Rational modification, synthesis and biological evaluation of N-substituted phthalazinone derivatives designed to target interleukine-15 protein
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Rational modification, synthesis and biological evaluation of \(N\)-substituted phthalazinone derivatives designed to target interleukine-15 protein.

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ABSTRACT

Interleukin (IL)-15 is a pleiotropic cytokine structurally close to IL-2 and sharing with the IL-2\(R\)\(\beta\) and \(\gamma\)c receptor (R) subunits. IL-15 plays important roles in innate and adaptative immunity, supporting the activation and proliferation of NK, NK-T, and CD8\(^{+}\) T cells. Overexpression of IL-15 has been shown to participate to the development of inflammatory and autoimmune diseases and diverse T cell malignancies. This study is in continuity of our previous work through which a family of small-molecule inhibitors impeding IL-15/IL-2\(R\)\(\beta\) interaction with sub-micromolar activity has been identified using pharmacophore-based virtual screening and hit optimization methods. With the aim to improve the efficacy and selectivity of our lead inhibitor, specific modifications have been introduced on the basis of optimized SAR and modelisation. The new series of compounds generated have been evaluated for their capacity to inhibit the proliferation as well as the down-stream signaling of IL-15-dependent cells and to bind to IL-15.

Graphical abstract:
Keywords: protein-protein interaction, interleukin-15, IL-15Rβ, IL-15Rβ inhibitor, inflammatory and autoimmune diseases.

1. INTRODUCTION

Interleukin(IL)-15 is a proinflammatory cytokine, belonging to the IL-2 cytokine family, involved in the stimulation of T and natural killer (NK) cell activity. Dysregulation of IL-15 expression has been described in a range of autoimmune inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, alopecia areata, vitiligo, psoriasis, type 1 diabetes and celiac diseases and in patients with leukemia such as large granular lymphocyte (LGLL), cutaneous T cell lymphoma (CTCL) and human T cell lymphotropic virus I (HTLV-1) driven adult T cell leukemia (ATL). IL-15 binds a heterotrimeric receptor composed of a non-transducing, private α chain (IL-15Rα) and a IL-2Rβ/γc-signal transducing complex. The binding of IL-15 to IL-2Rβ/γc heterodimer induces JAK1 activation that subsequently phosphorylates STAT3 (p-Stat3) via the IL-2Rβ chain and JAK3 that subsequently phosphorylates STAT5 (p-Stat5) via the γc chain. Several approaches have been developed to inhibit IL-15 activity, including soluble IL-15Rα, IL-15 mutants, IL-15- or IL-15R-directed monoclonal antibodies and fusion proteins, IL-2 mutant or peptide modified to block IL-15 interaction with its receptors (H9-RETR, BNZ-1 and 2) and JAK inhibitors. Smith et al. have reported the use of a soluble form of the murine IL-15Rα chain (sIL-15Rα) to investigate the contribution of IL-15 in the rejection of fully vascularized cardiac allografts in a murine model. Administration of sIL-15Rα to CBA/Ca (H-
2k) recipients completely prevented rejection of minor histocompatibility complex-mismatched B10.BR (H-2k) heart grafts and led to a state of donor specific immunologic tolerance\textsuperscript{14}. In addition, administration of sIL-15R\textsubscript{D} profoundly suppressed the development of collagen-induced arthritis in mice.\textsuperscript{8} Soluble IL-15R\textsubscript{D} also prevented psoriasis\textsuperscript{15} as well as allergic inflammation\textsuperscript{16}. In this context, Amgen has developed an anti-IL-15 antibody (AMG-714 or HuMax-IL15) targeting IL-15 in the region involved in \( \gamma \)c chain binding. AMG-714 has first shown efficacy in a xenograft model of human psoriasis and rheumatoid arthritis\textsuperscript{17} as well as coeliac disease\textsuperscript{18}. In psoriasis, it led to a better clinical outcome than the standard cyclosporine A therapy. Likewise, a humanized antibody (Hu-Mik-Beta-1) specific for IL-2R\( \beta \) was developed and was shown to prolong cardiac allograft survival in cynomolgus monkeys.\textsuperscript{19} 20 21 This antibody is currently being tested in patients with refractory coeliac disease and HTLV-1-associated tropical spastic paraparesis (HAM/TSP) (clinicaltrials.gov). As an alternative and challenging approach, design of original small molecule inhibitors of protein-protein interactions (PPIs) has recently been applied to target IL-15/IL-15R interfaces. Some potent small-molecule inhibitors of the IL-2/IL-2R\( \beta \) interface have already been identified by chemical or DNA-encoded chemical library screening, fragment-based approaches or virtual library screening followed by chemical optimizations.\textsuperscript{22} 23 24 25 26 Concerning IL-15/IL-15R interfaces, a study using \textit{in silico} pharmacophore-based virtual screening methods directed to IL-15/IL-15R\textsubscript{D} interface has revealed putative IL-15 inhibitors in an \textit{in vitro} primary cellular assay among which cefazolin, a first-generation cephalosporin antibiotic.\textsuperscript{27} However, it turned that cefazolin was not selective to IL-15 but was rather a broad common gamma chain cytokine inhibitor.\textsuperscript{28}

Meanwhile, using a similar approach coupled to hit optimization, we discovered original low MW non-peptidic inhibitors targeting the IL-15/IL-2R\( \beta \) interface.\textsuperscript{29} In this previous work, we designed a novel family of IL-15 inhibitors with a thio-triazolyl-methyl-phthalazinone backbone bearing variable alkyl amide fragments. Starting from selected hit derivative 1, identified through the virtual screening strategy targeting the IL-2R\( \beta \) binding site of IL-15, SAR investigation has given access to a potent lead 2 and other derived analogues showing inhibitory activities against IL-15 with IC\textsubscript{50} values ranging between 50 to 150 nM in cell-based experiments (p-Stat5). Unfortunately, none of these IL-15 inhibitors expressed selectivity versus the competitive IL-2.

Based on our docking model (Fig. 1), we proposed to further investigate some options to improve the inhibitory potency of the compounds introducing specific modifications directed
by a modelisation design progress. This process led us to target the triazole or phthalazinone heterocycle cores by substitution of the \( N\)-Me with \( N\)-alkylated baits, offering a terminal hydrophilic function, in order to probe complementary hydrogen bond interactions with the polar asparagine side chain residues of the IL-15 protein (Fig. 1B). For this purpose, two novel series of analogues of compounds 1 and 2, such as \( N\)-substituted triazole (Fig. 2, Series 1) and phthalazinone (Fig. 2, Series 2) derivatives have been targeted. Furthermore, based on the inhibitor potency of benzamide 2 and its alkoxy or benzoate analogs 2A, 2B and 2C, which gave a good activity in a cell proliferation assay and \( IC_{50} \) at a nanomolar range in a p-Stat5 assay (Fig. 2, Series 3), we proposed to complete our initial series\(^9\) with a compound bearing a benzoyl substituent giving also access to a benzophenone derivative which could be regarded as a potential photocrosslinker tool\(^{10}\) (Fig. 2, R\textsubscript{3} modulation).

**Fig. 1.** Binding mode of compound 1. (A) Connolly surface of IL-15 coloured according to the hydrophobicity index of the exposed residues (hydrophobic in brown; hydrophilic in blue) showing the docking mode of compound 1 and hydrophilic areas surrounding the phthalazinone-triazole moiety\(^9\). (B) Ribbon drawing of the predicted binding mode of compound 1 on IL-15 showing IL-15 residues in the vicinity of the triazole and the phthalazinone heterocycles of 1. Binding interactions are depicted (dashed lines: green = hydrogen bond, light green = carbon hydrogen bond, pink = hydrophobic interaction).
2. RESULTS AND DISCUSSION

2.1 Chemistry

To access to the first series of N-substituted triazole analogues of compounds 1 or 2, we chose to condense the dihydro-phthalazine hydrazide 3 with an isothiocyanate diethylacetal reagent providing a masked carbonyl group. So, isothiocyanate 4 was condensed with hydrazide 3 to give the N-substituted mercapto-triazole derivative 5 in 80% yield. This strategy allowed the access to the desired targeted compounds 10 to 14 from this common precursor (Scheme 1). Both alkylating agents 6 (n = 1) and 7 (n = 7) efficiently reacted by nucleophilic
substitution with the thiol 5 in basic conditions, without identification of the N-substituted regioisomer. The resulting acetal derivatives 8 and 9 were then subjected to hydrolysis in acidic conditions to yield the corresponding aldehydes 10 and 11, respectively. The targeted aldehyde 10 with the short linker chain (n = 1) was obtained with a good 95 % yield after 2 hours stirring at a temperature of 40 °C. The poor solubility of the acetal 9, bearing a longer linker chain (n = 7), in THF or acetone gave aldehyde 11 in a moderate 59 % yield after completion of the reaction (24 hours). We also observed that in these acidic conditions retro-Michael process occurred leading to the NH free analogue 12. Intermediate 10 was either reduced with sodium borohydride in an ethanolic medium to furnish the alcohol 13 (67%), or oxidized in Dalcanale conditions31 into the corresponding carboxylic acid 14 (60%). However, reduction or oxidation of the aldehyde 11 into the corresponding alcohol or carboxylic acid derivatives was not successful due to poor solubility and slow progress of the reaction in both conditions. Consequently, efficient purification of the reaction products by flash column chromatography failed.
To access to the targeted N-substituted triazolyl analogues of the lead 2 (n = 7), we turned back to a linear synthesis starting from hydrazide 3 which was condensed with the isothiocyanate reagents 15 and 16 bearing a terminal alcohol or amino function, respectively (Scheme 2). Following this strategy, mercapto-triazole derivatives 17 and 18 were efficiently obtained in 80 % yields and subsequently alkylated with bromo amide fragment 7 leading to the silylated...
intermediate 19 and the targeted N-dimethylamino propyl analogue 20 in 95% yields. Finally, the alcohol 21 was quantitatively isolated after desilylation of 19 by Olah’s reagent prior to be oxidized into the corresponding acid 22 using TEMPO/DAIB system.

Scheme 2. Synthesis of the N-triazolyl dimethyl propylamine 20, N-triazolyl propanol 21 and N-triazolyl propionionic acid 22 compounds (n = 7).

The second series of N-substituted phthalazinone analogues of compounds 1 or 2 was then investigated. For the short alkyl chain series (n = 1), the strategy involved the dihydro-phthalazinone carboxylic ester 24 quantitatively obtained by the condensation of one equivalent of hydrazine hydrate with the conjugated ester 23 (Scheme 3). Here again a reagent bearing an acetal function regarded as a suitable promoter of a key aldehyde was preliminary introduced on the phthalazinone ring. Thereby, commercial bromo acetal 25 was used to alkylate the phthalazinone prior inducing the formation of the triazol ring in a two steps procedure involving hydrazine hydrate addition followed by a cyclisation with methylisothiocyanate (89 % overall yield). Thioalkylation under Williamson-type conditions in the presence of the bromo amide reagent 6 afforded the key intermediate 28. Once again, the hydrolysis of the acetal 27 in aqueous HCl medium failed to give aldehyde 29, however, success was encountered in 81 % yield using APTS (0.5 equiv.) in an acetone/water solvent mixture, at 60 °C overnight. Starting from the aldehyde 29, both targeted alcohol 30 and carboxylic acid
31 analogues (n = 1) were obtained in the same reduction and oxidation conditions, as previously described for the N-substituted triazolyl analogues. Alternatively, reductive amination of 29 with dimethylamine in the presence of sodium borohydride also afforded N-dimethylaminopropyl analogue 32 conveniently.

Scheme 3. Synthesis of the N-phthalazino propionaldehyde 29 and derivatives 30-32 (n = 1).

To overcome problems highlighted in precedent attempts, a linear strategy was adopted to reach the targeted N-substituted phthalazino compounds bearing heptyl-amido linker (n = 7). Starting from the precursor 24, N-alkylation was performed with protected bromopropanol 33 or mesylated N-dimethylaminopropanol 34. Alkylation of 24 monitored by K$_2$CO$_3$ as base did not work well and sodium hydride was required to improve the yield of alkylated phthalazinones 35 and 36 to 60 and 75 %, respectively (Scheme 4). The formation of the N-methylmercaptotriazole heterocycle was efficient in two steps giving the corresponding thiols 37 (70 %) and 38 (89 %), from 35 and 36, respectively. Here again the thio-alkylation of thiol 37 and 38 using the bromo reagent 7, in Williamson-type conditions proceeded smoothly giving...
the protected intermediate 39 and the dimethylaminopropyl phthalazinone analogue 40 in moderate 52 and 47 % yields after 24 h of stirring at room temperature. From the protected alcohol derivative 39, desilylation using Olah’s reagent quantitatively led to the targeted N-triazolyl propanol compound 41 from which a first oxidation with Dess-Martin periodinane (DMP) reagent afforded the aldehyde analogue 42 and a second oxidation step in Dalcanale conditions gave the carboxylic acid 43.

Scheme 4. Linear synthesis of the N-phthalazino substituted compounds with the longer linker chain (n = 7): 40 (N-dimethylaminopropyl residue), 41 (N-propanol residue), 42 (N-propionaldehyde residue), and 43 (N-propionic acid residue).

