Chlorocarbonylsulfenyl Chloride Cyclizations Towards Piperidin-3-yl-oxathiazol-2-ones as Potential Covalent Inhibitors of Threonine Proteases

Marko Jukič,1 Katarina Grabrijan,1 Selmir Kadić,1 Fernando Juan de Lera Garrido,1,2 Izidor Sosič,1 Stanislav Gobec1 and Aleš Obreza1,*

1 University of Ljubljana, Faculty of Pharmacy, Department of medicinal chemistry; Aškerceva 7, SI–1000, Ljubljana, Slovenia
2 Universidad de Castilla–La Mancha (Albacete); Universidad de Castilla–La Mancha, Altagracia, 5013071 Ciudad Real, Spain

* Corresponding author: E-mail: ales.obreza@ffa.uni-lj.si
phone: +386 1 47 69 677; fax: +386 1 42 58 031

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Dedicated to Professor Emeritus Miha Tišler, University of Ljubljana, on the occasion of his 90th birthday.

Abstract

Using rescaffolding approach, we designed piperidine compounds decorated with an electrophilic oxathiazol-2-one moiety that is known to confer selectivity towards threonine proteases. Our efforts to prepare products according to the published procedures were not successful. Furthermore we identified major side products containing nitrile functional group, resulting from carboxamide dehydration. We systematically optimized reaction conditions towards our desired products to identify heating of carboxamides with chlorocarbonylsulfenyl chloride and sodium carbonate as base in dioxide at 100 °C. Our efforts culminated in the preparation of a small series of piperidin-3-yl-oxathiazol-2-ones that are suitable for further biological evaluation.

Keywords: Cyclization, amide dehydration, oxathiazole-2-one, threonine protease, covalent inhibitors, irreversible inhibition

1. Introduction

Proteases play key roles in complex biological systems and in multiple structural and signalling pathways. They constitute a historically important field in medicinal chemistry and continue to represent a source of potential drug targets. They are involved in the pathology of hypertension, autoimmune and inflammatory diseases, reperfusion injury, blood clotting disorders, HIV and other viral infections, parasitic and bacterial infections, and last but not least, cancer.1 Protease inhibitors are not valuable only as potential drugs but also as experimental tools for structural biology,2 as they can be used as molecular probes in the elucidation of protease structures and protease pathway mechanisms.3 Recently, databases of proteases (sometimes also termed peptidases, proteinases or proteolytic enzymes) have been established as a resource in this immense research field; namely the Merops database with over 4000 individual entries.4

Our research efforts are mainly focused on the N-terminal threonine proteases that form stable covalent acyl-enzyme complexes and are subsequently hydrolyzed to afford product peptides. Threonine proteases constitute 99 entries in the Merops database, where we specifically study the threonine-type endopeptidases, such as the proteasomes.5 The proteasomes consist of a central proteolytic unit, known as the 20S proteasome, and the 19S regulators, which together make up a 26S structure (Figure 1). The constitutive isoform of the proteasome is expressed in all eukaryotic cells while its immunomodulatory isoform, the immunoproteasome, is mainly expressed in cells associated with the immune system, such as lymphocytes and
monocytes.\textsuperscript{5-6} The constitutive proteasome contains three enzymatically active subunits, namely the b1c (caspase-like), the b2c (trypsin-like), and the b5c (chymotrypsin-like) that are embedded into a barrel-shaped structure consisting of four rings of β-subunits and α-subunits in an abba order. The immunoproteasome has essentially the same overall structure, only the catalytically active subunits of cCP are replaced by their counterparts b1i, b2i, and b5i (Figure 1). The 20S proteasome core particle of both isoforms is a protease of 720 kDa and 28 individual subunits and is responsible for essential proteolytic degradation during cellular inflammatory and oxidative stress.\textsuperscript{7} Immunoproteasome is also important for the generation of peptides for antigen presentation; moreover, recent studies also suggest a pleiotropic role in cellular function of the immunoproteasome.\textsuperscript{8-10}

