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Discovery of a Small-Molecule Inhibitor of Interleukin 15: Pharmacophore-Based Virtual Screening and Hit Optimization

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ABSTRACT

Interleukin (IL)-15 is a pleiotropic cytokine, which is structurally close to IL-2 and shares with it the IL-2 β and γ receptor (R) subunits. By promoting the activation and proliferation of NK, NK-T and CD8+ T cells, IL-15 plays important roles in innate and adaptative immunity. Moreover, the association of high levels of IL-15 expression with inflammatory and auto-immune diseases has led to the development of various antagonistic approaches targeting IL-15. This study is an original approach aimed at discovering small-molecule inhibitors impeding IL-15/IL-15R interaction. A pharmacophore and docking-based virtual screening of compound libraries led to the selection of 240 high-scoring compounds, 36 of which were found to bind IL-15, to inhibit the binding of IL-15 to the IL-2Rβ chain and/or the proliferation of IL-15-dependent cells. One of them was selected as a hit, optimized by a structure-activity relationship approach, leading to the first small-molecule IL-15 inhibitor with sub-micromolar activity.

Keywords: Cytokine, protein-protein interaction, inhibitor, interleukin-15, inflammatory and autoimmune diseases

1. INTRODUCTION

First identified as a T cell growth factor, Interleukin (IL)-15 is a 14-15 kDa cytokine belonging to the IL-2 cytokine family and involved in the differentiation and proliferation of NK and T cells.[1, 2] IL-15 binds a trimeric IL-15 receptor (IL-15R) complex formed by a specific receptor chain (IL-15Rα) combined with the β and γ chains of the IL-2R, acting as signal transducing components. Binding of IL-15 to IL-2Rβ/γ induces Jak1 and Jak3 activation and subsequently phosphorylation of Stat3 and Stat5 (where Jak and Stat stand for Janus kinase and signal transducer and activators of transcription, respectively). High levels of IL-15 expression have been associated with the pathogenesis of autoimmune and inflammatory diseases like Crohn's disease,[3] psoriasis,[4] leukemias,[5] rheumatoid arthritis (RA)[6] and graft rejection,[7, 8] resulting in the IL-15 system being a target of interest for the treatment of these diseases. Several biological approaches have validated this concept *in vivo*, including the use of soluble forms of IL-15Rα, antagonist IL-15 mutants, IL-15- or IL-15R-directed monoclonal antibodies and fusion proteins that have proved effective in reducing RA in animal models.[9-11] However, their therapeutic benefit in human diseases has not yet been demonstrated and further studies and clinical trials are required in human systems to evaluate their safety and efficacy.[12] In this context, the design of small-molecule inhibitors of protein-protein interactions (PPIs) is an emerging and challenging research area[13, 14] which could be envisaged as an alternative approach to target the IL-15/IL-15R interfaces.

In fact, potent small-molecule inhibitors of the IL-2/IL-2Rα interface have already been identified using fragment-based approaches[15] or library screening[16] combined with chemistry optimization. Moreover, the targeting of the IL-2Rβ chain by siRNA has been shown to be effective in reducing disease severity in adjuvant-induced arthritis in rats,[17] highlighting the interest of IL-2Rβ as a therapeutic target for the treatment of RA. To date, no small-molecule inhibitor of the IL-15 system has been reported, nor has the targeting of the IL-2Rβ interface

been explored by a PPI approach. This work is the first attempt to discover such low MW non-peptidic inhibitors, by targeting the IL-15/IL-2Rβ interface. For this purpose, a pharmacophore model was built (Figure 1) based on IL-15 specific residues[18, 19] involved in this interface, and was used to screen virtually commercial and academic chemical databases (ca. 160,000 compounds). The resulting filtered libraries were subsequently docked to the binding site of IL-15 with IL-2Rβ, and the best-docked compounds were retained. On the basis of experimental *in vitro* properties such as the capacity to bind to IL-15, to reduce the binding of IL-15 to IL-2Rβ or to inhibit the proliferation of IL-15-dependent cells, one hit was chosen among 240 purchased compounds. After chemical optimization of its structure, this led to the development of a low MW inhibitor of the IL-15/IL-15R system with sub-micromolar efficiency.

2. RESULTS AND DISCUSSION

2.1 Pharmacophore-based and docking-based virtual screening. In order to disrupt IL-15/IL-15R recognition and impede IL-15 signaling, our approach was to use small MW molecules to target one of the IL-15 domains that are interacting with the signal transducing receptor chains IL-2Rβ or IL-2Rγ. The IL-15/IL-2Rγ interface was set aside to avoid specificity problems due to the sharing of this receptor chain by numerous other cytokine systems (IL-15, IL-2, IL-4, IL-7, IL-9 and IL-21).[20] Thus, we chose to target the IL-15/IL-2Rβ binding domain, a strategy that has not yet been described in the literature.

Our virtual screening strategy consisted of using pharmacophore searching and docking methods. The 3D structure of IL-15 (PDB code 2Z3Q)[21] was used to generate a pharmacophore model based on three residues Asp8, Asn65 and Leu69, previously shown by directed-mutagenesis studies[18, 19] or by homology to the IL-2/IL-2Rβ complex,[22, 23] to be involved in the binding of IL-15 to IL-2Rβ. Comparison of IL-15 and IL-2 structures after

superimposition of IL-15 onto IL-2 in the tetrameric complex structure IL-2:IL-2Rα/β/γ (PDB code 2B5I)[24] (available at the time of this work unlike that of the IL-15 tetrameric complex)[25], led us to consider (a) an additional orientation for the side chain of Asn65, similar to that of its homologous Asn88 in IL-2 (Figure 1A), and (b) the potential participation of two water molecules close to residues Asp8 and Asn65 (Figure 1B). The final model consisted of three main features (one projection of hydrogen bond donor (HBD) and one projection of hydrogen bond donor or acceptor (HBDA) centered on Asp8, and one hydrophobic in front of Ile68 and Leu69) completed by ten exclusion spheres centered on the main residues of the defined binding site. In addition, three accessory features (one projection of HBD centered on Asn65, considering two side chain orientations, and finally two projections of HBDA placed on both water molecules) were created (Figure 1C). The pharmacophore model was used to search a database of 160828 chemical compounds derived from commercial (Chembridge, Maybridge and Preswick) or academic libraries (National Cancer Institute (NCI) and the French national chemical library Chimiothèque Nationale). To be retained, a hit had to match at least the main features whereas the presence of the accessory features was not mandatory. A total of 24115 molecules were returned by the pharmacophore searching and were subjected to docking-based virtual screening using the LigandFit program implemented under Discovery Studio (DS) 3.0 (Accelrys Software Inc, San Diego, CA). More than 30% of the filtered molecules passed the docking stage and were ranked using 5 scoring functions. A consensus strategy was adopted (see Experimental Section) and the 240 top-ranked molecules were purchased (Table S1). They were first tested at a concentration of 100 μM for their ability to bind to IL-15 using the surface plasmon resonance (SPR) technology (Figure 2A) or to inhibit the binding of IL-15 to soluble IL-2Rβ in a Homogeneous Time Resolved Fluorescence (HTRF) assay (Figure 2C). Thirty-six compounds were found to bind to IL-15 as well as to our IL-15 superagonist RLI (a fusion protein linking IL-15 to the IL-15Rα sushi domain)[26] (Figure 2A

and B), thus discarding any binding of compounds to IL-15 at its interface with IL-15Rα. Some were also shown to reduce the FRET signal induced by the interaction between recombinant IL-2Rβ and IL-15 (Figure 2D). To evaluate their impact on a specific IL-15 functional effect, we further tested these hits for their ability to inhibit IL-15-dependent cell proliferation. Most of them were inhibitors and five of them showed IC_{50} values around or below 10 μM (Figure S2). One of these, named compound **1** (Figure 3) was selected for chemical optimization. Its binding mode to the IL-15 structure was predicted by a molecular docking technique using LigandFit and C-Docker programs. The interactions[27] involved, as predicted by the last fine docking step were π-alkyl hydrophobic interactions with residues Ile68 and Leu69, an amide-π stacked interaction with the Ser7 and Asp8 peptidic bond, a hydrogen bond (HB) with Asp8 and a carbon HB with Asn65 (Figure 4A).

2.2 Synthesis. Among the identified hits, the chemical structure of compound **1** emerged as a good platform to initiate synthetic modifications to improve its biological activity. The chemical structure of this compound can be regarded as the association of three fragments: a dihydro-phthalazinone tethered to a triazole moiety, which can be considered the main core of the molecule, a terminal aromatic group and an alkyl linker chain to join both parts of the molecule through S-C bond and amide bond formation (Figure 3). To the best of our knowledge, the synthesis of compound **1** has never been described in the literature, and we began our synthetic work to define the best strategy to access this hit. A good and combinatorial approach consisted of condensing anilines with a carboxylic acid derived from the *S*-alkylation of the main dihydro-phthalazinone core, with bromoacetic acid (Figure 3, route 1). Although this carboxylic acid derivative could easily be prepared with a 60% yield in Williamson-type alkylation conditions,[28] all attempts to access to the amide **1** in common coupling conditions with carbodiimide reagents[29, 30] and 2,4-dichloroaniline failed. We thus

turned to a second strategy, exploring the *S*-alkylation of the dihydro-phthalazinone main core with a variety of halogenoalkylamides previously prepared (Figure 3, route 2).

The key dihydro-phthalazinone intermediates **4** and **5** were prepared from conjugated ester **2-*E*** obtained by Wittig olefination of phthalic anhydride with the (carbethoxymethylidene)triphenylphosphorane.[31] Successive additions of methylhydrazine and hydrazine[32] in a one-pot two-step manner afforded the phthalazinone hydrazide **3** in good yield**.** This latter intermediate was condensed with methyl isothiocyanate[33] to give the mercapto-triazole main intermediate **4** or with CS_2 in the presence of KOH to afford oxadiazole **5**[34] (Scheme 1).

Next, halogenoalkylamide linkers **6-24** and **27-38** were obtained from commercially available bromoalkyl carboxylic acids activated as their acid chloride and coupled to the selected amines, mainly substituted anilines, but also hydrophobic amines such as 1-adamantylamine or 1-naphthylamine (Scheme 2). To substitute hydrophobic halogenes at the terminal aromatic part of the molecule with hydrophilic substituents, dimethoxy compound **24** was subjected to a demethylation step in the presence of BBr_3 to give the phenolic derivatives **25** and **26** (Scheme 3). Because all attempts to prepare the *N*-methylamide analog **47** later from the hit **1** failed, we had to introduce the *N*-methyl group earlier by methylation of the alkylating agent **6** with an excess of methyl iodide. This alkylation step was also reluctant and iodo amide **39** was isolated in poor yield (Scheme 2). However, it should be noted that the amide compound **43** bearing a longer alkyl linker chain than **1**, could easily be *N*-methylated in mild conditions (NaH, MeI, DMF, 0 °C) to give derivative **63** in 74 % yield.

In a final step, hit **1** and its analogs **41-54**, **56**, **64**, **66-78** and **86-90** were obtained by *S*-alkylation of the phathalazinone intermediate **4** with the appropriate bromoalkylamide reagents (Scheme 4 and Schemes 5a and 5b). In some cases, a small amount of *N*-alkylation of the phthalazinone intermediate **4** could be observed due to the tautomeric properties of the

3-mercapto-1,2,4-triazole ring.[35] In similar conditions, oxadiazole analogs **85** and **91** (Scheme 5c) were prepared starting from the thiol **5**.

Variations at the terminal arylamide part of some compounds were achieved starting from aryl ether or benzoate compounds. Following demethylation of compounds **51** and **74** in the presence of BBr_3, phenol intermediates **57** and **79** could react with suitable alkylating agents to give the corresponding phenolic ether analogs **58-60** and **80-82** in good yields (Scheme 6). On the other hand benzoic acid derivatives **61-62** and **83** were obtained from hydrolysis of the corresponding esters **52-53** and **75** with LiOH (Scheme 7).

Because the 1,2,3-triazole ring system has received considerable interest in medicinal chemistry due to its versatile potential to interact with biological systems through dipolar interactions and hydrogen bonding,[36] we opted for the formation of this heterocycle to replace the terminal amide bond function or to be introduced into a long alkyl chain linker. For this purpose, we prepared the azide **40** (Scheme 2) and the alkyne **55**, which were involved in a Huisgen 1,3-dipolar cycloaddition under copper catalysis[37] and gave access to compound **84** with 60% yield. Starting from commercially available 1-azidoadamantane, the intermediate **55** reacted in the same conditions to form compound **56**, the 1,2,3-triazole analog of *N*-1-adamantyl amide **54** (Scheme 8).

2.3 Structure-activity analyses. To date, to the best of our knowledge, no IL-15 selective inhibitor with low MW has been described in the literature. This work therefore opens the way to the first *in vitro* SAR study to provide crucial information on the key binding interactions of IL-15 with its IL-2Rβ binding site. Each fragment of compound **1** could be subjected to pertinent modifications but, among the available options, we focused this preliminary work on the study of the functional impact of the length of the alkyl linker and of the structure of the terminal amidic group. The effectiveness of the novel compounds was

evaluated using the IL-15-dependent cell proliferation assay and, for the best of them, by SPR technology and the Stat5 phosphorylation assay.

The binding properties of the compounds were first monitored by SPR. Given that the binding domains of IL-15 and IL-2 with IL-2Rβ show high similarity, we chose to study compounds activity systematically on both cytokine systems to provide the opportunity to detect potential selective compounds rapidly. In the SPR assay, the compounds were flowed over a chip's surface, on which RLI or IL-2 had previously been coupled. Binding of compounds induced a change in mass on the chip, which was detected by the change in reflectance units (RU). Direct interaction of compound **1** with immobilized RLI or IL-2 gave similar K_d values in the ten micromolar range (48 and 10 μM, respectively). In the case of RLI, consisting of IL-15 bound to IL-15Rα, the binding of compound **1** could not have occured on the IL-15 binding domain to IL-15Rα because the latter was no longer accessible.

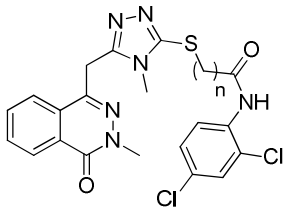
For further classic SAR exploration, we preferred to perform a functional and cellular assay with the aim of identifying a potential drug candidate. For this purpose, we first tested our hit **1** analogs, compounds **41**-**54** and **56**-**91**, for their potency in inhibiting cell proliferation induced by a fixed concentration (close to their respective EC_{50} values) of RLI (100 pM) or IL-2 (1.5 nM), on 32Dβ cells that express IL-2Rβ/γ. RLI was preferred to IL-15 because of its better efficacy (Figure S3).[38, 39] In addition, the main compounds were checked for their lack of cytotoxicity (Figure S4).

2.3.1 Probing the linker to interact with a lipophilic pocket. Based on our binding model (Figure 4), it appeared that the main phthalazinone-triazole core of compound **1** was involved in major interactions with specific residues of the binding site, such as π-alkyl hydrophobic interactions with Ile68 and Leu69, and carbon HB with Asn65. Furthermore, the model revealed a large hydrophobic patch formed by residues Val3, Ile6, Val104 and Ile111, close to the predicted location of the terminal aromatic group of compound **1** and which could be

targeted for compound optimization. We reasoned that considering the bis-cyclic aromatic part to be the main anchorage of the hit **1,** lengthening the alkyl chain could increase the affinity and activity through additive interactions with the lipophilic channel. We therefore prepared compounds **41** to **46**, which are analogs of compound **1** bearing an alkyl chain with 3 to 15 methylene groups.

In the proliferation cellular assay, the IC_{50} value followed an inverted bell-shaped curve when the length of the alkyl chain was increased (Figure S5), decreasing from 17.3 μM (n = 1) to 1.9 μM (n = 7) and increasing back to 7.3 μM (n = 15) (Table 1). Increasing the alkyl chain therefore led to a gain of one order of magnitude in activity potency and identified compounds **43-45** as potential leads (Table 1). We prepared compound **46** with a long chain (n = 15) to test the limit of such chemical modification. Surprisingly, compound **46** kept the activity, suggesting a good adaptation of this long flexible lipophilic chain probably in a folded form. However, no IL-15 versus IL-2 selectivity could be observed at this stage and the best IC_{50} values of 1.9 and 1.1 μM were obtained with compound **43** for the inhibition of IL-15- and IL-2-induced cell proliferation respectively.

Table 1. *In vitro* inhibition of 32Dβ cell proliferation with compound 1 and its homologs 41-46.



1 and 41-46

Cpd	n	IC_{50} (μM)[a]	
		RLI[b]	IL-2[c]
1	1	17.3	11.6

41	3	9.8	11.2
42	5	4.1	9.8
43	7	1.9	1.1
44	9	2.3	1.6
45	10	3.3	4.4
46	15	7.3	4.6

[a] All values are the mean of two or more independent assays. [b] Inhibition of 32Dβ cell proliferation induced by RLI (0.1 nM). [c] Inhibition of 32Dβ cell proliferation induced by IL-2 (1.5 nM).

2.3.2 SAR study around the terminal amidic part of the molecules. The terminal amidic part of the molecules, was predicted to form a stacking interaction between its substituted terminal phenyl ring and the Ser7 and Asp8 peptidic bond and an H bond between its amide function and the carboxyl function of Asp8 of IL-15. Accordingly, the *N*-methylation of the amide function (Table 2, compound **47**) decreased the inhibitory effect. Unexpectedly, the substitution of both 2,4-chloro substituents with various halogens such as bromide or fluoride (Table 2, compounds **48** and **49**) gave the same results. We next focused on the para position of the terminal phenyl group, which appeared not far from lipophilic residues such as Ile6 and Val104 in the structure of IL-15 (Figure 4B). We therefore introduced hydrophobic substituents such as iodide (Table 2 and Figure 5A, compound **50**) or alkoxy substituents with variable alkyl chains (Table 2, compounds **51**, **58**-**60**). To complete the series, we also selected the adamantane moiety as an interesting lipophilic and steric system to substitute the terminal phenyl group (Table 2, compound **54**). Uniformly, no improvement in the activity was observed compared to compound **1,** and neither with compound **56** (Figure 5A), with a triazole ring in place of the amide function in **54**. Finally, to try to favor HB interaction with Lys11, carboxylic and ester functions were introduced into the ortho (compounds **52** and **61**) or meta (compounds **53** and **62**) positions of the aromatic phenyl cycle but such modifications were detrimental for the activity because no more effect was observed (Figure 5A). In this series, all attempts to improve the biological results failed. Because the IC_{50} value of the hit

compound **1** was close to the limit of sensitivity of the cell proliferation assay, the SAR study of that series of compounds (n = 1) could not be refined up to 30 μM (Figure S6).

We then performed modulations on compounds **43** (n = 7) and **45** (n = 10) by varying the terminal amidic part of the molecules. As both compounds had a longer aliphatic linker, it could be expected that this lipophilic part of the molecule would interact more efficiently with the hydrophobic channel formed by residues Val3, Ile111, Ile6 and Val104 of IL-15. The investigation first focused on the 2- and 4-chloro substituents of the phenyl group as explored with the hit **1.** From the 2,4-dichlorophenyl analog **45** (n = 10), deletion of one chloro substituent in the para (compound **86**) or ortho (compound **87**) positions of the phenyl group afforded compounds that were three times less potent, what was not observed with the monochloro analog **71** of compound **43** (n = 7), which retained a good antiproliferative activity (IC_{50} = 0.8 μM, Figure 5B). Substitution of the phenyl ring of **45** with hydrophobic and bulky groups, such as the naphthyl and adamantyl rings retained the activity (Table 2, compounds **88** and **89**, Figure 5C) but, surprisingly, deletion of this hydrophobic amidic part of the molecule in compound **45** to give the *S*-decyl analog **90** did not dramatically affect the results (IC_{50} around 10 μM, Figure 5C).

We then turned our attention to the SAR on the terminal phenyl group of our best lead compound **43** (n = 7). Replacement of one or both chloro substituents with bromide or fluoride (compounds **64** and **66**), with an apolar group such as methyl (compounds **67**) or a substituent such as methoxy (compounds **68** and **69**) was uniformly tolerated by both IL-15 and IL-2 systems, with IC_{50} values in the micromolar range (Table 2). Deletion of the chloro substituent at the 2-position of the phenyl ring to give the 4-chloro phenyl compound **71** or analogs bearing apolar or polar systems (compounds **72**-**74**) also showed similar activity (Table 2, Figure 5D). The azide analog **73** has been developed as a potential partner for a cross-linking study with the IL-15 protein (data not shown). At this stage we could conclude

that the terminal amidic part of the leads **43** and **45** bearing a long alkyl chain did not appear to be crucial for anchorage on the binding site. Nevertheless, simple *N*-methylation of the amide function of our lead compound **43** (compound **63**) as well as 2- and 4- mono- or di-substitution with a hydroxyl function (compounds **70**, **75** and **79**), caused an approximately 10-fold decrease in the biological activity (Table 2). These results observed with phenolic compounds suggested that binding to the protein was highly sensitive to acidic functions. This was confirmed with the ester analog **76**, whose acidic form **83** showed no effect. Moreover, the ethyl ester **76** was identified as one of the best products tested with an IC_{50} value of 0.8 μM (Table 2).

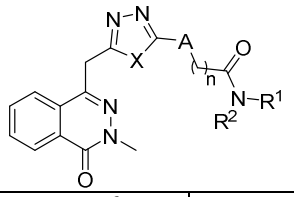
Starting with the inactive phenol analog **79**, alkylation with short alkyl chains such as methyl, propyl, isopropyl or isobutyl restored the activity (compounds **74**, **80**-**82**, Table 2 and Figure 5E). Furthermore as observed with compound **45**, substitution of the phenyl ring with a bulky lipophilic substituent such as naphthyl (compounds **77** and **88**) or adamantyl (compounds 78 and 89) preserved the activity and uniformly higher efficiency was observed with analogs with the seven-membered chain (Table 2, Figure 5C).

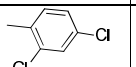
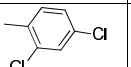
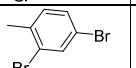
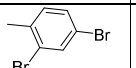
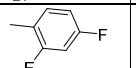
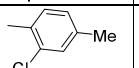
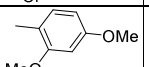
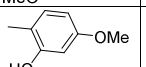
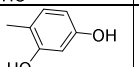
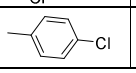
2.3.3 Modification of the mercapto triazole part of compounds 43 and 45. Suspecting potential biological oxidative events in cellular assays, we reacted the dibromo compound **64** in a chemical oxidation step with meta-chloroperbenzoic acid to afford the sulfone derivative **65**. Interestingly, the same results were obtained with the alkylsulfide parent **64** and its sulfone **65**, on IL-15 as well as on IL-2. As observed in our binding model (Figure 4), the sulfur atom is surrounded by two asparagine residues, Asn65 and Asn4, which constitute a favorable environment for the oxygen of **65** to make HB.

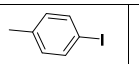
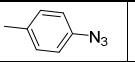
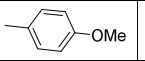
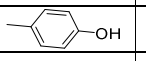
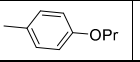
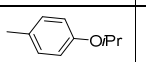
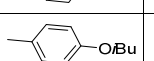
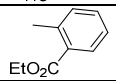
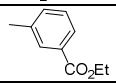
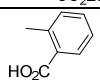
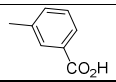
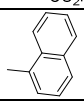
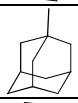
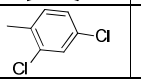
However, in relation to the model, a carbon HB with Asn65 and the methyl group of the *N*-methyl triazole moiety was predicted. Consequently, we wanted to study the substitution of this *N*-methyl system with an oxygen atom and so prepared the oxadiazole analogs **85** and **91**

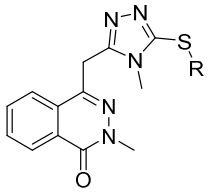
of the corresponding triazole compounds **43** and **45**. No drastic decrease of the IC_{50} values was observed and oxadiazole **85** appeared as one of the best compounds tested, with an IC_{50} of 0.8 µM on both interleukines (Table 2, Figure 5F). The formation of a HB bond between the amine function of Asn65 residue and the oxygen of the oxadiazole ring, could explain this result. Because no IL-15 versus IL-2 selectivity could be observed, we didn't go further with the oxadiazole series.

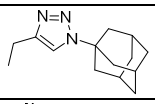
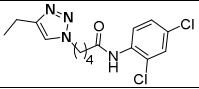
Table 2. SAR study with analogs of the selected compounds 1, 43 and 45.



