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COMMUNICATION

Modified peptide inhibitors of the Keap1-Nrf2 protein-protein interaction incorporating unnatural amino acids

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Abstract: Non-covalent inhibitors of the Keap1-Nrf2 protein-protein interaction (PPI) have therapeutic potential in a range of disease states including neurodegenerative diseases (Parkinson's and Alzheimer's diseases), chronic obstructive pulmonary disease and various inflammatory conditions. By stalling Keap1-mediated ubiquitination of Nrf2, such compounds can enhance Nrf2 transcriptional activity and activate the expression of a range of genes with antioxidant response elements (AREs) in their promoter regions. Keap1 inhibitors have been identified based on peptide and small molecule templates. In this paper we develop the SAR of the peptide series and identify a group of ligands incorporating unnatural amino acids that demonstrate improved binding affinity in fluorescence polarisation, differential scanning fluorimetry and isothermal titration calorimetry assays. These modified peptides have the potential for further development into peptidomimetic chemical probes to explore the role of Nrf2 in disease and as potential lead structures for drug development.

Increasing the activity of the transcription factor Nrf2 is an inducible cellular response to a range of inputs including redox and electrophilic stress, and various intracellular stimuli.[1] Nrf2 activation results in increased expression of a large battery of genes with antioxidant response elements in their promoter regions.[2] These include proteins associated with redox homeostasis (*e.g.* thioredoxin, thioredoxin reductase), phase I and II metabolism (*e.g.* NAD(P)H quinone oxidoreductase-1 (NQO1), glutathione synthesis and conjugation enzymes) and proteins involved in autophagy (*e.g.* sequestosome-1/p62, NDP52) amongst others.[1, 3] Increasing Nrf2 activity has been proposed as a potential disease-modifying intervention in neurodegenerative conditions such as Parkinson's and Alzheimer's disease and various inflammatory conditions.[4] The main negative regulators of Nrf2 activity are the proteins Keap1 and β-TrCP that target Nrf2 for ubiquitination and proteosomal degradation by interacting with degrons in the Neh2 and Neh6 domains of Nrf2 respectively.[5] Keap1 is the major regulator and its interaction with Nrf2 has been studied extensively.[6] Inhibition of the direct protein-protein interaction (PPI) between Keap1 and Nrf2 has been proposed as an intervention that can increase Nrf2 transcriptional activity by stalling its ubiquitination and turnover in the cell, thus prolonging its half-life (Figure 1).[4] Recently, several approaches to inhibit the PPI between the *C*-terminal Keap1 Kelch domain and the Neh2 domain of Nrf2 have been described.[7] One of the early approaches to develop Keap1 inhibitors was based on the native peptide sequences of Nrf2 (**1** and **2**) and related proteins (p62 **3**, prothymosin-α) that interact with the Kelch domain of Keap1.[8] Such peptides have a high affinity for Keap1, with the most active examples having IC_{50}s in the nanomolar range. Small molecules based on sulphonamide scaffolds (*e.g.* **4**) have been described with **4b** demonstrating low nanomolar activity in competitive binding assays with Keap1.[9] Other diverse structures identified through high-throughput screening and SAR studies are generally less active.[10] Most of these compounds are capable of inducing the expression of Nrf2 target genes in cells, although these effects are observed at micromolar concentrations in most cases.

Peptides represent orthogonal lead structures to known small molecule Keap1 inhibitors and have the potential to be developed into drug- or chemical probe-like leads for further development. Indeed, co-crystallization studies have demonstrated that small molecule inhibitors and peptide ligands bind to subtly different conformations of the Keap1 protein, supporting the notion that divergent SAR profiles are feasible. We and others have shown that peptides based upon the high affinity Nrf2 ETGE sequence have proven to be the most active in Keap1 binding assays, and hybrid sequences based upon the ETGE/p62 consensus sequences e.g. **5** have provided the best short (7mer) interacting sequences.[8, 11] However, these peptides are polar and have limited activity in cell-based assays of Nrf2 induction. Modest enhancement of cellular activity can be achieved by conjugation of the peptides to fatty acids[12] or TAT sequences,[13] however further development of the underlying peptide SAR is required in order to address the membrane permeability of the structures.

