Lead optimization of phthalazinone Phosphodiesterasas inhibitors as novel antitrypanosomal compounds

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ABSTRACT

Human African trypanosomiasis is causing thousands of deaths every year in the rural areas of Africa. In this manuscript we describe the optimization of a family of phtalazinone derivatives. Phosphodiesterases have emerged as attractive molecular targets for a novel treatment for a variety of neglected parasitic diseases. Compound **1** resulted to be a potent TbrPDEB1 inhibitor with interesting activity against *T. brucei* in a phenotypic screen. Derivative **1** was studied in an acute *in vivo* mouse disease model but unfortunately showed no efficacy. Its low metabolic stability is certainly one of the reasons. We report structural modifications to achieve compounds with an improved metabolic stability while maintaining high potency against TbrPDEB1 and *T. brucei*. Compound **14**, presented a good microsomal stability in mouse and human microsomes and will be studied in the future in an acute *in vivo T. brucei* mouse model.

INTRODUCTION

Human African trypanosomiasis (HAT), or African sleeping sickness, is caused by the unicellular parasites of the species *Trypanosoma brucei* (*T.brucei*). The vector that propagates this disease is the tsetse fly, found mainly in rural Africa. The disease is divided in two different subtypes: gambian sleeping sickness (*Trypanosoma brucei gambiense*) and rhodesian sleeping sickness (*Trypanosoma brucei rhodesiense*). The first one is chronic and can take years until fatality while the second one is an acute illness causing death within weeks or months after the infection.[1] In both forms, the late stage of the disease is characterized by the entrance of the parasite into the central nervous system with fatal consequences. Without treatment, the disease will progress until the death of the patient. Although the two forms affect human beings, 98% of the cases of sleeping sickness are caused by *T.brucei gambiense*, which predominates in central and western African countries.[2]

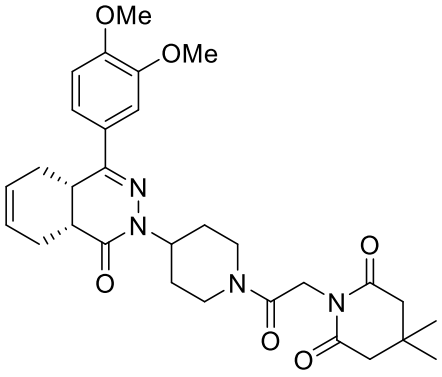
HAT belongs to the family of neglected tropical diseases (NTDs); these sicknesses contribute to a high level of morbidity and mortality in the third world countries (550000 deaths per year).[3] The investment from the pharmaceutical companies to achieve new molecules capable to cure those diseases is not enough. For this reason research from public institutions to develop new drugs, which are safe and easy to administer is crucial.

To develop new molecules able to cure this disease, a public-private consortium has been established funded by the European Union. This project named 'Parasite-specific cyclic nucleotide phosphodiesterase inhibitors to target neglected parasitic diseases' has validated parasitic cyclic nucleotide phosphodiesterases as valuable targets for drug discovery and has identified parasite-specific features of the PDE active sites.[4]

PDEs have emerged as attractive molecular targets for a novel treatment for a variety of neglected parasitic diseases, including African trypanosomiasis, Chagas disease, and malaria.[5]

The genome of *T.brucei* presents five trypanosomal cyclic nucleotide phosphodiesterases.[6] For sleeping sickness, TbrPDEB1 and TbrPDEB2 were genetically validated as drug targets.[7] These enzymes are different from the human form since they have a sub-pocket, named as the parasite specific pocket or the P-pocket, in the substrate binding site.[8] This pocket potentially offers an area that can be targeted to impart selectivity, avoiding side effects related to inhibition of the human off-targets. For instance, inhibition of hPDE4 yields side effects such as nausea and emesis.[9] Moreover, inhibition of human PDE4 (hPDE4) attenates the immune system via inhibition of TNFα which is certainly undesirable in rural Africa. [10, 11, 12]