				IC_{50} (µM)[a] n = 1			IC_{50} (µM)[a] n = 7			IC_{50} (µM)[a] n = 10		
X	**A**	**R1**	**R2**	**Cpd**	**RLI[b]**	**IL-2[c]**	**Cpd**	**RLI[b]**	**IL-2[c]**	**Cpd**	**RLI[b]**	**IL-2[c]**
NMe	S	H		**1**	17.3 ± 2.5	11.6 ± 1.6	**43**	1.9 ± 0.4	1.1 ± 0.2	**45**	3.3 ± 0.4	4.4 ± 1.3
NMe	S	Me		**47**	> 30	NE	**63[d]**	11.9 ± 3.6	8.4 ± 0.9		-	-
NMe	S	H		**48**	> 30	ND	**64**	1.5 ± 0.4	1.3 ± 0.5		-	-
NMe	**SO_2**	H			-	-	**65[e]**	1.4 ± 0.4	1.6 ± 1.1		-	-
NMe	S	H		**49**	NE	ND	**66**	2.3 ± 0.9	1.0 ± 0.7		-	-
NMe	S	H			-	-	**67**	2.3 ± 1.0	1.7 ± 0.5		-	-
NMe	S	H			-	-	**68**	1.1 ± 0.2	0.9 ± 0.5		-	-
NMe	S	H			-	-	**69**	1.3 ± 0.4	1.0 ± 0.2		-	-
NMe	S	H			-	-	**70**	17[f]	21[f]		-	-
NMe	S	H			-	-		-	-	**86**	15[f]	ND
NMe	S	H			-	-	**71**	0.8 ± 0.2	0.8 ± 0.2	**87**	20[f]	ND

NMe	S	H		**50**	NE	NE	**72**	1.3 ± 0.5	0.8 ± 0.4		-	-
NMe	S	H			-	-	**73**	1.8 ± 0.6	1.1 ± 0.3		-	-
NMe	S	H		**51**	NE	NE	**74**	3.9 ± 1.8	3.2 ± 1.2			
NMe	S	H		**57**	NE	NE	**79**	> 30	> 30			
NMe	S	H		**58**	> 30	> 30	**80**	1.6 ± 0.8	0.9 ± 0.7			
NMe	S	H		**59**	> 30	> 30	**81**	1.1 ± 0.5	0.6 ± 0.2		-	-
NMe	S	H		**60**	≈ 30	≈ 30	**82**	4.8 ± 1.9	3.0 ± 1.9		-	-
NMe	S	H			-	-	**75**	27[f]	23[f]		-	-
NMe	S	H		**52**	≈ 30	≈ 30		-	-		-	-
NMe	S	H		**53**	NE	NE	**76**	0.8 ± 0.1	0.6 ± 0.4		-	-
NMe	S	H		**61**	NE	NE		-	-		-	-
NMe	S	H		**62**	NE	NE	**83**	> 30	ND		-	-
NMe	S	H			-	-	**77**	1.9 ± 0.2	1.9 ± 0.4	**88**	6.6 ± 1.7	8.5[f]
NMe	S	H		**54**	NE	NE	**78**	1.6 ± 0.2	1.8 ± 0.3	**89**	3.8 ± 0.7	4.9 ± 1.4
O	S	H			-	-	**85**	0.8 ± 0.3	0.8 ± 0.5	**91**	8.7 ± 0.6	7.5[f]



R	Cpd	IC_{50} (μM)[a] RLI[b]	IC_{50} (μM)[a] IL-2[c]
	**56**	≈ 30	≈ 30
	**84**	> 30	> 30
	**90**	7.6 ± 1.5	10[f]

[a] All values are the mean of at least two independent assays. [b] Inhibition of 32Dβ cell proliferation induced by RLI (100 pM). [c] Inhibition of 32Dβ cell proliferation induced by IL-2 (1.5 nM). [d] Compound **63** was prepared by *N*-methylation of amide **43** in DMF, in the presence of NaH (1 equiv) and MeI (2 equiv). [e] Compound **65** was prepared by *S*-oxidation of thioether **64** in dichloromethane in the presence of 5 equivalents of *m*CPBA. ND: not determined, NE: no effect.

2.3.4 Measurement of direct interaction and pharmacological effect of selected compounds on IL-15 and IL-2 systems. We further analyzed our best compounds using two supplementary cytokine specific assays. We first compared them based on their direct binding to RLI or IL-2 using the SPR technology and then measured their potency in reducing the phosphorylation of Stat5, a signal transducer that is activated when IL-15 or IL-2 binds to its respective receptor expressed at the surface of NK-92 cells (Figure S7).

The advantage of the p-Stat5 assay over the cell proliferation assay used in the first SAR study, is that the readout is obtained over a short time (1 h), close to the initial cytokine receptor binding event, and does not result from the sum of several downstream signaling reactions, occurring in cascade and/or in parallel. Thus, the p-Stat5 assay represents a specific biological test more adapted to a fine study of SAR in a series of compounds.

The great majority of the selected compounds displayed K_d values for RLI binding of around 10-20 μM, close to those for IL-2 binding, when measured (Table 3). The compound displaying a somewhat weaker affinity (78 μM) in this series (n = 7) was the adamantyl derivative **78**. This was probably due to the bulky nature of the tricyclic moiety, compared to the aromatic phenyl one. Interestingly, lengthening the central alkyl linker to give the adamantyl analog **89** (n = 10), decreased the K_d value to 16 μM, suggesting a better hydrophobic interaction with the binding site on IL-15, possibly near the hydrophobic patch formed with residues Val3, Ile111, Ile6 and Val104.

Regarding the *in vitro* functional p-Stat5 results, the IC_{50} values measured were mainly around one order of magnitude lower than those found in the cell proliferation test (Table 3). This could be explained by the shorter incubation time (1 h versus 2.5 days for p-Stat5 and cell proliferation, respectively), which could have a different impact on compound integrity and efficiency. Overall, these biological results in accordance with those previously obtained

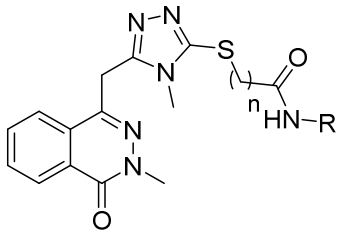
in the cell proliferation assay, confirmed the gain in efficacy by lengthening the central alkyl chain of compound **1** or **54**, to afford the dichlorophenyl analogs **43** and **45** or the adamantyl analogs **78** and **89**, respectively (Figure 5G). Here too, SAR studies with both series (n = 7 and n = 10), did not reveal a unique lead compound (Table 3). However, the best results were obtained with the phenoxy derivatives **80** and **82** and the ethyl ester analog **76** with p-Stat5 inhibitory potencies of around 50 nM (Table 3). In the ether series, compounds with alkyl chains having fewer than three extended carbons with or without ramification, such as methoxy **74** and isopropoxy **81**, were the least efficient (Figure 5H). The ester **76** could be classified as the best compound in this study with p-Stat5 inhibitory efficiencies of 57 and 43 nM with IL-15 and IL-2 respectively, and a cell proliferation IC_{50} of 0.8μM with both cytokines.

Taken together, these results led to the discovery of the first small-molecule inhibitors of the IL-15 system showing efficacies in the fifty nanomolar range in a p-Stat5 assay and in the micromolar range in a cell proliferation assay as observed with the ester **76** and the alkoxy derivatives **80** and **82.** These compounds also proved to be effective on the IL-2 system. It should be noted that similar affinity or activity values were described in earlier studies for non-peptidic small-molecule inhibitors of IL-2 however, in that case, targeting specifically the IL-2/IL-2Rα interface. [15, 16, 40, 41]

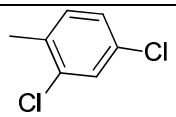
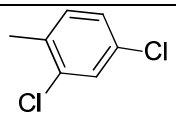
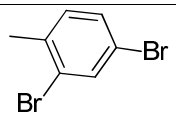
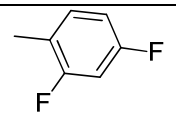
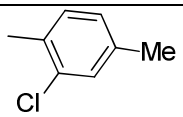
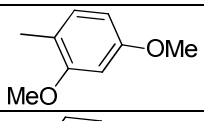
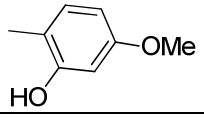
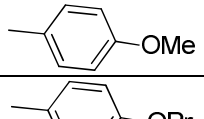
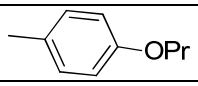
Ongoing clinical trials are being conducted with humanized Mik-Beta-1 antibody (Hu-Mik-Beta1) directed against IL-2Rβ and which targets both IL-15 and IL-2 systems. This antibody is currently tested in patients with arthritis, celiac disease, multiple sclerosis and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[42, 43] (clinicaltrials.gov), and has also been previously shown to prolong primate cardiac allograft survival.[44] Taking advantage of their combined profile, the kind of inhibitors described in this

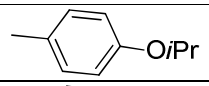
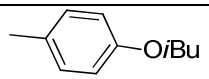
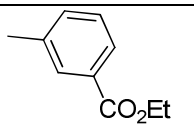
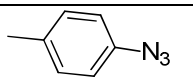
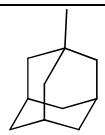
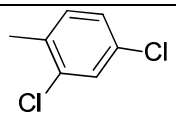
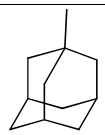
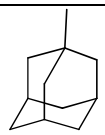
study may be of therapeutic value in pathologies where both cytokines have been shown to be involved such as RA,[17] HAM/TSP, inflammatory bowel disease, or multiple sclerosis.[45]

Table 3. Pharmacological data of selected potent compounds[a].



n = 1 : **1, 54**
n = 7 : **43, 64, 66-69 and 73, 74, 76, 78, 80-82**
n = 10 **: 45, 89**

R	n	Cpd	Direct interaction SPR (Kd µM)		Inhibition of p-Stat5 in NK92 cells (IC_{50} nM)	
			RLI linked	IL-2 linked	IL-15 (50pM)	IL-2 (250pM)
	1	**1**	48.4	10.5	9110 ± 1470	14070 ± 2170
	7	**43**	19.1	8.0	153 ± 30	160 ± 46
		**64**	11.6	10.0	92 ± 19	83 ± 20
		**66**	ND[b]	ND	110 ± 18	76 ± 10
		**67**	24.1	ND	76 ± 6	69 ± 6
		**68**	19.6	27.6	205 ± 10	162 ± 24
		**69**	21.2	12.5	360 ± 6	226 ± 17
		**74**	26.0	ND	201 ± 12	204 ± 28
		**80**	19.0	ND	40 ± 5	53 ± 1

		**81**	20.9	ND	93 ± 13	89 ± 6
		**82**	25.0	ND	55 ± 7	58 ± 14
		**76**	21.8	23.1	57 ± 2	43 ± 10
		**73**	42.4	ND	100 ± 20	73 ± 8
		**78**	78	ND	210 ± 11	152 ± 21
	10	**45**	ND	ND	142 ± 10	159 ± 16
		**89**	16.0	ND	124 ± 8	59 ± 5
	1	**54**	ND	ND	>30000	>30000

[a] All values are the mean of two or more independent assays. [b] ND = not determined.

3. CONCLUSION

In the present study, we report the development process of hit optimization from structure-based drug virtual screening to small-molecule compounds, blocking the interaction of IL-15 to its receptor with sub-micromolar efficiency. This is the first and successful attempt to target the IL-15 system by a PPI inhibitor approach, which opens the way for the identification of new classes of therapeutic drugs for IL-15 related pathologies such as RA or graft rejection. Binding and biological cell proliferation assays led to the selection of one hit among a number of compounds initially selected by virtual screening. This hit has an inhibitory activity in the ten micromolar range, and was used as a starting template for lead discovery. Pharmacomodulation studies around the linker chain and the terminal amidic part of the hit

led to inhibitors with IC_{50} values close to 50 nM in cell-based experiments, nevertheless showing the same activity on the IL-2 system. Having now identified the first class of small-molecule inhibitors of the IL-15/IL15R system, we are currently investigating the development of analogs with higher IL-15 selectivity, to rule out possible side effects to specific IL-2 blockade, such as the decrease of regulatory T cells.

4. EXPERIMENTAL SECTION

4.1. Computational study

Pharmacophore modeling. At the time of this work, the structure of IL-15 in complex with IL-2Rβ was not yet available. The X-ray crystal structure of IL-15 extracted from the structure of the IL-15/IL-15Rα complex (PDB code 2Z3Q)[21] was thus used. The protein structure was firstly prepared by adding the hydrogen atoms, removing the water molecules, and inserting the missing loop regions using the Prepare Protein tool within DS3.0. After a series of energy minimization carried out with CHARMM force field and Steepest Descent algorithm implemented into DS3.0, the protein was used for generating a pharmacophore model using the Structure Based Pharmacophore tool within DS3.0. Pharmacophore models were created from the binding site of IL-15 with IL-15Rβ based on "hot spots" residues (Asp8, Asn65 and Leu69). The superimposition of IL-15 onto IL-2 in the tetrameric complex structure IL-2:IL-2Rα/β/γ (PDB code 2B5I)[24], had led us to consider an additional orientation for the side chain of residue Asn65 of IL-15, similar to that of its homologous IL-2 residue Asn88. Moreover, the X-ray crystal structure of IL-2:IL-2R complex shows two water molecules involved in the hydrogen bonding networks between Asp20 and Asn88 (IL-2 counterpart of Asp8 and Asn65 of IL-15) and the side chains of IL-2Rβ. Thus, two water molecules close to residues Asp8 and Asn65 of IL-15 were also taken into consideration. The pharmacophore model was built using hydrogen bond donor (HBD), hydrogen bond donor

and acceptor (HBDA) and HY (hydrophobic) features on the basis of the four IL-15 residues Asp8, Asn65, Ile68 and Leu69. Two HBD projections were centered on OD1 atom within residues Asp8 and Asn65 (two orientations of its side chain considered) and a HBDA projection on OD2 atom within residue Asp8, and all were located within a sphere of 2.2 Å radius. The HY feature was manually created at distances of 5.062, 4.554 and 6.346 Å from carbon alpha (CA) atoms of residues Ile68, Leu69 and Asn65 respectively. Exclusion spheres of 1.6 Å radius were generated, centered on carbon beta (CB) atoms of residues Asn1, Asn4, Ser7, Asp8, Thr62, Asn65, Ile68, Leu69 and Asn72 and on carbon gamma (CG) atom of Lys11. Two HBDA projections were placed on water molecules 147 and 148. Finally, virtual screening was done using as main features HBD and HBDA projections on Asp8, the HY feature and the exclusion spheres and as accessory features the HBD projection on Asn65 and HBDA projections on water molecules. A hit had to match at least the main features while the presence of the accessory features was not mandatory.

***Virtual screening*.** The chemical library for virtual screening was obtained by merging commercial (Chembridge, Maybridge, Preswick) and academic collections (the Diversity set II from the NCI and the French national chemical library Chimiothèque Nationale). Duplicate structures were removed and 3D coordinates (using Catalyst) were generated using the Prepare ligand tool within DS3.0. A multi-conformation ligand database was then created using Catalyst within the Build 3D Database tool under DS3.0. The query was performed using the Search 3D Database tool with the FAST search method under DS3.0. Hits were screened for Fit values and those with a Fit value more than 1 were then taken for molecular docking analysis.

***Molecular docking*.** Molecular docking studies were performed using LigandFit option of receptor-ligand interactions protocol section available in DS3.0. Initially the protein was prepared by adding the hydrogen atoms, removing the water molecules and inserting the

missing loop regions and then was minimized using CHARMM as described above. The protein molecule thus prepared and minimized was defined as the total receptor. Four docking conditions (4 targets: IL-15 protein with residue Asn65 side chain in its initial orientation (as referred to structure 2Z3Q) or in an orientation close to that of its IL-2 homolog Asn88 (from structure 2B5I), as well as each of these targets with 2 water molecules) were conducted in parallel. For each condition, the ligand molecules retained by the pharmacophore model were docked into a binding site facing residues Asn1, Asn4, Ser7, Asp8, Lys11, Thr62, Asn65, Ile68, Leu69 and Asn72 of IL-15 structures. The volumes of the binding site were 1396, 1538, 1481 and 1523 $Å^3$ and contained 11171, 12304, 11851 and 12188 points respectively to the 4 targets described above. Docking was performed using CFF as the energy grid. A penalty of 200 kcal/mol/atom was set up to reduce the dock score of poses that occurred outside of the binding site. The conformational search of the ligand poses was performed by the Monte Carlo trial method. Maximum internal energy was set at 10000 kcal/mol. A short rigid body minimization was then performed (steepest descent and Broyden Fletcher Goldfarb Shanno (BFGS) minimizations). Twenty poses were saved for each ligand after docking and 100 steps of BFGS rigid body minimization were then carried out. Scoring was performed with five scoring functions: LigScore1, Ligscore2,[46] PLP1, PLP2[47] and PMF.[48, 49] CFF force field was used for LigScore calculations. Among the 24115 compounds retained after the pharmacophore-based search, 7991 were actually docked to the targets. To improve the screening accuracy, a consensus strategy was adopted after dividing compounds in subsets based on MW (MW $\leq$ 600 and > 600 Da) and number of rotatable bonds (rotatable bonds $\leq$ 5 and > 5). Compounds with a consensus score equal to 5 (i.e. in the TOP 40 % for each scoring function) were retained within each subset for each docking condition. All together, 1588 compounds were thereby retained. They were ranked by rescoring them against each 4 targets and summing the four consensus score obtained (maximal score = 20).

The top-ranked 20 % were selected and represented 315 compounds, 240 of which were actually available and purchased for biological testing.

Binding mode and molecular interactions analysis of compound 1. In order to determine the potential binding mode of compound 1, we performed a refinement step on the top 50 poses obtained from two more docking runs, one using LigandFit and the other using C-Docker, another docking program implemented under DS3.0. This refinement procedure consisted in a simulated annealing refinement (2000 steps at 700K, 5000 steps at 300K) followed by a final full forcefield minimization using CHARMM (100 steps of steepest descent followed by conjugate gradient with gradient tolerance of 0.01). The 100 poses retained with the two docking runs were compared in term of interaction energy and those with the most favorable binding were further visually validated for their proper docking. The top 10 poses belonged to the same cluster and one of them has been selected.

Images for the figures 1 and 4 were captured from DS3.0 visualizer and treated with Adobe Photoshop CS4 software.

4.2 Chemistry. *General.* Melting points were determined on a Stuart melting point SMP3 apparatus. All solvents used were reagent grade and TLC was performed on silica-covered aluminum sheets (Kieselgel $60F_{254}$, MERCK). Eluted TLC was revealed using UV radiation (λ = 254 nm), or molybdate solution. Flash column chromatography was performed on silica gel 60 ACC 40-63 μm (SDS-CarloErba). NMR spectra were recorded on a BRUKER AC300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) or on a BRUKER 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) at room temperature, on samples dissolved in an appropriate deuterated solvent. References of tetramethylsilane (TMS) for ^{1}H and deuterated solvent signal for ^{13}C were used. Chemical displacement values (δ) are expressed in parts per million (ppm), and coupling constants (J) in Hertz (Hz). Clearly identified proton and carbon were specified (for dihydrophthalazinone moiety as *Phthal* and for triazole moiety as *Triaz*). Low-resolution

mass spectra (MS in Da unit) were recorded in the CEISAM laboratory on a Thermo-Finnigan DSQII quadripolar at 70 eV (CI with NH_3 gas) or on a Waters Xevo G2-XS QTOF. High-Resolution Mass Spectrometry (HRMS in Da unit) analyses were recorded on an LC-Q-TOF (Synapt-G2 HDMS, Waters) in the IRS-UN center (Mass Spectrometry platform, Nantes) or on a MALDI -TOF-TOF apparatus (Autoflex III from Bruker) in the INRA center (BIBS platform, Nantes). The purity of the tested compounds was determined by analytical UPLC-MS (Acquity H-Class- Xevo G2-XS Qtof) using C18 reverse-phase column [BEH (1.7 µm, 2.1 × 50 mm)] and was over 95%. The compounds described in Tables 1-3 were checked for the presence of PAINS substructures as described by Baell and Holloway,[50] using FAF-Drugs3[51] server and no PAINS substructures were identified except in **73** which was only developed for the purpose of carrying out cross-linking studies with IL-15 protein .

Synthesis and chemical characteristics of the halogenoalkylamides **6-39** and the azidoalkylamide **40** are detailed in the Supporting Information.

General Synthetic Procedure 1 for the Synthesis of Bromoalkylamides. The commercial bromoacid reagent (1 eq) was dissolved in anhydrous DCM and oxalyl chloride (1.2 eq) was added at room temperature. A catalytic amount of DMF was then added. The mixture was stirred under argon atmosphere during 3 h. Carboxylic acid complete conversion to acyl chloride was determined by ^{1}H NMR in deuterated benzene after concentration in vacuum. The acyl chloride was taken up in anhydrous THF and the amine (0.85 eq) was then added at 0 °C, followed by triethylamine (2.5 eq). After completion of the reaction, water was added and the aqueous phase was extracted with Et_2O (the aqueous layer was acidified to pH = 2 for compounds with phenol function). The organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The expected bromoalkylamide product was then purified by flash column chromatography on silica gel.

*General Synthetic Procedure 2 for the S-alkylation of 4-((5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (**4**) and 4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-methylphthalazin-1(2H)-one (**5**) with halogenoalkyles or halogenoalkylamides to give compounds **1**, **41-54**, **64**, **66-78**, **86-91.*** To a suspension of dry K_2CO_3 (3 eq, 3 mmol) in DMF (8 mL) was added thiol **4** (1 mmol). The mixture was stirred for 15 min and a solution of bromoalkylamide (1.5 eq) in CH_2Cl_2 (1 mmol.mL^{-1}) was added. The mixture was then stirred at room temperature until starting material disappeared (2 h to 18 h). After completion of the reaction, a saturated solution of ammonium chloride was added. The mixture was extracted twice with ethyl acetate. The organic layer was washed several times with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel.

*N-(2,4-dichlorophenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**1**).* Following procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 2-bromo-*N*-(2,4-dichlorophenyl)acetamide **6** (3 mmol, 0.84 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **1** in 81% yield, as a white powder; mp 201.5 - 202 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.0 (bs, 1H, NH), 8.45 (dd, J = 7.8 and J = 1.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.23 (d, J = 8.8 Hz, 1H, H_{ar}), 8.19 (dd, J =7.8 and J = 1.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.82 (ddd, J = 7.9, J = 7.8 and J = 1.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.77 (ddd, J = 7.9, J = 7.8 and J = 1.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.31 (d, J = 2.4 Hz, 1H, H_{ar}), 7.18 (dd, J = 8.8 and J = 2.4 Hz, 1H, H_{ar}), 4.49 (s, 2H, CH_2), 4.05 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$)) δ 167.1 (CONH), 159.5 (CONMe), 152.9 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.5 ($C_{IV\text{-}C=N}$), 134.0 (C_{IV}),

133.5 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 129.6 (C_{IV}), 129.1 (CH_{ar}), 129.0 (C_{IV}), 128.2 (C_{IV}); 127.6 (CH_{ar}), 127.3 ($CH_{ar\text{-}Phthal}$), 125.1 ($CH_{ar\text{-}Phthal}$), 124.7 (C_{IV}), 123.2 (CH_{ar}), 39.5 (NMe_{Phthal}), 36.0 (CH_2), 30.8 (NMe), 30.02 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{21}H_{19}Cl_2N_6O_2S$ $(M+H)^+$ 489.0662, found 489.0637.

*2-(3-Methyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetohydrazide (**3**)*.[31] To a solution of ethyl [(*E*)-3-oxo-1,3-dihydroisobenzofuran-1-ylidene]acetate **2-*E*** (13 mmol, 2.83 g,) in EtOH (15 mL), was added dropwise methylhydrazine (13 mmol, 0.52 mL). The mixture was then stirred for 30 min and hydrazine monohydrate was added dropwise (6 eq, 78 mmol, 4.45 mL). The solution was finally heated at reflux during 3h and the resulting suspension was cooled to 20 °C and filtered. The white powder obtained was washed with Et_2O to give 94% of hydrazide **3** which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (bs, 1H, NH), 8.28 (d, J = 7.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.92 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.85 (m, J = 4.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 4.27 (bs, 2H, NH_2), 3.77 (s, 2H, CH_2), 3.70 (s, 3H, NMe). HRMS (MALDI: DHB, PEG 600) calcd for $C_{11}H_{13}N_4O_2$ $(M+H)^+$ 233.1033, found 233.1035.

*4-((5-Mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (**4**)*. To a suspension of hydrazide **3** (12 mmol, 2.8 g) in EtOH (28 mL) was added methylisothiocyanate (5 eq, 60 mmol, 4.54 g), triethylamine (5 eq, 60 mmol, 8 mL) and the reaction mixture was heated to reflux during 8 h. The resulting suspension was cooled to 20 °C, filtered and washed with Et_2O to give compound **4** with 99% yield as a yellowish powder. This one was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.56 (bs, 1H, SH), 8.30 (m, 1H, $H_{ar\text{-}Phthal}$), 7.97-7.84 (m, 3H, 3 x $H_{ar\text{-}Phthal}$), 4.51 (s, 2H, CH_2), 3.67 (s, 3H, NMe_{Phthal}), 3.49 (s, 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (C_{IV}-

$_{SH}$), 158.4 (CONMe), 149.6 ($C_{IV-C=N}$), 140.3 ($C_{IV-C=N}$), 133.2 ($CH_{ar-Phthal}$), 131.9 ($CH_{ar-Phthal}$), 128.7 (C_{IV}), 127.1 (C_{IV}), 126.0 ($CH_{ar-Phthal}$), 125.6 ($CH_{ar-Phthal}$), 39.0 (NMe_{Phthal}), 30.2 (NMe), 28.3 (CH_2). HRMS (ESI^+): calcd for $C_{13}H_{14}N_5OS$ $(M+H)^+$ 288.0914, found 288.0919.