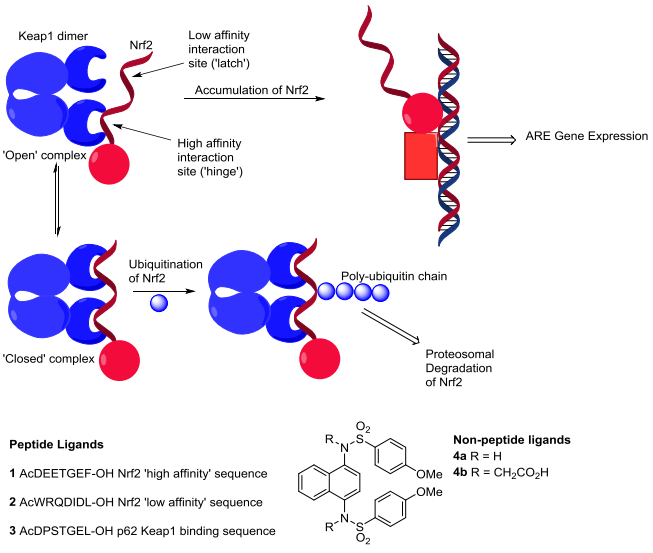

Figure 1. Schematic representation of the Keap1-Nrf2 'hinge and latch' interaction mechanism. Peptides **1** and **2** match the high and low affinity sequences of Nrf2 respectively and **3** is derived from the Keap1 binding protein p62. Compound **4** is an example of a small molecule inhibitor of the Keap1-Nrf2 interaction.

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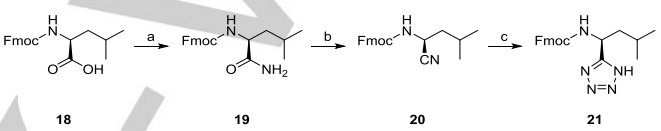
In this manuscript, we describe some of our recent work to characterize the binding behavior of short 7mer Keap1-interactive peptides using X-ray crystallography and to explore the SAR of analogues that incorporate unnatural amino acids. We anticipate that any increases in binding could be used to offset removal or substitution of charged residues from the peptide sequence in subsequent molecules, thus progressing towards more cell-permeable derivatives. We show that the incorporation of unnatural amino acids at key points in the sequence results in peptides with improved or maintained binding affinity (measured by FP, DSF or ITC) and we rationalise some of these changes using *in silico* structural substitutions using the new crystal structures as templates.

Table 1. FP IC_{50} and DSF ΔTm values for Keap1 peptide inhibitors.

Cpd[a]	Sequence	FP IC_{50} (nM)	FP Calc. K_i (nM)	ΔT_m (°C)
1	Ac-Asp-Glu-Glu-Thr-Gly-Glu-Phe-OH[a]	5390 ± 580[a]	2265	1.95 ± 0.1
5	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Leu-OH[a]	115 ± 13[a]	48	n/d[b]
6	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Leu-NH-*i*Pentyl	745 ± 126	313	2.0 ± 0.1
7	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Leu-NH-Bn	888 ± 28	373	2.5 ± 0.1
8	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Leu-TET	374 ± 31	157	6.2 ± 0.3
9	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Tle-OH	235 ± 16	99	5.8 ± 0.6
10	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Thi-OH	578 ± 25	243	5.3 ± 0.2
11	Ac-Asp-Pro-Glu-Thr-Gly-Glu-Cha-OH	85 ± 14	36	5.8 ± 0.9
12	Ac-Asp-Pro-Glu-hPhe-Gly-Glu-Leu-OH	20%[c]	n/d[b]	0.8 ± 0.2
13	Ac-Asp-Pro-Glu-Bap-Gly-Glu-Leu-OH	27%[c]	n/d[b]	0.5 ± 0.1
14	Ac-Asp-Pro-Glu-Asn-Gly-Glu-Leu-OH	3036 ± 310	1276	0.7 ± 0.2
15	Ac-Asp-Thp-Glu-Thr-Gly-Glu-Leu-OH	89 ± 6	37	8.3 ± 0.3
16	Ac-Asp-Pip-Glu-Thr-Gly-Glu-Leu-OH	1063 ± 280	447	1.8 ± 0.2
17	Ac-Asp-Thp-Glu-Thr-Gly-Glu-Cha-OH	31 ± 3.7	13	n/d[b]