There is an amounting body of research on the small-molecule inhibitors of proteasomes.\textsuperscript{5,11} Both marketed medicines, bortezomib and carfilzomib, equally inhibit the catalytically active β5 subunits of the constitutive proteasome and the immunoproteasome. The combined inhibition of both isoforms leads to cytotoxicity that limits the clinical application of these broad spectrum proteasome inhibitors.\textsuperscript{6} In addition, many of the investigational compounds are peptide-like compounds and this represents a serious limitation to their metabolic stability and bioavailability.\textsuperscript{5} To overcome these problems, multiple approaches can be found in literature: design of reversible proteasome inhibitors,\textsuperscript{12} use of structural differences in the binding sites of both proteasomes in structure-based drug design,\textsuperscript{13,14} design of highly selective and hydrolytically more stable peptidic compounds,\textsuperscript{15} design of highly selective non-peptidic compounds,\textsuperscript{16} use of non-catalytic residues or allosteric sites in inhibitor design,\textsuperscript{17} and the design of selective electrophilic warheads.\textsuperscript{18} The majority of these compounds are covalent irreversible inhibitors bearing an electrophilic warhead that is capable of reacting with the N-terminal threonine residue in the catalytic active site of the examined protease.\textsuperscript{5,11} Electrophilic warheads belong to structural classes of aldehydes, α,β′–epoxyketones, α–keto aldehydes, β–lactones, vinyl sulfones, Michael-acceptor systems, and boronates.\textsuperscript{19} The active interest in this field is clearly represented by a very recent publication,\textsuperscript{19} where a new mechanism for an existing warhead was reported, i.e. the formation of 1,4-oxazepane upon reaction of an α,β′–epoxyketone warhead with the N-terminal threonine rather than the previously reported morpholine ring.\textsuperscript{14,19} Such new developments provide invaluable data for the design of novel and selective irreversible inhibitors of threonine proteases.

In order to design targeted covalent inhibitors of threonine protease, we sought to examine the available electrophilic warheads.\textsuperscript{20} We were in particular interested in compounds that could provide a suitable reactivity and selectivity towards threonine proteases. Recently, oxathiazol-2-one moiety was identified in a high-throughput screening campaign as a promising candidate.\textsuperscript{21} The proposed mechanism of the covalent modification of N-terminal threonine induced by this electrophilic fragment is depicted in Figure 2 and proceeds through cyclocarbonylation.\textsuperscript{18,21} In current paper we describe an optimized synthetic approach towards oxathiazol-2-one electrophilic war-
head in compounds with basic nitrogen atom and the preparation of a focused library of piperidin-3-yl-oxathiazol-2-ones that are suitable for further biological evaluation.

2. Results and Discussion

We designed our compounds on the basis of their synthetic accessibility and their potential to be modified accordingly during further optimizations. Therefore, we selected a piperidine central core derivatized with an electrophilic oxathiazol-2-one warhead that could confer the selectivity towards threonine proteases as reported beforehand (Figure 3).18,21

We started the synthesis with the alkylation of nipecotamide employing a set of alkyl bromides in DMF as a solvent and Na2CO3 as a base to obtain compounds 2a, 2b and 2c–e. In the case of compound 2f, alkylation with p-nitrobenzylbromide was followed by hydrogenation in MeOH with final acylation using benzyl chloride. The key step in the synthesis was the cyclization of suitably substituted nipecotamides 2a–f into piperidin-3-yl-oxathiazol-2-ones 7–3e using chlorocarboxylsulfenyl chloride as a reagent (Figure 4). This synthetic approach was reported by Gryder et al. when they described the synthesis of the oxathiazol-2-one analogue of bortezomib. The penultimate carboxamide dipeptide was successfully transformed into the oxathiazol-2-one-bortezomib in high yield by using chlorocarboxylsulfenyl chloride in refluxing THF.22

Despite our numerous attempts to obtain the final oxathiazol-2-ones 3a–f by following the original procedure no product could be isolated. Initial experiments in refluxing THF resulted in a complex mixture of products.23 If the experiments were performed at lower temperature (0 °C, room temperature), no apparent conversion was observed. Our first modification of the original procedure was to use relatively nonpolar and system-inert toluene as a solvent that could provide an alternative reactant/intermediate stabilization pattern and would enable a broader temperature sweep. This system was also described by Gurjar et al. where they heated the mixture of amide and chlorocarboxylsulfenyl chloride in toluene from 60 to 90 °C until the settlement of HCl evolution, followed by 1 h of reflux; this yielded > 50% of isolated oxathiazol-2-one.23 No conversion was observed in our case at lower temperatures (0 °C, room temperature) with a formation of complex mixture of products at 60 °C and reflux conditions. Further experiments using pyridine as solvent afforded similar results. Nevertheless, a difference in reaction scope can be observed as besides previously mentioned report by Gryder et al.,22 literature only describes a relatively simple case of benzamide cyclization towards final 5-phenyl-1,3,4-oxathiazol-2-one. In our case, the reaction incorporated a piperidin-3-yl central scaffold (compounds 2a–e) containing an additional basic centre. We also conducted a thor-

Figure 2. Oxathiazol-2-one electrophilic warhead and its interaction mechanism with the N-terminal threonine in the active site

Figure 3. Design of piperidin-3-yl-oxathiazol-2-ones as potential covalent inhibitors of threonine proteases.
ough separation of complex product mixtures in the case of cyclization of compound \( \text{2a} \) and identified a dominant side product (> 30% yield) flanked by a myriad of other chemical species that could not be obtained at a significant quantity. The dominant side product was identified when examining its \(^{13}\text{C} \) NMR spectrum. Namely, the carbon atom of the carboxamide \( \text{2a} \) can be found as expected at 178.3 ppm (400 MHz, DMSO-\( d_6 \)), whereas the carbon of the dominant side product species was found upfield at 121.8 ppm. When recording IR spectrum, a marked peak at 2240 cm\(^{-1} \) was found indicating the presence of a nitrile functionality; the formation of the side product 1-benzylpiperidine-3-carbonitrile \( \text{4a} \) (Figure 5) was then further confirmed by HRMS. The nature of this reaction outcome can be rationalized as presented in Figure 5.