In previous investigations carried out by de Koning et al, a phenylpyridazinone derivative was discovered from a high throughput screening campaign (PPS54019, compound A) and validated as TbrPDEB1 and TbrPDEB2 inhibitor.[13] This compound (now renamed as NPD-001), that had previously been designed as hPDE4 inhibitor[14], has been used as hit to develop new families of TbrPDE inhibitors. It has recently been published the identification of the first series of selective TbrPDEB1 inhibitors over hPDE4 isoforms.[15] A new family of PDE inhibitors was discovered,[16] from this family, compound **1** (Fig. 1), was further studied resulting to be a potent TbrPDEB1 inhibitor with interesting activity against *T. brucei* in a phenotypic screening. Derivative **1** was studied in an acute *in vivo* mouse disease model on swiss mice infected with 10^4 trypanosomes *T. brucei* at a daily dosing of 50 mg/kg i.p. or 50 mg/kg p.o.; but unfortunately showed no efficacy. Its low metabolic stability is certainly one of the reasons (Fig.1-table). In this paper, we report structural modifications to achieve compounds with an improved metabolic stability while maintaining high potency against TbrPDEB1 and *T. brucei*.



1

pIC_{50} (Tbr)	pIC_{50}(MRC5)	pKi(TbrPDEB1)	pIC_{50}(hPDE4)	mouse microsomal stability
6.35	4.35	7	9	6.7% after 30 min

Figure 1. Structure of the TbrPDEB1 inhibitor, compound **1.** In the table, the values of different parameters are shown. For instance, this compound was evaluated in a phenotypic panel including Tbrucei and MRC5 to study if the compound is selective over human cells. In addition, it was evaluated on the isolated enzymes TbrPDEB1 and hPDE4, resulting non-selective over the human form of the PDE4 enzyme. On the other hand, the mouse microsomal stability reveals the need to improve the stability of this compound.

To get a better understanding of the metabolic hot spots in compound **1**, the theoretical metabolites resulting from phase 1 metabolism were predicted by using the Meteor program (Figure 2).

