Design and Synthesis of Novel Small-Molecule Inhibitors of the Keap1-Nrf2 PPI

Sigtryggur Bjarki Sigtryggsson

Department of Chemistry - BMC
University of Uppsala
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Sigtryggur Bjarki Sigtryggsson

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Supervisor
Prof. Jan Kihlberg

Department of Chemistry - BMC
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Abstract

Reactive oxygen species (ROS) are formed in cells under oxidative stress. These ROS can have damaging effects on DNA and other essential cellular components. ROS play a critical role in inflammatory diseases and in cancers. Under basal conditions, a cellular antioxidant defence mechanism balances out the formation of ROS species, that under dissregulated conditions can harm the cell. The major components of the defence mechanism are Keap1, Nrf2 and the antioxidant response element (ARE). Once Nrf2 is activated it can translocate into the nucleus where it interacts with the ARE to regulate transcription of antioxidant enzymes and cytoprotective proteins. The Keap1-Nrf2 protein-protein interaction makes for an interesting target against inflammation and cancer. In this project, the synthesis of a previously known small-molecule inhibitor was successfully reproduced. Furthermore, four analogues were designed and synthesized, where a key step in the synthesis was the successful application of a copper catalysed reaction between complex tetrahydroisoquinoline substrates and reactive terminal alkynes. The novel target compounds were synthesized in overall yields of ~1%. The compounds’ biological activity was determined by surface plasmon resonance experiments. Unfortunately, the novel compounds showed lower affinity in comparison to the reference compound.
Popular Scientific Description

Oxygen is essential for maintaining life, as it helps in creating energy for our cells. However, this is not a faultless system. Oxidative stress is when there is an imbalance between highly reactive oxygen compounds and antioxidants in the body. When the cells are under oxidative stress, harmful oxygen compounds are formed as a side effect in greater amount than the antioxidants can fight off. These harmful compounds are called reactive oxygen species (ROS) and can react with various other molecules in the body, e.g. leading to damage to our DNA. Fortunately, our cells have developed a defence system to defend against the ROS. Nrf2 is one of three major components in the defence system, the other two being the protein Keap1, which interacts with and releases Nrf2, and the antioxidant response element (ARE). Under normal conditions Nrf2 will be degraded to avoid unnecessary build up. When the cells are under oxidative stress the defence system is activated and Keap1 releases Nrf2, resulting in transcription of genes that prevents the ROS from building up.

As oxidative stress has been linked to many diseases such as inflammatory diseases and cancer, a great interest has been shown for the design and development of a drug capable of tackling the oxidative stress. As Keap1 and Nrf2 are essential components of the cells defence system, inhibiting the Keap1 Nrf2 interaction is of interest as a possible treatment for the aforementioned diseases. If successful, Nrf2 will then be released in higher amount than without the drug and have greater potential for fighting off the ROS. It is best if the drug fits perfectly with Keap1 to get a better interaction and avoid potential off target side effects. In this regard, Keap1 can be thought of as a glove and the drug as a hand that fits the glove perfectly. Targeting Keap1 shows promise as a treatment against inflammation and cancer. In this project, the possibility of modifying one of the already made compounds that fits into Keap1 was explored to make a new and improved drug.
In dedication of Póló.
# Table of Contents

Table of Figures ........................................................................................................ iv

Table of Schemes ......................................................................................................... v

Table of Tables ............................................................................................................ vi

Abbreviations ............................................................................................................... vii

Acknowledgements ...................................................................................................... ix

1 Introduction ............................................................................................................. 1
   1.1 Keap1–Nrf2 Protein–Protein Interaction ............................................................ 1
   1.2 Previously discovered inhibitors ...................................................................... 4
      1.2.1 Covalent inhibitors .................................................................................. 4
      1.2.2 First reported non-covalent inhibitor ....................................................... 5
      1.2.3 Other reported non-covalent inhibitors ................................................... 7
   1.3 Surface Plasmon Resonance ............................................................................. 7

2 Aim of the Project .................................................................................................. 10

3 Results and Discussions ....................................................................................... 12
   3.1 Computational studies ..................................................................................... 12
      3.1.1 Design of Target Molecules .................................................................. 12
      3.1.2 Docking results ...................................................................................... 13
   3.2 Synthesis of the reference molecule (9) ........................................................ 14
   3.3 Original synthetic strategy ............................................................................. 16
      3.3.1 Synthesis of building block A – tetrahydroisoquinoline moiety ............ 16
      3.3.2 Synthesis of building block B – isooindoline moiety ............................. 17
      3.3.3 Attempted N-alkylation of building block B ........................................... 19
   3.4 Revised synthetic strategy ............................................................................. 22
      3.4.1 Design of the new synthetic strategy ...................................................... 22
      3.4.2 Synthesis of the target compounds ......................................................... 22
   3.5 SPR assays ..................................................................................................... 26

4 Conclusion and Future Outlooks .......................................................................... 27

5 Experimental ........................................................................................................ 29
   5.1 General methods ............................................................................................ 29
   5.2 Experimental .................................................................................................. 29
      5.2.1 Methyl (S)-2-(2-oxo-4-phenyloxazolidin-3-yl)acetate (1) [3] ............... 29
      5.2.2 (S)-2-(2-oxo-4-phenyloxazolidin-3-yl)acetic acid (2) [3] ................. 30
      5.2.3 (S)-1,10b-dihydro-3H-oxazolo[4,3-a]isoquinoline-3,6(5H)-dione (3) [3] .... 30
      5.2.4 (S)-1,5,6,10b-tetrahydro-3H-oxazolo[4,3-a]isoquinolin-3-one (4) [3] ....... 31
      5.2.5 (S)-(1,2,3,4-tetrahydroisoquinolin-1ul)methanol (5) [3] ................... 31
      5.2.6 tert-butyl (S)-1-(hydroxymethyl)-3,4-dihydroisoquinoline-2(1H)-
          carboxylate (6) [19] ............................................................................... 32
5.2.7 tert-butyl (S)-1-((1,3-dioxoisindolin-2-yl)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (7)\[20] 32
5.2.8 methyl (S,S,R)-2-((S)-1-((1,3-dioxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylate (8)\[3] 33
5.2.9 (S,S,R)-2-((S)-1-((1,3-dioxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (9)\[3] 34
5.2.10 tert-butyl (S)-1-(((methylsulfonyl)oxy)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (10)\[21] 34
5.2.11 tert-butyl (S)-1-((tosyloxy)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (11) 35
5.2.12 tert-butyl (S)-1-((iodomethyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (12) 36
5.2.13 (S)-1,5,6,10b-tetrahydro-[1,2,3]oxathiazolo[4,3-a]isoquinoline,3,3-dioxide (13)\[3] 36
5.2.14 1-phenylprop-2-yn-1-one (14)\[23] 37
5.2.15 Benzyl propiolate (15)\[23] 37
5.2.16 (Z)-3-((2-oxo-2-phenylethyllidene)isoindolin-1-one (16)\[12] 38
5.2.17 Benzyl (Z)-2-((3-oxoisindolin-1-ylidene)acetate (17)\[12] 38
5.2.18 Benzyl (E)-2-((3-oxoisindolin-1-ylidene)acetate (18)\[12] 39
5.2.19 tert-butyl (S)-1-(azidomethyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (19) 39
5.2.20 tert-butyl (S)-1-((1,3-dioxoisindolin-2-yl)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (20)\[21] 40
5.2.21 tert-butyl (S)-1-((2-iodobenzamido)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (21) 40
5.2.22 tert-butyl (S)-1-((1-benzylidene-3-oxoisindolin-2-yl)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (22)\[16] 41
5.2.23 tert-butyl (S)-1-(((2-benzyl)-2-oxoethylidene)-3-oxoisindolin-2-yl)methyl)-3,4-dihydroisooquinoline-2(1H)-carboxylate (23)\[16] 42
5.2.24 methyl (S,S,R)-2-((S)-1-((1-benzylidene-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylate (24) 43
5.2.25 methyl (S,S,R)-2-((S)-1-((1-((2-benzyl)-2-oxoethylidene)-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylate (25) 44
5.2.26 (1S,2R)-2-((S)-1-((1-benzylidene-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (26) 44
5.2.27 (1S,2R)-2-((S)-1-((1-carboxymethylene)-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (27) 45
5.2.28 methyl (1S,2R)-2-((S)-1-((2-iodobenzamido)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylate (28) 46
5.2.29 (1S,2R)-2-((S)-1-((2-iodobenzamido)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (29) 47
5.2.30 (1S,2R)-2-((S)-1-((1-oxo-3-(2-oxo-2-phenylethyllidene)isoindolin-2-yl)methyl)-1,2,3,4-tetrahydroisooquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (30)\[16] 47
5.2.31 (1S,2R)-2-((S)-1-((1-(2-(benzyl)oxy)-2-oxoethylidene)-3-oxoisooindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (31) [16] .......................................................... 48

5.3 General Experimental procedure for the attempted N-alkylation ................................................................. 49
5.3.1 Mitsunobu reaction ............................................................................................................................................ 49
5.3.2 N-alkylation with mesyl, tosyl or iodo as leaving group ................................................................. 49
5.3.3 N-alkylation with sulfamidate as leaving group ......................................................................................... 49
5.3.4 N-alkylation with 4-nitrobenzyl bromide and 1-iodopropane as electrophiles .................................................. 49

5.4 Keap1 binding assays ........................................................................................................................................... 50
5.4.1 SPR inhibition in solution assay (ISA) for Keap1 ....................................................................................... 50

Bibliography .................................................................................................................................................................. 51

Appendix A .................................................................................................................................................................. 53
NMR spectra of important and novel compounds .......................................................................................... 53

Appendix B .................................................................................................................................................................. 84
MM-GBSA binding free energy results of original set of compounds ................................................. 84
Table of Figures

Figure 1.1. The Keap1-Nrf2-ARE pathway ................................................................. 3

Figure 1.2. i) Surface overview in hydrophobic colouring of the Keap1 binding site with subpockets P1-P5 labelled ................................................................. 4

Figure 1.3. Schematic representation of a SPR biosensor ........................................... 8

Figure 3.1. Co-crystal structure of the Keap1-9 complex ........................................... 12
Table of Schemes

Scheme 1.1. Previously discovered covalent small-molecule Keap1 inhibitors .................. 5
Scheme 1.2. Illustration of analogues of Compound 9 prepared ........................................ 6
Scheme 1.3. Three examples of previously synthesized compounds and the binding constants. ................................................................................................................. 7
Scheme 2.1. 1,2,3,4-Tetrahydroisoquinoline cored ligand (9) ........................................ 10
Scheme 2.2. Retrosynthesis of the designed ligand derivatives ........................................ 10
Scheme 2.3. The designed and synthesized novel inhibitors .............................................. 11
Scheme 3.1. Synthetic route towards the reference compound, 9 .................................... 14
Scheme 3.2. Synthetic route towards building block A with a leaving group .................. 16
Scheme 3.3. The isoindoline moiety .................................................................................. 17
Scheme 3.4. Synthetic route towards building block B .................................................. 17
Scheme 3.5. Synthetic route towards phenyl propiolate ................................................. 18
Scheme 3.6. Reactions tested for the N-alkylation of building block B ............................. 19
Scheme 3.7. Reported ring closure to afford the desired isoindoline derivative ............... 22
Scheme 3.8. Retrosynthesis of the same product as suggested by the N-alkylation approach ............................................................................................................. 22
Scheme 3.9. Synthetic route towards two final compounds ........................................... 23
Scheme 3.10. Synthetic route towards two final compounds .......................................... 24
Scheme 3.11. Proposed mechanism of the copper catalysed ring closure ....................... 25
## Table of Tables

Table 3.1. Summary of computed MM-GBSA binding free energy of synthesized compounds. ........................................................................................................................................... 13

Table 3.2. Reaction conditions of the attempted $N$-alkylations. ...................................................... 21

Table 3.3. SPR binding affinity results. .............................................................................................................. 26

Table 4.1. Summary of the synthesized target molecules and overall yields.............................. 28
## Abbreviations

Abbreviations which appear within the thesis

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
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<tr>
<td>15C5</td>
<td>15-crown-5</td>
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<tr>
<td>ARE</td>
<td>Antioxidant response element</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlated spectroscopy</td>
</tr>
<tr>
<td>DBAD</td>
<td>Di-tert-butyl azodicarboxylate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal effective concentration</td>
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<td>EDC·HCl</td>
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<td>Electronspray ionization</td>
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<td>EtOAc</td>
<td>Ethyl acetate</td>
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<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation</td>
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<td>HPLC</td>
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<td>HSQC</td>
<td>Heteronuclear single quantum coherence</td>
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<td>HTS</td>
<td>High-throughput screening</td>
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<td>Hertz</td>
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<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
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<td>Inhibition in solution assay</td>
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<tr>
<td>ITC</td>
<td>Isothermal titration calorimetry</td>
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<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
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<tr>
<td>Keap1</td>
<td>Kelch-like ECH-associated protein 1</td>
</tr>
<tr>
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<td>Molecular mechanics/generalized born surface area</td>
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<td>Neh</td>
<td>Nrf2-ECH homology</td>
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<td>NMR</td>
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<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>Nrf2</td>
<td>Nuclear factor erythroid 2–related factor 2</td>
</tr>
<tr>
<td>PPI</td>
<td>Protein–protein interaction</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SAR</td>
<td>Structure-activity relationship</td>
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<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
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<tr>
<td>TEA</td>
<td>Triethylamine</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>VT NMR</td>
<td>Variable-temperature NMR</td>
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<tr>
<td>br s</td>
<td>Broad singlet</td>
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<td>d</td>
<td>Doublet</td>
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<tr>
<td>Symbol</td>
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<td>dt</td>
<td>Doublet of triplets</td>
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<tr>
<td>m</td>
<td>Multiplet</td>
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1 Introduction

All living systems rely on oxygen for their cellular energy metabolism, however, these processes do not come without side effects, as they produce reactive oxygen species (ROS) when under oxidative stress.\cite{1} Oxidative stress is the disproportion between biochemical processes leading to the formation of oxidative and electrophilic species and the processes responsible for their elimination. Oxidative stress thus leads to excess in reactive oxygen species. These species can be induced from either exogenous oxidative sources, such as carcinogenic chemicals, or endogenous oxidative sources from cellular processes such as cellular signalling and metabolism. Since ROS can lead to oxidative damage to DNA and to other essential cellular components, the body has established antioxidative and cytoprotective mechanism against numerous forms of oxidative stress.\cite{2}

Studies have shown that ROS have an important role in various diseases, such as cancers and inflammatory diseases.\cite{1} In order to protect cells from oxidative stress and ROS, the cells utilize a cellular defence mechanism, to which there are three major regulatory components. These are Kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2) and the antioxidant response element (ARE). Nrf2 is a transcription factor responsible for the regulation of cytoprotective genes through interaction with ARE of the Keap1-Nrf2-ARE signalling pathway. ARE is a short DNA sequence, a so-called enhancer element. When it comes to protecting against oxidative stress this system plays a vital role.\cite{1}

Additionally, the Keap1-Nrf2-ARE signalling pathway also plays an important role in inflammation and carcinogenesis.\cite{1} Recently, it has become an attractive strategy to target the Keap1-Nrf2 protein-protein interaction (PPI) by developing inhibitors that can serve as a preventive or therapeutic agent for many diseases that involve oxidative stress and inflammation. Previous studies have already shown that this PPI constitutes a promising target against inflammation and as chemopreventive agents for cancer.\cite{1, 3}

Targeting PPIs such as Keap1-Nrf2 can be a challenging task.\cite{4} PPIs usually have a very large interacting surface which often are quite shallow and spread over a wide area. For these reasons, PPIs have generally been thought of as very difficult drug targets and the term “undruggable” has even been coined for these interactions. Recently, however, small-molecular inhibitors have been shown to be capable of interrupting certain classes of PPIs, including globular PPIs such as Keap1-Nrf2.\cite{4} As the activation of Nrf2 through covalent inhibition of Keap1 might cause unpredictable and potentially devastating side effects, non-covalent activation of Nrf2 is the desired route which has the potential for a lower risk of toxicity.\cite{5}

1.1 Keap1–Nrf2 Protein–Protein Interaction

Nrf2 is a transcription factor that occurs naturally in the cell defence mechanism.\cite{3} Nrf2 is capable of regulating ARE and mediate transcription of numerous antioxidants and protective genes which are responsible for counteracting the damaging effects caused by ROS.\cite{3}
Nrf2 consists of 605 amino acids and is defined into six domains termed Nrf2-ECH homology (Neh) 1-6. The domain responsible for Keap1 binding is the Neh2 which is a negative regulatory domain at the N-terminus of Nrf2. Neh2 is therefore the most important domain of Nrf2 in relation to the Keap1-Nrf2 PPI. It contains the two key motifs for the Keap1-Nrf2 PPIs, ETGE and DLG, named after their respective amino acid sequences.

The Keap1 protein consists of three domains, two of which are of main interest here; the intervening region (IVR) and the Kelch domain. The IVR domain consists of highly reactive cysteine residues which act as sensors to oxidative stress. In the Keap1-Nrf2 PPI, two Kelch domains of Keap1 interact with the Neh2 domain of a singular Nrf2 protein. It has been shown that ETGE binds more strongly than DLG to the Kelch domain ($K_d = 19 \text{ nM vs } 1 \text{ µM}$, respectively), and therefore the two interactions are sometimes referred to as the “hinge” and the “latch”, respectively. The two motifs have been found to be essential for the Keap1-dependent suppression of Nrf2. It has been hypothesized that the Keap1-Nrf2 system works almost at saturated levels, thus by inhibiting Keap1 ever so slightly would allow Nrf2 to build up.