In our reaction system, the dehydration process is facilitated by the primary amide \( \text{2a} \) (Figure 5) that readily couples with the chlorocarbonylsulfenyl chloride to form an active intermediate (Figure 5). The coupling is followed by rapid elimination that is catalyzed either with the starting substituted piperidine as a base or is assisted by other bases in the reaction system (such as pyridine) to form the corresponding nitrile \( \text{4a} \) (Figure 5). Indeed, similar dehydrations of primary carboxamides using an acidic reagent such as \( \text{POCl}_3, \text{SOCl}_2 \) are well documented in literature.\(^{24,25} \)

More recent, chemoselective and milder methods were
also reported, where ethyl dichlorophosphate/DBU system or methyl (carboxysulfamoyl)triethylammonium hydroxide (Burgess reagent) were used as the dehydrating reagents. In addition, Vilsmeier reagents, bromodimethylsulphonium bromide (BDMS), PdCl₂ in aqueous acetonitrile, Swern oxidation conditions and other catalytic or alternative methods using silanes, silazanes, chlorosilanes, alkoxysilanes, and aminosilanes were also described. The myriad of reaction side products that was observed is a consequence of multitude of side reactions that can occur during dehydration reactions, such as thermal decomposition of the formed oxathiazol-2-one and hydrolysis reactions (Figure 6). The formed oxathiazol-2-one can also take part in the 1,3-dipolar nitrile sulfide cycloaddition reaction with available nitrile to obtain thiadiazoles as side products. The nitrile sulphide is

![Figure 6. Possible side reaction pathways in the synthesis of piperidin-3-yl-oxathiazol-2-ones.](image-url)

**Table 1. Synthesis of piperidin-3-yl-oxathiazol-2-ones 3a–f.**

<table>
<thead>
<tr>
<th>Cpd. No</th>
<th>Product</th>
<th>Yield (η) (%)*</th>
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<td>68</td>
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<tr>
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<td>33</td>
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<tr>
<td>3e</td>
<td><img src="image-url" alt="Image" /></td>
<td>43</td>
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<tr>
<td>3f</td>
<td><img src="image-url" alt="Image" /></td>
<td>16</td>
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</tbody>
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* Yield after purification using column chromatography (SiO₂ support with n–hexane:EtOAc solvent system as an eluent).
generated in situ by thermal decomposition of oxathiazol-2-one.\textsuperscript{35} Nitrile sulphides are short-lived species prone to fragmentation and can take part in further cyclloaditions.\textsuperscript{36–38}

After initial unsuccessful attempts to prepare the desired compounds 3a–f, we turned our attention to microwave-assisted report on flow-chemistry synthesis of oxathiazol-2-one in dioxane at 200 °C and residence time of 1 min in a flow reactor reported by Öhrngren et al.\textsuperscript{39} On this basis, we modified the reaction procedure and dissolved the carboxamides 2a–f (Figure 4) in dry dioxane (27 mL/1 mmol carboxamide), used an excess of solid Na\textsubscript{2}CO\textsubscript{3} (5 eq) and chlorocarbonsulphenyl chloride (2 eq), and stirred the reaction mixture at 100 °C for 16 h under argon to obtain the desired oxathiazol-2-ones 3a–f (Figure 4) in 16 to 68% yields (Table 1).

### 3. Experimental

Chemicals from commercial sources were used without further purification. Anhydrous THF, DCM, and Et\textsubscript{3}N were dried and purified by distillation over Na, K\textsubscript{2}CO\textsubscript{3}, and KOH, respectively. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60F\textsubscript{254}) plates (0.25 mm). Column chromatography was performed on Merck silica gel 60 (Merck, particle size 0.040–0.063 mm). Melting points were determined on a Reichert hot stage microscope and are uncorrected. \textsuperscript{1}H-, COSY-, HMOC- and \textsuperscript{13}C-NMR spectra were recorded on a Bruker AVANCE DPX\textsubscript{400} spectrometer in CDCl\textsubscript{3} or DMSO–d\textsubscript{6} solution with TMS as internal standard. Chemical shifts are reported in ppm (δ) downfield from TMS. All the coupling constants (J) are in hertz. IR spectra were recorded on a PerkinElmer Spectrum BX System FT-IR spectrometer. Mass spectra were obtained with a VG–Analytical Autospec Q mass spectrometer with ESI ionization (MS Center). Mass spectra were obtained with a VG–Analytical Autospec Q mass spectrometer with ESI ionization (MS Center). Jukič et al.: Chlorocarbonylsulphenyl Chloride Cyclizations ...

