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Synthesis and optimisation of P_3 substituted vinyl sulfone-based inhibitors as anti-trypanosomal agents

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ABSTRACT

A series of lysine-based vinyl sulfone peptidomimetics were synthesised and evaluated for anti-trypanosomal activity against bloodstream forms of *T. brucei*. This focused set of compounds, varying in the P_3 position, were accessed in a divergent manner from a common intermediate (ammonium salt **8**). Several P_3 analogues exhibited sub-micromolar EC_{50} values, with thiourea **14**, urea **15** and amide **21** representing the most potent anti-trypanosomal derivatives of the series. In order to establish an *in vitro* selectivity index the most active anti-trypanosomal compounds were also assessed for their impact on cell viability and cytotoxity effects in mammalian cells. Encouragingly, all compounds only reduced cellular metabolic activity in mammalian cells to a modest level and little, or no cytotoxicity, was observed with the series.

1. Introduction

In 1992, it was reported that vinyl sulfones were inhibitors of the cysteine protease papain.[1] Since then, compounds containing this functional group have emerged as valuable members of a toolkit of electrophilic agents (including, for example, maleimides etc.) with the ability to interact with and derivatise biological molecules.[2] It is now well-appreciated that this occurs *via* the S-conjugate addition of vinyl sulfones with nucleophilic cysteine residues and, since Hanzlik's initial report,[1] several publications have appeared featuring the use of vinyl sulfone-based molecules as inhibitors of enzymes and chemical probes.[3] Efforts, most notably from the collective research groups of McKerrow, Rosenthal, Roush and Renslo,[4] have identified thiophilic peptidyl vinyl sulfones **1** and **2** as lead structures to combat infections caused by parasitic organisms.[5] HYPERLINK "SPS:refid::bib5"

Specifically, regarding anti-parasitic activity, numerous studies have implicated trypanosomal cysteinyl proteases as targets for peptidyl vinyl sulfones.[6] S-Derivatisation of these enzymes, which are critical to the survival of the bloodstream form of the parasite, leads to cell death.[6–7] We have developed a series of lysine-based compounds (e.g. **3**, Figure 1) stemming from McKerrow's previous work (e.g. **1** and **2**) and have shown that members of this family exhibited potent anti-trypanosomal activity *in vitro*.[8] To probe the $P_{1'}$-P_2 regions of the cysteinyl protease binding domain, a series of compounds with various substitutions at positions R^2, R^3 and R^4 were prepared. However, to date, we have not similarly varied the identity of the Cbz substituent. This is of interest since substituents in this position protrude into the large P_3 region of the cysteinyl protease[7] binding pocket and in principle, additional binding interactions and/or selectivity might be achieved following optimisation of the group penetrating this region. With this in mind, it is important to note that the well-established methods for Cbz removal offer the possibility to explore the P_3 region from a common intermediate in a

Abbreviations: Ac, acetyl; $[\alpha]_D$, specific rotation (measured at 589 nm); app, apparent; Ar, aromatic; Bu, butyl; Cbz, carboxybenzyl (benzyloxycarbonyl); d, doublet; dd, doublet of doublets; DIPEA, diisopropylethylamine; DMSO, dimethylsulfoxide; *c*-Hex, cyclohexane; CI, confidence interval; clogP, calculated partition coefficient (log_{10}); EC_{50}, effective concentration half maximal; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ES, electrospray; Et, ethyl; EtOAc, ethyl acetate; equiv, equivalent; g/mg, gram/milligram; GI_{50}, growth inhibition half maximal; H, hour(s); HaCaT, human keratinocyte cells; HOBt, hydroxybenzotriazole; HRMS, high resolution mass spectrometry; IR, infrared (spectroscopy); *J*, coupling constant; LD_{50}, lethal dose half maximal; LDH, lactate dehydrogenase; M, molarity ($moldm^{-3}$); m, multiplet; Me, methyl; mL/μL, millilitre/microlitre; M.p, melting point; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic resonance; Ph, phenyl; ppm, parts per million; q, quartet; R_f, retention factor; rt, room temperature (approx. 18 °C); S.D, standard deviation; t, triplet.

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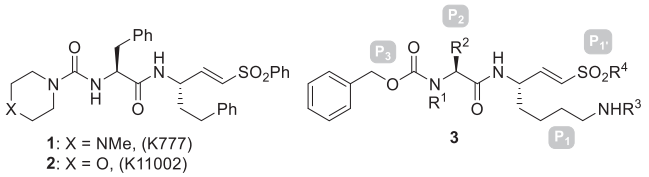


Fig. 1. K777(**1**) and K11002 (**2**): Benchmark vinyl sulfone inhibitors and our lysine-based scaffold **3**.

divergent manner.

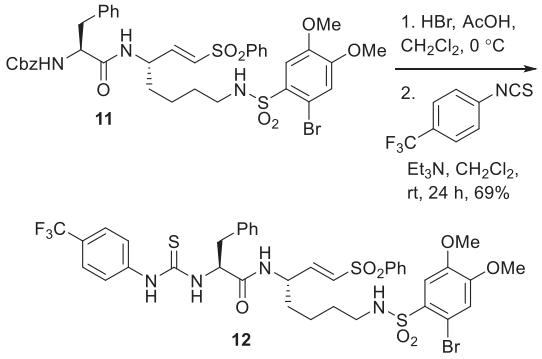
Previous approaches to access P_3 substituted analogues of vinyl sulfone inhibitors (of the type **6**) involve coupling strategies whereby an amino acid derivative (**4**) and vinyl sulfone ammonium salt (**5**) are united, whereby the desired P_3 substituent (R^1) is pre-installed (Scheme 1).[3a,4a,h,i] Whilst convergent, from the perspective of rapidly identifying an ideal P_3 substituent, this approach is limited.

Previously, we have investigated the Cbz-deprotection of ε-amino naphthalene sulfonamide **7**, a compound which itself exhibited promising anti-trypanosomal activity.[8] Treatment of **7** with HBr in acetic acid gave ammonium bromide salt **8** which was isolated and was fully characterised. Upon treatment with just base **8** gradually underwent a diastereoselective intramolecular *N*-conjugate addition, forming 6-membered ring adduct **9** in moderate yield.[8b] Although **8** forms **9** in the absence of external electrophiles, the cyclisation took 24 h to reach completion. We, therefore, felt that ammonium salt **8** potentially offered a means to access P_3 modified vinyl sulfone inhibitors of the type **10**. In the current work, attempts to improve activity of our lead vinyl sulfone compound **7** involving treatment of **8** with a variety of electrophiles and subsequent biological evaluation of the resultant analogues, is reported.

2. Chemistry

In order to probe if ammonium salts, such as **8**, would undergo the planned derivatisation, a test reaction sequence was performed using compound **11** (Scheme 2). Standard HBr-Cbz deprotection, followed by direct treatment of the resultant ammonium bromide salt with an aromatic isothiocyanate,[9] in the presence of base, led to the isolation of thiourea **12** in reasonable yield.

Based on this promising result, the similar synthesis of P_3 analogues of compound **7** was considered. As shown in Scheme 3, thioureas **13**, **14** were accessed following the reaction of ammonium salt **8** with the corresponding isothiocyanates. Identical use of isocyanates gave compounds **15** to **17** and we considered that established physicochemical



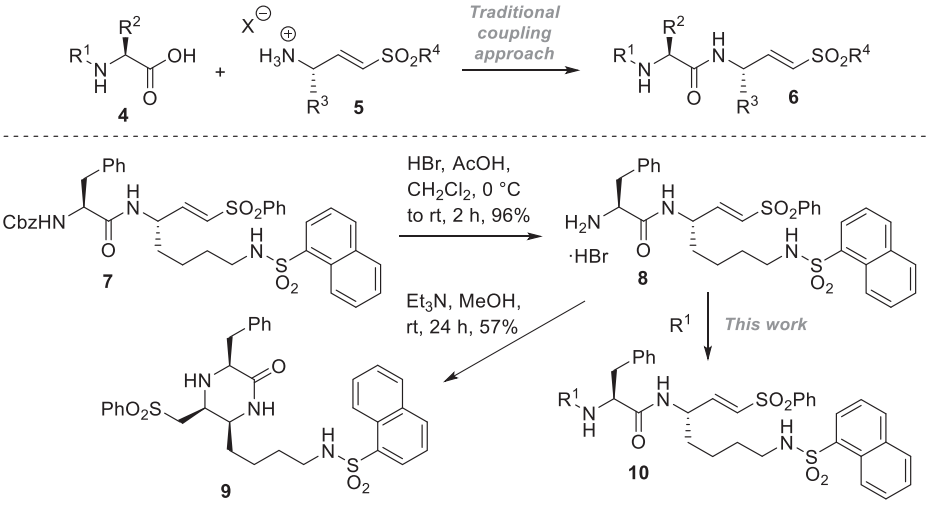
Scheme 2. Sequential vinyl sulfone-Cbz-deprotection-thiourea formation: Synthesis of **12**.

differences between the thiourea and urea functional groups would be of interest.[10] Alternatively, *N*-methyl piperazine and morpholine ureas **18** and **19** were accessed in one-pot using initially carbonyldiimidazole and subsequently the respective cyclic secondary amines.[11] This type of P_3-interacting substituents are found in K777 (**1**) and K11002 (**2**) (see Figure 1) and served to provide an interesting comparison with the lysine derived vinyl sulfone inhibitors in our series.[4] Attempts to synthesise the corresponding thioureas using 1,1′-thiocarbonyldiimidazole, under otherwise identical conditions, were unsuccessful. Reaction of **8** with tosyl chloride gave the sulfonamide analogue **20**. Amide **21** was accessed from the substituted benzoyl chloride, whereas amides **22** and **23** were prepared following EDCI based couplings, derivatives chosen to resemble P_3 substituents recently reported.[4h,j] Overall, as shown in Scheme 3, resulting yields indicated that these reactions were inefficient with the accompanied production of side-products, including piperazinone **9**. However, all analogues, **13** to **23**, were isolated with high chemical purity.