In the cytoplasm, Keap1, is responsible for regulating Nrf2 under basal conditions. Keap1 binding to Nrf2 leads to ubiquitination and proteasomal degradation of Nrf2 in the cytosol, thus maintaining a lower level of Nrf2. Under oxidative stress, ROS react with and modify the sulfhydryl groups of specific cysteine residues on Keap1, the so-called sensors, (i.e., Cys151, Cys257, Cys273, Cys288, and Cys297). Under these conditions, Keap1 acts as a redox sensor and regulator and prevents ubiquitination of Nrf2 by altering the Keap1-Nrf2 PPI via conformational changes taking place in Keap1. This leads to translocation of Nrf2 into the nucleus once Nrf2 dissociates from Keap1 (Figure 1.1). In the nucleus, Nrf2 forms heterodimers with transcriptional regulatory proteins, which subsequently brings about transcription of cytoprotective enzymes, through interactions with the ARE.

The two interfaces involved in Keap1 binding to Nrf2, Kelch-DLG and Kelch-ETGE, differ considerably in the size of their buried surfaces, which are ~780 Å² and ~550 Å², respectively. They are, however, both much smaller compared to the general size of the buried surface of classical PPIs, which are typically in the range of 1000-6000 Å². The Kelch protein adopts a concave binding surface when interacting with Neh2 of Nrf2 which is similar to small-molecule binding pockets of traditional targets. The Kelch binding pocket has been classified into five spaces, which have been labelled P1-P5 (Figure 1.2, i). P1 and P2 constitute mostly polar residues, Arg380, Arg415, Arg483, Asn382, Ser363 and Ser508, whereas, P4 and P5 constitute mostly non-polar residues, Gln530, Phe577, Tyr334, Tyr525, and Tyr572. Lastly, P3 constitutes both small polar and non-polar residues, Ala556, Gly509, Gly571, Gly603, Ser555, and Ser602 (Figure 1.2, ii). As evident from the aforementioned information, the Kelch binding pocket is polar and basic as it contains several positively charged arginine residues, which form crucial salt bridges with carboxylic acid residues of the ETGE and DLG motifs. Previous studies have shown that it is of uppermost importance to occupy both P1 and P2 and that they can be regarded as hot spots of the Kelch binding pocket.
Figure 1.1. The Keap1-Nrf2-ARE pathway. A) Under basal conditions Nrf2 is targeted for ubiquitination which leads to ubiquitinated Nrf2 to be released from the Keap1-Nrf2 complex and later B) for the Nrf2 protein to undergo proteasomal degradation; C) Under induced conditions, according to the “hinge” and “latch” mechanism the Keap1-Nrf2 PPI is interrupted, D) Nrf2 is liberated which E) leads to translocation to the nucleus where it interacts with ARE and leads to transcription of cytoprotective genes. Adopted from [2].

Keap1-Nrf2 inhibition could lead to increased levels of Nrf2 directly by help releasing Nrf2 from the induced state. To date, it is not known which interaction would be disrupted when targeting Keap1, Keap1-Nrf2 ETGE or Keap1-Nrf2 DLG or both. It has, however, been shown that disrupting only the DLG motif, the weaker interaction, should be sufficient to inhibit Nrf2 suppression. [4]
**Figure 1.2.** Surface overview of the Keap1 binding site, blue: positively charge residues, red: negatively charged residues, and grey: neutral residues; i) Keap1 binding site with subpockets P1-P5 labelled, ii) The same surface overview with the addition of a few of the key amino acids involved in Keap1-Nrf2 interaction.

## 1.2 Previously discovered inhibitors

### 1.2.1 Covalent inhibitors

Many small-molecule Keap1-Nrf2 PPI inhibitors have been successfully developed as potential therapeutics.\[1^\] There are already a few known Nrf2-activation/ARE-inducing agents in human clinical trials\[6^\] as a cancer preventing treatment and for conditions involving inflammation. These compounds can be categorized into two groups, electrophilic agents which covalently inhibit Keap1 through reactive cysteine residues and non-covalent inhibitors. Examples of the covalent inhibitors include natural products, such as sulforaphane and curcumin (Scheme 1.1 bottom) and synthetic compounds, such as dimethyl fumarate and bardoxolone (Scheme 1.1 top). Dimethyl fumarate is converted to monomethyl fumarate during absorption which reacts with Cys151 of Keap1 which subsequently leads to gene transactivation mediated by the Nrf2 activation. Bardoxolone also reacts with Cys151 of Keap1. Electrophilic compounds have the potential to cause unwanted side effects by reacting unspecifically with many different proteins as their reactivity is quite unpredictable and therefore difficult to control. Non-covalent inhibitors of the Keap1-Nrf2 PPI are potentially safer and therefore might prove to be a more attractive approach.\[1^, 4, 5\]
1.2.2 First reported non-covalent inhibitor

High-throughput screening (HTS) of the commercially available MLPCN library using homogeneous fluorescence polarization (FP) competition assay was used to identify the first non-covalent small-molecular Keap1-Nrf2 inhibitor by Hu et al.\textsuperscript{[1]} The hit molecule from this assay, compound 9, has also been found to be active in functional cell assays and has the ability to promote the translocation of Nrf2 into the nucleus of human bone osteosarcoma epithelial cells and there induce downstream ARE activation.\textsuperscript{[4]}

In one experiment, in order to analyse the binding mode of compound 9, it was exposed to a high concentration of glutathione that was used to mimic cysteine residues. As no thiol addition or decomposition could be detected over 48 h, it was concluded that 9 inhibits the Keap1-Nrf2 PPI in a non-covalent manner, making it a first-in-class direct inhibitor of the Keap1-Nrf2 interaction.\textsuperscript{[1]}

The work presented in this thesis is centred around compound 9 as it was used as a starting point to design and synthesize novel analogues of 9.

Compound 9 has eight diastereomers, the one shown in the margin has been found to be the most active.\textsuperscript{[1]} This isomer of compound 9 has furthermore been found to have at least 100-fold the potency compared to the other stereoisomers. Through SPR competition assay, the binding constant ($K_d$) of 9 towards Keap1 was found to be 1.0 µM. The small-molecule has been shown to be both cell permeable and can inhibit the Keap1-Nrf2 PPI with a half maximal effective concentration ($EC_{50}$) of 18 µM. To show that 9 is a feasible starting point for further optimization to discover and develop a more potent direct inhibitor of Keap1-Nrf2 PPI, several analogues were synthesized to establish a structure-activity relationship, SAR. It has been shown that the free acid on the cyclohexane moiety is needed for the binding to Keap1 as the corresponding methyl esters showed no activity. Likewise, the corresponding amide was 20 times less active. It has also been shown that the optimal number of carbon atoms between

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\textbf{Scheme 1.1.} Previously discovered covalent small-molecule Keap1 inhibitors. Top: synthetic compounds. Bottom: natural products.
the tetrahydroisoquinoline and the phthalimide moieties is a single carbon, methylene, as additional carbons rendered the molecule inactive. Finally, substitution of the phthalimide moiety for a lactam derivative has been shown to be possible with only a small reduction in the affinity for Keap1 (Scheme 1.2).[1] Substituting the carboxylic acid for a tetrazole was found to be applicable with only a small reduction in affinity (Scheme 1.2).[3]

The co-crystal structure of the Keap1-9 complex revealed important information about the binding.[4] Jnoff et al. showed that one of the arginine amino acids, Arg415, present in the Keap1 binding pocket adopts a different conformation when inhibited by compound 9 as compared with that observed in the Keap1-Nrf2 structure.[3] Interestingly, it was observed in the Keap1-9 co-crystal structure that the bulkier amide substituent on the cyclohexane moiety adopts the sterically less favourable axial conformation. Furthermore, the co-crystal structure shows that the tetrahydroisoquinoline moiety of 9 reaches further into the central pore of the binding pocket, whereas the free acid of the cyclohexane and the phthalimide moiety extend outward into P1-5 and form interactions with Arg415, Asn414, Ser602, and Tyr572. Furthermore, it has been shown that when occupied by the inhibitor, the side chains of Arg380 and Arg415 need to make way for 9 by re-orienting away from the central pore. The free acid on 9 acts as a hydrogen bond acceptor and forms hydrogen bonds with Arg415 and Asn414 and fits neatly into a narrow opening formed by the amino acids. Furthermore, one of the two carbonyls of the phthalimide moiety forms hydrogen bonds with Ser602 while the other carbonyl forms a water-mediated hydrogen bond with Ser555 in addition to also being hydrogen bonded to Ser508 through double water-mediation. The phenyl ring of the phthalimide moiety forms a face-to-face π-stacking with Tyr572.[3] Compared with the Keap1-Nrf2 complex, the Keap1-9 complex is strengthened by two π-cation interaction between tetrahydroisoquinoline and Arg415 and the phthalimide moiety and Arg380.[1]

There is room for changes of compound 9. Since the tetrahydroisoquinoline moiety sits above the central pore it is possible to extend the molecule further into the pore by introducing substituent to the phenyl group of the moiety. This could yield new favourable interactions and hence increase the inhibitor’s affinity toward Keap1. Jnoff et al. suggested introduction of a lipophilic substituent of a suitable shape and size to increase van der Waals interactions in an attempt to improve the affinity.[3] Alternatively, another idea that was introduced was to extend a part of the compound further into the central pore with the intent of forming additional hydrogen bonds with the residues in the pore or forming new hydrophobic interactions. Methylation, of the lactam analogue, was successfully introduced to position 3 of the aromatic ring of the tetrahydroisoquinoline moiety and showed only a
slight reduction in affinity with a half maximal inhibitory concentration (IC₅₀) of 7.1 µM compared with compound 9 with IC₅₀ of 2.3 µM (Scheme 1.2).[³]

1.2.3 Other reported non-covalent inhibitors

Other small molecule inhibitors have been discovered and their binding orientation analysed using X-ray crystallography of the corresponding co-crystal structures.[⁴] A symmetric 1,4-diaminonaphthalene cored molecule (Scheme 1.3, left) was found to have very similar binding affinity to compound 9 (Kᵥd = 1.7 µM compared to 1.0 µM for compound 9). The compound was also found to occupy the P3 subpocket of Keap1 with its naphthalene moiety and the P4 and P5 pockets with its two anisole substituents. However, the two hot spots of the Kelch binding pocket, the polar P1 and P2, remained unoccupied compared with compound 9 where P2 is occupied. Derivatives of 1,4-diaminonaphthalene core-1 have been synthesized with impressive results. 1,4-diaminonaphthalene core-2 was found to have much higher affinity toward Keap1 compared to its analogue, yielding a Kᵥd = 3.6 nM (Scheme 1.3, middle). This result highly suggests that the two newly introduced carboxyl acid substituents on 1,4-diaminonaphthalene core-2 interact with important arginine residues in P1 and P2, Arg415 and Arg483, respectively. Furthermore, substitution of the carboxylic acid with a bioisostere, tetrazole, maintained a low nanomolar activity which indicates that a substituent of similar properties to the carboxylic acid is essential for successful binding.[⁴] Finally, inhibitors with a 3-phenylpropanoic core have also been discovered (Scheme 1.3, right). The 3-phenylpropanoic acid core was found to be active towards Keap1, with Kᵥd = 1.3 nM. It was developed for chronic obstructive pulmonary disease and does require oral administration as it can be inhaled.[⁴]

![Scheme 1.3. Three examples of previously synthesized compounds and the binding constants.](image)

1.3 Surface Plasmon Resonance

Surface plasmon resonance (SPR) is a powerful technique for detection of biomolecular interactions.[⁷] It is an optical based technique that allows for real-time measurements of molecular interactions in a label free manner. Since no labelling is needed, sample preparation takes shorter time and there is no risk of the label interfering with the interaction. The output of SPR analysis gives comprehensive evidence about the binding event and gives both an affinity value and kinetic parameters for association and dissociation of the compound. This information is especially informative for SAR studies. Compared with other technologies which do not use labels, such as, titration or scanning calorimetry, reduced amount of sample is needed when utilizing SPR biosensors and they have higher throughput.
Overall, SPR is a well established technique that is commonly used in the pharmaceutical industry for drug discovery and development e.g. for initial screening of compounds.\[7\]

SPR biosensors consist of a liquid phase, where analytes flow in solution, and a solid phase (sensor surface) which consists of a disposable sensor chip where a biomolecule of interest is immobilized on a thin metal surface.\[8\] During a measurement, the analyte solution flows over the sensor surface allowing interactions between the immobilized biomolecule and the analyte to take place (Figure 1.3). The technique is based on the phenomenon of SPR where light waves (photons) are transferred into electron waves (plasmons). In SPR biosensors, this is achieved by applying an incident light to the thin metal layer of the sensor surface. A part of the incident light couples with the electrons in the metal layer, creating plasmon resonance. Since the SPR is sensitive to changes in the refractive index of the medium near the surface, any binding interaction taking place between an analyte in solution and the immobilized biomolecule, is detected. As the mass accumulates on the surface during complex formation, the refractive index change is monitored in real-time through the changes that occur in the angle of the reflected light.\[6, 7\]

**Figure 1.3.** Schematic representation of a SPR biosensor. An incident light is applied to the sensor chip surface through a high refractive glass prism. As analytes in solution flow by the sensor surface, any interactions between the analyte molecules and the immobilized biomolecules on the sensor surface are detected through the changes in the refractive index that affect the SPR on the surface. The changes in the refractive index result in changes in the angle of the reflected light that hits the light detector. Adopted from \[7\]

It is important that the binding site of the immobilized ligand remains accessible.\[7\] This can be achieved by minimising steric constraints and nonspecific interactions by utilizing a surface which contains a non-crosslinked hydrogel when performing the experiment. Studies have shown that there is no measurable difference between the obtained thermodynamic parameters afforded from three different methods, SPR, isothermal calorimetry and stopped-flow fluorescence.\[9\] These results clearly indicate that immobilisation of the biomolecule on the surface has no effect on the binding constants which confirms the reliability of SPR biosensors as a biophysical method in drug discovery. SPR biosensors can be used for studies of a variety of different types of molecular interactions, receptors, digomers, antibodies, enzymes, albumin, and membrane surfaces, and they can be used for target identification and characterisation to support clinical studies. SPR biosensors have high sensitivity rendering them capable of monitoring compounds as small as fragments (>90 Da) and they are capable measuring affinities in the milli- to picomolar range with a typical detection level of < 1 µg/mL. The SPR biosensor surface has furthermore been shown to successfully be
able to mimic membrane environments *in vitro*. By utilizing lipid layers of varying structure and fluidity, it is possible to use the biosensor to estimate compounds permeability.[7]
2 Aim of the Project

The aim of the project was to reproduce the synthesis of compound 9, a reported inhibitor of the Keap1-Nrf2 PPI (Scheme 2.1, left).\(^3\) In addition to this, using the target-bound crystal structure of compound 9, a set of analogues was designed and synthesized (Scheme 2.1, right). The analogues were designed to form interactions with an important arginine residue in the P1 subpocket of Keap1, as well as to displace a water molecule present in the binding pocket (Figure 3.1).

These analogues were chosen as they were able to reach into the P1 subpocket of Keap1, according to the docking performed, and they were synthetically feasible.

The synthetic route initially planned was to introduce an isoindoline derivative as building block B via N-alkylation (Scheme 2.2), where the alcohol of building block A had been transformed into a proper leaving group. Subsequently, building block C could be introduced via amide coupling, followed by hydrolysis of the methyl ester to yield the target compounds.

Due to problems with the synthesis, a new set of compounds was designed and synthesized (Scheme 2.3). These were chosen as the docking was promising.
Scheme 2.3. *The designed and synthesized novel inhibitors.*

Lastly, the synthesised compounds were to be tested in a Keap1 binding assay and their affinities to be compared with the reference, compound 9.¹

¹ Assays carried out by specialists at Astra Zeneca, Gothenburg.
3 Results and Discussions

3.1 Computational studies

3.1.1 Design of Target Molecules

The co-crystal structure of the Keap1-9 complex was used as a starting point for the docking of the designed compounds. The ligand design was centred on modifications of the isoindoline moiety while the rest of the molecule was left unchanged to probe its effect on Keap1 binding (Scheme 2.1). The analogues were designed to occupy more space in the P1 subpocket of the active site (Figure 3.1, red) and new interactions with key amino acid residues (Arg415 and Ser508) could potentially be established by extending the molecule further into the P1 subpocket. The newly designed molecules were docked, and their free energy of binding was estimated for their most favourable position with the use of the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method. This force-field based method uses snapshots acquired from molecular dynamics simulations to approximate free energy of binding from the use of solvation models and solvent accessibility methods.\(^\text{[10]}\) This method was used as it is the fastest method available.\(^\text{[11]}\) An average of the computed binding free energies of the five poses for each molecule yields the final estimation of the binding free energy.

\[9-1 = -80.90 \text{ kcal/mol}\]
\[9-2 = -72.725 \text{ kcal/mol}\]
\[(E)-26 = -81.52 \text{ kcal/mol}\]
\[(E)-26 = -85.95 \text{ kcal/mol}\]

\[^2\text{Figure provided by a group member: Dr. Vasanthanathan Poongavanam}\]
## 3.1.2 Docking results

Free energies of binding were computed for the original set of compounds (Appendix B) as well for the synthesized compounds. The binding free energies were computed both with and without solvent molecules present in the binding site (Table 3.1). The solvent molecules can assist in establishing invaluable interactions. Therefore, it is of interest to obtain estimated energies which indicate if it should be aimed to displace a solvent molecule or not.