#### 1-(4-Nitrobenzyl)piperidine-3-carboxamide (2b)

To a solution of piperidine-3-carboxamide (500 mg, 3.90 mmol) in 50 mL DMF, solid Na\textsubscript{2}CO\textsubscript{3} (460 mg, 4.33 mmol) and 4-nitrobenzyl bromide (1.69 g, 7.82 mmol) were added. The reaction was stirred at 100 °C overnight. DMF was removed under reduced pressure, the residue dissolved in EtOAc (30 mL) and extracted with 0.5 M HCl (2 × 15 mL). The pH of combined aqueous phases was adjusted to 8 with NaHCO\textsubscript{3} and extracted with EtOAc (4 × 30 mL). Combined organic phases were washed with H\textsubscript{2}O (1 × 30 mL), brine (1 × 30 mL) and dried over Na\textsubscript{2}SO\textsubscript{4}. The volatiles were removed under reduced pressure to give compound 2b as pale orange solid. Yield = 93%; TLC (EtOAc:MeOH = 2:1), R\textsubscript{f} = 0.63; m.p. 111–114 °C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 1.33–1.36 (m, 1H, H–5), 1.43–1.48 (m, 1H, H–4), 1.60–1.65 (m, 1H, H–5), 1.72–1.76 (m, 1H, H–4), 1.91–2.09 (m, 2H, H–6 and H–2), 2.30–2.33 (m, 1H, H–6), 2.69–2.78 (m, 2H, H–2 and H–3), 3.55–3.63 (m, 2H, CH\textsubscript{2}), 6.76 (br s, 1H, NH\textsubscript{2}), 7.27 (br s, 1H, NH\textsubscript{2}), 7.56–7.60 (m, 2H, H–2' and H–6'), 8.19 (dd, J = 2.0, 4.8 Hz, 2H, H–3' and H–5'); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) δ 24.4 (C–3'), 27.0 (C–5'), 27.0 (C–3'), 42.3 (C–3'), 53.2 (C–6'), 55.8 (C–2), 61.4 (CH\textsubscript{2}), 123.3 (C–3' and C–5'), 129.6 (C–2' and C–6'), 146.5 (C–1'–C–5'), 175.4 (OCNH\textsubscript{2}); IR (ATR) ν 3333, 3180, 2926, 2787, 1644, 1605, 1512, 1421, 1349, 1249, 1204, 1166, 1102, 1048, 989, 862, 797, 736,720 cm–1; MS m/z (relative intensity): 261.97 (M–H, 100).

#### 1-(4-Chlorobenzyl)piperidine-3-carboxamide (2c)

To a solution of piperidine-3-carboxamide (500 mg, 3.90 mmol) in 50 mL DMF, solid Na\textsubscript{2}CO\textsubscript{3} (460 mg, 4.33 mmol) and 3-chlorobenzyl bromide (1.64 g, 7.99 mmol) were added. The reaction was stirred at 100 °C overnight. DMF was removed under reduced pressure, the residue dissolved in EtOAc (30 mL) and extracted with 0.5 M HCl (2 × 15 mL). The pH of combined aqueous phases was adjusted to 8 with NaHCO\textsubscript{3} and extracted with EtOAc (4 × 30 mL). Combined organic phases were washed with H\textsubscript{2}O (1 × 30 mL), brine (1 × 30 mL) and dried over Na\textsubscript{2}SO\textsubscript{4}. The volatiles were removed under reduced pressure to give compound 2c as pale orange solid. Yield = 53%; TLC (EtOAc:MeOH = 2:1), R\textsubscript{f} = 0.60; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 1.55–1.61 (m, 1H, H–5), 1.65–1.77 (m, 2H, H–4 and H–5), 1.82–1.84 (m, 1H, H–4), 2.26 (s, 1H, H–6), 2.44–2.53 (m, 2H, H–2 and H–6), 2.59 (s, 1H, H–2), 2.68–2.78 (m, 2H, H–2 and H–3), 3.44 (dd, J = 4.4, 13.2 Hz, 2H, CH\textsubscript{2}), 6.75 (br s, 1H, NH\textsubscript{2}), 7.22–7.34 (m, 6H, ArH and NH\textsubscript{2}); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) δ 22.8 (C–3'), 27.0 (C–5'), 41.8 (C–3), 53.8 (C–6), 55.0 (C–2), 63.5 (CH\textsubscript{2}), 127.4 (C–4'), 128.4 (C–3' and C–5'), 129.2 (C–2' and C–6'), 137.6 (C–1'), 178.0 (OCNH\textsubscript{2}); IR (ATR) ν 3385, 3180, 2926, 2787, 1644, 1604, 1512, 1421, 1349, 1249, 1204, 1166, 1102, 1048, 989, 862, 797, 736,720 cm–1; MS m/z (relative intensity): 240.95 (M+Na, 100), 219.03 (M+H, 30).
(s, 1H, H–3), 3.46 (s, 2H, CH₂), 6.07 (br s, 1H, NH₃), 7.14–7.16 (m, 1H, NH), 7.24–7.26 (m, 4H, ArH); ¹³C NMR (400 MHz, CDCl₃) δ 22.9 (C–5), 26.9 (C–4), 41.9 (C–3), 53.7 (C–6), 55.1 (C–2), 62.9 (CH₂), 127.2, 127.6 (C–3’ and C–5’), 129.1, 129.7 (C–2’ and C–6’), 134.3 (C–4’), 139.8 (C–1’), 177.8 (CONH₂); IR (ATR) ν 3364, 3187, 2929, 2811, 2227, 1646, 1611, 1569, 1486, 1411, 1350, 1264, 1210, 1165, 1089, 1002, 941, 849, 830, 731 cm⁻¹; MS m/z (relative intensity): 283.71 (M+Na, 100), 261.78 (M+H, 20).