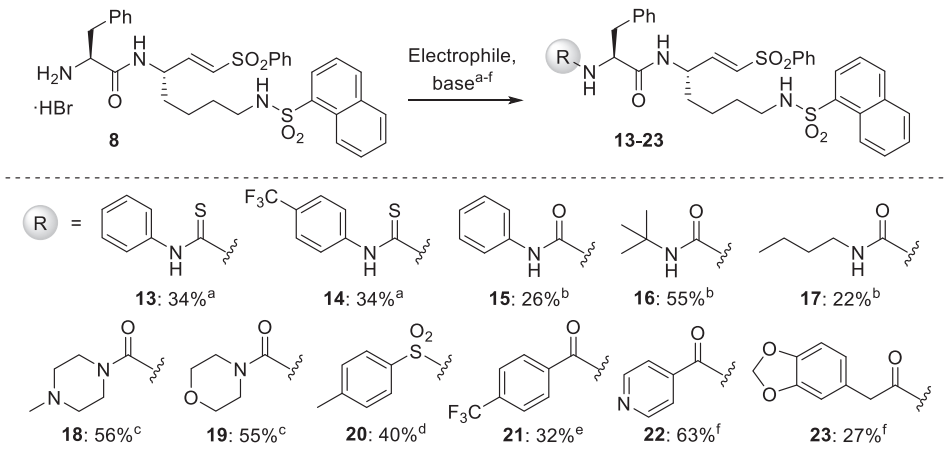
Azide analogue, **25**, was synthesised using the diazo-transfer procedure reported by Goddard-Borger and co-workers.[12] This reaction proved problematic, again, due to competing intramolecular cyclisation forming **9**; nevertheless, **25** could be isolated in modest yield. From **25**, peptidomimetic[13] triazoles **26** and **27** were accessed using Click chemistry (Scheme 4).[14–15]

3. Biology

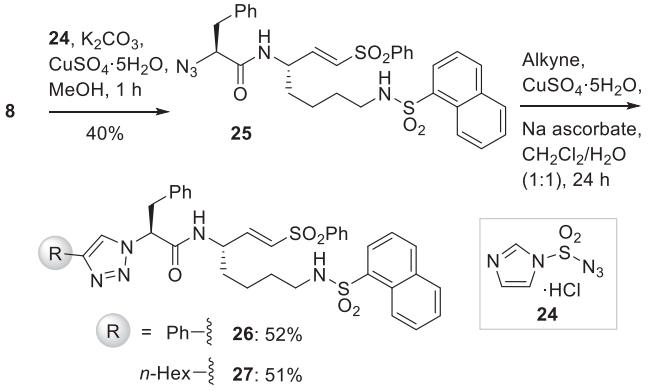
Using a previously reported method,[16] *T. b. brucei* cells were



Scheme 1. Traditional approach to P_3 substituted vinyl sulfone inhibitor of type **6**. Cbz deprotection/intramolecular cyclisation sequence and proposed intermolecular functionalisation.



Scheme 3. Thiourea, urea, sulfonamide and amide based P_3 derivatives **13**–**23** [Conditions: [a]Isothiocyanate, Et_3N, CH_2Cl_2, rt, 1–2 h; [b]Isocyanate, Et_3N, CH_2Cl_2, rt, 5–18 h; [c]CDI, Et_3N, CH_2Cl_2, 40 °C, 1 h; then amine, 40 °C, 1 h; [d]TsCl, Et_3N, CH_2Cl_2, rt, 1 h; [e]ArCOCl, Et_3N, CH_2Cl_2, rt, 18 h; [f]$ArCO_2H$, EDCI·HCl, HOBt·H_2O, iPr_2EtN, CH_2Cl_2, rt, 18 h].



Scheme 4. Azide **25** and triazole based analogues **26** and **27**.

incubated with varying concentrations of each compound in the series and assessed for their ability to disrupt the life-cycle of trypanosomes *via* a standard Alamar Blue assay (Table 1).[8,16] As shown in Entry 1, compound **7** has an EC_{50} value of 0.74 μM and is the figure used as a benchmark for values determined in the current work. Interestingly, the ammonium salt **8**, lacking the carbamate P_3 substituent, possessed some activity (Entry 2). This was a somewhat unexpected finding since it was expected that the ammonium salt would be too polar for efficient cellular uptake. Unsurprisingly, the secondary cyclic amine **9**, resulting from a diastereoselective intramolecular *N*-conjugate addition, consequently lacking the vinyl sulfone motif, exhibits only negligible anti-trypanosomal activity (Entry 3). Thioureas **12**–**14**, in contrast, exhibited sub-micromolar activity with the additional lipophilicity, conferred through inclusion of a *para*-trifluoromethyl group, providing slightly enhanced anti-trypanosomal activity (Entries 4 to 6). Moving from the P_3 thiourea substituents to their urea counterparts, urea **15** proved slightly more active than the corresponding thioureas (Entry 7). As Entries 8 and 9 indicate, in comparison, *t*-butyl and *n*–butyl urea derivatives, **16** and **17**, are less active (significantly so in the case of **16**). *N*-Methyl piperazine **18** and morpholine **19** derivatives, synthesised to mimic K777 (**1**) and K11002 (**2**), were also active producing EC_{50} values of 1.19 μM and 0.90 μM respectively (Entries 10 and 11). However, these compounds display reduced activity compared to their less substituted, secondary urea analogues. Sulfonamide **20** is active (Entry 12) with an EC_{50} value of 0.99 μM but again this figure is higher than observed for the secondary urea and thioureas (Entries 4–7 and 9). In contrast, trifluoromethyl substituted amide **21** (Entry 13) exhibited an EC_{50} of 0.24 μM, a value which is approximately equipotent to the most active ureas – compounds **14** and **15**.

Table 1
Anti-trypanosomal activity for P_3 analogues of lead vinyl sulfone **7**.

Entry	Compound	EC_{50} (μM)[a]	95% CI for the EC_{50} (μM)[b]	clogP[c]
1	**7**	0.74[d]	ND	6.98 (±0.72)
2	**8**	3.43	3.27 to 3.61	4.52 (±0.59)[e]
3	**9**	>5	ND	3.90 (±0.56)[e]
4	**12**	0.39	0.24 to 0.63	7.16 (±0.72)
5	**13**	0.48	0.3 to 0.75	6.14 (±0.65)
6	**14**	0.22	0.15 to 0.33	7.15 (±0.69)
7	**15**	0.15	0.09 to 0.25	6.36 (±0.65)
8	**16**	>5	ND	5.46 (±0.66)
9	**17**	0.64	0.43 to 0.95	5.83 (±0.66)
10	**18**	1.19	0.69 to 2.02	4.94 (±0.70)[e]
11	**19**	0.90	0.75 to 1.08	4.51 (±0.69)
12	**20**	0.99	0.80 to 1.25	6.68 (±0.64)
13	**21**	0.24	0.18 to 0.32	7.22 (±0.75)
14	**22**	0.71	0.53 to 0.96	4.99 (±0.74)[e]
15	**23**	0.80	0.64 to 1.02	6.36 (±0.71)
16	**25**	>5	ND	5.45 (±0.59)
17	**26**	1.12	0.97 to 1.31	6.62 (±0.69)
18	**27**	1.05	0.96 to 1.16	7.98 (±0.68)

[a] Response of *T. b. brucei* (2×10^5 cells/mL) to exposure of varying concentrations of each compound determined by non-linear regression analysis of curve-fitting using the equation $Y = 100/[1 + 10(\log EC_{50}\text{-}X)$ *Hill slope] and variable slope (four parameter)
[b] CI = Confidence Interval.
[c] Calculated using ACDlabs/ChemSketch (Freeware).
[d] Value taken from Ref. 8b.
[e] Value for free amine. ND = Not Determined.

As Entries 14 and 15 indicate, isonicotinamide **22** and 1,3-dioxane **23** are active but slightly less active than **21**. Interestingly, Garcia-Salcedo *et al.* have recently shown that nicotinamide itself inhibits the lysosomal cathepsin b-like protease, blocking endocytosis and thereby causing trypanosomal cell death at millimolar concentrations.[17] Azide **25**, a more lipophilic analogue of ammonium salt **8** shows reduced activity at > 5 μM (Entry 16), compared with an EC_{50} for the ammonium salt **8** of 3.43 μM (Entry 2). Triazoles **26** and **27**, designed to protrude lipophilic aryl and alkyl chains into the putative P_3 region of this compound class' biological target, are slightly more active than **25** but less active than ureas **14**, **15**, and also amide **21**, possessing one, or two H-bond donors, and a (thio)carbonyl H-bond acceptor group (Entries 17 and 18). Representative dose–response curves for the survival of *T. b. brucei* cells with vinyl sulfones **12**, **14**, **15**, **18** and **21** are shown in Figure 2.

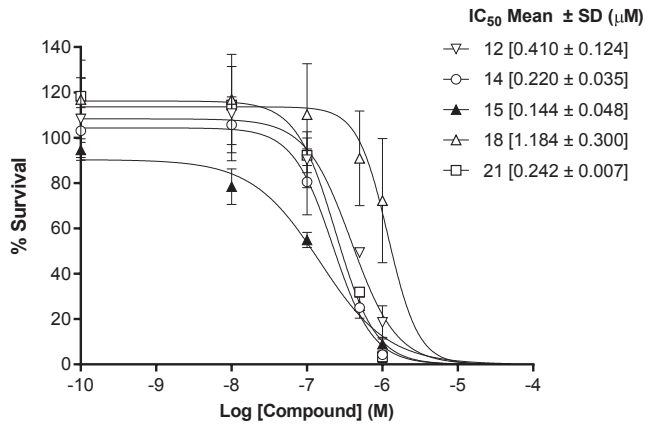


Fig. 2. Representative dose-response curves for *T. b. brucei* (2×10^5 cells/mL) on exposure to varying concentrations of compounds **12**, **14**, **15**, **18** and **21**. The values represent the mean ± S.D. for two independent experiments performed in triplicate.