### Table 3.1. Summary of computed MM-GBSA binding free energy of synthesized compounds. Energies displayed are the results for the best and worst pose computed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MM-GBSA [kcal/mol]</th>
<th>Active site with water</th>
<th>Active site without water</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td></td>
<td>-80.90</td>
<td>-72.73</td>
</tr>
<tr>
<td>(E)-26</td>
<td></td>
<td>-81.15</td>
<td>-66.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-81.52</td>
<td>-85.95</td>
</tr>
<tr>
<td>(Z)-26</td>
<td></td>
<td>-85.47</td>
<td>-42.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-66.67</td>
<td>-26.47</td>
</tr>
<tr>
<td>(E)-30</td>
<td></td>
<td>-54.92</td>
<td>-78.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-35.00</td>
<td>-77.38</td>
</tr>
<tr>
<td>(Z)-30</td>
<td></td>
<td>-65.14</td>
<td>-41.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-56.70</td>
<td></td>
</tr>
<tr>
<td>(E)-31</td>
<td></td>
<td>-82.84</td>
<td>-81.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-77.17</td>
<td>-77.49</td>
</tr>
<tr>
<td>(Z)-31</td>
<td></td>
<td>-95.90</td>
<td>-66.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-89.05</td>
<td>-37.85</td>
</tr>
</tbody>
</table>

The results indicate that some of the synthesized compounds have the potential to have increased affinity toward Keap1. The results suggest that for (Z)-26 the water molecule in the active site is essential to obtain reasonable binding. Results for (E)-30 showed the opposite, a more favourable binding energy was obtained without water molecule present. The results for (E)-31 were very similar both with and without water molecule. According to these results, (Z)-31 with water molecule present in the active site has the highest potential for increased affinity toward Keap1 compared to 9.

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3 Docking was performed by a group member: Dr. Vasanthanathan Poongavanam
3.2 Synthesis of the reference molecule (9)

The synthesis of the reference molecule, 9, was carried out in a similar manner to that previously reported (Scheme 3.1). However, some minor modifications were made based on the availability of compounds (Scheme 3.1, red).

Scheme 3.1. Synthetic route towards the reference compound, 9. Reagents and conditions: a) methyl 2-bromoacetate, K$_2$CO$_3$, DMF, rt, 1.5 h; b) 2 M KOH, THF, 10 min, then 12 M HCl; c) SOCl$_2$, DCM, DMF, reflux, 1 h, then AlCl$_3$, DCM, rt, 1 h, then H$_2$O; d) H$_2$, Pd/C, MeOH, 5 bar, rt, 18 h; e) 5 M KOH, MeOH, reflux, 2 h; f) Boc$_2$O, TEA, THF, rt, 1 h; g) PPh$_3$, DBAD, THF, rt, 30 min, then phthalimide and 6, rt, 3 h; h) 4 M HCl, rt, 1 h, 7a treated as an intermediate; i) (1R,2S)-2-((benzoyl)carbonyl)cyclohexane-1-carboxylic acid, EDC·HCl, HOBt·H$_2$O, DIPEA, rt, 18 h, then 40 °C, 24 h; j) H$_2$, Pd/C, MeOH/THF (2:1), 5 bar, rt, 24 h. Indicated in red is what differs from the literature. *Purification by preparative HPLC performed twice.

The synthesis started from (S)-4-phenyloxazolidin-2-one, which is the product acquired after the first reaction as reported by Jnoff et al.[3] The compound is commercially available and one step in the synthetic route is saved. (S)-4-phenyloxazolidin-2-one was N-alkylated by treatment with methyl bromoacetate to yield compound 1, which was subsequently hydrolysed to give the free acid 2. Methyl bromoacetate was used rather than ethyl bromoacetate as it was available in the lab. Friedel-Crafts acylation of 2 resulted in the tetrahydroisoquinolinone derivative 3, which was subsequently reduced by catalytic
hydrogenation to yield 4. The cyclic carbamate moiety of 4 was cleaved by basic hydrolysis to afford tetrahydroisoquinoline 5. Tert-Butyloxycarbonyl (Boc) protection of the free amine of compound 5 afforded 6. A Boc protective group was used instead of reported benzyl protective group because it is less time consuming and does not require catalytic hydrogenolysis. The introduction of a Boc protective group was introduced with Boc anhydride in the presence of triethylamine (TEA). With the Mitsunobu reaction, the phthalimide moiety was introduced to afford 7. The Boc protective group was then removed with 4 M HCl to give the intermediate 7a, which was subsequently subjected to coupling with (1R,2S)-2-(benzyloxycarbonyl)cyclohexane-1-carboxylic acid (ee 80%) to afford amide 8. Finally, hydrogenolysis of the benzyl protective group successfully afforded the reference compound 9.

The amide coupling to obtain 8 was successfully carried out by using HOBt and EDC instead of using T3P as reported in the literature. T3P reaction was not tested for this specific reaction. T3P was tested for isoindoline derivatives, however, the HOBt/EDC reaction gave a better yield (Chapter 3.4.2).

Initially compound 9 was to be synthesized via amide coupling of 7a with (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid and the methyl ester subsequently cleaved to obtain the acid 9. The reported benzyl ester substrate is not commercially available and is synthesized from the strongly allergenic cis-hexahydroisobenzofuran-1,3-dione. Therefore, the commercially available methyl ester substrate was used. The target molecule was, however, not obtained from hydrolysis of the methyl ester. After the first attempt, the methyl ester was still clearly observed by 1H NMR analysis. LC-MS analysis detected a M+1 peak, however, a more intense M+18 peak was also found, which indicates addition of a water molecule. The methyl and the aromatic signals appeared more downfield compared with the starting material. A second attempt with higher concentration of base was tested. LC-MS analysis detected a M+1 peak and a more intense M+18/36 peak. Although 1H NMR analysis confirmed cleavage of the methyl ester, the spectrum was not in agreement with the literature. The literature conditions were reproduced with benzyl ester substrate (ee 80%) which was available in the lab. The amide coupling was carried out successfully, following hydrogenolysis of the benzyl to afford the target compound. LC-MS analysis found only the M+1 peak and 1H NMR analysis confirmed the compound.
3.3 Original synthetic strategy

The original strategy relied on a synthetic approach similar to the one employed for the reference compound, 9. In detail, the different building blocks were to be separately synthesized and then connected via N-alkylation of the isoindoline derivative. However, for that to work, the primary alcohol of building block A needed to be converted into a suitable leaving group (Scheme 3.2).

3.3.1 Synthesis of building block A – tetrahydroisoquinoline moiety

Compounds 5 and 6 possess a primary alcohol which should undergo SN2 reaction with a nucleophile once transformed into a leaving group. Three different compounds, 10-12, were synthesized from compound 6 with mesylate, tosylate, and iodide as leaving groups (Scheme 3.2, ii). Additionally, compound 5 was converted into a cyclic sulfamidate, 13, which acts as a leaving group (Scheme 3.2, iii).

\[ \text{Compounds 5 and 6} \]

\[ \text{Reagents and conditions: a) methansulfonyl chloride, TEA, DCM, rt, 30 min; b) p-toluenesulfonyl chloride, TEA, DCM, rt, 22 h; c) } I_2, PPh_3, \text{imidazole, THF, rt, 1 h; d) imidazole, TEA, SOCl}_2, \text{DCM, 1 h 0 }^\circ\text{C, then H}_2\text{O, then NaIO}_4, \text{RuCl}_3, \text{H}_2\text{O, H}_2\text{O, EtOAc, rt, 2.5 h.} \]
3.3.2 Synthesis of building block B – isoindoline moiety

Building block B constitutes the isoindoline derivatives (Scheme 3.3).

Scheme 3.3. The isoindoline moiety.

The terminal alkynes were synthesized prior to their reaction with N-hydroxyphthalimide to afford the desired building blocks B. Compound 14 was prepared via oxidation of the corresponding alcohol utilizing activated manganese(IV) oxide. Compound 15 was prepared via alkylation of propiolic acid. The building blocks (Z)-16, (Z)-17, and (E)-18 were then synthesized from the pre-made terminal alkynes and N-hydroxyphthalimide. By reducing the temperature and reaction period, the E isomer could be synthesized selectively (Scheme 3.4, iii).

Scheme 3.4. Synthetic route towards building block B. Reagents and conditions: a) MnO₂, THF, rt, 20 h; b) benzyl bromide, K₂CO₃, DMF, rt, 18 h; c) 14, K₂CO₃, DMF, 60 °C, 17 h; d) 15, K₂CO₃, DMF, 100 °C, 18 h; e) 15, K₂CO₃, DMF, 60 °C, 3 h.

The benzyl propiolate, 15, was chosen instead of the originally planned phenyl propiolate since the synthesis of the latter was unsuccessful (Scheme 3.5). The desired phenyl propiolate was not observed via LC-MS nor any traces recovered from purification. However, the by-product, generated by a Michael addition of the phenyl propiolate was the only isolated product. This was confirmed by LC-MS and NMR analysis.
Scheme 3.5. Synthetic route towards phenyl propiolate. Phenyl propiolate was not observed using the literature conditions, red crossed over arrow.\textsuperscript{[14]} However, the Michael addition product was observed, bottom black arrow.\textsuperscript{[13]}

Due to difficulties in the synthesis and isolation of the products, the other two original building blocks B (Scheme 2.1, B2 and B4) were abandoned. The focus was set on synthesizing (Z)-17 as it was afforded in higher yields than the other two compounds. Therefore, this compound was used for optimizing the reaction conditions for the N-alkylation of building block B with A.
### 3.3.3 Attempted N-alkylation of building block B

Various reaction conditions were tested for N-alkylation of building block B (Table 3.2). As a first attempt the Mitsunobu reaction was tried, with the same conditions as used for the synthesis of 7 (Scheme 3.6, i). No reaction was observed after 24 h (Table 3.2 entry 1).

After deprotonation of (Z)-17, the electrophile was introduced in excess (Scheme 3.6, ii). No reaction was observed by LC-MS and TLC. Where purification was performed, only (Z)-17, eluted (Table 3.2, entries 2-6). As a result of these failed reactions it was decided to test the nucleophilicity of (Z)-17 (Table 3.2, entry 7). 4-Nitrobenzyl bromide was chosen as electrophile for the test reaction since it was reported in the literature to be successful with similar substrates (Scheme 3.6, iii).\(^{[12]}\) However, after 6 h there were no sign of N-alkylation occurring, only the separate substrates were observed. A common trend among the first set of reactions was that after introducing NaH to the system, a precipitate was formed, most likely the sodium salt of compound (Z)-17. To keep the substrate in solution it was necessary to remove the sodium counterion. This was achieved by complexing the counterion by...
utilizing the cyclic ether 15-crown-5 (15C5), specific for the sodium cation. The reaction with 4-nitrobenzyl bromide was then repeated with the same conditions as before with the addition of the crown ether, showing completion after 2 h (Table 3.2, entry 8). NMR analysis of the crude product confirmed that the reaction had proceeded, and N-alkylated product was afforded. After discovering that the crown ether was essential for the reaction to proceed, (Z)-17 was tested against a less activated alkyl halide (Table 3.2, entry 9). After 24 h N-alkylation was observed, however, only to low extent. After reacting for additional 4 days the reaction had reached completion (Scheme 3.6, iii). This proved that the N-alkylation was feasible, and the previous reactions were then repeated with the new reaction conditions, using compounds 13, 10, and 11 as the electrophile (Table 3.2, entries 10-13). However, no reaction was observed to have occurred (Scheme 3.6, ii). Again, only (Z)-17 was observed. Since it had been observed that N-alkylation could be achieved, a reaction was carried out to test the electrophilicity of building block A. Compound 11, however, was observed to undergo nucleophilic substitution with sodium azide in an overnight reaction (Table 3.2, entry 14). Compound 10 was also observed to undergo nucleophilic substitution with sodium azide in DMF, while the reaction did, however, not proceed in THF (Table 3.2, entries 15 and 16). This showed that building block A and B could undergo nucleophilic substitution and N-alkylation, respectively.

To evaluate further conditions, the reaction between (Z)-17 and 11 was repeated following a different procedure (Table 3.2, entry 17). A solution of (Z)-17, NaH and crown ether was added dropwise to a solution of 11 and monitored by LC-MS at regular intervals. Again, no reaction was observed to have taken place, only (Z)-17 was observed. Entry 17 was repeated, this time with compound 12 as the electrophile (Table 3.2, entry 17). No reaction was observed to have taken place. An attempt was tested where the N-H was deprotonated to make it a better nucleophile (Table 3.2, entry 19). A solution of (Z)-17, NaH and crown ether was added dropwise to a solution of compound 6, PPh₃, and di-tert-butyl-azodicarboxylate (DBAD) and reacted at rt for 3 days, then at 90 °C for additional 4 days. Only trace amounts of N-alkylation product were observed by LC-MS. As a last attempt, the N-alkylation of (Z)-17 was attempted with 1 m NaHMDS in THF as base, following the same procedure as before (Table 3.2, entries 20-21). In both cases no reaction was observed after 18 h. Crown ether was introduced to the systems in same equivalents to the base. After additional 24 h, traces of N-alkylation product were observed for the reaction with iodopropane. No reaction was observed for compound 10 as the electrophile. Both systems were then heated to 50 °C and after additional 24 h, the reaction with iodopropane showed roughly 50% completion, as judged by LC-MS. The reaction with compound 10, however, did not show any conversion.

The strategy of separately synthesizing building block A and B and combining the two through N-alkylation was unsuccessful. Therefore, a new strategy was required.
Table 3.2. Reaction conditions of the attempted N-alkylation (Scheme 3.6). Equivalent relative to nucleophile.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Electrophile Compound (eq)</th>
<th>Nucleophile Compound</th>
<th>Reaction conditions (eq)[d]</th>
<th>Reaction time [h]</th>
<th>Product detected (Yes/No)[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>(Z)-17</td>
<td>PPh₃, DBAD, THF, rt</td>
<td>24</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>10 (1.4)</td>
<td>(Z)-17</td>
<td>NaH, THF, rt (1.0)</td>
<td>72</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>10 (1.0)</td>
<td>(Z)-17</td>
<td>NaH, THF, rt (1.2)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>12 (1.0)</td>
<td>(Z)-17</td>
<td>NaH, THF, rt (1.2)</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>10 (1.1)</td>
<td>(Z)-17</td>
<td>NaH, DMF, rt (1.0)</td>
<td>18</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>13 (0.8)</td>
<td>(Z)-17</td>
<td>NaH, DMF, 70 °C (1.0)</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>4-nitrobenzyl bromide (2.0)</td>
<td>(Z)-17</td>
<td>NaH, THF, 40 °C (2.0)</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>4-nitrobenzyl bromide (2.0)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, THF, 40 °C (2.0)</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>1-iodopropane (2.5)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, THF, rt (1.1)</td>
<td>120</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>13 (1.2)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, THF, 40 °C (2.0)</td>
<td>24</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>10 (2.0)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, THF, 70 °C (2.0)</td>
<td>18</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>10 (0.4)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, 60 °C, DMF (1.0)</td>
<td>0.5</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>11 (1.1)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, DMF, 70 °C (1.1)</td>
<td>22</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>11 (0.4)</td>
<td>NaN₃</td>
<td>DMSO, 45 °C</td>
<td>24</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>10 (0.4)</td>
<td>NaN₃</td>
<td>DMF, 60 °C</td>
<td>24</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>10 (0.4)</td>
<td>NaN₃</td>
<td>THF, 60 °C</td>
<td>24</td>
<td>No</td>
</tr>
<tr>
<td>17</td>
<td>11 (1.1)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, DMF, rt (1.1)</td>
<td>72</td>
<td>No</td>
</tr>
<tr>
<td>18</td>
<td>12 (2.0)</td>
<td>(Z)-17</td>
<td>NaH, 15C5, DMF, rt (1.1)</td>
<td>96</td>
<td>No</td>
</tr>
<tr>
<td>19</td>
<td>6 (1.0)</td>
<td>(Z)-17</td>
<td>NaH, 15C5 / PPh₃, DBAD, THF, rt-90 °C (1.1 / 1.6)</td>
<td>168</td>
<td>No</td>
</tr>
<tr>
<td>20</td>
<td>1-iodopropane (2.5)</td>
<td>(Z)-17</td>
<td>NaNHMD'S, 15C5, THF, rt-50 °C (1.1)</td>
<td>72</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>10 (2.5)</td>
<td>(Z)-17</td>
<td>NaNHMD'S, 15C5, THF, rt-50 °C (1.1)</td>
<td>72</td>
<td>No</td>
</tr>
</tbody>
</table>

[a] Detection by LC-MS analysis. [b] Reaction worked up and purified, only building block B recovered, no indications of N-alkylation. [c] NMR analysis. [d] Dry DMF used.
3.4 Revised synthetic strategy

3.4.1 Design of the new synthetic strategy

Synthesis of similar compounds to building block B have been reported via catalytic copper reaction of phenylacetylene and o-iodobenzamide (Scheme 3.7).\textsuperscript{[16]} It is noteworthy that this type of isoindoline formation has only been reported for phenylacetylene. Below is described how the reaction conditions were applied to a more complex o-iodobenzamide substrate and more complicated alkynes constituting a ketone and an ester, respectively.

\[ \text{phenylacetylene} + \text{o-iodobenzamide} \xrightarrow{\text{Cu catalyst}} \text{desired isoindoline derivative} \]

Scheme 3.7. Reported ring closure to afford the desired isoindoline derivative.\textsuperscript{[16]}

A new strategy was formulated where the suggested product of the attempted N-alkylation would be afforded by applying the copper reaction to the o-iodobenzamide substrate with phenyl acetylene and other terminal alkynes. The o-benzamide substrate would be synthesized via amide coupling between o-iodobenzoic acid and the primary amine of building block A, which can be synthesized from the corresponding primary alcohol, 6 (Scheme 3.8).

\[ \text{amine} + \text{o-iodobenzoic acid} \xrightarrow{\text{HOBt and EDC}} \text{desired product} \]

Scheme 3.8. Retrosynthesis of the same product as suggested by the N-alkylation approach.