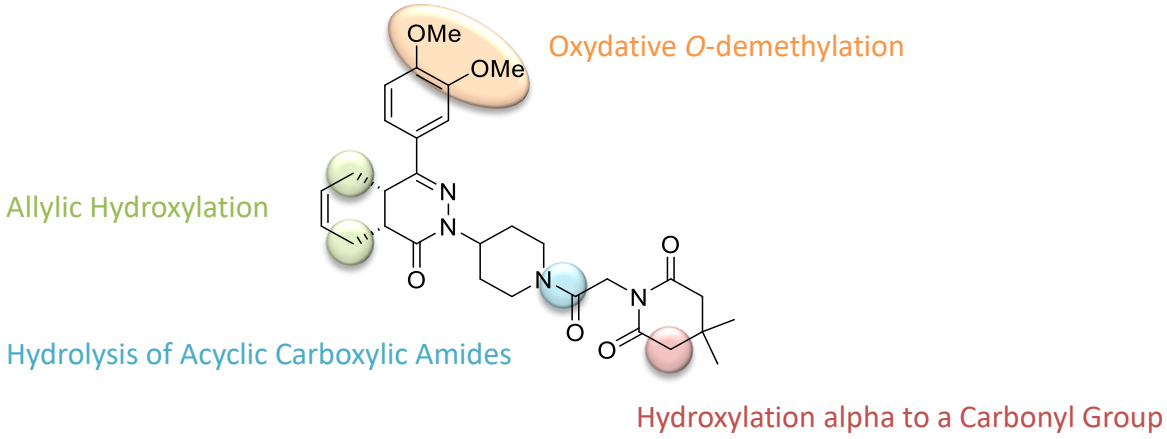


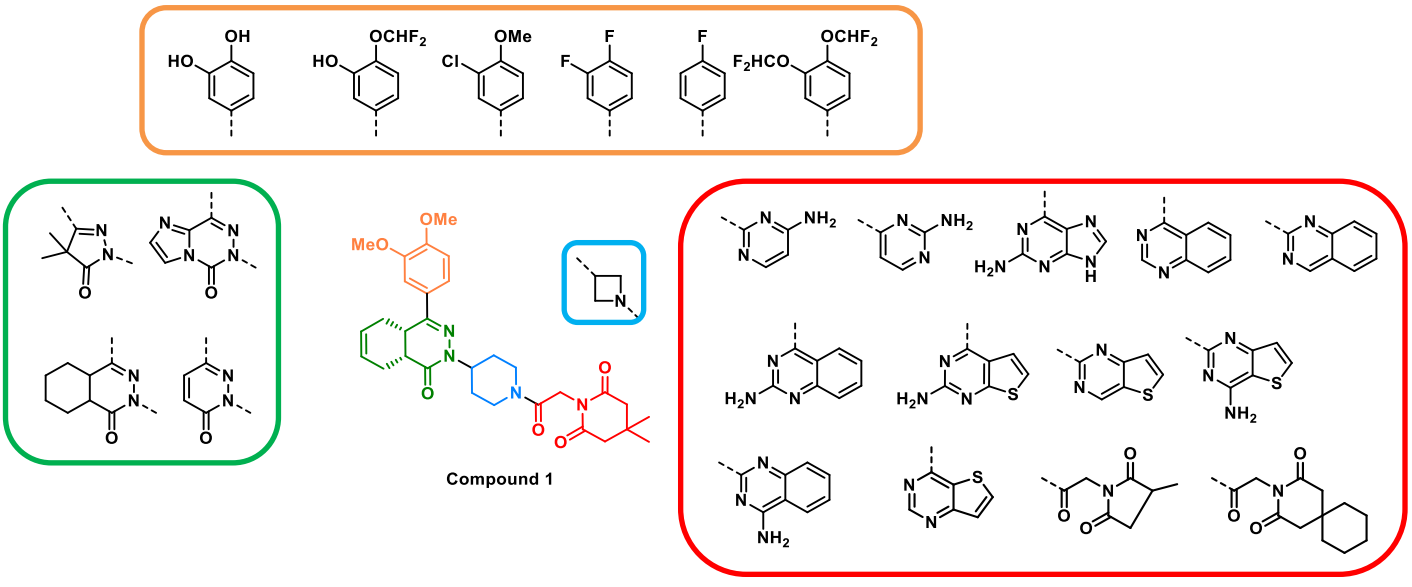
Figure 2. Main metabolic hotspots predicted by the Meteor program.

RESULTS

Chemistry

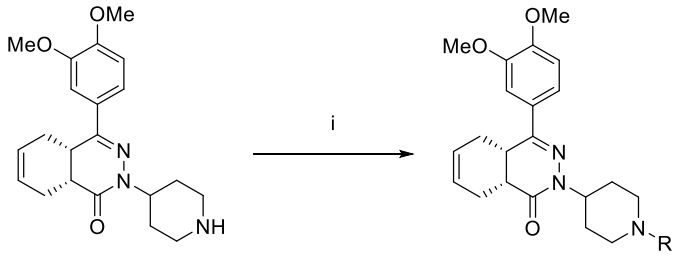
Based on the metabolic hotspots of compound **1**, we designed different molecules to improve the metabolic stability (Fig. 3).

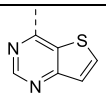
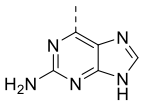
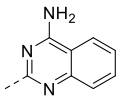
Figure 3. Derivatives of compound **1**, designed to improve metabolic stability.

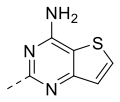
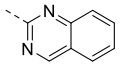
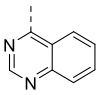
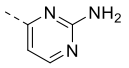
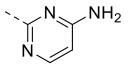
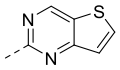
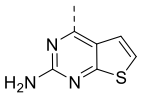
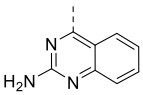


Modification of the methoxy groups to avoid *O*-demethylation was carried out by introducing fluorine or chlorine atoms. Changes in the core of the molecule were also performed to study the importance of the phthalazinone scaffold in the activity and stability. The saturated form of the phthalazinone was obtained to avoid allylic hydroxylation. Diverse heterocycles were introduced mimicking a purine moiety, to benefit from an active uptake in the parasite by the nucleoside P2 transport system.[17-19]

Different synthetic approaches were followed depending on the final compound. In general, (4a*S*, 8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one was used as starting material (Scheme 1), to introduce the different heterocycles using *N*-alkylation.

