, *J* = 7.5 Hz and 0.7 Hz, 1H), 7.45 (dd, *J* = 7.9 Hz and 0.9 Hz, 1H), 7.37 (bt, *J* = 7.7 Hz, 1H), 6.61 (bd, *J* = 7.0 Hz, 1H), 4.65 (d, *J* = 18.2 Hz, 1H), 4.42 (p, *J* = 7.2 Hz, 1H), 4.34 (d, *J* = 18.2 Hz, 1H), 4.35 (d, *J* = 11.2 Hz, 1H), 3.67 (s, 3H), 2.47 – 2.34 (m, 1H), 1.30 (d, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 6.5 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.86, 169.28, 168.18, 140.07, 133.94, 131.71, 129.72, 129.42, 122.24, 61.90, 52.43, 48.13, 46.97, 27.98, 19.31, 19.02, 17.81; LC/MS (ESI): 351.1 [M-H]⁻, 353.1 [M+H]⁺; yellow oil. Yield = 37%.

(2S)-2-[(2S)-2-(5-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoate (47)

¹H NMR (CDCl₃, 400 MHz): δ = 7.71 (dd, *J* = 7.8 Hz and 1.0 Hz, 1H), 7.40 – 7.36 (m, 2H), 6.79 (bd, *J* = 7.2 Hz, 1H), 4.65 (d, *J* = 17.9 Hz, 1H), 4.41 (p, *J* = 7.2 Hz, 1H), 4.36 (d, *J* = 11.1 Hz, 1H), 4.34 (d, *J* = 17.8 Hz, 1H), 3.67 (s, 3H), 2.40 – 2.30 (m, 1H), 1.30 (d, *J* = 7.3 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.80 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.85, 169.35, 168.05, 143.44, 138.15, 130.43, 128.69, 125.04, 123.37, 61.87, 52.43, 48.11, 47.32, 27.99, 19.31, 18.97, 17.79; LC/MS (ESI): 351.1 [M-H]⁻, 353.2 [M+H]⁺; white solid. Yield = 32%.

(2S)-2-[(2S)-2-(5-chloro-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoate (48)

¹H NMR (CDCl₃, 400 MHz): δ = 7.65 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.52 (m, 2H), 6.68 (bd, *J* = 7.0 Hz, 1H), 4.63 (d, *J* = 17.9 Hz, 1H), 4.41 (p, *J* = 7.2 Hz, 1H), 4.36 – 4.30 (m, 2H), 3.67 (s, 3H), 2.42 – 2.28 (m, 1H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.80 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.84, 169.29, 168.15, 143.65, 131.54, 130.88, 126.50, 126.33, 125.24, 61.87, 52.43, 48.11, 47.24, 27.97, 19.31, 18.96, 17.80; LC/MS (ESI): 395.1/397.1 [M-H]⁻, 397.1/399.1 [M+H]⁺; white solid. Yield = 43%.

methyl (2S)-[(2S)-(6-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-methylbutanamido]propanoate (49)

¹H NMR (CDCl₃, 400 MHz): δ = 8.00 (d, *J* = 1.6 Hz, 1H), 7.69 (dd, *J* = 8.1 Hz and 1.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 6.85 (bd, *J* = 7.1 Hz, 1H), 4.70 (d, *J* = 17.8 Hz, 1H), 4.50 (q, *J* = 7.2 Hz, 1H), 4.45 (d, *J* = 11.2 Hz, 1H), 4.40 (d, *J* = 17.9 Hz, 1H), 3.76 (s, 3H), 2.49-2.38 (m, 1H), 1.39 (d, *J* = 7.2 Hz, 3H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.71, 169.28, 167.63, 140.47, 134.65, 133.99, 127.04, 124.50, 122.07, 61.99, 52.40, 48.11, 47.48, 27.97, 19.30, 18.95, 17.80; LC/MS (ESI): 395.1/397.1 [M-H]⁻; 396.8/398.9 [M+H]⁺; white solid. Yield = 62%.

General procedure for the synthesis of isoindolinone intermediates (55-63)

To a stirred solution of the commercial methyl 2-(bromomethyl)benzoate **54** (1 eq) and the corresponding *L*-amino acid methyl ester hydrochloride derivative (1.2 eq) (200 mg, 1 eq) in dry DMF (2 mL), *N,N*-diisopropylethylamine (3 eq) was added. The mixture was stirred under argon atmosphere during 5h at 80°C, cooled and then concentrated. The resulting residue was diluted in ethyl acetate, washed twice with 1N HCl, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with cyclohexane-ethyl acetate (9:1 to 7:3).

ethyl (2S)-3-(4-methoxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (55)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.5 Hz, 1H), 7.45 (td, *J* = 7.4 Hz and 1.2 Hz, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.7 Hz, 2H), 5.30 (dd, *J* = 10.3 Hz and 6.0 Hz, 1H), 4.51 (d, *J* = 16.5 Hz, 1H), 4.26 (d, *J* = 16.5 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 3H), 3.36 (dd, *J* = 14.7 Hz and 6.0 Hz, 1H), 3.05 (dd, *J* = 14.7 Hz and 10.3 Hz, 1H), 1.15 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.00, 168.94, 158.46, 141.70, 131.95, 131.55, 129.55, 128.28, 127.92, 123.95, 122.78, 114.09, 61.47, 55.18, 54.79, 47.46, 35.11, 14.14; LC/MS (ESI): 340.2 [M+H]⁺; yellow oil. Yield = 57%.

ethyl (2S)-3-(4-methylphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (56)

¹H NMR (CD₃OD, 400 MHz): δ = 7.60 (bd, *J* = 7.6 Hz, 1H), 7.48 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.41 (bd, *J* = 7.6 Hz, 1H), 7.36 (bd, *J* = 7.4 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 7.9 Hz, 2H), 5.13 (dd, *J* = 11.0 Hz and 5.4 Hz, 1H), 4.38 (s, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.34 (dd, *J* = 14.5 Hz and 5.4 Hz, 1H), 3.11 (dd, *J* = 14.5 Hz and 11.0 Hz, 1H), 2.12 (s, 3H), 1.13 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 170.51, 169.72, 142.15, 136.23, 133.57, 131.81, 131.19, 128.86, 128.20, 127.75, 122.86 (2C), 61.30, 55.65, 47.97, 34.62, 19.60, 13.02; LC/MS (ESI): 324.1 [M+H]⁺; yellow oil. Yield = 83%.

ethyl (2S)-3-(4-methylphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (57)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.5 Hz, 1H), 7.46 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.36 (bd, *J* = 7.5 Hz, 1H), 7.34 (bd, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 5.30 (dd, *J* = 10.4 Hz and 5.9 Hz, 1H), 4.44 (d, *J* = 16.4 Hz, 1H), 4.23 (d, *J* = 16.4 Hz, 1H), 3.65 (s, 3H), 3.38 (dd, *J* = 14.8 Hz and 5.8 Hz, 1H), 3.08 (dd, *J* = 14.8 Hz and 10.4 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.04, 168.92, 141.47, 135.36, 131.84, 131.76, 131.72, 130.26, 128.10, 124.06, 122.84, 120.90, 54.39, 52.54, 47.51, 35.24; LC/MS (ESI): 374.1/376.1 [M+H]⁺; yellow oil. Yield = 30%.

methyl (2S)-3-(naphthalen-2-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (58)

¹H NMR (CDCl₃, 400 MHz): δ = 7.73 (bd, *J* = 7.6 Hz, 1H), 7.71 – 7.64 (m, 3H), 7.60 (bs, 1H), 7.42 (td, *J* = 7.4 Hz and 1.2 Hz, 1H), 7.36 – 7.29 (m, 5H), 5.47 (dd, *J* = 10.3 Hz and 5.9 Hz, 1H), 4.48 (d, *J* = 16.4 Hz, 1H), 4.29 (d, *J* = 16.4 Hz, 1H), 3.66 (s, 3H), 3.60 (dd, *J* = 14.8 Hz and 5.9 Hz, 1H), 3.29 (dd, *J* = 14.8 Hz and 10.4 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.40, 168.98, 141.61, 133.86, 133.47, 132.42, 131.86, 131.62, 128.50, 127.97, 127.63, 127.61, 127.22, 126.58, 126.13, 125.72, 123.98, 122.81, 54.55, 52.50, 47.59, 36.03; LC/MS (ESI): 346.2 [M+H]⁺; yellow oil. Yield = 65%.

methyl (2S)-3-(benzylsulfanyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (59)

¹H NMR (CDCl₃, 400 MHz): δ = 7.83 (bd, *J* = 7.5 Hz, 1H), 7.50 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.42 (bd, *J* = 7.4 Hz, 1H), 7.37 (bd, *J* = 7.5 Hz, 1H), 7.29 – 7.16 (m, 5H), 5.19 (dd, *J* = 10.5 Hz and 5.2 Hz, 1H), 4.47 (d, *J* = 16.3 Hz, 1H), 4.26 (d, *J* = 16.3 Hz, 1H), 3.72 (d, *J* = 13.5 Hz, 1H), 3.67 (d, *J* = 13.5 Hz, 1H), 3.65 (s, 3H), 3.04 (dd, *J* = 14.0 Hz and 5.2 Hz, 1H), 2.76 (dd, *J* = 14.0 Hz and 10.5 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 170.56, 169.13, 141.77, 137.40, 131.79, 131.71, 129.04, 128.63, 128.06, 127.25, 124.11, 122.91, 52.58, 52.54, 47.12, 35.60, 30.85; LC/MS (ESI): 342.1 [M+H]⁺; yellow oil. Yield = 74%.

methyl (2S)-3-(benzylsulfanyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (60)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.5 Hz, 1H), 7.46 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.38 (bd, *J* = 7.5 Hz, 1H), 7.34 (bd, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 2H), 7.09 (d, *J* = 8.5 Hz, 2H), 5.30 (dd, *J* = 10.4 Hz and 5.9 Hz, 1H), 4.44 (d, *J* = 16.4 Hz, 1H), 4.23 (d, *J* = 16.4 Hz, 1H), 3.65 (s, 3H), 3.40 (dd, *J* = 14.8 Hz and 5.9 Hz, 1H), 3.09 (dd, *J* = 14.8 Hz and 10.4 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.06, 168.93, 141.48, 134.82, 132.82, 131.76, 131.72, 129.90, 128.89, 128.09, 124.05, 122.83, 54.47, 52.53, 47.51, 35.18; LC/MS (ESI): 330.1 [M+H]⁺; yellow oil. Yield = 99%.

ethyl (2S)-3-(4-cyanophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (61)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.6 Hz, 1H), 7.51 – 7.45 (m, 3H), 7.39 (bd, *J* = 7.5 Hz, 1H), 7.35 (bd, *J* = 7.5 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 5.34 (dd, *J* = 10.2 Hz and 6.0 Hz, 1H), 4.47 (d, *J* = 16.4 Hz, 1H), 4.22 (d, *J* = 16.4 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.48 (dd, *J* = 14.8 Hz and 6.0 Hz, 1H), 3.18 (dd, *J* = 14.8 Hz and 10.2 Hz, 1H), 1.16 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃,

101 MHz): δ = 170.19, 168.92, 142.12, 141.34, 132.48, 131.88, 131.54, 129.46, 128.20, 124.09, 122.85, 118.63, 111.02, 61.85, 54.22, 47.43, 35.97, 14.13; LC/MS (ESI): 335.1 [M+H]⁺; yellow oil. Yield = 57%.

methyl (2S)-3-(4-hydroxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (62)

¹H NMR (CDCl₃, 400 MHz): δ = 7.71 (d, *J* = 7.6 Hz, 1H), 7.41 (td, *J* = 7.4 Hz and 1.2 Hz, 1H), 7.32 (d, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 7.3 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 5.45 (dd, *J* = 11.5 Hz and 4.9 Hz, 1H), 4.54 (d, *J* = 16.7 Hz, 1H), 4.29 (d, *J* = 16.7 Hz, 1H), 3.67 (s, 3H), 3.40 (dd, *J* = 15.0 Hz and 4.9 Hz, 1H), 3.00 (dd, *J* = 15.0 Hz and 11.6 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.34, 169.85, 155.86, 141.58, 131.88, 131.33, 129.25, 128.14, 126.70, 124.18, 122.77, 115.78, 54.54, 52.56, 47.35, 34.93; LC/MS (ESI): 312.2 [M+H]⁺, 310.1 [M-H]⁻; yellow solid. Yield = 71%.

methyl (2S)-3-(1H-indol-3-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (63)

¹H NMR (CDCl₃, 400 MHz): δ = 8.05 (bs, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.42 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.34 (bt, *J* = 7.1 Hz, 1H), 7.28 (bd, *J* = 7.5 Hz, 1H), 7.25 (dt, *J* = 7.9 Hz and 0.9 Hz, 1H), 7.10 (ddd, *J* = 7.9 Hz, 7.1 Hz and 1.2 Hz, 1H), 7.05 (ddd, *J* = 7.9 Hz, 7.0 Hz and 1.0 Hz, 1H), 6.96 (bs, 1H), 5.48 (dd, *J* = 10.8 Hz and 5.2 Hz, 1H), 4.47 (d, *J* = 16.6 Hz, 1H), 4.23 (d, *J* = 16.6 Hz, 1H), 3.67 (s, 3H), 3.51 (ddd, *J* = 15.8 Hz, 5.2 Hz and 1.2 Hz, 1H), 3.33 (ddd, *J* = 15.8 Hz, 10.8 Hz and 0.9 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.72, 169.12, 141.76, 136.10, 131.98, 131.61, 127.95, 127.22, 123.88, 122.88, 122.24, 121.87, 119.57, 118.27, 111.26, 110.62, 53.57, 52.49, 47.23, 25.77; LC/MS (ESI): 335.2 [M+H]⁺, 333.1 [M-H]⁻; yellow solid. Yield = 73%.

General procedure for the synthesis of carboxylic acid intermediates (64-72) and derivatives (82-90)

To a stirred solution of ester intermediate derivative (**55-63** and **73-81**) (1eq) in THF (2 ml) at 0°C, aqueous solution of LiOH (4 eq, 0.5 ml) was added dropwise. The resulting mixture was stirred 1 h at room temperature. After completion, THF was removed in vacuo. Then, 1N HCl solution was added dropwise until pH 2-3 and the aqueous layer was extracted with ethyl acetate. The organic layer was washed once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo to afford a pure compound (**64-68**, **70-72** and **82-90**). Compound **69** was purified by column chromatography eluting with dichloromethane-MeOH (95:5 à 9:1).

(2S)-3-(4-methoxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (64)

¹H NMR (CD₃OD, 400 MHz): δ = 7.60 (bd, *J* = 7.6 Hz, 1H), 7.48 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.42 (bd, *J* = 7.5 Hz, 1H), 7.38 – 7.33 (m, 1H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.67 (d, *J* = 8.7 Hz, 2H), 5.11 (dd, *J* = 11.5 Hz and 4.8 Hz, 1H), 4.47 (d, *J* = 17.4 Hz, 1H), 4.36 (d, *J* = 17.4 Hz, 1H), 3.59 (s, 3H), 3.36 (dd, *J* = 14.8 Hz and 4.8 Hz, 1H), 3.09 (dd, *J* = 14.7 Hz and 11.5 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 172.33, 169.84, 158.58, 142.17, 131.71, 131.34, 129.23, 128.89, 127.69, 122.83, 122.82, 113.60, 55.57, 54.15, 47.88, 34.25; LC/MS (ESI): 312.1 [M+H]⁺, 310.1 [M-H]⁻; white solid. Yield = 89%.