To access to the free NH- phthalazinone analogue 46 of the lead compound 2, a similar strategy was applied starting from compound 23 via the key intermediate 44 obtained in 82 % yield in a refluxing alcoholic solution of hydrazine. The expected analogue 46 was then obtained in 26 % overall yield following a same three steps reaction sequence previously described (Scheme 5). Finally, the targeted benzophenone analogue 49 was quantitatively obtained after alkylation of the intermediate 47\textsuperscript{29} with the iodinated amidic chain 48 (Scheme 6).
Scheme 5. Synthesis of the NH-phthalazinone analogue 46.

Scheme 6. Synthesis of the benzophenone analogue 49.

2.2 Structure-activity analyses

In a previous work, we identified the first IL-15 small molecules inhibitors by a pharmacomodulation study focused on the terminal amide part of our hit 1 and the linker chain relying it to the triazole-phthalazinone main core of the molecule. Several novel compounds with IC$_{50}$ values ranging between 50 and 150 nM in cell-based experiments (p-Stat5), were identified and among them a compound 2 (n=7) homolog of the hit 1 (n=1) appeared as the first promising inhibitor candidate. Nevertheless, any of these inhibitors disclosed IL-15 selectivity, displaying the same inhibitory activity on the IL-2 protein. Based on the physicochemical properties of the exposed residues to IL-2Rβ binding site on IL-15 and IL-2 and on the docking of compound 1 to IL-15 (Fig. 1), we have pursued our SAR study towards higher IL-15 inhibitor efficiency and selectivity. Exploring the protein surfaces close to the triazole and phthalazinone moieties of compound 1 (Fig. 1A), we envisioned to graft hydrophilic substituents on chemically modulable nitrogens of both heterocycles. Besides these modifications, we also prepared a benzophenone analogue of compound 2, in order to complete our previous SAR study$^{29}$ with a bulky terminal amide fragment.
The effectiveness of the novel compounds was evaluated using the IL-15- and IL-2-dependent cell proliferation and Stat5 phosphorylation assays and, for some of them, their binding to IL-15 was determined by SPR technology.

2.2.1 In vitro inhibition of cell proliferation and Stat5 phosphorylation

First of all, similar IC\(_{50}\) values were retrieved for the reference compounds 1 and 2, as compared with those previously published\(^2\) both in the cell proliferation and p-Stat5 assays (IC\(_{50}\) = 22.5 and 4.5 \(\mu\)M for 1, respectively and IC\(_{50}\) = 2.2 and 0.19 \(\mu\)M for 2, respectively), ascertaining the good stability of the two compounds as well as of the two cell lines used (Tables 1 and 2).

In the predicted binding mode of the hit 1 (Fig. 1B), the methyl substituent of the triazole heterocycle was found to share hydrogen bond with the carbamoyl group of the residue asparagine 65 (N65) of IL-15, and the methyl substituent of the phthalazinone moiety seemed to favor hydrophobic interactions with the isoleucine 68 and leucine 69 residues of IL-15. Moreover asparagine residues (Fig 1B, residues N1, N4 and N72) were also pointing in the vicinity of the the phthalazinone moiety. To experimentally sustain the binding mode of this family of inhibitors, we chose to change the N-methyl group with hydrophilic substituents sequentially on the triazole or phthalazinone heterocycles to develop two series of analogues of compounds 1 and 2 (Fig. 2, series 1 and series 2, respectively).

The targeted analogues of hit 1 bearing the shorter chain (n = 1), uniformly gave no inhibition of cell proliferation (Table 1) as well as p-Stat5 (Table 2). One exception was the N-triazolyl substituted analogue 10, with an aldehyde residue, giving a similar inhibitor potency (IC\(_{50}\) values of 6.0 and 4.2 \(\mu\)M, respectively) than the hit 1.

As noticed in our previous SAR study,\(^2\) some analogues of lead 2 having a longer alkyl chain (n= 7), appeared among the most interesting molecules. In these novel series, introduction of an acetal group (compound 9) on the N-substituent of the triazole, a protected or not protected hydroxyl function on either of the heterocyles (compounds 19, 39 or compounds 21, 41) were found deleterious since low or no inhibitory effect was measured either in the cell proliferation and p-Stat5 assays (Tables 1 and 2). Some improvement was observed with the introduction of a carboxylic function as depicted on the triazole analogue 22 (IC\(_{50}\) = 9.5 \(\mu\)M for p-Stat5) but not for the phthalazinone analogue 43 (IC\(_{50}\) > 30 \(\mu\)M for p-Stat5). Furthermore, the dimethylaminopropyl residue gave similar results in the cell proliferation assay (IC\(_{50}\) = 2-4 \(\mu\)M) whatever grafted on the triazole (compound 20) or on the phthalazinone (compound 40) heterocycles (Table 1) albeit no inhibitory effect of these compounds was detectable in the p-
Stat5 assay (Table 2, IC_{50} > 30 μM). This discrepancy could reflect a non-specific effect of these compounds affecting the proliferation of the cells independently to Stat5 signaling pathway and consequently of IL-15 or IL-2 stimulation.

As observed with the propionaldehyde derivative 10 in series 1, the analogue 11 with a seven carbons chain appeared as the most efficient inhibitor in this series with its NH-free triazolyl analogue 12, which gave p-Stat5 inhibition of 280 nM compared to 710 nM for 11. Because this NH-free triazolyl analogue 12 derived from the propionaldehyde 11 following a retro-Michael process, some doubt remains on the biological stability of the aldehyde 11 and so its efficiency level in these bioassays. At least, compounds 11 and the NH-analogue 12 behaved similarly to compound 2 suggesting the involvement of plausible hydrogen bonds with the carbamoyl function of asparagine N65, as depicted in the binding model (Fig. 1B). Interestingly, in the N-substituted phthalazinone series 2, the NH-free analogue 46 appeared absolutely inactive in cell proliferation and Stat5 assays and 42 with the propionaldehyde chain in the range of 10 μM in both bioassays (Tables 1 and 2).

The methyl group on the phthalazinone heterocycle (series 2) of compound 2 seems to have an essential role since its substitution with more polar groups (compounds 39, 40, 41, 43) or its withdrawal (compound 46) led to compounds unable to inhibit IL-15-dependent cell proliferation as well as Stat5 phosphorylation (Table 2, IC_{50} > 30 μM). These results can strengthen the hypothetic binding model which pointed on the interaction of lipophilic residues such as isoleucine 68 and leucine 69 with the N-methyl-phthalazinone moiety.

Satisfyingly, data obtained from cell proliferation assays correlate those found in the p-Stat5 assay, highlighting that the proliferative inhibitory effects measured for IL-15 and IL-2 with these analogues resulted from the inhibition of these cytokines signaling pathway. Unfortunately, no selectivity of IL-15 versus IL-2 could be obtained with these novel series of derivatives.

Finally the substitution of the terminal 2,4-dichlorophenyl group of compound 2 with a benzophenone moiety led to the compound 49 which displayed efficiency in the same range of its parent 2 (Tables 1-2, Fig. 3). It can be classified such as the alkoxy analogues 2A and 2B or the carboxylate analogue 2C (Fig.2), among the best representatives of this family with an IC_{50} of 50 nM in the p-Stat5 bioassay with no selectivity observed versus IL-2 (IC_{50} = 80 nM). However, such compounds may be of therapeutic interest in pathologies where both cytokines are known to be involved.37
Table 1. Effect of the compounds on 32Dβ cell proliferation.

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<th>Inhibition of 32β cell proliferation</th>
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<th>RLIᵇ</th>
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Table 2. Effect of the compounds on Stat5 phosphorylation in NK-92 cells.

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\(^a\) All values are the mean of at least three independent experiments. \(^b\) RLI (100 pM). \(^c\) IL-2 (1.5 nM).
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* All values are the mean of at least three independent experiments. *b* IL-15 at 50 pM. *c* IL-2 at 250 pM.
Fig. 3. Inhibition of IL-15-dependent cell proliferation and Stat5 phosphorylation. (A-B) 32Dβ cell proliferation induced by a fixed concentration of the IL-15 agonist RLI (100pM) and increasing concentrations of indicated compounds was assessed by the Alamar Blue reduction assay. Data showed the effect of indicated compounds calculated as the percentage of the proliferation response induced by 100 pM of RLI. (C-D) Phosphorylation of Stat5 was evaluated in NK-92 cells stimulated for 1 h by a fixed concentration of IL-15 (50 pM) after a 30 min incubation with increasing concentrations of indicated compounds. Data showed the effect of indicated compounds calculated as the percentage of the p-Stat5 response induced by 50 pM of IL-15. All data are the mean ± SEM of at least 3 independent experiments.

2.2.2 Binding of selected compounds to IL-15.

To complete our study, we focussed on the binding of selected compounds 2, 11-12, 41-42, 46 and 49 to IL-15 using SPR technology. The affinity of these compounds for IL-15 was determined and compared to their pharmacological profile measured in the p-Stat5 assay (Table 3). Thus, recombinant IL-15 was covalently immobilized to sensor chips and the binding at increasing concentrations of the compounds was monitored. Satisfyingly, we could observe that the NH-free phthalazinone compound 46 lacking activity in both cell proliferation and p-Stat5 assays did not bind to IL-15 protein. However, the propanol analogue 41 with similar profile was found to be able to bind to IL-15 with a Kd value of 3.9 µM. Its behaviour must probably proceed by a different and less specific mode than that of the lead compound 2, since it was not efficient enough to impair the functional effects of the cytokine.
With the efficient $N$-Me inhibitors such as 2, the benzophenone analogue 49, and the NH-triazolyl analogue 12 the protein affinity values were good and close to 2-3 µM. The compounds bearing an aldehyde function such as 11 and 42, displayed higher Kd values (Kd = 5.9 and 7.8 µM, respectively) in agreement with their IC50 values in the p-Stat5 assay. Moreover, these data allow us to confirm that aldehyde 11 is able to bind to the IL-15 protein attesting that the observed biological results (Tables 1 and 2) do not result from its optional metabolite 12.

Table 3. IL-15 binding (Kd) of compounds 2, 11-12, 41, 42, 46 and 49 associated with their p-Stat5 inhibitory effect (IC50).^a

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<th>R1</th>
<th>R2</th>
<th>Cpd</th>
<th>Inhibition of p-Stat5 in NK-92 cells</th>
<th>Direct interaction SPR</th>
<th>IL-15 IC50 (µM)</th>
<th>IL-15 binding Kd (µM)</th>
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<td>no binding</td>
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<td>11</td>
<td>0.71 ± 0.15</td>
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3. CONCLUSION

Starting from IL-15 inhibitors identified in our first SAR study and based on docking structural data obtained with our hit 1, we have developed two novel series of potential IL-15 inhibitors with specific modifications on the N-triazole or N-phthalazinone heterocycles and we also completed our initial series. Twenty-five compounds have been prepared and tested in two functional bioassays, cell proliferation and p-Stat5 bioassay. In the novel series, the best compounds were found in the N-triazole modified family, with the nitrogen substituted with a propionaldehyde chain or free. We also identified a novel benzophenone analogue of our lead compound 2, with a very good functional activity. These compounds were confirmed as good binders of the targeted protein IL-15. Despite our efforts, we did not succeed in improving IL-15 selectivity in respect with IL-2. However, such inhibitors targeting both IL-15 and IL-2 may be of therapeutic interest in pathologies such as HAM/TSP, CTCL, LGLL and HTLV-1 driven ATL, for which these two cytokines have been shown to be involved.

4. EXPERIMENTAL SECTION

4.1 Chemistry.

General. All solvents used were reagent grade and TLC was performed on silica-covered aluminum sheets (Kieselgel 60F254, MERCK). Eluted TLC was revealed using UV radiation ($\lambda = 254$ nm), or molybdate solution. Flash column chromatography was performed on silica gel 60 ACC 40-63 $\mu$m (SDS-CarloErba). NMR spectra were recorded on an Avance I 300 MHz Bruker, on an Avance III 400 MHz Bruker and an Avance III 500 MHz Bruker 500 at room
temperature, on samples dissolved in an appropriate deuterated solvent. References of tetramethyilsilane (TMS) for $^1$H and deuterated solvent signal for $^{13}$C were used. Chemical displacement values ($\delta$) are expressed in parts per million (ppm), and coupling constants (J) in Hertz (Hz). High-Resolution Mass Spectrometry (HRMS in Da unit) analyses were recorded on a Xevo-Q-Tof Waters in the CEISAM Laboratory (AMaCC platform, Nantes) or on a LC-Q-TOF (Synapt-G2 HDMS, Waters) in the IRS-UN center (Mass Spectrometry platform, Nantes).

4.1.1. 1,1-dioethoxy-3-isothiocyanatopropane (4).
To a solution of 3,3-dioethoxypropan-1-amine (1 eq, 11 mmol, 1.78 mL) and anhydrous triethylamine (3.3 eq, 33 mmol, 5 mL) in THF (1.1 mol.L$^{-1}$) at 0 °C under argon atmosphere was added carbon disulfide (1 eq, 11 mmol, 0.66 mL) over a period of 30 min and then stirred at r.t for 2 hours. The mixture was cooled to 0 °C using an ice-bath before tosyl chloride (1.1 eq, 12.1 mmol, 2.3 g) was added in one portion, and the reaction was stirred at r.t for another 30 min. 1 N HCl (10 mL) and tert-butyl methyl ether (MTBE) (10 mL) were added to the mixture. The aqueous layer was extracted with MTBE (10 mL) and the combined organic layers dried over Na$_2$SO$_4$, filtered and concentrated. The crude oil was purified by flash column chromatography on silica gel (PE/AcOEt, 98:2 to 96:4) to afford the desired product 4 as a colorless oil (9.68 mmol, 1.83 g, 88%). HRMS (ESI$^+$): calcd for C$_8$H$_{15}$N$_2$O$_3$S [M+Na]$^+$ m/z 212.0716; found 212.0709.