4-((5-Mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-methylphthalazin-1(2H)-one ***(5)****.* The compound was prepared as earlier described for the *N*-allyl analog.[34] To a suspension of hydrazide **3** (1 mmol, 0.23 g) in CS_2 (10 mL) was added KOH (2.5 eq, 2.5 mmol) and the reaction mixture was heated to reflux under vigorous stirring during 3 h. It was cooled to 10 °C and the precipitate formed was filtered, washed with Et_2O. The powder was stirred in hot ethanol and filtered to give the compound **5** with 65% yield as a grey powder. This one was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (m, 1H, $H_{ar-Phthal}$), 7.98 (m, 3H, 3 x $H_{ar-Phthal}$), 4.49 (s, 2H, CH_2), 3.70 (s, 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2 (C_{IV-SH}), 160.5 (CO), 158.3 ($C_{IV-C=N}$), 139.7 ($C_{IV-C=N}$), 133.4 ($CH_{ar-Phthal}$), 132.1 ($CH_{ar-Phthal}$), 128.5 (C_{IV}), 127.1, (C_{IV}) 126.2 ($CH_{ar-Phthal}$), 125.4 ($CH_{ar-Phthal}$), 45.4 (NMe), 29.1 (CH_2). HRMS (ESI^-): calcd for $C_{12}H_9N_4O_2S$ $(M-H)^-$ 273.0446, found 273.0445.

N-(2,4-dichlorophenyl)-4-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)butanamide ***(41)*****.** Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 4-bromo-*N*-(2,4-dichlorophenyl)butanamide **7** (3 mmol), purification by column chromatography on silica gel (DCM/MeOH; 95:5) afforded compound **41** in 72% yield, as a white powder; mp 195 - 196 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (m, 1H, $H_{ar-Phthal}$), 8.17 (m, 2H, $H_{ar-Phthal}$ and H_{ar}), 8.01 (m, 1H, H_{ar}), 7.77 (ddd,*J* = 8.2 and *J* = 7.3 and *J* = 1.5 Hz, 1H, $H_{ar-Phthal}$), 7.72 (ddd,*J* = 8.2, *J* = 7.8 and *J* = 1.3 Hz, 1H,

$H_{ar\text{-}Phthal}$), 7.31 (d, J = 2.3 Hz, 1H, H_{ar}), 7.19 (dd, J = 8.9 and J = 2.4 Hz, 1H, H_{ar}), 4.44 (s, 2H, CH_2), 3.77 (s, 3H, NMe_{Phthal}), 3.55 (s, 3H, NMe), 3.27 (t, J = 7.1 Hz, 2H, SCH_2), 2.61 (t, J = 7.1 Hz, 2H, CH_2CO), 2.17 (tt, J = 7.1 Hz, J = 7.1 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CONH), 159.5 (CONMe), 152.2 ($C_{IV\text{-}C=N}$), 151.5 ($C_{IV\text{-}C=N}$), 140.7 ($C_{IV\text{-}C=N}$), 133.4 (C_{IV}), 133.3 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.0 (C_{IV}), 128.9 (C_{IV}), 128.8 (C_{IV}), 127.8 (CH_{ar}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.2 ($CH_{ar\text{-}Phthal}$), 124.1 (C_{IV}), 123.2 (CH_{ar}), 39.5 (NMe_{Phthal}), 35.8 (CH_2), 32.2 (CH_2), 30.7 (NMe), 30.0 (CH_2), 25.6 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{23}H_{22}Cl_2N_6O_2SNa$ $(M+Na)^+$ 539.0794, found 539.0819.

*N-(2,4-dichlorophenyl)-6-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)hexanamide (**42**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 6-bromo-*N*-(2,4-dichlorophenyl)hexanamide **9** (3 mmol), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **42** in 56% yield, as a white powder; mp 184 - 185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (m, 1H, $H_{ar\text{-}Phthal}$), 8.27 (d, J = 8.9 Hz, 1H, H_{ar}), 8.20 (m, 1H, $H_{ar\text{-}Phthal}$), 7.78 (ddd, J = 7.8, J = 7.4 and J = 1.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.74 (ddd, J = 7.8 and J = 8.0 Hz, J = 1.3, 1H, $H_{ar\text{-}Phthal}$), 7.67 (bs, 1H, NH), 7.33 (d, J = 2.4 Hz, 1H, H_{ar}), 7.21 (dd, J = 8.9 and J = 2.4 Hz, 1H, H_{ar}), 4.46 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.18 (t, J = 7.3 Hz, 2H, SCH_2), 2.41 (t, J = 7.3 Hz, 2H, CH_2CO), 1.77 (m, 4H, 2 x CH_2), 1.51 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (CONH), 159.5 (CONMe), 152.0 ($C_{IV\text{-}C=N}$), 151.8 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.5 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.2 (C_{IV}), 129.1(C_{IV}), 128.8 (CH_{ar}), 128.1 (C_{IV}), 127.9 (CH_{ar}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 123.6 (C_{IV}), 122.8 (CH_{ar}), 39.5 (NMe_{Phthal}), 37.4 (CH_2), 32.7 (CH_2), 30.7 (NMe), 30.2 (CH_2),

29.2 (CH_2), 28.0 (CH_2), 24.8 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{25}H_{27}Cl_2N_6O_2S$ $(M+H)^+$ 545.1288, found 545.1281.

*N-(2,4-dichlorophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**43**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(2,4-dichlorophenyl)octanamide **10** (3 mmol, 1.09 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **43** in 53% yield, as a white powder; mp 165 - 166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.31 (d, J = 9.0 Hz, 1H, H_{ar}), 8.22 (m, 1H, $H_{ar\text{-}Phthal}$), 7.80 (m, 1H, $H_{ar\text{-}Phthal}$), 7.75 (m, 1H, $H_{ar\text{-}Phthal}$), 7.60 (bs, 1H, NH), 7.35 (d, J = 2.4 Hz, 1H, H_{ar}), 7.23 (dd, J = 9.0 and J = 2.4 Hz, 1H, H_{ar}), 4.47 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.18 (t, J = 7.3 Hz, 2H, SCH_2), 2.40 (t, J = 7.5 Hz, 2H, CH_2), 1.73 (m, 4H, 2 x CH_2), 1.48 – 1.29 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (CONH), 159.6 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.6 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 128.8 (CH_{ar}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 123.3 (C_{IV}), 122.6 (CH_{ar}), 39.5 (NMe_{Phthal}), 37.9 (CH_2), 33.1 (CH_2), 30.7 (NMe), 30.2 (CH_2), 29.5 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.4 (CH_2), 25.4 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{27}H_{31}Cl_2N_6O_2S$ $(M+H)^+$ 573.1601, found 573.1583.

*N-(2,4-dichlorophenyl)-10-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)decanamide (**44**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 10-bromo-*N*-(2,4-dichlorophenyl)decanamide **11** (3 mmol, 1.17 g), purification by column chromatography on

silica gel (DCM/MeOH; 98:2) afforded compound **44** in 84% yield, as a white powder; mp 124 - 126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (m, 1H, $H_{ar\text{-}Phthal}$), 8.32 (d, J = 8.8 Hz, 1H, H_{ar}), 8.21 (m, 1H, $H_{ar\text{-}Phthal}$), 7.79 (m, 1H, $H_{ar\text{-}Phthal}$), 7.74 (m, 1H, $H_{ar\text{-}Phthal}$), 7.61 (bs, 1H, NH), 7.35 (d, J = 2.4 Hz, 1H, H_{ar}), 7.22 (dd, J = 8.9 and J = 2.4 Hz, 1H, H_{ar}), 4.46 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.17 (t, J = 7.4 Hz, 2H, SCH_2), 2.41 (t, J = 7.5 Hz, 2H, CH_2CO), 1.70 (m, 4H, 2 x CH_2), 1.40-1.29 (m, 12H, 6 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (CONH), 159.5 (CONMe), 152.1 (C_{IV}), 152.0 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.6 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 129.0 (C_{IV}), 128.8 (CH_{ar}), 128.0 (C_{IV}), 127.9 ($CH_{ar\text{-}Phthal}$), 127.2 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 123.3 (C_{IV}), 122.6 (CH_{ar}), 122.5 (CH_{ar}), 39.5 (NMe_{Phthal}), 37.9 (CH_2CO), 33.2 (SCH_2), 30.7 (NMe), 30.2 (CH_2), 29.6 (CH_2), 29.6-28.6 (6 x CH_2), 25.5 (CH_2) HRMS (ESI$^+$): calcd for $C_{29}H_{35}Cl_2N_6O_2S$ $(M+H)^+$ 601.1913, found 601.1902.

*N-(2,4-dichlorophenyl)-11-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)undecanamide (**45**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 11-bromo-*N*-(2,4-dichlorophenyl)undecanamide **12** (3 mmol, 1.21 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **45** in 65% yield, as a white powder; mp 133 - 134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, J = 7.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.32 (d, J = 8.8 Hz, 1H, CH_{ar}), 8.21 (d, J = 7.9 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.79 (ddd, J = 8.2, J = 7.4 and J = 1.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.74 (ddd,J = 8.2, J = 7.8 and J = 1.2 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.60 (bs, 1H, NH), 7.35 (d, J = 2.4 Hz, 1H, H_{ar}), 7.22 (dd, J = 8.9 Hz, J = 2.4 Hz, 1H, H_{ar}), 4.46 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.17 (t, J = 7.4 Hz, 2H, SCH_2), 2.41 (t, J = 7.3 Hz, 2H, CH_2CO), 1.70 (m, 4H, 2 x CH_2), 1.4-1.18 (m, 12H, 6 x CH_2). ^{13}C NMR (75 MHz,

$CDCl_3$) δ 171.5 (CONH), 159.5 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.6 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 129.0 (C_{IV}), 128.8 (CH_{ar}), 128.0 (C_{IV}), 127.9 (CH_{ar}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 123.3 (C_{IV}), 122.5 (CH_{ar}), 39.5 (NMe_{Phthal}), 37.9 (CH_2CO), 33.2 (SCH_2), 30.7 (NMe), 30.2 (CH_2), 29.6-28.6 (6 x CH_2), 25.5 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{30}H_{37}Cl_2N_6O_2S$ $(M+H)^+$ 615.2070, found 615.2076.

*N-(2,4-dichlorophenyl)-16-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)hexadecanamide (**46**)* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 16-bromo-N-(2,4-dichlorophenyl)hexadecanamide **13** (3 mmol, 1.43 g), purification by column chromatography on silica gel (DCM/MeOH; 95:5) afforded compound **46** in 58% yield, as a white powder; mp 116 - 117.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (m, 1H, $H_{ar\text{-}Phthal}$), 8.36 (d, J = 8.9 Hz, 1H, CH_{ar}), 8.24 (m, 1H, $H_{ar\text{-}Phthal}$), 7.79 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.58 (bs, 1H, NH), 7.36 (d, J = 2.3 Hz, 1H, H_{ar}), 7.23 (dd, J = 8.9 and J = 2.4 Hz, 1H, H_{ar}), 4.54 (s, 2H, CH_2), 3.80 (s, 3H, NMe_{Phthal}), 3.56 (s, 3H, NMe), 3.21 (t, J = 7.4 Hz, 2H, SCH_2), 2.42 (t, J = 7.3 Hz, 2H, CH_2CO), 1.73 (m, 4H, 2 x CH_2), 1.4-1.23 (m, 18H, 9 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3 (CONH), 159.3 (CONMe), 151.9 ($C_{IV\text{-}C=N}$), 151.7 ($C_{IV\text{-}C=N}$), 140.6 ($C_{IV\text{-}C=N}$), 133.3 (C_{IV}), 133.2 ($CH_{ar\text{-}Phthal}$), 131.7 ($CH_{ar\text{-}Phthal}$), 128.9 (C_{IV}), 128.8 (C_{IV}), 128.5 (CH_{ar}), 127.8 (C_{IV}), 127.7 (CH_{ar}), 126.9 ($CH_{ar\text{-}Phthal}$), 125.2 ($CH_{ar\text{-}Phthal}$), 123.1 (C_{IV}), 122.3 (CH_{ar}), 39.3 (NMe_{Phthal}), 37.8 (CH_2CO), 33.0 (SCH_2), 30.5 (NMe), 30.0 (CH_2), 29.5 (CH_2), 29.4-29.3-29.2-29.1-29.0-28.6 (10 x CH_2), 25.4 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{35}H_{47}Cl_2N_6O_2S$ $(M+H)^+$ 685.2853, found 685.2824.

*N-(2,4-dichlorophenyl)-N-methyl-2-((4-methyl-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**47**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and *N*-(2,4-dichlorophenyl)-2-iodo-*N*-methylacetamide **39** (3 mmol, 1.02 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **47** in 52% yield, as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (dd, J = 7.7 and J = 1.4 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.17 (dd, J =7.4 and J = 1.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.51 (d, J = 2.4 Hz, 1H, H_{ar}), 7.25 (m, 2H, 2 x H_{ar}), 4.45 (m, 2H, CH_2), 3.95 (AB system, J = 15.4 Hz, 1H, C*H*H'), 3.80 (s, 3H, NMe_{Phthal}), 3.66 (AB system, J = 15.6 Hz, 1H, CH*H'*), 3.53 (s, 3H, NMe_{Triaz}), 3.28 (s, 3H, CONMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1 (CONH), 159.6 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 151.0 ($C_{IV\text{-}C=N}$), 140.7 ($C_{IV\text{-}C=N}$), 138.8 (C_{IV}), 135.8 (C_{IV}), 133.9 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 131.0 (CH_{ar}), 130.9 (CH_{ar}), 129.2 (CH_{ar}), 129.1 (C_{IV}), 129.1 (C_{IV}), 127.3 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 39.5 (NMe_{Phthal}), 36.9 (NMe), 36.7 (CH_2), 30.9 (NMe), 30.1 (CH_2). HRMS (ESI^+): calcd for $C_{22}H_{20}Cl_2N_6O_2NaS$ $(M+Na)^+$ 525.0643, found 525.0649.

*N-(2,4-dibromophenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**48**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 2-bromo-*N*-(2,4-dibromophenyl)acetamide **14** (3 mmol, 1.15 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **48** in 75% yield, as a white powder; mp 224 - 225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (bs, 1H, NH), 8.45 (m, 1H, $H_{ar\text{-}Phthal}$), 8.19 (m ,1H, $H_{ar\text{-}Phthal}$), 8.12 (d, J = 8.84 Hz, 1H, H_{ar}), 7.79 (m , 2H, 2 x $H_{ar\text{-}Phthal}$), 7.68 (d, J = 2.20 Hz, 1H, H_{ar}), 7.37 (dd, J = 8.90 and J = 2.2 Hz, 1H, H_{ar}), 4.49 (s, 2H, CH_2), 4,07 (s, 2H, CH_2),

3.80 (s, 3H, NMe_{Phthal}), 3.58 (s, 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1 (CONH), 159.5 (CONMe), 152.9 ($C_{IV-C=N}$), 151.9 ($C_{IV-C=N}$), 140.5 ($C_{IV-C=N}$), 135.6 (C_{IV}), 134.9 (CH_{ar}), 133.5 ($CH_{ar-Phthal}$), 132.2 ($CH_{ar-Phthal}$), 131.1 (CH_{ar}), 129.0 (C_{IV}), 128.1 (C_{IV}), 127.4 ($CH_{ar-Phthal}$), 125.1 ($CH_{ar-Phthal}$), 124.0 (CH_{ar}), 117.4 (C_{IV}), 115.0 (C_{IV}), 39.6 (NMe_{Phthal}), 36.0 (CH_2), 30.9 (NMe), 30.0 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{21}H_{19}Br_2N_6O_2S$ $(M+H)^+$ 576.9651, found 576.9640.

*N-(2,4-difluorophenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**49**).* Following procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (2 mmol, 0.58 g) and 2-bromo-*N*-(2,4-difluorophenyl)acetamide **15** (5 mmol, 1.2 g), purification by column chromatography on silica gel (DCM/MeOH; 99:1) afforded compound **49** in 21% yield, as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 10.28 (bs, 1H, NH), 8.45 (dd , J = 7.9 and J = 1.16Hz, 1H, $H_{ar-Phthal}$), 8.21 (d, J = 7.94 Hz, 1H, $H_{ar-Phthal}$), 8.15 (m, 1H, H_{ar}), 7.81 (m, 1H, $H_{ar-Phthal}$), 7.78 (m, 1H, $H_{ar-Phthal}$), 6.81 (m, 1H, H_{ar}), 6.79 (m, 1H, H_{ar}), 4.51 (s, 2H, CH_2), 3.97 (s, 2H, CH_2), 3.8 (s, 3H, NMe_{Phthal}), 3.58 (s, 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (CONH), 159.5 (CONMe), 158.9 (dd, J_{CF} = 244 Hz , J_{CF} = 11 Hz, C_{IV}), 153.3 (dd, J_{CF} = 247 Hz, J_{CF} = 11.5 Hz, C_{IV}), 152.8 ($C_{IV-C=N}$), 152.5 ($C_{IV-C=N}$), 140.4 ($C_{IV-C=N}$), 133.5 ($CH_{ar-Phthal}$), 132.1 ($CH_{ar-Phthal}$), 129 (C_{IV}), 128.2 (C_{IV}), 127.3 ($CH_{ar-Phthal}$), 125.1 ($CH_{ar-Phthal}$), 123.2 (d, J_{CF} = 8.94 Hz, CH_{ar}), 122.9 (dd; J_{CF} = 11.7 Hz, J_{CF} = 3.8Hz, C_{IV}), 111.1 (dd, J_{CF} = 21.63 Hz, J_{CF} = 3.36 Hz, CH_{ar}), 103.7 (dd, J_{CF} = 27.28 Hz, J_{CF} = 23.11 Hz, CH_{ar}), 39.5 (NMe_{Phthal}), 36.1 (CH_2), 31.0 (NMe), 30.0 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{21}H_{19}F_2N_6O_2S$ $(M+H)^+$ 457.1255, found 457.1250.

*N-(4-iodophenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**50**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (0.5 mmol, 0.15 g) and 2-bromo-*N*-(4-iodophenyl)acetamide **16** (1.1 eq, 0.6 mmol), purification by stirring the precipitate in dichloromethane followed by filtration, afforded **50** in 52% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.53 (s, 1 H, NH), 8.29 (m, 1H, $H_{ar\text{-}Phthal}$), 8.05 (m, 1H, $H_{ar\text{-}Phthal}$), 7.88 (m, 2H, $H_{ar\text{-}Phthal}$), 7.63 (m, 2H, H_{ar}), 7.39 (m, 2H, H_{ar}), 4.53 (s, 2H, CH_2), 4.02 (s, 2H, CH_2CO), 3.65 (s, 3H, NMe_{Phthal}), 3.59 (s, 3H, NMe). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1 (CONH), 158.4 (CONMe), 153.0 ($C_{IV\text{-}C=N}$), 149.1 ($C_{IV\text{-}C=N}$), 141.1 ($C_{IV\text{-}C=N}$), 138.7 (C_{IV}), 137.3 (CH_{ar}), 133.2 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 128.9 (C_{IV}), 127.2 (C_{IV}), 126.0 ($CH_{ar\text{-}Phthal}$), 125.8 ($CH_{ar\text{-}Phthal}$), 121.2 (CH_{ar}), 87.0 ($C_{IV\text{-}C\text{-}I}$), 38.9 (NMe_{Phthal}), 37.7 (CH_2CO), 30.6 (NMe), 28.8 (CH_2). HRMS (ESI^+) calcd for $C_{21}H_{19}N_6O_2SINa$ $(M+Na)^+$ 569.0227, found 569.0228.

*N-(4-methoxyphenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**51**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 2-bromo-*N*-(4-methoxyphenyl)acetamide **17** (1.5 mmol, 0.36 g), purification by column chromatography on silica gel (DCM/MeOH; 97:3) afforded compound **51** in 80% yield, as a white powder; mp 246-247 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.05 (bs, 1H, NH), 8.45 (dd, J = 7.4 Hz, J = 1.5Hz, 1H, $H_{ar\text{-}Phthal}$), 8.17 (dd, J = 7.4 Hz, J = 1.2 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.81 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.44 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.80 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.49 (s, 2H, CH_2), 3.91 (s, 2H, CH_2); 3.80 (s, 3H, NMe_{Phthal}), 3.76 (s, 3H, OMe); 3.59 (s, 3H, NMe). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3 (CONH), 159.5 (CONMe), 156.4 (C_{IV}), 152.8 ($C_{IV\text{-}C=N}$), 152.8 ($C_{IV\text{-}$

$_{C=N}$), 140.4 ($C_{IV-C=N}$), 133.5 ($CH_{ar-Phthal}$), 132.1 ($CH_{ar-Phthal}$), 131.5 (C_{IV}), 129.0 (C_{IV}), 128.1 (C_{IV}), 127.4 ($CH_{ar-Phthal}$), 125.0 ($CH_{ar-Phthal}$), 121.5 (2 x CH_{ar}), 114.1 (2 x CH_{ar}), 55.6 (OMe), 39.6 (NMe $_{Phthal}$), 36.3 (CH_2), 31.0 (NMe), 29.9 (CH_2). HRMS (ESI^+): calcd for $C_{22}H_{22}N_6O_3NaS$ $(M+Na)^+$ 473.1372, found 473.1363.

*Ethyl 2-(2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydroPhthalazine-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamido)benzoate (**52**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and ethyl 3-(2-bromoacetamido)benzoate **18** (2 mmol, 0.58 g), purification by column chromatography on silica gel (DCM/MeOH; 97:3) afforded compound **52** in 61% yield, as a white powder; mp 165 - 166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.52 (bs, 1H, NH), 8.59 (dd , J = 8.53 and J = 0.92 Hz, 1H, H_{ar}), 8.43 (m , 1H, $H_{ar-Phthal}$), 8.18 (m, 1H, $H_{ar-Phthal}$), 7.99 (dd, J = 8.05 and J = 1.59 Hz, H_{ar}), 7.75 (m, 2H, 2 x $H_{ar-Phthal}$), 7.50 (m, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 4.47 (s, 2H, CH_2), 4.30 (q, J = 8.14 Hz, 2H, CH_2CH_3), 4.16 (s, 2H, CH_2), 3.79 (s, 3H, NMe $_{Phthal}$), 3.64 (s, 3H, NMe), 1.38 (t, J = 8.0 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1 (*C*OOEt), 165.9 (CONH), 159.6 (CONMe), 152.5 ($C_{IV-C=N}$), 150.5 ($C_{IV-C=N}$), 140.9 (C_{IV}), 140.7 ($C_{IV-C=N}$), 134.5 (CH_{ar}), 133.4 ($CH_{ar-Phthal}$), 132.0 ($CH_{ar-Phthal}$), 130.9 (CH_{ar}), 129.1 (C_{IV}), 128.1 (C_{IV}), 127.2 ($CH_{ar-Phthal}$), 125.3 ($CH_{ar-Phthal}$), 123.2 (CH_{ar}), 120.7 (CH_{ar}), 116.0 (C_{IV}), 61.6 (CH_2CH_3), 39.5 (NMe_{Phthal}), 38.3 (CH_2), 31.0 (NMe), 30.2 (CH_2), 14.3 (CH_2CH_3). HRMS (MALDI: DHB, PEG 600): calcd for $C_{24}H_{24}N_6O_4NaS$ $(M+Na)^+$ 515.1472, found 515.1462.

*Ethyl 3-(2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydroPhthalazine-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamido)benzoate (**53**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-

one **4** (1 mmol, 0.29 g), and ethyl 3-(2-bromoacetamido)benzoate **19** (1.5 mmol, 0.42 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **53** in 68% yield, as a white powder; mp 211 - 212 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.36 (bs, 1H, NH), 8.45 (m , 1H, $H_{ar\text{-}Phthal}$), 8.18 (m, 2H, H_{ar} and $H_{ar\text{-}Phthal}$), 7.79 (m , 4H, 2 x H_{ar} and 2 x $H_{ar\text{-}Phthal}$), 7.34 (dd, 2H, J = 8.0 Hz, 1H, H_{ar}), 4.5 (s, 2H, CH_2), 4.36 (q , J = 8.14 Hz, 2H, CH_2CH_3), 3.95 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.64 (s, 3H, NMe), 1.38 (t, J = 7.16 Hz, 3H, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.8 (*C*ONH), 166.4 (COOEt or CONH), 159.6 (CONMe or COOEt), 152.8 ($C_{IV\text{-}C=N}$), 152.7 ($C_{IV\text{-}C=N}$), 140.72 ($C_{IV\text{-}C=N}$), 138.4 (C_{IV}), 133.5 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 131.5 (C_{IV}), 129.1 (C_{IV}), 129.0 (CH_{ar}), 128.2 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 127.4 ($CH_{ar\text{-}Phthal}$), 125.5 (CH_{ar}), 125.0 ($CH_{ar\text{-}Phthal}$), 124.4 (CH_{ar}), 121 (CH_{ar}), 61.2 (CH_2CH_3), 39.6 (NMe_{Phthal}), 36.5 (CH_2), 31.0 (NMe), 29.9 (CH_2), 14.5 (CH_2CH_3). HRMS (MALDI: DHB, PEG 600) calcd for $C_{24}H_{24}N_6O_4NaS$ $(M+Na)^+$ 515.1472, found 515.1467.