(2S)-3-(4-methylphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (65)

¹H NMR (CD₃OD, 400 MHz): δ = 7.60 (bd, *J* = 7.6 Hz, 1H), 7.47 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.41 (bd, *J* = 7.5 Hz, 1H), 7.35 (td, *J* = 7.4 Hz and 0.9 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 7.9 Hz, 2H), 5.14 (dd, *J* = 11.5 Hz and 4.8 Hz, 1H), 4.45 (d, *J* = 17.3 Hz, 1H), 4.35 (d, *J* = 17.3 Hz, 1H), 3.39 (dd, *J* = 14.7 Hz and 4.8 Hz, 1H), 3.12 (dd, *J* = 14.7 Hz and 11.5 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 172.33, 169.84, 158.58, 142.17, 131.71, 131.34, 129.23, 128.89, 127.69, 122.83, 122.82, 113.60, 55.57, 54.15, 47.88, 34.25; LC/MS (ESI): 296.2 [M+H]⁺, 294.1 [M-H]⁻; white solid. Yield = 70%.

(2S)-3-(4-bromophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (66)

¹H NMR (CD₃OD, 400 MHz): δ = 7.61 (bd, *J* = 7.6 Hz, 1H), 7.51 – 7.47 (td, *J* = 7.6 Hz and 1.1 Hz, 1H), 7.43 (bd, *J* = 7.5 Hz, 1H), 7.37 (td, *J* = 7.4 Hz and 1.0 Hz, 1H), 7.26 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 5.15 (dd, *J* = 11.4 Hz and 4.9 Hz, 1H), 4.44 (d, *J* = 17.3 Hz, 1H), 4.38 (d, *J* = 17.3 Hz, 1H), 3.39 (dd, *J* = 14.7 Hz and 4.9 Hz, 1H), 3.15 (dd, *J* = 14.7 Hz and 11.4 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 171.82, 169.84, 142.09, 136.49, 131.83, 131.25, 131.17, 130.31, 127.77, 122.90, 122.88, 120.16, 55.19, 47.88, 34.43; LC/MS (ESI): 360.1/362.0 [M+H]⁺, 358.1/360.1 [M-H]⁻; white solid. Yield = 82%.

(2S)-3-(naphthalen-2-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (67)

¹H NMR (CD₃OD, 400 MHz): δ = 7.67 – 7.54 (m, 5H), 7.47 – 7.41 (m, 1H), 7.39 (bd, *J* = 7.4 Hz, 1H), 7.34 – 7.26 (m, 4H), 5.28 (dd, *J* = 11.4 Hz and 4.8 Hz, 1H), 4.50 (d, *J* = 17.4 Hz, 1H), 4.43 (d, *J* = 17.4 Hz, 1H), 3.61 (dd, *J* = 14.8 Hz and 4.6 Hz, 1H), 3.33 (dd, *J* = 14.7 Hz and 11.4 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 172.22, 169.88, 142.11, 134.65, 133.56, 132.45, 131.72, 131.24, 127.87, 127.67, 127.17, 127.10, 126.89, 126.38, 125.69, 125.25, 122.83, 122.80, 77.88, 55.37, 35.25; LC/MS (ESI): 332.2 [M+H]⁺, 330.1 [M-H]⁻; white solid. Yield = 91%.

(2S)-3-(benzylsulfanyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (68)

¹H NMR (CD₃OD, 400 MHz): δ = 7.71 (bd, *J* = 7.6 Hz, 1H), 7.54 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.46 (bd, *J* = 7.6 Hz, 1H), 7.42 (bt, *J* = 7.5 Hz, 1H), 7.25 – 7.09 (m, 5H), 4.99 (dd, *J* = 11.4 Hz and 4.5 Hz, 1H), 4.48 (d, *J* = 17.1 Hz, 1H), 4.29 (d, *J* = 17.1 Hz, 1H), 3.70 (d, *J* = 13.3 Hz, 1H), 3.66 (d, *J* = 13.3 Hz, 1H), 3.09 (dd, *J* = 14.1 Hz and 4.6 Hz, 1H), 2.87 (dd, *J* = 14.1 Hz and 11.5 Hz, 1H). ¹³C

NMR (CD₃OD, 101 MHz): δ = 171.28, 170.13, 142.37, 137.90, 131.88, 131.25, 128.71, 128.16, 127.78, 126.77, 123.01, 122.95, 52.99, 47.30, 34.82, 30.28; LC/MS (ESI): 328.1 [M+H]⁺, 326.1 [M-H]⁻; yellow solid. Yield = 98%.

(2S)-3-(4-chlorophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (69)

¹H NMR (CD₃OD, 400 MHz): δ = 7.61 (bd, J = 7.6 Hz, 1H), 7.49 (td, J = 7.5 Hz and 1.1 Hz, 1H), 7.43 (bd, J = 7.5 Hz, 1H), 7.36 (bt, J = 7.4 Hz, 1H), 7.14 (d, J = 7.4 Hz, 2H), 7.11 (d, J = 7.4 Hz, 2H), 5.15 (dd, J = 11.4 Hz and 4.9 Hz, 1H), 4.44 (d, J = 17.3 Hz, 1H), 4.38 (d, J = 17.3 Hz, 1H), 3.41 (dd, J = 14.7 Hz and 4.9 Hz, 1H), 3.15 (dd, J = 14.7 Hz and 11.4 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 171.81, 169.83, 142.10, 135.98, 132.24, 131.83, 131.17, 129.95, 128.24, 127.77, 122.90, 122.88, 55.24, 48.09, 34.35; LC/MS (ESI): 316.1 [M+H]⁺, 314.1 [M-H]⁻; white solid. Yield = 75%.

(2S)-3-(4-cyanophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (70)

¹H NMR (CD₃OD, 400 MHz): δ = 7.60 (bd, J = 7.6 Hz, 1H), 7.51 – 7.47 (m, 3H), 7.43 (bd, J = 7.5 Hz, 1H), 7.39 – 7.34 (m, 3H), 5.20 (dd, J = 11.3 Hz and 4.9 Hz, 1H), 4.45 (d, J = 17.3 Hz, 1H), 4.39 (d, J = 17.3 Hz, 1H), 3.52 (dd, J = 14.7 Hz and 4.9 Hz, 1H), 3.27 (dd, J = 14.7 Hz and 11.4 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 171.57, 169.82, 143.38, 142.06, 132.00, 131.89, 131.08, 129.52, 127.81, 122.92 (2C), 118.18, 110.29, 55.00, 47.88, 35.08; LC/MS (ESI): 307.1 [M+H]⁺, 305.1 [M-H]⁻; white solid. Yield = 82%.

(2S)-3-(4-hydroxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (71)

¹H NMR (CD₃OD, 400 MHz): δ = 7.60 (d, J = 7.6 Hz, 1H), 7.47 (td, J = 7.5, 1.1 Hz, 1H), 7.43 – 7.38 (m, 1H), 7.38 – 7.31 (m, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.55 – 6.51 (d, J = 8.5 Hz, 2H), 5.09 (dd, J = 11.4, 4.9 Hz, 1H), 4.39 (dd, J = 17.3 Hz, 2H), 3.32 (dd, J = 14.7, 4.9 Hz, 1H), 3.06 (dd, J = 14.7, 11.4 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz): δ = 172.25, 169.86, 155.88, 142.18, 131.71, 131.33, 129.24, 127.68, 127.59, 122.84, 122.81, 114.98, 55.56, 47.86, 34.26; LC/MS (ESI): 298.1 [M+H]⁺, 296.1 [M-H]⁻; yellow solid. Yield = 85%.

(2S)-3-(1H-indol-3-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (72)

¹H NMR (CDCl₃, 400 MHz): δ = 8.15 (bs, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.39 (td, J = 7.5 Hz and 1.2 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H), 7.24 – 7.19 (m, 2H), 7.11–7.05 (m, 1H), 7.05 – 6.99 (m, 1H), 6.92 (bs, 1H), 5.38 (dd, J = 10.9 Hz and 4.9 Hz, 1H), 4.36 (d, J = 16.9 Hz, 1H), 4.20 (d, J = 16.9 Hz, 1H), 3.53 (dd, J = 15.7 Hz and 4.9 Hz, 1H), 3.39 (dd, J = 15.7 Hz and 11.0 Hz, 1H). ¹³C NMR (CDCl₃, 101 MHz): δ = 173.57, 170.26, 141.85, 136.10, 131.91, 131.58, 128.06, 127.10, 123.91, 122.84, 122.20, 119.55, 118.18, 111.35, 110.32, 54.98, 48.30, 25.55; LC/MS (ESI): 321.1 [M+H]⁺, 319.1 [M-H]⁻; brown solid. Yield = 52%.

(2S)-2-[(2S)-3-(4-methoxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (82)

¹H NMR (CD₃OD, 400 MHz): δ = 7.72 (bd, J = 7.6 Hz, 1H), 7.60 (td, J = 7.4 Hz and 1.1 Hz, 1H), 7.55 (bd, J = 7.4 Hz, 1H), 7.47 (td, J = 7.6 Hz and 1.0 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H), 6.79 (d, J = 8.7 Hz, 2H), 5.27 (dd, J = 10.7 Hz and 5.4 Hz, 1H), 4.75 (d, J = 17.7 Hz, 1H), 4.56 (d, J = 17.7 Hz, 1H), 4.37 (q, J = 7.2 Hz, 1H), 3.71 (s, 3H), 3.39 (dd, J = 14.7 Hz and 5.5 Hz, 1H), 3.18 (dd, J = 14.7 Hz and 10.7 Hz, 1H), 1.41 (d, J = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.46, 170.88, 169.65, 158.59, 142.34, 131.69, 131.30, 129.36, 128.57, 127.64, 122.82, 122.80, 113.59, 55.80, 54.15, 48.17, 47.88, 34.61, 16.14; LC/MS (ESI): 383.2 [M+H]⁺, 381.2 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₁H₂₃N₂O₅ [M+H]⁺ 383.1601, found 383.1612; white solid. Yield = 77%.

(2S)-2-[(2S)-3-(4-methylphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (83)

¹H NMR (CD₃OD, 400 MHz): δ = 7.61 (d, J = 7.6 Hz, 1H), 7.50 – 7.39 (m, 2H), 7.38 – 7.31 (m, 1H), 7.08 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 8.7 Hz, 2H), 5.15 (dd, J = 10.7 Hz and 5.5 Hz, 1H), 4.63 (d, J = 17.7 Hz, 1H), 4.44 (d, J = 17.6 Hz, 1H), 4.26 (q, J = 7.2 Hz, 1H), 3.59 (s, 3H), 3.26 (dd, J = 14.7 Hz and 5.4 Hz, 1H), 3.06 (dd, J = 14.7 Hz and 10.7 Hz, 1H), 1.29 (d, J = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 170.85, 170.16, 169.65, 142.32, 136.08, 133.63, 131.68, 131.29, 128.82, 128.51, 128.22, 127.63, 122.84, 55.87, 47.88, 35.14, 29.36, 19.63, 16.22; LC/MS (ESI): 367.2 [M+H]⁺, 365.2 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₁H₂₃N₂O₄ [M+H]⁺ 367.1652, found 367.1670; white solid. Yield = 66%.

(2S)-2-[(2S)-3-(4-bromophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (84)

¹H NMR (CD₃OD, 400 MHz): δ = 7.73 (bd, J = 7.7 Hz, 1H), 7.61 (td, J = 7.6 Hz and 1.0 Hz, 1H), 7.56 (bd, J = 7.4 Hz, 1H), 7.48 (td, J = 7.7 Hz and 1.0 Hz, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 5.30 (dd, J = 10.3 Hz and 5.7 Hz, 1H), 4.76 (d, J = 17.7 Hz, 1H), 4.57 (d, J = 17.7 Hz, 1H), 4.38 (q, J = 7.3 Hz, 1H), 3.41 (dd, J = 14.6 Hz and 5.6 Hz, 1H), 3.21 (dd, J = 14.6 Hz and 10.3 Hz, 1H), 1.41 (d, J = 7.4 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.26, 170.43, 169.58, 142.26, 136.15, 131.79, 131.23, 131.15, 130.45, 127.72, 122.88, 122.86, 120.18, 55.38, 48.11, 47.79, 34.88, 16.05; LC/MS (ESI): 431.1/433.1 [M+H]⁺, 429.0/431.0 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₀H₂₀BrN₂O₄ [M+H]⁺ 431.0601, found 431.0608; white solid. Yield = 36%.

(2S)-2-[(2S)-3-(naphthalen-2-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (85)

¹H NMR (CD₃OD, 400 MHz): δ = 7.66 – 7.54 (m, 5H), 7.46 – 7.38 (m, 2H), 7.35 – 7.26 (m, 4H), 5.33 (dd, J = 10.5 Hz and 5.5 Hz, 1H), 4.67 (d, J = 17.7 Hz, 1H), 4.51 (d, J = 17.7 Hz, 1H), 4.30 (q, J = 7.3 Hz, 1H), 3.50 (dd, J = 14.7 Hz and 5.4 Hz, 1H), 3.30 (dd, J = 14.7 Hz and 10.6 Hz, 1H), 1.31 (d, J = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.38, 170.78, 169.67, 142.30, 134.37,

133.57, 132.46, 131.70, 131.20, 127.83, 127.63, 127.14, 127.13, 127.01, 126.53, 125.63, 125.22, 122.81, 122.78, 55.56, 48.18, 47.92, 35.60, 16.10; LC/MS (ESI): 403.2 [M+H]⁺, 401.2 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₄H₂₃N₂O₄ [M+H]⁺ 403.1652, found 403.1668; white solid. Yield = 91%.

(2S)-2-[(2S)-3-(benzylsulfanyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (86)

¹H NMR (CD₃OD, 400 MHz): δ = 7.72 (dd, *J* = 7.6 Hz and 1.2 Hz, 1H), 7.52 (m, 1H), 7.43 (m, 2H), 7.23 (m, 2H), 7.17 (d, *J* = 7.3 Hz, 2H), 7.12 (m, 1H), 5.04 (m, 1H), 4.53 (d, *J* = 17.4 Hz, 1H), 4.28 (m, 1H), 3.70 (s, 2H), 3.01 (dd, *J* = 14.0 Hz and 5.9 Hz, 1H), 2.78 (m, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.20, 169.83, 169.47, 142.49, 138.04, 131.80, 131.22, 128.74, 128.14, 127.74, 126.72, 122.99, 122.95, 53.42, 48.11, 47.29, 35.08, 30.82, 16.07; LC/MS (ESI): 399.2 [M+H]⁺, 397.2 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₁H₂₃N₂O₄S [M+H]⁺ 399.1373, found 399.1411; white solid. Yield = 99%.

(2S)-2-[3-(4-chlorophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (87)

¹H NMR (CD₃OD, 400 MHz): δ = 7.74 (d, *J* = 7.7 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.50 – 7.45 (m, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 5.30 (dd, *J* = 14.7 Hz and 5.4 Hz, 1H), 4.77 (d, *J* = 17.7 Hz, 1H), 4.57 (d, *J* = 17.7 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 1H), 3.41 (dd, *J* = 14.6 Hz and 5.7 Hz, 1H), 3.22 (dd, *J* = 14.7 Hz and 10.3 Hz, 1H), 1.41 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.29, 170.42, 169.58, 142.27, 136.13, 131.79, 131.24, 131.15, 130.46, 127.72, 122.88, 122.87, 120.18, 55.39, 48.12, 47.89, 34.88, 16.06; LC/MS (ESI): 387.1 [M+H]⁺, 385.1 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₀H₂₀ClN₂O₄ [M+H]⁺ 387.1106, found 387.1119; white solid. Yield = 98%.