4.1.2. 4-[(4-(3,3-dioethoxypropyl)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-2-methylphthalazin-1(2H)-one (5).
To a suspension of (4-(3,3-dioethoxypropyl)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (5) (1 eq, 0.82 mmol, 155 mg) followed by triethylamine (5 eq, 41.1 mmol, 5.57 mL). The mixture was refluxed overnight under argon atmosphere. After completion of the reaction, the mixture was allowed to cool to room temperature, concentrated, and directly purified by flash column chromatography on silica gel (DCM/ACOEt, 50:50) to afford the desired product 5 as a white powder (0.66 mmol, 265 mg, 80%). HRMS (ESI$^+$): calcd for C$_9$H$_{16}$N$_2$O$_3$S [M+Na]$^+$ m/z 246.1570; found 246.1566.

4.1.3. N-(2,4-dichlorophenyl)-2-[(4-(3,3-dioethoxypropyl)-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio]acetamide (8).
To a solution of thiol 5 (1 eq, 0.57 mmol, 230 mg) in DMF (0.06 mol.L⁻¹) under argon atmosphere at room temperature was added K₂CO₃ (1.5 eq, 0.86 mmol, 118 mg) and a solution of α-bromoamide 6 (1.1 eq, 0.63 mmol, 177 mg) in DMF (0.7 mol.L⁻¹) was added. The mixture was then stirred at room temperature for 1 h until completion. A saturated solution of ammonium chloride was added and the product was extracted twice with AcOEt. The organic layer was washed several times with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH, 98:2) to afford the desired compound 8 as a white powder (0.55 mmol, 318 mg, 96%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 10.13 (s, 1H, NH), 8.50-8.39 (m, 1H, H_ar-Phthal), 8.26-8.15 (m, 2H, H_ar-Phthal and H_n), 7.85-7.73 (m, 2H, 2 x H_ar-Phthal), 7.30 (d, 3J = 2.3 Hz, 1H, H_a), 7.18 (dd, 3J = 8.9 Hz, 4J = 2.3 Hz, 1H, H_a), 4.54 (s, 2H, CH₂), 4.50 (t, 3J = 4.8 Hz, 1H, CH), 4.13-4.01 (m, 4H, NCH₂ and SCH₂), 3.80 (s, 3H, NMe), 3.66-3.54 (m, 2H, 2 x OCH₂), 3.49-3.38 (m, 2H, 2 x OCH₂), 2.02-1.88 (m, 2H, NCH₂CH₂), 1.15 (t, 3J = 7.0 Hz, 6H, 2 x CH₃). ¹³C NMR (75MHz, CDCl₃): δ(ppm) 167.3 (CO), 159.5 (CO), 152.9 (CIV-C=O), 151.5 (CIV-C=O), 140.9 (CIV-C=O), 134.0 (CIV), 133.4 (C-ar-Phthal), 132.0 (Car-Phthal), 129.5 (CIV), 129.1 (CIV and Car), 128.0 (CIV), 127.5 (Car), 127.3 (Car-Phthal), 125.2 (Car-Phthal), 124.8 (CIV), 123.2 (Car), 100.1 (CH), 62.1 (2 x CH₂), 40.1 (NCH₂), 39.5 (NMe), 36.0 (SCH₂), 33.1 (NCH₂CH₂), 29.9 (CH₃), 15.3 (2 x CH₃). HRMS (ESI⁺): calcd for C₂₇H₃₁Cl₂N₅O₄S [M+Na⁺] m/z 605.1499; found: 605.1503.

4.1.4. N-(2,4-dichlorophenyl)-8-((4-(3,3-diethoxypropyl)-5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (9).

To a solution of thiol 5 (1 eq, 0.46 mmol, 187 mg) in anhydrous DMF (0.08 mol.L⁻¹) at room temperature under argon atmosphere was added K₂CO₃ (1.5 eq, 0.69 mmol, 95 mg), followed by a solution of bromoamide 7 (1.1 eq, 0.51 mmol, 188 mg) in DMF at 0 °C. The mixture was stirred for 4 h at room temperature, quenched with water and extracted with AcOEt. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The product 9 was obtained in quantitative yield without any purification. ¹H NMR (400 MHz, CDCl₃): δ(ppm) 8.43 (dd, 3J = 7.7 Hz, 4J = 1.2 Hz, 1H, H_ar-Phthal), 8.32 (d, 3J = 8.7 Hz , 1H, H_ar), 8.22 (d, 3J = 7.7 Hz, 1H, H_ar-Phthal), 7.77 (m, 2H, H_ar-Phthal), 7.60 (bs, 1H, NH), 7.36 (d, 3J = 2.35 Hz, 1H, H_ar), 7.23 (dd, 3J = 2.35 Hz, 4J = 8.88 Hz, 1H, H_ar), 4.53 (s, 2H, CH₂), 4.50 (t, 3J = 5.2 Hz, 1H, CH), 4.05 (t, 2H, NCH₂), 3.80 (s, 3H, NMe), 3.61 (m, 2H, 2 x OCH₂), 3.45 (m, 2H, 2 x OCH₂), 3.23 (t, 3J = 7.3 Hz, 2H, SCH₂), 2.40 (t, 3J = 7.30 Hz, 2H, CH₂CO), 1.93 (m, 2H, NCH₂CH₂), 1.72 (m, 4H, 2 x CH₂), 1.37 (m, 6H, 3 x CH₃), 1.17 (t, 6H, 2 x CH₃). ¹³C NMR (100 MHz, CDCl₃): δ(ppm) 171.4 (CO); 159.6 (CO), 151.9 (CIV-C=O), 151.8 (CIV-C=O), 141.1 (CIV-C=O), 133.6 (Car-Phthal), 133.4 (CIV), 131.9 (Car-Phthal), 129.2 (CIV), 128.8 (Car), 128.0 (2 x CIV), 128.0 (Car), 127.2 (Car-Phthal), 125.5 (CIV), 123.3 (CIV), 122.6 (Car), 100.2 (CH), 61.9 (2 x OCH₂), 40.1 (NCH₂), 39.5 (NMe), 37.9 (CH₂CO), 33.3 (NCH₂CH₂), 33.1 (SCH₂), 30.0 (CH₂), 29.5 (2 x CH₂), 29.0 (CH₂), 28.5 (CH₂), 28.5 (CH₂), 25.4 (CH₂), 15.4 (2 x CH₃). HRMS (ESI⁺): calcd for C₃₃H₃₉N₁₀O₄S₂ [M+H⁺] m/z 689.2444; found 689.2448.

4.1.5. N-(2,4-dichlorophenyl)-2-((5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (10).

To a solution of ketal 8 (1 eq, 0.17 mmol, 100 mg) in THF (0.04 mol.L⁻¹) at room temperature was added a 1N solution of hydrochloric acid (33% v/v). The mixture was stirred 2 h at 40 °C and quenched by addition of a saturated solution of Na₂CO₃ until pH 7. The product was extracted with DCM. The organic layers were combined and washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH, 97:3) to afford the desired compound 10 as a white powder (0.12 mmol, 64 mg, 71%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 9.97 (s, 1H, NH), 9.77 (s, 1H, CHO), 8.47-8.42 (m, 1H, H_ar-Phthal), 8.23-8.16 (m, 2H, H_ar-Phthal and H_n), 7.86-7.75 (m, 2H, H_ar-Phthal),
were rem
mixture was quenched by addition of a saturated solution of ammonium chloride. The volatiles

1H NMR (400 MHz, CDCl3): δ (ppm) 9.71 (s, 1H, CHO), 8.42-8.39 (m, 1H, Har-Phtal), 8.28 (d, J = 6.6 Hz, 1H, Har), 8.21-8.19 (m, 1H, Har-Phtal), 7.81-7.72 (m, 2H, 2 x Har-Phtal), 7.62 (s, 1H, NH), 7.33 (d, J = 2.2 Hz, 1H, Har), 7.21 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H, Har), 4.55 (s, 2H, CH2), 4.27 (t, J = 7.1 Hz, 2H, NCH2), 3.75 (s, 3H, NMe), 3.21 (t, J = 7.1 Hz, 2H, CH2S), 2.81 (t, J = 7.2 Hz, 2H, CH2CO), 2.39 (t, J = 7.4 Hz, 2H, CH2CONH), 1.77-1.65 (m, 4H, 2 x CH2), 1.42-1.34 (s, 6H, 3 x CH3). 13C NMR (100 MHz, CDCl3): δ (ppm) 197.7 (CHO), 171.2 (CONH), 159.3 (C=O), 151.7 (CIV-C-N), 151.3 (CIV-C-N), 141.0 (CIV-C-N), 133.4 (Civ), 133.3 (CHar-Phtal), 131.9 (CHar-Phtal), 128.9 (Civ), 128.6 (Civ), 127.8 (Civ), 127.7 (CHar-Phtal), 127.0 (Civ), 125.3 (CHar-Phtal), 122.5 (Civ), 42.9 (NCH2), 39.3 (NMe), 37.6 (NCH2CH2), 37.2 (SCH2), 33.0 (CH2), 29.9 (CH2), 28.9 (CH2), 28.6 (CH2), 28.3 (CH2), 25.1 (CH2). Two quaternary carbon atoms are missing. HRMS (ESI+): calcd for C_{25}H_{23}Cl_{2}N_{4}O_{3}S [M+H]^+ m/z 615.1712, found 615.1719. Compound 12: 1H NMR (400 MHz, CDCl3): δ (ppm) 8.30-8.27 (m, 1H, Har-Phtal), 8.25 (br s, 1H, NH), 7.81-7.79 (m, 1H, Har), 7.72-7.63 (m, 3H, 3 x Har-Phtal), 7.33 (d, J = 2.4 Hz, 1H, Har), 7.19 (dd, J = 8.8 Hz, J = 2.3 Hz, 1H, Har), 4.42 (s, 2H, CH2), 3.67 (s, 3H, NMe), 3.07 (t, J = 7.2 Hz, 2H, CH2S), 2.38 (t, J = 7.3 Hz, 2H, CH2CONH), 1.72-1.60 (m, 4H, 2 x CH2), 1.38-1.24 (s, 6H, 3 x CH3). 13C NMR (100 MHz, CDCl3): δ (ppm) 171.6 (CONH), 159.6 (C=O), 157.3 (CIV-C-N), 142.22 (CIV-C-N), 133.4 (Civ), 133.2 (CHar-Phtal), 131.7 (CHar-Phtal), 129.2 (Civ), 128.8 (Civ), 127.9 (Civ), 127.8 (CHar-Phtal), 127.1 (Civ), 125.1 (CHar-Phtal), 123.5 (Civ), 122.7 (Civ), 39.5 (NMe), 37.8 (SCH2), 32.7 (CH2), 31.7 (CH2), 29.5 (CH2), 28.9 (CH2), 28.7 (CH2), 28.3 (CH2), 25.3 (CH2). Two quaternary carbon atoms are missing. HRMS (ESI+): calcd for C_{26}H_{25}Cl_{2}N_{4}O_{3}S [M+H]^+ m/z 559.1450, found 559.1464.

4.1.7. N-(2,4-dichlorophenyl)-2-(((4-(3-hydroxypropyl)-5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (13).

To a solution of aldehyde 10 (1 eq, 0.15 mmol, 80 mg) in ethanol (0.03 mol.L⁻¹) at 0 °C the mixture was quenched by addition of a saturated solution of ammonium chloride. The volatiles were removed and the crude was diluted in DCM, washed with brine, dried over Na₂SO₄ and
To a suspension of hydrazide 13 (0.11 mmol, 67%). 

\[
\text{H NMR (400 MHz, DMSO-d6)}: \delta (ppm) 9.97 (s, 1H, NH), 8.31-8.27 (m, 1H, Har-Phthal), 8.05-8.01 (m, 1H, Har-Phthal), 7.92-7.83 (m, 2H, Har-Phthal), 7.79 (d, \text{^3}J = 8.8 Hz, 1H, Har), 7.63 (d, \text{^3}J = 2.4 Hz, 1H, Har), 7.39 (dd, \text{^3}J = 8.8 Hz, \text{^4}J = 2.4 Hz, 1H, Har), 4.68 (t, \text{^3}J = 4.9 Hz, 1H, OH), 4.55 (s, 2H, CH2), 4.16 (s, 2H, SCH2), 4.09 (t, \text{^3}J = 7.4 Hz, 2H, NCH2), 3.67 (s, 3H, NMe), 3.45-3.39 (m, 2H, CH2OH), 1.82-1.74 (m, 2H, NCH2CH2). \] 

\[ ^{13}C \text{ NMR (100 MHz, DMSO-d6)}: \delta (ppm) 166.6 (C=O), 158.3 (C=O), 152.7 (C_{IV-C-N}), 149.2 (C_{IV-C-N}), 141.3 (C_{IV-C-N}), 133.8 (CIV), 133.1 (CHar-Phthal), 131.8 (CHar-Phthal), 129.3 (CIV), 128.9 (CHa), 128.8 (CIV), 127.6 (CHa), 127.1 (CIV), 126.5 (CIV), 126.0 (CHar and CHar-Phthal), 125.7 (CHar-Phthal), 57.5 (CH2OH), 41.2 (NCH2), 38.8 (NMe), 36.8 (SCH2), 32.1 (NCH2CH2), 28.6 (CH2). \] 

HRMS (ESI\(^{+}\)) calculated for C\(_{23}\)H\(_{23}\)O\(_3\)N\(_6\)Cl\(_3\)S \([M+H]^+\) \(m/z\): 533.0924, found 533.0919.