[a] Data from Hancock et al., 2012.[8a] [b] Not determined. [c] Percentage inhibition at 100 μM concentration of inhibitor. Estimated K_i values were calculated using the method described by Kenakin.[14]

We initially sought to investigate modifications on the *C*-terminal α-carboxylate of the Nrf2/p62 hybrid peptide **5** with the aim of improving Keap1 binding activity and reducing the overall net charge of the series. Peptides bearing a *C*-terminal *N*-isopentyl amide or *N*-benzyl amide (**6** and **7** respectively) were prepared by nucleophilic cleavage of the common, fully deprotected, peptide precursor Ac-DPETGEL from an HMBA resin. In addition, we synthesised the *C*-terminal 1*H*-tetrazole peptide **8** using a standard Fmoc-SPPS procedure that included the direct attachment of Fmoc-Leu-T **20** to a 2-chlorotrityl chloride resin as the loading step. The required building block **20** was prepared from the readily available Fmoc-Leu-OH **17** in a three-step solution phase synthetic procedure analogous to that reported previously for the corresponding Cbz-protected analogue.[15] Briefly, **18** was converted to the primary amide analogue **19** using Boc_2O and $(NH_4)_2CO_3$, then sequentially dehydrated with cyanuric chloride and subjected to [2+3] cycloaddition with NaN_3 to furnish **21** in 55% overall yield.



Scheme 1. Synthetic route to 1*H*-tetrazole **21**. Reagents and conditions: a) $(NH_4)_2CO_3$, Boc_2O, pyridine, MeCN, 0°C to rt, 16 h, 84%; b) Cyanuric chloride, DMF, 0°C to rt, 16 h, 98%; c) NaN_3, $ZnBr_2$, H_2O/*i*PrOH (2:1 v/v), reflux, 16h, 67%.

Previous work from our group has demonstrated that hydrophobic amino acids are generally well tolerated as *C*-terminal residues in ETGE-derived 7-mer peptides.[8a] This prompted us to synthesise analogues of **5** in which leucine is replaced by non-proteinogenic hydrophobic amino acids, as such modifications can increase the metabolic stability of peptides and lead to lipophilicity and potency improvements.[16] Peptides bearing *tert*-leucine (Tle) (peptide **9**), thienylalanine (Thi) (peptide **10**) or cyclohexylalanine (Cha) (peptide **11**), as *C*-terminal residues were synthesised using standard SPPS procedures on a 2-chlorotrityl chloride resin from commercially available *N*-Fmoc-protected amino acids.

Additionally, we investigated modifications to the central threonine residue of the optimised peptide sequence. According to the available crystallographic and molecular docking data,[12] the threonine side chain of the peptide occupies space above the central channel through the Keap1 Kelch domain with the methyl group pointing towards the pore. To explore the possibility of extending a portion of a modified side chain into this opening, we replaced threonine with the longer side chain amino acids homophenylalanine (hPhe) (peptide **12**) and 2-amino-3-benzamidopropanoic acid (Bap) (peptide **13**). We also substituted threonine with asparagine (peptide **14**) to mimic the ENGE motif of the Keap1-interacting protein prothymosin-α.[17]

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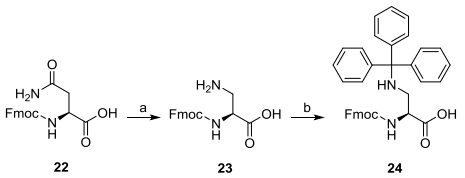

Scheme 2. Synthetic route to the N_β-trityl protected Dap **24**. Reagents and conditions: a) [*bis*(trifluoroacetoxy)iodo]benzene, pyridine, DMF/H_2O (2:1 v/v), rt, 18 h, 86%; b) TMS-Cl, DCM, reflux, 4 h, then Trt-Cl, pyridine, 0°C to rt, 2 h, 77%.