*N-((3s,5s,7s)-adamantan-1-yl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**54**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 2-bromo-*N*-(adamantyl)acetamide **20** (3 mmol, 1.05 g), purification by column chromatography on silica gel (DCM/MeOH; 95:5) afforded compound **54** in 81% yield, as a white powder; mp 134 - 136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, J = 7.2 and J = 1.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.46 (dd, J = 7.2 and J = 1.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.77 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.23 (bs, 1H, NH), 4.47 (s, 2H, CH_2), 3.78 (s, 3H, NMe_{Phthal}), 3.66 (m, 2H, CH_2), 3.57 (s, 3H, NMe), 1.99 (bs, 3H, 3 x CH), 1.91 (m, 6H, 3 x $CH_{2\text{-}adam}$), 1.56 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8 (CONH), 159.3 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 152.1 ($C_{IV\text{-}C=N}$), 140.2 ($C_{IV\text{-}C=N}$), 133.3 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}}$

$_{Phthal}$), 128.9 (C_{IV}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 124.9 ($CH_{ar\text{-}Phthal}$), 52.2 ($C_{IV\text{-}adam}$), 41.2 (3 x CH_2), 39.4 (NMe_{Phthal}), 37.7 (CH_2), 36.3 (3 x CH_2), 30.8 (NMe), 29.8 (SCH_2), 29.4 (3 x CH). HRMS (ESI^+): calcd for $C_{25}H_{31}N_6O_2S$ $(M+H)^+$ 479.2229, found 479.2231.

*2-Methyl-4-((4-methyl-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-3-yl)methyl)phthalazin-1(2H)-one (**55**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (2 mmol, 0.58 g) and propargyl bromide (5 eq, 10 mmol from a 80% solution in toluene), purification by column chromatography on silica gel (DCM/MeOH; 97:3) afforded compound **55** in 85% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.19 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.80 (d, J = 7.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.75 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 4.50 (s, 2H, CH_2), 3.87 (d, J = 2.6 Hz, 2H, SCH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.62 (s, 3H, NMe), 2.17 (t, J = 2.6 Hz, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7 (CO), 152.7 ($C_{IV\text{-}C=N}$), 150.1 ($C_{IV\text{-}C=N}$), 140.7 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 128.1 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 78.6 (C_{IV}), 72.7 (CH), 39.5 (NMe_{Phthal}), 31.1 (NMe), 30.3 (CH_2), 22.6 (CH_2). HRMS (ESI^+): calcd for $C_{16}H_{16}N_5OS$ $(M+H)^+$ 326.1076, found 326.1070.

*4-((5-(((1-((1s,3s)-Adamantan-1-yl)-1H-1,2,3-triazol-4-yl)methyl)thio)-4-methyl-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (**56**).* To a solution of alkyne **55** (0.21 mmol, 68 mg) and 1-azido adamantane (0.21 mmol, 60 mg) in *t*BuOH/ H_2O (1:1), was added sodium ascorbate (0.1 eq, 110 µL of a 0.2 M water solution) and $CuSO_4.5H_2O$ (0.01 eq, 10 µL of a 0.2 M water solution). After stirring at 60 °C during 20 h, a saturated solution of Na_2CO_3 was added and the aqueous phase was extracted with dichloromethane. The organic phase was washed with brine, dried over $MgSO_4$ and

concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (DCM/ petroleum ether; 90:10) to give compound **56** with 61% yield as a white powder; mp 197 - 198 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 7.8 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.20 (d, J = 7.8 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.69 (s, 1H, CH_{Triaz}), 4.51 (s, 2H, SCH_2), 4.48 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.52 (s, 3H, NMe), 2.15 (m, 3H, 3 x CH), 2.15 (m, 6H, 3 x CH_2), 1.76 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 159.5 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 151.6 ($C_{IV\text{-}C=N}$), 142.4 ($C_{IV\text{-}C=C}$), 140.4 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 128.2 (C_{IV}), 127.3 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 119.9 (*C*H=C), 59.8 (C_{IV}), 43.1 (3 x CH_2), 39.6 (NMe_{Phthal}), 36.0 (3 x CH_2), 30.9 (NMe), 30.0 (CH_2), 29.6 (3 x CH), 27.8 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{26}H_{31}N_8OS$ $(M+H)^+$ 503.2336, found 503.2336.

*N-(4-hydroxyphenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**57**).* To a solution of N-(4-methoxyphenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide **51** (1 eq) in anhydrous DCM (0.01 $mmol.mL^{-1}$) under argon atmosphere at 0 °C was added dropwise a solution of boron tribromide (6 eq of a 1M solution in DCM). The mixture was stirred at room temperature overnight. The mixture was then quenched by slow addition of MeOH at 0 °C and concentrated. The crude powder was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH; 95:5) to give **57** in 84% yield as a beige powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.02 (s, 1H, NH), 9.35 (bs, 1H, OH), 8.29 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.05 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.97-7.83 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.29 (d, J = 8.8 Hz, 2H, H_{ar}), 6.68 (d, J = 8.8 Hz, 2H, H_{ar}), 4.53 (s, 2H, CH_2), 3.96 (s, 2H, CH_2), 3.65 (s, 3H, NMe_{Phthal}), 3.59 (s, 3H, NMe). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.0 (CONH), 158.4 (CONMe), 153.6 (C_{IV}), 152.9 ($C_{IV\text{-}C=N}$), 149.4 ($C_{IV\text{-}C=N}$), 141.1 ($C_{IV\text{-}C=N}$), 133.1 ($CH_{ar\text{-}}$

$_{Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 130.4 (C_{IV}), 128.9 (C_{IV}), 127.2 (C_{IV}), 126.1 ($CH_{ar\text{-}Phthal}$), 125.8 ($CH_{ar\text{-}Phthal}$), 120.9 (2 x CH_{ar}), 115.1 (2 x CH_{ar}), 38.8 (NMe_{Phthal}), 37.7 (CH_2), 30.5 (NMe), 28.7 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{21}H_{21}N_6O_3S$ $(M+H)^+$ 437.1396, found 437.1390.

*2-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-propoxyphenyl)acetamide (**58**).* To a solution of phenol **57** (1 eq) in anhydrous DMF (0.05 $mmol.mL^{-1}$) under argon atmosphere at room temperature, was added 1-bromopropane (5 eq) followed by K_2CO_3 (5 eq). The mixture was then stirred at 60 °C overnight. The mixture was quenched at 0 °C by addition of a saturated solution of ammonium chloride and extracted twice with AcOEt, washed 5 times with a saturated solution of NaCl, dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH; 97:3) to give **58** in 69% yield as a beige powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (s, 1H, NH), 8.45 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.15 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.87-7.72 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.41 (d, J = 8.9 Hz, 2H, 2 x H_{ar}), 6.78 (d, J = 8.9 Hz, 2H, 2 x H_{ar}), 4.48 (s, 2H, CH_2), 3.90 (s, 2H, SCH_2), 3.86 (t, J = 6.6 Hz, 2H, OCH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.58 (s, 3H, NMe), 1.77 (quint, J = 7.1 Hz, 2H, CH_2CH_3), 1.00 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2 (CONH), 159.5 (CONMe), 155.9 (C_{IV}), 152.7 ($C_{IV\text{-}C=N}$), 140.4 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 131.3 (C_{IV}), 129.0 (C_{IV}), 128.1 (C_{IV}), 127.4 ($CH_{ar\text{-}Phthal}$), 125.0 ($CH_{ar\text{-}Phthal}$), 121.4 (2 x CH_{ar}), 114.8 (2 x CH_{ar}), 69.9 (OCH_2), 39.6 (NMe_{Phthal}), 36.3 (CH_2), 31.0 (NMe), 29.8 (CH_2), 22.7 (CH_2CH_3), 10.6 (CH_3). HRMS (ESI^+): calcd for $C_{24}H_{27}N_6O_3S$ $(M+H)^+$ 479.1865, found 479.1847.

*N-(4-isopropoxyphenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**59**)*. To a solution of phenol **57** (1 eq) in anhydrous DMF (0.05 mmol.mL^{-1}) under argon atmosphere was added 2-bromopropane (5 eq) followed by K_2CO_3 (5 eq). The mixture was then stirred at 60 °C overnight and quenched at 0 °C by addition of a saturated solution of ammonium chloride. The mixture was extracted twice with AcOEt, washed 5 times with a saturated solution of NaCl, dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH; 97:3) to give **59** in 63% yield as a beige powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H, NH), 8.43 (d, J = 7.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.13 (d, J = 7.7 Hz, 1H $H_{ar\text{-}Phthal}$), 7.82-7.72 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.39 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.76 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.48-4.41 (m, 3H, CH and CH_2), 3.90 (s, 2H, SCH_2), 3.77 (s, 3H, NMe_{Phthal}), 3.57 (s, 3H, NMe), 1.28 (d, J = 6.1 Hz, 6H, 2 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1 (CONH), 159.4 (CONMe), 154.7 (C_{IV}), 152.7 ($C_{IV\text{-}C=N}$), 152.6 ($C_{IV\text{-}C=N}$), 140.3 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.4 (C_{IV}), 129.0 (C_{IV}), 128.1 (C_{IV}), 127.3 ($CH_{ar\text{-}Phthal}$), 124.9 ($CH_{ar\text{-}Phthal}$), 121.4 (2 x CH_{ar}), 116.4 (2 x CH_{ar}), 70.4 (CH), 39.5 (NMe_{Phthal}), 36.5 (CH_2), 30.9 (NMe), 29.8 (CH_2), 22.1 (2 x CH_3). HRMS (ESI$^+$): calcd for $C_{24}H_{26}N_6O_3NaS$ $(M+Na)^+$ 501.1685, found 501.1666.

*N-(4-isobutoxyphenyl)-2-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**60**)*. To a solution of phenol **57** (1 eq) in anhydrous DMF (0.05 mmol.mL^{-1}) under argon atmosphere at room temperature was added 1-iodo-2-methylpropane (10 eq) followed by K_2CO_3 (10 eq). The mixture was then stirred at 60 °C overnight and quenched at 0 °C by addition of a saturated solution of ammonium chloride. The mixture was extracted twice with AcOEt, washed 5 times with a saturated solution of NaCl, dried over Na_2SO_4 and concentrated. The crude product was purified by flash column

chromatography on silica gel (DCM/MeOH; 97:3) to give **60** in 69 % yield as a yellowish brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H, NH), 8.45 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.16 (d, J = 7.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.85-7.75 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.41 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.78 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.48 (s, 2H, CH_2), 3.90 (s, 2H, SCH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.67 (d, J = 6.6 Hz, 2H, OCH_2), 3.58 (s, 3H, NMe), 2.04 (m, 1H, CH), 1.00 (d, J = 6.6 Hz, 6H, 2 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (CONH), 159.5 (CONMe), 156.2 (C_{IV}), 152.7 (2 x $C_{IV\text{-}C=N}$), 140.4 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 131.3 (C_{IV}), 129.0 (C_{IV}), 128.2 (C_{IV}), 127.4 ($CH_{ar\text{-}Phthal}$), 125.0 ($CH_{ar\text{-}Phthal}$), 121.5 (2 x CH_{ar}), 114.9 (2 x CH_{ar}), 74.9 (OCH_2), 39.5 (NMe_{Phthal}), 36.4 (CH_2), 31.0 (NMe), 29.9 (CH_2), 28.4 (CH), 19.4 (2 x CH_3). HRMS (ESI$^+$): calcd for $C_{25}H_{28}N_6O_3NaS$ $(M+Na)^+$ 515.1841, found 515.1825.

*2-(2-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydroPhthalazine-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamido) benzoic acid (**61**).* To a suspension of the ester **52** (0.3 mmol, 0.15 g) in dioxane (5 mL), LiOH (10 eq, 1M solution in water) was added and the mixture was stirred until starting material disappeared. The reaction was diluted with water (15 mL) and acidified to pH = 2. Concentration under reduced pressure gave a precipitate, which was filtered, washed with Et_2O, to give the pure compound **61** in 60% yield as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.79 (bs, 1H, NH), 8.43 (d, J = 8.5 Hz, 1H, H_{ar}), 8.28 (m, 1H, $H_{ar\text{-}Phthal}$), 8.02 (m, 1H, $H_{ar\text{-}Phthal}$), 7.96 (dd, J = 7.9 and J = 1.5 Hz, 1H, H_{ar}), 7.86 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.55 (ddd, J = 8.9, J = 7.9 and J = 1.6 Hz, 1H, H_{ar}), 7.15 (ddd, J = 8.4 Hz, J = 7.7 and J = 1.4 Hz, 1H, H_{ar}), 4.52 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 3.62 (s, 3H, NMe_{Phthal}), 3.60 (s, 3H, NMe). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 169.2 (COOH), 166.1 (CONH), 159.4 (CONMe), 152.9 ($C_{IV\text{-}C=N}$), 148.9 ($C_{IV\text{-}C=N}$), 141.1 ($C_{IV\text{-}C=N}$), 140.2 (C_{IV}), 133.7 (CH_{ar}), 133.1 ($CH_{ar\text{-}Phthal}$), 131.8 ($CH_{ar\text{-}Phthal}$), 131.1 (CH_{ar}), 128.8

(C_{IV}), 127.2 (C_{IV}), 126.0 ($CH_{ar\text{-}Phthal}$), 125.7 ($CH_{ar\text{-}Phthal}$), 123.0 (CH_{ar}), 119.8 (CH_{ar}), 117.3 (C_{IV}), 38.8 (NMe_{Phthal}), 37.5 (CH_2), 30.5 (NMe), 28.6 (CH_2). HRMS (ESI^+): calcd for $C_{22}H_{21}N_6O_4S$ $(M+H)^+$ 465.1340, found 465.1344.

*3-(2-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydroPhthalazine-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamido) benzoic acid (**62**).* To a suspension of the ester **53** (0.3 mmol, 0.15 g) in dioxane (5 mL), LiOH (10 eq, 1M solution in water) was added and the mixture was stirred until starting material disappeared. The reaction was diluted with water (15 mL) and acidified to pH = 2. Concentration under reduced pressure gave a precipitate which was filtered, washed with Et_2O, to give the pure compound **62** in 30% yield as a white powder. ^1H NMR (400 MHz, DMSO-d_6) δ 10.44 (bs, 1H, NH), 8.28 (m, 1H, $H_{ar\text{-}Phthal}$), 8.18 (s, 1H, H_{ar}), 8.05 (m, 1H, $H_{ar\text{-}Phthal}$), 7.87 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.72 (ddd, J = 8.13, J = 2.26 and J = 1.09 Hz, 1H, H_{ar}), 7.63 (m, 1H, H_{ar}), 7.42 (t, J = 7.9 Hz, 1H, H_{ar}), 4.53 (s, 2H, CH_2), 4.04 (s, 2H, CH_2), 3.65 (s , 3H, NMe_{Phthal}), 3.60 (s, 3H, NMe). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0 (COOH), 166.0 (CONH), 158.4 (CONMe), 152.9 ($C_{IV\text{-}C=N}$), 149.2 ($C_{IV\text{-}C=N}$), 141.1 ($C_{IV\text{-}C=N}$), 138.9 (C_{IV}), 133.1 ($CH_{ar\text{-}Phthal}$), 131.8 ($CH_{ar\text{-}Phthal}$), 131.4 (C_{IV}), 129.0 (CH_{ar}), 128.8 (C_{IV}), 127.1 (C_{IV}), 126.0 ($CH_{ar\text{-}Phthal}$), 125.7 ($CH_{ar\text{-}Phthal}$), 124.3 (CH_{ar}), 123.1 (CH_{ar}), 119.8 (CH_{ar}), 38.8 (NMe_{Phthal}), 37.7 (CH_2), 30.5 (NMe), 28.7 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{22}H_{20}N_6O_4NaS$ $(M+Na)^+$ 487.1159, found 487.1154.

*N-(2,4-dichlorophenyl)-N-methyl-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**63**).* To a solution of **43** (0.1 g, 0.17 mmol) in anhydrous DMF (3 mL) was added NaH (1.2 eq of a 60% suspension in oil) at 0 °C. Pure MeI (2 eq, 22 µL) was then added and after stirring during 2 h, the reaction was quenched with a saturated solution of NH_4Cl, and extracted twice with

dichloromethane, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **63** in 74% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (dd, J = 7.5 and J = 1.7 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.24 (dd, J = 7.3 and J = 1.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.51 (d, J = 2.3 Hz, 1H, H_{ar}), 7.32 (dd, J = 8.3 and J = 2.3 Hz, 1H, H_{ar}), 7.19 (d, J = 8.3 Hz, 1H, H_{ar}), 4.48 (s, 2H, CH_2), 3.80 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.15 (m, 5H, NMeCO and CH_2S), 1.93 (t, J = 7.5 Hz, 2H, CH_2CONH), 1.64 (m, 4H, 2 x CH_2), 1.24 (m, 6H, 3 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 173.0 (CH_2*C*ONMe), 159.6 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 151.9 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 140.1 (C_{IV}), 134.8 (C_{IV}), 134.1 (C_{IV}), 133.5 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 130.8 (CH_{ar}), 130.7 (CH_{ar}), 128.7 (CH_{ar}), 128.16 (C_{IV}), 127.7 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 39.5 (NMe_{Phthal}), 35.9 (SCH_2), 33.9 (CH_2CO), 33.1 (NMe_{Triaz}), 30.7 (NMe), 30.2 (CH_2), 20.5 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.5 (CH_2), 25.0 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{28}H_{33}Cl_2N_6O_2S$ $(M+H)^+$ 587.1763, found 587.1755.

*N-(2,4-dibromorophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**64**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(2,4-dibromophenyl)octanamide **21** (3 mmol, 1.36 g) purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **64** in 85% yield, as a white powder; mp 153.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (m, 1H, $H_{ar\text{-}Phthal}$), 8.23 (m, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phtha}$), 7.66 (d, J = 2.2 Hz, 1H, H_{ar}), 7.62 (bs, 1H, NH), 7.41 (dd, J = 8.7 and J = 2.2 Hz, 1H, H_{ar}), 4.51 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.55 (s, 3H, NMe), 3.20 (t, J = 7.3 Hz, 2H, SCH_2), 2.40 (t, J = 7.4 Hz, 2H, CH_2CO), 1.81-1.63 (m, 4H, 2 x CH_2), 1.46-1.34 (m, 6H, 3 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (CONH), 159.4 (CONMe), 152.1 ($C_{IV\text{-}}$

$_{C=N}$), 151.7 ($C_{IV-C=N}$), 140.5 ($C_{IV-C=N}$), 134.0 (C_{IV}), 134.3 (CH_{ar}), 133.3 ($CH_{ar\text{-}Phthal}$), 131.8 ($CH_{ar\text{-}Phthal}$), 131.3 (CH_{ar}), 129.9 (C_{IV}), 127.9 (C_{IV}), 127.0 ($CH_{ar\text{-}Phthal}$), 125.2 ($CH_{ar\text{-}Phthal}$), 122.9 (C_{IV}), 39.4 (NMe_{Phthal}), 37.7 (SCH_2), 32.9 (CH_2CO), 30.6 (NMe), 29.9 (CH_2), 29.3 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 25.2 (CH_2). HRMS (MALDI: DHB, PEG 600) calcd for $C_{27}H_{31}Br_2N_6O_2S$ $(M+H)^+$ 661.0590, found 661.0609.

*N-(2,4-dibromophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)sulfonyl)octanamide (**65**).* To a solution of thioether **64** in DCM (0.01 M) was added portion wise *meta*-chloroperbenzoic acid (5eq). After completion of the reaction, the mixture was washed several times with a saturated solution of Na_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The solid residue was purified by column chromatography on silica gel (DCM/MeOH; 98:2) to give compound **65** in 74% yield, as a white powder; mp 175 - 176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (m, 1H, $H_{ar\text{-}Phthal}$), 8.26 (m, 1H, $H_{ar\text{-}Phthal}$), 8.13 (m, 1H, $H_{ar\text{-}Phthal}$), 7.82 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.67 (d, J = 2.2 Hz, 1H, H_{ar}), 7.56 (bs, 1H, NH), 7.42 (dd, J = 8.7 and J = 2.2 Hz, 1H, H_{ar}), 4.55 (s, 2H, CH_2), 3.97 (s, 3H, NMe_{Phthal}), 3.79 (s, 3H, NMe), 3.62 (m, 2H, SCH_2), 2.41 (t, J = 7.4 Hz, 2H, CH_2CO), 1.95 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.38 (m, 4H, 2 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0 (CONH), 159.3 (CONMe), 153.9 ($C_{IV-C=N}$), 152.4 ($C_{IV-C=N}$), 139.7 ($C_{IV-C=N}$), 134.9 (C_{IV}), 134.3 (CH_{ar}), 133.4 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 131.4 (CH_{ar}), 128.7 (C_{IV}), 128.1 (C_{IV}), 127.4 ($CH_{ar\text{-}Phthal}$), 124.7 ($CH_{ar\text{-}Phthal}$), 122.9 (C_{IV}), 54.8 (SO_2CH_2), 39.4 (NMe_{Phthal}), 37.7 ($COCH_2$), 32.2 (NMe), 29.2 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 27.95 (CH_2), 25.1 (CH_2), 21.9 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{27}H_{31}Br_2N_6O_4S$ $(M+H)^+$ 693.0489, found 693.0492.

*N-(2,4-difluorophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**66**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(2,4-difluorophenyl)octanamide **22** (3 mmol, 1.00 g) purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **66** in 72% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (m, 1H, $H_{ar\text{-}Phthal}$), 8.16 (m, 2H, $H_{ar\text{-}Phthal}$ and H_{ar}), 7.77 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 6.82 (m, 2H, 2 x H_{ar}), 4.46 (s, 2H, CH_2), 3.77 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.15 (t, J = 7.03 Hz, 2H, SCH_2), 2.26 (t, J = 7.73 Hz, 2H, CH_2CO), 1.68 (m, 4H, 2 x CH_2), 1.45-1.25 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7 (CONH), 159.6 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 129.0 (C_{IV}), 127.9 (C_{IV}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.2 ($CH_{ar\text{-}Phthal}$), 123.4 (d, J_{CF} = 8.94 Hz, CH_{ar}), 111.2 (d, J_{CF} = 21.63 Hz, CH_{ar}), 103.6 (t, J_{CF} = 27.28 Hz, CH_{ar}), 39.5 (NMe_{Phthal}), 37.2 (CH_2), 33.5 (CH_2), 30.7 (NMe), 30.0 (CH_2), 29.4 (CH_2), 29.2-27.7 (3 x CH_2), 25.3 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{27}H_{31}F_2N_6O_2S$ $(M+H)^+$ 541.2192, found 541.2196.

*N-(2-chloro-4-methylphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**67**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g), and 8-bromo-*N*-(2-chloro-4-methylphenyl)octanamide **23** (3 mmol, 1.04 g) purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **67** in 74% yield, as a white powder; mp 151 - 152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.23 (m, 1H, $H_{ar\text{-}Phthal}$), 8.19 (d, J = 8.4 Hz, 1H, H_{ar}), 7.77 (m, 2H, 2 x $H_{ar\text{-}Phtha}$), 7.54 (bs, 1H, NH), 7.16 (d, J = 1.24 Hz, 1H, H_{ar}), 7.05 (dd, J = 8.4 and J = 1.9 Hz, 1H, H_{ar}), 4.49 (s, 2H, CH_2), 3.80 (s, 3H,

NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.18 (t, *J* = 7.3 Hz, 2H, SCH_2), 2.37 (t, *J* = 7.5 Hz, 2H, CH_2CO), 2.29 (s, 3H, CH_3), 1.72 (m, 4H, 2 x CH_2), 1.46-1.34 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (CONH), 159.4 (CONMe), 152.0 ($C_{IV-C=N}$), 151.8 ($C_{IV-C=N}$), 140.6 ($C_{IV-C=N}$), 133.3 ($CH_{ar-Phthal}$), 132.0 (C_{IV}), 131.8 ($CH_{ar-Phthal}$), 129.2 (C_{IV}), 129.0 (C_{IV}), 128.3 (CH_{ar}), 127.9 (C_{IV}), 127.0 ($CH_{ar-Phthal}$), 125.3 ($CH_{ar-Phthal}$), 121.7 (C_{IV}), 39.3 (NMe_{Phthal}), 37.8 (SCH_2), 33.0 (CH_2CO), 30.6 (NMe), 30.0 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 25.3 (CH_2), 20.6 (CH_3). HRMS (MALDI: DHB, PEG 600): calcd for $C_{28}H_{34}ClN_6O_2S$ $(M+H)^+$ 553.2147, found 553.2165.

*N-(2,4-difluorophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**68**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g), and 8-bromo-*N*-(2,4-dimethoxyphenyl)octanamide **24** (3 mmol, 1.07 g) purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **68** in 74% yield, as a white powder; mp 118 - 120 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 9.2 and *J* = 1.6 Hz, 1H, $H_{ar-Phthal}$), 8.23 (m, 2H, H_{ar} and $H_{ar-Phthal}$), 7.78 (m, 2H, 2 x $H_{ar-Phthal}$), 7.54 (bs, 1H, NH), 6.45 (m, 2H, 2 x H_{ar}), 4.52 (s, 2H, CH_2), 3.84 (s, 3H, OMe), 3.8 (s, 3H, NMe_{Phthal}), 3.78 (s, 3H, OMe), 3.55 (s, 3H, NMe), 3.20 (t, *J* = 7.3 Hz, 2H, SCH_2), 2.34 (t, *J* = 7.35 Hz, 2H, CH_2CO), 1.78-1.61 (m, 4H, 2 x CH_2), 1.46-1.25 (m, 6H, 3 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (CONH), 159.5 (CONMe), 156.3 (C_{IV}), 152.4 ($C_{IV-C=N}$), 151.9 ($C_{IV-C=N}$), 140.2 (C_{IV}), 140.6 ($C_{IV-C=N}$), 133.4 ($CH_{ar-Phthal}$), 133.5 ($CH_{ar-Phthal}$), 132.0 ($CH_{ar-Phthal}$), 129.1 (C_{IV}), 127.9 (C_{IV}), 127.2 ($CH_{ar-Phthal}$), 125.3 ($CH_{ar-Phthal}$), 121.4 (CH_{ar}), 120.7 (C_{IV}), 103.8 (CH_{ar}), 98.7 (CH_{ar}), 55.8 (OMe), 55.7 (OMe), 39.5 (NMe_{Phthal}), 37.9 (CH_2CO), 33.2 (SCH_2), 30.9 (NMe), 29.9 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.9 (CH_2),

28.5 (CH_2), 25.6 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{29}H_{37}N_6O_4S$ $(M+H)^+$ 565.2592, found 565.2574.