4.1.8. 3-(3-(2,4-dichlorophenyl)amino)-2-oxoethyl)thio)-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-4(3H)propanoic acid (14).

To a solution of aldehyde 10 (1 eq, 0.15 mmol, 80 mg) in a mixture of tert-butanol/water (3:1, 0.02 mol L\(^{-1}\)) at room temperature was added 2-methyl-2-butenone (50 eq, 7.5 mmol, 0.8 mL), followed by Na\(_2\)PO\(_4\) dihydrate (10 eq, 1.5 mmol, 235 mg). The mixture was stirred 20 min. before sodium chlorite (4.5 eq, 0.68 mmol, 61 mg) was added. The mixture was stirred 2 h. at room temperature and ethyl acetate was added followed by an aqueous 1N HCl solution. The aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with brine, dried over Na\(_2\)SO\(_4\) and concentrated to obtain the desired compound 14 as a white solid (0.09 mmol, 49 mg, 60%). 

\[ ^{1}H \text{ NMR (400 MHz, DMSO-d6)}: \delta (ppm) 9.97 (s, 1H, NH), 8.31-8.26 (m, 1H, Har-Phthal), 8.05-8.00 (m, 1H, Har-Phthal), 7.91-7.82 (m, 2H, Har-Phthal), 7.75 (d, \text{^3}J = 8.8 Hz, 1H, Har), 7.62 (d, \text{^3}J = 2.4 Hz, 1H, Har), 7.38 (dd, \text{^3}J = 8.8 Hz, \text{^4}J = 2.4 Hz, 1H, Har), 4.60 (s, 2H, CH2), 4.26 (t, \text{^3}J = 7.3 Hz, 2H, NCH2), 4.14 (s, 2H, SCH2), 3.65 (s, 3H, NMe), 2.70 (t, \text{^3}J = 7.3 Hz, 2H, NCH2CH2). \]

\[ ^{13}C \text{ NMR (100 MHz, DMSO-d6)}: \delta (ppm) 171.8 (C=O), 166.6 (C=O), 158.4 (C=O), 152.7 (C_{IV-C-N}), 149.2 (C_{IV-C-N}), 141.4 (C_{IV-C-N}), 133.8 (CIV), 131.1 (CHar-Phthal), 131.9 (CHar-Phthal), 129.4 (CIV), 128.9 (CHa), 128.8 (CIV), 127.7 (CHa), 127.2 (CIV), 126.7 (CIV), 126.3 (CHa), 126.1 (CHar-Phthal), 125.7 (CHar-Phthal), 40.2 (NCH2), 38.7 (NMe), 37.2 (SCH2), 33.6 (NCH2CH2), 28.7 (CH2). \] 

HRMS (ESI\(^{+}\)) calculated for C\(_{23}\)H\(_{23}\)O\(_3\)N\(_6\)Cl\(_3\)SNa \([M+Na]^+\) \(m/z\): 569.0536, found 569.0531.

4.1.9. 4-((4-(3-tert-butyldimethylsilyloxy)propyl)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (17).

To a suspension of hydrazide 3 (1 eq, 1.29 mmol, 300 mg) in EtOH (0.065 mol L\(^{-1}\)) was added 15 (1.28 eq, 1.64 mmol, 380 mg) and Et3N (4.5 eq, 5.81 mmol, 0.81 mL). The resulting mixture was heated at reflux for 14 h. The resulting solution was concentrated, the residue was solubilized in AcOEt and washed with brine. The organic layer was dried over Na\(_2\)SO\(_4\), filtered and concentrated. The crude product was purified by column chromatography on silica gel (cyclohexane/acetone, 50:50) to afford the thiol 17 as a white solid (1.01 mmol, 450 mg, 80%).

\[ ^{1}H \text{ NMR (300 MHz, CDCl3)}: \delta (ppm) 8.54-8.43 (m, 1H, Har-Phthal), 7.90-7.72 (m, 3H, 3 x Har-Phthal), 4.42 (s, 2H, CH2), 4.23 (t, \text{^3}J = 7.1 Hz, 2H, NCH2), 3.80 (s, 3H, NMe), 3.68 (t, \text{^3}J = 5.5 Hz, 2H, CH2O), 2.05 (m, 2H, NCH2CH2), 0.83 (s, 9H, 3 x CH3-Bu), 0.04 (s, 6H, 2 x SiCH3). \]

\[ ^{13}C \text{ NMR (75 MHz, CDCl3)}: \delta (ppm) 167.8 (C_{IV-C-N}), 159.5 (C=O), 139.9 (2 x C_{IV-C-N}), 133.3 (CHar-Phthal), 132.0 (CHar-Phthal), 128.9 (CIV), 128.1 (CIV), 127.6 (CHar-Phthal), 124.5 (CHar-Phthal), 59.7 (CH2O), 41.8 (NCH2), 39.6 (NMe), 30.7 (NCH2CH2), 29.4 (CH2), 25.9 (3 x CH3-Bu), 18.3 (CIV), -5.2 (2 x SiCH3). \] 

HRMS (ESI\(^{+}\)) calculated for C\(_{31}\)H\(_{31}\)N\(_{6}\)O\(_4\)SSi \([M+H]^+\) \(m/z\): 446.2036; found 446.2046.
4.1.10. 4-((4-(3-(dimethylamino)propyl)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (18).

To a suspension of hydrazide 3 (1 eq, 0.41 mmol, 95.5 mg) in EtOH (0.41 mol·L⁻¹) was added 16 (1.02 eq, 0.457 mmol, 65.9 mg) and Et₃N (4.5 eq, 1.85 mmol, 0.26 mL). The resulting mixture was heated to reflux 30 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue (brown solid) was solubilized in DCM, washed with brine and the organic layer was dried over Na₂SO₄, filtered and concentrated. The crude was purified by column chromatography on silica gel (DCM/MeOH-Et₂N (5%), 85:15) to afford the corresponding thiol 18 as a brown sticky solid (0.17 mmol, 60.0 mg, 54%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 8.60–8.37 (m, 1H, Har-Phthal), 7.99–7.60 (m, 3H, 3 x Har-Phthal), 4.47 (s, 2H, CH₂), 4.22–4.04 (m, 2H, NCH₂), 3.80 (s, 3H, NMe), 2.88 (s, 1H, SH), 2.33 (t, J = 6.7 Hz, 2H, CH₂(NMe₂)), 2.22 (s, 6H, NMe₂), 2.00 (p, J = 6.9 Hz, 2H, CH₂), ¹³C NMR (75 MHz, CDCl₃): δ(ppm) 167.9 (C=O), 149.2 (CIV-C-N), 140.1 (CIV-C-N), 133.3 (ChAr-Phthal), 132.0 (ChAr-Phthal), 128.9 (CIV), 128.0 (ChAr-Phthal/CIV), 127.5 (ChAr-Phthal/CIV), 124.6 (ChAr-Phthal/CIV), 55.9 (CH₂(NMe₂)), 46.0 (NMe), 45.1 (NMe), 42.6 (NCH₂), 39.6 (NMe₂), 29.8 (CH₂), 25.6 (CH₂). HRMS (ESI⁺): calcd for C₁₇H₂₂N₆O₃ [M+H⁺] m/z 359.1644; found 359.1654.

4.1.11. 8-((4-(3-(tert-butyl(dimethyl)silyl)oxy)propyl)-5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-((2,4-dichlorophenyl)octanamide (19).

To a suspension of mercapto-triazole 17 (1 eq, 0.65 mmol, 291 mg) was added bromoamide 7 (1.2 eq, 0.78 mmol, 287 mg) and K₂CO₃ (1.7 eq, 1.11 mmol, 153 mg) in DMF (0.065 mol·L⁻¹). After stirring 24 h at room temperature, the mixture was quenched with a saturated aqueous NH₄Cl solution. Brine was added and the product was extracted with DCM. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting yellow syrup was purified by column chromatography on silica gel (DCM/acetone, 50:50) to afford the product 19 as a colorless oil (0.48 mmol, 350 mg, 73%). ¹H NMR (500 MHz, CDCl₃): δ(ppm) 8.44 (dd, J = 7.9, 1.0 Hz, 1H, Har-Phthal), 8.34 (q, J = 9.0 Hz, 1H, Har), 8.24 (d, J = 7.5 Hz, 1H, Har-Phthal), 7.83–7.76 (m, 1H, Har-Phthal), 7.77–7.73 (m, 1H, Har-Phthal), 7.59 (s, 1H, NH), 7.36 (d, J = 2.4 Hz, 1H, Har), 7.24 (dd, J = 8.9, 2.4 Hz, 1H, Har), 4.51 (s, 2H, CH₂), 4.09 (t, J = 7.4 Hz, 2H, NCH₂), 3.79 (s, 3H, NMe), 3.62 (t, J = 5.6 Hz, 2H, CH₂O), 3.22 (d, J = 7.4 Hz, 2H, CH₂S), 2.40 (t, J = 7.5 Hz, 2H, CH₂CONH), 1.84–1.78 (m, 2H, CH₂), 1.77–1.68 (m, 4H, 2 x CH₂), 1.47–1.32 (m, 6H, 3 x CH₂), 0.88 (s, 9H, 3 x CH₃-tbut), 0.06 (s, 6H, 2 x SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ(ppm) 171.4 (CONH), 159.6 (C=O), 152.0 (CIV-C-N), 151.6 (CIV-C-N), 141.4 (CIV-Phthal), 133.6 (CIV-NHCO), 133.4 (ChAr-Phthal), 131.8 (ChAr-Phthal), 129.3 (CIV), 129.1 (CIV-C), 128.8 (ChAr), 128.1 (CIV), 128.0 (ChAr), 127.1 (ChAr-Phthal), 125.6 (ChAr-Phthal), 123.3 (CIV-C), 122.5 (ChAr), 59.5 (CH₂O), 41.4 (NCH₂), 39.4 (NMe), 37.9 (CH₂CONH), 33.0 (CH₂S), 32.7 (CH₂), 29.8 (CH₂), 29.5 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 28.5 (CH₂), 26.0 (3 x CH₃-tbut), 25.4 (CH₂), 18.3 (CIV), -5.2 (2 x SiCH₃). HRMS (ESI⁺): calcd for C₉₂H₉₀Cl₂N₁₂O₂S₆Si [M+H⁺]⁺ m/z 731.2734; found 731.2733.

4.1.12. N-(2,4-dichlorophenyl)-8-((4-(3-(dimethylamino)propyl)-5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (20).

To a suspension of mercapto-triazole 18 (1 eq, 0.446 mmol, 160 mg) was added bromoamide 7 (1.8 eq, 0.803 mmol, 295 mg) and K₂CO₃ (2 eq, 0.892 mmol, 123 mg) in DMF until dissolution. After stirring at room temperature to complete conversion, the resulting clear yellow solution was quenched with a saturated aqueous NH₄Cl solution. AcOEt was added and the organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/MeOH-Et₂N (5%), 90:10) to
afford the product 20 as a white solid (0.20 mmol, 288 mg, 45%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$(ppm) 8.43 (m, 1H, Har-Phthal), 8.33 (d, $J$ = 8.9 Hz, 1H, Har), 8.28–8.17 (m, 1H, Har-Phthal), 7.84–7.69 (m, 2H, 2 x Har-Phthal), 7.60 (s, 1H, NH), 7.36 (d, $J$ = 2.4 Hz, 1H, Har), 7.23 (m, 1H, Har), 4.53 (s, 2H, CH$_2$), 3.99 (m, 2H, NCH$_2$), 3.81 (s, 3H, NMe), 3.22 (m, 2H, CH$_2$S), 2.40 (t, $J$ = 7.5 Hz, 2H, CH$_2$CONH), 2.23 (t, $J$ = 6.7 Hz, 2H, CH$_2$NMe$_2$), 2.18 (s, 6H, 3 x CH$_2$), 1.73 (m, 6H, 3 x CH$_2$), 1.39 (m, 6H, 3 x CH$_2$). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$(ppm) 171.4 (CONH), 151.9 (C(IV-C=N)), 151.8 (C(IV-C=N)), 141.4 (C(IV-C=N)), 133.5 (C(IV-NH)), 133.4 (ChAr-Phthal), 131.9 (ChAr-Phthal), 129.2 (ChIV-Phthal), 129.0 (ChIV-C), 128.8 (Char), 128.0 (ChIV-Phthal), 128.0 (Ch$_2$), 127.1 (ChIV-Phthal), 125.6 (ChIV-Phthal), 123.2 (ChIV-C), 122.5 (Char), 56.1 (CH$_2$NMe$_2$), 45.4 (NMe$_2$), 42.3 (NCH$_2$), 39.5 (NMe$_2$), 37.9 (CH$_2$CONH), 33.1 (CH$_2$S), 30.2 (CH$_2$), 29.5 (CH$_2$), 29.1 (CH$_2$), 28.8 (CH$_2$), 28.5 (CH$_2$), 27.6 (CH$_2$), 25.4 (CH$_2$). HRMS (ESI$^+$): calcd for C$_{31}$H$_{39}$Cl$_2$N$_2$O$_5$S [M+H]$^+$ m/z 644.2339; found 644.2341.