1-(4-Benzamidobenzyl)piperidine-3-carboxamide (2f) Argon was bubbled into a solution of 2b (5.811 g, 17.6 mmol) in MeOH (70 mL) for 15 minutes. 10% Pd/C, unreduced, was then added and H₂ was bubbled into the stirred solution until the starting compound was no longer observed with TLC. Pd/C was filtered off and the solution concentrated in vacuo to yield crude product which was purified with column chromatography (EtOAc:MeOH = 2:1). Oil product was dissolved in DCM (30 mL). Et₃N (250 mg, 2.47 mmol) and benzoyl chloride (265 mg, 2.36 mmol) were added and the reaction mixture was stirred at room temperature overnight. DCM was removed under reduced pressure, the residue dissolved in EtOAc (30 mL) and extracted with 0.5 M HCl (1 × 10 mL). The pH of combined aqueous phases was adjusted to 8 with NaHCO₃ and extracted with EtOAc (4 × 30 mL). Combined organic phases were washed with H₂O (1 × 30 ml), brine (1 × 30 ml) and dried over Na₂SO₄. The volatiles were removed under reduced pressure to give compound 2d as a colourless oil. Yield = 16 %; TLC (EtOAc:MeOH = 2:1), Rₖ = 0.36; ¹³C NMR (400 MHz, DMSO-d₆) δ 1.06–1.63 (m, 1H, H–5), 1.64–1.70 (m, 3H, H–4 and H–5), 1.89–1.99 (m, 1H, H–6), 2.29–2.34 (m, 2H, H–2 and H–6), 2.67–2.76 (m, 1H, H–2); IR (ATR) ν 3403, 3183, 2936, 2797, 1715, 1647, 1434, 1415, 1273, 1239, 1199, 1165, 1112, 1086, 1027, 995, 964, 860, 806, 760, 754, 706 cm⁻¹.

1-(4-Cyano-2-fluorobenzyl)piperidine-3-carboxamide (2e) To a solution of piperidine-3-carboxamide (500 mg, 3.90 mmol) in 50 mL DMF, solid Na₂CO₃ (320 mg, 3.02 mmol) and methyl 4-(bromomethyl)benzoate (540 mg, 2.36 mmol) were added. The reaction was stirred at 100 °C overnight. DMF was removed under reduced pressure, the residue dissolved in EtOAc (30 mL) and extracted with 0.5 M HCl (2 × 15 mL). The pH of combined aqueous phases was adjusted to 8 with NaHCO₃ and extracted with EtOAc (2 × 15 mL). Combined organic phases were washed with H₂O (1 × 20 ml), brine (1 × 20 mL) and dried over Na₂SO₄. The volatiles were removed under reduced pressure to give compound 2e as pale yellow solid. Yield = 71 %; TLC (EtOAc:MeOH = 2:1), Rₖ = 0.66; mp. 126–128 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 1.32–1.42 (m, 1H, H–5), 1.44–1.49 (m, 1H, H–4), 1.59–1.64 (m, 1H, H–5), 1.71–1.75 (m, 1H, H–4), 1.88–1.95 (m, 1H, H–6), 1.98–2.03 (m, 1H, H–2), 2.29–2.35 (m, 1H, H–6), 2.68–2.71 (m, 1H, H–2), 2.74–2.78 (m, 1H, H–3), 3.52 (d, J₂,2 2.8 Hz, 2H, CH₂), 3.85 (s, 3H, CH₃), 6.77 (br s, 1H, NH), 7.27 (br s, 1H, NH), 7.44 (d, J 8.4 Hz, 2H, H–2’ and H–6’), 7.92 (dd, J 2.0, 4.8 Hz, 2H, H–3’ and H–5’); IR (ATR) ν 3403, 3183, 2936, 2797, 1715, 1647, 1434, 1415, 1273, 1239, 1199, 1165, 1112, 1086, 1027, 995, 964, 860, 806, 760, 754, 706 cm⁻¹.