Peptides **12** and **14** were prepared from commercially available Fmoc-protected amino acids, while **13** was synthesised using a modified Fmoc-SPPS procedure that included the on-resin modification of the peptide precursor containing an N_β-trityl protected (*S*)-2,3-diaminopropanoic acid (Dap) residue at the respective position. The required building block **24** was prepared from Fmoc-Asn-OH **22** according to the synthetic sequence outlined in Scheme 2. Hoffmann rearrangement of **22** using [*bis*(trifluoroacetoxy)iodo]benzene afforded Fmoc-Dap-OH **23**, which was in turn converted to **24** by installing a temporary TMS protection on the α-carboxylate group prior to treatment with trityl chloride. Following the synthesis of the fully protected peptide precursor on resin, the DAP trityl group was selectively removed by treatment with a dilute solution of TFA in DCM/TIS. The resulting free DAP amine was reacted with benzoyl chloride to afford peptide **13** after a simultaneous cleavage from the resin and global deprotection.

Scheme 3. Synthetic route to peptide **13**. Reagents and conditions: a) Fmoc-SPPS cycles; b) TFA/TIS/DCM (3:5:92 v/v/v), rt, 3 x 5 min; c) benzoyl chloride, pyridine, DMF, rt, 5 h; d) TFA/TIS/H_2O (95:2.5:2.5 v/v/v), rt, 3 h.

Additionally, we were interested in replacing proline with other cyclic amino acids in order to investigate how changes in the size and rigidity of the ring would affect the conformational stability of the peptide as well as its binding affinity for the Keap1 Kelch binding pocket. Peptides bearing the unnatural amino acids thiazolidine-4-carboxylic acid (Thp) (peptide **15**) or piperidine-2-carboxylic acid (Pip) (peptide **16**) were prepared following a standard Fmoc-SPPS procedure using commercially available building blocks.

The ability of the peptides to interact with the Kelch domain of Keap1 was determined using a previously described fluorescence polarisation (FP) assay.[8a] The native Nrf2 ETGE peptide **1** has an FP IC_{50} of 5.39 μM (calc. K_i 2.26 μM), while the Nrf2/p62 hybrid peptide **5** is more than 40-fold more active. Replacing the *C*-terminal carboxylate of **5** with either an *N*-isopentyl (peptide **6**) or an *N*-benzyl (peptide **7**) amide led to a moderate drop in activity (Table 1). These results are consistent with a recently reported molecular modelling study, attributing the reduced activity of peptides containing *C*-terminal amides to the absence of electrostatic interactions with Arg380.[18] On the other hand, substituting the *C*-terminal carboxylate with a 1*H*-tetrazole (peptide **8**), which has a similar pK_a value but is more lipophilic,[19] was better tolerated than the previous modifications, although a 3-fold drop in activity was recorded compared to the parent peptide **5**.

In addition to the changes in the α-carboxylate of leucine, we also synthesised a small set of peptides bearing modified side chains at the *C*-terminus. Replacing leucine with the shorter but bulkier side chain *tert*-leucine (peptide **9**) resulted in a 2-fold lower activity, while the aromatic thienylalanine (peptide **10**) was less well tolerated at this position, giving a 5-fold drop in binding affinity. Interestingly, changing the isobutyl sidechain group of leucine to cyclohexylmethyl (peptide **11**) led to a moderate potency improvement (FP IC_{50} 85 nM, calc. K_i 36 nM), an effect that could be attributed to an increase in hydrophobic contacts with the Keap1 binding pocket.