4.1.13. N-(2,4-dichlorophenyl)-8-((4-(3-hydroxypropyl)-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H,1,2,4-triazol-3-yl)thio)octanamide (21).

To a solution of the silylated alcohol 19 (1 eq, 0.219 mmol, 160 mg) in distilled THF (0.015 mol.L$^{-1}$) was carefully added a solution of HF 70 wt % in pyridine (75 eq, 16.40 mmol). The resulting colorless solution was stirred 16 h at room temperature. The reaction mixture was quenched with saturated aqueous NaHCO$_3$ solution and extracted with AcOEt. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/MeOH, 95:5) to afford the corresponding alcohol 21 as a white amorphous solid (0.19 mmol, 120 mg, 88%).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$(ppm) 8.48–8.39 (m, 1H, Har-Phthal), 8.33 (d, $J$ = 8.9 Hz, 1H, Har), 8.25 (d, $J$ = 7.5 Hz, 1H, Har-Phthal), 7.83–7.73 (m, 2H, 2 x Har-Phthal), 7.59 (s, 1H, NH), 7.37 (d, $J$ = 2.4 Hz, 1H, Har), 7.22 (m, 1H, Har), 4.52 (s, 2H, CH$_2$), 4.11 (t, $J$ = 7.3 Hz, 2H, NCH$_2$), 3.81 (s, 3H, NMe), 3.71–3.60 (m, 2H, CH$_2$OH), 3.23 (t, $J$ = 7.3 Hz, 2H, CH$_2$S), 2.41 (t, $J$ = 7.5 Hz, 2H, CH$_2$CONH), 1.81 (m, 6H, CH$_2$), 1.37 (s, 6H, CH$_2$). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$(ppm) 159.6 (C=O), 151.9 (C(IV-C=N)), 151.8 (C(IV-C=N)), 141.4 (C(IV-C=N)), 133.5 (C(IV-NH)), 133.4 (ChAr-Phthal), 131.9 (ChAr-Phthal), 129.2 (ChIV-Phthal), 129.0 (ChIV-C), 128.8 (Char), 128.0 (ChIV-Phthal), 128.0 (Ch$_2$), 127.1 (ChIV-Phthal), 125.6 (ChIV-Phthal), 123.2 (ChIV-C), 122.5 (Char), 56.1 (CH$_2$NMe$_2$), 45.4 (NMe$_2$), 42.3 (NCH$_2$), 39.5 (NMe$_2$), 37.9 (CH$_2$CONH), 33.1 (CH$_2$S), 30.2 (CH$_2$), 29.5 (CH$_2$), 29.0 (CH$_2$), 28.7 (CH$_2$), 28.4 (CH$_2$), 25.3 (CH$_2$). HRMS (ESI$^+$): calcd for C$_{25}$H$_{33}$Cl$_2$N$_2$O$_5$S [M+H]$^+$ m/z 617.1868; found 617.1868.

4.1.14. 3-(3-((8-((2,4-dichlorophenyl)amino)-8-oxooctyl)thio)-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H,1,2,4-triazol-4-yl)pipropanoic acid (22).

TEMPO (0.26 eq, 0.074 mmol, 11.64 mg) and BAIB (2.4 eq, 0.688 mmol, 221 mg) were added to a solution of alcohol 21 (1 eq, 0.286 mmol, 177 mg) in DCM/H$_2$O (1:1 mixture; 0.009 mol.L$^{-1}$) under inert atmosphere. After completion the mixture was quenched with aqueous solution of Na$_2$SO$_4$ (10%) and extracted with AcOEt. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/MeOH/AcOH (3%), 90:10) to afford the product 22 as a white syrup (0.190 mmol, 120 mg, 66%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$(ppm) 8.43 (d, $J$ = 7.0 Hz, 1H, Har-Phthal), 8.31 (d, $J$ = 8.7 Hz, 1H, Har), 8.18 (d, $J$ = 7.5 Hz, 1H, Har-Phthal), 7.89–7.68 (m, 2H, 2 x Har-Phthal), 7.63 (s, 1H, NH), 7.37 (d, $J$ = 2.4 Hz, 1H, Har), 7.24–7.21 (m, 1H, Har), 4.60 (s, 2H, CH$_2$), 4.27 (t, $J$ = 7.2 Hz, 2H, NCH$_2$), 3.78 (s, 3H, NMe), 3.25 (t, $J$ = 7.2 Hz, 2H, CH$_2$S), 2.72 (t, $J$ = 7.2 Hz, 2H, CH$_2$COOH), 2.41 (t, $J$ = 7.6 Hz, 2H, CH$_2$CONH), 1.82–1.59 (m, 4H, 2 x CH$_2$), 1.45–1.30 (m, 6H, 3 x CH$_2$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$(ppm) 172.9 (COOH), 171.7 (CONH), 159.7 (C=O), 152.2 (C(IV-C=N)), 151.7 (C(IV-C=N)), 141.4 (Ch$_2$), 133.5 (C(IV-NHCO)), 133.4 (ChAr-Phthal), 131.9 (ChAr-Phthal), 129.2 (ChIV-C), 129.1 (Ch$_2$), 128.8 (Ch$_2$),
128.1 (C\text{iv}), 128.0 (CH\text{ar}), 127.2 (CH\text{ar}), 125.4 (CH\text{ar-Phthal}), 123.5 (C\text{iv-Cl}), 122.8 (CH\text{ar}), 40.2 (NCH\text{3}), 39.5 (NMe), 37.9 (CH\text{2CONH}), 34.1 (CH\text{2COOH}), 33.3 (CH\text{2S}), 29.8 (CH\text{2}), 29.4 (CH\text{2}), 28.9 (CH\text{2}), 28.7 (CH\text{2}), 28.4 (CH\text{2}), 25.3 (CH\text{2}). HRMS (ESI\text{+}): calculated for C\text{59}H\text{51}N\text{3}O\text{8}S\text{2} [M+H]\text{+} m/z 631.1660; found 631.1661.

4.1.15. Ethyl 2-[(2-(1,3-dioxolan-2-yl)ethyl)-4-oxo-3,4-dihydrophthalazin-1-yl]acetate (26).

To a solution of ester 24 (1 eq, 8.6 mmol, 2 g) in DMF at 40 °C was added K\text{2}CO\text{3} (2 eq, 17 mmol, 2.3 g) under inert atmosphere. The mixture was stirred for 4 h. After completion of the reaction, the solution was allowed to cool to room temperature and the precipitate was filtered, washed with diethyl ether and extracted twice with DCM. The organic layer was washed with brine, dried over Na\text{2}SO\text{4}, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/isopropanol, 100:0 to 95:5) to afford the desired compound 26 as a yellow solid (6.41 mmol, 2.13 g, 75%). \text{1} H NMR (300 MHz, CDCl\text{3}): \text{δ} (ppm) 8.46 (dd, \text{J} = 6.6 Hz, \text{J} = 1.7 Hz, 1H, H\text{ar-Phthal}), 7.83-7.71 (m, 2H, H\text{ar-Phthal}), 7.68 (dd, \text{J} = 6.6 Hz, \text{J} = 1.7 Hz, 1H, H\text{ar-Phthal}), 5.03 (t, \text{J} = 4.6 Hz, 1H, CH), 4.37 (t, \text{J} = 7.3 Hz, 2H, NCH\text{2}], 4.18 (q, \text{J} = 7.1 Hz, 2H, CH\text{2}], 4.01-3.82 (m, 6H, CH\text{2}, 2 x CH\text{2-diox}], 2.27-2.18 (m, 2H, NCH\text{2}], 1.23 (t, \text{J} = 7.1 Hz, 3H, CH\text{3}), 1.20 (J = 6.6 Hz, 2H, NCH\text{2}], 102.8 (CH), 65.1 (2 x CH\text{2}], 45.6 (NCH\text{2}], 39.2 (CH\text{2}], 32.8 (NCH\text{2}], 14.3 (CH\text{3}). HRMS (ESI\text{+}) calculated for C\text{17}H\text{21}N\text{5}O\text{5} [M+Na]\text{+} m/z 333.1445; found 333.1447.

4.1.16. 2-[(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl]phthalazin-1(2H)-one (27).

To a solution of ester 26 (1 eq, 1.1 mmol, 362 mg) in ethanol (0.4 mol.L\text{−1}) at room temperature was slowly added hydrazine monohydrate (6 eq, 6.54 mmol, 320 μL). The mixture was refluxed for 4 h. After completion of the reaction, the solution was allowed to cool to room temperature and the precipitate was filtered, washed with diethyl ether and dried. The white solid hydrazide was used in the next step without further purification. \text{1} H NMR (300 MHz, DMSO-d\text{6}): \text{δ} (ppm) 9.34 (s, 1H, NH); 8.28 (d, \text{J} = 7.6 Hz, 1H, H\text{ar-Phthal}], 7.96-7.80 (m, 3H, H\text{ar-Phthal}], 4.93 (t, \text{J} = 4.5 Hz, 1H, CH), 4.40-4.10 (m, 4H, NCH\text{2}], NH\text{2}), 3.94-3.73 (m, 6H, 3 x CH\text{2}], 2.11-2.00 (m, 2H, NCH\text{2}], CH\text{2}), HRMS (ESI\text{+}) calculated for C\text{15}H\text{16}N\text{2}NaO\text{2} [M+Na]\text{+} m/z 319.1401; found 319.1402. To a suspension of the hydrazide previously obtained (1 eq, 0.98 mmol, 312 mg) in ethanol (0.33 mol.L\text{−1}) at room temperature under inert atmosphere were added successively methylisothiocyanate (5 eq, 4.9 mmol, 395 μL) followed by triethylamine (5 eq, 4.9 mmol, 689 μL). The mixture was refluxed overnight under argon atmosphere. After completion of the reaction, the solution was allowed to cool to room temperature and the precipitate was filtered, washed with diethyl ether and dried. The thiol 27 was obtained as a solid and used without further purification (0.87 mmol, 326 mg, 89%). \text{1} H NMR (300 MHz, DMSO-d\text{6}): \text{δ} (ppm) 13.56 (bs, 1H, SH), 8.30 (dd, \text{J} = 1.3 Hz, \text{J} = 7.6 Hz, 1H, H\text{ar-Phthal}], 8.07-8.00 (m, 1H, H\text{ar-Phthal}], 7.99-7.85 (m, 2H, H\text{ar-Phthal}], 4.86 (t, \text{J} = 4.5 Hz, 1H, CH), 4.52 (s, 2H, CH\text{2}], 4.17 (t, \text{J} = 7.3 Hz, 2H, NCH\text{2}], 3.91-3.71 (2m, 4H, 2 x CH\text{2}], 3.46 (s, 3H, NMe), 2.06-1.95 (m, 2H, NCH\text{2}], CH\text{2}). \text{1}C NMR (75MHz, DMSO-d\text{6}): \text{δ} (ppm) 167.0 (C \text{N=CH}], 158.0 (C=O), 149.5 (C \text{C=C}], 140.5 (C \text{C=CH}], 133.2 (C \text{ar-Phthal}], 131.9 (C \text{ar-Phthal}], 128.5 (C \text{iv}], 127.2 (C \text{iv}], 126.2 (C \text{ar-Phthal}], 125.4 (C \text{ar-Phthal}], 101.7 (CH], 64.3 (2 x CH\text{2-diox)], 45.6 (NCH\text{2}], 32.1 (NCH\text{2}], 30.1 (NMe), 28.9 (CH\text{2}). HRMS (ESI\text{+}) calculated for C\text{17}H\text{19}N\text{2}NaO\text{2}S [M+Na]\text{+} m/z 396.1101; found 396.1091.
To a solution of thiol 27 (1 eq, 0.83 mmol, 310 mg) in DMF (0.06 mol L⁻¹) under argon atmosphere was added K₂CO₃ (1.5 eq, 1.24 mmol, 172 mg). The mixture was stirred for 15 min. at room temperature before a solution of α-bromoamide 6 (1.1 eq, 0.91 mmol, 258 mg) in DMF (0.91 mol L⁻¹) was added. After completion of the reaction, a saturated solution of NH₄Cl was added. The mixture was extracted twice with AcOEt. The organic layer was washed with brine, dried over MgSO₄ and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH, 97:3) to afford the desired product 28 as a white powder (0.74 mmol, 425 mg, 89%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 10.08 (s, 1H, NH), 8.47-8.41 (m, 1H, Hα-NH), 8.26-8.18 (m, 2H, Hα and Hβ-NH), 7.85-7.73 (m, 2H, Hγ-NH), 7.31 (d, J= 2.4 Hz, 1H, Hβ-NH), 7.19 (dd, J= 8.9 Hz, J= 2.4 Hz, 1H, Hα-NH), 4.97 (t, J= 7.3 Hz, 2H, NCH₂), 4.05 (s, 2H, SCH₂), 3.97-3.79 (m, 4H, OCH₂CH₂), 3.56 (s, 3H, NMe), 2.21-2.13 (m, 2H, CH₂) ¹³C NMR (75 MHz, CDCl₃): δ(ppm) 167.2 (CONH), 159.1 (CONPhthal), 152.9 (CIV-C-N), 152.1 (CIV-C-N), 140.5 (CIV-C-N), 134.0 (CIV), 135.5 (CHα-NPhthal), 132.0 (CHα-NPhthal), 129.5 (CIV), 129.1 (CHβ), 128.9 (CIV), 128.2 (CIV), 127.6 (CHα), 127.5 (CHα), 125.1 (CHβ), 124.7 (CIV), 123.2 (CHα), 102.6 (C-O), 65.1 (2 x CH₂O), 46.5 (NCH₂), 36.0 (SCH₂), 32.9 (CH₂), 30.9 (NMe), 30.2 (CH₂). HRMS (ESI⁺) calcd for C₂₃H₂₄O₄NCl₂NaS [M+Na⁺] m/z 597.0855, found: 597.0882.