1-(4-Benzamidobenzyl)piperidine-3-carboxamide (2f) Argon was bubbled into a solution of 2b (5.811 g, 17.6 mmol) in MeOH (70 mL) for 15 minutes. 10% Pd/C, unreduced, was then added and H₂ was bubbled into the stirred solution until the starting compound was no longer observed with TLC. Pd/C was filtered off and the solution concentrated in vacuo to yield crude product which was purified with column chromatography (EtOAc:MeOH = 2:1). Oil product was dissolved in DCM (30 mL). Et₃N (250 mg, 2.47 mmol) and benzoyl chloride (265 mg, 2.36 mmol) were added and the reaction mixture was stirred at room temperature overnight. DCM was removed under reduced pressure, the residue dissolved in EtOAc (30 mL) and extracted with 0.5 M HCl (1 × 10 mL). The pH of combined aqueous phases was adjusted to 8 with NaHCO₃ and extracted with EtOAc (2 × 15 mL). Combined organic phases were washed with H₂O (1 × 20 ml), brine (1 × 20 mL) and dried over Na₂SO₄. The volatiles were removed under reduced pressure to give compound 2f as colourless oil. Yield = 16 %; TLC (EtOAc:MeOH = 2:1), Rₖ = 0.36; ¹³C NMR (400 MHz, DMSO-d₆) δ 1.06–1.63 (m, 1H, H–5), 1.64–1.70 (m, 3H, H–4 and H–5), 1.89–1.99 (m, 1H, H–6), 2.29–2.34 (m, 2H, H–2 and H–6), 2.67–2.76 (m, 1H, H–2), 2.78–2.99 (m, 1H, H–3), 3.01–3.51 (m, 2H, CH₂), 6.76 (br s, 1H, NH), 7.24–7.29 (m, 3H, H–2’ and H–6’ and NH₂), 7.52–7.62 (m, 3H, H–3’ and H–4’ and H–5’), 7.72–7.76 (d, 2H, H–2’ and H–5’), 7.95–7.97 (m, 2H, H–2” and H–6”), 10.26 (d, J 4.4 Hz, 1H, NH).

5-(1-Benzylpiperidin-3-yl)-1,3,4-oxathiazol-2-one (3a) To a solution of 1-benzylpiperidine-3-carboxamide (2a, 240 mg, 1.10 mmol) in dioxane (30 mL) in a three-necked flask, solid Na₂CO₃ (580 mg, 5.47 mmol) and chlorocarbonsulfonyl chloride (288 mg, 2.20 mmol) were added under argon. The reaction mixture was stirred at 100 °C overnight, cooled to room temperature and after the addition of Et₃N (0.75 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using hexane:EtOAc=3:1 as an eluent to give yellow oily product. Yield = 68%; TLC (hexane:EtOAc = 3:1), Rₖ = 0.56; ¹H NMR (400 MHz, DMSO-d₆) δ 1.50–1.56 (m, 1H, H–5), 1.70–1.72 (m, 1H, H–5), 1.88–1.93 (m, 1H, H–4), 2.08–2.12 (m, 1H, H–6), 2.20–2.25 (m, 1H, H–2).
5-(1-(4-Nitrobenzyl)piperidin-3-yl)-1,3,4-oxathiazol-2-one (3b)
To a solution of 1-(4-nitrobenzyl)piperidine-3-carboxamide (2b, 100 mg, 0.380 mmol) in dioxane (20 mL) in a three-necked flask, solid Na₂CO₃ (200 mg, 1.87 mmol) and chlorocarbonylsulfenyl chloride (100 mg, 0.760 mmol) were added under argon. The reaction mixture was stirred at 100 °C overnight, cooled to room temperature and after the addition of Et₃N (0.27 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using hexane:EtOAc = 2:1 as an eluent to give yellow oily product. Yield = 43%; TLC (hexane:EtOAc = 2:1), Rₑ = 0.38; ¹H NMR (400 MHz, DMSO-d₆) δ 1.51–1.53 (m, 2H, H–5, H–4), 1.72–1.75 (m, 1H, H–5), 1.91–1.94 (1H, H–4), 2.16 (s, 3H, CH₃), 2.65–2.68 (m, 1H, H–6), 2.89–2.92 (m, 2H, H–2 and H–3), 3.50 (d, J 2.8 Hz, 2H, CH₂), 7.45 (d, J 8.4 Hz, 2H, H–2' and H–6'), 7.93 (d, J 8.8 Hz, 2H, H–2' and H–6'–H–6); ¹³C NMR (400 MHz, DMSO-d₆) δ 24.1 (C–5), 26.9 (C–4), 38.3 (C–3), 53.5 (C–6), 54.9 (C–2), 61.5 (CH₃), 128.3 (C–2'–3'–4'), 130.1 (C–3' and C–5'), 144.0 (C–1'), 162.4 (CO), 166.1 (NCO), 174.2 (SCO); IR (ATR) ν 3339, 3160, 2938, 2820, 1758, 1666, 1599, 1493, 1467, 1450, 1350, 1320, 1287, 1282, 1254, 1157, 1105, 1075, 1046, 997, 930, 892, 876, 863, 808, 776, 717, 705, 682, 655, 569, 536, 516 cm⁻¹; HRMS–ESI (m/z): [M+H]⁺ calcd for C₁₄H₁₅N₃O₄S, 335.0615, found, 335.0621.