(2S)-2-[3-(4-cyanophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (88)

¹H NMR (CD₃OD, 400 MHz): δ = 7.59 (bd, *J* = 7.6 Hz, 1H), 7.60 – 7.46 (m, 1H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.36 – 7.31 (m, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.53 (d, *J* = 8.6 Hz, 2H), 5.13 (dd, *J* = 10.7 Hz and 5.4 Hz, 1H), 4.61 (d, *J* = 17.7 Hz, 1H), 4.43 (d, *J* = 17.7 Hz, 1H), 4.26 (q, *J* = 7.3 Hz, 1H), 3.26 – 3.23 (m, 1H), 3.02 (dd, *J* = 14.7 Hz and 10.7 Hz, 1H), 1.29 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.75, 170.88, 169.69, 155.83, 142.34, 131.67, 131.32, 129.36, 127.62, 127.38, 122.80, 122.29, 114.96, 55.94, 47.95, 47.68, 34.60, 16.97; LC/MS (ESI): 378.2 [M+H]⁺, 376.1 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₁H₂₀N₃O₄ [M+H]⁺ 378.1448, found 378.1467; white solid. Yield = 71%.

(2S)-2-[(2S)-3-(4-hydroxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (89)

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 8.50 (bd, *J* = 6.9 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.58 – 7.54 (m, 1H), 7.47 – 7.41 (m, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 5.18 (dd, *J* = 11.5 Hz and 4.3 Hz, 1H), 4.75 (d, *J* = 17.7 Hz, 1H), 4.44 (d, *J* = 17.7 Hz, 1H), 4.15 (p, *J* = 7.2 Hz, 1H), 3.20 (dd, *J* = 14.8 Hz and 4.2 Hz, 1H), 2.99 (dd, *J* = 14.8 Hz and 11.6 Hz, 1H), 1.28 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (DMSO-*d*₆, 101 MHz): δ = 174.41, 170.64, 168.13, 156.22, 142.61, 132.23, 131.86, 129.89, 128.18, 127.87, 123.81, 123.24, 115.55, 55.17, 48.39, 47.69, 35.12, 17.71; LC/MS (ESI): 369.2 [M+H]⁺, 367.1 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₀H₂₁N₂O₅ [M+H]⁺ 369.1445, found 369.1456; yellow solid. Yield = 53%.

(2S)-2-[(2S)-3-(3H-indol-3-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (90)

¹H NMR (CD₃OD, 400 MHz): δ = 7.61 (bd, *J* = 7.7 Hz, 1H), 7.57 (bd, *J* = 7.6 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.38 (bd, *J* = 7.5 Hz, 1H), 7.34 (bt, *J* = 7.4 Hz, 1H), 7.17 (bd, *J* = 8.0 Hz, 1H), 6.99 – 6.94 (m, 1H), 6.93 – 6.88 (m, 2H), 5.28 (dd, *J* = 10.2 Hz and 5.7 Hz, 1H), 4.62 (d, *J* = 17.7 Hz, 1H), 4.45 (d, *J* = 17.7 Hz, 1H), 4.29 (q, *J* = 7.3 Hz, 1H), 3.41 (ddd, *J* = 15.3 Hz, 5.7 Hz and 0.8 Hz, 1H), 3.33 (dd, *J* = 15.5 Hz and 10.2 Hz, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz): δ = 174.46, 171.31, 169.73, 142.41, 136.59, 131.62, 131.43, 127.59, 127.11, 122.81, 122.75, 122.33, 121.13, 118.45, 117.72, 110.89, 109.51, 56.94, 55.02, 47.79, 25.61, 16.13; LC/MS (ESI): 392.2 [M+H]⁺, 390.2 [M-H]⁻; HRMS (TOF, ESI⁺) calcd for C₂₂H₂₂N₃O₄ [M+H]⁺ 392.1605, found 392.1616; beige solid. Yield = 98%.

General procedure for the synthesis of methyl ester intermediates (73-81)

To a stirred solution of *L*-alanine methyl ester hydrochloride (1 eq) and the corresponding carboxylic acid derivative (64-72) (1 eq) in a mixture of dry dichloromethane / DMF (3:1), *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (2 eq) and triethylamine (2 eq) were added. The mixture was stirred under argon atmosphere during 18 h at room temperature. After completion, the resulting mixture was washed twice with 1N HCl solution, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 98:2).

methyl (2S)-2-[(2S)-3-(4-methoxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (73)

¹H NMR (CDCl₃, 400 MHz): δ = 7.76 (bd, *J* = 7.5 Hz, 1H), 7.46 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.39 (bd, *J* = 7.5 Hz, 1H), 7.34 (bd, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.7 Hz, 2H), 5.03 (dd, *J* = 9.0 Hz and 7.1 Hz, 1H), 4.45 (d, *J* = 17.2 Hz, 1H), 4.40 (p, *J* = 7.2 Hz, 1H), 4.35 (d, *J* = 17.2 Hz, 1H), 3.67 (s, 3H), 3.62 (s, 3H), 3.30 (dd, *J* = 14.5 Hz and 7.0 Hz, 1H), 3.11 (dd, *J* = 14.5 Hz and 9.1 Hz, 1H), 1.27 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.67, 169.45, 169.19, 158.44, 141.72, 131.88, 131.75, 129.75, 128.41, 128.05, 123.86, 122.90, 114.11, 56.64, 55.19, 52.42, 48.26, 48.08, 34.25, 18.04; LC/MS (ESI): 397.2 [M+H]⁺, 395.2 [M-H]⁻; white solid. Yield = 69%.

methyl (2S)-2-[3-(4-methylphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (74)

¹H NMR (CDCl₃, 400 MHz): δ = 7.76 (bd, *J* = 7.6 Hz, 1H), 7.49-7.43 (td, *J* = 7.5 Hz and 1.3 Hz, 1H), 7.38 (bd, *J* = 7.4 Hz, 1H), 7.34 (bd, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.98 (d, *J* = 7.8 Hz, 2H), 5.09 (m, 1H), 4.46 (d, *J* = 17.0 Hz, 1H), 4.42 (p, *J* = 7.2 Hz, 1H), 4.35 (d, *J* = 17.0 Hz, 1H), 3.62 (s, 3H), 3.35 (dd, *J* = 14.5 Hz and 7.3 Hz, 1H), 3.13 (dd, *J* = 14.5 Hz and 9.2 Hz, 1H), 2.19 (s, 3H), 1.27 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.77, 169.48, 169.37, 141.74, 136.38, 133.45, 131.87, 131.77, 129.38, 128.72, 128.04, 123.87, 122.93, 56.37, 52.41, 48.27, 48.04, 34.66, 21.01, 17.98; LC/MS (ESI): 381.2 [M+H]⁺, 379.1 [M-H]⁻; yellow solid. Yield = 25%.

methyl (2S)-2-[(2S)-3-(4-bromophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (75)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.5 Hz, 1H), 7.48 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.39 (bd, *J* = 7.3 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.83 (bd, *J* = 5.9 Hz, 1H), 5.08 (dd, *J* = 8.7 Hz and 7.4 Hz, 1H), 4.47 (d, *J* = 17.0 Hz, 1H), 4.39 (p, *J* = 7.2 Hz, 1H), 4.33 (d, *J* = 17.1 Hz, 1H), 3.62 (s, 3H), 3.32 (dd, *J* = 14.4 Hz and 7.2 Hz, 1H), 3.10 (dd, *J* = 14.5 Hz and 8.9 Hz, 1H), 1.26 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.61, 169.16, 169.06, 141.59, 135.52, 131.92, 131.78, 130.69, 130.54, 128.17, 123.92, 122.95, 120.81, 56.04, 52.49, 48.28, 47.94, 34.60, 17.94; LC/MS (ESI): 445.1/447.1 [M+H]⁺, 443.0/445.0 [M-H]⁻; brown solid. Yield = 75%.

methyl (2S)-2-[(2S)-3-(naphthalen-2-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (76)

¹H NMR (CDCl₃, 400 MHz): δ = 7.73 (bd, *J* = 7.5 Hz, 1H), 7.70 – 7.60 (m, 4H), 7.43 (td, *J* = 7.4 Hz and 1.0 Hz, 1H), 7.37 – 7.29 (m, 5H), 6.90 (bd, *J* = 7.1 Hz, 1H), 5.26 (dd, *J* = 8.7 Hz and 7.3 Hz, 1H), 4.53 (d, *J* = 17.1 Hz, 1H), 4.45 – 4.32 (m, 2H), 3.53 (dd, *J* = 14.4 Hz and 7.1 Hz, 1H), 3.48 (s, 3H), 3.32 (dd, *J* = 14.4 Hz and 8.9 Hz, 1H), 1.25 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.60, 169.45, 169.21, 141.73, 134.04, 133.49, 132.39, 131.80, 131.78, 128.42, 128.05, 127.63, 127.59, 127.47, 126.90, 126.06, 125.66, 123.85, 122.92, 56.22, 52.32, 48.27, 48.07, 35.46, 17.96; LC/MS (ESI): 417.2 [M+H]⁺, 415.2 [M-H]⁻; yellow oil. Yield = 58%.

methyl (2S)-2-[3-(benzylsulfanyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (77)

¹H NMR (CDCl₃, 400 MHz): δ = 7.82 (bd, *J* = 7.5 Hz, 1H), 7.50 (td, *J* = 7.5 Hz and 1.1 Hz, 1H), 7.42 (bd, *J* = 7.4 Hz, 1H), 7.36 (bd, *J* = 7.6 Hz, 1H), 7.31 – 7.21 (m, 5H), 6.74 (bd, *J* = 7.2 Hz, 1H), 4.90 (dd, *J* = 9.6 Hz and 6.2 Hz, 1H), 4.48 – 4.34 (m, 2H), 4.28 (d, *J* = 17.0 Hz, 1H), 3.70 (s, 2H), 3.66 (s, 3H), 2.99 (dd, *J* = 14.0 Hz and 6.2 Hz, 1H), 2.89 – 2.73 (m, 1H), 1.28 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.68, 169.30, 168.76, 141.79, 137.75, 131.97, 131.65, 130.15, 129.05, 128.67, 128.13, 127.26, 124.02, 123.05, 54.00, 52.51, 48.30, 47.36, 35.97, 30.26, 18.04; LC/MS (ESI): 413.2 [M+H]⁺, 411.2 [M-H]⁻; yellow foam. Yield = 53%.

methyl (2S)-2-[3-(4-chlorophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (78)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 8.1 Hz, 1H), 7.51 – 7.48 (m, 1H), 7.42-7.31 (m, 2H), 7.16 – 7.09 (m, 4H), 6.79 (bd, *J* = 7.3 Hz, 1H), 5.08 (dd, *J* = 8.6 Hz and 8.0 Hz, 1H), 4.45 (d, *J* = 17.0 Hz, 1H), 4.43-4.36 (m, 1H), 4.33 (d, *J* = 17.0 Hz, 1H), 3.62 (s, 3H), 3.35 (dd, *J* = 14.8 Hz and 7.4 Hz, 1H), 3.11 (dd, *J* = 14.5 Hz and 8.8 Hz, 1H), 2.89 (s, 1.26 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.72, 169.31, 169.06, 141.62, 135.18, 132.71, 131.90, 131.70, 130.30, 128.78, 128.16, 123.90, 122.95, 56.13, 52.47, 48.27, 47.90, 47.90, 34.51, 34.25, 17.98; LC/MS (ESI): 401.1 [M+H]⁺, 399.1 [M-H]⁻; yellow oil. Yield = 51%.

methyl (2S)-2-[3-(4-cyanophenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (79)

¹H NMR (CDCl₃, 400 MHz): δ = 7.75 (bd, *J* = 7.0 Hz, 1H), 7.49-7.43 (m, 3H), 7.43-7.34 (m, 2H), 7.34 (bd, *J* = 7.3 Hz, 2H), 6.76 (bd, *J* = 7.2 Hz, 1H), 5.20 – 5.07 (m, 1H), 4.50 – 4.28 (m, 3H), 3.63 (s, 3H), 3.44 (dd, *J* = 14.8 Hz and 7.6 Hz, 1H), 3.18 (dd, *J* = 14.5 Hz and 8.6 Hz, 1H), 2.90 (s, 3H), 1.27 (d, *J* = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.67, 169.35, 168.69, 142.42, 141.42, 132.47, 132.11, 131.46, 129.82, 128.32, 123.99, 123.03, 118.69, 110.93, 55.65, 52.53, 48.32, 47.76, 35.19, 17.93; LC/MS (ESI): 392.2 [M+H]⁺; white solid. Yield = 42%.

methyl (2S)-2-[(2S)-3-(4-hydroxyphenyl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (80)

¹H NMR (CDCl₃, 400 MHz): δ = 7.76 (d, *J* = 7.5 Hz, 1H), 7.48 – 7.43 (m, 1H), 7.39 – 7.33 (m, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 5.12 (dd, *J* = 9.4 Hz and 6.6 Hz, 1H), 4.57 (d, *J* = 17.2 Hz, 1H), 4.41 – 4.32 (m, 2H), 3.62 (s, 3H), 3.27 (dd, *J* = 14.7 Hz and 6.5 Hz, 1H), 3.08 (dd, *J* = 14.7 Hz and 9.4 Hz, 1H), 1.26 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.78, 169.58, 169.56, 155.08, 141.79, 131.91, 131.60, 129.68, 128.13, 127.72, 123.93, 122.90, 115.67, 56.14, 52.47, 48.26, 48.04, 34.44, 17.88; LC/MS (ESI): 383.2 [M+H]⁺, 381.2 [M-H]⁻; white solid. Yield = 30%.

methyl (2S)-2-[(2S)-3-(1H-indol-3-yl)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (81)

¹H NMR (CDCl₃, 400 MHz): δ = 8.21 (bs, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.42 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 8.00 Hz, 1H), 7.11-7.05 (m, 1H), 7.04 – 6.99 (m, 2H), 6.92 (bs, 1H), 5.28 (dd, *J* = 8.7 Hz and 7.0 Hz, 1H), 4.55 (d, *J* = 17.2 Hz, 1H), 4.40 (dd, *J* = 14.4 Hz and 7.2 Hz, 1H), 4.33 (d, *J* = 17.3 Hz, 1H), 3.59 (s, 3H), 3.38 – 3.33 (m, 2H), 1.26 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ = 171.87, 168.98, 168.27, 140.89, 135.09, 130.87, 130.69, 126.95, 126.22, 122.69, 121.94, 121.11, 121.07, 118.45, 117.39, 110.15, 109.55, 54.01, 51.36, 47.26, 46.70, 24.21, 16.83; LC/MS (ESI): 406.2 [M+H]⁺, 404.2 [M-H]⁻; yellow solid. Yield = 55%.