4.1.18. N-(2,4-dichlorophenyl)-2-((4-methyl-5-((4-oxo-3-(3-oxopropyl)-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (29).

To a solution of ketal 28 (1 eq, 0.18 mmol, 105 mg) in acetone/water mixture (9:1, 0.01 mmol L⁻¹) at room temperature was added para-toluene sulfonic acid (10 eq). The mixture was stirred overnight at 60°C. After completion of the reaction, the mixture was quenched by addition of a saturated aqueous NaHCO₃ solution. The product was extracted with DCM and the organic layers were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH, 97:3) to afford the desired aldehyde 29 (0.15 mmol, 77 mg, 81%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 10.04 (s, 1H, NH), 9.77 (t, J= 1.5 Hz, 1H, CHO), 8.45-8.40 (m, 1H, Hα-NPhthal), 8.24 (d, J= 8.9 Hz, 1H, Hα-NPhthal), 8.21-8.16 (m, 1H, Hβ-NPhthal), 7.87-7.76 (m, 2H, Hγ-NPhthal), 7.31 (d, J= 2.4 Hz, 1H, Hβ-NPhthal), 7.19 (dd, J= 8.9 Hz, J= 2.4 Hz, 1H, Hα-NPhthal), 4.53 (t, J= 6.4 Hz, 2H, NCH₂), 4.48 (s, 2H, CH₂), 4.05 (s, 2H, SCH₂), 3.53 (s, 3H, NMe), 2.91 (td, J= 1.5 Hz, J= 6.4 Hz, 2H, CH₂COH). ¹³C NMR (75 MHz, CDCl₃): δ(ppm) 199.9 (CONH), 167.1 (CONH), 159.2 (CONPhthal), 152.6 (CIV-C-N), 152.1 (CIV-C-N), 141.0 (CIV-C-N), 134.0 (CIV), 133.8 (CHα-NPhthal), 132.3 (CHα-NPhthal), 129.5 (CIV), 129.1 (CHβ), 128.8 (CIV), 128.0 (CIV), 127.6 (CHα), 127.5 (CHβ), 125.2 (CHα), 124.7 (CIV), 123.2 (CHα), 45.0 (NCH₂), 42.4 (CH₂COH), 36.1 (SCH₂), 30.8 (NMe), 30.0 (CH₂). HRMS (ESI⁺): calcd for C₂₃H₂₀Cl₂N₂O₄S Na⁺ [M+Na⁺] m/z 553.0592, found: 553.0610.

4.1.19. N-(2,4-dichlorophenyl)-2-((5-((3-oxo-3-propyl)-4-oxo-3,4-dihydropthalazin-1-ylmethyl)-3-methyl-4H-1,2,4-triazol-3-yl)thio)acetamide (30).

To a solution of the aldehyde 29 (1 eq, 0.15 mmol, 80 mg) in ethanol at 0°C was added sodium borohydride (2eq., 0.3 mmol, 12 mg). After stirring one hour at 0°C, the mixture was quenched by addition of a saturated aqueous NH₄Cl solution. The volatiles were removed and the crude was diluted in DCM, washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH, 100:0 to 97:3) to afford the desired alcohol 30 as a white powder (0.12 mmol, 64 mg, 80%). ¹H NMR (300 MHz, CDCl₃): δ(ppm) 10.02 (s, 1H, NH), 8.48-8.42 (m, 1H, Hα-NPhthal), 8.28 (m, 2H, Hα and Hβ-NPhthal), 7.89-7.76 (m, 2H, Hγ-NPhthal), 7.30 (d, J= 2.3 Hz, 1H, Hβ-NPhthal), 7.19 (dd, J= 8.9 Hz, J= 2.3 Hz, 1H, Hα-NPhthal), 4.52 (s, 2H, CH₂), 4.35 (t, J= 6.1 Hz, 2H, NCH₂), 4.05 (s, 2H, SCH₂), 3.60-3.51 (m, 5H, NMe and CH₂OH), 1.98 (m, 2H, CH₂). ¹³C NMR (100
To a solution of 4.1.22. E
C
45.0 (NMe
2
(CONH), 159.1 (CON
2
2.22 (s, 6H, NMe
Phthal
4.1.21. N-(2,4-dichlorophenyl)-2-((5-((3-(dimethylamino)propyl)-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)acetamide (32).

To a solution of aldehyde 29 (1 eq, 0.15 mmol, 80 mg) in MeOH (2 mL) at room temperature was added dimethylamine (1.1 eq, 0.16 mmol, 21 μL) followed by AcOH (1 eq.). The mixture was stirred at room temperature for 40 min before NaBH(OAc)₃ (1.2 eq, 0.18 mmol, 40 mg) was added. After completion, the solution was quenched with saturated aqueous NaHCO₃ solution and extracted with DCM, washed with brine, dried over Na₂SO₄ and concentrated. The crude was diluted with Et₂O, filtered and concentrated to afford the product 32 as a white powder (0.05 mmol, 28 mg, 33%). 1H NMR (300 MHz, CDCl₃): δ (ppm) 10.02 (s, 1 H, NH), 8.39 (d, J = 7.9 Hz, 1 H, Har-Phthal), 8.18-8.14 (m, 2H, Har and Har-Phthal), 7.78-7.73 (m, 2H, Har-Phthal), 7.27-7.25 (m, 1H, Har), 7.14 (dd, 1 H, J = 8.8, J = 2.1 Hz, Har), 4.47 (s, 2H, CH₂), 4.18 (t, 2H, J = 7.3 Hz, CH₂NMe₂), 4.01 (s, 2H, SCH₂), 3.54 (s, 3H, NMe), 2.41-2.36 (m, 2H, CH₂), 2.22 (s, 6H, NMe₂), 1.98-1.91 (m, 2H, CH₂). 13C NMR (75 MHz, CDCl₃): δ (ppm) 167.1 (CONH), 159.1 (CONPhthal), 152.8 (CIV-C-N), 151.9 (CIV-C-N), 140.7 (CIV-C-N), 133.9 (CIV), 133.5 (CH₂Phthal), 132.0 (CH₂Phthal), 129.4 (CIV), 129.0 (CH₃), 128.7 (CIV), 128.1 (CIV), 127.5 (CH₃), 127.3 (CH₂Phthal), 125.1 (CH₂Phthal), 124.6 (CIV), 123.1 (CH₃), 56.5 (CH₂NMe₂), 49.3 (CH₂N), 45.0 (NMe₂), 36.0 (SCH₂), 30.8 (NMe), 30.0 (CH₃), 26.5 (CH₂). HRMS (ESI⁺) calced for C₅₂H₃₅N₅O₅Na [M+Na]⁺ m/z 868.2450, found: 868.2448.

4.1.22. Ethyl2-(3-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-oxo-3,4-dihydrophthalazin-1-yl)acetate (35).

To a solution of 24 (1 eq, 5 mmol, 1.16g) in DMF (30 mL) was added NaH (1.5 eq, 0.3 g of a 60% suspension in oil) under argon, and after gas completion the bromoalkyne 33 (2 eq, 10 mmol, 2.53 g) in DMF solution (2 mL) was added. The mixture was stirred at 40°C during
48 h. then quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (cyclohexane/AcOEt, 100:0 to 70:30) to afford the desired ester 35 as an oil (2.95 mmol, 1.2 g, 60%). 1H NMR (300 MHz, CDCl3): δ (ppm) 8.48-8.45 (m, 1H, Har-Phthal), 7.81-7.73 (m, 2H, Har-Phthal), 7.69-7.67 (m, 1H, Har-Phthal), 4.30 (t, J = 7.2 Hz, 2H, NCH2), 4.18 (q, J = 7.1 Hz, 2H, CH2OEt), 3.96 (s, 2H, CH2CO2Et), 3.73 (t, J = 6.3 Hz, 2H, CH2OSi), 2.11-2.02 (m, 2H, CH3CH2), 1.24 (t, J = 7.1 Hz, 3H, CH3), 0.89 (s, 9H, 3 x CH3-tBu), -0.04 (s, 4H, 2 x SiCH3). 13C NMR (75 MHz, CDCl3): δ (ppm) 169.8 (C=O), 159.2 (C=O), 140.1 (Civ,C=N), 132.9 (CHar-Phthal), 131.3 (CHar-Phthal), 129.2 (Civ), 128.1 (Civ), 127.3 (CHar-Phthal), 124.3 (CHar-Phthal), 61.4 (CH2CH2), 60.71 (CH2O), 48.6 (NCH2), 39.0 (CH2), 31.6 (NCH2CH2), 25.9 (3 x CH3-tBu), 18.3 (Civ), 14.1 (CH3), -5.4 (2 x SiCH3). HRMS (ESI+) calcd for C21H33N2O4Si [M+H]+ m/z 405.2210, found 405.2199.

4.1.23. Ethyl 2-(3-(dimethylamino)propyl)-4-oxo-3,4-dihydropthalazin-1-yl)acetate (36).

To a solution of amino mesylate 34 in its HCl form (2 eq, 5 mmol, 1.08 g) in suspension in DMF (8 mL) was added NaH (3 eq, 0.6 g of a 60% suspension in oil) under argon, and after gas composition the ester 24 (1 eq, 2.5 mmol, 0.58g) in DMF (8 mL) was added. The mixture was stirred at 40°C overnight then quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/MeOH, 98:2 to 94:6) to afford the desired amine 36 as an oil (1.89 mmol, 0.6 g, 75%). 1H NMR (400 MHz, CDCl3): δ (ppm) 8.48-8.45 (m, 1H, Har), 7.81-7.73 (m, 2H, 2 x Har), 7.71-7.68 (m, 1H, Har), 4.27 (t, J = 7.2 Hz, 2H, NCH2), 4.19 (q, J = 7.1 Hz, 2H, CH2OEt), 3.96 (s, 2H, CH2CO2Et), 2.41, (t, 2H, J = 7.4 Hz, 2H, CH2NMe2), 2.25 (s, 6H, NMe2), 2.07-1.99 (m, CH2CH2), 1.24 (t, 3H, CH2CH2). 13C NMR (100 MHz, CDCl3): δ (ppm) 167.9 (COOEt), 159.2 (C=O), 140.2 (Civ), 132.9 (CH3a), 131.3 (CHa), 129.2 (Civ), 128.0 (Civ), 127.3 (CH3a), 124.3 (CH3a), 61.3 (CH2O), 56.8 (Me2NCH2), 49.3 (NCH2), 45.3 (2 x CH3), 39.0 (CH2COOEt), 26.5 (CH2), 14.1 (CH3CH2O). HRMS (ESI+) calcd for C17H24N4O3 [M+H]+ m/z 318.1818; found 318.1821.

4.1.24. 2-(3-(tert-butyl(dimethyl)silyloxy)propyl)-4-((5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl)phthalazin-1(2H)-one (37).

To a solution of ester 35 (1 eq, 0.98 mmol, 0.4 g) in ethanol (0.2 mol.L⁻¹) at room temperature was added hydrazine monohydrate (6 eq, 6 mmol, 300 μL). The mixture was stirred at 42°C during 24 h then a second portion of hydrazine monohydrate was added (6 eq, 6 mmol, 300 μL) and the mixture was refluxed overnight. After completion of the reaction, the solution was allowed to cool to room temperature and the precipitate was filtered, washed with ethylacetate and dried. The solid hydrazide (0.82 mmol, 0.32 g, 84%) was used in the next step without further purification. 1H NMR (400 MHz, DMSO-d6): δ (ppm) 9.33 (s, 1H, NH), 8.29–8.27 (m, 1H, CHa), 7.92–7.83 (m, 3H, CHa), 4.25 (s, 2H, NH2), 4.17 (t, J = 7.1 Hz, 2H, NCH2), 3.78 (s, 2H, CH2CO), 3.67 (t, J = 6.1 Hz, 2H, CH2O), 1.93 (m, 2H, CH2), 0.85 (s, 9H, 3 x CH3-tBu), 0.02 (s, 6H, 2 x SiCH3). HRMS (ESI+) calcd for C19H24N4O3Si [M+H]+ m/z 391.2165; found 391.2166. Under inert atmosphere, to a solution of the hydrazide previously obtained (1 eq, 0.394 mmol, 154 mg), methyl isothiocyanate (5.5 eq, 2.17 mmol, 158 mg) in ethanol (0.033 mol.L⁻¹) were added TEA (5 eq, 1.97 mmol, 0.275 mL). The mixture was refluxed 3 h then concentrated under reduced pressure. Et2O was added to the resulting residue followed by evaporation (3 times). The solid was then triturated in an Et2O/cyclohexane mixture. The thiol 37 was obtained by filtration as a white solid (0.359 mmol, 160 mg, 91%). 1H NMR (400 MHz, DMSO-d6): δ (ppm) 13.50 (s, 1H, SH), 8.49–8.21 (m, 1H, CHa), 8.05–8.00 (m, 1H, CHa), 7.97–7.83 (m, 2H, 2 x CHa), 4.50 (s, 2H, CH2), 4.14 (t, J = 7.1 Hz, 2H, NCH2), 3.63 (t, J = 6.1
Hz, 2H, CH₂O), 3.47 (s, 3H, NMe), 1.89 (m, 2H, CH₂), 0.82 (s, 9H, 3 x CH₃-tBu), -0.02 (s, 6H, 2 x SiCH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ(ppm) 167.0 (C=O), 158.1 (C=O), 149.4 (C=O), 140.4 (C=O), 132.2 (C=O), 128.5 (C=O), 127.3 (C=O), 126.2 (C=O), 125.5 (C=O), 60.2 (CH₂O), 47.4 (NCH₂), 31.1 (CH₂), 30.1 (NMe), 28.9 (CH₂), 27.7 (3 x CH₃-tBu), 17.8 (C=O), -5.4 (2 x SiCH₃). HRMS (ESI⁺): calcd for C₂₃H₂₃N₇O₅S'[M+H]' m/z 446.2038; found 446.2046.