The behaviour of compounds (**12**, **14**, **15**, **18** and **21**) were investigated in a human skin keratinocyte cell line (HaCaT) in order to assess the effects these compounds had on cell proliferation (GI_{50}) and cytotoxicity (LC_{50}).

Figure 3 highlights that using the lactate dehydrogenanse (LDH) assay[18] only negligible cytotoxicity was observed for the selected, more active compounds from the vinyl sulfone series. Encouragingly, only modest cytotoxicity can be seen at concentrations significantly higher than those required to kill trypanosomes (i.e. only at 100 μM). It should be mentioned that at this concentration compounds typically began to precipitate from solution and accordingly accurate LC_{50} figures could not be calculated.

Using the MTT assay[19] the effect of compounds **12**, **14**, **15**, **18** and **21** on cell proliferation were studied. As shown in the electronic supporting information (Figure S1) only data from compound **18**, containing the *N*-methyl piperazine, fitted a typical sigmoid dose–response curve enabling an accurate GI_{50} (28.67 ± 9.75 μM) to be calculated. This effect on growth can be compared to the anti-trypanosomal EC_{50} value of 1.19 μM. Other compounds demonstrated EC_{50} values in the range of 0.15 to 0.39 mM in the whole-cell parasite assay (see Table 1) and only inhibited cell growth by approximately 20% at concentrations above 10 μM in the MTT assay, i.e. approximately a 25 fold selectivity window. As observed in the LDH assay activities above 100 μM could not be measured due to issues with compound solubility at these concentrations. Taken together the findings from the LDH and MTT assays indicate that our compounds may only inhibit mammalian cell growth rather than being intrinsically cytotoxic.

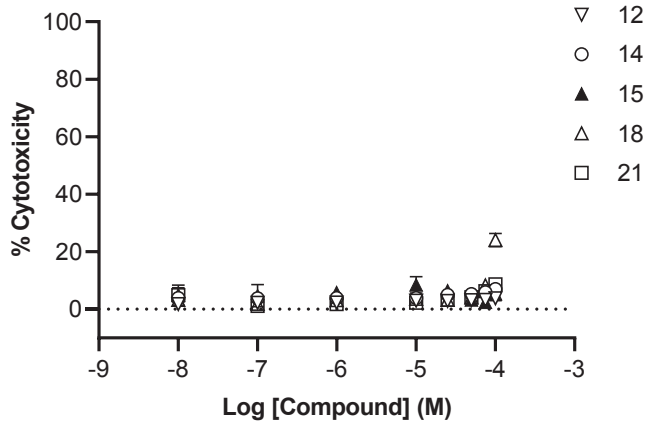


Fig. 3. Determination of cytoxicity *via* the lactate dehydrogenase (LDH) assay. Data are expressed as percentage of LDH release compared with the positive control. Each bar represents the mean ± S.D. of triplicate determinations from three independent experiments.

4. Conclusion

In conclusion we have disclosed results for a series of P_3 modified phenylalanine-lysine containing vinyl sulfones. Although the chemical yields were moderate, a wide range of structurally and electronically diverse analogues of our previously prepared, most active compound have been accessed from one common intermediate. Over the sixteen-member vinyl sulfone dipeptide-based family, most of the structural changes to the P_3 substituent were well tolerated in terms of anti-trypanosomal activity. However, optimum activity was observed for compounds with a lipophilic aromatic entity linked to the main vinyl sulfone dipeptide backbone with an H-bond donor: Secondary thiourea and urea analogues **12** to **15** and secondary trifluromethylbenzamide analogue **21**. The calculated log octanol/water partition coefficient (clogP) illustrates that there is no obvious relationship between total compound lipophilicity and activity in this assay, however, in general terms, increasing the lipophilicity of substituents at P_3 increases activity in the *in vitro* assays. Importantly, some members of the series (e.g. compound **12**) exhibited not only potent activity against trypanosomes but also high selectivity compared with the immortal keratinocyte cell line – HaCaT, and establishes the precident for further development of the series as anti-trypanosomal leads.[20]

5. Experimental

5.1. General experimental

^1H, ^{13}C and ^{19}F NMR spectra were recorded on Varian Unity 500 MHz and 400 MHz system spectrometers and coupling constants (*J*) are quoted in Hertz. High resolution mass spectra were carried out on a Waters/Micromass LCT ESI mass spectrometer in the electrospray positive (ES^+) mode with a time-of-flight analyser. Reported, experimentally found values are all within ± 5 ppm from the calculated values. Infrared spectra were recorded on a Varian Instruments Excalibur series FT-IR 3100 spectrometer. Melting points were recorded on a Gallenkamp electrothermal melting point apparatus. Optical rotation data was obtained with a Perkin Elmer Model 343 polarimeter and values are quoted in units of $10^{-1}degcm^2g^{-1}$. Reagents were obtained from commercial suppliers and were used without further purification. Thin-layer chromatography was performed on silica coated aluminium sheets and compounds were visualised with UV light and aqueous potassium permanganate, followed by heating. Ammonium bromide **8** was synthesised according to literature.[8b]

5.2. Trans-N-[7-{(2-Bromo-4,5-dimethoxyphenylsulfonamido)-1-(phenylsulfonyl)hept-1-en-3S-yl)-3-phenyl-2S-(3-(4-(trifluoromethyl) phenyl}thioureido]propanamide 12

A 33% solution of HBr in AcOH (1 mL) was added to a stirred solution of vinyl sulfone **11** (27 mg, 0.03 mmol, 1 equiv.) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture was stirred for 2 h warming slowly to rt. Solvent was removed *in vacuo* to give the crude product which was redissolved in CH_2Cl_2 (0.5 mL), precipitated with Et_2O (20 mL). The suspension was allowed to settle and the supernatant was removed by Pasteur pippette. The product was dried under high vaccuum to give the ammonium salt as a pink powder. In the same vessel, 4-(trifluoromethyl) phenyl isothiocyanate (7 mg, 0.03 mmol, 1.1 equiv.) was added followed by CH_2Cl_2 (1 mL) and the mixture was stirred. Et_3N (18 μL, 0.13 mmol, 4 equiv.) was then added and the mixture was left to stir overnight at room temperature. Solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave thiourea **12** (20 mg, 69%) as an off white solid. M.p. =

94–99 °C. R_f = 0.3 (*c*-Hex/EtOAc; 1:1). IR (film): ν_{max} = 3320, 3061, 2936, 1661, 1617, 1525, 1503, 1439, 1360, 1325, 1262, 1216, 1148, 1116, 1086, 1067, 1024 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.20–1.67 (m, 6H, CH_2) 2.79–2.90 (m, 2H, CH_2) 3.13 (dd, *J* = 14.0, 8.0 Hz, 1H, CH_2) 3.24 (dd, *J* = 14.0, 7.0 Hz, 1H, CH_2) 3.92 (s, 3H, CH_3) 3.94 (s, 3H, CH_3) 4.66–4.77 (m, 1H, CH) 5.28 (app. q, *J* = 7.5 Hz, 1H, CH) 5.84 (t, *J* = 6.0 Hz, 1H, NH) 6.10 (dd, *J* = 15.0, 2.0 Hz, 1H, CH) 6.65 (d, *J* = 9.0 Hz, 1H, NH) 6.81 (dd, *J* = 15.0, 4.0 Hz, 1H, CH) 6.99 (d, *J* = 7.5 Hz, 1H, NH) 7.15 (s, 1H, ArH) 7.18–7.25 (m, 5H, ArH) 7.33 (d, *J* = 8.5 Hz, 2H, ArH) 7.51 (d, *J* = 8.5 Hz, 2H, ArH) 7.54 (s, 1H, ArH) 7.57 (d, *J* = 8.0 Hz, 2H, ArH) 7.62–7.67 (m, 1H, ArH) 7.84 (d, *J* = 7.0 Hz, 2H, ArH) 8.58 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6 (CH_2) 27.4 (CH_2) 33.1 (CH_2) 37.3 (CH_2) 42.0 (CH_2) 48.6 (CH) 56.5 (CH_3) 56.6 (CH_3) 59.6 (CH) 111.2 (C) 113.9 (CH) 117.2 (CH) 123.5 (CH) 123.9 (q, J_{CF} = 272.0 Hz, CF_3) 126.5 (q, J_{CF} = 3.5 Hz, CH) 127.3 (CH) 127.5 (q, J_{CF} = 32.0 Hz, C) 127.7 (CH) 128.9 (CH) 129.2 (CH) 129.3 (CH) 129.4 (C) 130.4 (CH) 133.6 (CH) 136.1 (C) 139.9 (C) 140.4 (C) 145.5 (CH) 148.1 (C) 152.6 (C) 171.2 (CO) 180.2 (CS) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = ¯62.39 ppm. HRMS (ES^+) $C_{38}H_{40}N_4O_7NaBrF_3S_3$ (MNa^+) calcd. 919.1092; found 919.1069. $[\alpha]_D$ = ¯26 (*c* = 0.1, CH_2Cl_2).