General procedure for Suzuki synthesis of derivatives (91-98)

To a solution of compound **75** (100 mg, 1eq, 0.22 mmol) and corresponding boronic acid or ester derivative (1.3 eq, 0.29 mmol) in dry acetonitrile (7 mL) were added an aqueous solution of 1M K₂CO₃ (5 eq, 1.12 mmol, 1.1 mL) and [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium (II) (16 mg, 0.1 eq, 0.02 mmol). The resulting mixture was refluxed under argon atmosphere during 18 h, cooled and then concentrated. The resulting residue was diluted in ethyl acetate, washed twice with 1N HCl, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 8:2).

(2S)-2-[(2S)-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (91)

¹H NMR (CD₃OD, 400 MHz): δ = 7.61 (bd, *J* = 7.6 Hz, 1H), 7.47 (td, *J* = 7.0 Hz and 1.0 Hz, 1H), 7.44 (bd, *J* = 7.3 Hz, 1H), 7.39 – 7.30 (m, 3H), 7.29 – 7.24 (m, 3H), 7.21 – 7.16 (m, 1H), 6.99 (dd, *J* = 10.7 Hz and 8.4 Hz, 1H), 5.25 (dd, *J* = 10.6 Hz and 5.4 Hz, 1H), 4.67 (d, *J* = 17.6 Hz, 1H), 4.52 – 4.46 (m, 3H), 4.27 (q, *J* = 7.2 Hz, 1H), 3.40 (dd, *J* = 14.7 Hz and 5.3 Hz, 1H), 3.20 – 3.15 (m, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 178.96, 174.56, 173.65, 162.79 (d, ¹*J*_{CF} = 245.9 Hz), 146.25, 141.65 (d, ⁴*J*_{CF} = 3.5 Hz), 140.44, 138.19, 135.70, 135.20, 134.95 (d, ³*J*_{CF} = 9.5 Hz), 132.92 (d, ⁴*J*_{CF} = 3.4 Hz), 132.72 (d, ⁴*J*_{CF} = 3.0 Hz), 132.49, 132.31 (d, ²*J*_{CF} = 26.9 Hz), 132.18, 131.63, 131.28 (d, ³*J*_{CF} = 8.5 Hz), 126.81 (d, ⁴*J*_{CF} = 2.6 Hz), 119.37 (d, ²*J*_{CF} = 23.3 Hz), 66.99, 59.54, 51.88, 39.04, 20.27; LC/MS (ESI): 477.2 [M+H]⁺, 475.3 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₇H₂₆FN₂O₅ [M+H]⁺ 477.1820, found 477.1841; brown solid. Yield = 60%.

(2S)-2-[(2S)-3-{5'-cyano-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (92)

¹H NMR (CD₃OD, 400 MHz): δ = 7.72 (dd, *J* = 7.2 Hz and 2.1 Hz, 1H), 7.64 – 7.59 (m, 2H), 7.48 (td, *J* = 7.0 Hz and 1.0 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.38 – 7.29 (m, 5H), 7.24 (dd, *J* = 10.3 Hz and 8.6 Hz, 1H), 5.27 (dd, *J* = 10.5 Hz and 5.6 Hz, 1H), 4.68 (d, *J* = 17.7 Hz, 1H), 4.49 (d, *J* = 17.7 Hz, 1H), 4.27 (q, *J* = 7.2 Hz, 1H) 3.41 (dd, *J* = 14.7 Hz and 5.5 Hz, 1H), 3.24 – 3.16 (m, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 174.93, 170.47, 169.66, 162.02 (d, ¹*J*_{CF} = 256.4 Hz), 142.32, 137.73, 134.80 (d, ³*J*_{CF} = 4.9 Hz), 133.15 (d, ³*J*_{CF} = 10.0 Hz), 131.95, 131.78, 131.28, 130.29 (d, ²*J*_{CF} = 14.9 Hz) 128.91, 128.83 (d, ⁴*J*_{CF} = 2.9 Hz), 127.71, 122.87 (d, ⁴*J*_{CF} = 4.3 Hz), 117.52, 117.48 (d, ²*J*_{CF} = 24.8 Hz), 108.67 (d, ⁴*J*_{CF} = 3.8 Hz), 55.51, 48.09, 47.87, 35.12, 16.31; LC/MS (ESI): 472.4 [M+H]⁺, 470.4 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₇H₂₃FN₃O₄ [M+H]⁺ 472.1667, found 472.1692; brown solid. Yield = 53%.

(2S)-2-[(2S)-3-{2'-fluoro-5'-methoxy-[1,1'-biphenyl]-4-yl}-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (93)

¹H NMR (CD₃OD, 400 MHz): δ = 7.59 (d, *J* = 7.6 Hz, 1H), 7.46 (bt, *J* = 7.0 Hz, 1H), 7.41 (d, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 7.3 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 6.91 (t, *J* = 9.6 Hz, 1H), 6.78 – 6.68 (m, 2H), 5.26 (dd, *J* = 10.6 Hz and 5.4 Hz, 1H), 4.68 (d, *J* = 17.7 Hz, 1H), 4.47 (d, *J* = 17.6 Hz, 1H), 4.29 (q, *J* = 7.2 Hz, 1H), 3.64 (s, 3H), 3.38 (dd, *J* = 14.7 Hz and 5.3 Hz, 1H), 3.16 (dd, *J* = 14.7 Hz and 10.7 Hz, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 174.37, 170.76, 169.70, 155.99 (d, ⁴*J*_{CF} = 1.8 Hz), 153.98 (d, ¹*J*_{CF} = 239.0 Hz), 142.33, 136.52, 134.32, 131.74, 131.26, 128.91 (d, ²*J*_{CF} = 16.8 Hz), 128.77 (d, ⁴*J*_{CF} = 3.2 Hz), 128.51, 127.68, 122.85 (d, ³*J*_{CF} = 1.7 Hz), 116.09 (d, ²*J*_{CF} = 24.9 Hz), 114.93 (d, ⁴*J*_{CF} = 3.0 Hz), 113.64 (d, ³*J*_{CF} = 8.2 Hz), 55.48, 54.81, 48.11, 47.90, 35.16, 16.12; LC/MS (ESI): 477.2 [M+H]⁺; 475.2 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₇H₂₆FN₂O₅ [M+H]⁺ 477.1820, found 477.1845; brown solid. Yield = 18%.

(2S)-2-[(2S)-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[4-(4-oxo-4H-chromen-6-yl)phenyl]propanamido]propanoic acid (94)

¹H NMR (CD₃OD, 400 MHz): δ = 8.17 (d, *J* = 2.3 Hz, 1H), 8.07 (d, *J* = 6.0 Hz, 1H), 7.89 (dd, *J* = 8.8 Hz and 2.4 Hz, 1H), 7.61 (bd, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.50 – 7.42 (m, 4H), 7.36 (bd, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 2H), 6.28 (d, *J* = 6.0 Hz, 1H), 5.26 (dd, *J* = 10.5 Hz and 5.5 Hz, 1H), 4.67 (d, *J* = 17.6 Hz, 1H), 4.51 (d, *J* = 17.7 Hz, 1H), 4.27 (q, *J* = 7.3 Hz, 1H), 3.41 (dd, *J* = 14.6 Hz and 5.4 Hz, 1H), 3.25 – 3.15 (m, 1H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 178.54, 176.09, 170.47, 169.67, 157.08, 156.07, 142.33, 138.24, 137.52, 136.96, 132.86, 131.76, 131.26, 129.26, 127.70, 126.88, 124.26, 122.89, 122.85, 122.25, 118.74, 111.84, 55.64, 48.09, 47.88, 35.04, 16.42; LC/MS (ESI): 495.2 [M+H]⁺, 497.2 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₉H₂₅N₂O₆ [M+H]⁺ 497.1707, found 497.1729; brown solid. Yield = 47%.

(2S)-2-[(2S)-3-{5'-acetyl-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (95)

¹H NMR (CD₃OD, 400 MHz): δ = 7.93 (dd, *J* = 7.6 Hz and 2.3 Hz, 1H), 7.88 (ddd, *J* = 8.5 Hz, 4.7 Hz and 2.3 Hz, 1H), 7.61 (bd, *J* = 7.7 Hz, 1H), 7.48 (td, *J* = 7.0 Hz and 1.0 Hz, 1H), 7.44 (bd, *J* = 7.1 Hz, 1H), 7.38 – 7.29 (m, 3H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.16 (dd, *J* = 10.3 Hz and 8.6 Hz, 1H), 5.27 (dd, *J* = 10.6 Hz and 5.4 Hz, 1H), 4.68 (d, *J* = 17.7 Hz, 1H), 4.50 (d, *J* = 17.7 Hz, 1H), 4.28 (q, *J* = 7.2 Hz, 1H), 3.41 (dd, *J* = 14.7 Hz and 5.4 Hz, 1H), 3.24 – 3.17 (m, 1H), 2.49 (s, 3H), 1.31 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 197.53, 174.77, 170.60, 169.69, 162.61 (d, ¹*J*_{CF} = 255.1 Hz), 142.33, 137.16, 133.78 (d, ⁴*J*_{CF} = 3.0 Hz), 133.20, 131.77, 131.25, 131.16 (d, ³*J*_{CF} = 5.1 Hz), 129.48 (d, ³*J*_{CF} = 9.7 Hz), 128.99 (d, ²*J*_{CF} = 12.6 Hz), 128.83 (d, ⁴*J*_{CF} = 3.0 Hz), 128.76,

128.36 (d, $^3J_{CF}$ = 12.5 Hz), 127.70, 122.87 (d, $^4J_{CF}$ = 3.4 Hz), 116.05 (d, $^2J_{CF}$ = 24.0 Hz), 55.53, 48.10, 47.89, 35.13, 25.29, 16.26; LC/MS (ESI): 489.4 [M+H]⁺, 487.4 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₈H₂₆FN₂O₅ [M+H]⁺ 489.1820, found 489.1842; brown solid. Yield = 77%.

(2S)-2-[(2S)-3-[4-(2-methyl-3-oxo-2,3-dihydro-1H-isoindol-5-yl)phenyl]-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (**96**)

¹H NMR (CD₃OD, 400 MHz): δ = 7.78 (bd, *J* = 1.2 Hz, 1H), 7.66 (dd, *J* = 7.9 Hz and 1.7 Hz, 1H), 7.60 (bd, *J* = 7.7 Hz, 1H), 7.50 – 7.40 (m, 5H), 7.34 (bt, *J* = 7.0 Hz, 1H), 7.29 (bd, *J* = 8.2 Hz, 1H), 5.26 (dd, *J* = 10.5 Hz and 5.5 Hz, 1H), 4.68 (d, *J* = 17.6 Hz, 1H), 4.50 (d, *J* = 17.7 Hz, 1H), 4.39 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 1H), 3.40 (dd, *J* = 14.6 Hz and 5.5 Hz, 1H), 3.20 – 3.15 (m, 1H), 3.09 (s, 3H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 174.96, 170.57, 169.67, 169.32, 142.33, 140.93, 140.65, 138.55, 136.63, 132.68, 131.75, 131.27, 130.15, 129.13, 127.69, 126.91, 123.09, 122.88, 122.84, 120.60, 55.63, 51.80, 47.92, 47.88, 35.05, 28.27, 16.35; LC/MS (ESI): 498.2 [M+H]⁺, 496.2 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₉H₂₈N₃O₅ [M+H]⁺ 498.2023, found 498.2039; brown solid. Yield = 39%.

(2S)-2-[(2S)-3-{2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (**97**)

¹H NMR (CD₃OD, 400 MHz): δ = 7.62 (bd, *J* = 7.6 Hz, 1H), 7.48 (td, *J* = 7.0 and 1.0 Hz, 1H), 7.45 (bd, *J* = 7.1 Hz, 1H), 7.36 (td, *J* = 7.3 Hz and 1.2 Hz, 1H), 7.33 – 7.29 (m, 3H), 7.27 (bd, *J* = 8.36 Hz, 2H), 7.24 – 7.17 (m, 1H), 7.09 (td, *J* = 7.5 Hz and 1.2 Hz, 1H), 7.02 (ddd, *J* = 11.1 Hz, 8.2 Hz and 1.0 Hz, 1H), 5.25 (dd, *J* = 10.6 Hz and 5.4 Hz, 1H), 4.65 (d, *J* = 17.6 Hz, 1H), 4.50 (d, *J* = 17.6 Hz, 1H), 4.24 (q, *J* = 7.2 Hz, 1H), 3.41 (dd, *J* = 14.6 Hz and 5.3 Hz, 1H), 3.25 – 3.15 (m, 1H), 1.29 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 175.37, 170.54, 169.72, 159.65 (d, $^1J_{CF}$ = 246.4 Hz), 142.32, 136.48, 134.24, 131.76, 131.27, 130.37 (d, J_{CF} = 3.5 Hz), 128.81 (d, $^4J_{CF}$ = 2.8 Hz), 128.72, 128.53, 128.38, 127.70, 124.19 (d, $^4J_{CF}$ = 3.6 Hz), 122.88 (d, $^4J_{CF}$ = 3.1 Hz), 115.51 (d, $^2J_{CF}$ = 23.1 Hz), 55.66, 47.94, 47.88, 35.05, 16.43; LC/MS (ESI): 447.4 [M+H]⁺, 445.4 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₆H₂₄FN₂O₄ [M+H]⁺ 447.1714, found 447.1732; brown solid. Yield = 20%.

(2S)-2-[(2S)-3-{4'-acetamido-[1,1'-biphenyl]-4-yl}-2-(1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (**98**)

¹H NMR (CD₃OD, 400 MHz): δ = 7.59 (bd, *J* = 7.7 Hz, 1H), 7.48 – 7.31 (m, 9H), 7.22 (bd, *J* = 8.2 Hz, 2H), 5.24 (dd, *J* = 10.6 Hz and 5.4 Hz, 1H), 4.66 (d, *J* = 17.7 Hz, 1H), 4.46 (d, *J* = 17.7 Hz, 1H), 4.28 (q, *J* = 7.3 Hz, 1H), 3.36 (dd, *J* = 14.7 Hz and 5.3 Hz, 1H), 3.15 (dd, *J* = 14.7 Hz and 10.7 Hz, 1H), 2.01 (s, 3H), 1.30 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 174.72, 170.73, 170.22, 169.69, 142.32, 138.92, 137.77, 136.25, 135.70, 131.74, 131.27, 128.88, 127.68, 126.67, 126.42, 122.87, 122.84, 119.97, 55.60, 48.10, 47.92, 35.05, 22.43, 16.23; LC/MS (ESI): 484.2 [M+H]⁺, 486.2 [M-H]⁻; HRMS (TOF, ESI+) calcd for C₂₈H₂₈N₃O₅ [M+H]⁺ 486.2023, found 486.2042; brown solid. Yield = 51%.

Synthesis of methyl (2S)-3-(4-bromophenyl)-2-[[tert-butoxy]carbonyl]amino}propanoate (**100**)

To a solution of compound **99** (1.18 g, 1 eq, 4 mmol) in dry dichloromethane (20 mL) were added triethylamine (1.12 mL, 3 eq, 12.00 mmol) and slowly di-tert-butyl dicarbonate (2.76 ml, 3 eq, 12.00 mmol) at 0°C. The resulting mixture was stirred at room temperature under argon atmosphere during 2 h. After completion, the solution was washed twice with 1N HCl, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane.