Replacement of threonine with the longer side chain amino acids homophenylalanine (peptide **12**) and 2-amino-3-benzamidopropanoic acid (peptide **13**) resulted in a dramatic drop in activity that mirrors the previously reported effects of Ala or Val substitution.[11] On the other hand, substitution of threonine with asparagine (peptide **14**), which is present in the ENGE motif of the Keap1-interacting protein prothymosin-α,[28] was better tolerated, however the binding affinity of the respective peptide **14** was reduced by more than one order of magnitude compared to **5**. These results are consistent with a recent molecular modelling study suggesting that despite its similarity to the Nrf2 ETGE motif, the Keap1-interacting region of prothymosin-α is significantly more disordered.[29]

Substituting (*L*)-thiazolidine-4-carboxylic acid (thioproline) for proline (peptide **15**) resulted in an increased Keap1 binding activity and an FP IC_{50} of 89 nM (calc. K_i 37 nM). The Thp thiazolidine ring has a different rigidity and size compared to the pyrrolidine ring of proline,[30–32] and its incorporation into short peptides has been shown to induce a stabilisation of the *cis*-amide conformation, effects that could account for the affinity improvement recorded.[33,34] In contrast, changing proline to piperidine-2-carboxylic acid (peptide **16**) resulted in a more than 10-fold drop in binding activity (Table 1). Incorporating two unnatural amino acids in the peptide sequence (peptide 17) resulted in an improvement in the binding to Keap1 (IC_{50} 31 nM, calc. K_i 13 nM), suggesting that the substitutions may be partially additive in their effects on binding activity.

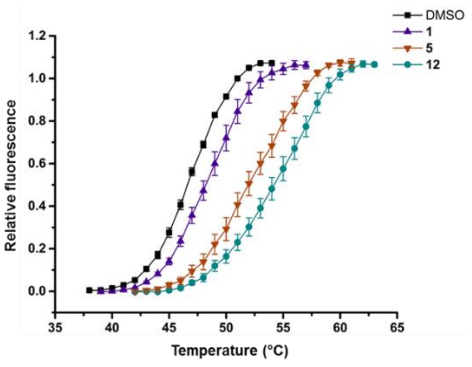

Figure 2. DSF melt curves for the Keap1 Kelch protein in the presence of DMSO control (■, coloured in black) or 10 μM concentrations of 1 (▲, coloured in purple), 8 (▼, coloured in orange) or 15 (●, coloured in cyan), n=4.

The binding activity of peptides **6** – **16** against the Keap1 Kelch domain was further characterised in a secondary differential scanning fluorimetry (DSF) screening assay at a fixed concentration of 10 μM (Table 1, Figure 2). The midpoint temperature of transition (T_m) for the Keap1 Kelch protein was estimated to be 47.0 ± 0.1 °C, while the Nrf2 ETGE peptide **1** that was used as a positive control induced a shift in the T_m (ΔT_m) of 1.5 ± 0.2 °C. Consistent with their weak binding profile in the FP assay, peptides **12**, **13** and **14** caused only a marginal thermal stabilisation (ΔT_m of ~ 0.5 °C) of the Keap1 Kelch protein in the DSF screen, while **6**, **7** and **16** that have FP IC_{50} values in the sub-micromolar to low micromolar concentration range were considerably more active (ΔT_m of ~ 2 °C). On the other hand, peptides **8** – **11** had a more profound effect on the Keap1 Kelch *T*m (ΔT_m of ~ 5 – 6 °C), which is in agreement with their increased potency as recorded in the FP assay. Interestingly, peptide **15** induced a ΔT_m of 8.3 °C, confirming its improved binding affinity compared to the other analogues of this series. Although it is difficult to compare the DSF and FP binding data directly (fixed concentration and variable concentration studies respectively), there is a reasonable correlation between the two sets of data (R^2 = 0.743 using a logarithmic fit) (Figure S1).