Methyl 4-((3-(2-oxo-1,3,4-oxathiazol-5-yl)piperidin-1-yl)methyl)benzoate (3d)
To a solution of methyl 4-((3-carbamoylpiperidin-1-yl)methyl)benzoate (2d, 300 mg, 1.09 mmol) in dioxane (30 mL) in a three-necked flask, solid Na₂CO₃ (570 mg, 5.38 mmol) and chlorocarbonylsulfenyl chloride (285 mg, 2.16 mmol) were added under argon. The reaction mixture was stirred at 100 °C overnight, cooled to room temperature and after the addition of Et₂N (0.75 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using hexane:EtOAc = 2:1 as an eluent to give yellow oily product. Yield = 43%; TLC (hexane:EtOAc = 2:1), Rₑ = 0.29; ¹H NMR (400 MHz, DMSO-d₆) δ 1.45–1.57 (m, 2H, H–2', H–5'), 1.72–1.75 (m, 1H, H–5), 1.91–1.95 (m, 1H, H–4), 2.09–2.17 (m, 1H, H–6), 2.24–2.34 (m, 1H, H–2), 2.65–2.68 (m, 1H, H–6), 2.87–2.96 (m, 2H, H–2 and H–3), 3.59 (s, 2H, CH₂), 3.85 (s, 3H, CH₃), 7.45 (d, J 8.4 Hz, 2H, H–2'–H–6'), 7.93 (dd, J 2.0, 4.8 Hz, 2H, H–3'–H–5'); ¹³C NMR (400 MHz, DMSO-d₆) δ 23.5 (C–5), 26.1 (C–4), 37.5 (CH₃), 52.0 (C–6), 53.0 (C–2), 54.5 (CH₂), 61.5 (CH₃), 128.3, 128.7, 128.8, 129.1, 129.1 (C–2,3,4′,5′,6′), 144.0 (C–1), 162.4 (CO), 166.1 (NCO), 174.2 (SCO); IR (ATR) δ 2964, 2810, 1759, 1717, 1609, 1434, 1415, 1395, 1349, 1309, 1275, 1190, 1173, 1106, 1049, 980, 828, 885, 801, 758, 731, 701, 650, 572, 538 cm⁻¹; HRMS–ESI (m/z): [M+H]⁺ calcd for C₁₄H₁₄ClN₂O₄, 353.1068, found, 353.1066.

3-Fluoro-4-((3-(2-oxo-1,3,4-oxathiazol-5-yl)piperidin-1-yl)methyl)benzonitrile (3e)
To a solution of 1-(4-cyano-2-fluorobenzyl)piperidine-3-carboxamide (2e, 300 mg, 1.15 mmol) in dioxane (30 mL) in a three-necked flask, solid Na₂CO₃ (608 mg, 5.74 mmol) and chlorocarbonylsulfenyl chloride (303 mg, 2.30 mmol) were added under argon. The reaction mixture was stirred at 100 °C overnight, cooled to room temperature and after the addition of Et₂N (0.75 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using hexane:EtOAc = 3:1 as an eluent to give yellow oily product. Yield = 43%; TLC (hexane:EtOAc = 3:1), Rₑ = 0.29; ¹H NMR (400 MHz, DMSO-d₆) δ 1.51–1.56 (m, 2H, H–2, H–5), 1.70–1.72 (m, 1H, H–5), 1.91–1.92 (m, 1H, H–4), 2.14–2.19 (m, 1H, H–6), 2.30–2.35 (m, 1H, H–2), 2.64–2.67 (m, 1H, H–6), 2.91–2.95 (m,
N-(4-((3-(2-oxo-1,3,4-oxathiazol-5-yl)piperidin-1-yl) methyl)phenyl)benzamide (3f)