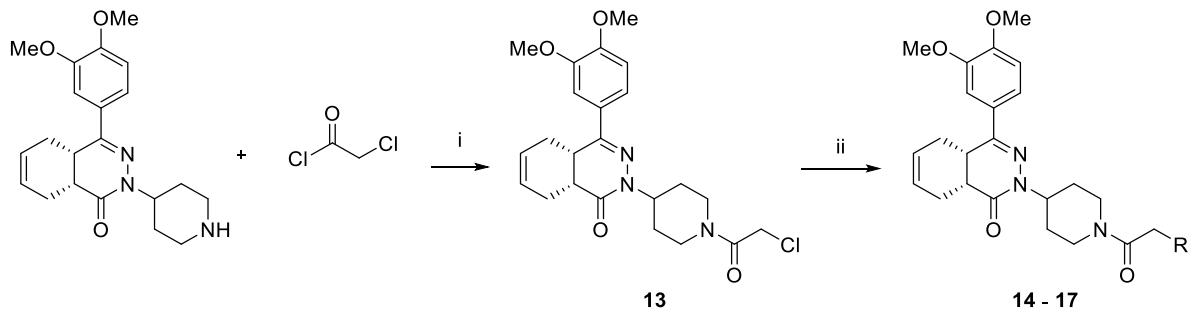


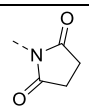
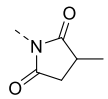
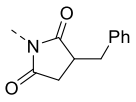
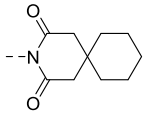
Comp.	R	Yield (%)
2		69
3		15
4		26

5		5
6		60
7		81
8		48
9		36
10		69
11		48
12		25

Scheme 1. Reaction conditions: i) heteroaryl chloride, Et_3N, K_2CO_3, DMF, 153 °C, 2 – 18 h.

Another modification concerning this part of the molecule is the size of the imide and its substitution pattern. These compounds were synthesized as previously described (Scheme 2).[20]



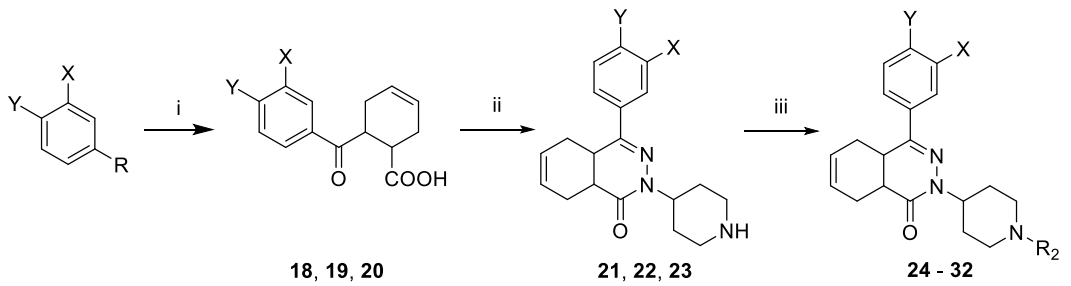
Comp.	R	Time (h)	T (°C)	Yield (%)[a]
14		18	20	50
15		18	20	26
16		18	60	19
17		1	153	47

[a] Calculated yield for the last step of the reaction.

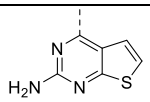
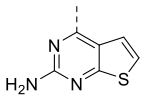
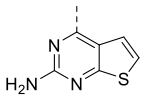
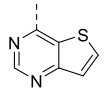
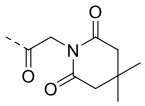
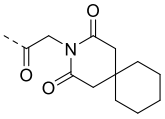
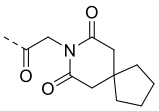
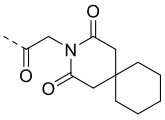
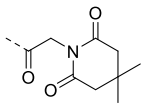
Scheme 2. Reaction conditions: i) Et_3N, K_2CO_3, THF, rt, 2 h. ii) imide derivative, K_2CO_3, DMF.