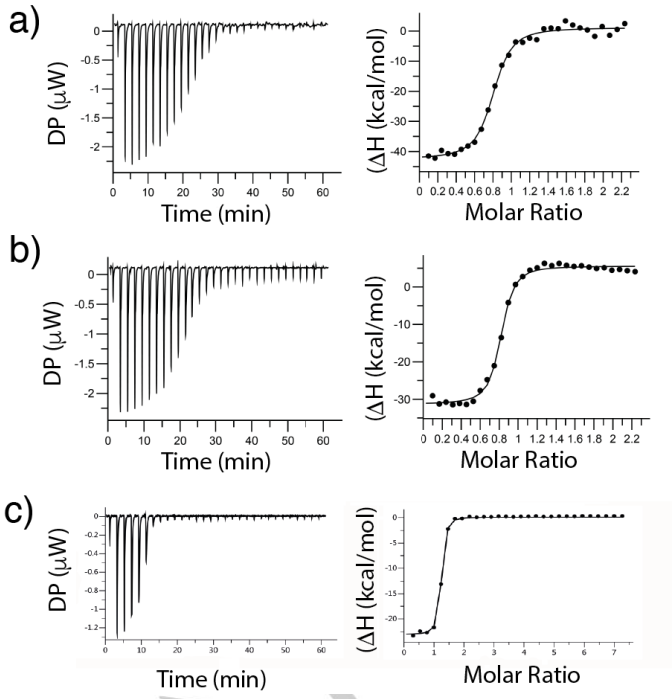

Figure 3. ITC analyses of peptide binding to Keap1. Raw (left panels) and normalized ITC data for titrations plotted versus the molar ratio of inhibitor−protein (right panels) demonstrating saturable exothermic reaction upon sequential additions of a) **5** c) **9** and d) **17** at 500 μM.

Motivated by the promising binding profiles observed in the FP and DSF assays, additional confirmation of the binding activity of the peptides was sought using isothermal titration calorimetry (ITC). In agreement with our previous report,[8a] peptide **5** had an ITC K_d of 250 nM (Figure 3a, Table 2), consistent with its FP IC_{50} of 115 nM (Table 1). Analysis of the thermodynamic profile of the interaction demonstrated a binding event that is enthalpy-driven (ΔH), with a small entropic ($T\Delta S$) penalty, possibly reflecting the stabilisation of the β-hairpin conformation of the peptide by the proline residue. On the other hand, replacing leucine of peptide **5** with *tert*-leucine **9** led to an approximately 2-fold drop in binding affinity that was characterised by an increased entropic penalty (Figure 3b, Table 2). In agreement with the FP data previously described, peptide **15** showed a good binding profile in the ITC assay with a calculated K_d of 310 nM (Figure 3c, Table 2). Peptide **11** with an FP IC_{50} of 89 nM gave a comparable ITC K_d of 75 nM and its analogue **17** incorporating both a thioproline and cyclohexylalanine had a K_d of 56 nM, similar to its FP IC_{50} of 31 nM. Compared to peptide **5**, improvements in the binding enthalpy recorded for **15** were offset by negative changes in the entropy of binding, resulting in a similar binding free energy (ΔG). The apparent large enthalpy/entropy compensation for peptide **17** warrants further investigation.

Table 2. Thermodynamic parameters extracted from the calorimetric evaluation of Keap1 binding with different peptides (n ≥ 2, T = 25°C)

Cpd	ITC K_d [μM]	ΔG [kcal/mol]	ΔH [kcal/mol]	$T\Delta S$ [kcal/mol]
5	0.25 ± 0.10	-9.03 ± 0.07	-9.07 ± 0.35	-0.03 ± 0.28
9	0.58 ± 0.09	-8.59 ± 0.12	-10.25 ± 0.21	-1.69 ± 0.34
11	0.075 ± 0.006	-9.57 ± 0.21	-9.83 ± 0.22	-0.29 ± 0.001
15	0.31 ± 0.01	-8.95 ± 0.17	-12.83 ± 0.33	-3.84 ± 0.31
17	0.056 ± 0.005	-9.89 ± 0.04	-23.2 ± 0.21	-13.25 ± 0.07

To date, co-crystallization studies with Keap1 and peptides have used relatively long linear sequences of 14-16 amino acids,[17, 20] and one 34mer sequence,[21] with only one example of a shorter cyclic 7mer[22] Nrf2-derived peptide. We have soaked human Keap1 Kelch domain crystals with peptides **1** and **5** to determine their bound conformations, particularly to observe the effect of shortening the *N*- and *C*-termini on the conformation of the peptide. The resulting crystal structures had resolutions of 2.92 Å (peptide **1**, PDB Ref: 6FMP) and 2.10 Å (peptide **5**, PDB Ref: 6FMQ). In each case the peptide occupied the binding pocket of one of the two Keap1 proteins in the unit cell (Figure S1a,b) and formed interactions with the vacant Keap1 Kelch domain via polar interactions between the *C*-terminal carboxylate of the peptide and Arg380 of the adjacent protein (Figure S1c,d). Otherwise, the peptide are oriented in a similar manner to the previously described ETGE 16mer peptide[20a] (Figure 4a,b), with the exception of the Asp sidechain being positioned differently due to rotation about the Cα-CO bond.