¹H NMR (CDCl₃, 400 MHz): δ = 7.34 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 8.3 Hz, 2H), 4.91 (bd, *J* = 7.2 Hz, 1H), 4.50 (dd, *J* = 13.3 Hz and 6.0 Hz, 1H), 3.64 (s, 3H), 3.02 (dd, *J* = 13.8 Hz and 5.7 Hz, 1H), 2.92 (dd, *J* = 13.8 Hz and 6.0 Hz, 1H), 1.35 (s, 9H). ¹³C NMR (CDCl₃, 101 MHz): δ = 172.06, 155.00, 135.09, 131.63, 131.03, 121.05, 80.09, 54.22, 52.33, 37.83, 28.28; LC/MS (ESI): 258.2 [M-H]⁻; white solid. Yield = 97%.

General procedure for Suzuki synthesis of derivatives (**101-103**)

To a solution of compound **100** (250 mg, 1 eq, 0.69 mmol) and corresponding boronic acid or ester derivative (1.5 eq, 1.04 mmol) in dry THF (8 mL) were added an aqueous solution of 1M Na₂CO₃ (3 eq, 2.09 mmol, 2 mL) and bis(triphenylphosphine)palladium (II) (48 mg, 0.1 eq, 0.06 mmol). The resulting mixture was stirred at 70°C under argon atmosphere during 1 h, cooled and then concentrated. The resulting residue was diluted in ethyl acetate, washed twice with 1N HCl, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 98:2).

methyl (2S)-2-[[tert-butoxy]carbonyl]amino}-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanoate (**101**)

¹H NMR (CDCl₃, 400 MHz): δ = 7.41 (dd, *J* = 8.2 Hz and 1.6 Hz, 2H), 7.36 (dd, *J* = 7.5 Hz and 2.1 Hz, 1H), 7.26 – 7.21 (m, 1H), 7.13 (bd, *J* = 8.0 Hz, 2H), 7.06 (dd, *J* = 10.4 Hz and 8.4 Hz, 1H), 4.95 (bd, *J* = 7.5 Hz, 1H), 4.64 (s, 2H), 4.59 – 4.50 (m, 1H), 3.67 (s, 3H), 3.10 (dd, *J* = 13.7 Hz and 5.7 Hz, 1H), 3.02 (dd, *J* = 13.7 Hz and 6.0 Hz, 1H), 1.35 (s, 9H). ¹³C NMR (CDCl₃, 101 MHz) δ = 172.33, 160.43 (d, $^1J_{CF}$ = 247.7 Hz), 156.54, 136.97 (d, $^4J_{CF}$ = 3.5 Hz), 135.66, 134.37, 129.45, 129.39 (d, $^4J_{CF}$ = 3.7 Hz), 129.14, 128.65 (d, $^2J_{CF}$ = 13.8 Hz), 128.57 (d, $^3J_{CF}$ = 12.0 Hz), 127.59 (d, $^3J_{CF}$ = 8.4 Hz), 116.24 (d, $^2J_{CF}$ = 23.3 Hz), 80.04, 64.67, 54.37, 52.30, 38.08, 28.31; LC/MS (ESI): 304.2 [M+H]⁺; yellow solid. Yield = 31%.

methyl (2S)-2-[[tert-butoxy]carbonyl]amino}-3-[4'-acetamido-[1,1'-biphenyl]-4-yl]propanoate (**102**)

¹H NMR (CD₃OD, 400 MHz): δ = 7.47 – 7.41 (m, 6H), 7.17 (d, *J* = 8.0 Hz, 2H), 4.29 (dd, *J* = 8.7 Hz and 5.7 Hz, 1H), 3.61 (s, 3H), 3.04 (dd, *J* = 13.7 Hz and 5.6 Hz, 1H), 2.84 (dd, *J* = 13.6 Hz and 9.0 Hz, 1H), 2.04 (s, 3H), 1.28 (s, 9H). ¹³C NMR (CD₃OD, 101 MHz) δ = 172.35, 168.30, 155.11, 132.19, 132.09, 129.77, 128.64, 128.52, 127.49, 126.95, 120.15, 77.23, 54.38, 52.28, 37.94, 28.31, 24.64; LC/MS (ESI): 313.2 [M+H]⁺; yellow solid. Yield = 76%.

methyl (2S)-2-[[tert-butoxy]carbonyl]amino-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanoate (103)

¹H NMR (CDCl₃, 400 MHz): δ = 7.99 (dd, *J* = 7.5 Hz and 2.3 Hz, 1H), 7.87 (ddd, *J* = 8.5 Hz, 4.7 Hz and 2.3 Hz, 1H), 7.43 (dd, *J* = 8.2 Hz and 1.5 Hz, 2H), 7.18 – 7.12 (m, 3H), 4.97 (bd, *J* = 8.3 Hz, 1H), 4.57 (d, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 3.12 (dd, *J* = 13.7 Hz and 5.6 Hz, 1H), 3.03 (dd, *J* = 13.7 Hz and 6.1 Hz, 1H), 2.55 (s, 3H), 1.36 (s, 9H). ¹³C NMR (CDCl₃, 101 MHz) δ = 197.49, 172.68, 162.67 (d, ¹*J*_{CF} = 255.0 Hz), 156.42, 137.36, 133.82 (d, ⁴*J*_{CF} = 3.2 Hz), 133.20, 131.14 (d, ³*J*_{CF} = 4.9 Hz), 129.56 (d, ³*J*_{CF} = 9.7 Hz), 129.26, 129.04 (d, ²*J*_{CF} = 14.5 Hz), 128.67 (d, ⁴*J*_{CF} = 2.5 Hz), 116.10 (d, ²*J*_{CF} = 24.1 Hz), 79.26, 55.03, 51.28, 37.04, 27.25, 25.31; LC/MS (ESI): 316.2 [M+H]⁺; yellow solid. Yield = 99%.

General procedure for the synthesis of ester amino acid derivatives (104-106)

To a solution of the corresponding Boc-protected amine **101-103** (1eq) in dichloromethane (3 mL) was slowly TFA (5 eq) at 0°C. The resulting mixture was stirred at room temperature during 4 h. Then, a solution of saturated NaHCO₃ was added dropwise until pH 7 and the aqueous layer was extracted twice with dichloromethane. The combining organic layer was washed once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (98:2 to 9:1).

methyl (2S)-2-amino-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanoate (104)

¹H NMR (CD₃OD, 400 MHz): δ = 7.48 (dd, *J* = 8.2 Hz and 1.5 Hz, 1H), 7.37 (dd, *J* = 6.5 Hz and 3.3 Hz, 1H), 7.33-7.16 m, 4H), 7.06 (dd, *J* = 10.7 Hz and 8.5 Hz, 1H), 4.53 (s, 2H), 4.27 (dd, *J* = 7.5 Hz and 6.0 Hz, 1H), 3.74 (s, 3H), 3.24 (dd, *J* = 14.2 Hz and 6.6 Hz, 1H), 3.11 (dd, *J* = 14.4 Hz and 7.6 Hz, 1H). ¹³C NMR (CD₃OD, 101 MHz) δ = 169.31, 158.90 (d, ¹*J*_{CF} = 245.5 Hz), 137.92 (d, ⁴*J*_{CF} = 3.2 Hz), 135.48, 133.57, 129.34 (d, ⁴*J*_{CF} = 3.1 Hz), 129.19, 128.98, 128.90 (d, ⁴*J*_{CF} = 3.5 Hz), 127.96 (d, ²*J*_{CF} = 13.7 Hz), 127.68 (d, ³*J*_{CF} = 8.4 Hz), 115.53 (d, ²*J*_{CF} = 23.4 Hz), 63.00, 53.78, 52.22, 35.89; LC/MS (ESI): 304.2 [M+H]⁺; yellow oil. Yield = 98%.

methyl (2S)-2-amino-3-[4'-acetamido-[1,1'-biphenyl]-4-yl]propanoate (105)

¹H NMR (CD₃OD, 400 MHz): δ = 7.52 (d, *J* = 8.7 Hz, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 3.65 (t, *J* = 6.5 Hz, 1H), 3.59 (s, 3H), 2.95 (dd, *J* = 13.5 Hz and 6.2 Hz, 1H), 2.86 (dd, *J* = 13.5 Hz and 6.9 Hz, 1H), 2.04 (s, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 174.84, 170.25, 139.05, 137.85, 136.36, 135.78, 129.46, 126.70, 126.40, 120.03, 55.24, 50.99, 39.89, 22.42; LC/MS (ESI): 311.2 [M-H]⁻; 313.2 [M+H]⁺; white solid. Yield = 34%.

methyl (2S)-2-amino-3-[4-(2-methyl-3-oxo-2,3-dihydro-1H-isoindol-5-yl)phenyl]propanoate (106)

¹H NMR (CD₃OD, 400 MHz): δ = 7.99 (dd, *J* = 7.6 Hz and 2.3 Hz, 1H), 7.93-7.89 (m, 1H), 7.42 (dd, *J* = 8.2 Hz and 1.6 Hz, 2H), 7.23 – 7.17 (m, 3H), 3.71 – 3.66 (m, 1H), 3.60 (s, 3H), 2.99 (dd, *J* = 13.5 Hz and 6.2 Hz, 1H), 2.89 (dd, *J* = 13.5 Hz and 7.0 Hz, 1H), 2.52 (s, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 197.49, 174.66, 162.66 (d, ¹*J*_{CF} = 254.9 Hz), 137.21, 133.84 (d, ⁴*J*_{CF} = 3.4 Hz), 133.31, 131.10 (d, ³*J*_{CF} = 5.0 Hz), 129.74, 129.61 (d, ³*J*_{CF} = 9.9 Hz), 129.32, 129.10 (d, ²*J*_{CF} = 13.2 Hz), 128.81 (d, ⁴*J*_{CF} = 2.9 Hz), 116.11 (d, ²*J*_{CF} = 24.0 Hz), 55.19, 51.06, 39.89, 25.31; LC/MS (ESI): 316.2 [M+H]⁺; yellow oil. Yield = 98%.

General procedure for the synthesis of isoindolinone intermediates (107-109) and (123)

To a stirred solution of the commercial methyl 4-bromo-2-(bromomethyl)benzoate **81** (1 eq) and the corresponding *L*-amino acid methyl ester derivative **104-106** or methyl (2S)-2-amino-3-(naphthalen-2-yl)propanoate hydrochloride (1.2 eq) in dry DMF (3 mL), *N,N*-diisopropylethylamine (3 eq) was added. The mixture was stirred under argon atmosphere during 3h-5h at 80°C, cooled and then concentrated. The resulting residue was diluted in ethyl acetate, washed twice with 1N HCl, once with water, once with brine, dried over Na₂SO₄, and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 98:2).

methyl (2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanoate (107)

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 7.65 (bd, *J* = 0.7 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.33 (dd, *J* = 8.2 Hz and 1.6 Hz, 2H), 7.28 (dd, *J* = 7.6 Hz and 2.2 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.21 – 7.17 (m, 1H), 7.00 (dd, *J* = 10.7 Hz and 8.4 Hz, 1H), 5.21 (dd, *J* = 11.0 Hz and 5.2 Hz, 1H), 4.48 (s, 2H), 4.42 (d, *J* = 3.9 Hz, 2H), 3.67 (s, 3H), 3.45 (dd, *J* = 14.6 Hz and 5.2 Hz, 1H), 3.25 – 3.17 (m, 1H). ¹³C NMR (DMSO-*d*₆, 101 MHz): δ = 170.67, 168.75, 158.85 (d, ¹*J*_{CF} = 246.0 Hz), 144.12, 137.76 (d, ⁴*J*_{CF} = 3.6 Hz), 136.31, 134.45, 131.30, 130.29, 128.95 (d, ⁴*J*_{CF} = 3.6 Hz), 128.85 (d, ⁴*J*_{CF} = 3.0 Hz), 128.58 (d, ³*J*_{CF} = 12.4 Hz), 128.49, 128.09 (d, ²*J*_{CF} = 13.7 Hz), 127.40 (d, ³*J*_{CF} = 8.4 Hz), 126.42, 126.39, 124.50, 115.44 (d, ²*J*_{CF} = 23.4 Hz), 63.03, 60.13, 55.55, 51.69, 34.63; LC/MS (ESI): 498.1/ 500.1 [M+H]⁺; yellow oil. Yield = 35%.

methyl (2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[4'-acetamido-[1,1'-biphenyl]-4-yl]propanoate (108)

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 7.85 (bd, *J* = 1.0 Hz, 1H), 7.68 – 7.55 (m, 6H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 5.25 (dd, *J* = 10.9 Hz and 5.2 Hz, 1H), 4.54 (d, *J* = 17.9 Hz, 1H), 4.43 (d, *J* = 17.9 Hz, 1H), 3.69 (s, 3H), 3.41 (dd, *J* = 14.5 Hz and

5.1 Hz, 1H), 3.25 (dd, $J = 14.5$ Hz and 11.0 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (DMSO- d_6 , 101 MHz): $\delta = 171.04, 168.67, 167.29, 144.58, 139.08, 138.39, 136.18, 134.53, 131.71, 130.99, 129.64, 127.33, 127.11, 126.58, 125.97, 125.36, 119.60, 55.33, 52.90, 47.70, 34.64, 24.45$; LC/MS (ESI): 507.0/509.1 [M+H] $^+$; white solid. Yield = 76%.

methyl (2S)-3-{5'-acetyl-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (109)

^1H NMR (CDCl $_3$, 400 MHz): $\delta = 7.94$ (dd, $J = 7.5$ Hz and 2.3 Hz, 1H), 7.84 (ddd, $J = 8.5$ Hz, 4.7 Hz and 2.3 Hz, 1H), 7.62 (d, $J = 8.7$ Hz, 1H), 7.52 – 7.49 (m, 2H), 7.39 (dd, $J = 8.1$ Hz and 1.4 Hz, 2H), 7.25 (d, $J = 8.2$ Hz, 2H), 7.12 (dd, $J = 10.0$ Hz and 8.6 Hz, 1H), 5.37 (dd, $J = 10.6$ Hz and 5.6 Hz, 1H), 4.49 (d, $J = 16.7$ Hz, 1H), 4.26 (d, $J = 16.7$ Hz, 1H), 3.69 (s, 3H), 3.49 (dd, $J = 14.9$ Hz and 5.6 Hz, 1H), 3.17 (dd, $J = 14.8$ Hz and 10.7 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (CDCl $_3$, 101 MHz): $\delta = 196.55, 171.05, 168.09, 162.66$ (d, $^1J_{\text{CF}} = 256.5$ Hz), 143.36, 136.31, 133.79 (d, $^4J_{\text{CF}} = 3.5$ Hz), 133.51, 131.58, 131.41 (d, $^3J_{\text{CF}} = 5.0$ Hz), 130.78, 129.70 (d, $^2J_{\text{CF}} = 18.3$ Hz), 129.38 (d, $^4J_{\text{CF}} = 2.9$ Hz), 128.79, 128.73, 128.52 (d, $^3J_{\text{CF}} = 12.0$ Hz), 126.51, 126.30, 125.41, 116.47 (d, $^2J_{\text{CF}} = 23.8$ Hz), 54.51, 52.62, 47.12, 35.46, 26.61; LC/MS (ESI): 542.1/ 544.1[M+H] $^+$; yellow solid. Yield = 27%.

methyl (2S)-3-(naphthalen-2-yl)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoate (119)

^1H NMR (CDCl $_3$, 400 MHz): $\delta = 7.71 - 7.64$ (m, 3H), 7.59 – 7.56 (m, 2H), 7.48 – 7.45 (m, 2H), 7.38 – 7.33 (m, 2H), 7.29 (dd, $J = 8.4$ Hz and 1.8 Hz, 1H), 5.44 (dd, $J = 10.5$ Hz and 5.7 Hz, 1H), 4.47 (d, $J = 16.7$ Hz, 1H), 4.25 (d, $J = 16.7$ Hz, 1H), 3.67 (s, 3H), 3.60 (dd, $J = 14.8$ Hz and 5.7 Hz, 1H), 3.27 (dd, $J = 14.8$ Hz and 10.6 Hz, 1H). ^{13}C NMR (CDCl $_3$, 101 MHz): $\delta = 171.20, 168.04, 143.36, 133.64, 133.45, 132.44, 131.48, 130.79, 128.58, 127.65, 127.59, 127.20, 126.44, 126.39, 126.23, 126.21, 125.80, 125.35, 54.58, 52.57, 47.15, 35.98$; LC/MS (ESI): not determined; yellow solid. Yield = 37%.