5.3. (S)-N-((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl) hept-1-en-3-yl)-3-phenyl-2-(3-phenylthioureido)propanamide 13

Ammonium salt **8** (19 mg, 0.03 mmol, 1 equiv.) was stirred with phenyl isothiocyanate (13 μL, 0.11 mmol, 4 equiv.) in CH_2Cl_2 (1 mL). Et_3N (15 μL, 0.11 mmol, 4 equiv.) was then added and the mixture was stirred for 1 h. Solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave thiourea **13** (7 mg, 34%) as a white solid. M.p. = 71–76 °C. R_f = 0.3 (*c*-Hex/EtOAc; 1:1). IR (film): ν_{max} = 3322, 2929, 1665, 1595, 1528, 1498, 1447, 1315, 1145, 1085, 805, 741 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.23–1.49 (m, 3H, CH_2) 1.52–1.66 (m, 3H, CH_2) 2.72–2.80 (m, 1H, CH_2) 2.94–3.02 (m, 1H, CH_2) 3.11 (dd, *J* = 14.0, 8.0 Hz, 1H, CH_2) 3.18 (dd, *J* = 14.0, 8.0 Hz, 1H, CH_2) 4.60–4.67 (m, 1H, CH) 5.29 (app. q, *J* = 8.0 Hz, 1H, CH) 6.08 (dd, *J* = 15.0, 2.0 Hz, 1H, CH) 6.31 (t, *J* = 5.5 Hz, 1H, NH) 6.49 (d, *J* = 8.5 Hz, 1H, NH) 6.79 (dd, *J* = 15.0, 4.0 Hz, 1H, CH) 6.96 (d, *J* = 8.5 Hz, 1H, NH) 7.04 (d, *J* = 7.5 Hz, 2H, ArH) 7.18–7.29 (m, 6H, ArH) 7.31–7.36 (m, 2H, ArH) 7.52–7.57 (m, 3H, ArH) 7.60–7.65 (m, 2H, ArH) 7.70–7.74 (m, 1H, ArH) 7.83 (d, *J* = 7.0 Hz, 2H, ArH) 7.95 (d, *J* = 8.0 Hz, 1H, ArH) 8.08 (d, *J* = 8.0 Hz, 1H, ArH) 8.24 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.78 (s, 1H, NH) 8.87 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): 22.1 (CH_2) 28.6 (CH_2) 32.2 (CH_2) 36.5 (CH_2) 41.8 (CH_2) 49.3 (CH) 59.1 (CH) 124.1 (CH) 124.6 (CH) 124.9 (CH) 127.0 (CH) 127.3 (CH) 127.35 (CH) 127.7 (CH) 128.1 (C) 128.5 (CH) 128.8 (CH) 129.0 (CH) 129.2 (CH) 129.3 (CH) 129.6 (CH) 130.1 (CH) 130.2 (CH) 133.5 (CH) 134.2 (C) 134.25 (C) 134.3 (C) 135.7 (C) 136.0 (C) 140.0 (C) 145.7 (CH) 170.8 (CO) 179.8 (CS) ppm. HRMS (ES^+) $C_{39}H_{40}N_4O_5NaS_3$ (MNa^+) calcd. 763.2059; found 763.2085. $[\alpha]_D$ = ¯52 (*c* = 0.1, CH_2Cl_2).

5.4. (S)-N-((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl) hept-1-en-3-yl)-3-phenyl-2-(3-(4-(trifluoromethyl)phenyl)thioureido) propanamide 14

Ammonium salt **8** (22 mg, 0.03 mmol, 1 equiv.) was stirred with 4-(trifluoromethyl)phenyl isothiocyanate (12 mg, 0.06 mmol, 2 equiv.) in CH_2Cl_2 (1 mL). Et_3N (12 μL, 0.09 mmol, 3 equiv.) was then added and the mixture was stirred for 2 h. Solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave thiourea **14** (8 mg, 34%) as a white solid. M.p. = 96–101 °C. R_f = 0.7 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3329, 3059, 2941, 1660, 1616, 1527, 1447, 1324, 1146, 1067, 738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.32–1.64 (m, 6H, CH_2) 2.78–2.92 (m, 2H, CH_2) 3.20 (dd, *J* = 14.0, 8.5 Hz, 1H, CH_2) 3.27 (dd, *J* = 14.0, 7.5 Hz, 1H, CH_2) 4.66–4.76 (m, 1H, CH) 5.34 (app. q, *J* = 8.0 Hz, 1H, CH) 5.95 (t, *J* = 5.5 Hz, 1H, NH) 6.03 (dd, *J* = 15.0, 2.0 Hz, 1H, CH) 6.67 (d, *J* = 9.0 Hz, 1H, NH) 6.79 (dd, *J* = 15.0, 4.0 Hz, 1H, CH) 6.98 (d, *J* = 8.5 Hz, 1H, NH) 7.19–7.29 (m, 6H, ArH) 7.32 (d, *J* = 8.5 Hz, 2H, ArH) 7.52 (d, *J* = 8.5 Hz, 2H, ArH) 7.53–7.59 (m, 2H, ArH) 7.61–7.67 (m, 2H, ArH) 7.73 (t, *J* = 7.5 Hz, 1H, ArH) 7.84 (d, *J* = 7.5 Hz, 2H, ArH) 7.97 (d, *J* = 8.0 Hz, 1H, ArH) 8.10 (d, *J* = 8.0 Hz, 1H, ArH) 8.21 (d, *J* = 7.5 Hz, 1H, ArH) 8.77 (d, *J* = 8.5 Hz, 1H, ArH) 8.80 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.5 (CH_2) 27.5 (CH_2) 33.3 (CH_2) 36.9 (CH_2) 41.9 (CH_2) 48.5 (CH) 59.4 (CH) 123.7 (CH) 123.8 (q, J_{CF} = 271.5 Hz, CF_3) 124.1 (CH) 124.3 (CH) 126.7 (q, J_{CF} = 3.5 Hz, CH) 127.1 (CH) 127.4 (CH) 127.7 (CH) 127.9 (q, J_{CF} = 33.0 Hz, C-CF_3) 128.0 (C) 128.6 (CH) 128.9 (CH) 129.2 (2 × CH) 129.3 (CH) 129.7 (CH) 130.4 (CH) 133.6 (CH) 133.8 (C) 134.3 (C) 134.5 (CH) 136.0 (C) 139.9 (C) 140.2 (C) 145.4 (CH) 171.2 (CO) 180.2 (CS) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = ¯62.47 ppm. HRMS (ES^+) $C_{40}H_{39}N_4O_5F_3NaS_3$ (MNa^+) calcd. 831.1932; found 831.1948. $[\alpha]_D$ = ¯36 (*c* = 0.1, CH_2Cl_2).

5.5. (S)-N-((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl) hept-1-en-3-yl)-3-phenyl-2-(3-phenylureido)propanamide 15

Ammonium bromide salt **8** (29 mg, 0.04 mmol, 1 equiv.) was stirred with phenyl isocyanate (23 μL, 0.21 mmol, 5 equiv.) in CH_2Cl_2 (1 mL). Et_3N (35 μL, 0.26 mmol, 6 equiv.) was added and the reaction mixture was stirred for 5 h after which time solvent was removed *in vacuo*. Purifcation by column chromatography (*c*-Hex/EtOAc; 2:1) gave urea **15** (8 mg, 26%) as a white solid. M.p. = 90–94 °C. R_f = 0.4 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3291, 3058, 2922, 2860, 1648, 1599, 1551, 1499, 1444, 1315, 1264, 1234, 1145, 1085, 1026, 805 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.16–1.44 (m, 6H, CH_2) 2.60–2.70 (m, 1H, CH_2) 2.88–2.98 (m, 1H, CH_2) 3.06–3.18 (m, 2H, CH_2) 4.43–4.54 (m, 1H, CH) 4.99–5.12 (m, 1H, CH) 5.96 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.23 (d, *J* = 9.0 Hz, 1H, NH) 6.65 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 6.89 (s (br), 1H, NH) 6.97 (t, *J* = 7.5 Hz, 1H, ArH) 7.16–7.22 (m, 3H, NH, ArH) 7.23–7.30 (m, 5H, NH, ArH) 7.35 (d, *J* = 8.0 Hz, 2H, ArH) 7.51–7.57 (m, 4H, ArH) 7.59–7.65 (m, 2H, ArH) 7.66–7.70 (m, 1H, ArH) 7.82 (m, 2H, ArH) 7.95 (d, *J* = 7.5 Hz, 1H, ArH) 8.08 (d, *J* = 8.0 Hz, 1H, ArH) 8.21 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH) 8.76 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 22.1 (CH_2) 28.6 (CH_2) 32.8 (CH_2) 39.5 (CH_2) 42.2 (CH_2) 49.0 (CH) 55.0 (CH) 120.0 (CH) 123.4 (CH) 124.1 (CH) 124.6 (CH) 127.05 (CH) 127.15 (CH) 127.7 (CH) 128.1 (C) 128.5 (CH) 128.8 (CH) 129.0 (CH) 129.05 (CH) 129.1 (CH) 129.3 (CH) 129.4 (CH) 130.2 (CH) 133.5 (CH) 134.2 (CH) 134.3 (C) 134.5 (C) 136.4 (C) 138.5 (C) 140.2 (C) 145.7 (CH) 155.8 (CO) 173.5 (CO) ppm. HRMS (ES^+) $C_{39}H_{40}N_4O_6NaS_2$ (MNa^+) calcd. 747.2287; found 747.2315. $[\alpha]_D$ = ¯27 (*c* = 0.1, CH_2Cl_2).

5.6. (S)-2-(3-(tert-Butyl)ureido)-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 16

Ammonium salt **8** (20 mg, 0.03 mmol, 1 equiv.) was stirred with *tert*-butyl isocyanate (7 μL, 0.06 mmol, 2 equiv.) in CH_2Cl_2 (1 mL). Et_3N (12 μL, 0.09 mmol, 3 equiv.) was added and the mixture was left to stir overnight. Solvent was removed *in vacuo* and the crude product was purified by column chromatography (*c*-Hex/EtOAc; 1:1) which gave urea **16** (11 mg, 54%) as a white solid. M.p. = 69–74 °C. R_f = 0.4 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3320, 2921, 1717, 1642, 1549, 1453, 1307, 1211, 1146, 1086 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.59 (m, 15H, ^tBu, CH_2) 2.66–2.74 (m, 1H, CH_2) 2.94–3.23 (m, 3H, CH_2) 4.63–4.82 (m, 2H, CH) 5.09 (s, 1H, NH) 5.63 (d, *J* = 9.0 Hz, 1H, NH) 6.02 (d, *J* = 15.0 Hz, 1H, CH) 6.15 (s (br), 1H, NH) 6.79 (dd, *J* = 15.0, 4.0 Hz, 1H, CH) 7.02 (s (br), 1H, NH) 7.11–7.35 (m, 5H, ArH) 7.46–7.75 (m, 6H, ArH) 7.83 (d, *J* = 8.0 Hz, 2H, ArH) 7.95 (d, *J* = 8.0 Hz, 1H, ArH) 8.07 (d, *J* = 8.0 Hz, 1H, ArH) 8.20 (d, *J* = 7.0 Hz, 1H, ArH) 8.73 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.3 (CH_2) 27.1 (CH_2) 29.3 (CH_3) 33.3 (CH_2) 37.7 (CH_2) 42.2 (CH_2) 47.8 (CH) 50.5 (C) 55.0 (CH) 124.1 (CH) 124.5 (CH) 126.8 (CH) 127.0 (CH) 127.7 (CH) 128.1 (C) 128.5 (CH) 128.7 (CH) 129.2 (CH) 129.3 (CH)

129.4 (CH) 129.5 (CH) 130.3 (CH) 133.5 (CH) 134.2 (CH) 134.3 (C) 134.5 (C) 137.0 (C) 140.1 (C) 146.0 (CH) 157.4 (CO) 174.0 (CO) ppm. HRMS (ES^+) $C_{37}H_{44}N_4O_6NaS_2$ (MNa^+) calcd. 727.2600; found 727.2607. $[\alpha]_D = -5$ ($c = 0.1$, CH_2Cl_2).