To a solution of 1-(4-benzimidobenzyl)piperidine-3-carboxamide (2f, 83 mg, 0.250 mmol) in dioxane (30 mL) in a three-necked flask, solid Na₂CO₃ (130 mg, 1.23 mmol) and chlorocarbonylsulfenyl chloride (65 mg, 0.916 mmol) was added under argon. The reaction mixture was stirred at 80 °C overnight, cooled to room temperature and after the addition of Et₃N (0.32 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using DCM:MeOH = 9:1 as an eluent to give a pure product. Yield = 66%; TLC (hexane:EtOAc = 3:1), Rₖ = 0.492 mmol; IR (ATR) ν 2943, 2804, 2766, 2240, 1499, 1439, 1154, 1093, 1072, 1011, 985, 959, 911, 868, 773, 604, 542, 510 cm⁻¹; MS m/z (relative intensity): 201.1 (M+Na, 100); HRMS–ESI (m/z): [M+H]^+ calcd for C₁₅H₁₄FN₃O₂S, 264.1379, found, 264.1379.

1-(4-Nitrobenzyl)piperidine-3-carbonitrile (4b)

In a three-necked flask 1-(4-nitrobenzyl)piperidine-3-carboxamide (2b, 100 mg, 0.380 mmol) was dissolved in pyridine (10 mL). The solution was cooled on ice and chlorocarbonylsulfenyl chloride (100 mg, 0.760 mmol) was added under argon. The reaction mixture was stirred at 80 °C overnight, cooled to room temperature and after the addition of Et₃N (0.32 mL) stirred for 15 minutes. The precipitate was filtered off and the residue concentrated in vacuo. Product was purified with column chromatography using DCM:MeOH = 9:1 as an eluent to give a brownish solid. Yield = 34%; TLC (DCM:MeOH = 9:1) Rₖ = 0.96; ¹H NMR (400 MHz, CDCl₃) δ 8.64–8.55 (m, 1H, H–5), 7.99–7.92 (m, 3H, H–5 and H–6), 2.46 (s, 2H, H–6), 2.63 (s, 2H, H–2), 2.81–2.84 (m, 1H, H–3), 3.63 (dd, J 6.8, 14.4 Hz, 2H, CH₂), 7.53 (d, 2H, H–2' and H–6'), 8.19 (dd, J 7.0, 4.8 Hz, 2H, H–3' and H–5'); ¹³C NMR (400 MHz, CDCl₃) δ 23.4 (C–5'), 27.4 (C–4'), 120.2 (C–3' and C–5'), 127.1 (C–2' and C–6'), 128.8 (C–2', C–6', C–3' and C–5'), 129.8 (C–4'), 131.9 (C–1' and C–1''), 134.9 (C–4'), 165.8 (NHCO); IR (ATR) ν 2945, 2802, 2766, 1655, 1615, 1523, 1458, 1410, 1376, 1319, 1258, 1167, 1098, 997, 973, 841, 809, 694 cm⁻¹; HRMS–ESI (m/z): [M+H]^+ calcd for C₁₅H₁₄FN₃O₂S, 264.1379, found, 264.1379.

4. Conclusion

Based on the previously reported oxathiazol-2-one-bearing and nonpeptidic inhibitors of the chymotrypsin-like (β5i) subunit of the immunoproteasome, we designed a novel series of piperidine-3-yl-oxathiazol-2-ones as potential covalent inhibitors of threonine proteases. Compounds were designed with a synthetically accessible piperidine central core derivatized with an oxathiazol-2-one bearing moiety. In lieu of previously reported synthetic approaches, we identified a synthetic protocol that enables the cyclization of carboxamides incorporating a basic centre into oxathiazol-2-ones. This straightforward protocol using chlorocarbonylsulfenyl chloride as a reagent in dioxane afforded the desired products in moderate to good yields. Thus, a vast chemical space of 5-substituted oxathiazol-2-ones can be explored and various chemical libraries of inhibitors of threonine proteases can be compiled.

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Conflict of interest
The authors declare they have no conflict of interest.

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Povzetek

Z zamenjavo molekulskega skeleta smo načrtovali spojine s piperidinskim jedrom, derivatiziranim z oksatiazol-2-on-skim elektrofilih smincem, ki omogoča selektivno zaviranje treoninskih proteaz. Sinteza produktov po postopkih, opisanih v literaturi, ni bila uspešna, poleg tega smo identificirali nitrile kot glavne stranske produkte, ki nastanejo pri dehidraciji karboksamidne funkcionalne skupine. S sistematično optimizacijo reakcijskih pogojev, smo s segrevanjem karboksamidov, klorokarbonilsulfenil klorida in natrijevega karbonata kot baze v dioksanu pri 100 °C pripravili serijo piperidin-3-il-oksatiazol-2-onov, primerno za nadaljnje biološko vrednotenje.