General procedure for the synthesis of carboxylic acid intermediates (110-112, 120) and derivatives (116-118 or SYNTi) and (122)

To a stirred solution of ester intermediate derivative (**107-109, 113-115, 119** and **121**) (1eq) in THF (2 ml) at 0°C, aqueous solution of LiOH (4 eq, 1 ml) was added dropwise. The resulting mixture was stirred 1 h at room temperature. After completion, THF was removed in vacuo. Then, 1N HCl solution was added dropwise until pH 2-3 and the aqueous layer was extracted with ethyl acetate. The organic layer was washed once with water, once with brine, dried over Na $_2$ SO $_4$, and evaporated in vacuo to afford a pure compound (**110-112, 116-118, 120** and **122**).

(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanoic acid (110)

^1H NMR (CD $_3$ OD, 400 MHz): $\delta = 7.77$ (bs, 1H), 7.66 – 7.62 (m, 2H), 7.62-7.55 (m, 1H), 7.45 (dd, $J = 8.2$ Hz and 1.4 Hz, 2H), 7.40 (dd, $J = 7.6$ Hz and 2.1 Hz, 1H), 7.36 (d, $J = 8.2$ Hz, 2H), 7.12 (dd, $J = 10.7$ Hz and 8.4 Hz, 1H), 5.33 (d, $J = 11.4$ Hz and 4.8 Hz, 1H), 4.61 (d, $J = 17.7$ Hz, 1H), 4.60 (s, 2H), 4.54 (d, $J = 17.6$ Hz, 1H), 3.60 (dd, $J = 14.7$ Hz and 4.8 Hz, 1H), 3.39 – 3.28 (m, 1H). ^{13}C NMR (CD $_3$ OD, 101 MHz) $\delta = 171.82, 168.89, 158.85$ (d, $^1J_{\text{CF}} = 245.9$ Hz), 144.13, 137.73 (d, $^4J_{\text{CF}} = 3.5$ Hz), 136.62, 134.35, 131.23, 130.41, 128.96 (d, $^4J_{\text{CF}} = 3.5$ Hz), 128.82 (d, $^4J_{\text{CF}} = 2.9$ Hz), 128.12 (d, $^3J_{\text{CF}} = 13.7$ Hz), 128.42, 128.12 (d, $^2J_{\text{CF}} = 13.7$ Hz), 127.37 (d, $^3J_{\text{CF}} = 8.6$ Hz), 126.38, 126.30, 124.46, 115.44 (d, $^2J_{\text{CF}} = 23.4$ Hz), 63.04, 55.36, 51.70, 34.67; LC/MS (ESI): 482.1/484.0 [M-H] $^-$, 484.1/486.1 [M+H] $^+$; white solid. Yield = 75%.

(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[4'-acetamido-[1,1'-biphenyl]-4-yl]propanoic acid (111)

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 13.23$ (bs, 1H), 9.99 (s, 1H), 7.85 (s, 1H), 7.66 (dd, $J = 8.1$ Hz and 1.3 Hz, 1H), 7.62 (bd, $J = 8.6$ Hz, 2H), 7.59 – 7.54 (m, 3H), 7.52 (bd, $J = 8.2$ Hz, 2H), 7.31 (bd, $J = 8.2$ Hz, 2H), 5.16 (dd, $J = 11.3$ Hz and 4.7 Hz, 1H), 4.52 (d, $J = 18.5$ Hz, 1H), 4.47 (d, $J = 18.4$ Hz, 1H), 3.42 (dd, $J = 14.7$ Hz and 4.7 Hz, 1H), 3.23 (dd, $J = 14.6$ Hz and 11.4 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (DMSO- d_6 , 101 MHz) $\delta = 172.21, 168.75, 167.42, 144.62, 139.16, 138.30, 136.64, 134.58, 131.63, 131.23, 129.55, 127.28, 127.10, 126.57, 125.82, 125.29, 119.69, 55.36, 47.58, 34.71, 24.50$; LC/MS (ESI): 491.1/493.1 [M-H] $^-$, 493.1/495.1 [M+H] $^+$; white solid. Yield = 54%.

(2S)-3-{5'-acetyl-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (112)

^1H NMR (CD $_3$ OD, 400 MHz): $\delta = 7.94$ (dd, $J = 7.6$ Hz and 2.3 Hz, 1H), 7.89 (ddd, $J = 8.5$ Hz, 4.7 Hz and 2.3 Hz, 1H), 7.66 (bs, 1H), 7.57 – 7.49 (m, 2H), 7.37 (d, $J = 8.1$ Hz and 1.3 Hz, 2H), 7.29 (d, $J = 8.2$ Hz, 2H), 7.17 (dd, $J = 10.3$ Hz and 8.6 Hz, 1H), 5.22 (dd, $J = 11.4, 4.8$ Hz, 1H), 4.50 (d, $J = 17.6$ Hz, 1H), 4.43 (d, $J = 17.7$ Hz, 1H), 3.50 (dd, $J = 14.7$ Hz and 4.8 Hz, 1H), 3.28 – 3.23 (m, 1H), 2.50 (s, 3H). ^{13}C NMR (CD $_3$ OD, 101 MHz) $\delta = 197.51, 171.77, 168.88, 162.60$ (d, $^1J_{\text{CF}} = 255.1$ Hz), 144.13, 137.29, 133.79 (d, $^4J_{\text{CF}} = 3.5$ Hz), 133.29, 131.24, 131.15 (d, $^3J_{\text{CF}} = 5.0$ Hz), 130.41, 129.52 (d, $^3J_{\text{CF}} = 9.7$ Hz), 128.88 (d, $^4J_{\text{CF}} = 2.9$ Hz), 128.73, 128.63, 128.52, 126.38, 126.30, 124.47, 116.07 (d, $^2J_{\text{CF}} = 24.0$ Hz), 55.32, 34.68, 29.49, 25.29; LC/MS (ESI): 493.9/495.9 [M-H] $^-$, 496.0/498.1 [M+H] $^+$; beige solid. Yield = 55%.

(2S)-2-[(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanamido]propanoic acid (116)

^1H NMR (CD $_3$ OD, 400 MHz): $\delta = 7.66$ (bs, 1H), 7.52 – 7.50 (m, 2H), 7.49 – 7.43 (m, 1H), 7.34 – 7.31 (m, 2H), 7.26 (bd, $J = 8.3$ Hz, 2H), 7.22 – 7.17 (m, 1H), 7.00 (dd, $J = 10.7$ Hz and 8.4 Hz, 1H), 5.24 (dd, $J = 10.6$ Hz and 5.4 Hz, 1H), 4.68 (d, $J = 18.1$ Hz, 1H), 4.49 (d, $J = 18.0$ Hz, 1H), 4.48 (s, 2H), 4.28 (q, $J = 7.2$ Hz, 1H), 3.39 (dd, $J = 14.7$ Hz and 5.4 Hz, 1H), 3.16 (dd, $J = 10.3$ Hz and 4.4

Hz, 1H), 1.31 (d, $J = 7.4$ Hz, 3H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 174.51, 170.54, 168.67, 158.86$ (d, $^1J_{\text{CF}} = 245.6$ Hz), 144.33, 137.71 (d, $^4J_{\text{CF}} = 3.5$ Hz), 136.38, 134.33, 131.15, 130.42, 128.98 (d, $^4J_{\text{CF}} = 3.5$ Hz), 128.80 (d, $^4J_{\text{CF}} = 3.0$ Hz), 128.64, 128.54, 128.18 (d, $^2J_{\text{CF}} = 13.8$ Hz), 127.36 (d, $^3J_{\text{CF}} = 8.4$ Hz), 126.36, 126.24, 124.41, 115.43 (d, $^2J_{\text{CF}} = 23.5$ Hz), 63.05, 55.64, 48.09, 47.88, 35.12, 16.16; LC/MS (ESI): 553.1/555.1 [M-H]⁻; 555.1/557.1 [M+H]⁺; HRMS (TOF, ESI+) calcd for $\text{C}_{27}\text{H}_{25}\text{BrFN}_2\text{O}_5$ [M+H]⁺ 555.0925, found 555.0908; white solid. Yield = 78%.

(2S)-2-[(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-{4'-acetamido-[1,1'-biphenyl]-4-yl}propanamido]propanoic acid (117)

^1H NMR (CD_3OD , 400 MHz): $\delta = 7.66$ (bs, 1H), 7.53 – 7.45 (m, 4H), 7.42 – 7.36 (m, 4H), 7.23 (d, $J = 8.2$ Hz, 2H), 5.22 (dd, $J = 10.7$ Hz and 5.3 Hz, 1H), 4.68 (d, $J = 17.9$ Hz, 1H), 4.49 (d, $J = 18.0$ Hz, 1H), 4.27 (q, $J = 7.2$ Hz, 1H), 3.37 (dd, $J = 14.6$ Hz and 5.2 Hz, 1H), 3.13 (dd, $J = 14.8$ Hz and 4.2 Hz, 1H), 2.02 (s, 3H), 1.30 (d, $J = 7.3$ Hz, 3H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 170.54, 170.23, 168.65, 144.32, 139.00, 137.79, 136.26, 135.61, 131.15, 130.44, 128.88, 126.68, 126.44, 126.36, 126.23, 124.40, 119.97, 55.70, 48.09, 47.87, 35.04, 22.40, 16.22$; LC/MS (ESI): 562.1/564.1 [M-H]⁻; 564.1/566.0 [M+H]⁺; HRMS (TOF, ESI+) calcd for $\text{C}_{28}\text{H}_{27}\text{BrN}_3\text{O}_5$ [M+H]⁺ 564.1129, found 564.1121; white solid. Yield = 55%.

(2S)-2-[(2S)-3-{5'-acetyl-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (118 or SYNTI)

^1H NMR (CD_3OD , 400 MHz): $\delta = 7.94$ (dd, $J = 7.5$ Hz and 2.3 Hz, 1H), 7.89 (ddd, $J = 8.5$ Hz, 4.7 Hz and 2.3 Hz, 1H), 7.66 (bs, 1H), 7.55 – 7.50 (m, 2H), 7.37 – 7.35 (m, 2H), 7.31 (bd, $J = 8.3$ Hz, 2H), 7.17 (dd, $J = 10.3$ Hz and 8.6 Hz, 1H), 5.26 (dd, $J = 10.6$ Hz and 5.4 Hz, 1H), 4.70 (d, $J = 18.1$ Hz, 1H), 4.50 (d, $J = 18.0$ Hz, 1H), 4.29 (q, $J = 7.3$ Hz, 1H), 3.40 (dd, $J = 14.7$ Hz and 5.4 Hz, 1H), 3.19 – 3.14 (m, 1H), 2.50 (s, 3H), 1.31 (d, $J = 7.3$ Hz, 3H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 197.55, 170.27, 168.65, 162.62$ (d, $^1J_{\text{CF}} = 255.0$ Hz), 144.32, 137.12, 133.79 (d, $^4J_{\text{CF}} = 3.2$ Hz), 133.25, 131.23, 131.16, 130.44, 129.47 (d, $^3J_{\text{CF}} = 9.8$ Hz), 128.85 (d, $^4J_{\text{CF}} = 2.8$ Hz), 128.80, 128.76, 128.58 (d, $^2J_{\text{CF}} = 12.2$ Hz), 126.38, 126.24, 124.42, 116.06 (d, $^2J_{\text{CF}} = 24.0$ Hz), 55.71, 48.44, 47.87, 35.08, 25.29, 16.44; LC/MS (ESI): 565.1/567.1 [M-H]⁻; 567.1/569.1 [M+H]⁺; HRMS (TOF, ESI+) calcd for $\text{C}_{28}\text{H}_{25}\text{BrFN}_2\text{O}_5$ [M+H]⁺ 567.0925, found 567.0923; white solid. Yield = 73%.

(2S)-3-(naphthalen-2-yl)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanoic acid (120)

^1H NMR (CD_3OD , 400 MHz): $\delta = 7.79$ – 7.70 (m, 5H), 7.61 (dd, $J = 8.2$ Hz and 1.6 Hz, 1H), 7.56 (bd, $J = 8.0$ Hz, 1H), 7.45 – 7.38 (m, 3H), 5.39 (dd, $J = 11.4$ Hz and 4.8 Hz, 1H), 4.60 (d, $J = 17.8$ Hz, 1H), 4.54 (d, $J = 17.7$ Hz, 1H), 3.71 (dd, $J = 14.7$ Hz and 4.8 Hz, 1H), 3.44 (dd, $J = 14.7$ Hz and 11.4 Hz, 1H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 172.12, 168.82, 144.09, 134.60, 133.56, 132.46, 131.14, 130.40, 127.90, 127.17, 127.10, 126.89, 126.36, 126.28, 126.19, 125.71, 125.27, 124.38, 55.55, 47.48, 35.24$; LC/MS (ESI): 408.0/410.1 [M-H]⁻; 410.1/412.1 [M+H]⁺; white solid. Yield = 76%.

(2S)-2-[(2S)-3-(naphthalen-2-yl)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoic acid (122)

^1H NMR (CD_3OD , 400 MHz): $\delta = 7.79$ – 7.70 (m, 5H), 7.62 – 7.58 (m, 2H), 7.34 – 7.25 (m, 3H), 5.43 (dd, $J = 10.6$ Hz and 5.4 Hz, 1H), 4.79 (d, $J = 18.0$ Hz, 1H), 4.64 (d, $J = 18.1$ Hz, 1H), 4.41 (q, $J = 7.3$ Hz, 1H), 3.62 (dd, $J = 14.7$ Hz and 5.3 Hz, 1H), 3.40 (dd, $J = 14.7$ Hz and 10.6 Hz, 1H), 1.43 (d, $J = 7.3$ Hz, 3H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 174.33, 170.62, 168.62, 144.26, 134.26, 133.56, 132.47, 131.09, 130.35, 127.87, 127.16, 127.14, 127.02, 126.51, 126.29, 126.18, 125.67, 125.26, 124.34, 55.64, 48.16, 47.88, 35.60, 16.06$; LC/MS (ESI): 481.1/483.1 [M+H]⁺; HRMS (TOF, ESI+) calcd for $\text{C}_{24}\text{H}_{22}\text{BrN}_2\text{O}_4$ [M+H]⁺ 481.0757, found 481.0757; white solid. Yield = 97%.