3.4.2 Synthesis of the target compounds

Scheme 3.9 illustrates the synthesis of two of the desired products, 22 and 23. Compound 10 undergoes a S_N2 reaction to afford the azide 19, which was subsequently subjected to catalytic hydrogenation to afford the primary amine 20. Compound 21 was synthesized by amide coupling between amine 20 and o-iodobenzoic acid, using a combination of HOBt and EDC as coupling agents. Compounds 22 and 23 were then synthesized via copper catalysed ring closure. The catalytic ring closure resulted in a mixture of E and Z isomers of the newly formed double bond, ratio 6:4 and 7:3 for 22 and 23, respectively, as calculated...
The copper catalyzed isoindoline formation was followed by Boc deprotection and amide coupling with (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid using HOBt and EDC, to afford amides 24 and 25. Hydrolysis of the methyl ester afforded the final products 26 and 27 as mixtures of E and Z isomers in ratio 8:2 for both compounds. The hydrolysis of methyl ester 24 was successful, the hydrolysis of 25, however, resulted in hydrolysis of both the methyl and benzyl ester. In both cases it was not possible to separate the isomers, and both products were isolated as a mixture of E and Z isomers, ratio 6:4 for both compounds. Initially, the intent was to only hydrolyse the methyl ester of compound 25. However, LC-MS analysis indicated that the desired benzyl ester was also undergoing hydrolysis. Therefore, it was decided to increase the reaction time and introduce more base to the reaction mixture to drive the hydrolysis of both esters forward. This resulted in the diacid, compound 27.

Scheme 3.9. Synthetic route towards two final compounds. Reagents and conditions: a) NaN₃, DMF, rt, 20 h; b) H₂, Pd/C, MeOH, 5 bar, rt, 18 h; c) o-iodobenzoic acid, EDC∙HCl, HOBr∙H₂O, DIPEA, DMF, rt, 18 h; d) terminal alkyne, Cu(OAc)₂∙H₂O, K₂CO₃, DMF, 60 °C, 18 h; e) 4 M HCl in dioxane then (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid, EDC∙HCl, HOBr∙H₂O, DIPEA, DMF, rt, 18 h; f) 1 M LiOH∙H₂O, LiOH∙H₂O (s), MeOH, rt, 24-72 h. **Purification by preparative HPLC.}

This result clearly indicated that having the second ester on the isoindoline moiety causes problems with the hydrolysis. Therefore, a different route was taken to synthesize the final two compounds, 30 and 31. In the new route the copper catalysed ring closure was carried
out as the final step to overcome the problematic hydrolysis of the methyl ester (Scheme 3.10). Compound 21 was Boc deprotected and the resulting free amine was subjected to amide coupling with (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid to afford amide 28, whose methyl ester was subsequently cleaved to afford acid 29. As a final step, the catalytic copper reaction was carried out to provide compounds 30 and 31. Compounds 30 and 31 were obtained as a mixture of isomers (major:minor 2:1) around the newly formed C-C double bond. This approach however afforded the desired compounds in low yields. LC-MS analysis indicated the significant formation of by-product, roughly 1:1 with the desired compound. The by-product was isolated in 7 mg yield. $^1$H NMR analysis showed the same number of protons as in the starting material minus the acid proton. This could indicate formation of a macrocycle through intramolecular ester formation.

![Scheme 3.10](image)

**Scheme 3.10.** Synthetic route towards two final compounds. Reagents and conditions: a) $4 \text{M} \text{HCl}$ in dioxane, then (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid, EDC·HCl, HOBr·H$_2$O, DIPEA, DMF, rt, 18 h; b) 1 M LiOH·H$_2$O, LiOH·H$_2$O (s), 35 °C, 48 h; c) compound 14, Cu(OAc)$_2$·H$_2$O, K$_2$CO$_3$, DMF, 60 °C, 18 h; d) compound 15, Cu(OAc)$_2$·H$_2$O, K$_2$CO$_3$, DMF, 60 °C, 18 h. *Purification by preparative HPLC performed twice. ** Purification by preparative HPLC.

Utilizing HOBt and EDC to achieve amide coupling is not the only feasible route. As reported for the synthesis of the reference compound 9, the coupling was performed with 2,4,6-Tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P) solution.$^3$ The reaction was monitored by LC-MS and TLC and deemed to have reached completion once no more amine could be detected in the reaction mixture. The consumption of T3P itself could also be monitored this way as it is UV active. After aqueous workup no more T3P could be observed by either LC-MS or TLC. The only peak observed was the one corresponding to the desired mass. However, the reaction only afforded the product in 5 % yield, even though the completion of the reaction was confirmed by LC-MS and TLC. LC-MS analysis of the crude and the aqueous phase after liquid-liquid extraction indicated that all the product had been successfully extracted from the aqueous phase. Utilizing HOBt/EDC
for the amide coupling instead afforded the desired compound in 92% yield. Therefore, it was decided to apply these conditions for the amine to amide couplings.

The copper catalysed ring closure was reported with a ligand, 2,2'-biimidazole.\textsuperscript{[16]} The reaction was carried out both in presence and absence of the ligand, however, no difference in reaction rate or yields was observed. LC-MS analysis did not indicate any difference in the ratio between the two isomers. The reaction was also tested at 100 °C to probe the temperature effect on the $E$:Z selectivity. However, no changes in the reaction outcome were observed. Scheme 3.11 illustrates the proposed reaction mechanism. Step A, the base deprotonates the acetylene which is coordinated with the copper. Step B, Sonogashira type reaction which releases the catalyst. Step C and D, deprotonation of the amide which subsequently attacks the C-C triple bond which has been coordinated by the copper. Step E, protonation releases the copper catalyst and affords the product.\textsuperscript{[16]}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme3_11.png}
\caption{Scheme 3.11. Proposed mechanism of the copper catalysed ring closure adopted from.\textsuperscript{[16]}}
\end{figure}
3.5 SPR assays

The biological activity of the target compounds was tested using SPR inhibition in solution assay (ISA). In the experiment, Nrf2 is immobilized on the surface, Keap1 is flowed through which interacts with Nrf2. The small-molecule is then flowed through to compete for the Keap1 interaction. The reference compound, 9, showed micro molar affinity, similar to what previously had been reported ($K_d = 1.0 \, \mu M$).\cite{4} The synthesized analogues were all found to have two orders of magnitude weaker affinity toward Keap1 (Table 3.3).

*Table 3.3. ISA results showing the obtained affinities for the compounds binding to Keap1.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_d$ [$\mu M$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>26</td>
<td>157.4 ± 9.9</td>
</tr>
<tr>
<td>27</td>
<td>312.7 ± 89</td>
</tr>
<tr>
<td>30</td>
<td>480.3 ± 100</td>
</tr>
<tr>
<td>31</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>
4 Conclusion and Future Outlooks

The aim of the project was to design and synthesize novel derivatives of a known small-molecule inhibitor of the Keap1-Nrf2 PPI. The original set of compounds proved problematic to synthesize and were all abandoned with one exception (Scheme 2.1, B3). It was discovered that N-alkylation of building block B was feasible with 4-nitrobenzyl bromide and the less activated 1-iodopropane. However, N-alkylation was only obtained in the presence of a crown ether. Despite discovering the necessity of the crown ether, the N-alkylation was not observed for the desired electrophile. A new approach was formulated which was successfully applied and yielded a set of novel compounds. This approach included a copper catalysed isoindoline formation which has to date only been reported for phenylacetylene. This study has shown that these reaction conditions can be applied to more complex substrates and more reactive terminal alkynes. The catalytic reaction, however, resulted in a mixture of E and Z isomers. Furthermore, there was a considerable difference in the obtained yield when using the reported phenylacetylenes compared with the corresponding benzyl ester when synthesizing 22 (92%) and 23 (63%), respectively. Starting from a much more complex substrate, which includes a free acid, yields obtained were very poor, 30 (5%) and 31 (13%). A total of four novel potential small-molecule Keap1-Nrf2 PPI inhibitors were successfully synthesized (Table 4.1). Results from the biological activity experiments show that none of the synthesized derivatives were active. The results obtained for the reference, 9, were in agreement with what has previously been reported.\[4\]

All compounds were initially identified with LC-MS. Separation of the novel compounds by diastereomers is needed as well as full characterization by NMR analysis. Some experiments that might help with the characterization of the compounds include recording of the spectra with a narrower spectral width as it would result in higher resolution especially for 2D spectra. Furthermore, recording a N-H HMBC might provide significant insight to the characterisation and by performing a variable-temperature NMR (VT NMR) experiment it would be possible to identify the compounds coalescence temperature and hence obtain simpler spectra. This can help distinguishing between if the compounds are truly rotamers or if they are diastereomers. It is also possible to utilize a 1D gradient NOE experiment to distinguish between if what is observed is actually rotamers or if they are diastereomers.\[17\]
### Table 4.1. Summary of the synthesized target molecules and overall yields.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Structure</th>
<th>Number of steps</th>
<th>Isomeric ratio(^a)</th>
<th>Yield [%]</th>
<th>(K_d) [µM]</th>
<th>ISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td><img src="image1" alt="Structure" /></td>
<td>9</td>
<td>n.a.</td>
<td>10.3</td>
<td>1.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td><img src="image2" alt="Structure" /></td>
<td>13</td>
<td>8:2</td>
<td>0.94</td>
<td>157.4 ± 9.9</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td><img src="image3" alt="Structure" /></td>
<td>13</td>
<td>8:2</td>
<td>0.54</td>
<td>312.7 ± 89</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td><img src="image4" alt="Structure" /></td>
<td>13</td>
<td>8:2</td>
<td>0.48</td>
<td>480.3 ± 100</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td><img src="image5" alt="Structure" /></td>
<td>13</td>
<td>8:2</td>
<td>1.24</td>
<td>&gt;500</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Calculated by NMR analysis.
5 Experimental

5.1 General methods

All reagents were purchased from Sigma Aldrich, Fluorochem, and VWR International. The solvents, DCM, DMF, ethyl acetate, and methanol were purchased from VWR International. THF and anhydrous DMF were purchased from Sigma Aldrich. All non-aqueous reactions were carried out in oven dried glassware under an inert atmosphere of argon. For the evaporation of solvents, a Büchi R-114 rotary evaporator was used. All reactions were monitored via LC-MS and/or TLC. The LC-MS instrument was equipped with an Agilent 1100 series HPLC with a C18 Atlantis T3 column (3.0x50.0 mm, 5 µm) using acetonitrile/deionized water as mobile phase, with a flow rate of 0.75 mL/min over 7.5 min and a Waters micromass ZQ (model code: MM1) mass spectrometer with electrospray ionization mode as detector. TLC silica gel 60 F254 plates, purchased from VWR International, were used and visualised under UV light, at 254 nm. Hydrogenation was performed in a Parr hydrogenator (series 5100). Purification by flash column chromatography was done using a Biogel® Isolera™ instrument using Luknova SuperSep™ prepacked silica flash columns (40-60 µm). Purification of final compounds were carried out using a preparative reversed-phase HPLC, a Gilson HPLC, on a Kromasil C8 column (250x21.2 mm, 5 µm) equipped with Gilson 322 pump, UV/Visible-156 detector, with detection at 214 and 254 nm, and 202 collector using acetonitrile/distilled water gradients as mobile phase with a flow rate of 15 mL/min.

1H, 13C, COSY, HSQC, HMBC nuclear magnetic resonance spectra were recorded at 298 K on a Varian spectrometer at 400 MHz or 100 MHz, on a Bruker 500 spectrometer at 500 MHz or 126 MHz, or on a Bruker Avance III spectrometer at 600 MHz or at 151 MHz. For all spectra, CDCl3 or DMSO-d6 was used, the residual signal of the respective solvent was used as an internal standard, δH 7.26 ppm and δC 77.0 ppm, and δH 2.50 ppm and δC 39.5 ppm for CDCl3 and DMSO-d6, respectively.

5.2 Experimental

5.2.1 Methyl (S)-2-(2-oxo-4-phenyloxazolidin-3-yl)acetate (1)[3]

\[
\text{\includegraphics[width=1in]{methyl-2-oxo-4-phenyloxazolidin-3-yl-acetate.png}}
\]

To a solution of (S)-4-phenyloxazolidin-2-one (4.89 g, 30.0 mmol, 1.0 eq) and 2-bromoacacetate (2.93 mL, 33.0 mmol, 1.1 eq) dissolved in DMF (50 mL) was added K2CO3 (11.6 g, 83.6 mmol, 3.0 eq). The resulting mixture was stirred for 1.5 h. The reaction mixture was diluted with EtOAc (250 mL) and washed with H2O (3x150 mL) and brine (50 mL). The organic phase was dried over Na2SO4 and concentrated in vacuo. The crude product was afforded as a yellow oil in quantitative yield and used without further purification in the next step.
The $^1$H NMR spectrum is in agreement with the reported one.$^{[18]}$

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.45 – 7.36 (m, 3H), 7.32 – 7.25 (m, 2H), 5.06 (t, $J = 8.4$ Hz, 1H), 4.71 (t, $J = 8.8$ Hz, 1H), 4.29 (d, $J = 18.0$ Hz, 1H), 4.15 (t, $J = 8.4$ Hz, 1H), 3.71 (s, 3H), 3.39 (d, $J = 18.0$ Hz, 1H).

LCMS (ESI) calcd for C$_{12}$H$_{14}$NO$_4$ $^+ \ [M-H]^+$ 236.1, found 236.3. $R_t = 3.20$ min.

5.2.2 (S)-2-(2-oxo-4-phenyloxazolidin-3-yl)acetic acid (2)$^{[3]}$

![Structure of (S)-2-(2-oxo-4-phenyloxazolidin-3-yl)acetic acid (2)]

To a solution of 1 (6.89 g, 29.3 mmol, 1.0 eq) dissolved in THF (70 mL) was added 2 M KOH (70 mL). The reaction mixture was stirred vigorously for 10 min. THF was evaporated in vacuo and the resulting solution quenched with 12 M HCl (12 mL). To the aqueous phase was added EtOAc (150 mL) and the organic phase separated. The aqueous phase was extracted with additional EtOAc (2x 50 mL) and the combined organic layers washed with brine (50 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. The product was afforded as a pale-yellow oil in quantitative yield and used without further purification in the next step.

The $^1$H NMR spectrum is in agreement with the reported one.$^{[3]}$

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.93 (br s, 1H), 7.48 – 7.29 (m, 5H), 4.99 (t, $J = 8.5$ Hz, 1H), 4.72 (t, $J = 8.8$ Hz, 1H), 4.04 (t, $J = 8.5$ Hz, 1H), 3.99 (d, $J = 18.0$ Hz, 1H), 3.25 (d, $J = 18.0$ Hz, 1H).

LCMS (ESI) calcd for C$_{11}$H$_{12}$NO$_4$ $^+ \ [M-H]^+$ 222.1, found 222.2. $R_t = 2.72$ min.

5.2.3 (S)-1,10b-dihydro-3H-oxazolo[4,3-a]isoquinoline-3,6(5H)-dione (3)$^{[3]}$

![Structure of (S)-1,10b-dihydro-3H-oxazolo[4,3-a]isoquinoline-3,6(5H)-dione (3)]

To a solution of 2 (6.54 g, 29.6 mmol, 1.0 eq) dissolved in DCM (25 mL) was added SOCl$_2$ (8.63 mL, 118 mmol, 4.0 eq) and DMF (3 drops). The reaction mixture was heated under reflux for 1 h. The volatiles were then removed in vacuo. The resulting residue was dissolved in DCM (60 mL) and added slowly to a suspension of AlCl$_3$ (19.7 g, 148 mmol, 5.0 eq) in DCM (120 mL). The resulting mixture stirred for 1 h. The reaction mixture was quenched with H$_2$O (240 mL). The organic phase was separated, and the aqueous phase extracted with DCM (2x 50 mL). The combined organic layers were washed with NaHCO$_3$ (60 mL) and brine (50 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in
vacuo. The crude product was afforded as an off-white solid (6.01 g, 29.6 mmol, quantitative) and used without further purification in the next step.

The $^1$H NMR spectrum is in agreement with the reported one.[3]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 8.13 (d, $J = 7.7$ Hz, 1H), 7.68 (t, $J = 7.7$ Hz, 1H), 7.51 (t, $J = 7.7$ Hz, 1H), 7.20 (d, $J = 7.7$ Hz, 1H), 5.22 (dd, $J = 8.6, 5.9$ Hz, 1H), 4.93 (t, $J = 8.6$ Hz, 1H), 4.71 (d, $J = 18.5$ Hz, 1H), 4.48 (dd, $J = 8.6, 5.9$ Hz, 1H), 3.99 (d, $J = 18.5$ Hz, 1H).

LCMS (ESI) calcd for C$_{11}$H$_{10}$NO$_3$ $[M-H]^+$ 204.1, found 204.2. $R_t = 2.52$ min.

5.2.4 (S)-1,5,6,10b-tetrahydro-3H-oxazolo[4,3-a]isoquinolin-3-one (4)[3]

A 50 mL round bottom flask was charged with compound 3 (2.40 g, 11.8 mmol, 1.0 eq) and Pd/C (30 wt%, 800 mg). The flask was evacuated and refilled with argon three times, followed by addition of MeOH (15 mL). H$_2$ atmosphere was introduced via purging of the system three times with hydrogen in a Parr hydrogenator and maintained at 5 bar. LC-MS analysis showed complete conversion after stirring at room temperature for 18 h. The reaction mixture was filtered through Celite. The crude residue was purified via flash chromatography on a silica gel column using 10-100% EtOAc/Hexane as eluent to give the title compound (2.01 g, 10.6 mmol, 90%) as a white solid.

The $^1$H NMR spectrum is in agreement with the reported one.[3]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.28 – 7.23 (m, 2H), 7.19 – 7.15 (m, 1H), 7.03 – 6.97 (m, 1H), 5.02 (dd, $J = 8.5, 6.6$ Hz, 1H), 4.81 (t, $J = 8.5$ Hz, 1H), 4.20 (dd, $J = 8.5, 6.6$ Hz, 1H), 4.10 (ddd, $J = 13.2, 6.4, 1.9$ Hz, 1H), 3.24 (dd, $J = 13.2, 11.6, 4.2$ Hz, 1H), 3.12 – 3.01 (m, 1H), 2.75 (ddd, $J = 16.2, 4.9, 1.9$ Hz, 1H).

LCMS (ESI) calcd for C$_{11}$H$_{12}$NO$_2$ $[M-H]^+$ 190.1, found 190.2. $R_t = 3.08$ min.