*N-(2-hydroxy-4-methoxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**69**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g) and 8-bromo-*N*-(2-hydroxy-4-methoxyphenyl)octanamide **26** (1.5 mmol, 0.52 g) purification by column chromatography on silica gel (DCM/MeOH; 95:5 to 90:10) afforded compound **69** in 48% yield, as a white powder; mp 154 - 155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, H, OH), 8.44 (m, 1H, $H_{ar\text{-}Phthal}$), 8.10 (dd, 1H, J = 6.4 and J = 1.7 Hz, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.08 (d, J = 8.7 Hz, 1H, H_{ar}), 6.56 (d, J = 2.7 Hz, 1H, H_{ar}), 6.32 (dd, J = 8.7 and J = 2.8 Hz, 1H, H_{ar}), 4.49 (s, 2H, CH_2), 3.78 (s, 3H, NMe_{Phthal}), 3.73 (s, 3H, OMe), 3.59 (s, 3H, NMe), 3.19 (t, J = 7.3 Hz, 2H, SCH_2), 2.43 (t, J = 7.35 Hz, 2H, CH_2CO), 1.72 (m, 4H, 2 x CH_2), 1.45-1.34 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3 (CONH), 159.4 (CONMe), 158.6 (C_{IV}), 152.9 ($C_{IV\text{-}C=N}$), 150.2 ($C_{IV\text{-}C=N}$), 140.1 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 128.7 (C_{IV}), 127.9 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 124.8 ($CH_{ar\text{-}Phthal}$), 123.1 (CH_{ar}), 119.6 (C_{IV}), 106.2 (CH_{ar}), 104.2 (CH_{ar}), 55.4 (OMe), 39.4 (NMe_{Phthal}), 36.4 (CH_2CO), 32.7 (SCH_2), 30.9 (NMe), 28.5 (CH_2), 28.8 (CH_2), 27.9 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 25.2 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{28}H_{35}N_6O_4S$ $(M+H)^+$ 551.2435, found 551.2441.

*N-(2,4-dihydroxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**70**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(2,4-dihydroxy-

phenyl)octanamide **25** (2 mmol, 0.66 g) purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **70** in 40% yield, as a pink powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (bs, 1H, NH), 9.13 (s, 1H, OH), 9.08 (s, 1H, OH), 8.30 (d, J = 7.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.06 (d, J = 7.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.89 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.20 (d, J = 8.6 Hz, 1H, H_{ar}), 6.28 (d, J = 2.6 Hz, 1H, H_{ar}), 6.17 (dd, J = 8.6 and J = 2.7 Hz, 1H, H_{ar}), 4.53 (s, 2H, CH_2), 3.66 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.06 (t, J = 7.2 Hz, 2H, SCH_2), 2.29 (t, J = 7.5 Hz, 2H, CH_2CO), 1.64-1.51 (m, 4H, 2 x CH_2), 1.39-1.24 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.5 (CONH), 158.3 (C_{IV}), 154.9 (C_{IV}), 152.5 ($C_{IV\text{-}C=N}$), 149.5 (C_{IV}), 146.6 (C_{IV}), 141.04 ($C_{IV\text{-}C=N}$), 133.0 ($CH_{ar\text{-}Phthal}$), 131.75 ($CH_{ar\text{-}Phthal}$), 128.7 (C_{IV}), 127.8 (C_{IV}), 125.9 ($CH_{ar\text{-}Phthal}$), 125.6 ($CH_{ar\text{-}Phthal}$), 123.9 (C_{IV}), 117.9 (C_{IV}), 105.8 (CH_{ar}), 103.3 (CH_{ar}), 38.7 (NMe_{Phthal}), 35.4 (CH_2CO), 32.6 (SCH_2), 30.2 (NMe), 28.9 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28 (CH_2), 27.6 (CH_2), 25.1 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{27}H_{33}N_6O_4S$ $(M+H)^+$ 537.2279, found 537.2302.

*N-(4-chlorophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**71**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (0.7 mmol, 0.20 g) and 8-bromo-*N*-(4-chlorophenyl)octanamide **27** (0.84 mmol, 0.28 g) purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **71** in 31% yield, as a yellowish powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (br s, 1H, NH), 8.42 (m, 1H, $H_{ar\text{-}Phthal}$), 8.13 (m, 1H, $H_{ar\text{-}Phthal}$), 7.76 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.54 (d, J = 8.8 Hz, 2H, 2 x H_{ar}), 7.19 (d, J = 8.8 Hz, 2H, 2 x H_{ar}), 4.50 (s, 2H, CH_2), 3.78 (s, 3H, NMe_{Phthal}), 3.56 (s, 3H, NMe), 3.15 (t, J = 7.27, 2H, SCH_2), 2.30 (t, J = 7.37, 2H, CH_2CO), 1.61-1.72 (m, 4H, 2 x CH_2), 1.25-1.61 (m, 6H, 3 x CH_2). RMN ^{13}C (75 MHz, $CDCl_3$) δ 172.0 (CONH), 159.5 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 152.1 ($C_{IV\text{-}C=N}$), 140.5 (C_{IV}), 137.3 (C_{IV}), 133.5

($CH_{ar\text{-}Phthal}$), 132.1 ($CH_{ar\text{-}Phthal}$), 128.9 (CH_{ar}), 128.7 (C_{IV}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.1 ($CH_{ar\text{-}Phthal}$), 121.1 (CH_{ar}), 39.6 (NMe_{Phthal}), 37.5 (CH_2CO), 33.0 (SCH_2), 31.0 (NMe), 29.9 (CH_2), 29.2 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 27.8 (CH_2), 25.2 (CH_2). HRMS (ESI^+): calcd for $C_{27}H_{32}N_6O_2SCl$ $(M+H)^+$ 539.1990, found 539.1999.

*N-(4-iodophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**72**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (0.43 mmol, 0.12 g), and 8-bromo-*N*-(4-iodophenyl)octanamide **28** (1.1 eq, 0.20 g). The crude precipitate was taken up in dichloromethane, and the product was filtrered and washed with petroleum ether to give **72** in 49% yield, as a white powder; mp 120 - 121 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H, NH), 8.34 (m, 1H, $H_{ar\text{-}Phthal}$), 8.1 (m, 1H, $H_{ar\text{-}Phthal}$), 7.9 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.60 (m, 2H, 2 x H_{ar}), 7.41 (m, 2H, H_{ar}), 4.53 (s, 2H, CH_2), 3.66 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.05 (t, J = 7.2 Hz, 2H, SCH_2), 2.27 (t, J = 7.4 Hz, 2H, CH_2CO), 1.60-1.55 (m, 4H, 2 x CH_2), 1.35-1.26 (m, 6H, CH_2). RMN ^{13}C (75 MHz, DMSO-d_6) δ 171.4 (CONH), 158.4 (CONMe), 152.6 ($C_{IV\text{-}C=N}$), 149.6 ($C_{IV\text{-}C=N}$), 141.2 ($C_{IV\text{-}C=N}$), 139.1 (C_{IV}), 137.3 (CH_{ar}), 133.2 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 128.9 (C_{IV}), 127.2 (C_{IV}), 126.1 ($CH_{ar\text{-}Phthal}$), 125.7 ($CH_{ar\text{-}Phthal}$), 121.2 (CH_{ar}), 86.2 ($C_{IV\text{-}C\text{-}I}$), 38.9 (NMe_{Phthal}), 36.4 (CH_2CO), 32.7 (SCH_2), 30.4 (NMe), 29.0 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 27.7 (CH_2), 24.9 (CH_2). HRMS (ESI^+): calcd for $C_{27}H_{32}N_6O_2SI$ $(M+H)^+$ 631.1346, found 631.1319.

*N-(4-azidophenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**73**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(4-azidophenyl)octanamide **32** (1.5 mmol, 0.51 g),

purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **73** in 87% yield, as a white powder; mp 96.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.19 (m, 1H, $H_{ar\text{-}Phthal}$), 7.95 (bs, 1H, NH), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.57 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.93 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.48 (s, 2H, CH_2), 3.78 (s, 3H, NMe_{Phthal}), 3.57 (s, 3H, NMe), 3.16 (t, J = 7.2 Hz, 2H, SCH_2), 2.30 (t, J = 7.7 Hz, 2H, CH_2CO), 1.73-1.65 (m, 4H, 2 x CH_2), 1.43-1.28 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7 (CONH), 159.6 (CONMe), 152.2 (C_{IV}), 152.1 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 135.6 (C_{IV}), 135.5 (CH_{ar})133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 128.1 (C_{IV}), 127.3 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.4 (2 x CH_{ar}), 119.5 (2 x CH_{ar}), 39.5 (NMe_{Phthal}), 37.6 (CH_2CO), 33.1 (SCH_2), 30.8 (NMe), 30.2 (CH_2), 29.3 (CH_2), 28.6 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 25.3 (CH_2). HRMS (ESI^+): calcd for $C_{27}H_{32}N_9O_2S$ $(M+H)^+$ 546.2400, found 546.2403.

*N-(4-methoxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**74**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(4-methoxyphenyl)octanamide **29** (3 mmol, 1.00 g), purification by column chromatography on silica gel (DCM/MeOH; 96:4) afforded compound **74** in 89% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (m, 1H, $H_{ar\text{-}Phthal}$), 8.18 (m, 1H, $H_{ar\text{-}Phthal}$), 7.82-7.72 (m, 3H, NH and 2 x $H_{ar\text{-}Phthal}$), 7.44 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.81 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.47 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.76 (s, 3H, OMe), 3.55 (s, 3H, NMe), 3.17 (t, J = 7.3 Hz, 2H, SCH_2), 2.28 (t, J = 7.8 Hz, 2H, CH_2CO), 1.75-1.61 (m, 4H, 2 x CH_2), 1.46-1.20 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (CONH), 159.6 (CONMe), 156.3 (C_{IV}), 152.1 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.6 (C_{IV}), 129.1 (C_{IV}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.8 (2 x CH_{ar}), 114.2 (2 x CH_{ar}), 55.6 (OMe), 39.5 (NMe_{Phthal}),

37.5 (CH_2CO), 33.1 (SCH_2), 30.8 (NMe), 30.2 (CH_2), 29.4 (CH_2), 28.8 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 25.5 (CH_2). HRMS (ESI^+): calcd for $C_{28}H_{35}N_6O_3S$ $(M+H)^+$ 535.2491, found 535.2500.

*N-(2-hydroxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**75**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g) and 8-bromo-*N*-(2-hydroxyphenyl)octanamide **30** (2 mmol, 0.63 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **75** in 65% yield, as a white powder; mp 180.2 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, J = 7.6 and J = 1.2 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.08 (d, 1H, $H_{ar\text{-}Phthal}$), 7.76 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.40 (d, J = 8 Hz, 1H, 2 x H_{ar}), 6.96 (t, J = 8.2 Hz, 1H, H_{ar}), 6.88 (d, J = 8 Hz, 1H, H_{ar}), 6.77 (t, J = 7.3 Hz, 1H, H_{ar}), 4.45 (s, 2H, CH_2), 3.74 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.11 (t, J = 7.3 Hz, 2H, SCH_2), 2.36 (t, J = 7.4 Hz, 2H, CH_2CO), 1.66 (m, 4H, 2 x CH_2), 1.44-1.32 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2 (CONH), 159.6 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 151.9 ($C_{IV\text{-}C=N}$), 140.7 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 128.7 (C_{IV}), 127.7 (C_{IV}), 126.9 (CH_{ar}), 126.1 (C_{IV}), 125.7 ($CH_{ar\text{-}Phthal}$), 124.8 ($CH_{ar\text{-}Phthal}$), 121.6 (CH_{ar}), 120.0 (CH_{ar}), 117.7 (CH_{ar}), 39.3 (NMe_{Phthal}), 36.6 (CH_2CO), 32.9 (SCH_2), 30.7 (NMe), 29.4 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 25.3 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{27}H_{33}N_6O_3S$ $(M+H)^+$ 521.2329, found 521.2353.

*Ethyl 3-(8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamido)benzoate (**76**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g), and ethyl 3-(8-bromo octanamido)benzoate **31** (3 mmol, 1.11 g),

purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **76** in 72 % yield, as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (m, 1H, $H_{ar\text{-}Phthal}$), 8.34 (bs, 1H, NH), 8.16 (m, 1H, $H_{ar\text{-}Phthal}$), 8.06 (s, 1H, H_{ar}), 7.94 (d, J = 7.8 Hz, 1H, H_{ar}), 7.74 (m, 3H, H_{ar} and 2 x $H_{ar\text{-}Phthal}$), 7.33 (t, J = 7.9 Hz, 1H, H_{ar}), 4.50 (s, 2H, CH_2), 4.32 (q, J = 7.0 Hz, 2H, CH_2CH_3), 3.78 (s, 3H, $NMe\text{-}_{Phthal}$), 3.57 (s, 3H, NMe), 3.16 (t, J = 7.3 Hz, 2H, SCH_2), 2.35 (t, J = 7.4 Hz 2H, CH2CO), 1.69 (m, 4H, 2 x CH_2), 1.37 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.4-1.30 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1 (CONH), 166.4 (*C*OOEt), 159.5 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 152.1 ($C_{IV\text{-}C=N}$), 140.7 ($C_{IV\text{-}C=N}$), 138.8 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.2 (C_{IV}), 129.1 (C_{IV}), 129.0 (CH_{ar}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.2 ($CH_{ar\text{-}Phthal}$), 124.9 (CH_{ar}), 124.4 (CH_{ar}), 120.7 (CH_{ar}), 61.1 (CH_2CH_3), 39.5 (NMe_{Phthal}), 37.6 (CH_2CO), 33.1 (SCH_2), 30.8 (NMe), 30.0 (CH_2), 29.4 (2 x CH_2), 28.7 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 25.3 (2 x CH_2), 14.4 (CH_2CH_3). HRMS (MALDI: DHB, PEG 600): calcd for $C_{3O}H_{37}N_6O_2S$ $(M+H)^+$ 577.2592, found 577.2608.

8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-(naphthalen-1-yl)octanamide (77). Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (0.7 mmol, 0.20 g) and 8-bromo-*N*-(1-naphthalen-1-yl)octanamide **33** (0.84 mmol, 0.29 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **77** in 44% yield, as a yellowish powder; mp 162 - 163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (m, 1H, $H_{ar\text{-}Phthal}$), 8.18 (bs, 1H, NH), 8.05 (m, 1H, $H_{ar\text{-}Phthal}$), 7.9-7.65 (m, 6H, 6 x Har), 7.43 (m, 3H, 3 x Har), 4.39 (s, 2H, CH_2), 3.75 (s, 3H, NMe_{Phthal}), 3.51 (s, 3H, NMe), 3.22 (t, J = 7.1 Hz, 2H, SCH_2), 2.53 (t, J = 7.3 Hz, 2H, CH_2CO), 1.82-1.73 (m, 4H, 2 x CH_2), 1.48-1.41 (m, 6H, 3 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3 (CONH), 159.4 (CONMe), 152.3 (C_{IV}), 151.8 (C_{IV}), 140.3 (C_{IV}), 134.1 (C_{IV}), 133.3 (CH_{ar}), 132.6 (CH_{ar}), 131.9 (CH_{ar}),

128.8 (CH_{ar}), 128.6 (CH_{ar}), 127.8 ($CH_{ar\text{-}Phthal}$), 127.5 ($CH_{ar\text{-}Phthal}$), 127.0 ($CH_{ar\text{-}Phthal}$), 126.1 (CH_{ar}), 125.9 (CH_{ar}), 125.7 (CH_{ar}), 125.0 (CH_{ar}), 121.4 (CH_{ar}), 121.2 (CH_{ar}), 39.4 (NMe_{Phthal}), 37.4 (CH_2CO), 32.9 (SCH_2), 30.7 (NMe), 29.5 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 28.3 (CH_2), 27.9 (CH_2), 25.5 (CH_2). HRMS (ESI^+): calcd for $C_{31}H_{35}N_9O_2S$ $(M+H)^+$ 555.2537, found 555.2545.

*N-((3s,5s,7s)-adamantan-1-yl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**78**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **4** (1 mmol, 0.29 g)and 8-bromo-*N*-(adamantan-1-yl)octanamide **34** (3 mmol, 1.06 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **78** in 75% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (m, 1H, $H_{ar\text{-}Phthal}$), 8.18 (m, 1H, $H_{ar\text{-}Phthal}$), 7.76 (m, 1H, $H_{ar\text{-}Phthal}$), 7.71 (m ,1H, $H_{ar\text{-}Phthal}$), 5.26 (bs, 1H, NH), 4.44 (s, 2H, CH_2), 3.75 (s, 3H, NMe_{Phthal}), 3.51 (s, 3H, NMe), 3.12 (t, J = 7.1 Hz, 2H, SCH_2), 2.00 (m, 5H, CH_2CO and 3 x CH_{adam}), 1.94 (m, 6H, 3 x $CH_{2\text{-}adam}$), 1.65 (m, 2H, CH_2), 1.61 (m, 6H, 3 x $CH_{2\text{-}adam}$), 1.51 (m, 2H, CH_2), 1.42-1.13 (m, 6H, 3 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4 (CONH), 159.5 (CONMe), 151.9 ($C_{IV\text{-}C=N}$), 151.8 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.3 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 127.8 (C_{IV}), 127.0 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 51.7 ($C_{IV\text{-}adam}$), 41.7 (3 x $CH_{2\ adam}$), 39.8 (NMe_{Phthal}), 37.6 (CH_2), 36.4 (3 x $CH_{2\ adam}$), 33.0 (SCH_2), 30.6 (NMe), 30.2 (CH_2), 30.3-27.6 (3 x CH_2), 29.4 (3 x CH_{adam}), 29.3 (CH_2), 25.7 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{31}H_{43}N_6O_2S$ $(M+H)^+$ 563.3163, found 563.3163.

*N-(4-hydroxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**79**)*. To a solution of benzamide **74** (1 eq) in

anhydrous DCM (0.01 mmol.mL^{-1}) under argon atmosphere at 0 °C was added dropwise a solution of boron tribromide in DCM (8 eq). The mixture was then stirred at room temperature overnight. The mixture was quenched by slow addition of MeOH at 0 °C and concentrated. The crude powder was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH; 95:5) to give **79** in 94% yield as a beige powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H, OH), 8.31 (dd, J = 7.6 and J = 1.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.07 (d, J = 7.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.98-7.87 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.34 (d, J = 8.9 Hz, 2H, 2 x H_{ar}), 6.66 (d, J = 8.9 Hz, 2 x H_{ar}), 4.70 (s, 2H, CH_2), 3.65 (s, 3H, NMe_{Phthal}), 3.64 (s, 3H, NMe), 3.17 (t, J = 7.2 Hz, 2H, SCH_2), 2.22 (t, J = 7.4 Hz, 2H, CH_2CO), 1.65 (quint, J = 7.2 Hz, 2H, SCH_2CH_2), 1.55 (quint, J = 7.0 Hz, 2H, CH_2), 1.42-1.34 (m, 2H, CH_2), 1.32-1.24 (m, 4H, 2 x CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4 (CONH), 158.4 (CONMe), 153.0 (C_{IV}), 152.9 ($C_{IV\text{-}C=N}$), 151.6 ($C_{IV\text{-}C=N}$), 139.9 ($C_{IV\text{-}C=N}$), 133.2 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.0 (C_{IV}), 128.6 (C_{IV}), 127.2 (C_{IV}), 126.1 ($CH_{ar\text{-}Phthal}$), 125.5 ($CH_{ar\text{-}Phthal}$), 120.8 (2 x CH_{ar}), 114.9 (2 x CH_{ar}), 38.8 (NMe_{Phthal}), 36.1 (CH_2), 32.7 (CH_2), 31.2 (NMe), 28.8 (CH_2), 28.5 (CH_2), 28.1 (2 x CH_2), 27.6 (CH_2), 25.1 (CH_2). HRMS (ESI$^+$): calcd for $C_{27}H_{33}N_6O_3S$ $(M+H)^+$ 521.2335, found 521.2349.

*8-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-propoxyphenyl)octanamide (**80**).* To a solution of phenol **79** (1 eq) in anhydrous DMF (0.04 mmol.mL^{-1}) under argon atmosphere was added dropwise 1-bromopropane (5 eq) followed by K_2CO_3 (5 eq). The mixture was then stirred at 60 °C overnight and quenched at 0 °C by addition of a saturated solution of ammonium chloride. The aqueous phase was extracted twice with AcOEt, and the organic phase was washed 5 times with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH; 95:5) to give **80** in 59% yield as a white powder; mp 117 - 118

°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (dd, J = 6.8 and J = 2.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.18 (dd, J = 6.1 and J = 2.1 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.84 (bs, 1H, NH), 7.81-7.71 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.42 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.80 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.47 (s, 2H, CH_2), 3.86 (t, J = 6.6 Hz, 2H, OCH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.55 (s, 3H, NMe), 3.15 (t, J = 7.3 Hz, 2H, SCH_2), 2.28 (t, J = 7.7 Hz, 2H, $COCH_2$), 1.83-1.73 (m, 2H, CH_2), 1.72-1.62 (m, 4H, 2 x CH_2), 1.42-1.26 (m, 6H, 3 x CH_2), 1.00 (t, J = 7.5Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5 (CONH), 159.6 (CONMe), 155.8 (C_{IV}), 152.1 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.4 (C_{IV}), 129.0 (C_{IV}), 128.0 (C_{IV}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.7 (2 x CH_{ar}), 114.8 (2 x CH_{ar}), 69.9 (OCH_2), 39.5 (NMe_{Phthal}), 37.5 (CH_2), 33.1 (CH_2), 30.8 (NMe), 30.1 (CH_2), 29.4 (CH_2), 28.9 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 25.5 (CH_2), 22.7 (CH_2), 10.6 (CH_3). HRMS (ESI^+): calcd for $C_{30}H_{39}N_6O_3S$ $(M+H)^+$ 563.2804; found 563.2793.

*N-(4-isopropoxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**81**)*. To a solution of phenol **79** (1 eq) in anhydrous DMF (0.04 mmol.mL^{-1}) under argon atmosphere was added dropwise 2-bromopropane (5 eq) followed by K_2CO_3 (5 eq). The mixture was then stirred at 60 °C overnight and quenched at 0 °C by addition of a saturated solution of ammonium chloride. The aqueous phase was extracted twice with AcOEt, and the organic phase was washed 5 times with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (DCM/MeOH; 95:5) to give **81** in 90% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (dd, J = 6.9 and J = 2.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.19 (d, J = 6.9 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.81-7.71 (m, 3H, NH and 2 x $H_{ar\text{-}Phthal}$), 7.42 (d, J = 8.9 Hz, 2H, 2 x Har), 6.80 (d, J = 8.9 Hz, 2H, 2 x Har), 4.51-4.42 (m, 3H, CH_2 and CH), 3.79 (s, 3H, NMe_{Phthal}), 3.55 (s, 3H, NMe), 3.15 (t, J = 7.3 Hz, 2H, SCH_2), 2.28 (t, J = 7.5 Hz, 2H, CH_2CO), 1.73-1.62 (m, 4H, 2 x CH_2),

1.41-1.27 (m, 12H, 3 x CH_2 and 2 x CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5 (CONH), 159.6 (CONMe), 154.5 (C_{IV}), 152.0 (2 x $C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.4 (C_{IV}), 129.0 (C_{IV}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.7 (2 x CH_{ar}), 116.5 (2 x CH_{ar}), 70.4 (CH-O), 39.5 (NMe_{Phthal}), 37.5 (CH_2), 33.1 (CH_2), 30.8 (NMe), 30.2 (CH_2), 29.4 (CH_2), 28.9 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 25.5 (CH_2), 22.2 (2 x CH_3). HRMS (ESI^+): calcd for $C_{30}H_{38}N_6NaO_3S$ $(M+Na)^+$ 585.2624, found 585.2631.