4.1.25. 2-(3-(dimethylamino)propyl)-4-((5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl)phthalazin-1(2H)-one (38).

To a solution of the ester 36 (1 eq, 1.57 mmol, 0.5 g) in ethanol (0.2 mol L⁻¹) at room temperature was added hydrazine monohydrate (0.031 mol L⁻¹) at room temperature. The resulting yellow crude was taken up in Et₂O, the precipitate was filtered and washed with Et₂O. The THF 38 was recovered as a white solid (0.335 mmol, 120 mg, 89%). ¹H NMR (400 MHz, CDC1₃): δ(ppm) 8.51–8.33 (m, 1H, CH₂), 7.99–7.86 (m, 1H, CH₂), 7.85–7.63 (m, 2H, 2 x CH₂), 4.35 (s, 2H, CH₂), 4.21 (t, J = 7.2 Hz, 2H, NCH₂), 3.54 (s, 3H, NMe), 2.60–2.38 (m, 2H, CH₂NMe₂), 2.29 (t, 6H, NMe₂), 2.11–1.78 (m, 2H, CH₂). ¹³C NMR (100 MHz, CDC1₃): δ(ppm) 168.5 (C=O), 159.2 (C=O), 148.9 (C=O), 139.8 (C=O), 133.4 (C=O), 132.0 (C=O), 128.7 (C=O), 128.2 (C=O), 127.6 (C=O), 124.4 (C=O), 56.5 (C=O), 49.4 (NCH₂), 44.9 (NMe₂), 31.1 (NMe), 30.2 (CH₂), 26.3 (CH₂). HRMS (ESI⁺): calcd for C₁₇H₁₅O₄S'[M+H]' m/z 359.1646; found 359.1654.

4.1.26. 8-((3-(3-(tert-butyldimethylsilyl)oxy)propyl)-4-oxo-3,4-dihydrophthalazin-1-yl)(methyl)-4-methyl-4H-1,2,4-triazol-3-ylthio)-N-(2,4-dichlorophenyl)octanamide (39).

The mercapto-triazole 37 (1 eq, 0.269 mmol, 120 mg) and bromoamide 7 (1.5 eq, 0.303 mmol, 56 mg) were dissolved in DMF and stirred overnight. The mixture was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting crude was purified by column chromatography on silica gel (DCM/MeOH, 85:15). The desired product 39 was obtained as a colorless syrup (0.191 mmol, 140 mg, 71%). ¹H NMR (400 MHz, CDC1₃): δ(ppm) 8.47–8.39 (m, 1H, Har-Phtal), 8.33 (d, J = 8.9 Hz, 1H, Har), 8.30–8.23 (m, 1H, Har-Phtal), 7.83–7.70 (m, 2H, 2 x Har-Phtal), 7.60 (s, 1H, NH), 7.36 (d, J = 2.4 Hz, 1H, Har), 7.23 (dd, J = 8.9, 2.4 Hz, 1H, Har), 4.48 (s, 2H, CH₂), 4.28 (t, J = 7.3 Hz, 2H, NCH₂), 3.73 (t, J = 6.1 Hz, 2H, CH₂O), 3.54 (s, 3H, NMe), 3.19 (t, J = 7.4 Hz, 2H, CH₂S), 2.40 (t, J = 7.5 Hz, 2H, CH₂CONH), 2.14–1.96 (m, 2H, CH₂), 1.80–1.65 (m, 4H, 2 x CH₂), 1.48–1.30 (m, 6H, 3 x CH₂), 0.89 (s, 9H, 3 x CH₃-tBu), 0.04 (s, 6H, 2 x SMe). ¹³C NMR (100 MHz, CDC1₃): δ(ppm) 171.4 (CONH), 159.3 (C=O), 152.1 (C=O), 152.0 (C=O), 140.8 (C=O), 133.6 (C=O), 133.4 (C=O), 131.9 (C=O), 129.1 (C=O), 128.9 (C=O), 128.9 (C=O), 128.3 (C=O), 128.0 (C=O), 127.3 (C=O), 125.5 (C=O), 123.3 (C=O), 122.6 (C=O), 60.8 (CH₂O), 48.8
N-(2,4-dichlorophenyl)-8-((5-((3-(3-dimethylamino)propyl)-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)octanamide (40).

The mercapto-triazole 38 (1 eq, 0.181 mmol, 65 mg) and K₂CO₃ (1.5 eq, 0.272 mmol, 37 mg) were dissolved in DMF and stirred at room temperature overnight. The resulting yellow solution was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting crude was purified by column chromatography on silica gel (DCM/MeOH, 85:15) to give the desired product 40 as a white powder (0.085 mmol, 55 mg, 47%).

HRMS (ESI²⁺): calcd for C₃₅H₃₈N₂O₃S₂Cl₂ [M+H]⁺ m/z 731.2723; found 731.2733.

N-(2,4-dichlorophenyl)-8-((5-((3-(3-hydroxypropyl)-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4-methyl-4H-1,2,4-triazol-3-yl)thio)octanamide (41).

To a solution of the silylated alcohol 39 (1 eq, 0.672 mmol, 480 mg) in distilled THF (0.034 mol.L⁻¹) was carefully added a solution of HF (70 wt % in pyridine, 0.9 mL). The resulting colorless solution was stirred 4 h at room temperature. The reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the corresponding alcohol 41 as a white solid (0.648 mmol, 400 mg, 98%).

HRMS (ESI²⁺): calcd for C₃₁H₄₀N₂O₃S₂Cl₂ [M+H]⁺ m/z 644.2343; found 644.2341.

N-(2,4-dichlorophenyl)-8-((4-methyl-5-((4-oxo-3-(3-oxopropyl)-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (42).

To a solution of the alcohol 41 (1 eq, 0.162 mmol, 100 mg) in DCM (0.008 mol.L⁻¹) was added Dess Martin periodinane (DMP) (2 eq, 0.323 mmol, 140 mg). The resulting white suspension
was allowed to stir at room temperature for 20 h. Incomplete conversion was observed (1H NMR), and an additional portion of DMP (2 eq, 0.323 mmol, 140 mg) was added and DMSO (20 mL) to increase solubility. Upon full conversion (40 h), the reaction mixture was quenched with saturated solutions of Na2S2O3 (10 mL) and NaHCO3 (10 mL). The organic layer was washed with brine and water. The organic layer was dried over Na2SO4, filtered and concentrated under reduced pressure to afford the corresponding aldehyde 42 as a white solid (0.120 mmol, 74 mg, 74%). 1H NMR (400 MHz, CDCl3): δ(ppm) 9.81 (t, J = 1.6 Hz, 1H, Hα), 8.48–8.38 (m, 1H, Har-Pthal), 8.33 (d, J = 8.9 Hz, 1H, Hα), 8.27–8.18 (m, 1H, Har-Pthal), 7.89–7.73 (m, 2H, 2 x Har-Pthal), 7.61 (s, 1H, NH), 7.37 (d, J = 2.4 Hz, 1H, Har), 7.28–7.19 (m, 1H, Hα), 4.55 (t, J = 6.5 Hz, 3H, NCH2), 4.50 (s, 2H, CH2), 3.52 (s, 3H, NMe), 3.22 (t, J = 7.3 Hz, 2H, CH2S), 2.94 (td, J = 6.5, 1.6 Hz, 2H, CH2CHO), 2.41 (t, J = 7.5 Hz, 2H, CH2CONH), 1.74 (m, 4H, 2 x CH2), 1.49–1.33 (m, 6H, 3 x CH2). 13C NMR (100 MHz, CDCl3): δ(ppm) 200.0 (COH), 171.4 (CONH), 159.3 (C=O), 152.2 (CIV-triazole), 151.7 (CIV-triazole), 141.4 (CIV), 133.8 (CHar-Pthal), 133.6 (CIV-COH), 132.2 (CHar-Pthal), 129.1 (CIV-Cl), 128.9 (CIV), 128.8 (2 x CHar), 128.0 (CIV), 127.3 (CHar-Pthal), 125.5 (CHar-Pthal), 123.3 (CIV-Cl), 122.6 (CHar), 45.1 (NCH2), 42.5 (CH2CHO), 37.9 (CH2CONH), 33.1 (CH2S), 30.7 (NMe), 30.2 (CH2), 29.5 (CH2), 29.0 (CH2), 28.8 (CH2), 28.4 (CH2), 25.4 (CH2). HRMS (ESI’): calcd for C20H23N6O4SCl2 [M+H]+ m/z 615.1712; found 615.1719.

4.1.30. 3-(4-(5-((8-((2,4-dichlorophenyl)amino)-8-oxoctylthio)acetyl)-4-methyl-4H-1,2,4-triazol-3-yl)methyl)-1-oxophthalazin-2(1H)-ylpropanoic acid (43). To a solution of aldehyde 42 (1 eq, 0.113 mmol, 70 mg) in t-BuOH/THF 1:1 (0.011 mol L−1) was added DMSO, 2-methyl-2-buten (1 mL) and water (5 mL). Then an aqueous solution of NaHPO4 (15 eq) and NaClO2 (9 eq, 1.02 mmol, 92.6 mg) were added. The solution was stirred at room temperature during 16 h and diluted with a saturated aqueous NH4Cl solution (30 mL), then extracted with DCM. The combined organic layers were washed with brine, water, dried over Na2SO4, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silical gel (DCM/Methanol, 96:4) to afford the carboxylic acid 43 as a white powder (0.043 mmol, 27 mg, 37%). 1H NMR (400 MHz, CDCl3): δ(ppm) 8.47–8.36 (m, 1H, Har-Pthal), 8.30 (d, J = 9.0 Hz, 1H, Hα), 8.16–8.09 (m, 1H, Har-Pthal), 7.84–7.72 (m, 2H, 2 x Har-Pthal), 7.65 (s, 1H, NH), 7.36 (d, J = 2.4 Hz, 1H, Har), 7.23 (dd, J = 8.9, 2.4 Hz, 1H, Hα), 4.54–4.34 (m, 4H, CH2 and NCH2), 3.52 (s, 3H, NMe), 3.17 (t, J = 7.3 Hz, 2H, CH2S), 2.82 (t, J = 6.7 Hz, 2H, CH2COOH), 2.42 (t, J = 7.5 Hz, 2H, CH2CONH), 1.70 (m, 4H, 2 x CH2), 1.47–1.30 (m, 6H, 3 x CH2). 13C NMR (100 MHz, CDCl3): δ(ppm) 173.9 (COOH), 171.7 (CONH), 159.2 (C=O), 152.3 (CIV-C=O), 151.9 (CIV-C=O), 141.1 (CIV-Pthal), 133.7 (CHar-Pthal), 133.5 (CIV), 132.1 (CHar-Pthal), 129.3 (CIV-Cl), 128.9 (CIV), 128.9 (CIV), 128.8 (CHar), 127.9 (CHα), 127.4 (CHα-Pthal), 121.5 (CHα-Pthal), 123.5 (CIV-Cl), 122.8 (CHα), 46.7 (NCH2), 37.8 (CH2CONH), 33.2 (CH2S), 33.0 (CH2COOH), 30.8 (NMe), 30.5 (CH2), 29.8 (CH2), 29.4 (CH2), 28.9 (CH2), 28.3 (CH2), 25.3 (CH2). HRMS (ESI’): calcd for C20H23N6O4S [M+H]+ m/z 629.1492; found 629.1505.