5.2.5 (S)-(1,2,3,4-tetrahydroisoquinolin-1ul)methanol (5)[3]

To a solution of 4 (1.91 g, 10.1 mmol, 1.0 eq) dissolved in MeOH (7 mL) was added 5 M KOH (10.1 mL). The mixture was then heated under reflux for 2 h. After cooling, the reaction mixture was diluted with brine (150 mL) and the aqueous layer was extracted with DCM (4x70 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The obtained orange solid (1.62 g, 9.94 mmol, 98%) was used in the next step without further purification.
The $^1$H NMR spectrum is in agreement with the reported one.[3]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.19 – 7.13 (m, 2H), 7.13 – 7.05 (m, 2H), 4.05 (dd, $J$ = 9.3, 4.3 Hz, 1H), 3.79 (dd, $J$ = 10.9, 4.3 Hz, 1H), 3.64 (dd, $J$ = 10.9, 9.3 Hz, 1H), 3.15 – 3.00 (m, 2H), 2.79 – 2.74 (m, 2H), 2.72 (br s, 2H).

LCMS (ESI) calcd for C$_{10}$H$_{14}$NO$^+$ [M-H]$^+$ 164.1, found 164.3. $R_t$ = 1.57 min.

5.2.6 tert-butyl (S)-1-(hydroxymethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (6)[19]

![Chemical Structure]

To a solution of 5 (1.70 g, 10.4 mmol, 1.0 eq) dissolved in THF (10 mL) was added Boc anhydride (2.95 g, 13.5 mmol, 1.3 eq) and TEA (1.89 mL, 13.5 mmol, 1.3 eq). The resulting mixture was stirred for 1 h. The volatiles were removed under reduced pressure and the residue was dissolved in EtOAc (200 mL) and washed with 1 M HCl (3x100 mL) and brine (100 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 10-80% EtOAc/Hexane as eluent to give the title compound (2.45g, 9.31 mmol, 90%) as a pale-yellow oil.

The $^1$H NMR spectrum is in agreement with the reported one.[19]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.24 – 7.11 (m, 4H), 5.27 (br s, 1H), 3.95 (br s, 1H), 3.86 (dd, $J$ = 11.3, 4.4 Hz, 1H), 3.79 (dd, $J$ = 11.3, 8.6 Hz, 1H), 3.42 (br s, 1H), 2.95 – 2.83 (m, 1H), 2.78 (dt, $J$ = 16.1, 4.7 Hz, 1H), 2.49 (br s, 1H), 1.49 (s, 9H).

LCMS (ESI) calcd for C$_{15}$H$_{22}$NO$_3$ $^{+}$ [M-H]$^+$ 264.2, found 264.3. $R_t$ = 3.77 min.

5.2.7 tert-butyl (S)-1-((1,3-dioxoisindolin-2-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (7)[20]

![Chemical Structure]

To a solution of PPh$_3$ (890 mg, 3.39 mmol, 1.5 eq) dissolved in THF (15 mL) was added DBAD (781 mg, 3.39 mmol, 1.5 eq). The resulting mixture stirred for 30 min. Phthalimide (333 mg, 2.26 mmol, 1.0 eq) was added followed by addition of 6 (595 mg, 2.26 mmol, 1.0 eq) dissolved in THF (5 mL) and stirred 18 h. The volatiles were removed under reduced pressure. The crude residue was purified via flash chromatography on a silica gel column
using 7-60% EtOAc/Hexane as eluent to give the title compound in quantitative yield (444 mg, 1.13 mmol, 50%) as a white solid.

The $^1$H NMR spectrum is in agreement with the reported one.$^{[20]}$

**$^1$H NMR (400 MHz, CDCl$_3$, mixture of 2 rotamers in ratio 6:4) $\delta$ ppm**

7.88 (dd, $J = 5.4$, 3.1 Hz, 1H), 7.85 (dd, $J = 5.4$, 3.1 Hz, 1H), 7.74 (dd, $J = 5.5$, 3.1 Hz, 1H), 7.68 (dd, $J = 5.5$, 3.1 Hz, 1H), 7.40 – 7.33 (m, 1H), 7.29 – 7.16 (m, 3H), 5.59 (dd, $J = 11.1$, 3.7 Hz, 0.4H), 5.43 (dd, $J = 11.1$, 3.7 Hz, 0.4H), 4.25 (dd, $J = 14.0$, 6.0 Hz, 0.6H), 4.07 (ddd, $J = 18.4$, 14.0, 11.1 Hz, 1H), 3.99 (dt, $J = 14.0$, 4.9 Hz, 0.4H), 3.86 (td, $J = 14.0$, 3.7 Hz, 1H), 3.48 (ddd, $J = 14.0$, 10.3, 4.9 Hz, 0.4H), 3.39 (ddd, $J = 14.0$, 11.4, 3.8 Hz, 0.6H), 3.02 – 2.75 (m, 2H), 1.12 (s, 3.6H), 1.03 (s, 5.4H).

**LCMS (ESI)**

calcld for C$_{23}$H$_{25}$N$_2$O$_4$ $^+[M-H]^+$ 393.2, found 393.2. R$_t$ = 4.91 min.

**5.2.8 methyl (1S,2R)-2-(((S)-1-((1,3-dioxoisooindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylate (8)$^{[3]}$**

Compound 7 (887 mg, 2.26 mmol, 1.0 eq) was dissolved in 6 M HCl in isopropanol (10 mL) and MeOH (5 mL) and stirred for 1 h. The volatiles were removed under reduced pressure and the resulting residue was dissolved in EtOAc (300 mL). The organic phase was washed with sat. NaHCO$_3$ (3x100 mL) and brine (100 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. To a solution of the resulting residue (262 mg, 0.671 mmol, 1.0 eq) dissolved in DMF (3 mL) was added (1R,2S)-2-((benzyl oxy)carbonyl)cyclohexane-1-carboxylic acid (210 mg, 0.801 mmol, 80% ee, 1.2 eq), EDC·HCl (232 mg, 1.21 mmol, 1.8 eq), HOBt·H$_2$O (185 mg, 1.21 mmol, 1.8 eq) and DIPEA (0.25 mL, 1.4 mmol, 2.1 eq). The resulting mixture was stirred for 18 h. Additional acid was added (100 mg, 0.40 mmol, 0.6 eq) and the resulting mixture stirred at 40 °C for additional 24 h. The reaction mixture was diluted with EtOAc (70 mL) and washed with 1 M HCl (2x30 mL), sat. NaHCO$_3$ (2x30 mL) and brine (2x30 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give the title compound (330 mg, 0.615 mmol, 92%) as a white solid.

The $^1$H NMR spectrum is in agreement with the reported one.$^{[3]}$

**$^1$H NMR (400 MHz, CDCl$_3$, mixture of 2 diastereomers in ratio 8:2) $\delta$ ppm**

7.85 – 7.78 (m, 2H), 7.71 – 7.65 (m, 2H), 7.40 (d, $J = 7.1$ Hz, 1H), 7.38 – 7.27 (m, 1H), 7.25 – 7.10 (m, 3H), 7.09 – 6.99 (m, 4H), 6.13 (dd, $J = 11.2$, 3.7 Hz, 0.2H), 6.07 (dd, $J = 11.2$, 3.7 Hz, 0.8H), 4.83 (s, 1.6H), 4.77 (s, 0.4H), 4.06 – 4.01 (m, 1H), 3.89 (dd, $J = 14.1$, 3.7, 1H), 3.85 – 3.79 (m, 1H), 3.71 (dd, $J = 14.1$, 11.3, 4.2 Hz, 1H), 3.23 (q, $J = 4.6$ Hz, 1H), 2.85 – 2.65 (m,
2H), 2.37 (dt, J = 10.2, 4.7 Hz, 1H), 2.13 – 2.00 (m, 1H), 1.83 – 1.68 (m, 2H), 1.54 – 1.45 (m, 1H), 1.43 – 1.33 (m, 1H), 1.27 – 1.24 (m, 1H), 1.13 – 0.99 (m, 2H).

**LCMS (ESI)** calcd for C$_{33}$H$_{33}$N$_2$O$_5^+$ [M-H]$^+$ 537.2, found 537.3. $R_t = 5.14$ min.

**5.2.9** (1$S$,2$R$)-2-(((S)-1-((1,3-dioxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (9)[3]

![Chemical Structure of 9]

A 50 mL round bottom flask was charged with compound 8 (270 mg, 0.513 mmol, 1.0 eq) and added Pd/C (30 wt%, 90 mg). The flask was evacuated and refilled with argon three times, followed by addition of MeOH/THF (10/5 mL). H$_2$ atmosphere was introduced via purging of the system three times with hydrogen in a Parr hydrogenator and maintained at 5 bar. LC-MS analysis showed complete conversion after stirring at room temperature for 24 h. The reaction mixture was filtered through Celite. The crude residue was purified via reverse phase HPLC using 30-85% MeCN/H$_2$O as eluent to afford the title compound (41 mg, 93 µmol, 18%, $R_t$=22.2 min) as a white solid.

The $^1$H NMR spectrum is in agreement with the reported one.[3]

$^1$H NMR (500 MHz, CDCl$_3$, mixture of 2 rotamers in ratio 8.5:1.5) δ ppm 7.88 (dd, J = 5.4, 3.0 Hz, 0.3H), 7.84 (dd, J = 5.4, 3.0 Hz, 1.7H), 7.77 (dd, J = 5.5, 3.0 Hz, 0.3H), 7.73 (dd, J = 5.5, 3.0 Hz, 1.7H), 7.41 (dd, J = 7.7, 1.5 Hz, 1H), 7.34 – 7.25 (m, 2H), 7.19 (d, J = 7.4 Hz, 1H), 6.08 (dd, J = 11.2, 3.6 Hz, 0.85H), 6.04 (dd, J = 11.0, 3.4 Hz, 0.15H), 4.10 (dd, J = 14.3, 11.2 Hz, 1H), 3.96 (dd, J = 14.3, 3.7 Hz, 1H), 3.93 – 3.78 (m, 2H), 3.09 – 2.78 (m, 2H), 2.64 – 2.60 (m, 1H), 2.35 – 2.10 (m, 2H), 1.63 – 1.22 (m, 6H).

**LCMS (ESI)** calcd for C$_{26}$H$_{27}$N$_2$O$_5^+$ [M-H]$^+$ 447.2, found 447.2. $R_t = 4.08$ min.

**5.2.10** tert-butyl (S)-1-(((methylsulfonyl)oxy)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (10)[21]

![Chemical Structure of 10]

To a solution of 6 (100 mg, 0.380 mmol, 1.0 eq) dissolved in DCM (1.5 mL) was added methanesulfonyl chloride (44 µL, 0.57 mmol, 1.5 eq) and TEA (80 µL, 0.57 mmol, 1.5 eq). The resulting mixture was stirred for 30 min. The reaction mixture was diluted with EtOAc (50 mL) and washed with H$_2$O (3x40 mL) and brine (40 mL). The organic phase was dried.
over Na₂SO₄ and concentrated in vacuo. The crude product was afforded as an orange oil in quantitative yield and used as acquired without further purification in the next step.

¹H NMR (600 MHz, CDCl₃, mixture of two rotamers in 5:5:4.5 ratio) δ ppm 7.25 – 7.13 (m, 4H, H-1/2/3/6), 5.47 – 5.42 (m, 0.55H, H-10), 5.37 – 5.32 (m, 0.45H, H-10) 4.52 – 4.31 (m, 2H, H-11/11′), 4.19 – 4.12 (m, 0.55H, H-8/8′), 3.96 – 3.87 (m, 0.45H, H-8/8′), 3.50 – 3.40 (m, 0.55H, H-8/8′), 3.36 – 3.27 (m, 0.45H, H-8/8′), 2.99 – 2.91 (m, 0.9H, H-7/7′), 2.89 (s, 1.7H, H-13), 2.87 (s, 1.3H, H-13), 2.84 – 2.74 (m, 1.1H, H-7/7′), 1.51 (s, 4H, H-19/20/21), 1.49 (s, 5H, H-19/20/21).

³C NMR (151 MHz, CDCl₃, mixture of two rotamers) δ ppm 155.0 and 154.5 (1C, C-22), 135.6 and 135.5 (1C, C-4), 132.1 and 131.7 (1C, C-5), 129.3 and 129.0 (1C, C-1/2/3/6), 127.8 and 127.7 (1C, C-2/3/6), 127.4 and 127.3 (1C, C-1/2/3/6), 126.5, and 126.4 (1C, C-1/2/3/6), 80.8 and 80.4 (1C, C-18), 71.0 and 70.7 (1C, C-11), 54.0 and 53.2 (1C, C-10), 39.5 and 37.8 (1C, C-8), 37.7 and 37.5 (1C, C-13), 28.6 (1C, C-7) 28.4 (3C, C-19/20/21).


5.2.11 tert-butyl (S)-1-((tosyloxy)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (11)

![Structure of compound 11]

To a solution of 6 (375 mg, 1.43 mmol, 1.0 eq) dissolved in DCM (7 mL) was added p-toluenesulfonyl chloride (410 mg, 2.15 mmol, 1.5 eq) and TEA (0.3 mL) stirred for 22 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with H₂O (3x100 mL) and brine (100 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 2-20% EtOAc/Hexane as eluent to give the title compound (507 mg, 1.21 mmol, 85%) as a colourless oil.

¹H NMR (600 MHz, CDCl₃, mixture of two rotamers in 6:4 ratio) δ ppm 7.68 (d, J = 8.0 Hz, 1H, H-24/28), 7.62 (d, J = 7.9 Hz, 1H, H-24/28), 7.30 (d, J = 8.0 Hz, 1H, H-25/27), 7.25 (d, J = 7.9 Hz, 1H, H-25/27), 7.21 – 7.14 (m, 1H, H-1/2/3/6), 7.11 (d, J = 7.9 Hz, 2H, H-1/2/3/6), 7.04 – 6.98 (m, 1H, H-1/2/3/6), 5.35 – 5.28 (m, 1H, H-10), 4.32 (dd, J = 10.3, 4.6 Hz, 0.4H, H-11/11′), 4.25 (t, J = 10.3, 5.7 Hz, 0.4H, H-11/11′), 4.18 (dd, J = 10.3, 4.6 Hz, 0.6H, H-11/11′), 4.12 (dd, J = 10.3, 7.8 Hz, 0.6H, H-11/11′), 4.06 – 3.99 (m, 0.6H, H-8/8′), 3.81 (dt, J = 12.3, 5.2 Hz, 0.4H, H-8/8′), 3.39 (ddd, J = 13.6, 8.0, 4.6 Hz, 0.4H, H-8/8′), 2.98 (td, J = 13.6, 10.4, 4.6 Hz, 0.6H, H-8/8′), 2.92 – 2.61 (m, 2H, H-7/7′), 2.43 (s, 3H, H-29), 1.47 (s, 9H, H-19/20/21).

³C NMR (151 MHz, CDCl₃, mixture of two rotamers) δ ppm 154.8 and 154.4 (1C, C-28), 144.8 and 144.5 (1C, C-26), 135.5 (1C, C-4), 132.8 and 132.6 (1C, C-13), 132.2 and 131.6 (1C, C-5), 129.8 and 129.7 (2C, C-25/27), 129.2 and 128.7 (1C, C-3), 127.9 and 127.8 (2C, C-24/28), 127.6 and 127.3 (1C, C-1/2/6), 127.2 and 127.1 (1C, C-1/2/6), 126.4 and 126.3 (1C, C-1/2/6), 80.6 and 80.2 (1C, C-18), 71.6 and 70.7 (1C, C-11), 53.6 and 52.9 (1C, C-
5.2.12 tert-butyl (S)-1-(iodomethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (12)

To a solution of 6 (0.50 g, 1.9 mmol, 1.0 eq) dissolved in THF (15 mL) was added I₂ (0.92 g, 3.61 mmol, 1.9 eq), PPh₃ (1.15 g, 4.37 mmol, 2.3 eq) and imidazole (0.26 g, 3.8 mmol, 2.0 eq). The resulting mixture was stirred for 1 h. The volatiles were removed under reduced pressure and the resulting residue dissolved in EtOAc (100 mL) and washed with H₂O (100 mL) and brine (100 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 1-10% EtOAc/Hexane as eluent to give the title compound (395 mg, 1.06 mmol, 56%) as a colourless oil with triphenylphosphine oxide impurities.

¹H NMR (500 MHz, CDCl₃, mixture of two rotamers in 6:4 ratio) δ ppm 7.28 – 7.16 (m, 3H, H-1/2/6), 7.15 (d, J = 7.1 Hz, 1H, H-3), 5.37 (t, J = 7.0 Hz, 0.4H, H-10), 5.24 (t, J = 7.0 Hz, 0.6H, H-10), 4.18 – 4.09 (m, 0.6H, H-8/8’), 3.94 – 3.85 (m, 0.4H, H-8/8’), 3.60 – 3.46 (m, 2H, H-7/7’), 3.46 – 3.37 (m, 0.4H, H-8/8’), 3.32 – 3.21 (m, 0.6H, H-8/8’), 2.96 – 2.70 (m, 2H, H-7/7’), 1.52 (s, 5.4H, H-15/16/17), 1.51 (s, 3.6H, H-15/16/17).

¹³C NMR (126 MHz, CDCl₃, mixture of two rotamers) δ ppm 154.8 and 154.4 (1C, C-18), 135.2 and 135.0 (1C, C-5), 134.8 and 134.5 (1C, C-4), 129.3 and 128.9 (1C, C-3), 127.9 and 127.7 (1C, C-1/2), 127.6 and 127.5 (1C, C-6), 126.3 (1C, C-1/2), 80.7 and 80.2 (1C, C-14), 55.6 and 54.6 (1C, C-10), 38.9 and 37.0 (1C, C-8), 28.5 (3C, C-15/16/17), 28.7 (1C, C-7), 10.3 and 9.9 (1C, C-11).

LCMS (ESI) calcd for C₂₂H₂₈NO₅S⁺ [M-H]⁺ 418.2, found 418.1. Rₜ = 5.08 min.