Subsequently, we examined the crystal structures to determine how the various structural changes made in our current study may be accommodated in the binding pocket (Figure 4). Peptide **1** forms electrostatic and/or hydrogen bond interactions with Arg380, Asn382, Arg483, Gln530, Tyr525 and Ser602 (Figure 4a) and peptide **5** forms an additional interaction with Arg415, but lacks the glutamate sidechain that interacts with Tyr525 (Figure 4b). Peptide **5** forms a tighter interaction with Keap1, probably due to the conformational restriction introduced by the Glu > Pro

substitution which restricts the mobility of the peptide backbone. *In silico* structural replacements within the crystal structure to convert **5** to **8** (*C*-terminal carboxylate to tetrazole) suggest that the larger tetrazole, in its deprotonated form, can occupy a similar space and could form hydrogen bond interactions with Asn382 (Figure 5a *cf*. 5b). This is consistent with its comparable binding affinity to Keap1 observed in the FP and ITC experiments (Tables 1 and 2). Similarly, the larger thioproline present in **15** could be accommodated in place of proline without clashing with the edge of the binding pocket (Figure 5c *cf*. 5d). The limited structural distortion resulting from changing the proline to thioproline suggests that the network of polar interactions formed by **15** in the modified structure could be similar to that of the other peptides, so the nature of the different enthalpy and entropy contributions from **15** *vs*. **5** in the ITC study (Table 2) requires further investigation.

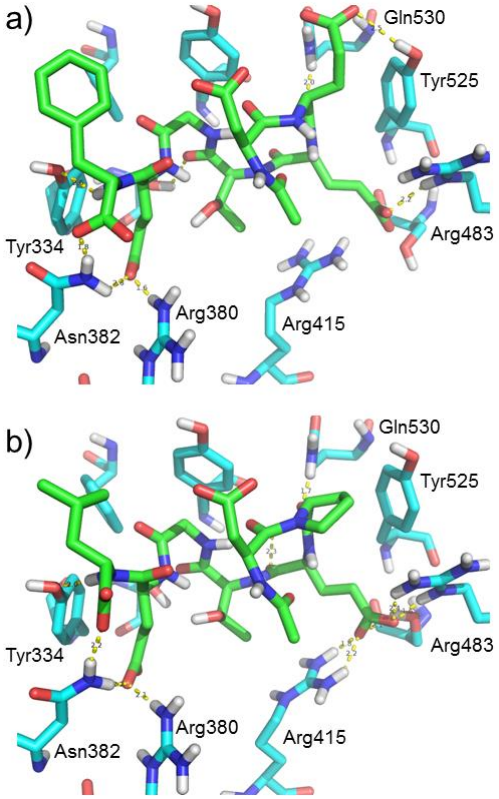

Figure 4. Interactions between peptides **1** and **5** and the Keap1 Kelch domain. a. Peptide **1** – Keap1 Kelch domain (PDB Ref 6FMP) and b. peptide **5** – Keap1 Kelch domain (PDB Ref 6FMQ) structures respectively, selected protein residues are shown cyan and peptide residues in green.

The new peptide structures described in this study provide further insights into the structural requirements for binding of this class of molecule to Keap1. It is notable that the structural changes that improve or maintain binding affinity are relatively modular: proline to thioproline (**5** *vs*. **15**), leucine to *tert*-leucine or cyclohexyl-alanine (**5** *vs*. **8** or **11**), and *C*-terminal carboxylate to tetrazole (**5** *vs*. **8**). Thus, combining two or more of these changes in a single molecule may be advantageous. The improved activity of peptide **17** which incorporates both a thioproline and a cyclohexylalanine appears to support this. We would anticipate that incorporating extensions of the *N*-terminal acetyl group (*e.g.* to steroyl) as we described previously[12] would be expected to yield a peptide with improved binding affinity and, potentially, improved biological stability and permeability.