5.7. (S)-2-(3-Butylureido)-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 17

Ammonium bromide salt **8** (27 mg, 0.04 mmol, 1 equiv.) was stirred with *n*-butyl isocyanate (20 μL, 0.17 mmol, 4 equiv.) in CH_2Cl_2 (1 mL). Et_3N (24 μL, 0.17 mmol, 4 equiv.) was added and the mixture was left to stir overnight. Solvent was removed *in vacuo* to give the crude product which was purified by column chromatography (*c*-Hex/EtOAc; 1:1) giving urea **17** (6 mg, 22%) as a white solid. M.p. = 76–82 °C. $R_f = 0.3$ (*c*-Hex/EtOAc; 1:1). IR (film): $\nu_{max} = 3308, 2956, 2932, 2863, 1638, 1561, 1509, 1447, 1307, 1145, 1085, 981\ cm^{-1}$. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, J = 7.5 Hz, 3H, CH_3) 1.24–1.38 (m, 6H, CH_2) 1.39–1.55 (m, 4H, CH_2) 2.71–2.80 (m, 1H, CH_2) 2.88–3.00 (m, 1H, CH_2) 3.01–3.19 (m, 4H, CH_2) 4.60–4.77 (m, 2H, CH) 5.27 (s, 1H, NH) 5.62 (s, 1H, NH) 5.96 (d, J = 15.0 Hz, 1H, CH) 6.43 (s (br), 1H, NH) 6.77 (dd, J = 15.0, 4.0 Hz, 1H, CH) 6.89 (s (br), 1H, NH) 7.10–7.24 (m, 5H, ArH) 7.51–7.58 (m, 3H, ArH) 7.59–7.65 (m, 2H, ArH) 7.68 (t, J = 8.0 Hz, 1H, ArH) 7.83 (d, J = 7.5 Hz, 2H, ArH) 7.94 (d, J = 8.0 Hz, 1H, ArH) 8.06 (d, J = 8.0 Hz, 1H, ArH) 8.20 (d, J = 7.5 Hz, 1H, ArH) 8.74 (d, J = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9 (CH_3) 20.1 (CH_2) 21.5 (CH_2) 27.7 (CH_2) 32.1 (CH_2) 33.5 (CH_2) 38.6 (CH_2) 40.5 (CH_2) 42.3 (CH_2) 48.3 (CH) 55.7 (CH) 124.3 (CH) 124.7 (CH) 127.05 (CH) 127.1 (CH) 127.8 (CH) 128.3 (C) 128.5 (CH) 128.7 (CH) 129.2 (CH) 129.3 (CH) 129.4 (CH) 129.5 (CH) 130.2 (CH) 133.6 (CH) 134.3 (CH) 134.5 (C) 134.9 (C) 137.0 (C) 140.3 (C) 146.2 (CH) 158.4 (CO) 173.5 (CO) ppm. HRMS (ES^+) $C_{37}H_{44}N_4O_6NaS_2$ (MNa^+) calcd. 727.2600; found 727.2634. $[\alpha]_D = -17$ ($c = 0.1$, CH_2Cl_2).

5.8. 4-Methyl-N-((S)-1-(((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)amino)-1-oxo-3-phenylpropan-2-yl) piperazine-1-carboxamide 18

In a small screw cap vial, ammonium salt **8** (25 mg, 0.04 mmol, 1 equiv.) was stirred in CH_2Cl_2 (0.5 mL). Et_3N (6 μL, 0.04 mmol, 1.1 equiv.) was added followed by carbonyldiimidazole (7 mg, 0.04 mmol, 1.1 equiv.). The reaction mixture was then warmed with a water bath to 40 °C for 1 h. *N*-Methyl piperazine (5 μL, 0.04 mmol, 1.2 equiv.) was then added and stirring continued at 40 °C for 1 h. Direct purification by column chromatography (CH_2Cl_2/MeOH; 15:1) gave urea **18** (15 mg, 56%) as a white solid. M.p. = 69–75 °C. $R_f = 0.3$ (CH_2Cl_2/MeOH; 10:1). IR (film): $\nu_{max} = 3270, 3060, 2940, 2863, 2800, 1657, 1625, 1533, 1446, 1412, 1306, 1264, 1239, 1201, 1145, 1085, 1001, 805\ cm^{-1}$. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.17–1.37 (m, 4H, CH_2) 1.39–1.51 (m, 2H, CH_2) 2.28 (s, 3H, CH_3) 2.29–2.34 (m, 2H, CH_2) 2.35–2.43 (m, 2H, CH_2) 2.75–2.81 (m, 1H, CH_2) 2.95–3.04 (m, 1H, CH_2) 3.09 (dd, J = 13.5, 8.0 Hz, 1H, CH_2) 3.17 (dd, J = 13.5, 7.5 Hz, 1H, CH_2) 3.31–3.38 (m, 2H, CH_2) 3.39–3.46 (m, 2H, CH_2) 4.56 (app. q, J = 8.0 Hz, 1H, CH) 4.61–4.69 (m, 1H, CH) 5.60 (d, J = 8.0 Hz 1H, NH) 5.98–6.03 (m, 1H, NH) 6.10 (dd, J = 15.0, 2.0 Hz, 1H, CH) 6.74 (dd, J = 15.0, 4.0 Hz, 1H, CH) 6.78 (d, J = 9.5 Hz, 1H, NH) 7.15–7.25 (m, 5H, ArH) 7.52–7.58 (m, 4H, ArH) 7.62–7.69 (m, 2H, ArH) 7.85 (d, J = 7.0 Hz, 2H, ArH) 7.92 (d, J = 8.0 Hz, 1H, CH) 8.06 (d, J = 8.0 Hz, 1H, CH) 8.23 (dd, J = 7.5, 1.0 Hz, 1H, CH) 8.78 (d, J = 8.5 Hz, 1H, CH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.1 (CH_2) 27.2 (CH_2) 32.2 (CH_2) 37.4 (CH_2) 42.2 (CH_2) 43.8 (CH_2) 46.0 (CH_3) 47.9 (CH) 54.5 (CH_2) 56.1 (CH) 124.2 (CH) 124.6 (CH) 126.9 (CH) 127.0 (CH) 127.7 (CH) 128.1 (C) 128.2 (CH) 128.7 (CH) 129.15 (CH) 129.25 (CH) 129.32 (2 × CH) 130.2 (CH) 133.5 (CH) 134.1 (CH) 134.3 (C) 135.1 (C) 137.0 (C) 140.1 (C) 145.8 (CH) 157.5 (CO) 173.3 (CO) ppm. HRMS (ES^+) $C_{38}H_{45}N_5O_6NaS_2$ (MNa^+) calcd. 754.2709; found 754.2723. $[\alpha]_D = -26$ ($c = 0.1$, CH_2Cl_2).

5.9. N-((S)-1-(((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)amino)-1-oxo-3-phenylpropan-2-yl) morpholine-4-carboxamide 19

In a small screw cap vial, ammonium salt **8** (21 mg, 0.03 mmol, 1 equiv.) was stirred in CH_2Cl_2 (0.5 mL). Et_3N (5 μL, 0.04 mmol, 1.2 equiv.) was added followed by carbonyldiimidazole (8 mg, 0.04 mmol, 1.2 equiv.). The reaction mixture was then warmed with a water bath to 40 °C for 1 h. Morpholine (3 μL, 0.04 mmol, 1.2 equiv.) was then added and stirring continued at 40 °C for 1 h. The reaction mixture was then purified directly by column chromatography (EtOAc) to give urea **19** (12 mg, 55%) as a white solid. M.p. = 74–78 °C. $R_f = 0.3$ (EtOAc). IR (film): $\nu_{max} = 3270, 3065, 2927, 2859, 1661, 1625, 1535, 1446, 1305, 1262, 1145, 1118, 1085, 994\ cm^{-1}$. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.22–1.41 (m, 4H, CH_2) 1.43–1.55 (m, 2H, CH_2) 2.75–2.81 (m, 1H, CH_2) 2.97–3.06 (m, 1H, CH_2) 3.13 (dd, J = 14.0, 8.0 Hz, 1H, CH_2) 3.21 (dd, J = 14.0, 7.5 Hz, 1H, CH_2) 3.31 (ddd, J = 13.0, 6.5, 3.5 Hz, 2H, CH_2) 3.40 (ddd, J = 13.0, 6.5, 3.5 Hz, 2H, CH_2) 3.59 (ddd, J = 11.5, 6.5, 3.5 Hz, 2H, CH_2) 3.66 (ddd, J = 11.5, 6.5, 3.5 Hz, 2H, CH_2) 4.53 (app. q, J = 8.0 Hz, 1H, CH) 4.68–4.76 (m, 1H, CH) 5.66 (d, J = 8.0 Hz, 1H, NH) 5.71 (dd, J = 8.0, 2.5 Hz, 1H, NH) 6.14 (dd, J = 15.0, 2.0 Hz, 1H, CH) 6.65 (d, J = 9.5 Hz, 1H, NH) 6.77 (dd, J = 15.0, 4.0 Hz, 1H, CH) 7.16–7.28 (m, 5H, ArH) 7.51–7.60 (m, 4H, ArH) 7.63–7.67 (m, 1H, ArH) 7.68–7.73 (m, 1H, ArH) 7.86 (d, J = 7.0 Hz, 2H, ArH) 7.93 (d, J = 8.0 Hz, 1H, ArH) 8.07 (d, J = 8.0 Hz, 1H, ArH) 8.23 (dd, J = 7.5,1.0 Hz, 1H, ArH) 8.77 (d, J = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.8 (CH_2) 27.0 (CH_2) 33.2 (CH_2) 37.2 (CH_2) 42.2 (CH_2) 44.1 (CH_2) 47.7 (CH) 56.1 (CH) 66.5 (CH_2) 124.2 (CH) 124.5 (CH) 126.9 (CH) 127.0 (CH) 127.7 (CH) 128.1 (C) 128.2 (CH) 128.7 (CH) 129.19 (CH) 129.24 (CH) 129.27 (CH) 129.33 (CH) 130.3 (CH) 133.5 (CH) 134.2 (CH) 134.3 (C) 134.9 (C) 137.0 (C) 140.1 (C) 145.7 (CH) 157.7 (CO) 173.3 (CO) ppm. HRMS (ES^+) $C_{37}H_{42}N_4O_7NaS_2$ (MNa^+) calcd. 741.2393; found 741.2427. $[\alpha]_D = -16$ ($c = 0.1$, CH_2Cl_2).