General procedure for the synthesis of methyl ester intermediates (113-115 and 121)

To a stirred solution of *L*-alanine methyl ester hydrochloride (1 eq) and the corresponding carboxylic acid derivative (**110-112** and **120**) (1 eq) in a mixture of dry dichloromethane/DMF (2 mL/0.5 mL), *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (2 eq) and triethylamine (2 eq) were added. The mixture was stirred under argon atmosphere during 8-18 h at room temperature. After completion, the resulting mixture was washed twice with 1N HCl solution, once with water, once with brine, dried over Na_2SO_4 , and evaporated in vacuo. Crude was purified by column chromatography eluting with dichloromethane-methanol (100 to 98:2).

methyl (2S)-2-[(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-[2'-fluoro-5'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl]propanamido]propanoate (113)

^1H NMR (CD_3OD , 400 MHz): $\delta = 7.66$ (bs, 1H), 7.53 – 7.50 (m, 2H), 7.49 – 7.42 (m, 1H), 7.34 (dd, $J = 8.2$ Hz and 1.4 Hz, 2H), 7.27 (d, $J = 8.2$ Hz, 2H), 7.22 – 7.17 (m, 1H), 7.01 (dd, $J = 10.7$ Hz and 8.4 Hz, 1H), 5.23 (dd, $J = 10.0$ Hz and 6.0 Hz, 1H), 4.67 (d, $J = 18.0$ Hz, 1H), 4.50 (d, $J = 17.8$ Hz, 1H), 4.49 (s, 2H), 4.30 (q, $J = 7.3$ Hz, 1H), 3.58 (s, 3H), 3.37 (dd, $J = 14.5$ Hz and 6.0 Hz, 1H), 3.14 (dd, $J = 14.5$ Hz and 10.1 Hz, 1H), 1.28 (d, $J = 7.3$ Hz, 3H). ^{13}C NMR (CD_3OD , 101 MHz) $\delta = 172.95, 170.55, 168.58, 158.86$ (d, $^1J_{\text{CF}} = 245.7$ Hz), 144.32, 137.75 (d, $^4J_{\text{CF}} = 3.4$ Hz), 136.27, 134.35, 131.17, 130.41, 128.96 (d, $^4J_{\text{CF}} = 3.5$ Hz), 128.80 (d, $^4J_{\text{CF}} = 3.0$ Hz), 128.62, 128.52, 128.16 (d, $^2J_{\text{CF}} = 13.8$ Hz), 127.37 (d, $^3J_{\text{CF}} = 8.5$ Hz), 126.37, 126.26, 124.41, 115.45 (d, $^2J_{\text{CF}} = 23.5$ Hz), 63.04, 55.55, 51.38, 48.09, 47.88, 35.23, 15.81; LC/MS (ESI): 569.1/571.1 [M+H]⁺; yellow solid. Yield = 30%.

methyl (2S)-2-[(2S)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)-3-{4'-acetamido-[1,1'-biphenyl]-4-yl}propanamido]propanoate (114)

¹H NMR (CD₃OD, 400 MHz): δ = 7.66 (bs, 1H), 7.58 – 7.43 (m, 6H), 7.42 – 7.36 (m, 3H), 7.24 (bd, *J* = 8.2 Hz, 2H), 5.22 (dd, *J* = 10.2 Hz and 5.8 Hz, 1H), 4.68 (d, *J* = 18.0 Hz, 1H), 4.50 (d, *J* = 18.0 Hz, 1H), 4.30 (q, *J* = 7.3 Hz, 1H), 3.58 (s, 3H), 3.35 (dd, *J* = 14.6 Hz and 6.0 Hz, 1H), 3.12 (dd, *J* = 14.6 Hz and 10.2 Hz, 1H), 2.03 (s, 3H), 1.28 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD, 101 MHz) δ = 172.95, 170.62, 170.26, 168.59, 144.33, 139.00, 137.80, 136.24, 135.50, 131.17, 130.42, 128.96, 126.67, 126.44, 126.37, 126.25, 124.40, 120.01, 55.60, 51.38, 48.09, 47.88, 35.14, 22.40, 15.81; LC/MS (ESI): 578.2/580.2 [M+H]⁺; white solid. Yield = 43%.

methyl (2S)-2-[(2S)-3-{5'-acetyl-2'-fluoro-[1,1'-biphenyl]-4-yl}-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (115)

¹H NMR (CDCl₃, 400 MHz): δ = 7.94 (dd, *J* = 7.5 Hz and 2.3 Hz, 1H), 7.84 (ddd, *J* = 8.5 Hz, 4.7 Hz and 2.3 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.53 (bs, 1H), 7.39 (dd, *J* = 8.1 Hz and 1.4 Hz, 2H), 7.27 (bd, *J* = 8.2 Hz, 2H), 7.13 (dd, *J* = 10.0 Hz and 8.6 Hz, 1H), 6.72 (bd, *J* = 7.2 Hz, 1H), 5.13 (dd, *J* = 8.9 Hz and 7.1 Hz, 1H), 4.51 (d, *J* = 17.3 Hz, 1H), 4.41 (p, *J* = 7.1 Hz, 1H), 4.36 (d, *J* = 17.4 Hz, 1H), 3.62 (s, 3H), 3.41 (dd, *J* = 14.9 Hz and 6.7 Hz, 1H), 3.20 (dd, *J* = 14.6 Hz and 9.0 Hz, 1H), 2.53 (s, 3H), 1.28 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz) δ = 196.54, 172.63, 169.00, 168.25, 162.66 (d, ¹*J*_{CF} = 256.5 Hz), 143.41, 136.45, 133.80 (d, ⁴*J*_{CF} = 3.2 Hz), 133.43, 131.67, 131.42 (d, ³*J*_{CF} = 4.9 Hz), 130.74, 129.55 (d, ³*J*_{CF} = 9.5 Hz), 129.36 (d, ⁴*J*_{CF} = 2.9 Hz), 128.98, 128.86, 128.54 (d, ²*J*_{CF} = 12.1 Hz), 126.69, 126.38, 125.29, 116.47 (d, ²*J*_{CF} = 23.8 Hz), 56.08, 52.50, 48.30, 47.47, 34.84, 26.61, 18.05; LC/MS (ESI): 580.9/583.4 [M+H]⁺; yellow solid. Yield = 50%.

methyl (2S)-2-[(2S)-3-(naphthalen-2-yl)-2-(5-bromo-1-oxo-2,3-dihydro-1H-isoindol-2-yl)propanamido]propanoate (121)

¹H NMR (CDCl₃, 400 MHz): δ = 7.72 – 7.64 (m, 3H), 7.62 – 7.58 (m, 2H), 7.53 – 7.48 (m, 2H), 7.38 – 7.33 (m, 2H), 7.31 (dd, *J* = 8.4 Hz and 1.7 Hz, 1H), 6.65 (bd, *J* = 6.9 Hz, 1H), 5.18 (dd, *J* = 8.7 Hz and 7.4 Hz, 1H), 4.52 (d, *J* = 17.3 Hz, 1H), 4.43 – 4.34 (m, 2H), 3.55 – 3.47 (m, 1H), 3.5 (s, 3H), 3.30 (dd, *J* = 14.4 Hz and 8.9 Hz, 1H), 1.26 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 101 MHz) δ = 172.53, 169.11, 168.22, 143.44, 133.74, 133.49, 132.42, 131.59, 130.76, 128.53, 127.62 (2C), 127.47, 126.75, 126.59, 126.33, 126.16, 125.76, 125.23, 56.27, 52.38, 48.27, 47.56, 35.42, 18.07; LC/MS (ESI): 493.0/495.1 [M-H]⁻; white solid. Yield = 98%.

Virtual screening for hit identification stage

The virtual screening workflow was based on a strategy previously described ⁶. It combines three robust computational approaches: Molecular Dynamic simulations (MDS), molecular docking and pharmacophore-based filtering. The workflow is depicted in Figure 1A. Briefly, molecular dynamics simulations in explicit aqueous solvent, starting from X-ray structures, were performed with the Charmm force-field to create a set of additional protein conformations for the subsequent ensemble molecular docking. Then, dozen millions output poses from molecular docking were filtered out using the pharmacophore concept to extract compounds able to mimic the binding mode of endogenous peptide binders.

Molecular dynamics simulations

MD simulations were performed starting from 4 PDB structures of the second PDZ-domain of syntenin: the apo form (1N99) and 3 structures with bound peptides (1V1T, 1W9E, 4Z33). Of note, bound peptide from 1W9E structure includes a large hydrophobic terminal residue that triggers slight conformational changes in the structure (endogenous syndecan ligands have an alanine residue as C-terminal moiety). Finally, two histidine tautomers (HSD and HSE) were considered for H208, leading to 8 independent MD simulations.

The CHARMM-GUI web services (<http://www.charmm-gui.org>) were used to generate inputs and scripts to run the MD simulations from the reference PDB structures. CHARMM version 33 was used to perform the MD simulations. Briefly, the system was solvated with a pre-equilibrated solvation box of TIP3P water molecules around the protein. Chloride and potassium ions were then added to neutralize the system. Finally, periodic boundary conditions were automatically applied based on the system shape and size. For each considered system, unfavorable contacts were first removed by a short energy minimization protocol (max 5000 minimization cycles), followed by equilibration and production simulations. Equilibration stage consisted of two small 0.5ns simulations (time step 1 fs) with then without constraints on the protein. Production stage consisted of 50 ns simulation (time step 2 fs) without any constraints. Snapshots of the coordinates were saved every 250 steps (0.5 ps) leading to 100000 instantaneous conformations for each MD trajectory. Trajectories were analyzed using the VMD tool using both built-in and in house Tcl scripts to verify their consistency (e.g.; convergence over time using RMSD criteria over all C-alpha atoms after superimposition on all frames in the same 3D referential).

Then, 1 frame was extracted every 10 frames for the subsequent pocket detection analysis using the MDpocket tool ¹⁷.

Molecular docking

The PDB files from the ensemble set were prepared using Sybyl-X. The binding site was defined as all residues with at least one atom within 10 Å radius from the reference bound peptide from 1V1T structure. Surflex (<https://www.jainlab.org/>) was

used as the docking engine and default values were used to generate the intermediate “protomol” (abstract definition of binding site features that was used by Surflex during the docking process). Redocking simulations of reference peptides (2 to 5 C-terminal residues) were beforehand performed to validate the docking protocol. This control study was used to determine the best docking parameters for the target. These reference ligands were successfully redocked into the PDZ domain of syntenin with good RMSD values: lower than 1 Å between crystallized conformation and predicted pose for short peptides; and less than 2 Å for long peptide versions). Importantly, key interactions between C-terminal carboxylate and carboxylate binding loop were accurately reproduced, in addition to known hydrogen bond interactions between both backbone chains. A collection of 479K molecules was created from the Clean ZINC database (<https://zinc.docking.org/>). First, 371K compounds were extracted from the Clean ZINC database after clustering-based search to promote structure diversity. Then, an additional subset of 108K compounds was added to enrich the original set with carboxylate-containing compounds. It was expected that carboxylate-containing compounds would mimic the C-terminal end of reference bound peptides. Finally, the set of 479K compounds was virtually screened over the ensemble of 9 conformations using Surflex¹⁸. A maximum of 10 poses were generated by the docking algorithm for each compound, leading to 43M output poses.

Pharmacophore-based filtering

The goal of this stage was to identify organic compounds able to mimic more or less strictly the binding mode of reference bound peptides. The Unity package from Sybyl-X was used as the pharmacophore screening engine.

It should be noted that the full strict pharmacophore filter, with all possible features mimicking the reference peptide, retrieved no compound. In practice, only reference peptides were fished out. Two pharmacophore filters were designed starting from reference X-ray structures. The first pharmacophore filter was short but strict and required the 3 canonical hydrogen bonds with the carboxylate binding loop (V209, G210, F211) and 2 additional hydrogen bonds with F211 and F213 backbone from syntenin. The second pharmacophore was a modified version of the full strict pharmacophore in which some features were now optional. For example, only 3 out of the 5 hydrogen bonds from canonical binding mode were required. One hydrophobic feature mimicking the hydrophobic moiety from C-terminal residue was also mandatory. Finally, one last hydrophobic feature was required in the vicinity of H208 or I212 or F213.

After the pharmacophore filter stage, 4k poses were extracted out of the 43M docking poses. This set was then refined by only considering docking poses with a score threshold better than “6”: It should be noted that some compounds were retrieved several times, as different poses of the same ligand matched the pharmacophore or they were successfully docked in different conformations from the docking ensemble. About 1k poses were finally considered and visually inspected leading to the final subset of 249 poses, of which 102 poses (41%) were identified from the 4 original X-ray conformations and 147 poses (59%) came from the MD-based conformations. These poses corresponded to 182 unique compounds.

Final selection of compounds to be tested

While compounds from ZINC database were perfectly defined from stereo-chemistry point of view, the commercially available compounds can be racemic mixtures. Therefore, compounds with more than one undefined stereocenter were discarded. In addition, several compounds were either very expensive or not available at the time of purchase. In the end, 86 compounds were purchased and evaluated experimentally using an HTRF assay as putative inhibitors of the syntenin-syndecan PPI interaction.

Molecular Modeling for hit-to-lead stage

H2L program relied on our previously reported DOTS strategy². Briefly, it requires the structure of the protein-hit complex (SBDD context) and consists in two main stages: library design followed by constrained docking. The role of the design stage is to generate accessible compounds that can be described as putative optimizations of the original compound. Then, the role of the docking stage is to prioritize the most promising compounds in order to catch additional interactions between the protein and the ligand.

Design of the focused libraries

The creation of the virtual focused libraries relied on the procedure described in the DOTS methodology². Briefly, a focused library was generated starting from an activated substructure, a collection of commercially available BBs and a set of in silico encoded chemistry reactions. This chemistry-driven strategy is expected to design accessible compounds with high reaction yields. Two diverse chemical libraries of accessible compounds were designed in this H2L context.

The first focused library was created by virtually encoding the Scheme 3. Commercially available residue-like BBs with protected carboxylate (ester function) were used to generate theoretically accessible compounds with varied sidechains. 1070 compounds were initially created, out of which 766 were kept after the filtering stage. The goal of the library was to explore the chemical space around compound **E5** (or **19**) by replacing its isopropyl group by other substituents.

Similarly, a second focused library was generated using the DOTS workflow to explore the chemical space from the *para*-position of compound **29** (*para*-Br analog, compound **84**) using a Suzuki reaction and compatible commercially available BBs. 3963 compounds were initially created, out of which 1,610 have been retained after the filtering stage. The goal of the library was to explore the chemical space around compound **29** by adding an additional ring with various decorations to fill up the nearby cavity and to catch additional interactions between the protein and the ligand.

The filtering stage consisted in several fully automated post-processing steps to extract a subset of duplicate-free compounds with a focus on structures with reasonable physicochemical properties. More precisely, a structural check was performed with StructureChecker from ChemAxon (<http://www.chemaxon.com>) to eliminate compounds with any structural warning. Duplicates and compounds that contain at least one undesired element (other than C, H, N, O, P, S, F, Cl, Br or I) were also discarded. Usual threshold values for common physico-chemical descriptors (molecular weight ≤ 550 , cLogP ≤ 5.5 , tPSA ≤ 150 Å², H-Bond acceptors ≤ 10 , H-Bond donors ≤ 5 , rotational bond count ≤ 14 , and formal charge count ≤ 2) were used to automatically extract a subset of compounds with reasonable properties. The remaining compounds were subjected to a post-processing: major microspecies, partial charges, force-field atomic types, and single 3D-conformer were computed for each compound. A maximum common substructure (MCS) algorithm was also used to flag atoms that were already present in the compound **19**. These atoms will be constrained in the subsequent virtual screening stage (see below). The mapping of force-field atomic types was done using programs from AmberTools (<http://ambermd.org>), while all the other steps were performed using in house tools relying on the ChemAxon Java API.