*N-(4-isobutoxyphenyl)-8-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamide (**82**)*. To a solution of phenol **79** (1 eq) in anhydrous DMF (0.04 $mmol.mL^{-1}$) under argon atmosphere was added dropwise 1-iodo-2-methyl propane (10 eq) followed by K_2CO_3 (10 eq). The mixture was then stirred at 60 °C overnight and quenched at 0 °C by addition of a saturated solution of ammonium chloride. The aqueous phase was extracted twice with AcOEt, and the organic phase was washed 5 times with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (DCM/MeOH; 97:3) to give **82** in 83% yield as a beige powder. ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (dd, J = 6.9 and J = 2.3 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.18 (d, J = 6.6 Hz, 1H, $H_{ar\text{-}Phthal}$), 7.85 (bs, 1H, NH), 7.80-7.71 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.41 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 6.79 (d, J = 9.0 Hz, 2H, 2 x H_{ar}), 4.46 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.66 (d, J = 7.2 Hz, 2H, OCH_2), 3.55 (s, 3H, NMe), 3.15 (t, J = 7.2 Hz, 2H, SCH_2), 2.28 (t, J = 7.2 Hz, 2H, CH_2CO), 1.99 (m, 1H, CH), 1.74-1.59 (m, 4H, 2 x CH_2), 1.43-1.25 (m, 6H, 3 x CH_2), 0.99 (d, J = 6.7 Hz, 6H, 2 x CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5 (CONH), 159.6 (CONMe), 156.0 (C_{IV}), 152.0 (2 x $C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.0 ($CH_{ar\text{-}Phthal}$), 131.4 (C_{IV}), 129.0 (C_{IV}), 128.0 (C_{IV}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.7 (2 x CH_{ar}), 114.8 (2 x CH_{ar}), 74.8 (OCH_2), 39.5 (NMe_{Phthal}), 37.5 (CH_2CO), 33.1 (SCH_2), 30.7 (NMe), 30.1

(CH_2), 29.4 (CH_2), 28.8 (CH_2), 28.4 (CH_2), 28.4 (CH), 28.1 (CH_2), 25.4 (CH_2), 19.4 (2 x CH_3). HRMS (ESI^+): calcd for $C_{31}H_{40}N_6O_3NaS$ $(M+Na)^+$ 599.2780, found 599.2800.

*3-(8-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)octanamido)benzoic acid (**83**).* To a suspension of the ester **76** (0.3 mmol, 0.10 g) in dioxane (5 mL), LiOH (10 eq, 1M solution in water) was added and the mixture was stirred until starting material disappeared. The reaction was diluted with water (15 mL) and acidified to pH = 2. Elimination of volatils under reduced pressure gave a precipitate, which was filtered, washed with Et_2O, to give the pure compound **83** in 50% yield as a white powder. ^1H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H, NH), 8.3 (m, 1H, $H_{ar\text{-}Phthal}$), 8.22 (s, 1H H_{ar}), 8.06 (m, 1H, $H_{ar\text{-}Phthal}$), 8.06 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.79 (d, J = 8.1 Hz, 1H, H_{ar}), 7.59 (d, 1H, J = 8.1 Hz, 1H, H_{ar}), 7.39 (t, J = 8.1 Hz, 1H, H_{ar}), 4.53 (s, 2H, CH_2), 3.66 (s, 3H, NMe_{Phthal}), 3.55 (s, 3H, NMe), 3.06 (t, J = 7.2 Hz, 2H, SCH_2), 2.30 (t, J = 7.3 Hz 2H, CH_2CO), 1.67-1.56 (m, 4H, 2 x CH_2), 1.38-1.20 (m, 6H, 3 x CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3 (CONH), 158.3 (CONMe), 152.5 ($C_{IV\text{-}C=N}$), 149.5 ($C_{IV\text{-}C=N}$), 141.04 ($C_{IV\text{-}C=N}$), 139.3 (C_{IV}), 133.0 ($CH_{ar\text{-}Phthal}$), 131.7 ($CH_{ar\text{-}Phthal}$), 128.2 (2 x CH_{ar}), 127.1 (C_{IV}), 125.8 (C_{IV}), 125.6 ($CH_{ar\text{-}Phthal}$), 125.6 (CH_{ar}), 123.6 (CH_{ar}), 122.8 (C_{IV}), 119.67 (CH_{ar}), 39.5 (NMe_{Phthal}), 36.2 (CH_2CO), 32.6 (SCH_2), 30.2 (NMe), 28.9 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 27.5 (CH_2), 24.79 (CH_2). HRMS (ESI^+): calcd for $C_{28}H_{32}N_6O_2NaS$ $(M+Na)^+$ 571.2098, found 571.2121.

*N-(2,4-dichlorophenyl)-5-(4-(((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)pentanamide (**84**).* To a solution of alkyne **55** (0.21 mmol, 68 mg) and azide **40** (0.21 mmol, 60 mg) in *t*BuOH/ H_2O (1.1), was added sodium ascorbate (0.1 eq, 110 µL, of a 0.2 M water solution) and

$CuSO_4.5H_2O$ (0.01 eq, 10 µL of a 0.2 M water solution). After stirring at 60 °C during 20 h, a saturated solution of Na_2CO_3 was added and the aqueous phase was extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (DCM/ petroleum ether; 99:1 to 96:4) to give compound **84** with 60% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (dd, J = 7.5 and J = 1.5 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.22 (d, J = 8.8 Hz, 1H, H_{ar}), 8.21 (m, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 3H, 2 x $H_{ar\text{-}Phthal}$ and H_{ar}), 7.69 (s, 1H, CH_{Triaz}), 7.34 (d, J = 2.4 Hz, 1H, H_{ar}), 7.20 (dd, J = 8.9 Hz and J = 2.4 Hz, 1H, H_{ar}), 4.47 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 4.28 (t, J = 7.4 Hz, 2H, NCH_2), 3.78 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 2.41 (t, J = 7.5 Hz, 2H, CH_2CO), 1.92 (m, 6H, 3 x CH_2), 1.68 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (CONH), 159.4 (CONMe), 152.3 ($C_{IV\text{-}C=N}$), 143.5 ($C_{IV\text{-}C=N}$), 140.4 ($C_{IV\text{-}C=N}$), 133.3 ($CH_{ar\text{-}Phthal}$), 133.2 (CH_{arl}, 131.8 ($CH_{ar\text{-}Phthal}$), 128.8 (CH), 128.7 (CH_{ar}), 127.9 (CH_{ar}), 127.8 (CH_{ar}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.0 (C_{IV}), 123.4 (CH), 123.3 (CH_{ar}), 122.7 (CH_{ar}), 49.8 (CH_2-Ntriaz), 39.4 (NMe_{Phthal}), 36.5 (CH_2CO), 30.7 (NMe), 29.7 (CH_2), 27.2 (CH_2S), 22.0 (CH_2). HRMS (ESI^+): calcd for $C_{27}H_{28}Cl_2N_9O_2S$ $(M+H)^+$ 612.1458, found 612.1460.

*N-(2,4-dichlorophenyl)-8-((5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-1,3,4-oxadiazol-2-yl)thio)octanamide (**85**).* Following general procedure 2 and starting from 4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-methylphthalazin-1(2*H*)-one **5** (0.55 mmol, 0.15 g), and 8-bromo-*N*-(2,4-dichlorophenyl)octanamide **10** (0.66 mmol, 0.24 g), purification by column chromatography on silica gel (DCM/ethyl acetate; 90:10) afforded **85** in 20% yield, as an beige powder; mp 61 - 63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.30 (d, J = 8.5 Hz, 1H, H_{ar}), 7.72-7.85 (m, 3H, 3 x $H_{ar\text{-}Phthal}$), 7.60 (bs, 1H, NH), 7.34 (d, J = 2.4 Hz, 1H, H_{ar}), 7.21 (dd, J = 8.6 and J = 2.4 Hz, 1H, H_{ar}), 4.48 (s, 2H,

CH_2), 3.80 (s, 3H, NMe_{Phthal}), 3.17 (t, J = 7.5 Hz, 2H, SCH_2), 2.39 (t, J = 7.2 Hz, 2H, CH_2CO), 1.79-1.65 (m, 4H, 2 x CH_2), 1.45-1.28 (m, 6H, 3 x CH_2). RMN ^{13}C (75 MHz, $CDCl_3$) δ 171.3 (CONH), 165.6 ($C_{IV\text{-}C=N}$), 164.1 ($C_{IV\text{-}C=N}$), 159.5 (CONMe), 139.2 ($C_{IV\text{-}C=N}$), 133.4 (CH_{ar}), 133.3 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.0, 128.8, 128.7, 127.9 (2 x CH_{ar}-, $CH_{ar\text{-}Phthal}$, C_{IV}), 127.4 ($CH_{ar\text{-}Phthal}$), 124.3 ($CH_{ar\text{-}Phthal}$), 123.2 (C_{IV}), 122.5 (CH_{ar}), 39.5 (CH_3), 37.8 (CH_2CO), 32.5 (SCH_2), 29.9 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.7 (CH_2), 28.4 (CH_2), 25.3 (CH_2). HRMS (ESI^+): calcd for $C_{26}H_{28}Cl_2N_5O_3S$ $(M+H)^+$ 560.1284, found 560.1286.

*N-(2-chlorophenyl)-11-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)undecanamide (**86**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g), and 11-bromo-*N*-(2-chlorophenyl)undecanamide **35** (3 mmol, 1.12 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **86** in 42% yield, as a white powder; mp 129 - 130 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (dd, J = 7.5 Hz and J = 1.0 Hz, 1H, $H_{ar\text{-}Phthal}$), 8.35 (d, 1H, CH_{ar}), 8.21 (m, 1H, $H_{ar\text{-}Phthal}$), 7.75 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 77.65 (bs, 1H, NH), 7.33 (dd, J = 7.3 and J = 1.3 Hz, 1H, H_{ar}), 7.24 (t, J = 8.2 Hz, 1H, H_{ar}), 7.01 (t, J = 8 Hz, 1H, H_{ar}), 4.47 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.17 (t, J = 7.3 Hz, 2H, SCH_2), 2.41 (t, J = 7.4 Hz, 2H, CH_2CO), 1.70 (m, 4H, 2 x CH_2), 1.32 (m, 12H, 6 x CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5 (CONH), 159.5 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 151.8 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 134.7 (C_{IV}), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV} and CH_{ar}), 127.9 (C_{IV}), 127.8 (C_{IV}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 124.5 (CH_{ar}), 122.7 (C_{IV}), 121.8 (CH_{ar}), 39.5 (NMe_{Phthal}), 37.9 (CH_2CO), 33.2 (SCH_2), 30.7 (NMe), 30.2 (CH_2), 29.6 (CH_2), 29.6-28.6 (6 x CH_2), 25.6 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{30}H_{38}ClN_6O_2S$ $(M+H)^+$ 581.2460, found 581.2449.

*N-(4-chlorophenyl)-11-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)undecanamide (**87**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g), and 11-bromo-*N*-(4-chlorophenyl)undecanamide (3 mmol, 1.12 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **87** in 54% yield, as a white powder; mp 168 - 169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.17 (m, 1H, $H_{ar\text{-}Phthal}$), 7.94 (bs, 1H, NH), 7.75 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.49 (d, J = 7.5 Hz, 2H, 2 x H_{ar}), 7.22 (d, J = 8.9 Hz, 2H, 2 x H_{ar}), 4.47 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.17 (t, J = 7.4 Hz, 2H, SCH_2), 2.32 (t, J = 7.5 Hz, 2H, CH_2CO), 1.69 (m, 4H, 2 x CH_2), 1.43-1.15 (m, 12H, 6 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (CONH), 159.6 (CONMe), 152.2 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 137.7 (C_{IV}), 133.5 ($CH_{ar\text{-}Phthal}$), 132 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 120 (2 x CH_{ar}), 128.1 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.3 ($CH_{ar\text{-}Phthal}$), 121.2 (2 x CH_{ar}), 39.5 (NMe_{Phthal}), 37.7 (CH_2CO), 33.3 (SCH_2), 30.8 (NMe), 30.1 (CH_2), 29.5 (CH_2), 29.6-28.6 (6 x CH_2), 25.6 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{30}H_{38}ClN_6O_2S$ $(M+H)^+$ 581.2460, found 581.2434.

*11-((4-Methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-(naphthalen-1-yl)undecanamide (**88**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g), and 11-bromo-*N*-(naphthalen-1-yl)undecanamide **37** (3 mmol, 1.95 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **88** in 65% yield, as a white powder; mp 136 - 137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (m, 1H, $H_{ar\text{-}Phthal}$), 8.11 (m, 1H, $H_{ar\text{-}Phthal}$), 8.00-7.79 (m, 3H, 3 x H_{ar}), 7.74 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 7.65 (m, 1H, H_{ar}), 7.45 (m, 3H, 3 x H_{ar}),

4.40 (s, 2H, CH_2), 3.77 (s, 3H, NMe_{Phthal}), 3.51 (s, 3H, NMe), 3.17 (t, J = 7.4 Hz, 2H, SCH_2), 2.53 (m, 2H, CH_2CO), 1.91-1.63 (m, 4H, 2 x CH_2), 1.50-1.04 (m, 12H, 6 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (CONH), 159.6 (CONMe), 152.2 ($C_{IV\text{-}C=N}$), 152.0 ($C_{IV\text{-}C=N}$), 140.8 ($C_{IV\text{-}C=N}$), 134.3 ($C_{IV\text{-}napht}$), 133.4 ($CH_{ar\text{-}Phthal}$), 132.7 ($C_{IV\text{-}napht}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.0 (C_{IV}), 128.8 ($CH_{ar\text{-}napht}$), 128.0 (C_{IV}), 127.5, 127.2, 126.2, 126.0, 125.8, 125.3, 121.4, 124.0 (8 x C_{ar}), 39.5 (NMe_{Phthal}), 37.7 (CH_2CO), 33.3 (SCH_2), 30.7 (NMe), 30.1 (CH_2), 29.6 (CH_2), 29.5-28.4 (5 x CH_2), 25.9 (CH_2). HRMS (MALDI: DHB, PEG 600) calcd for $C_{34}H_{41}N_6O_2S$ $(M+H)^+$ 597.3006, found 597.2998.

*N-((3s,5s,7s)-adamantan-1-yl)-11-((4-methyl-5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-4H-1,2,4-triazol-3-yl)thio)undecanamide (**89**)*. Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g), and *N*-((3s,5s,7s)-adamantan-1-yl)-11-bromoundecanamide **37** (3 mmol, 1.17 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **89** in 75% yield, as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 8.43 (m, 1H, $H_{ar\text{-}Phthal}$), 8.23 (m, 1H, $H_{ar\text{-}Phthal}$), 7.78 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 4.96 (br, 1H, NH), 4.48 (s, 2H, CH_2), 3.80 (s, 3H, NMe_{Phthal}), 3.54 (s, 3H, NMe), 3.18 (t, J = 7.3 Hz, 2H, SCH_2), 2.05 (m, 5H, CH_2 and 3 x CH_{adam}), 1.98 (m, 6H, 3 x $CH_{2\text{-}adam}$), 1.71 (m, 2H, CH_2), 1.67 (m, 6H, 3 x $CH_{2\text{-}adam}$), 1.56 (m, 2H, CH_2), 1.39 (m, 2H, CH_2), 1.25 (m, 10H, 5 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (CONH), 159.6 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 151.9 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.5 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.1 (C_{IV}), 128.0 (C_{IV}), 127.2 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 51.9 ($C_{IV\text{-}adam}$), 41.8 (3 x $CH_{2\text{-}adam}$), 39.5 (NMe_{Phthal}), 37.9 ($COCH_2$), 36.5 (3 x $CH_{2\text{-}adam}$), 33.3 (SCH_2), 30.7 (C_{IV}), 30.3 (NMe), 29.6 (CH_2), 29.5-28.8 (7 x CH_2), 25.9 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{34}H_{49}N_6O_2S$ $(M+H)^+$ 605.3632, found 605.3623.

*4-((5-(Decylthio)-4-methyl-4H-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2H)-one (**90**).* Following general procedure 2 and starting from 4-((5-mercapto-4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-methylphthalazin-1(2*H*)-one **1** (1 mmol, 0.29 g), and 1-bromo decane (3 mmol, 0.66 g), purification by column chromatography on silica gel (DCM/MeOH; 98:2) afforded compound **90** in 84% yield, as a white powder; mp 108 - 109 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (m, 1H, $H_{ar\text{-}Phthal}$), 8.22 (m, 1H, $H_{ar\text{-}Phthal}$), 7.76 (m, 2H, 2 x $H_{ar\text{-}Phthal}$), 4.46 (s, 2H, CH_2), 3.79 (s, 3H, NMe_{Phthal}), 3.53 (s, 3H, NMe), 3.18 (t, J = 7.4 Hz, 2H, SCH_2), 1.71 (m, 2H, SCH_2CH_2), 1.39 (m, 2H, CH_2CH_3), 1.32-1.16 (m, 12H, 6 x CH_2), 0.85 (t, J = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6 (CONMe), 152.1 ($C_{IV\text{-}C=N}$), 151.9 ($C_{IV\text{-}C=N}$), 140.9 ($C_{IV\text{-}C=N}$), 133.4 ($CH_{ar\text{-}Phthal}$), 131.9 ($CH_{ar\text{-}Phthal}$), 129.2 (C_{IV}), 128.1 (C_{IV}), 127.1 ($CH_{ar\text{-}Phthal}$), 125.4 ($CH_{ar\text{-}Phthal}$), 39.5 (NMe_{Phthal}), 33.3 (SCH_2), 32.0-29.0 (5 x CH_2), 30.7 (NMe), 29.6 (SCH_2CH_2), 28.8 (CH_2), 22.8 (CH_2), 14.2 (CH_3). HRMS (ESI^+) calcd for $C_{23}H_{34}N_5OS$ $(M+H)^+$ 428.2478, found 428.2467.

*N-(2,4-dichlorophenyl)-11-((5-((3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)methyl)-1,3,4-oxadiazol-2-yl)thio)undecanamide (**91**).* Following general procedure 2 and starting 4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-methylphthalazin-1(2*H*)-one **5** (1 mmol, 0.27 g), and 11-bromo-*N*-(2,4-dichlorophenyl)undecanamide **12** (3 mmol, 1.21 g), purification by column chromatography on silica gel (DCM/MeOH; 90:10) afforded compound **91** in 65% yield, as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (m, 1H, $H_{ar\text{-}Phthal}$), 8.34 (d, J = 8.8 Hz, 1H, H_{ar}), 7.86-7.57 (m, 3H, 2 x $H_{ar\text{-}Phthal}$ and H_{ar}), 7.57 (bs, 1H, NH), 7.36 (d, J = 2.3 Hz, 1H, H_{ar}), 7.24 (dd, J = 8.9 Hz, J = 2.4 Hz, 1H, H_{ar}), 4.49 (s, 2H, CH_2), 3.82 (s, 3H, NMe_{Phthal}), 3.19 (t, J = 7.3 Hz, 2H, SCH_2), 2.41 (t, J = 7.4 Hz, 2H, CH_2CO), 1.79-1.69 (m, 4H, 2 x CH_2), 1.4-1.27 (m, 12H, 6 x CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3 (CONH),

165.6 ($C_{IV-C=N}$), 163.9 ($C_{IV-C=N}$), 159.4 (CONMe), 139.1 ($C_{IV-C=N}$), 133.4 (C_{IV}), 133.2 ($CH_{ar-Phthal}$), 131.8 ($CH_{ar-Phthal}$), 128.9 (C_{IV}), 128.8 (C_{IV}), 128.6 (CH_{ar}), 128.0 (C_{IV}), 127.9 (CH_{ar}), 127.3 ($CH_{ar-Phthal}$), 124.2 ($CH_{ar-Phthal}$), 122.3 (C_{IV}), 39.4 (NMe_{Phthal}), 37.8 (CH_2CO), 33.5 (SCH_2), 29.8 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.1 (CH_2), 28.5 (6 x CH_2), 25.4 (CH_2). HRMS (MALDI: DHB, PEG 600): calcd for $C_{30}H_{37}Cl_2N_6O_2S$ $(M+H)^+$ 615.2070, found 615.2076.

4.3 Biological assays

Cell lines, recombinant proteins and antibodies. The 32Dβ and NK-92 cell lines were used in that study and cultured as described previously.[38, 52] Recombinant human IL-15, recombinant murine IL-3 were obtained from Peprotech, Inc. and recombinant human IL-2 from Chiron. RLI was produced as described previously.[26] Recombinant IL-2Rβ (224-2B-025/CF) was purchased from R&D Systems. CF1 antibody was from Beckman Coulter.

SPR experiment. The SPR experiments were performed at 25 °C with a BIAcore 3000 biosensor (GE Healthcare, Chalfont St Giles, UK). Recombinant IL-15, RLI or IL-2 were covalently immobilized to CM5 sensor chips using the amine coupling method in accordance with the manufacturer's instructions, and the binding of compounds at 100 μM or at increasing concentrations prepared in PBS, 1% or 5% DMSO was monitored. Analysis of sensorgrams was performed using BIAeval 4.1 software.

HTRF assays. HTRF assays were performed using as donor molecule Lumi4®-Tb-NHS cryptate (Tb) conjugated to CF1 mouse anti-human IL-2Rβ mAb, and as acceptor molecule D2 conjugated to Streptavidin (Streptavidin-D2) (HTRF® Dye labeling kits, Cisbio). The donor excitation at 320 nm leads to energy emission at 620 nm that induces in close proximity (d<10 nm) excitation of the acceptor and energy emission at 665 nm. The assays were carried out in a white 384 well Small Volume™ HiBase Polystyrene Microplate (Greiner) at room

temperature. After a 30-min pre-incubation period of biotinylated human IL-15 (Fluorokine®, R&D Systems) with compounds, recombinant human IL-2Rβ was added for an additional 30-min incubation time. Finally, the donor CF1-Tb and the acceptor Streptavidin-D2 were added to the mixture and incubated for 30 min. Final concentrations were 100 µM for compounds and 20 nM for biotinylated IL-15, IL-2Rβ, CF1-Tb and Streptavidin-D2 in PBS, 0.1% BSA 1% DMSO. Fluorescence signals were read with a Mithras reader (Berthold) at excitation 320 nm and emission 620 nm and 665 nm, and the HTRF was calculated as normalized fluorescence transfer value (ΔF):

$$\Delta F = \frac{(F665nmSample/F620nmSample)-(F665nmcontrol/F620nmcontrol)}{(F665nmcontrol/F620nmcontrol)}$$

Proliferation assays. The proliferation response of 32Dβ cells to RLI 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 fixed or increasing concentrations of compounds or the vehicle (culture medium, DMSO 0.1%, final concentration). Alamar blue (10 µl) was added to each well and, after a 6h-incubation period at 37 °C, the emitted fluorescence at 590 nm under excitation at 560 nm was measured using Fluoroskan Ascent FL reader (Thermo Electro Corporation). Compounds were tested in triplicates in at least three independent experiments.

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 or increasing concentrations of IL-15 or IL-2 that have been or not preincubated for

30 min with increasing concentrations of chemical compounds or the vehicle (PBS, BSA 0.1% DMSO 0.6%, final concentration). 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). Compounds were tested in duplicates in at least three independent experiments.

***Cytotoxicity assay*.** The cytotoxicity of the main compounds was evaluated on 32Dβ cells using the CytoTox-FluorTM Cytoxicity Assay kit from Promega. Briefly, $1x10^4$ cells were plated in 100 µl and cultured in the medium supplemented with vehicle (culture medium, DMSO 0.1%, final concentration) or compounds at a concentration of 11 µM for 3 h. The bis-AAF-R110 Substrate was then added in each well and the dead-cell protease activity was detected 30 min later by measuring the fluorescence signals with a Mithras reader (Berthold) at excitation 485 nm and emission 520 nm. Conditions with medium and with medium supplemented with digitonin were used for negative and positive controls respectively. Compounds were tested in triplicates in two independent experiments.

ASSOCIATED CONTENT

Supporting Information

Docking scores of compounds selected from virtual screening; *in vitro* inhibitory effect of 5 compounds selected from virtual screening; Dose-response curves of the effect of cytokines on 32Dβ cell proliferation; Evaluation of the cytotoxicity of some compounds; SAR of **1** and its homologs **41**-**46**; SAR of **1** and its analogs **47**-**54** and **56**-**62**; SAR of **43** and **45** and their analogs **47**-**54** and **56**-**62**; Dose-response curves of the effect of cytokines on p-Stat5 in NK-92 cells; Synthesis and chemical characteristics of the halogenoalkylamides **6-39** and the azidoalkylamide **40** ; NMR spectra of the tested compounds **1** and **41**-**91**.

Molecular Formula Strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

IL, Interleukin; R, Receptor; Stat, Signal Transducers and Activators of Transcription; RA, Rheumatoid Arthritis; PPIs, Protein-Protein Interactions; SPR, Surface Plasmon Resonance; HTRF, Homogeneous Time Resolved Fluorescence; Tb, Lumi4®-Tb-NHS cryptate; HB, hydrogen bond.

REFERENCES

(1) Grabstein, K. H.; Eisenman, J.; Shanebeck, K.; Rauch, C.; Srinivasan, S.; Fung, V.; Beers, C.; Richardson, J.; Schoenborn, M. A.; Ahdieh, M.; Johnson, L.; Alderson, M. R.; Watson, J. D.; Anderson, D. M.; Giri, J. G. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science* **1994,** *264*, 965-968.

(2) Burton, J. D.; Bamford, R. N.; Peters, C.; Grant, A. J.; Kurys, G.; Goldman, C. K.; Brennan, J.; Roessler, E.; Waldmann, T. A. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. U. S. A.* **1994,** *91*, 4935-4939.

(3) Kirman, I.; Nielsen, O. H. Increased numbers of interleukin-15-expressing cells in active ulcerative colitis. *Am. J. Gastroenterol.* **1996,** *91*, 1789-1794.

(4) Ruckert, R.; Asadullah, K.; Seifert, M.; Budagian, V. M.; Arnold, R.; Trombotto, C.; Paus, R.; Bulfone-Paus, S. Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? *J. Immunol.* **2000,** *165*, 2240-2250.

(5) Yamada, Y.; Kamihira, S. Pathological roles of interleukin-15 in adult T-cell leukemia. *Leuk. Lymphoma* **1999,** *35*, 37-45.

(6) McInnes, I. B.; Liew, F. Y. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. *Immunol. Today* **1998,** *19*, 75-79.