5.10. (S)-2-(4-Methylphenylsulfonamido)-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 20

Ammonium salt **8** (32 mg, 0.05 mmol, 1 equiv.) was stirred with 4-toluenesulfonyl chloride (41 mg, 0.22 mmol, 5 equiv.) in CH_2Cl_2 (1 mL). Et_3N (30 μL, 0.22 mmol, 5 equiv.) was then added and the reaction mixture was stirred for 1 h. Solvent was removed *in vacuo* to give the crude product. Direct purifcation by column chromatography (*c*-Hex/EtOAc; 1:1) gave sulfonamide **20** (14 mg, 40%) as a white solid. M.p. = 69–71 °C. $R_f = 0.7$ (*c*-Hex/EtOAc; 1:2). IR (film): $\nu_{max} = 3336, 3060, 2930, 2861, 1668, 1597, 1537, 1447, 1307, 1147, 1087, 956, 810, 734\ cm^{-1}$. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.21–1.57 (m, 6H, CH_2) 2.39 (s, 3H, CH_3) 2.81 (dd, J = 14.0, 8.5 Hz, 1H, CH_2) 2.83–2.94 (m, 2H, CH_2) 3.01 (dd, J = 14.0, 5.5 Hz, 1H, CH_2) 3.80–3.86 (m, 1H, CH) 4.54–4.61 (m, 1H, CH) 5.30 (t, J = 6.20, 1H, NH) 5.63 (d, J = 6.5 Hz, 1H, NH) 6.51 (dd, J = 15.0, 2.0 Hz, 1H, CH) 6.70 (d, J = 9.0 Hz, 1H, NH) 6.80 (dd, J = 15.0, 4.5 Hz, 1H, CH) 6.89 (d, J = 7.0, 2H, ArH) 7.04 (t, J = 7.5 Hz, 2H, ArH) 7.10 (d, J = 7.5 Hz, 1H, ArH) 7.12 (d, J = 8.0 Hz, 2H, ArH) 7.43 (d, J = 8.5 Hz, 2H, ArH) 7.53 (d, J = 7.5 Hz, 2H, ArH) 7.56 (d, J = 8.5 Hz, 1H, ArH) 7.57–7.63 (m, 2H, ArH) 7.64–7.67 (m, 1H, ArH) 7.88 (d, J = 7.0 Hz, 2H, ArH) 7.95 (d, J = 8.0 Hz, 1H, ArH) 8.08 (d, J = 8.0 Hz, 1H, ArH) 8.25 (dd, J = 7.5, 1.0 Hz, 1H, ArH) 8.67 (d, J = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.6 (CH_3) 21.8 (CH_2) 28.5 (CH_2) 32.7 (CH_2) 38.1 (CH_2) 42.3 (CH_2) 49.4 (CH) 58.2 (CH) 124.2 (CH) 124.3 (CH) 127.0 (CH) 127.1 (2 × CH) 127.7 (CH) 128.1 (C) 128.5 (CH) 128.8 (CH) 129.0 (CH) 129.2 (CH) 129.3 (CH) 129.6 (CH) 129.8 (CH) 130.5 (CH) 133.4 (CH) 134.25 (C) 134.3 (CH) 134.6 (C) 135.3 (C) 135.35 (C) 140.2 (C) 143.8 (C) 145.3 (CH) 170.5 (CO) ppm. HRMS (ES^+) $C_{39}H_{41}N_3O_7NaS_3$ (MNa^+) calcd. 782.2004; found 782.2010. $[\alpha]_D = -55$ ($c = 0.1$, CH_2Cl_2).

5.11. N-((S)-1-(((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)-4-(trifluoromethyl)benzamide 21

Ammonium salt **8** (30 mg, 0.04 mmol, 1 equiv.) was stirred with 4-(trifluoromethyl)benzoyl chloride (32 μL, 0.22 mmol, 5 equiv.) in CH_2Cl_2 (1 mL). Et_3N (37 μL, 0.26 mmol, 6 equiv.) was then added and the reaction mixture was left to stir overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. The crude residue was purified by column chromatography (*c*-Hex/EtOAc; 1:1) to give amide **21** (11 mg, 32%) as a white solid. M.p. = 159–161 °C. R_f = 0.5 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3287, 2926, 1699, 1661, 1643, 1537, 1446, 1324, 1260, 1134, 1065, 847, 808, 754 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3/CD_3OD$ 3:2, CD_3OD reference): δ = 1.09–1.48 (m, 6H, CH_2) 2.70–2.87 (m, 2H, CH_2) 3.03 (dd, *J* = 13.5, 7.0 Hz, 1H, CH_2) 3.11 (dd, *J* = 13.5, 8.5 Hz, 1H, CH_2) 4.37–4.44 (m, 1H, CH) 4.67 (dd, *J* = 8.5, 7.0 Hz, 1H, CH) 5.83 (dd, *J* = 15.0, 2.0 Hz, 1H, CH) 6.65 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.08–7.21 (m, 5H, ArH) 7.44–7.49 (m, 1H, ArH) 7.52–7.62 (m, 6H, ArH) 7.63–7.69 (m, 1H, ArH) 7.81 (d, *J* = 7.0 Hz, 2H, ArH) 7.84 (d, *J* = 8.0 Hz, 2H, ArH) 7.91 (dd, *J* = 7.5, 2.0 Hz, 1H, ArH) 8.03 (d, *J* = 8.0 Hz, 1H, ArH) 8.08 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH) 8.61 (d, *J* = 8.5 Hz, 1H, ArH) ppm. *Note: The three NH signals were not observed in the ^1H NMR spectrum. However a very dilute $CDCl_3$ ^1H NMR spectrum clearly shows the three NH signals*. ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 3:2, CD_3OD reference): δ = 22.6 (CH_2) 29.1 (CH_2) 33.3 (CH_2) 38.3 (CH_2) 42.6 (CH_2) 49.8 (CH) 56.4 (CH) 124.2 (q, J_{CF} = 272.0 Hz, CF_3) 124.6 (CH) 125.0 (CH) 125.9 (q, J_{CF} = 3.5 Hz, CH) 127.3 (CH) 127.6 (q, J_{CF} = 32.0 Hz, C^-CF_3) 127.7 (CH) 128.0 (CH) 128.4 (CH) 128.5 (CH) 128.6 (C) 129.1 (CH) 129.5 (q, J_{CF} = 2.0 Hz, CH) 129.6 (CH) 129.9 (CH) 130.2 (CH) 133.9 (CH) 134.2 (CH) 134.6 (CH) 134.8 (C) 135.6 (C) 136.8 (C) 137.5 (C) 140.5 (C) 146.5 (CH) 167.7 (CO) 172.2 (CO) ppm. ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ 3:2): δ = ⁻59.67 ppm. HRMS (ES^+) $C_{40}H_{38}N_3O_6NaS_2F_3$ (MNa^+) calcd. 800.2052; found 800.2040. $[\alpha]_D$ = ⁻17 (*c* = 0.1, MeOH).