Constrained docking of the focused libraries

The virtual library of 2,376 compounds was screened into the Synt-PDZ2/**29** X-ray structure, using a constrained docking approach ², in order to select the best putative optimized compounds. The top 200 compounds were extracted on the basis of the force field-based interaction energy. Both water and water-free binding site variations of Synt-PDZ2/**29** structure were considered to either catch potential water-mediated hydrogen bonds or to virtually displace crystallographic waters.

The shared core from the original compound was constrained during the simulation to maintain the reference binding mode and to mimic the growing strategy from fragment-based drug discovery. The sampling stage mainly consisted in three independent simulations of 400 generations with a population of 30 individuals. All saved poses were merged into one file before switching to the post-processing stage that involved the minimization of all non-redundant poses while unlocking all ligand atoms. The energy used to rank all screened compounds was equal to the best energy of the complex minus the energy of the best conformer of the free ligand. The latter was obtained by performing three independent simulations with the same parameters on the ligand alone. The goal was to compute a potential energy difference between bound and free forms to estimate the interaction energy while considering the strain energy of the ligand.

SeeSAR-based ranking of top compounds from VS

This subset of the best molecules was ultimately refined using SeeSAR software (<http://biosolveit.de>). Briefly, this computer-aided design tool relies on the HYDE method ¹⁴ to evaluate affinity contributions to the binding for each ligand atom. SeeSAR was used in this context to reassess docking poses from VS. The main goal was to discard molecules with either poorly predicted affinity (mainly unfavourable desolvation penalties from either ligand or protein side) or geometry warnings (poor dihedral angles and/or slight intra/inter clashes).

HTRF assay

Production of GST-syntenin full length for HTRF experiments

Competent *E. coli* (ER2566 strain) cells were transformed with the human syntenin1 pGEX-5X expression vector (GE Healthcare). Expression of N-terminally GST-tagged syntenin-1 full length was induced overnight at 30 °C and by the addition of 0.4mM IPTG (Fisher Scientific). Protein was purified using GSTrap4B columns 28-4017-45 (GE Healthcare).

Production of N-terminal GST-tagged GRASP55 fusion proteins and N-terminal GST-tagged Erbin were accomplished by an induction 18 h at 25°C with 0.4 mM IPTG in *E. coli* BL21 (DE3) bacteria cells transformed with the purified pDEST15-GRASP55 or pDEST15-Erbin expression plasmids (Invitrogen). Fusion proteins were recovered from the cell lysates by conventional affinity chromatography on Glutathione resin using an Akta Explorer system (Amersham Pharm Biotech).

HTRF screen

HTRF assays were performed in white 96Well Small Volume™HiBase Polystyrene Microplates (Greiner) with a total working volume of 100µL, as described previously ¹⁹. Briefly, compounds were firstly dispensed into the wells at 400µM (in 25 mM Hepes pH 7.4, 150 mM NaCl and 0.1% BSA ultrapure) for the primary screen or with serial DMSO dilutions for IC₅₀ measurement assays. Primary screening assays have been performed in monoplicate while IC₅₀ measurements were performed in triplicates. Control experiments were performed with a biotinylated-GST to verify that compounds were not interfering with the FRET signal in a non-specific manner. All HTRF reagents were purchased from CisBio Bioassays and reconstituted

according to the supplier protocols. The plates were spin for 3 min at 500g and then incubated at 4°C for 16 h. The HTRF signals were recorded on a POLAR star Omega plate reader (BMG Labtech) with an excitation filter at 337 nm and fluorescence wavelength measurement at 620 and 665 nm, an integration delay of 60µs and an integration time of 400µs. Results were analyzed with a two-wavelength signal ratio: [intensity (665 nm)/intensity (620 nm)]*10⁴. Percentage of inhibition was calculated using the following equation: % inhibition = [(compound signal)-(min signal)]/[(max signal)-(min signal)]*100, where 'max signal' is the signal ratio with the compound vehicle alone (DMSO) and 'min signal' the signal ratio without protein B. For IC₅₀ measurements, values were normalized and fitted with Prism (GraphPad software) using the following equation: $Y=100/(1+((X/IC_{50})^{Hill\ slope}))$.

Isothermal Titration Calorimetry

Syntenin samples prepared for crystallography experiments were directly used for isothermal titration calorimetry (ITC) after an additional step of buffer exchange in 10 mM Hepes, 150 mM NaCl, pH 7.5 using a PD10 column (GE Healthcare). The compound from a 50mM stock solution in 100% DMSO was directly diluted in the buffer exchange buffer. Titrations were carried out on a MicroCal ITC200 microcalorimeter (MALVERN) and each experiment was designed using various titrant/analyte concentrations as shown Table S2 and using 15 syringe injections of 2.3µL SYNTi. An additional first 0.4 µL injection was included in the titration protocol to remove air bubbles trapped in the syringe prior to titration and to take into account syringe predilution in the cell during equilibration. Raw data were scaled after setting the zero to the titration saturation heat value. Integrated raw ITC data were fitted to a one-site nonlinear least squares fit model using the MicroCal Origin plugin as implemented in Origin 7 (Origin Lab). Finally, ΔG and TΔS values were calculated from the fitted ΔH and K_A values using the equations ΔG = -R.T.ln(KA) and ΔG = ΔH - TΔS. The final K_D value was calculated from averaging the 3 performed independent experiments.

X-ray crystallography

6xHis-syntenin (aa 113-273) production and purification for crystallization studies

Competent E.coli (ER2566 strain) cells were transformed with the pETM-11 vector (EMBL Heidelberg) allowing expression of N-terminally His-tagged proteins. Bacteria were plated on LB agar plates with 50 µg/ml Kanamycin. The culture and induction were performed in TYB medium (5-6 hours induction at 30°C after addition of 0.5 mM IPTG). The pellet was re-suspended in 30 ml of lysis buffer (25 mM Hepes pH 7.4, 150 mM NaCl, 1 µg/ml DNase I Recombinant RNase free (Qiagen), 0.013 g Lysozyme (Sigma) and 1 tablet of protease inhibitor EDTA free (Roche)) and kept on ice for 30 min. After sonication, the suspension was transferred to JA 20 tubes and centrifuged at 12000 g at 4°C for 45 min. The supernatant was applied to a HistrapTMHP columns 17-5247-01 (GE Healthcare) equilibrated with 20 ml Buffer A (25 mM Hepes pH 7.4, 150 mM NaCl and 20 mM Imidazole). The purification was performed on the Akta Explorer (Amersham Pharm Biotech). His-tagged proteins were eluted with 30 ml of an imidazole gradient (20- 500 mM, Buffer B, 25 mM Hepes pH 7.4, 150 mM NaCl and 500 mM Imidazole). 6xHis-tagged proteins were fully recovered within the first 10 ml of elution. The His-tag was removed using TEV protease at 4°C for 16 h for crystallization trials. Purification was finalised using size exclusion gel chromatography with a HiLoad 16/600 Superdex 75pg (GE Healthcare) column in buffer containing 10 mM Hepes pH 7.4, 150 mM NaCl and 1mM DTT.

Crystallization of syntenin PDZ protein and compound soaking

Crystallization trials were performed using the hanging drop vapor diffusion method at 4 °C. The apo - syntenin PDZ protein crystallized in 0.1M Sodium Acetate pH 4.6/0.2 M Ammonium Acetate and 22% PEG 3350 at a protein concentration of 6 mg.ml⁻¹. syntenin PDZ crystals were soaked in mother liquors containing 5 mM of compound (5% DMSO) for 4 hours at 18°C. Crystals were flash-frozen in mother liquor containing 10% glycerol. Diffraction data of syntenin PDZ - soaked compounds were collected at the European Synchrotron radiation Facility (ID30A and IB30B) and at SOLEIL synchrotron (Proxima 1 and Proxima 2) and processed using X-ray Detector Software (XDS). **Compound E5 (19)**. The space group was assigned to P1 with four proteins and four **E5** compounds per asymmetric unit (PDB code 8AAI). The phase was solved by molecular replacement using the structure of the PDB entry 1W9E as a template and the program PHASER. Models were rebuilt using COOT and refined with Refmac. This dataset showed translational Non-Crystallographic Symmetry (TNCS) that was handled by PHASER during the molecular replacement step. **Compound 29**. The space group was assigned to P212121 with two proteins and two **29** compounds per asymmetric unit (PDB code 8AAK). The phase was solved by molecular replacement using the structure of the PDB entry 1W9E as a template and the program PHASER. Models were rebuilt using COOT and refined with Refmac then with autoBUSTER. This dataset showed translational Non-Crystallographic Symmetry (TNCS) that was handled by PHASER during the molecular replacement step. **Compound 95**. The space group was assigned to C2 with two proteins and two **95** compound per asymmetric unit (PDB code 8AAO). We used Pipedream ²⁰, a tool specifically designed for linking

data processing with AutoPROC, rigid-body replacement with Phaser, and structure refinement with autoBUSTER. Using this automated pipeline, images were processed and scaled using XDS²¹ and SCALA. The structure of syntenin PDZ protein (PDB code 1N99) were used as template models in the limited molecular replacement procedure. Iterative cycles of model building were done with the program COOT. **Compound SYNTi (or 118)**. The space group was assigned to P1 with two proteins and two **SYNTi** compounds per asymmetric unit (PDB code 8AAP). The phase was solved by molecular replacement using the structure of the PDB entry 1N99 as a template and the program PHASER. Models were rebuilt using COOT and refined with autoBUSTER. Compounds restraints were generated with grade²² and Mogul²³ using RM1 semi-empirical QM methods.

Cell-based assays

Culture and reagents

MDA-MB-231 cell lines were purchased from the American Type culture collection (Manassas, VA, USA). Cells were grown in DMEM-F12 (Invitrogen) supplemented with 10% fetal calf serum (FCS) (Eurobio) at 37°C under 5% CO₂. syntenin CRISPR/Cas9 knockout (SyntKO) MDA-MB-231 cells were generated following the procedures as previously described (Imjeti et al., 2017). Briefly, SDCBP-specific gRNA oligos sgrNASDCBP1 targeting exon1 (F: CACCgCTATCCCTCACGATGGAAGT; R: AAACACTTCCATCGTGAGGGATAGc) were cloned into the pX458 two-in-one CRISPR targeting vector (Addgene, Cambridge, MA, USA), individually transfected into MDA-MB-231 cells and sorted for GFP expression after 48 h. Single cell clones were grown and screened by western blotting for syntenin expression. One individual clone has been used in the *in vitro* experiments.

Cancer cell viability

The effects of the compounds **30**, **E5** and **SYNTi** were tested both on MDA-MB-231 and MDA-MB-231 SyntKO cells. Briefly, the cells were treated with increasing concentrations of the compounds for 48 h and early/late apoptosis was measured by using the FITC Annexin V apoptosis detection kit with 7-AAD (Biolegend, San Diego, CA, USA) according to the manufacturer. Annexin V positive but 7-AAD negative (early apoptotic cells) and Annexin V positive and 7-AAD positive (late-stage apoptosis) was determined by using FACS LSRII flow cytometer (BD Biosciences) and data were analyzed with Flowjo software (Tree Star).

Single cell migration assay

25,000 cells were seed on collagen precoated 6-wells plate. Cells were monitored using live cell imaging using Metamorph 7.8.1 and followed for 24 hours. Cell tracking was performed manually for all selected cells in 1/2 of the field-of-view at two different X-Y positions for at least 2 independent experiments by serial selection of the center of the cell nucleus at each time point during the time series using the Plugin "Manual Tracking" provided in the ImageJ software. The values for the assessment of migration speed, velocity and directionality were obtained using ImageJ software. Migration speed was determined by the ratio between the total distance and duration of cell migration. Euclidean distance corresponds to the distance between the positions of the cells at the beginning and the end of the experiment²⁴.

ASSOCIATED CONTENT

Supporting Information. A Molecular Formula Strings is provided with associated IC₅₀ and LE values (CSV). An Additional SI is provided (PDF) containing HTRF primary screening data, additional figures illustrating HTRF secondary screening data, dose response curves, ITC thermograms (complemented with a table), effects of **E5** & **SYNTi** on MDA-MB-231 breast cancer cell survival and on cell migration speed, HPLC traces and ¹H-NMR & ¹³C-NMR Spectra final compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Accession codes

PDB codes for Syntenin PDZ-2 domain with bound **E5** (**19**) is 8AAL, with bound **29** is 8AAK, with bound **95** is 8AAO and with bound **SYNTi** (**118**) is 8AAP.

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‡ Karine Barral and Xavier Morelli contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

L. Hoffer analyzed the structural data, performed the virtual screening (MDS and docking), carried out the computer-aided design of organic compounds during the optimization stage and wrote the manuscript; M. Garcia performed chemistry experiments and associated analyses; R. Leblanc carried out HTRF and cell biological work; M. Feracci contributed to crystallization studies and associated calculations ; S. Betzi performed ITC experiments ; K. Ben Yaala performed protein production and purification ; Avasi M. Daulat carried out cell biological work; P. Zimmermann conceived cell biology experiments; P. Roche helped with the in silico analyses; K. Barral contributed to structure–activity relationship studies, crystallization experiments, X-ray complex structures resolution, and organic syntheses; K. Barral and X. Morelli supervised, conceived the project and wrote the manuscript.

ACKNOWLEDGMENT

This work was supported by grants from the National Research Agency (ANR, Investissements d’Avenir, A*MIDEX project ANR-11-IDEX-0001-02), the Institut National du Cancer (INCa, subvention 2013-105), the French National Research Agency (ANR-18-CE13-0017, Project SynTEV), the French Infrastructure for Integrated Structural Biology (FRISBI) ANR-10-INSB-05-01 with the use of the AFMB laboratory X-ray facility. The authors acknowledge the European Synchrotron Radiation Facility (ESRF) as well as the SOLEIL synchrotron (Proxima 1 and Proxima 2 beamlines) for provision of synchrotron radiation facilities. M. Garcia was the recipient of La Ligue, French Foundation for Cancer Research. R. Leblanc was the recipient of the Fondation ARC, French Foundation for Cancer Research. We would also like to thank the platforms ‘HiTS’, the core flow cytometry and the Datacenter IT and Scientific Computing from CRCM.

ABBREVIATIONS

BB, building block; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DOTS, diversity-oriented target-focused synthesis; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; H2L, hit-to-lead; HTRF, Homogeneous Time Resolved Fluorescence; IC50, inhibitory concentration needed to decrease by 50% the enzyme activity; LC/MS, liquid chromatography / mass spectroscopy; LE, ligand efficiency; MDS, molecular dynamic simulation; MW, molecular weight; NMR, nuclear magnetic resonance; PDB, protein data bank; PPI, protein-protein interaction; RMSD, root-mean-square deviation; rt, room temperature; SAR, structure-activity relationship; SBDD, structure-based drug design; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; VdW, Van der Waals; VS, virtual screening.

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