5.2.13 (S)-1,5,6,10b-tetrahydro-[1,2,3]oxathiazolo[4,3-a]isoquinoline 3,3-dioxide (13)³

To a solution of 5 (200 mg, 1.23 mmol, 1.0 eq) dissolved in DCM (4 mL) at 0 °C was added imidazole (335 mg, 4.92 mmol, 4.0 eq) and TEA (0.40 mL, 2.90 mmol, 2.3 eq) followed by dropwise addition of SOCl₂ (0.11 mL, 1.5 mmol, 1.2 eq) in DCM (1.5 mL). The resulting mixture was stirred for 1 h. The reaction mixture was quenched with H₂O (10 mL). The organic layer was separated, the aqueous layer extracted with DCM (3×20 mL). The combined organic layers were washed with H₂O (40 mL), dried over Na₂SO₄ and
concentrated in vacuo. To a solution of the resulting residue dissolved in EtOAc (15 mL) was added NaIO₄ (289 mg, 1.35 mmol, 1.1 eq), RuCl₃·H₂O (4 mg, 0.02 mmol, 0.005 eq) and H₂O (3 mL). The resulting mixture was stirred for 2.5 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with H₂O (2x100 mL) and brine (100 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude product was afforded as a brown solid (213 mg, 0.951 mmol, 77%).

The ¹H NMR spectrum is in agreement with the reported one.[³]

¹H NMR (400 MHz, CDCl₃) δ ppm 7.35 – 7.19 (m, 3H), 7.03 (d, J = 6.9 Hz, 1H), 5.15 (dd, J = 9.8, 6.8 Hz, 1H), 4.85 (ddt, J = 7.8, 6.8, 1.0 Hz, 1H), 4.27 – 4.18 (m, 1H), 3.56 – 3.42 (m, 2H), 3.10 – 2.93 (m, 2H).


5.2.14 1-phenylprop-2-yn-1-one (14) [²²]

To a solution of 1-phenylprop-2-yn-1-ol (2.60 mL, 21.4 mmol, 1.0 eq) dissolved in THF (60 mL) was added MnO₂ (9.30 g, 107 mmol, 5.0 eq). The resulting mixture was stirred for 20 h. The reaction mixture was filtered through Celite. The crude residue was purified via flash chromatography on a silica gel column using 4-40% EtOAc/Hexane as eluent to give the title compound (1.39 g, 10.7 mmol, 50%) as a pale-yellow liquid.

The ¹H NMR spectrum is in agreement with the reported one.[²²]

¹H NMR (400 MHz, CDCl₃) δ ppm 8.21 – 8.14 (m, 2H), 7.64 (t, J = 7.4 Hz, 1H), 7.51 (t, J = 7.6 Hz, 2H), 3.43 (s, 1H).

LCMS (ESI) calcd for C₉H₇O⁺ [M-H]⁺ 131.0, found 130.1. Rᵣ = 3.72 min

5.2.15 Benzyl propiolate (15) [²³]

To a solution of propionic acid (4.0 g, 57 mmol, 1.0 eq) dissolved in DMF (100 mL) was added K₂CO₃ (11.8 g, 85.5 mmol, 1.5 eq) in small portions, followed by addition of benzyl bromide (7.46 mL, 62.7 mmol, 1.1 eq). The resulting mixture was stirred for 18 h. The reaction mixture was diluted with EtOAc (400 mL) and washed with H₂O (3x400 mL) and brine (400 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 1-10% EtOAc/Hexane as eluent to give the title compound (8.2 g, 51 mmol, 90%) as a colourless liquid.
The $^1$H NMR spectrum is in agreement with the reported one.[23]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.41 – 7.33 (m, 5H), 5.23 (s, 2H), 2.89 (s, 1H).

LCMS (ESI) calcd for C$_{10}$H$_9$O$_2$ $[M-H]^+$ 161.1, found 161.2. $R_t$ = 4.03 min.

5.2.16 (Z)-3-(2-oxo-2-phenylethylidene)isoindolin-1-one (16)[12]

![Chemical structure](image)

To a solution of N-hydroxyphthalimide (146 mg, 0.895 mmol, 1.0 eq) dissolved in DMF (1 mL) was added a solution of 14 (139 mg, 1.07 mmol, 1.2 eq) dissolved in DMF (2 mL) followed by addition of K$_2$CO$_3$ (26 mg, 0.18 mmol, 0.2 eq). The resulting mixture was stirred at 60 °C for 17 h. After allowing cooling to room temperature, the reaction mixture was diluted with EtOAc (30 mL) and washed with H$_2$O (3x25 mL) and brine (20 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 3-30% EtOAc/Hexane as eluent to give the title compound (67 mg, 0.27 mmol, 30%) as a yellow solid.

The $^1$H NMR spectrum is in agreement with the reported one.[12]

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.92 (s, 1H), 8.36 (d, $J$ = 7.6 Hz, 1H), 8.21 (d, $J$ = 7.6 Hz, 2H), 7.88 – 7.79 (m, 2H), 7.73 (t, $J$ = 7.4 Hz, 1H), 7.68 (t, $J$ = 7.4 Hz, 1H), 7.60 (t, $J$ = 7.3 Hz, 2H), 7.41 (s, 1H).

LCMS (ESI) calcd for C$_{16}$H$_{12}$NO$_2$ $[M-H]^+$ 250.1, found 250.2. $R_t$ = 4.16 min.

5.2.17 Benzyl (Z)-2-(3-oxoisooindolin-1-ylidene)acetate (17)[12]

![Chemical structure](image)

To a solution of N-hydroxyphthalimide (1.03 g, 6.24 mmol, 1.0 eq) dissolved in DMF (30 mL) was added 15 (1.20 g, 7.49 mmol, 1.2 eq) followed by addition of K$_2$CO$_3$ (0.18 g, 1.3 mmol, 0.2 eq). The resulting mixture was stirred at 100 °C for 18 h. After allowing cooling to room temperature, the reaction mixture was diluted with EtOAc (150 mL) and washed with H$_2$O (100 mL) and brine (2x100 mL). The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 5-25% EtOAc/Hexane as eluent to give the title compound (1.20 g, 4.30 mmol, 69%) as a white solid.
The $^1$H NMR spectrum is in agreement with the reported one.\cite{12}

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 9.63 (br s, 1H), 7.91 – 7.86 (m, 1H), 7.70 – 7.59 (m, 3H), 7.45 – 7.32 (m, 5H), 5.83 (s, 1H), 5.26 (s, 2H).

LCMS (ESI) calcd for C$_{17}$H$_{14}$NO$_3$ $^\text{+}$ [M-H]$^+$ 280.1, found 280.2. $R_t$ = 4.39 min.

5.2.18 Benzyl (E)-2-(3-oxoisindolin-1-ylidene)acetate (18)\cite{12}

\begin{center}
\includegraphics[width=0.2\textwidth]{benzyl.png}
\end{center}

To a solution of $N$-hydroxyphthalimide (255 mg, 1.56 mmol, 1 eq) dissolved in DMF (6 mL) was added 15 (300 mg, 1.87 mmol, 1.2 eq) followed by addition of K$_2$CO$_3$ (44 mg, 0.32 mmol, 0.2 eq). The reaction mixture was stirred at 60 °C for 3 h. After allowing cooling to room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with H$_2$O (3x80 mL) and brine (80 mL) and dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 5-40% EtOAc/Hexane as eluent to give the title compound (170 mg, 0.61 mmol, 40%) as a pale-yellow solid. A 9:1 mixture was obtained and only the major product described.

The $^1$H NMR spectrum is in agreement with the reported one.\cite{12}

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 9.09 (dt, $J$ = 7.9, 1.1 Hz, 1H), 7.91 – 7.84 (m, 1H), 7.67 (dtt, $J$ = 28.5, 7.4, 1.2 Hz, 2H), 7.45 – 7.31 (m, 5H), 5.82 (s, 1H), 5.26 (s, 2H).

LCMS (ESI) calcd for C$_{17}$H$_{14}$NO$_3$ $^\text{+}$ [M-H]$^+$ 280.1, found 280.2. $R_t$ = 4.22 min.

5.2.19 tert-butyl (S)-1-(azidomethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (19)

\begin{center}
\includegraphics[width=0.2\textwidth]{tert.png}
\end{center}

To a solution of 10 (4.50 g, 13.6 mmol, 1.0 eq) dissolved in DMF (50 mL) was added NaN$_3$ (2.20 g, 33.9 mmol, 2.5 eq). The resulting mixture was stirred for 20 h. The reaction mixture was diluted with EtOAc (200 mL) and washed with brine (4x100 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 2-20% EtOAc/Hexane as eluent to give the title compound (1.57 g, 5.46 mmol, 41%) as a colourless oil.

$^1$H NMR (600 MHz, CDCl$_3$, mixture of 2 rotamers in ratio 1:1) $\delta$ ppm 7.24 – 7.19 (m, 2H, H-1/2/3/6), 7.18 – 7.13 (m, 2H, H-1/2/3/6), 5.38 – 5.31 (m, 0.5H, H-10), 5.24 – 5.18 (m, 0.5H, H-10), 4.22 – 4.13 (m, 0.5H, H-8/8'), 3.98 – 3.87 (m, 0.5H, H-8/8'), 3.68 – 3.40 (m,
$2.5, H-8/8'/11/11'$, 3.33 – 3.22 (m, 0.5H, H-8/8'), 3.01 – 2.73 (m, 2H, H-7/7'), 1.51 (s, 9H, H-19/20/21).

$^{13}$C NMR (151 MHz, CDCl$_3$, mixture of 2 rotamers) $\delta$ ppm 155.0 and 154.5 (1C, C-15), 135.3 and 135.2 (1C, C-4/5), 133.9 and 133.6 (1C, C-4/5), 129.3 and 128.8 (1C, C-1/2/3/6), 127.4 and 127.3 (1C, C-1/2/3/6), 127.1 (1C, C-1/2/3/6), 126.5 and 126.4 (1C, C-1/2/3/6), 80.6 and 80.3 (1C, C-18), 55.3 (1C, C-11), 54.7 and 53.9 (1C, C-10), 39.5 and 37.5 (1C, C-8), 28.8 (1C, C-7), 28.42 (3C, C-19/20/21).

LCMS (ESI) calcd for C$_{15}$H$_{21}$N$_{5}$O$_{2}$ $^{+}$ [M-H]$^{+}$ 289.2, found 289.2. $R_t$ = 4.82 min

5.2.20 tert-butyl (S)-1-(aminomethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (20)$^{[21]}$

A 50 mL round bottom flask was charged with compound 19 (1.55 mg, 5.38 mmol, 1.0 eq) and Pd/C (30 wt%, 500 mg). The flask was evacuated and refilled with argon three times, followed by addition of MeOH (10 mL). H$_2$ atmosphere was introduced via purging of the system three times with hydrogen in a Parr hydrogenator and maintained at (5 bar). LC-MS analysis showed complete conversion after stirring at room temperature for 18 h. The reaction mixture was filtered through Celite. The crude residue was purified via flash chromatography on a silica gel column using 1-12% MeOH/DCM as eluent to give the title compound (1.00 g, 3.81 mmol, 71 %) as a yellow oil.

The $^1$H NMR spectrum is in agreement with the reported one.$^{[20]}$

$^1$H NMR (400 MHz, CDCl$_3$, mixture of 2 rotamers in ratio 1:1) $\delta$ 7.29 – 7.08 (m, 4H), 5.07 – 4.95 (m, 0.5H), 4.95 – 4.84 (m, 0.5H), 4.05 – 3.91 (m, 0.5H), 3.89 – 3.76 (m, 0.5H), 3.37 – 3.09 (m, 1H), 3.08 – 2.65 (m, 4H), 1.49 (s, 9H).

LCMS (ESI) calcd for C$_{15}$H$_{23}$N$_{2}$O$_{2}$ $^{+}$ [M-H]$^{+}$ 263.2, found 263.3. $R_t$ = 2.53 min

5.2.21 tert-butyl (S)-1-((2-iodobenzamido)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (21)

To a solution of 20 (0.97 g, 3.7 mmol, 1.0 eq) dissolved in DMF (12 mL) was added 2-iodobenzoic acid (1.10 g, 4.44 mmol, 1.2 eq), EDC·HCl (1.28 g, 6.66 mmol, 1.8 eq), HOBt·H$_2$O (0.90 g, 6.66 mmol, 1.8 eq) and DIPEA (1.35 mL, 7.76 mmol. 2.1 eq). The
resulting mixture was stirred for 18 h. The resulting mixture was diluted with EtOAc (150 mL) and washed with 1 M HCl (2x100 mL), sat. NaHCO₃ (2x100 mL) and brine (2x100 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give the title compound (1.76 g, 3.58 mmol, 96%) as a pale-yellow oil.

¹H NMR (500 MHz, CDCl₃, mixture of 2 rotamers in ratio 6:4) δ ppm 7.87 (d, J = 7.9 Hz, 1H, H-17), 7.43 (dd, J = 7.5, 1.7 Hz, 1H, H-20), 7.39–7.31 (m, 1H, H-3), 7.12 – 7.05 (m, 1H, H-18), 6.78 (br s, 1H, H-12), 5.49 – 5.34 (m, 0.6H, H-10), 5.34 – 5.16 (m, 0.4H, H-10), 4.32 – 3.94 (m, 1H, H-8/8'), 3.94 – 3.56 (m, 2H, H-11/11'), 3.48 – 3.16 (m, 1H, H-8/8'), 3.03 – 2.89 (m, 1H, H-7/7'), 2.89 – 2.70 (m, 1H, H-7/7'), 2.89 – 2.70 (m, 1H, H-7/7'), 1.42 (br s, 9H, H-26/27/28).

¹³C NMR (126 MHz, CDCl₃, mixture of 2 rotamers) δ ppm 169.4 (1C, C-13), 156.5 (1C, C-22), 141.9 (1C, C-14), 140.0 (1C, C-17), 134.8 (1C, C-5), 134.0 (1C, C-4), 131.1 (1C, C-18), 129.0 (1C, C-3), 128.2 (1C, C-20), 128.0 (1C, C-19), 127.3 (1C, C-2), 127.2 (1C, C-6), 126.5 (1C, C-1), 92.7 (1C, C-16), 80.5 (1C, C-25), 54.3 and 53.3 (1C, C-10), 45.7 and 44.7 (1C, C-11), 39.0 and 37.3 (1C, C-8), 28.5 (1C, C-7), 28.4 (3C, C-26/27/28).

LCMS (ESI) calcd for C₂₂H₂₆InO₃⁺ [M-H]⁺ 493.1, found 493.1. Rᵣ = 4.62 min

5.2.22 tert-butyl (S)-1-(((1-benzylidene-3-oxoisooindolin-2-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (22)[16]

To a solution of 21 (159 mg, 0.323 mmol, 1.0 eq) dissolved in DMF (1 mL) was added phenylacetylene (40 mL, 0.39 mmol, 1.2 eq), Cu(OAc)₂·H₂O (12 mg, 0.060 mmol, 0.2 eq) and K₂CO₃ (90 mg, 0.65 mmol, 2.0 eq). The resulting mixture was stirred at 60 °C for 18 h. After allowing cooling to room temperature, the reaction mixture was diluted with EtOAc (50 mL) and washed with brine (3x50 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give a mixture of isomers (138 mg, 0.30 mmol, 92%, ratio 6:4) as a yellow oil.

¹H NMR (500 MHz, CDCl₃, mixture of two isomers in ratio 6:4) δ ppm 7.88 (d, J = 7.5 Hz, 0.6H), 7.83 (d, J = 7.5 Hz, 0.4H), 7.79 (dd, J = 7.8, 4.6 Hz, 1H), 7.63 (td, J = 7.5, 1.2 Hz, 0.8H), 7.61 – 7.54 (m, 1.2H), 7.53 – 7.40 (m, 6H), 7.11 – 6.97 (m, 2H), 6.95 (s, 0.4H), 6.90 (t, J = 7.5 Hz, 0.6H), 6.84 (t, J = 7.5 Hz, 0.4H), 6.82 (s, 0.6H), 5.06 (dd, J = 11.5, 3.4 Hz, 0.4H), 4.95 (dd, J = 11.5, 3.4 Hz, 0.6H), 4.42 (dd, J = 14.6, 11.3 Hz, 1H), 4.08 (dd, J = 14.2, 5.9 Hz, 0.6H), 3.92 (dd, J = 14.2, 6.7, 2.4 Hz, 0.4H), 3.82 (dd, J = 14.2, 3.2 Hz, 0.4H), 3.72 (dd, J = 14.7, 3.5 Hz, 0.6H), 3.36 – 3.27 (m, 0.6H), 3.09 – 2.80 (m, 0.4H), 2.85 – 2.68
(m, 1H), 2.60 (dt, J = 16.3, 3.4 Hz, 0.4H), 2.52 (dt, J = 16.3, 3.4 Hz, 0.6H), 1.04 (s, 5.4H), 1.03 (s, 3.6H).

$^1$C NMR (126 MHz, CDCl$_3$, mixture of two isomers) δ ppm 169.3 and 168.7 (1C), 154.1 (1C), 139.1 and 138.6 (1C), 135.6 (1C), 135.3 (1C), 135.2 and 135.1 (1C), 134.9 and 134.8 (1C), 134.2 and 134.0 (1C), 132.1 and 131.9 (1C), 130.5 and 130.3 (1C), 129.2 and 129.2 (1C), 128.9 and 128.8 (1C), 128.6 (1C), 128.2 and 128.1 (1C), 127.7 and 127.5 (1C), 126.9 and 126.8 (1C), 126.7 and 126.5 (1C), 125.7 (1C), 123.7 and 123.2 (1C), 119.6 and 119.3 (1C), 106.7 and 106.0 (1C), 79.6 and 79.1 (1C), 53.9 and 51.9 (1C), 44.5 and 44.1 (1C), 37.9 and 36.4 (1C), 28.6 and 28.1 (1C), 27.8 (3C).