It is well known that methoxyphenyl derivatives can be rapidly metabolized through oxidative *O*-demethylation during Phase I metabolism. In an attempt to avoid this, the two methoxy substituents were replaced by different halogen atoms or by introducing a difluoromethoxy moiety. Fluorine atoms have been used extensively in medicinal chemistry programmes to improve ADME properties of molecules.[21-23]

The introduction of a fluorine or chlorine atom in the phenyl ring was performed following the reactions described in Scheme 3.[14]



18, **19**, **20** **21**, **22**, **23** **24 - 32**

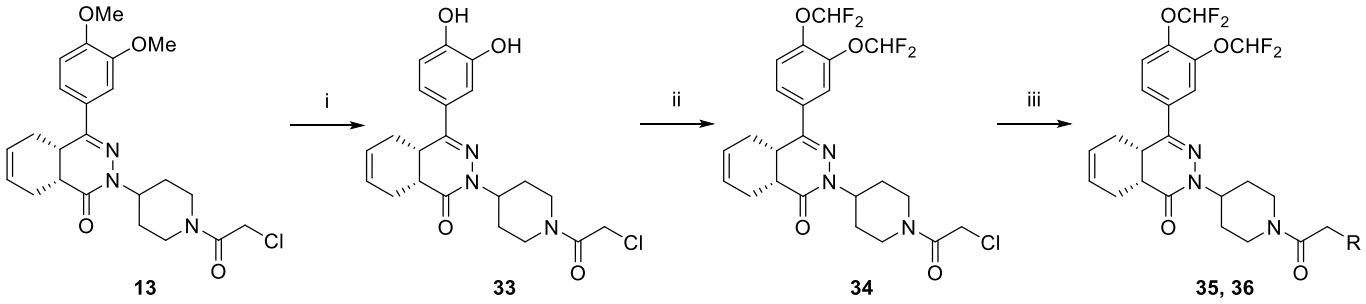
Comp.	X	Y	R	Yield (%)[a]
24	Cl	OMe		80
25	H	F		16
26	F	F		32
27	F	F		22
28	H	F		26
29	H	F		26
30	H	F		23
31	F	F		44
32	F	F		5

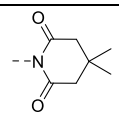
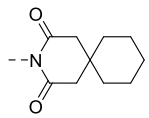
[a] Calculated yield for the last step of the reaction.

Scheme 3. Reaction conditions: When X = Cl, Y = OMe and R = H: i) $AlCl_3$, DCM, rt, overnight; ii) 4-hydrazinylpiperidine dihychloride, Et_3N, EtOH, 80 °C, overnight; iii) 4-chloro-2-aminothieno[2,3-*d*]pyrimidine, K_2CO_3, DMF, 120 °C, 16 h.

When X = H and Y = F or X = Y = F, R = MgBr: i) (3aR,7aS)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione, THF, rt, 16 h; ii) 4-hydrazinylpiperidine dihydrochloride, Et_3N, EtOH, 80 °C, 16 h; iii) heteroaryl chloride, K_2CO_3, DMF, 120 °C, 3 – 16 h or 2-chloroacetyl chloride, Et_3N, DCM, 0 °C, 30 min followed by 2,6-dione derivative, K_2CO_3, DMF, 100 - 120 °C, 1 - 16 h.

Furthermore, difluoromethoxy moieties were explored instead of the methoxy groups. This reaction was performed as previously described (Scheme 4).[24] A demethylation in presence of BBr_3 was carried out, followed by difluoromethylation of the diphenol derivative.