(7) Pavlakis, M.; Strehlau, J.; Lipman, M.; Shapiro, M.; Maslinski, W.; Strom, T. B. Intragraft IL-15 transcripts are increased in human renal allograft rejection. *Transplantation* **1996,** *62*, 543-545.

(8) Manfro, R. C.; Roy-Chaudhury, P.; Zheng, X. X.; Steiger, J.; Nickerson, P. W.; Li, Y.; Maslinski, W.; Strom, T. B. Interleukin-15 gene transcripts are present in rejecting islet allografts. *Transplant. Proc.* **1997,** *29*, 1077-1078.

(9) Ruchatz, H.; Leung, B. P.; Wei, X. Q.; McInnes, I. B.; Liew, F. Y. Soluble IL-15 receptor α-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. *J. Immunol.* **1998,** *160*, 5654-5660.

(10) Ferrari-Lacraz, S.; Zanelli, E.; Neuberg, M.; Donskoy, E.; Kim, Y. S.; Zheng, X. X.; Hancock, W. W.; Maslinski, W.; Li, X. C.; Strom, T. B.; Moll, T. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. *J. Immunol.* **2004,** *173*, 5818-5826.

(11) Wang, D.; Deng, X.; Leng, X.; Mao, X. Interleukin-15 receptor-directed immunotoxins atteunuate disease severity in rat adjuvant arthritis. *Mol. Immunol.* **2010,** *47*, 1535-1543.

(12) Yang, X. K.; Xu, W. D.; Leng, R. X.; Liang, Y.; Liu, Y. Y.; Fang, X. Y.; Feng, C. C.; Li, R.; Cen, H.; Pan, H. F.; Ye, D. Q. Therapeutic potential of IL-15 in rheumatoid arthritis. *Hum. Immunol.* **2015,** *76*, 812-818.

(13) Sheng, C.; Dong, G.; Miao, Z.; Zhang, W.; Wang, W. State-of-the-art strategies for targeting protein-protein interactions by small-molecule inhibitors. *Chem. Soc. Rev.* **2015,** *44*, 8238-8259.

(14) Villoutreix, B. O.; Kuenemann, M. A.; Poyet, J. L.; Bruzzoni-Giovanelli, H.; Labbe, C.; Lagorce, D.; Sperandio, O.; Miteva, M. A. Drug-Like Protein-Protein Interaction Modulators: Challenges and Opportunities for Drug Discovery and Chemical Biology. *Mol. Inform.* **2014,** *33*, 414-437.

(15) Raimundo, B. C.; Oslob, J. D.; Braisted, A. C.; Hyde, J.; McDowell, R. S.; Randal, M.; Waal, N. D.; Wilkinson, J.; Yu, C. H.; Arkin, M. R. Integrating fragment assembly and biophysical methods in the chemical advancement of small-molecule antagonists of IL-2: an approach for inhibiting protein-protein interactions. *J. Med. Chem.* **2004,** *47*, 3111-3130.

(16) Kalsoom, S.; Rashid, U.; Shaukat, A.; Abdalla, O. M.; Hussain, K.; Khan, W.; Nazir, S.; Mesaik, M. A.; Haq, Z.; Ansari, F. L. In vitro and in silico exploration of IL-2 inhibition by small drug-like molecules. *Med. Chem. Res.* **2013,** *22*, 5739-5751.

(17) Zhang, T.; Bai, X.; Mao, X. Systemic delivery of small interfering RNA targeting the interleukin-2/15 receptor β chain prevents disease progression in experimental arthritis. *PLoS One* **2013,** *8*, e78619.

(18) Pettit, D. K.; Bonnert, T. P.; Eisenman, J.; Srinivasan, S.; Paxton, R.; Beers, C.; Lynch, D.; Miller, B.; Yost, J.; Grabstein, K. H.; Gombotz, W. R. Structure-function studies of interleukin 15 using site-specific mutagenesis, polyethylene glycol conjugation, and homology modeling. *J. Biol. Chem.* **1997,** *272*, 2312-2318.

(19) Bernard, J.; Harb, C.; Mortier, E.; Quemener, A.; Meloen, R. H.; Vermot-Desroches, C.; Wijdeness, J.; van Dijken, P.; Grotzinger, J.; Slootstra, J. W.; Plet, A.; Jacques, Y. Identification of an interleukin-15α receptor-binding site on human interleukin-15. *J. Biol. Chem.* **2004,** *279*, 24313-24322.

(20) Pulliam, S. R.; Uzhachenko, R. V.; Adunyah, S. E.; Shanker, A. Common gamma chain cytokines in combinatorial immune strategies against cancer. *Immunol. Lett.* **2016,** *169*, 61-72.

(21) Chirifu, M.; Hayashi, C.; Nakamura, T.; Toma, S.; Shuto, T.; Kai, H.; Yamagata, Y.; Davis, S. J.; Ikemizu, S. Crystal structure of the IL-15-IL-15Rα complex, a cytokine-receptor unit presented in trans. *Nat. Immunol.* **2007,** *8*, 1001-1007.

(22) Shanafelt, A. B.; Lin, Y.; Shanafelt, M. C.; Forte, C. P.; Dubois-Stringfellow, N.; Carter, C.; Gibbons, J. A.; Cheng, S. L.; Delaria, K. A.; Fleischer, R.; Greve, J. M.; Gundel, R.; Harris, K.; Kelly, R.; Koh, B.; Li, Y.; Lantz, L.; Mak, P.; Neyer, L.; Plym, M. J.; Roczniak, S.; Serban, D.; Thrift, J.; Tsuchiyama, L.; Wetzel, M.; Wong, M.; Zolotorev, A. A T-cell-selective interleukin 2 mutein exhibits potent antitumor activity and is well tolerated in vivo. *Nat. Biotechnol.* **2000,** *18*, 1197-1202.

(23) Collins, L.; Tsien, W. H.; Seals, C.; Hakimi, J.; Weber, D.; Bailon, P.; Hoskings, J.; Greene, W. C.; Toome, V.; Ju, G. Identification of specific residues of human interleukin 2 that affect binding to the 70-kDa subunit (p70) of the interleukin 2 receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1988,** *85*, 7709-7713.

(24) Wang, X.; Rickert, M.; Garcia, K. C. Structure of the quaternary complex of interleukin-2 with its α, β, and γc receptors. *Science* **2005,** *310*, 1159-1163.

(25) Ring, A. M.; Lin, J. X.; Feng, D.; Mitra, S.; Rickert, M.; Bowman, G. R.; Pande, V. S.; Li, P.; Moraga, I.; Spolski, R.; Ozkan, E.; Leonard, W. J.; Garcia, K. C. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. *Nat. Immunol.* **2012,** *13*, 1187-1195.

(26) Mortier, E.; Quemener, A.; Vusio, P.; Lorenzen, I.; Boublik, Y.; Grotzinger, J.; Plet, A.; Jacques, Y. Soluble interleukin-15 receptor α (IL-15Rα)-sushi as a selective and potent agonist of IL-15 action through IL-15Rβ/γ. Hyperagonist IL-15 x IL-15Rα fusion proteins. *J. Biol. Chem.* **2006,** *281*, 1612-1619.

(27) Bissantz, C.; Kuhn, B.; Stahl, M. A medicinal chemist's guide to molecular interactions. *J. Med. Chem.* **2010,** *53*, 5061-5084.

(28) Polucci, P.; Magnaghi, P.; Angiolini, M.; Asa, D.; Avanzi, N.; Badari, A.; Bertrand, J.; Casale, E.; Cauteruccio, S.; Cirla, A.; Cozzi, L.; Galvani, A.; Jackson, P. K.; Liu, Y.; Magnuson, S.; Malgesini, B.; Nuvoloni, S.; Orrenius, C.; Sirtori, F. R.; Riceputi, L.; Rizzi, S.; Trucchi, B.; O'Brien, T.; Isacchi, A.; Donati, D.; D'Alessio, R. Alkylsulfanyl-1,2,4-triazoles, a new class of allosteric valosine containing protein inhibitors. Synthesis and structure-activity relationships. *J. Med. Chem.* **2013,** *56*, 437-450.

(29) Carpino, L. A.; El-Faham, A. The diisopropylcarbodiimide/1-hydroxy-7-azabenzotriazole system: segment coupling and stepwise peptide assembly. *Tetrahedron* **1999,** *55*, 6813-6330.

(30) Montalbetti, C. A. G. N.; Falque, V. Amide bond formation and peptide coupling. *Tetrahedron* **2005,** *61*, 10827-10852.

(31) Johnson, C. R.; Gorla, S. K.; Kavitha, M.; Zhang, M.; Liu, X.; Striepen, B.; Mead, J. R.; Cuny, G. D.; Hedstrom, L. Phthalazinone inhibitors of inosine-5'-monophosphate dehydrogenase from Cryptosporidium parvum. *Bioorg. Med. Chem. Lett.* **2013,** *23*, 1004-1007.

(32) Sridhara, A. M.; Reddy, K. R.; Keshavayya, J.; Goud, P. S.; Somashekar, B. C.; Bose, P.; Peethambar, S. K.; Gaddam, S. K. Synthesis and antimicrobial activity of 2-substituted [4-(1,3,4-oxadiazol-2-yl methyl)] phthalazin-1(2H)-one derivatives. *Eur. J. Med. Chem.* **2010,** *45*, 4983-4989.

(33) Plech, T.; Luszczki, J. J.; Wujec, M.; Flieger, J.; Pizon, M. Synthesis, characterization and preliminary anticonvulsant evaluation of some 4-alkyl-1,2,4-triazoles. *Eur. J. Med. Chem.* **2013,** *60*, 208-215.

(34) Sridhara, A. M.; Venugopala, K. R.; Keshavayya, J.; Ambika, D. S.; Goud, S.; Peethambar, S. K. Synthesis and antimicrobial studies of some new 3-isoxazoline substituted phthalazine methylsulfonyl oxadiazoles. *E-Journal of Chemistry* **2011,** *8*, 1022-1029.

(35) Shaker, M. R. The chemistry of mercapto- and thione- substituted 1,2,4-triazoles and their utility in heterocyclic synthesis. *ARKIVOC* **2006,** *ix*, 59-112.

(36) Kolb, H. C.; Sharpless, K. B. The growing impact of click chemistry on drug discovery. *Drug Discovery Today* **2003,** *8*, 1128-1137.

(37) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew Chem. Int. Ed. Engl.* **2002,** *41*, 2596-2599.

(38) Vincent, M.; Bessard, A.; Cochonneau, D.; Teppaz, G.; Sole, V.; Maillasson, M.; Birkle, S.; Garrigue-Antar, L.; Quemener, A.; Jacques, Y. Tumor targeting of the IL-15 superagonist RLI by an anti-GD2 antibody strongly enhances its antitumor potency. *Int. J. Cancer* **2013,** *133*, 757-765.

(39) Vincent, M.; Teppaz, G.; Lajoie, L.; Sole, V.; Bessard, A.; Maillasson, M.; Loisel, S.; Bechard, D.; Clemenceau, B.; Thibault, G.; Garrigue-Antar, L.; Jacques, Y.; Quemener, A. Highly potent anti-CD20-RLI immunocytokine targeting established human B lymphoma in SCID mouse. *MAbs* **2014,** *6*, 1026-1037.

(40) Tilley, J. W.; Chen, L.; Fry, D. C.; Emerson, S. D.; Powers, G. D.; Biondi, D.; Varnell, T.; Trilles, R.; Guthrie, R.; Mennone, F.; Kaplan, G.; LeMahieu, R. A.; Carson, M.; Han, R.-J.; Liu, C.-M.; Palermo, R.; Ju, G. Identification of a small molecule inhibitor of the IL-2/IL-2Rα receptor interaction which binds to IL-2. *J. Am. Chem. Soc.* **1997,** *119*, 7589-7590.

(41) Geng, B.; Fleming, P. R.; Umlauf, S.; Lin, A.; Pallai, P. V. The synthesis and selective IL-2 inhibitory activity of bis piperazine-phenol Mannich adducts. *Bioorg. Med. Chem. Lett.* **2002,** *12*, 775-778.

(42) Waldmann, T. A. Targeting the interleukin-15/interleukin-15 receptor system in inflammatory autoimmune diseases. *Arthritis Res. Ther.* **2004,** *6*, 174-177.

(43) Waldmann, T. A. Targeting the interleukin-15 system in rheumatoid arthritis. *Arthritis Rheum.* **2005,** *52*, 2585-2588.

(44) Tinubu, S. A.; Hakimi, J.; Kondas, J. A.; Bailon, P.; Familletti, P. C.; Spence, C.; Crittenden, M. D.; Parenteau, G. L.; Dirbas, F. M.; Tsudo, M. Humanized antibody directed to the IL-2 receptor beta-chain prolongs primate cardiac allograft survival. *J. Immunol.* **1994,** *153*, 4330-4338.

(45) Nata, T.; Basheer, A.; Cocchi, F.; van Besien, R.; Massoud, R.; Jacobson, S.; Azimi, N.; Tagaya, Y. Targeting the binding interface on a shared receptor subunit of a cytokine family enables the inhibition of multiple member cytokines with selectable target spectrum. *J. Biol. Chem.* **2015,** *290*, 22338-22351.

(46) Krammer, A.; Kirchhoff, P. D.; Jiang, X.; Venkatachalam, C. M.; Waldman, M. LigScore: a novel scoring function for predicting binding affinities. *J. Mol. Graph. Model.* **2005,** *23*, 395-407.

(47) Gehlhaar, D. K.; Verkhivker, G. M.; Rejto, P. A.; Sherman, C. J.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. Molecular recognition of the inhibitor AG-1343 by HIV-1 protease: conformationally flexible docking by evolutionary programming. *Chem. Biol.* **1995,** *2*, 317-324.

(48) Muegge, I.; Martin, Y. C. A general and fast scoring function for protein-ligand interactions: a simplified potential approach. *J. Med. Chem.* **1999,** *42*, 791-804.

(49) Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman, M. LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites. *J. Mol. Graph. Model.* **2003,** *21*, 289-307.

(50) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010,** *53*, 2719-2740.

(51) Lagorce, D.; Sperandio, O.; Baell, J. B.; Miteva, M. A.; Villoutreix, B. O. FAF-Drugs3: a web server for compound property calculation and chemical library design. *Nucleic Acids Res.* **2015,** *43*, W200-207.

(52) Tamzalit, F.; Barbieux, I.; Plet, A.; Heim, J.; Nedellec, S.; Morisseau, S.; Jacques, Y.; Mortier, E. IL-15.IL-15Rα complex shedding following trans-presentation is essential for the survival of IL-15 responding NK and T cells. *Proc. Natl. Acad. Sci. U. S. A.* **2014,** *111*, 8565-8570.

FIGURE AND SCHEME LEGENDS

Figure 1. IL-2/IL-15 – IL-2Rβ interfaces, pharmacophore model and binding mode of compound 1. (A) Ribbon diagram of IL-2 in complex with IL-2Rβ (PDB code 2B5I) and (B) of IL-15 (PDB code 2Z3Q) showing the side chains of Asp20 (D20), Asn88 (N88) and Ile92 (I92) of IL-2 involved in the hydrogen bonding networks to 2 water molecules and Gln70 (Q70) and Tyr134 (Y134) of IL-2Rβ, and the homologous residues Asp8 (D8), Asn65 (N65) and Leu69 (L69) of IL-15. (C) Pharmacophore model used for database searches. HB donor projection (HBD) indicated as pink spheres, HB donor or acceptor (HBDA) projection as a purple sphere, hydrophobic feature (HY) as a cyan sphere and exclusion spheres in gray.

Figure 2. SPR analysis and HTRF assays. (A) SPR sensorgrams of binding to immobilized IL-15 with one concentration of 100 μM or (B) increasing concentrations (3.125, 6.25, 12.5, 25, 50 and 100 μM) of one positive compound. (C) HTRF principle with Lumi4®-Tb-NHS cryptate (Tb) and D2 as donor and acceptor, respectively. If the labeled soluble IL-2Rβ binds to biotinylated IL-15, the two fluorophores will be in close proximity to allow FRET to occur, which results in a specific long-lived fluorescence emission of D2 at 665 nm. A chemical inhibitor binding to IL-15 will modify the intensity of the fluorescence emission. (D) An example of HTRF screening of a hundred small molecular compounds against IL-15/IL-2Rβ interaction. Data are normalized based on negative (○) and positive (□) controls.

Figure 3: **Structure of the selected hit 1 with three modulable fragments and both chemical disconnections considered (route 1 and route 2).**

Figure 4. Binding mode of compound 1. (A) Binding mode of compound 1 as predicted by LigandFit and C-Docker docking simulation. Compound **1** made π-alkyl hydrophobic

interactions with residue Ile68 (I68) and Leu69 (L69), an amide-π stacked interaction with Asp8 (D8) and Ser7 (S7) peptidic bond, an HB with Asp8 (D8) and a carbon HB with Asn65 (N65, the distance between the hydrogen and acceptor atoms is 2.690 Å). (B) Two views of the Connolly surface of IL-15 colored according to the hydrophobicity index of the exposed residues (hydrophobic in brown; hydrophilic in blue) showing the environment of compound **1**.

Figure 5. Inhibition of IL-15-dependent cell proliferation and Stat5 phosphorylation. (A-F) 32Dβ cell proliferation induced by a fixed concentration of RLI (100 pM) and increasing concentrations of indicated compounds was assessed by the Alamar Blue reduction assay. Data show the inhibitory effect of indicated compounds calculated as the percentage of reduction of the proliferative effect of RLI (100 pM). (G-H) 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 show the inhibitory effect of indicated compounds calculated as the percentage of reduction of the p-Stat5 response induced by 50 pM of IL-15. All data are the mean ± SEM of at least 3 independent experiments.

Scheme 1. Synthesis of the phathalazinone main cores 4, and 5 of the targeted compounds[a]. [a]Reagents and conditions: a) Ph_3P=CHCOOEt (1 equiv), toluene, 3 h, then reflux 30 min, 90% yield; b) $MeNHNH_2$ (1 equiv), EtOH, rt, 30 min, then $NH_2NH_2.H_2O$ (4 equiv), reflux, 2 h, 97% yield; c) MeNCS (5 equiv), Et_3N (5 equiv), EtOH, reflux, 5 h, 99% yield; d) CS_2, KOH (2.5 equiv), reflux, 3 h, 65% yield.

Scheme 2. Synthesis of the halogenoalkylamide reagents 6-39 and the azide reagent 40[a]. [a]Reagents and conditions: a) $(COCl)_2$ (1.2 equiv), DMF cat, DCM, rt, 18 h; b) RNH_2, Et_3N (5 equiv), THF, rt, 3 h to 24 h, 20 to 93%; c) MeI (5 equiv), K_2CO_3 (1.2 equiv), DMF, 40 °C, 4 h, 8%; d) NaN_3 (5 equiv), DMF, 80 °C, 1 h, 72%.

Scheme 3. Demethylation of amide 24 with BBr_3.

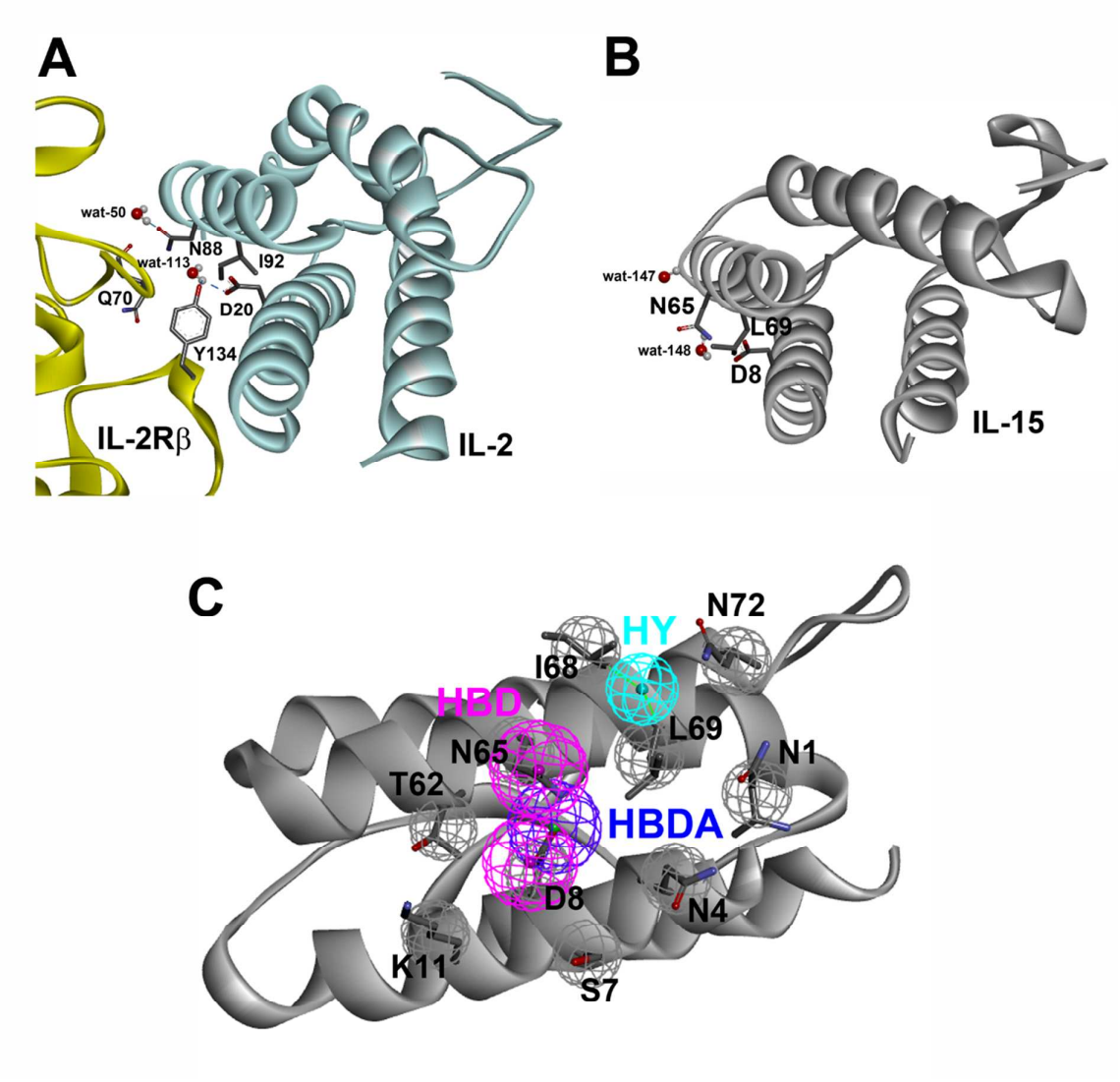
Scheme 4. *S*-alkylation step of thiol 4, with the bromoalkylamides 6 to 38.

Scheme 5. *S*-alkylation step of thiols 4 and 5 with alkyl halides.

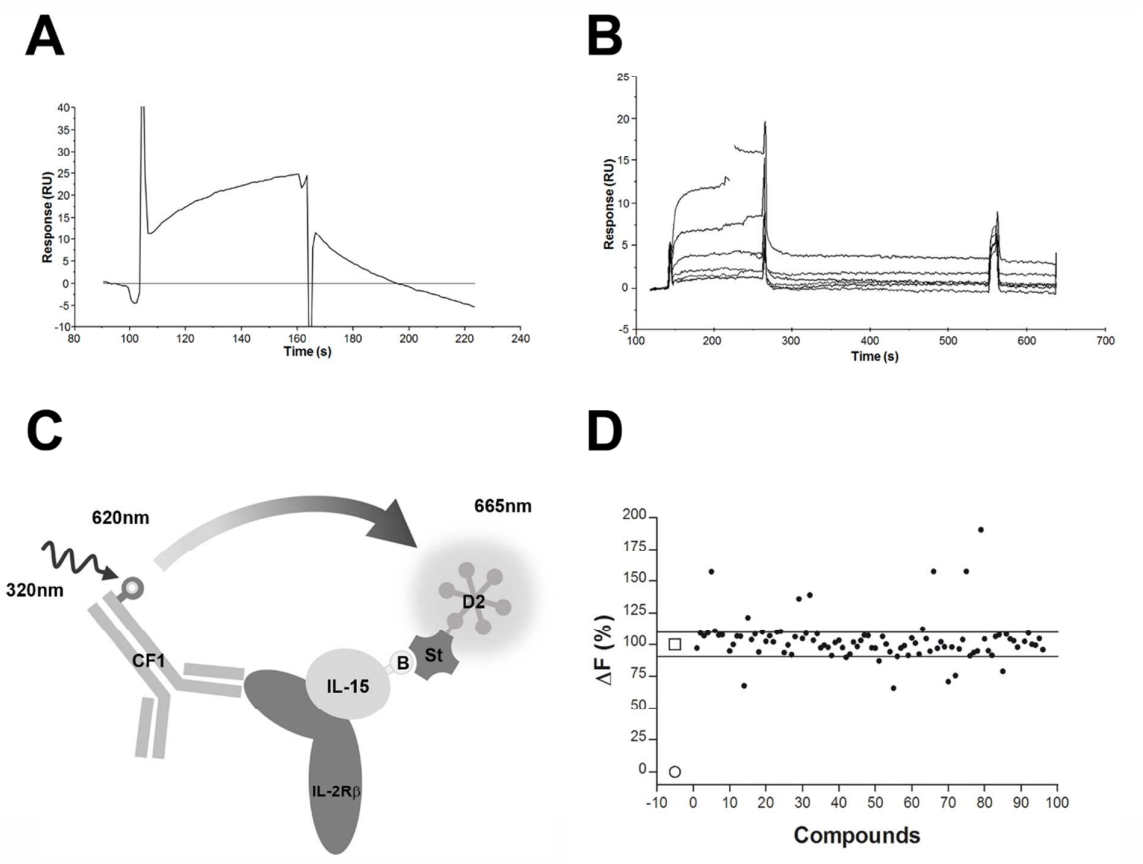
Scheme 6. Demethylation step of compounds 51 and 74, to access to the targeted final hydroxy analogs 57 and 79, and alkoxy analogs 58-60 and 80-82.