LCMS (ESI) calcd for C$_{30}$H$_{31}$N$_2$O$_3$ [M-H]$^+$ 467.2, found 467.2. R$_t$ = 5.46 min

5.2.23 tert-butyl (S)-1-((1-(2-(benzyloxy)-2-oxoethylidene)-3-oxoisindolin-2-yl)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (23)$^{[16]}$

A procedure identical to that described for compound 22 was followed using 21 (249 mg, 0.506 mmol, 1.0 eq), 15 (98 mg, 0.61 mmol, 1.2 eq), Cu(OAc)$_2$·H$_2$O (20 mg, 0.10 mmol, 0.2 eq) and K$_2$CO$_3$ (140 mg, 1.01 mmol, 2.0 eq) in DMF (2 mL). The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give a mixture of isomers (168 mg, 0.32 mmol, 63%, ratio 2:1) as a dark red oil.

$^1$H NMR (600 MHz, CDCl$_3$, mixture of two isomers in ratio 7:3) δ ppm 9.14 (dd, J = 11.8, 7.9 Hz, 1H), 7.84 (d, J = 7.4 Hz, 0.7H), 7.78 (d, J = 7.4 Hz, 0.3H), 7.68 (t, J = 7.5 Hz, 0.7H), 7.64 (t, J = 7.5 Hz, 0.3H), 7.60 (t, J = 7.4 Hz, 0.7H), 7.54 (t, J = 7.4 Hz, 0.3H), 7.50 – 7.29 (m, 5H), 7.24 – 7.13 (m, 4H), 6.14 (s, 0.3H), 5.94 (s, 0.7H), 5.54 (dd, J = 10.3, 3.9 Hz, 0.3H), 5.42 (dd, J = 10.3, 3.9 Hz, 0.7H), 5.37 – 5.30 (m, 1H), 5.37 – 5.30 (m, 0.7H), 5.29 (s, 1.6H), 5.28 – 5.24 (m, 0.3H), 4.71 (s, 0.4H), 4.37 – 4.19 (m, 1H), 3.96 – 3.76 (m, 1H), 3.75 – 3.55 (m, 0.3H), 3.54 – 3.40 (m, 0.7H), 3.02 – 2.72 (m, 2H), 1.02 (s, 9H).

$^1$C NMR (151 MHz, CDCl$_3$, mixture of two isomers) δ ppm 167.4 and 167.2 (1C), 165.9 and 165.7 (1C), 154.9 and 154.2 (1C), 149.0 (1C), 136.0 (1C), 135.9 and 135.2 (1C), 134.0 and 133.92 (1C), 133.85 and 133.5 (1C), 133.3 and 133.1 (1C), 131.4 and 131.0 (1C), 129.8 and 128.3 (1C), 129.5 (1C), 128.8 and 128.6 (2C), 128.34 and 128.31 (2C), 128.26 and 128.2 (2C), 127.4 and 127.3 (1C), 127.2 and 127.0 (1C), 126.4 and 126.3 (1C), 123.4 and 123.0 (1C), 98.1 and 97.6 (1C) 80.1 and 79.8 (1C), 66.4 and 66.5 (1C), 52.8 and 51.8 (1C), 43.4 and 43.2 (1C), 39.2 and 37.1 (1C), 28.3 and 28.1 (1C), 27.8 (3C).

LCMS (ESI) calcd for C$_{32}$H$_{33}$N$_2$O$_5$+ [M-H]$^+$ 525.2, found 525.3. R$_t$ = 5.60 min
5.2.24 methyl (1S,2R)-2-((S)-1-((1-benzyldiene-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylate (24)

Compound 22 (124 mg, 0.266 mmol, 1.0 eq) was dissolved in 4 M HCl in dioxane (4 mL) and stirred for 1 h at rt. The volatiles were removed under reduced pressure and the resulting residue was dissolved in EtOAc (70 mL). The organic phase was washed with sat. NaHCO₃ (3x20 mL) and brine (20 mL), dried over Na₂SO₄ and concentrated in vacuo. To a solution of the resulting residue dissolved in DMF (1 mL) was added (1R,2S)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid (54 mg, 0.29 mmol, 1.2 eq), EDC·HCl (82 mg, 0.43 mmol, 1.8 eq), HOBt·H₂O (66 mg, 0.43 mmol, 1.8 eq) and DIPEA (0.088 mL, 0.50 mmol, 2.1 eq). The resulting mixture was stirred for 18 h at rt. The reaction mixture was diluted with EtOAc (50 mL) and washed with 1 M HCl (2x30 mL), sat. NaHCO₃ (2x30 mL) and brine (2x30 mL). The organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give an isomeric mixture of the title compound (105 mg, 0.196 mmol, 74%, ratio 6:4) as an orange oil.

¹H NMR (500 MHz, CDCl₃, mixture of two isomers in ratio 6:4) δ ppm 7.88 (d, J = 7.6 Hz, 0.4H), 7.71 (d, J = 7.5 Hz, 0.6H), 7.58 – 7.34 (m, 8H), 7.23 – 7.15 (m, 2H), 7.10 (s, 0.6H), 7.06 – 6.80 (m, 2H), 6.46 (s, 0.4H), 5.99 (t, J = 7.5 Hz, 1H), 4.43 – 3.98 (m, 3H), 3.80 (dt, J = 12.4, 5.6 Hz, 1H), 3.42 (s, 1.8H), 3.22 (s, 1.2H), 3.19 – 2.89 (m, 3H), 2.88 – 2.28 (m, 1H), 2.18 – 1.89 (m, 1H), 1.85 – 1.04 (m, 7H).

¹³C NMR (126 MHz, CDCl₃, mixture of two isomers) δ ppm 174.7 and 173.6 (1C), 173.8 and 173.7 (1C), 167.1 and 166.7 (1C), 136.4 and 136.3 (1C), 135.6 and 135.2 (1C), 135.0 and 134.6 (1C), 134.4 and 134.2 (1C), 133.7 (1C), 132.0 (1C), 130.2 and 130.1 (1C), 129.8 (1C), 129.6 and 129.5 (1C), 129.4 (1C), 128.9 and 128.8 (1C), 128.6 (1C), 128.4 (1C), 128.1 (1C), 127.7 and 127.6 (1C), 127.4 and 127.3 (1C), 126.4 and 126.3 (1C), 123.2 and 123.1 (1C), 111.6 and 110.5 (1C), 55.7 and 53.8 (1C), 51.2 and 50.8 (1C), 44.5 and 42.7 (1C), 42.3 and 42.1 (1C), 40.9 and 40.6 (1C), 36.2 (1C), 29.3 and 28.1 (1C), 26.5 and 26.1 (1C), 26.0 and 25.8 (1C), 23.3 and 23.2 (1C), 22.9 and 22.6 (1C).

LCMS (ESI) calcd for C₃₄H₃₅N₂O₄⁺ [M-H]⁺ 535.3, found 535.3. Rₜ = 5.19 min
5.2.25  methyl (1\(S\),2\(R\))-2-((\(S\))-1-((1-(2-(benzyloxy)-2-oxoethylidene)-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylate (25)

A procedure identical to that described for compound 24 was followed using 23 (160 mg, 0.305 mmol, 1.0 eq), 6 \(m\) HCl in isopropanol (4 mL) and DCM (2 mL) for 2 h. Then, (\(IR\),2\(S\))-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid (69 mg, 0.53 mmol, 1.2 eq), EDC·HCl (105 mg, 0.549 mmol, 1.8 eq), HOBt·H\(2\)O (84 mg, 0.55 mmol, 1.8 eq) and DIPEA (0.11 mL, 0.64 mmol, 2.1 eq) in DMF (1 mL). The crude residue was purified via flash chromatography on a silica gel column using 10-80% EtOAc/Hexane as eluent to give an isomeric mixture (58 mg, 0.10 mmol, 33\%, ratio 6:4) as a pale-yellow oil.

\(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of two isomers in ratio 6:4) \(\delta\) ppm 9.15 – 9.06 (m, 1H), 8.08 – 7.98 (m, 1H), 7.89 – 7.31 (m, 7H), 7.24 – 6.88 (m, 4H), 6.44 (s, 0.6H), 6.10 (s, 0.4H) 5.89 – 5.81 (m, 1H), 5.32 (s, 1.2H), 5.31 (s, 0.8H), 4.21 – 3.84 (m, 2H), 3.79 (s, 1.8H), 3.30 (s, 1.2H), 3.26 – 2.71 (m, 5H), 2.46 – 1.19 (m, 9H).

\(^{13}\)C NMR (126 MHz, CDCl\(_3\), mixture of two isomers) \(\delta\) ppm 174.7 (1C), 173.8 (1C), 169.8 (1C), 167.3 and 166.1 (1C), 148.7 and 148.6 (1C), 136.2 (1C), 134.9 and 134.4 (1C), 133.9 and 133.6 (1C), 133.2 and 132.9 (1C), 131.1 and 131.0 (1C), 130.0 (1C), 129.7 and 129.5 (1C), 129.2 and 129.1 (1C), 128.64 and 128.61 (1C), 128.55 and 128.4 (1C), 128.13 and 128.08 (1C), 127.5 and 127.3 (1C), 126.5 (1C), 124.7 (1C), 123.1 (1C), 120.3 (1C), 116.3 and 115.4 (1C), 109.0 and 99.0 (1C), 66.5 and 66.4 (1C), 66.2 (1C), 52.3 and 51.6 (1C), 51.1 and 50.6 (1C), 44.4 and 43.0 (1C), 42.7 and 42.4 (1C), 41.9 and 40.9 (1C), 40.5 and 40.2 (1C), 29.79 and 29.2 (1C), 28.1 (1C), 26.2 and 25.8 (1C), 23.5 and 23.2 (1C), 23.0 (1C).

LCMS (ESI) calcd for C\(_{36}\)H\(_{37}\)N\(_2\)O\(_6\)\(^+\) [M-H]\(^+\) 593.3, found 593.2. \(R_t = 5.46\) min

5.2.26  (1\(S\),2\(R\))-2-((\(S\))-1-((1-benzylidene-3-oxoisindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (26)
To a solution of 24 (120 mg, 0.224 mmol, 1.0 eq) dissolved in MeOH (2 mL) was added 1 M LiOH·H₂O (1.5 mL). The resulting mixture was stirred for 4 h. Additional LiOH·H₂O (46 mg, 1.1 mmol, 4.8 eq) was then added and the reaction mixture was stirred at rt for additional 72 h. The reaction mixture was quenched with 1 M HCl (10 mL) and extracted with DCM (3x20 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via reverse phase HPLC using 45-95% MeCN/H₂O as eluent to afford a mixture of isomers of the title compound (10 mg, 19 μmol, 8 %, Rt=25.0 min, ratio 8:2) as a yellow oil.

1H NMR (500 MHz, CDCl₃, mixture of two isomers in ratio 8:2) δ ppm 7.84 – 7.69 (m, 1H), 7.69 – 7.39 (m, 6H), 7.39 – 7.30 (m, 1H), 7.30 – 7.21 (m, 4H), 7.15 – 7.04 (m, 1H), 6.96 (s, 0.2H), 6.88 (s, 0.8H), 6.19 (dd, J = 10.1, 4.6 Hz, 0.8H), 5.55 (dd, J = 10.1, 4.6 Hz, 0.2H), 4.71 – 4.38 (m, 1H), 4.20 – 4.07 (m, 1H), 3.97 (dd, J = 14.7, 4.1 Hz, 1H), 3.92 – 3.67 (m, 1H), 3.15 (dt, J = 16.2, 4.1 Hz, 1H), 3.09 – 2.25 (m, 1H), 2.24 – 2.14 (m, 1H), 1.83 – 1.21 (m, 6H).

13C NMR (126 MHz, CDCl₃, mixture of two isomers) δ ppm 176.9 and 176.4 (1C), 173.1 and 172.8 (1C), 169.3 and 167.2 (1C), 138.9 (1C), 136.2 (1C), 135.21 (1C), 134.9 and 134.8 (1C), 133.6 (1C), 133.0 and 132.9 (1C), 132.2 (1C), 130.3 (1C), 129.6 (1C), 129.4 (1C), 129.24 and 129.19 (1C), 128.9 and 128.83 (1C), 128.77 and 128.6 (1C), 128.0 and 127.8 (1C), 127.2 and 126.92 (1C), 126.87 and 126.4 (1C), 123.6 (1C), 122.7 and 122.6 (1C), 119.9 (1C), 111.0 and 107.8 (1C), 51.8 and 51.6 (1C), 44.0 and 43.5 (1C), 43.3 and 42.9 (1C), 42.2 and 41.7 (1C), 41.2 and 40.5 (1C), 29.7 and 29.4 (1C), 29.2 (1C), 27.0 and 26.9 (1C), 26.1 and 25.8 (1C), 21.9 and 21.6 (1C).


5.2.27 (1S,2R)-2-((S)-1-((1-(carboxymethylene)-3-oxoisooindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (27)

To a solution of 25 (43 mg, 0.073 mmol, 1.0 eq) dissolved in MeOH (2 mL) was added 1 M LiOH·H₂O (1.5 mL). The resulting mixture was stirred for 24 h at rt. The reaction mixture was quenched with 1 M HCl (10 mL) and extracted with DCM (3x30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via reverse phase HPLC using 30-85% MeCN/H₂O as eluent to afford a mixture of isomers of the title compound (5.3 mg, 11 μmol, 15 %, Rₜ=18.7 min, ratio 8:2) as a yellow solid.

1H NMR (500 MHz, CDCl₃, mixture of two isomers in ratio 8:2) δ ppm 9.04 (d, J = 7.3 Hz, 0.2H), 8.91 (d, J = 7.6 Hz, 0.8H), 7.91 – 7.36 (m, 5H), 7.24 – 7.09 (m, 1H), 7.05 – 6.75
(m, 1H), 6.44 (s, 0.8H), 6.19 (s, 0.2H), 5.89 – 5.71 (m, 1H), 4.06 – 3.89 (m, 3H), 3.84 – 3.72 (m, 1H), 3.29 – 2.88 (m, 4H), 2.82 – 2.63 (m, 1H), 2.58 – 2.43 (m, 1H), 2.38 – 2.15 (m, 1H), 2.13 – 1.89 (m, 1H), 1.87 – 1.37 (m, 4H).

$^{13}$C NMR (126 MHz, CDCl$_3$, mixture of two isomers) $\delta$ ppm 178.6 and 147.8 (1C), 170.3 (1C), 166.8 (1C), 163.0 (1C), 149.2 and 148.9 (1C), 134.5 and 133.9 (1C), 133.34 (1C), 133.31 (1C), 133.2 (1C), 131.2 (1C), 129.1 (1C), 128.6 and 128.4 (1C), 128.0 (1C), 127.7 (1C), 127.1 (1C), 126.7 (1C), 126.1 (1C), 129.9 (1C), 99.0 (1C), 59.5 (1C), 50.9 (1C), 42.7 (1C), 42.0 (1C), 41.6 and 41.5 (1C), 38.1 (1C), 31.9 and 31.2 (1C), 29.7 (1C), 29.2 (1C), 26.2 (1C).

LCMS (ESI) calcd for C$_{28}$H$_{29}$N$_2$O$_6$ $\text{[M-H]}^+$ 489.2, found 489.2. $R_t$ = 3.82 min.

5.2.28 methyl (1$S$,2$R$)-2-((S)-1-((2-iodobenzamido)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylate (28)

![Chemical structure](image)

A procedure identical to that described for compound 24 was followed using 21 (160 mg, 0.325 mmol, 1.0 eq), 4 M HCl in dioxane, added ($R$,$S$)-2-(methoxycarbonyl)cyclohexane-1-carboxylic acid (73 mg, 0.39 mmol, 1.2 eq), EDC-HCl (111 mg, 0.585 mmol, 1.8 eq), HOBr·H$_2$O (89 mg, 0.58 mmol, 1.8 eq) and DIPEA (0.12 mL, 0.68 mmol, 2.1 eq). The crude residue was purified via flash chromatography on a silica gel column using 7-60% EtOAc/Hexane as eluent to give the title compound (108 mg, 0.193 mmol, 60%) as a pale-yellow oil.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm 7.84 (dd, $J = 8.0$, 1.1 Hz, 1H), 7.51 (dd, $J = 7.6$, 1.7 Hz, 1H), 7.35 (dd, $J = 7.5$, 1.2 Hz, 1H), 7.32 (dt, $J = 7.3$, 1.6 Hz, 1H), 7.26 – 7.21 (m, 1H), 7.20 (dd, $J = 7.3$, 1.6 Hz, 1H), 7.14 (dd, $J = 7.4$, 1.6 Hz, 1H), 7.05 (td, $J = 7.7$, 1.7 Hz, 1H), 6.99 (br s, 1H), 5.95 (dd, $J = 11.1$, 3.4 Hz, 1H), 4.08 – 3.96 (m, 2H), 3.75 – 3.60 (m, 2H), 3.28 – 3.21 (m, 1H), 3.17 (s, 3H), (ddd, $J = 16.9$, 11.4, 5.6 Hz, 1H), 2.91 (dt, $J = 16.9$, 3.2 Hz, 1H), 2.53 (dt, $J = 9.4$, 4.5 Hz, 1H), 2.31 – 2.20 (m, 1H), 2.01 – 1.25 (m, 7H).

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 175.4, 174.7, 169.6, 142.2, 139.7, 134.2, 133.5, 130.7, 128.7, 128.6, 127.9, 127.9, 127.2, 126.7, 93.0, 51.9, 51.4, 45.3, 43.6, 39.7, 38.2, 29.5, 26.9, 25.4, 24.2, 22.1.

LCMS (ESI) calcd for C$_{28}$H$_{30}$N$_2$O$_4$ $\text{[M-H]}^+$ 561.1 found 561.3. $R_t$ = 4.49 min.
5.2.29  (1S,2R)-2-((S)-1-((2-iodobenzamido)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (29)

![Chemical Structure]

To a solution of 28 (105 mg, 0.187 mmol, 1.0 eq) dissolved in MeOH (2 mL) was added 1 M LiOH·H₂O (1 mL) and LiOH·H₂O (75 mg, 1.8 mmol, 9.4 eq). The reaction mixture was stirred at 35 °C for 48 h. The reaction mixture was quenched with 1 M HCl (10 mL), the aqueous phase was extracted with DCM (3x50 mL). The combined organic layers were washed with brine (50 mL), dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified via flash chromatography on a silica gel column using 1-10% MeOH/DCM as eluent to afford the title compound (95 mg, 0.17 mmol, 92%) as an off-white solid.