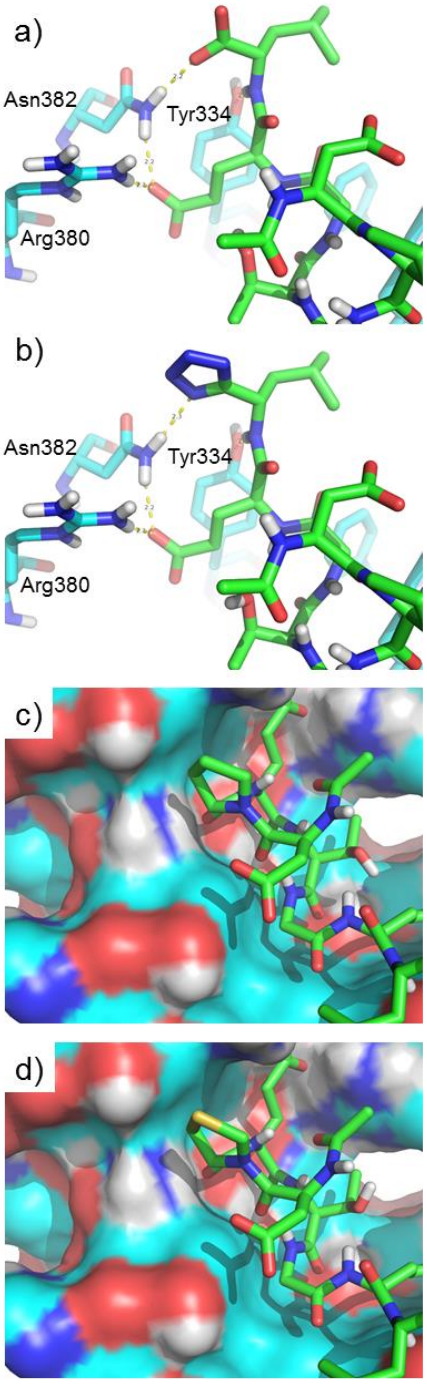

Figure 5. Bound conformations of peptides in complex with the Keap1 Kelch domain. a. Peptide **5** forms polar interactions via its C-terminal carboxylate; b. the modelled conformation of peptide **8** suggests a tetrazole can mimic such interactions; c. the proline residue of peptide **5** occupies space near the surface of the binding pocket; d. the modelled conformation of peptide **15** suggests that the thioproline reside can be accomodated at this site. Peptides are represented as green sticks; Keap1 is represented as cyan sticks with selected residues labelled (a., b.) or as a surface (c., d.); polar interactions are shown as yellow dotted lines.

Experimental Section

All synthetic procedures, characterisation data for all new compounds (^{1}H & ^{13}C NMR, HPLC, LC-MS, HRMS) and biophysical techniques (FP and DSF assays, ITC, and molecular modelling) are detailed in the ESI.†

Acknowledgements

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Conflict of interest

GW, JG and NDG are founders of Keregen Therapeutics Ltd, an SME with an interest in developing small molecule inducers of Nrf2.

Keywords: Keap1 • Nrf2 • peptides • unnatural amino acids • protein-protein interaction

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Entry for the Table of Contents

COMMUNICATION

Compound **17**

Ac-Asp-Thp-Glu-Thr-Gly-Glu-Cha-OH

Keap1 Binding Properties:

FP IC_{50} 31 nM

ITC K_d 56 nM

*Dr. Nikolaos D. Georgakopoulos, Dr. Sandeep K. Talapatra, Dr. Jemma Gatliff, Prof. Frank Kozielski and Dr. Geoff Wells**

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Modified peptide inhibitors of the Keap1-Nrf2 protein-protein interaction incorporating unnatural amino acids