4.1.31. 2-(4-oxo-3,4-dihydropthalazin-1-yl)acetohydrazide (44) To a suspension of 23 (1 eq, 3.44 g, 15.7 mmol) in EtOH was added hydrazine monohydrate (1 eq, 15.7 mmol, 775 μL) at room temperature. The solution was stirred for 45 min, then a second portion of hydrazine was added (4 eq, 62.8 mmol, 3.1 mL) and the mixture was refluxed during 14 h. The solution was then cooled to room temperature and the solid was filtered and washed with Et2O to afford the desired hydrazide 44 as a white powder (12.9 mmol, 2.81 g, 82%). 1H NMR (300 MHz, DMSO-d6): δ(ppm) 12.75 (bs, 1H, NH), 9.36 (br, 1H, NH), 8.24 (m, 1H, Har-Pthal), 7.91–7.84 (m, 3H, Har-Pthal), 4.28 (bs, 2H, NH2), 3.75 (s, 2H, CH2). 13C NMR (75 MHz, DMSO-d6): δ(ppm) 167.9 (C=O), 159.5 (CO-Pthal), 141.9 (CIV), 133.3 (CHar-Pthal), 131.5 (CHar-
CH

silica gel (DCM/AcOEt, 50:50) to afford the N
washed with water and brine. The organic layer was
dried
added
reduced pressure and a solution of
oxalyl chloride (2 eq, 2.24 mmol, 0.197 mL)
To a solution of octanoic acid (1 eq, 1.12 mmol, 250 mg) in DCM (0.11
mmol, 0.84g), and the mixture was heated at reflux during 3 h. Then DBU (1 eq, 2.2 mmol, 0.35g) was added and heating was maintained during another 3 h. The reaction was cooled to room temperature and the precipitate was filtered and washed with EtOH and Et₂O to afford the desired thiol 45 as a white powder (12.9 mmol, 2.81 g, 69%). ¹H NMR (300 MHz, DMSO-
δ(ppm) 13.58 (s, 1H, H); 12.58 (s, 1H, NH); 8.27 (m, 1H, H₄Phthal); 8.0-7.85(m, 3H, H₄Phthal)
100)
δ(ppm) 13.58 (s, 1H, SH); 12.58 (s, 1H, NH); 8.27 (m, 1H, H₄Phthal); 8.0-7.85(m, 3H, H₄Phthal)
δ(ppm) 166.9 (CIV); 159.3 (C=O); 149.6 (CIV); 140.9 (CIV); 133.6 (CH₄Phthal); 131.9 (CH₄Phthal); 129.1 (CIV); 127.6 (CIV); 125.9 (CH₄Phthal); 125.5 (CH₄Phthal); 30.1 (CH₃); 28.1 (CH₂). HRMS (ESI⁺): calec for
C₂H₁₂N₃O₅ [M+H]⁺ m/z 274.0763; found 274.0752.

N-(2,4-dichlorophenyl)-8-(((4-methyl-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (46)
To a solution of thioli 45 (1 eq, 0.7 mmol, 0.2 g), in dry DMF, bromoamide 7 (1 eq, 0.7 mmol, 0.255 mmol) and K₂CO₃ (1.2eq, 0.8 mmol, 0.109 mg) were added. The mixture was then stirred for 48 h, then quenched with a saturated aqueous NH₄Cl solution and extracted with DCM. The organic layer was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/MeOH, 95:5) to afford the desired product 46 as a white powder (0.34 mmol, 190 mg, 49%). ¹H NMR (300 MHz, DMSO-
δ(ppm) 12.55 (s, 1H, NHPhthal); 9.52 (s, 1H, NH); 8.27-8.24 (m, 1H, H₄Phthal); 8.07-8.04 (m, 1H, H₄); 7.95-7.85(m, 2H, H₄Phthal); 7.68 (d, J = 8.7 Hz, 1H, H₄Phthal); 7.63 (d, J = 2.4 Hz, m, 1H, H₄); 7.6-7.40 (m, 1H, H₄); 4.51 (s, 2H, CH₂); 3.47 (s, 3H, NMe); 3.07 (t, J = 7.2 Hz, 2H, SCH₂); 2.36 (t, 2H, J = 7.2 Hz, CH₂CO); 1.64-1.54 (m, 4H, 2 x CH₂); 1.35-1.28 (m, 6H, 3 x CH₃). ¹³C NMR (75 MHz, DMSO-
δ(ppm) 171.7 (CONH), 159.4 (C=O), 152.7 (CIV-C=N), 151.6 (CIV-C=N), 141.6 (CIV-Phthal), 134.2 (CIV-NHC(O)), 133.4 (CH₄Phthal), 131.7 (CH₄Phthal), 129.2 (CIV), 129.1 (CIV-C), 128.8 (CH₄), 128.1 (CIV), 127.7 (CH₄), 127.5 (CH₄Phthal), 125.9 (CH₄Phthal), 123.3 (CIV-C), 125.6 (CH₄), 39.5 (NMe), 32.5 (CH₂CONH), 30.2 (CH₂S), 29.1 (CH₃), 28.6 (CH₂), 28.4 (CH₂), 28.2 (CH₂), 27.8 (CH₂), 25.0 (CH₂). Four quaternary carbon atoms are missing and the carbon atom of NMe is in the solvent signal. HRMS (ESI⁺): calec for C₂H₂₅Cl₂N₂O₅S [M+H]⁺ m/z 559.1447; found 559.1450.

N-(4-benzoylphenyl)-8-iodooctanamide (48)
To a solution of octanoic acid (1 eq, 1.12 mmol, 250 mg) in DCM (0.11 mmol.mL⁻¹) was added oxaly chloride (2 eq, 2.24 mmol, 0.197 mL) with some drops of DMF at 0°C. The resulting clear solution was stirred until completion of the reaction. The mixture was concentrated under reduced pressure and a solution of 4-amino benzophenone (1.2 eq, 1.34 mmol, 265 mg), EtuN (2 eq, 2.24 mmol, 0.312 mL) and DMAP (0.05 eq. 0.056 mmol, 7 mg) in DCM (5 mL) was added slowly and then stirred at room temperature. The mixture was diluted with DCM and washed with water and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (DCM/AcOEt, 50:50) to afford the N-(4-benzoylphenyl)-8-bromoocatanamide as a white-yellowish solid (0.831 mmol, 327 mg, 74%). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.85 – 7.79 (m, 2H, 2 x CH₂), 7.79 – 7.71 (m, 2H, 2 x CH₂), 7.68 – 7.62 (m, 2H, 2 x CH₂), 7.62 – 7.53 (m, 1H, CH₃), 7.53 – 7.44 (m, 2H, 2 x CH₂), 7.39 (s, 1H, NH), 3.41 (t, J = 6.8 Hz, 2H, CH₂), 2.51 – 2.35 (m, 2H, CH₂CONH), 1.92 – 1.81 (m, 2H, CH₂), 1.81 – 1.66 (m, 2H, CH₂), 1.49 – 1.37 (m, 6H, 3 x CH₂). HRMS (ESI⁺): calec for C₂₁H₂₅NO₂Br [M+H]⁺ m/z 402.1059;
found 402.1069. NaI (2.25 eq, 14.54 mmol, 2.18 g) was added to a solution of bromo compound previously obtained (1 eq, 6.46 mmol, 2.60 g) in acetone (0.7 mol.L⁻¹). The solution was allowed to reflux during 2 h then concentrated. The residue was solubilized in DCM and NaI/NaBr salts were filtered off. The organic layer was then concentrated under reduced pressure to afford the expected iodo compound 48 as a yellow viscous oil (6.45 mmol, 2.90 g, quant.). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.30 (s, 1H, NH), 7.81 – 7.72 (m, 4H, 4 x CH₃-Phthal), 7.68 (d, J = 8.8 Hz, 2H, 2 x CH₃), 7.61 – 7.52 (m, 1H, CH₃), 7.50 – 7.37 (m, 2H, 2 x CH₃), 3.14 (t, J = 7.0 Hz, 2H, CH₂), 2.38 (t, J = 7.5 Hz, 2H, CH₂-CONH), 1.82 – 1.73 (m, 2H, CH₂), 1.70 (m, 2H, CH₂), 1.34 (m 6H, 3 x CH₃). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 196.1 (C=O), 172.2 (CONH), 142.5 (CIV-NH CO), 137.8 (CIV-CO), 132.7 (CIV-CO), 132.4 (CH₂), 131.7 (2 x CH₃), 129.9 (2 x CH₃), 128.4 (2 x CH₃), 118.9 (2 x CH₃), 37.7 (CH₂CONH), 33.4 (CH₂), 30.3 (CH₂), 29.1 (CH₃), 28.3 (CH₃), 25.4 (CH₂), 7.3 (CH₂). HRMS (ESI⁺): calcd for C₂₁H₂₉NO₂I [M+H]⁺ m/z 450.0922; found 450.0930.

4.1.35. N-(4-benzylphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydropthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-ylthio)octanamide (49)

In a reactor tube was added K₂CO₃ (2.2 eq, 0.43 mmol, 59 mg) to a solution of thiol 47 (1 eq, 0.19 mmol, 56 mg) and iodo compound 48 (1.3 eq, 0.253 mmol, 114 mg) in DMF (0.05 mol.L⁻¹). The solution was allowed to stir at 40 °C during 1h30, then at room temperature during 12 h. Upon full conversion the mixture was diluted in AcOEt (15 mL), washed with water and brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (DCM/MeOH, 100:0 to 95:5) to afford the corresponding compound 49 as white powder (0.197 mmol, 120 mg, 99 %). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 9.02 (s, 1H, NH), 8.55 – 8.31 (m, 1H, CH₃-Phthal), 8.22 – 8.08 (m, 1H, CH₃-Phthal), 7.81 – 7.68 (m, 8H, 2 x CH₃-Phthal, 3 x CH₃), 7.60 – 7.51 (m, 1H, CH₃), 7.49 – 7.40 (m, 2H, 2 x CH₃), 4.48 (s, 2H, CH₂), 3.77 (s, 3H, NMe₃), 3.58 (s, 3H, NMe₃), 3.12 (t, J = 7.3 Hz, 2H, CH₂), 2.40 – 2.29 (m, 2H, CH₂-CONH), 1.66 (td, J = 7.4, 5.1 Hz, 4H, 2 x CH₂), 1.36 (m, 2H, CH₂), 1.26 (m, 4H, 2 x CH₂). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 195.9 (CO_benzo), 172.5 (CONH), 159.5 (C=O), 152.2 (CIV), 152.1 (CIV), 142.9 (CIV-NH CO), 140.7 (CIV-s), 138.1 (CIV-CO), 133.4 (CH₃-Phthal), 132.5 (CH₉-Phthal), 132.3 (CIV-CO), 132.1 (CH₉), 131.6 (2 x CH₉), 129.9 (2 x CH₃), 128.4 (2 x CH₉), 128.0 (CIV), 127.2 (CH₉-Phthal), 125.1 (CH₉-Phthal), 118.9 (2 x CH₉-Phthal), 39.6 (NMe₂-Phthal), 37.6 (CH₂CONH), 33.1 (CH₂-s), 30.9 (NMe₃), 30.0 (CH₃), 29.3 (CH₃), 28.7 (CH₂), 28.2 (CH₂), 27.9 (CH₂), 25.2 (CH₂). HRMS (ESI⁺): calcd for C₃₄H₃₇N₅O₅S [M+H]⁺ m/z 609.2650; found 609.2648.

4.2 Biological assays

4.2.1 Cell lines, recombinant proteins and antibodies. The 32Dβ and NK-92 cell lines were used in that study and cultured as described previously. Recombinant human IL-15, recombinant murine IL-3 were obtained from Peprotech, Inc. and recombinant human IL-2 from Chiron. RLI, an IL-15 agonist was produced as described previously.
4.2.2 **Proliferation assays.** The proliferation response of 32Dβ cells to RLI\(^3\) was assessed by Alamar blue reduction assay (AbDSerotec). Cells were starved in the culture medium without cytokine for 4 h. Cells (1x10\(^4\)) were cultured for 2.5 days in the medium supplemented with 100 pM RLI or 1.5 nM IL-2, previously preincubated for 30 min with increasing concentrations of compounds. Cell proliferation was revealed by adding Alamar blue (10 µl) to each well and, after a 6h-incubation period at 37 °C, by measuring the emitted fluorescence at 590 nm under excitation at 560 nm using an EnSpire Multimode Plate Reader (Perkin Elmer).

4.2.3 **P-Stat5 assays.** Exponentially-growing NK-92 cells were washed and serum-starved overnight to reduce basal phosphorylation. Cells (2x10\(^5\)) were then stimulated at 37 °C for 1 h with fixed concentrations of IL-15 or IL-2 that have been preincubated for 30 min with increasing concentrations of chemical compounds. At the end of the stimulation, cells were lysed and Stat5 phosphorylation was measured according to the manufacturer’s instructions using p-Stat5 AlphaScreen Surefire kit (Perkin Elmer Life Sciences). The signal in the wells was then detected using an EnSpire Multimode Plate Reader (Perkin Elmer).

4.2.4 **SPR experiment.** The SPR experiments were performed at 25°C on a Biacore T200 biosensor (GE Healthcare, Chalfont St Giles, UK). Recombinant IL-15 was covalently immobilized to CM5 sensor chips using the amine coupling method in accordance with the manufacturer’s instructions, and the binding of compounds at increasing concentrations, prepared in HBSEP/DMSO (0.1 %) was monitored. The Biacore T200 Evaluation 3.0 software (GE Healthcare) was used to run experiments and for data analysis.

**ASSOCIATED CONTENT**

Supplementary data
1H NMR and 13C NMR spectra of the compounds 1-2, 8-14, 19-22, 28-32, 39-43, 46 and 49, molecular properties of compounds 1, 2, 2A, 2B, 2C, 49, 12, 46, 11, 41, 42, and SPR sensorgrams of compounds 2, 11, 12, 41, 42, 46 and 49 are provided in Supplementary Material.

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS USED
IL, Interleukin; R, Receptor; NK, Natural Killer; LGLL, Large Granular lymphocyte; CTCL, Cutaneous T Cell Lymphoma; HTLV-1, Human T Cell Lymphotropic Virus 1; ATL, Adult T cell Leukemia; Stat, Signal Transducers and Activators of Transcription; HAM/TSP, HTLV-1-Associated Tropical Spastic paraparesis; PPIs, Protein-Protein Interactions; SPR, Surface Plasmon Resonance.

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