Scheme 7. Hydrolysis of benzoate derivatives 52-53 and 75.

Scheme 8. Synthesis of the 1,2,3-triazole derivatives 56 and 84[a]. [a]Reagents and conditions: a) $CuSO_4.5\ H_2O$ (0.01 equiv), sodium ascorbate (0.1 equiv), *t*BuOH/ H_2O (1:1), 60 °C, 20 h, **56** (61%) and **84** (60%).



Quéméner et al., Figure 1.



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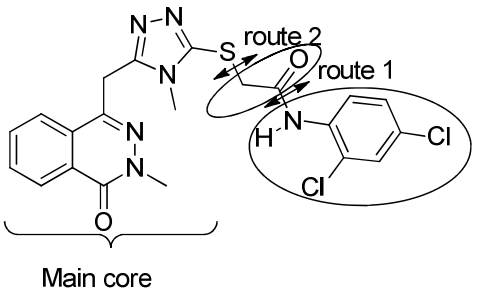

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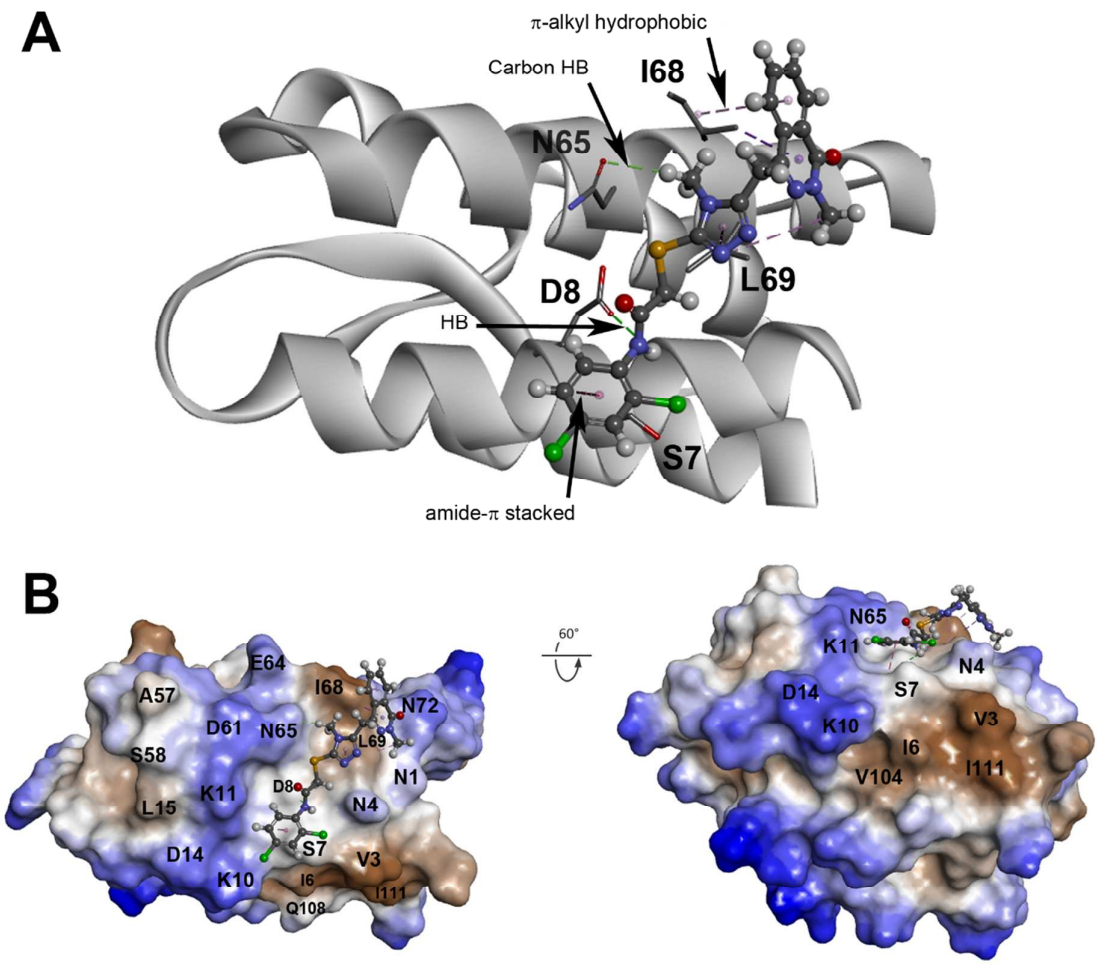

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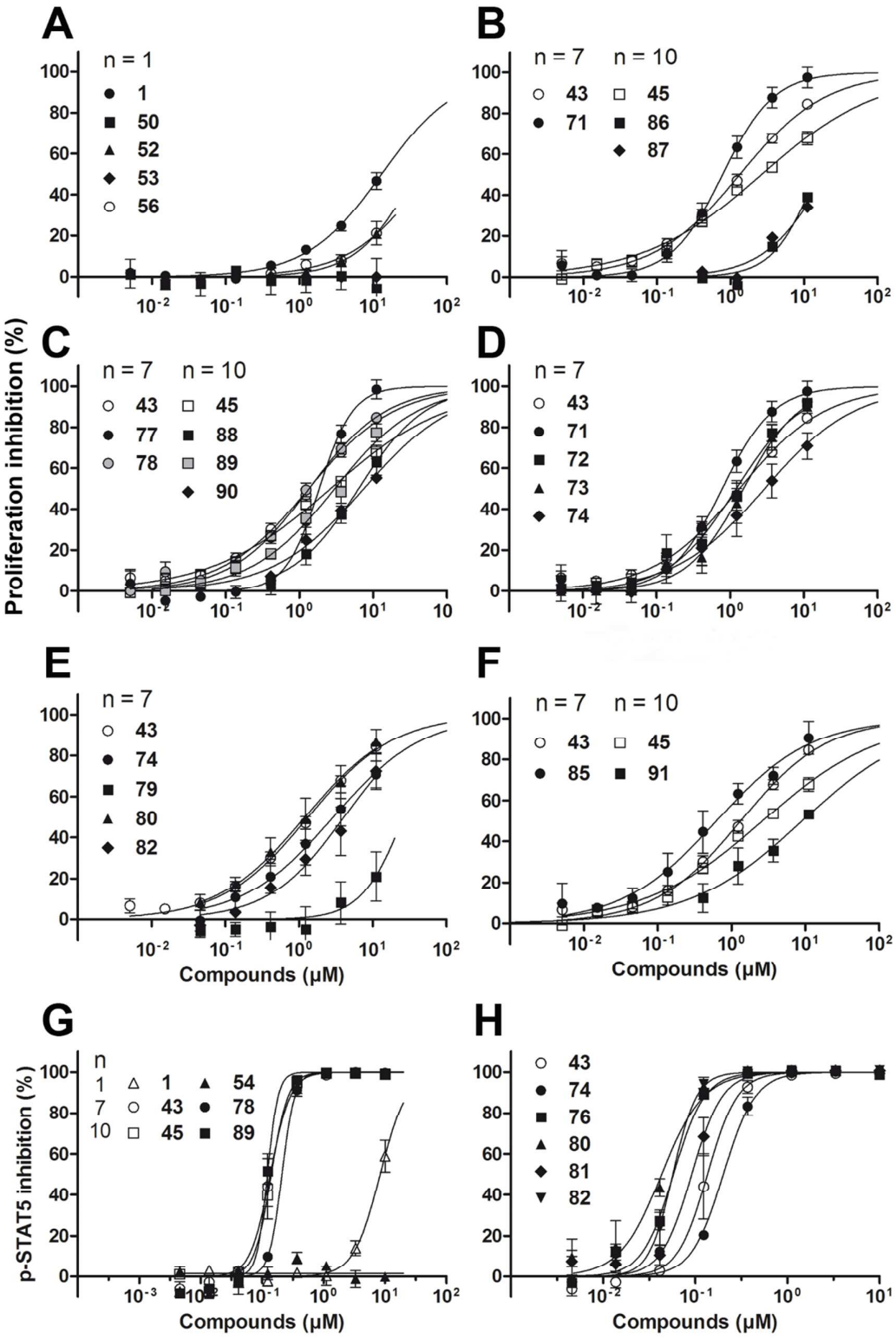
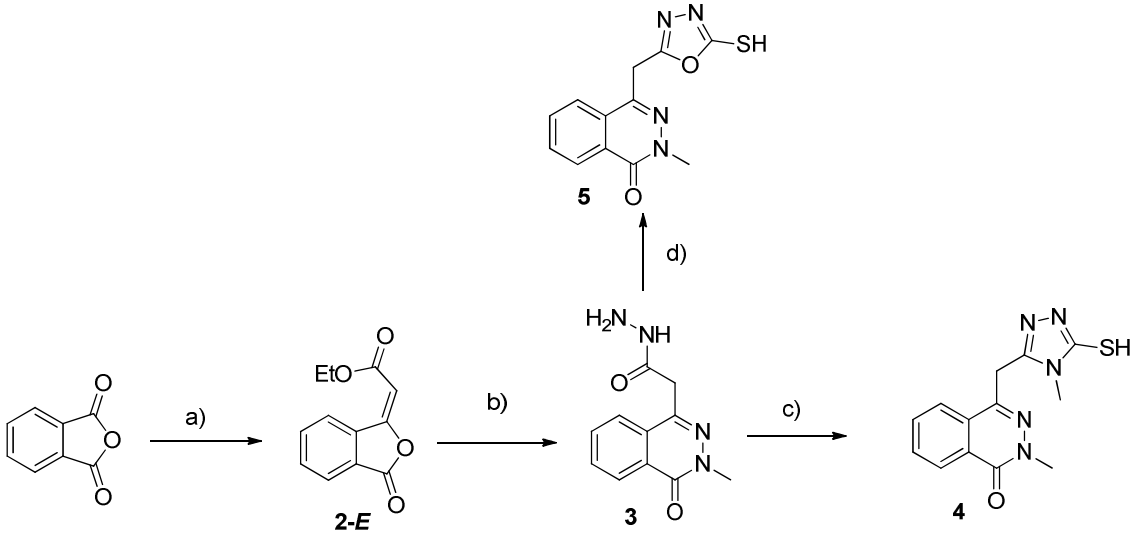


Quéméner et al., Figure 5.



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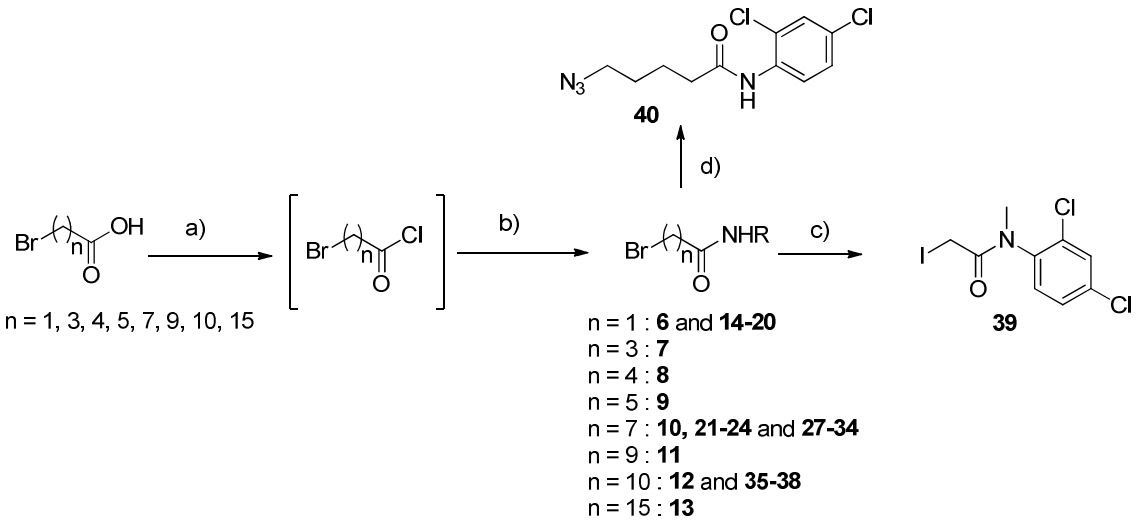

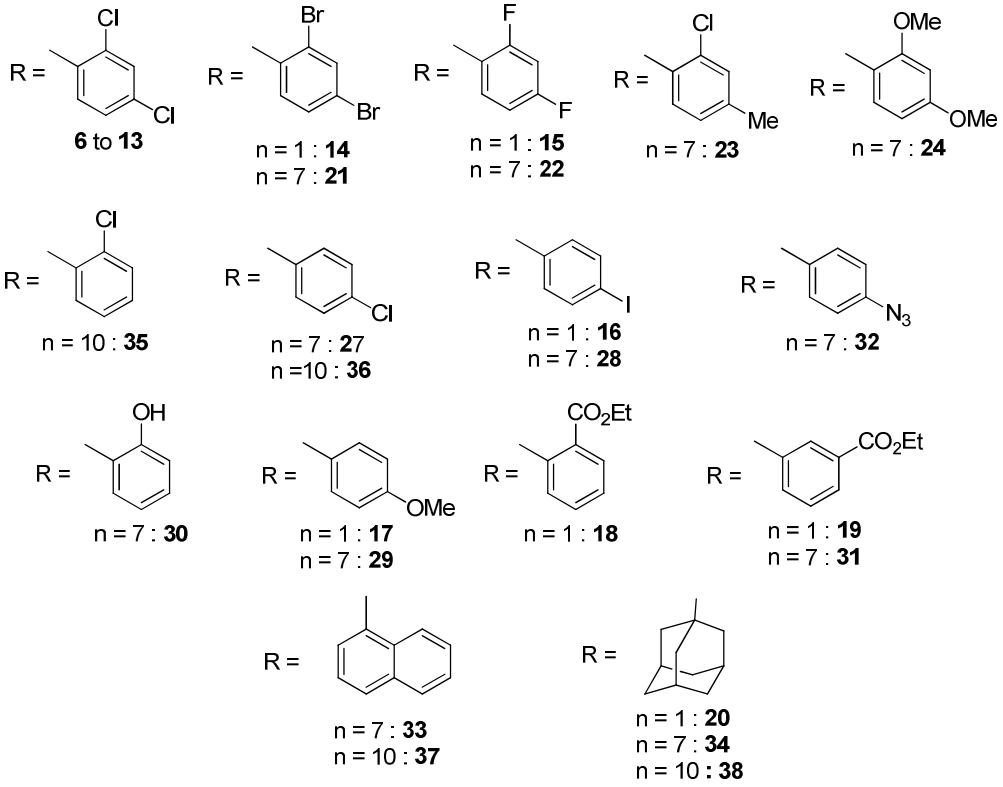

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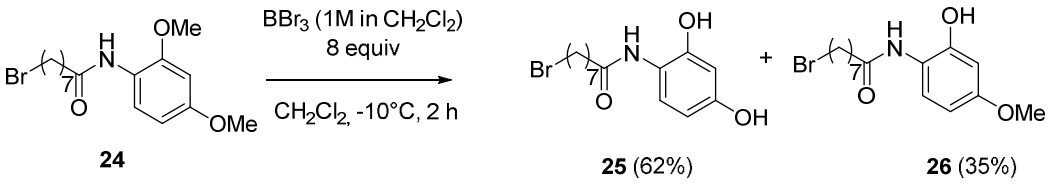
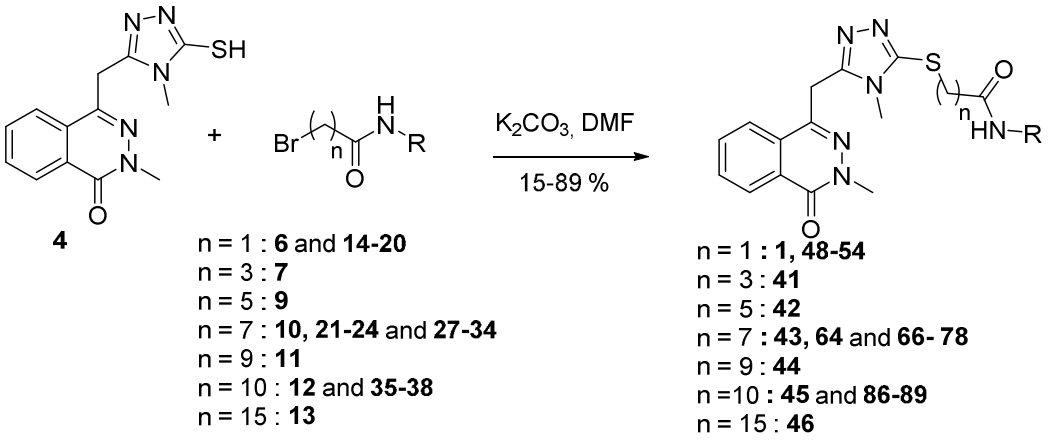


24 **25** (62%) **26** (35%)

Quéméner et al., Scheme 3.



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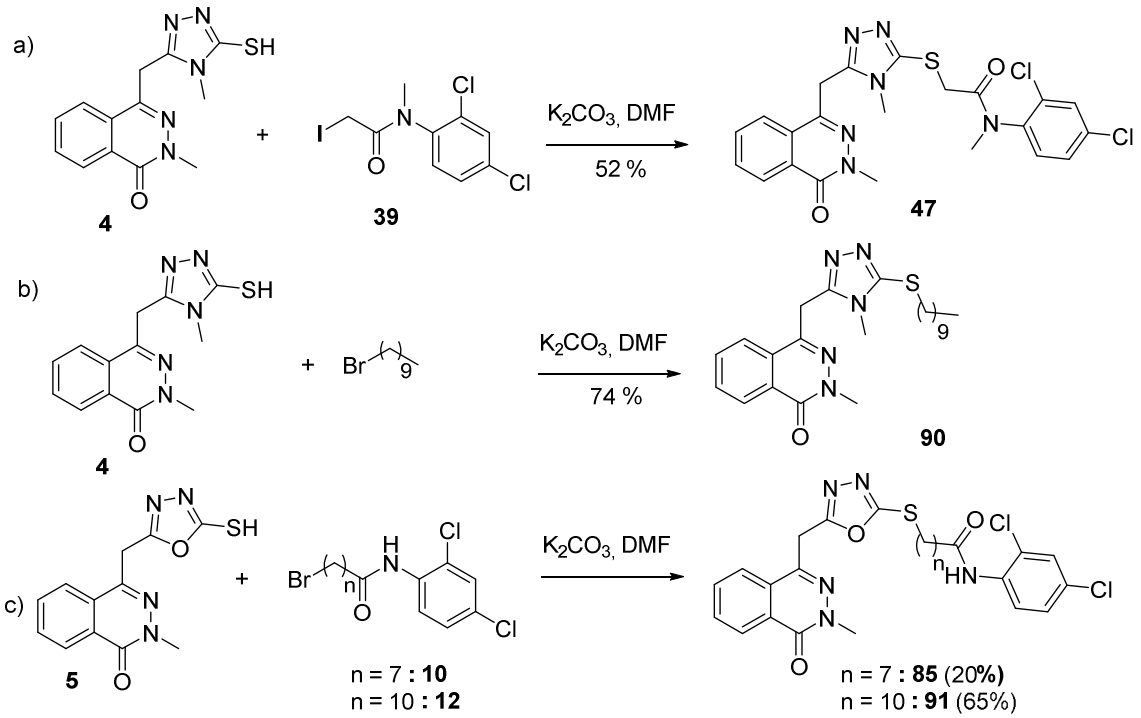
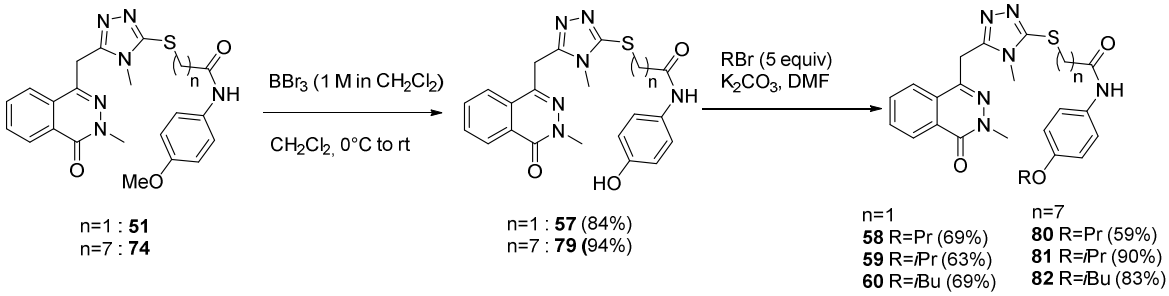


Quéméner et al., Scheme 5.



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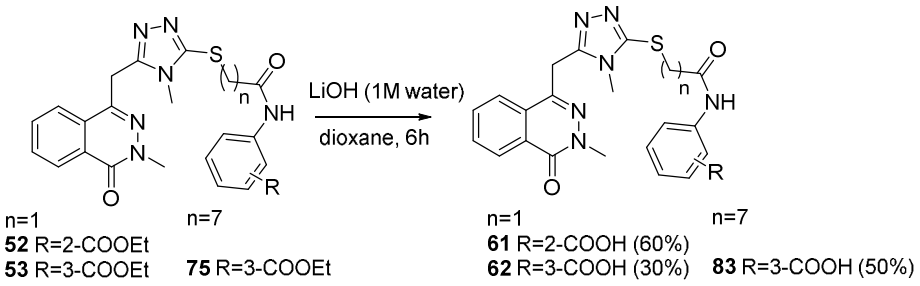

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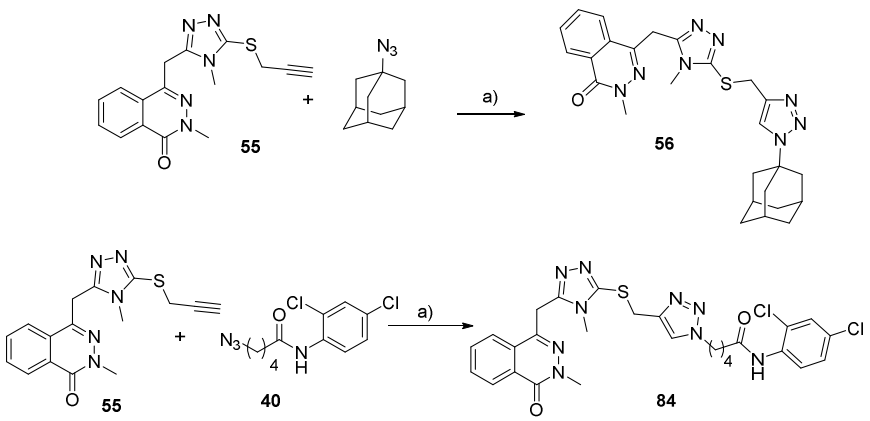

Quéméner et al., Scheme 8.

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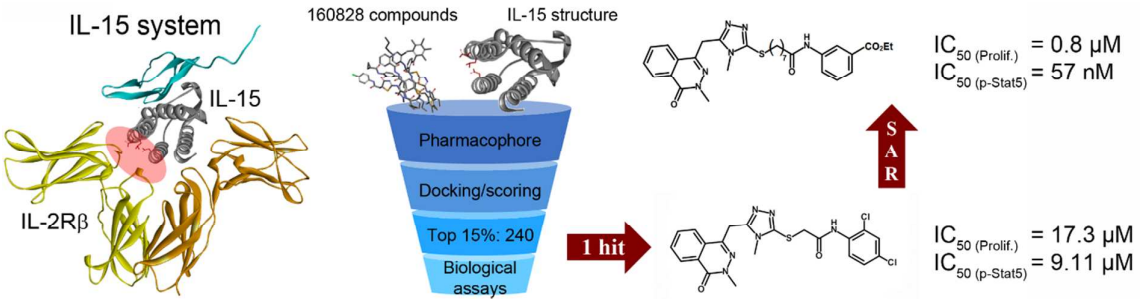

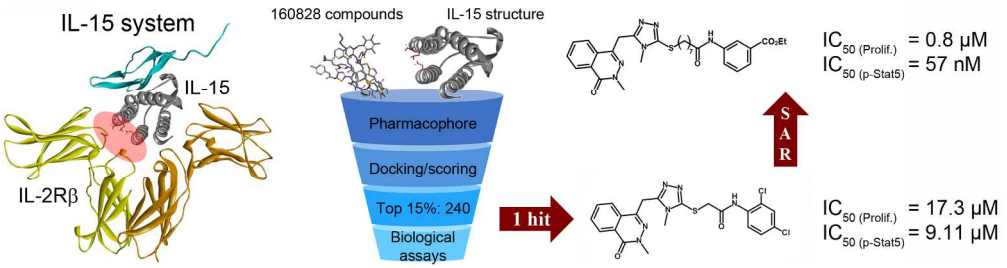

Discovery of a Small-Molecule Inhibitor of IL-15: Pharmacophore-Based Virtual Screening and Hit Optimization

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