5.12. N-((S)-1-(((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)amino)-1-oxo-3-phenylpropan-2-yl) isonicotinamide 22

Isonicotinic acid (5 mg, 0.04 mmol, 1.2 equiv.) was stirred with EDCI·HCl (7 mg, 0.04 mmol, 1.1 equiv.) and HOBt·H_2O (6 mg, 0.04 mmol, 1.2 equiv.) in CH_2Cl_2 (1 mL) for 10 min. Ammonium salt **8** (23 mg, 0.03 mmol, 1 equiv.) was then added followed by DIPEA (13 μL, 0.07 mmol, 2.2 equiv.) and the reaction mixture was stirred overnight. Solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (EtOAc) gave amide **22** (15 mg, 63%) as a white solid. M.p. = 84–88 °C. R_f = 0.2 (EtOAc). IR (film): ν_{max} = 3279, 3055, 2927, 2863, 1649, 1537, 1446, 1410, 1318, 1146, 1085 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.21–1.57 (m, 6H, CH_2) 2.71–2.80 (m, 1H, CH_2) 2.85–2.96 (m, 1H, CH_2) 3.21 (dd, *J* = 13.5, 7.5 Hz, 1H, CH_2) 3.28 (dd, *J* = 13.5, 7.5 Hz, 1H, CH_2) 4.62–4.72 (m, 1H, CH) 4.87 (app. q, *J* = 7.5 Hz, 1H, CH) 5.60–5.66 (m, 1H, NH) 6.14 (dd, *J* = 15.0, 2.0 Hz, 1H, CH) 6.58 (d, *J* = 9.0 Hz, 1H, NH) 6.78 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.15–7.28 (m, 5H, ArH) 7.48 (m, 9H, ArH + NH) 7.87 (d, *J* = 7.0 Hz, 2H, ArH) 7.94 (d, *J* = 7.5 Hz, 1H, ArH) 8.06 (d, *J* = 8.0 Hz, 1H, ArH) 8.12 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.56–8.73 (m, 3H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.1 (CH_2) 27.5 (CH_2) 32.9 (CH_2) 37.4 (CH_2) 42.0 (CH_2) 48.5 (CH) 55.7 (CH) 121.1 (CH) 124.2 (CH) 124.3 (CH) 126.9 (CH) 127.3 (CH) 127.7 (CH) 128.0 (C) 128.3 (CH) 128.9 (CH) 129.19 (CH) 129.23 (CH) 129.3 (CH) 129.4 (CH) 130.5 (CH) 133.6 (CH) 134.3 (CH) 134.6 (C) 136.2 (2 × C) 140.0 (C) 140.2 (C) 145.3 (CH) 150.6 (CH) 166.3 (CO) 171.2 (CO) ppm. HRMS (ES^+) $C_{38}H_{38}N_4O_6NaS_2$ (MNa^+) calcd. 733.2130; found 733.2121. $[\alpha]_D$ = ⁻8 (*c* = 0.1, CH_2Cl_2).

5.13. (S)-2-(2-(Benzo[d][1,3]dioxol-5-yl)acetamido)-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 23

3,4-(Methylenedioxy)phenylacetic acid (12 mg, 0.06 mmol, 2 equiv.) was stirred with EDCI·HCl (11 mg, 0.06 mmol, 2 equiv.) and HOBt·H_2O (10 mg, 0.06 mmol, 2 equiv.) in CH_2Cl_2 (1 mL) for 10 min. Ammonium salt **8** (20 mg, 0.03 mmol, 1 equiv.) was then added followed by DIPEA (15 μL, 0.09 mmol, 3 equiv.) and the reaction mixture was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with 1 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered and solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave amide **23** (6 mg, 27%) as a white solid. M.p. = 58–62 °C. R_f = 0.3 (*c*-Hex/EtOAc; 1:1). IR (film): ν_{max} = 3285, 3065, 2928, 1646, 1544, 1503, 1445, 1316, 1245, 1146, 1086, 1038, 930, 738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.54 (m, 6H, CH_2) 2.72–2.82 (m, 1H, CH_2) 2.90–3.04 (m, 2H, CH_2) 3.09 (dd, *J* = 14.0, 7.5 Hz, 1H, CH_2) 3.45 (d, *J* = 15.0 Hz, 1H, CH_2) 3.50 (d, *J* = 15.0 Hz, 1H, CH_2) 4.56–4.67 (m, 2H, CH) 5.66–5.71 (m, 1H, NH) 5.93 (s, 2H, CH_2) 6.04 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.38 (d, *J* = 9.0 Hz, 1H, NH) 6.60–6.67 (m, 3H, ArH) 6.70 (d, *J* = 8.0 Hz, 1H, NH) 6.73 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.09–7.13 (m, 2H, ArH) 7.16–7.23 (m, 3H, ArH) 7.51–7.73 (m, 6H, ArH) 7.84 (d, *J* = 7.5 Hz, 2H, ArH) 7.94 (d, *J* = 8.0 Hz, 1H, ArH) 8.07 (d, *J* = 8.5 Hz, 1H, ArH) 8.24 (d, *J* = 7.5 Hz, 1H, ArH) 8.74 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.1 (CH_2) 27.5 (CH_2) 33.1 (CH_2) 37.0 (CH_2) 42.2 (CH_2) 42.8 (CH_2) 48.1 (CH) 55.1 (CH) 101.0 (CH_2) 108.5 (CH) 109.7 (CH) 122.6 (CH) 124.2 (CH) 124.5 (CH) 126.9 (CH) 127.1 (CH) 127.7 (CH) 127.9 (C) 128.1 (C) 128.3 (CH) 128.7 (CH) 129.1 (CH) 129.15 (CH) 129.3 (CH) 129.4 (CH) 129.4 (CH) 130.3 (CH) 133.6 (CH) 134.2 (CH) 134.3 (C) 134.9 (C) 136.2 (C) 140.0 (C) 145.4 (CH) 146.8 (C) 147.9 (C) 171.5 (CO) 172.4 (CO) ppm. HRMS (ES^+) $C_{41}H_{41}N_3O_8NaS_2$ (MNa^+) calcd. 790.2233; found 790.2212. $[\alpha]_D$ = ⁻26 (*c* = 0.1, CH_2Cl_2).

5.14. (S)-2-Azido-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 25

Ammonium salt **8** (81 mg, 0.12 mmol, 1 equiv.) was stirred with imidazole-1-sulfonyl azide hydrochloride salt **24** (106 mg, 0.51 mmol, 4 equiv.) in MeOH (1 mL). K_2CO_3 (69 mg, 0.50 mmol, 4 equiv.) was added followed by $CuSO_4·5H_2O$ (1 mg, 0.004 mmol, 0.03 equiv.) and the reaction mixture was left to stir overnight at room temperature. Solvent was removed *in vacuo* to give a crude residue which was dissolved in EtOAc (20 mL), washed with water (2 × 10 mL), brine (10 mL), dried over $MgSO_4$, filtered and solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave azide **25** (32 mg, 40%) as a white crystalline solid. M.p. = 55–60 °C. R_f = 0.3 (*c*-Hex/EtOAc; 1:1). IR (film): ν_{max} = 3314, 2937, 2114, 1712, 1670, 1526, 1447, 1309, 1182, 1147, 1085, 1005 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.99–1.11 (m, 2H, CH_2) 1.22–1.39 (m, 4H, CH_2) 2.77–2.86 (m, 2H, CH_2) 3.03 (dd, *J* = 14.0, 7.0 Hz, 1H, CH_2) 3.22 (dd, *J* = 14.0, 5.0 Hz, 1H, CH_2) 4.16 (dd, *J* = 7.0, 5.0 Hz, 1H, CH) 4.43–4.50 (m, 1H, CH) 5.02 (t, *J* = 6.0 Hz, 1H, NH) 6.20 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.28 (d, *J* = 8.5 Hz, 1H, NH) 6.75 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 7.12–7.25 (m, 5H, ArH) 7.53–7.58 (m, 3H, ArH) 7.59–7.69 (m, 3H, ArH) 7.85 (d, *J* = 7.0 Hz, 2H, ArH) 7.96 (d, *J* = 8.0 Hz, 1H, ArH) 8.08 (d, *J* = 8.0 Hz, 1H, ArH) 8.25 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.65 (d, *J* = 8.0 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.0 (CH_2) 28.8 (CH_2) 32.9 (CH_2) 38.0 (CH_2) 42.5 (CH_2) 49.2 (CH) 64.8 (CH) 124.2 (CH) 124.3 (CH) 127.0 (CH) 127.3 (CH) 127.7 (CH) 128.1 (C) 128.4 (CH) 128.6 (CH) 129.2 (CH) 129.4 (CH) 129.5 (CH) 129.7 (CH) 130.6 (CH) 133.7 (CH) 134.3 (C) 134.4 (CH) 134.5 (C) 135.6 (C) 139.8 (C) 145.1 (CH) 168.3 (CO) ppm. HRMS (ES^+) $C_{32}H_{33}N_5O_5NaS_2$ (MNa^+) calcd. 654.1821; found 654.1812. $[\alpha]_D$ = +41 (*c* = 0.1, CH_2Cl_2).

5.15. (S)-N-((S,E)-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl) hept-1-en-3-yl)-3-phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl)propanamide 26

Azide **25** (10 mg, 0.02 mmol, 1 equiv.) was stirred with phenylacetylene (3 μL, 0.03 mmol, 1.8 equiv.), $CuSO_4{\cdot}5H_2O$ (0.2 mg, 8 μmol, 0.05 equiv, i.e. 20 μL of a 10 mg/mL stock solution in H_2O) in CH_2Cl_2/H_2O; 1:1 (0.6 mL). Sodium ascorbate (1 mg, 0.005 mmol, 0.3 equiv.) was added and the reaction mixture was stirred vigourously for 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and water (1 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave triazole **26** (6 mg, 52%) as a white solid. M.p. = 196–198 °C. R_f = 0.5 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3346, 3208, 1677, 1528, 1446, 1316, 1260, 1202, 1145, 1080, 947, 799, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3/CD_3OD$ 3:2, CD_3OD reference): δ = 1.00–1.14 (m, 2H, CH_2) 1.18–1.37 (m, 4H, CH_2) 2.64–2.78 (m, 2H, CH_2) 3.27 (dd, *J* = 13.0, 6.5 Hz, 1H, CH_2) 3.47 (dd, *J* = 13.0, 9.5 Hz, 1H, CH_2) 4.28–4.35 (m, 1H, CH) 5.46 (dd, *J* = 9.5, 6.5 Hz, 1H, CH) 5.73 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.60 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.10–7.19 (m, 5H, ArH) 7.31 (t, *J* = 7.5 Hz, 1H, ArH) 7.37 (d, *J* = 7.5 Hz, 2H, ArH) 7.39–7.41 (m, 1H, NH) 7.46 (t, *J* = 8.0 Hz, 1H, ArH) 7.51–7.68 (m, 6H, ArH) 7.74 (d, *J* = 7.0 Hz, 2H, ArH) 7.79 (d, *J* = 7.0 Hz, 2H, ArH) 7.89 (d, *J* = 8.5 Hz, 1H, ArH) 8.00 (d, *J* = 8.5 Hz, 1H, ArH) 8.11 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.21 (s, 1H, ArH) 8.59 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 3:2, CD_3OD reference): δ = 22.5 (CH_2) 29.0 (CH_2) 33.1 (CH_2) 39.2 (CH_2) 42.4 (CH_2) 49.5 (CH) 65.1 (CH) 119.8 (CH) 124.4 (CH) 124.7 (CH) 125.9 (CH) 127.1 (CH) 127.8 (CH) 128.0 (C) 128.4 (CH) 128.45 (CH) 128.8 (CH) 129.1 (CH) 129.2 (CH) 129.3 (CH) 129.35 (CH) 129.5 (CH) 129.7 (CH) 130.2 (CH) 130.3 (C) 134.1 (CH) 134.4 (CH) 134.6 (C) 135.0 (C) 135.2 (C) 140.1 (C) 145.7 (CH) 148.2 (C) 167.8 (CO) ppm. HRMS (ES^+) $C_{40}H_{39}N_5O_5NaS_2$ (MNa^+) calcd. 756.2290; found 756.2296. $[\alpha]_D$ = -14 (*c* = 0.1, EtOAc).