**1H NMR** (600 MHz, CDCl₃, mixture of two rotamers in ratio 6:4) δ ppm 7.86 (d, J = 7.7 Hz, 0.6H), 7.83 (d, J = 7.8 Hz, 0.4H), 7.45 – 7.30 (m, 3H), 7.28 – 7.11 (m, 3H), 7.05 (dt, J = 25.4, 7.8 Hz, 1H), 6.96 – 6.83 (m, 0.6H), 6.80 – 6.63 (m, 0.4H), 5.95 – 5.76 (m, 1H), 4.14 – 3.43 (m, 4H), 3.09 – 2.78 (m, 3H), 2.65 – 2.53 (m, 1H), 2.34 – 2.07 (m, 1H), 1.88 – 1.72 (m, 3H), 1.53 – 1.20 (m, 4H).

**13C NMR** (151 MHz, CDCl₃, mixture of two rotamers) δ ppm 177.9 and 176.3 (1C), 175.80 and 175.7 (1C), 169.3 (1C), 141.8 and 141.19 (1C), 140.15 and 139.8 (1C), 134.1 and 133.81 (1C), 133.5 and 133.4 (1C), 131.3 and 130.8 (1C), 128.9, 128.8 and 128.7 (1C), 128.4 and 128.3 (1C), 128.1 and 128.0 (1C), 127.6 and 127.5 (1C), 127.3 (1C), 126.9 and 126.8 (1C), 93.0 and 92.8 (1C), 52.9 and 52.7 (1C), 45.4 (1C), 45.0 and 44.9 (1C), 42.8 and 42.3 (1C), 40.6 and 40.0 (1C), 29.7 (1C), 29.3 (1C), 28.8 (1C), 27.1 (1C), 25.33 and 25.29(1C).


5.2.30  (1S,2R)-2-((S)-1-((1-oxo-3-(2-oxo-2-phenylethylidene)isoindolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (30)

![Chemical Structure]

A procedure identical to that described for compound 22 was followed using 29 (400 mg, 0.732 mmol, 1.0 eq), compound 14 (115 mg, 0.884 mmol, 1.2 eq) Cu(OAc)₂·H₂O (30 mg,
0.15 mmol, 0.2 eq), and K$_2$CO$_3$ (303 mg, 2.19 mmol, 3.0 eq) in DMF (5 mL). The reaction mixture was quenched with 6 M HCl (30 mL) and diluted with EtOAc (150 mL). The organic phase was washed with brine (3x50 mL) and dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via reverse phase HPLC using 40-85% MeCN/H$_2$O as eluent to afford a mixture of isomers of the title compound (20 mg, 36 µmol, 5 %, R$_f$=33.0 min) as a yellow oil and (10 mg, 15 µmol, 2 %, R$_f$=29.0 min) as a yellow oil in ration 8:2.

$^1$H NMR (500 MHz, CDCl$_3$, mixture of two isomers in ratio 8:2) $\delta$ ppm 8.96 (d, $J$ = 7.7 Hz, 1H), 8.28 (d, $J$ = 7.0 Hz, 1.6H), 8.19 (d, $J$ = 7.5 Hz, 0.4H), 7.88 – 7.37 (m, 8H), 7.24 – 6.93 (m, 3H), 6.90 – 6.80 (m, 1H), 5.93 – 5.84 (m, 0.8H), 5.80 (dd, $J$ = 11.5, 3.9 Hz, 0.2H), 4.89 – 4.63 (m, 1H), 4.27 – 4.00 (m, 2H), 3.79 – 3.72 (m, 1H), 3.36 – 1.94 (m, 6H), 1.85 – 1.15 (m, 6H).

$^{13}$C NMR (126 MHz, CDCl$_3$, mixture of two isomers) $\delta$ ppm 190.9 and 189.8 (1C), 176.6 and 176.4 (1C), 172.7 (1C), 169.3 and 167.3 (1C), 147.4 and 144.2 (1C), 139.1 (1C), 138.6 and 138.3 (1C), 134.2 and 134.0 (1C), 133.6 and 133.5 (1C), 133.4 and 133.0 (1C), 132.89, 132.86 and 132.8 (1C), 131.5 and 131.2 (1C), 129.5 and 129.0 (1C), 128.9 (1C), 128.83 (2C), 128.76 and 128.6 (1C), 128.2 and 128.1 (1C), 127.8 and 127.6 (1C), 127.5 and 127.2 (1C), 127.0 and 126.9 (1C), 123.3 and 123.1 (1C), 120.6 (1C), 104.5 and 102.0 (1C), 51.9 and 51.6 (1C), 44.9 and 43.2 (1C), 42.9 and 42.4 (1C), 42.1 and 42.0 (1C), 40.7 (1C), 29.8 and 29.7 (1C), 29.4 and 29.1 (1C), 26.94 and 26.89 (1C), 26.0 and 24.5 (1C), 23.0 and 21.7 (1C).

LCMS (ESI) calcd for C$_{34}$H$_{33}$N$_2$O$_5^+$ [M-H]$^+$ 549.2 found 549.3. R$_t$ = 4.92 and 5.12 min.

5.2.31 (1S,2R)-2-(((S)-1-((1-(2-(benzyloxy)-2-oxoethylidene)-3-oxoisodolin-2-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclohexane-1-carboxylic acid (31)[16]

A procedure identical to that described for compound 22 was followed using compound 29 (80 mg, 0.15 mmol, 1.0 eq), compound 15 (29 mg, 0.18 mmol, 1.2 eq) Cu(OAc)$_2$·H$_2$O (6 mg, 30 µmol, 0.2 eq), and K$_2$CO$_3$ (61 mg, 0.44 mmol, 3.0 eq) in DMF (1 mL). The reaction mixture was quenched with 1 M HCl (10 mL) and diluted with EtOAc (150 mL). The organic phase was washed with brine (3x50 mL) and dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via reverse phase HPLC using 45-95% MeCN/H$_2$O as eluent to afford a mixture of isomers of the title compound (11 mg, 19 µmol, 13 %, R$_t$=25.3 min, ratio 8:2) as an orange oil.

$^1$H NMR (500 MHz, CDCl$_3$, mixture of two isomers in ratio 8:2) $\delta$ ppm 9.09 (d, $J$ = 7.9 Hz, 1H), 7.80 – 7.28 (m, 8H), 7.25 – 7.12 (m, 4H), 6.14 (s, 0.8H), 6.07 (s, 0.2H), 6.00 – 5.93
(m, 1H), 5.40 – 5.25 (m, 2H), 4.40 – 3.61 (m, 4H), 3.17 – 2.78 (m, 2H), 2.46 – 2.22 (m, 2H), 1.86 – 1.13 (m, 8H).

$^{13}$C NMR (126 MHz, CDCl$_3$, mixture of two isomers) $\delta$ ppm 176.9 and 176.6 (1C), 173.1 and 172.7 (1C), 167.7 (1C), 165.6 (1C), 148.4 (1C), 138.3 (1C), 136.0 (1C), 133.8 and 133.69 (1C), 133.65 and 133.5 (1C), 133.5 and 133.1 (1C), 132.6 (1C), 131.5 and 131.3 (1C), 129.2 (1C), 128.8 (1C), 128.7 (1C), 128.5 (1C), 128.4 (1C), 128.3 (1C), 128.0 and 127.9 (1C), 127.4 and 127.3 (1C), 127.0 (1C), 123.1 and 122.9 (1C), 120.6 (1C), 98.7 (1C), 66.7 and 66.5 (1C), 51.7 (1C), 43.1 and 42.9 (1C), 42.3 and 42.0 (1C), 41.4 (1C), 40.9 (1C), 29.7 (1C), 29.1 (1C), 26.9 and 26.8 (1C), 26.0 and 25.5 (1C), 22.1 and 21.7 (1C).

LCMS (ESI) calcd for C$_{35}$H$_{35}$N$_2$O$_6^+$ [M-H]$^+$ 579.2, found 579.3. R$_t$ = 4.99 min.

5.3 General Experimental procedure for the attempted $N$–alkylation

5.3.1 Mitsunobu reaction

To a solution of PPh$_3$ (29 mg, 0.11 mmol, 1.5 eq) dissolved in THF (0.5 mL) was added DBAD (25 mg, 0.11 mmol, 1.5 eq). The resulting mixture was stirred for 30 min. (Z)-17 (20 mg, 72 µmol, 1.0 eq) was added followed by addition of 6 (19 mg, 72 µmol, 1.0 eq) dissolved in THF (0.5 mL) and the resulting mixture was stirred 18 h at rt. LC-MS analysis indicated no reaction.

5.3.2 $N$-alkylation with mesyl, tosyl or iodo as leaving group

To a solution of (Z)-17 (1.0 eq) dissolved in DMF or THF was added a non–nucleophilic base (1.0–2.0 eq) and 15-crown-5 (0-2.0 eq). The resulting mixture was stirred at rt for 30 min. Electrophile 10, 11 or 12 (1.0-2.0 eq) was introduced and stirring continued at the indicated temperature for the required amount of time. Reaction detection via LC-MS. The reaction mixture was diluted in EtOAc. The organic phase was washed with H$_2$O and brine and dried over Na$_2$SO$_4$ and concentrate in vacuo. No product was detected.

5.3.3 $N$-alkylation with sulfamidate as leaving group

To a solution of (Z)-17 (1.0 eq) dissolved in DMF or THF was added a non–nucleophilic base (1.0-2.0 eq) and 15-crown-5 (0-2.0 eq). The resulting mixture was stirred at rt for 30 min. Compound 13 (0.8-1.2 eq) was introduced and stirring continued at the indicated temperature for the required amount of time. 6 M HCl was introduced and stirring continued for additional 2 h. The reaction mixture was diluted in DCM. The organic phase was washed with sat. Na$_2$CO$_3$ and brine and dried over Na$_2$SO$_4$ and concentrated in vacuo. No product was detected.

5.3.4 $N$-alkylation with 4-nitrobenzyl bromide and 1-iodopropane as electrophiles

To a solution of (Z)-17 (1.0 eq) dissolved in THF was added a non–nucleophilic base (1.0-2.0 eq) and 15-crown-5 (0-2.0 eq). The resulting mixture stirred at rt for 30 min. 4-nitrobenzyl bromide (2.0 eq), 1-iodopropane or 10 (2.5 eq) was introduced and stirring continued at the indicated temperature for the required amount of time. The reaction mixture
was diluted in EtOAc. The organic phase was washed with H$_2$O and brine and dried over Na$_2$SO$_4$ and concentrated in vacuo.

5.4 Keap1 binding assays$^4$

5.4.1 SPR inhibition in solution assay (ISA) for Keap1

The SPR ISA was performed in analogy to the protocol developed by Chen et al.$^{[24]}$ with the following differences. Instead of a biotinylated peptide, a lysine-tagged version of the Nrf2-peptide (KKKAFQAQLQLDEETGEFL) was utilized for tethering to the sensor surface. For the covalent tethering, a CM5 biosensor (GE Healthcare – research grade) was employed using HBS-P (10mM HEPES, 150 mM NaCl, 0.05%(v/v) Tween 20, 1 mM TCEP, pH 7.40) as continuous flow buffer at a rate of 10 μL/min at 20°C on a BIAcore 3000 optical biosensor unit (GE Healthcare). The surface was activated by injecting a mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) for 7 min, followed by an injection of 50 μM Nrf2-peptide in 10mM Na-Acetate pH 4.0 for 2 min. Any reactive groups still present on the surface were deactivated by a 7min injection of 0.1 M ethanolamine hydrochloride-NaOH pH 8.5. Peptide immobilization levels have been typically between 400-600 RU to ensure mass transport limitation and thus a protein concentration-dependent response.

For the detection of active compounds, a solution of 25 nM Keap1 (Kelch domain = aa321 – aa609) was prepared in running buffer and preincubated with compounds at either constant or varying concentrations. These mixtures have been subsequently injected over the peptide-modified biosensor and control samples devoid of compounds have been used to determine the remaining free protein concentration of Keap1 in response to compound binding. For this, the initial association slopes of the binding sensorgrams have been measured (interval 5-15 s after sample injection) for each sample, followed by a regeneration of the sensor for the next cycle through a 45 s injection of 50 mM Tris/Cl, 0.25% SDS, 5 mM TCEP, pH 7.5. Results have been either reported as % inhibition in relation to the control samples or as KD-values for concentration-response experiments. In the latter case this was achieved by a non-linear regression analysis of the concentration response data which is normalized by the control samples representing 0% inhibition.

$^4$ Keap1 binding assay provided and performed by collaborators at AstraZeneca, Gothenburg.
Bibliography


Appendix A

NMR spectra of important and novel compounds

Figure A. 1. $^1$H NMR of compound 10.

Figure A. 2. Predicted $^1$H NMR of two rotomers of compound 10.
Figure A. 3. $^{13}$C NMR of compound 10.

Figure A. 4. Predicted $^{13}$C NMR of compound 10.
Figure A. 5. $^1H$ NMR of compound 11.

Figure A. 6. Predicted $^1H$ NMR of two rotomers of compound 11.
Figure A. 7. $^{13}$C NMR of compound 11.

Figure A. 8. Predicted $^{13}$C NMR of compound 11.
Figure A. 9. $^1$H NMR of compound 12. Triphenylphosphine oxide impurities labelled as PPh$_3$O.

Figure A. 10. Predicted $^1$H NMR of two rotomers of compound 12.
Figure A. 11. $^{13}$C NMR of compound 12. Triphenylphosphine oxide impurity peaks indicated in grey.

Figure A. 12. Predicted $^{13}$C NMR of compound 12.
Figure A. 13. $^1$H NMR of compound 13.

Figure A. 14. Predicted $^1$H NMR of compound 13.
Figure A. 15. $^1$H NMR of compound 19.

Figure A. 16. Predicted $^1$H NMR of two rotomers of compound 19.
Figure A. 17. $^{13}$C NMR of compound 19.

Figure A. 18. Predicted $^{13}$C NMR of compound 19.
Figure A. 19. $^1$H NMR of compound 21.

Figure A. 20. Predicted $^1$H NMR of two rotomers of compound 21.
Figure A. 21. $^{13}$C NMR of compound 21.

Figure A. 22. Predicted $^{13}$C NMR of compound 21.
Figure A. 23. $^1$H NMR of compound 22.

Figure A. 24. Predicted $^1$H NMR of two diastereomers of compound 22.
Figure A. 25. $^{13}$C NMR of compound 22.

Figure A. 26. Predicted $^{13}$C NMR of compound 22.
**Figure A. 27.** Impure $^1H$ NMR of compound 23.

**Figure A. 28.** Predicted $^1H$ NMR of two diastereomers of compound 23.
Figure A. 29. Impure $^{13}$C NMR of compound 23.

Figure A. 30. Predicted $^{13}$C NMR of compound 23.
Figure A. 31. Impure $^1$H NMR of compound 24.

Figure A. 32. Predicted $^1$H NMR of two diastereomers of compound 24.
Figure A. 33. Impure $^{13}$C NMR of compound 24.

Figure A. 34. Predicted $^{13}$C NMR of compound 24.
Figure A. 35. Impure $^1$H NMR of compound 25.

Figure A. 36. Predicted $^1$H NMR of two diastereomers of compound 25.
Figure A. 37. Impure $^{13}$C NMR of compound 25.

Figure A. 38. Predicted $^{13}$C NMR of compound 25.
Figure A. 39. Impure $^1$H NMR of compound 26.

Figure A. 40. Predicted $^1$H NMR of two diastereomers of compound 26.
Figure A. 41. Impure $^{13}$C NMR of compound 26.

Figure A. 42. Predicted $^{13}$C NMR of compound 26.
Figure A. 43. Impure $^1$H NMR of compound 27.

Figure A. 44. Predicted $^1$H NMR of two diastereomers of compound 27.
Figure A. 45. Impure $^{13}$C NMR of compound 27.

Figure A. 46. Predicted $^{13}$C NMR of compound 27.
Figure A. 47. Impure $^1H$ NMR of compound 28.

Figure A. 48. Predicted $^1H$ NMR of compound 28.
Figure A. 49. Impure $^{13}$C NMR of compound 28.

Figure A. 50. Predicted $^{13}$C NMR of compound 28.
Figure A. 51. Impure $^1$H NMR of compound 29.

Figure A. 52. Predicted $^1$H NMR of two diastereomers of compound 29.
Figure A. 53. Impure $^{13}$C NMR of compound 29.

Figure A. 54. Predicted $^{13}$C NMR of compound 29.
Figure A. 55. Impure $^1$H NMR of compound 30.

Figure A. 56. Predicted $^1$H NMR of two diastereomers of compound 30.
Figure A. 57. Impure $^{13}$C NMR of compound 30.

Figure A. 58. Predicted $^{13}$C NMR of compound 30.
Figure A. 59. Impure $^1$H NMR of compound 31.

Figure A. 60. Predicted $^1$H NMR of two diastereomers of compound 31.
Figure A. 61. Impure $^{13}$C NMR of compound 31.

Figure A. 62. Predicted $^{13}$C NMR of compound 31.
# Appendix B

**MM-GBSA binding free energy results of original set of compounds**

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Ph

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Ph

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-90.0

Ph

-98.90

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Ph

-84.3

-66.34

Ph

-71.20

-59.02

Ph

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-52.78

Ph

-66.44

-66.34

Ph

-63.71

-47.87
\[
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& \quad -48.09 & -55.34 \\
& \quad -74.37 & -71.15 \\
& \quad -73.81 & -61.19 \\
& \quad -76.6 & -63.45 \\
& \quad -70.20 & -52.75
\end{align*}
\]