5.16. (S)-2-(4-Hexyl-1H-1,2,3-triazol-1-yl)-N-((S,E)-7-(naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-phenylpropanamide 27

Azide **25** (10 mg, 0.02 mmol, 1 equiv.) was stirred with 1-octyne (3 μL, 0.02 mmol, 1.3 equiv.), $CuSO_4{\cdot}5H_2O$ (0.2 mg, 8 μmol, 0.05 equiv, i.e. 20 μL of a 10 mg/mL stock solution in H_2O) in CH_2Cl_2/H_2O; 1:1 (0.6 mL). Sodium ascorbate (1 mg, 0.005 mmol, 0.3 equiv.) was added and the reaction mixture was stirred vigourously for 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and water (1 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. Purification by column chromatography (*c*-Hex/EtOAc; 1:1) gave triazole **27** (6 mg, 51%) as a white solid. M.p. = 62–65 °C. R_f = 0.5 (*c*-Hex/EtOAc; 1:2). IR (film): ν_{max} = 3310, 3053, 2928, 2857, 1678, 1546, 1447, 1317, 1201, 1146, 1085 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 6.5 Hz, 3H, CH_3) 1.10–1.48 (m, 12H, CH_2) 1.53–1.64 (m, 2H, CH_2) 2.63 (t, *J* = 7.5 Hz, 2H, CH_2) 2.73–2.91 (m, 2H, CH_2) 3.31 (dd, *J* = 13.5, 8.0 Hz, 1H, CH_2) 3.51 (dd, *J* = 13.5, 8.0 Hz, 1H, CH_2) 4.43–4.52 (m, 1H, CH) 5.29–5.37 (m, 2H, CH + NH) 5.95 (d, *J* = 15.0 Hz, 1H, CH) 6.68 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 6.82 (d, *J* = 8.0 Hz, 1H, NH) 7.00–7.07 (m, 2H, ArH) 7.13–7.18 (m, 3H, ArH) 7.39 (s, 1H, ArH) 7.51–7.68 (m, 6H, ArH) 7.82 (d, *J* = 7.5 Hz, 2H, ArH) 7.94 (d, *J* = 8.0 Hz, 1H, ArH) 8.06 (d, *J* = 8.0 Hz, 1H, ArH) 8.24 (d, *J* = 7.5 Hz, 1H, ArH) 8.67 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1 (CH_3) 21.9 (CH_2) 22.5 (CH_2) 25.6 (CH_2) 28.6 (CH_2) 28.8 (CH_2) 29.2 (CH_2) 31.5 (CH_2) 32.7 (CH_2) 39.2 (CH_2) 42.4 (CH_2) 49.7 (CH) 65.6 (CH) 121.3 (CH) 124.2 (CH) 124.4 (CH) 126.9 (CH) 127.5 (CH) 127.6 (CH) 128.1 (C) 128.4 (CH) 128.8 (CH) 128.9 (CH) 129.1 (CH) 129.3 (CH) 129.7 (CH) 130.5 (CH) 133.6 (CH) 134.3 (C, CH) 134.6 (C) 135.1 (C) 140.0 (C) 144.9 (CH) 148.6 (C) 167.5 (CO) ppm. HRMS (ES^+) $C_{40}H_{47}N_5O_5NaS_2$ (MNa^+) calcd. 764.2916; found 764.2945. $[\alpha]_D$ = -20 (*c* = 0.1, CH_2Cl_2).

5.17. Trypanosome cell culture

The bloodstream form *Trypanosome brucei brucei* MITat1.1 strain was used for assessment of compound sensitivity *in vitro*. As described,[8] *T. b. brucei* was cultured in antibiotic-free HMI-9 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biosera, UK). Trypanosomes were subcultured at the appropriate dilutions (1:10) every 24 h in fresh HMI-9 medium to ensure log growth phase. The cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.

5.18. Trypanosomal viability assays

The effect of each final compound in the series on parasite growth was determined using the Alamar Blue cell viability assay. This assay was performed in triplicate according to Räz *et al.*[16] Briefly, *T. b. brucei* cells (strain MIT at 1.1) were seeded in 96-well plates at a density of 2 × 10^5 cells/mL in 100 μL media in the presence of varying concentrations of predicted inhibitors (5 μM, 1 μM, 500 nm, 100 nM, 10 nM, 100 pM) or DMSO alone. A further 30 μL of media was added to each well. After 6 h, 15 μL of Alamar Blue (Invitrogen) was added to the cells and incubation continued so that the total incubation time was 24 h. Absorbances at 540 and 595 nm were measured using a SpectraMax M3 Microplate Reader (Molecular Devices), and EC_{50} values were calculated using the GraphPad Prism 8 software.

5.19. HaCaT cell culture

Human Skin Keratinocyte cell line HaCaT was used to assess the effects of cell proliferation and cytotoxicity of a range of compounds. Stocks were tested for mycoplasma contamination (Lonza MycoAlert™ LT07-218).

5.20. Mammalian cell sub-culturing

HaCaT cells were established in Dubecco's Eagle Modified Media with L-glutamine (DMEM) with 10% fetal calf serum. Volumes used are for 75 cm^2 flask; cell culture medium was removed and discarded (virkon). Briefly, cell layers were gently rinsed with 0.25% (w/v) trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. Then 2.0 to 3.0 mL of trypsin-EDTA solution was added per flask and cells observed under an inverted microscope until cell layer is dispersed (usually within 5 to 15 min). Cells that are difficult to detach were placed at 37 °C to facilitate dispersal (cells were not agitated during the detachment process in order to avoid clumping). Thereafter, 6–8.0 mL of complete growth medium was added to neutralise the trypsin solution and cells were aspirated by gently pipetting. Appropriate aliquots of the cell suspension were added to new culture vessels with fresh media [A cell suspension, as described above, was prepared then resuspended in media after centrifugation and counted using haemocytometer to determine correct seeding densities]. Cultures were established between 2 × 10^3 and 1 × 10^4 viable cells/cm^2 and did not exceed 7 × 10^4 cells/cm^2. All cells were incubated at 37 °C with 5% CO_2 atmosphere and maintained at a cell confluence of between 30% and 90%. Prior to testing compounds with MTT and LDH, compounds were tested for cross reactivity to LDH and MTT reagents. No atypical reaction with compounds tested and assay reagents was evident.

5.21. LDH assay

The LDH assay,[18] quantitatively measuring lactate dehydrogenase (LDH) released into the media from damaged cells, assesses the number

of cells undergoing apoptosis, necrosis, and other forms of cellular damage. LDH assay was carried out using the Peirce LDH Cytotoxicity Kit (Thermo Scientific #88954), according to manufacturer's specifications. In brief, for LDH analysis 50 μL of transferred media after cell treatment was added to 50 μL of reaction buffer (1:1 ratio) in a flat-bottomed plate and incubated at room temperature in the dark for 30 min. After the incubation time 50 μL of stop buffer was added and the absorbance was read at 490 nm and 680 nm. Results were normalised to negative untreated (spontaneous LDH release) and positive 10X Lysis Buffer (maximum LDH release) in order to report a % cytotoxicity. Statistical analysis was carried out using GraphPad Prism 8, results were normalised to positive and negative controls and nonlinear regression was carried out on MTT results using log(inhibitor) vs normalised response. To ensure statistical validity, cell assays were repeated in triplicate, *i.e.* three times on different days with different cell stocks with similar passage numbers.

5.22. MTT assay

The MTT assay, performed as described by Mosmann,[19] was used to assess the viability of cells post-treatment with all compounds in the series. MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) a yellow tetrazole is readily incorporated by viable cells and reduced by the mitochondrial enzyme succinate dehydrogenase to form the insoluble purple coloured formazan compound. Viability, connected with the quantification of formazan, was measured by absorbance between 520 and 600 nm. For all MTT assays, 100 μL of cells in media were seeded per well ($1x10^5$ cells/mL) in a 96 well plate and incubated for 24 h. After the 24 h the media was gently removed and cells were washed 3 times with sterile PBS, being careful not disrupt the cell monolayer. Drug compounds were provided at 10 mM in DMSO, and were diluted to 100 μM in DMEM complete media and sterile filtered using a 0.22 μm filter, next samples were incubated in triplicate wells with positive (0.1% v/v Triton-X100 in PBS), and negative (untreated) controls included in each plate. Following 24 h exposure to all solutions, media was removed and 50 μL added to a new 96 well plate for use in the LDH assay if required. Then 100 μL of MTT solution (0.5 mg/mL MTT in complete media) was next added to each well without washing, or disturbing the cell monolayer, and incubated for 3 h. The MTT solution was carefully removed without disturbing any crystals that formed and 100 μL DMSO was added to each well to solubilise the formazan crystals. The plate was transferred to an orbital shaker which was shaken for 15 mins. Absorbance was then measured at 590 nm on a ThermoScientific Multiskan GO plate reader.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people, or organisations that could inappropriately influence our work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2020.115774. Supporting information: Copies of ^1H and ^{13}C and ^{19}F NMR spectra and the graph for the MTT assay are available electronically via.

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