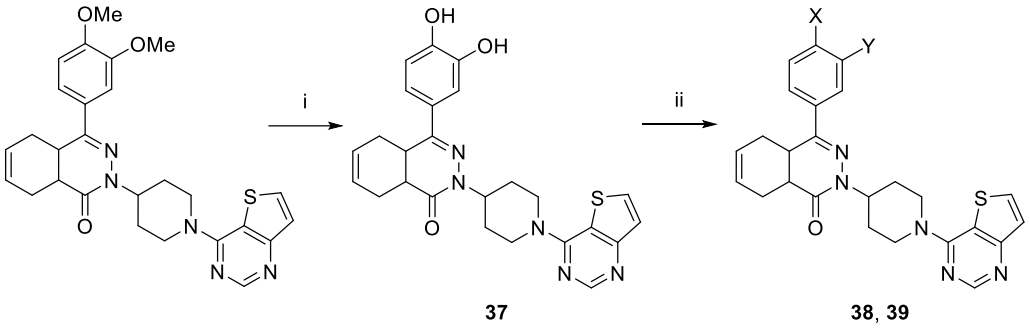


Comp.	R	Yield (%)[a]
35		10
36		6

[a] Calculated yield for the last step of the reaction.

Scheme 4. Reaction conditions: i) BBr_3, DCM, -40 °C, 2 h; ii) diethyl (bromodifluoromethyl)phosphonate, KOH, acetonitrile/water, -40 °C, 45 min; iii) 2,6-dione derivative, K_2CO_3, DMF, 120 °C, 16 h.

This modification was also performed for some of the heterocycle substituted derivatives, leading to three different compounds (Scheme 5).



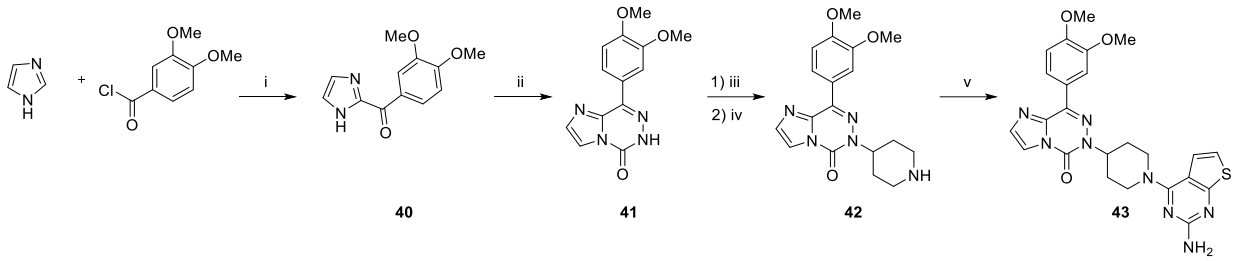
Comp.	X	Y	Yield (%)[a]
37	OH	OH	99
38	$OCHF_2$	$OCHF_2$	2
39	$OCHF_2$	OH	2

[a] Calculated yield for the last step of the reaction.

Scheme 5. Reaction conditions: i) BBr_3, DCM, -40 °C, 2 h; ii) diethyl (bromodifluoromethyl)phosphonate, KOH, acetonitrile/water, 45 – 120 min at -40 °C and 2 – 16 h at rt

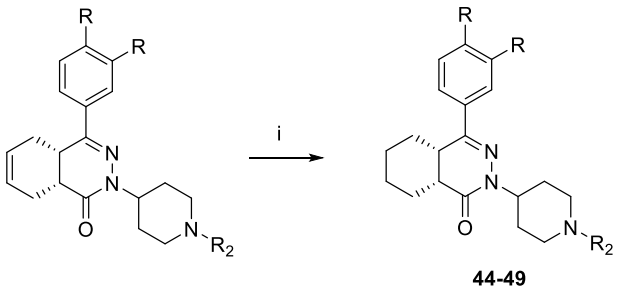
The phthalazinone core was also replaced aiming to remove the different stereocenters and to improve synthetic feasibility. The imidazotriazinone was synthesized following the procedure

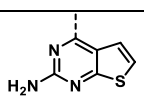
already described.[25] After the reaction of the imidazole and the acyl chloride, the cyclization of the compound in the presence of *p*-toluensulfonic acid was carried out. Once the new core scaffold was obtained, the molecule was modified as previously (Scheme 6).

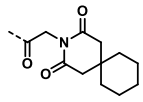
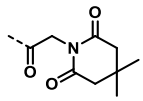
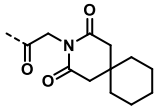
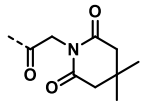
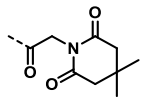


Scheme 6. Reaction conditions: i) Et_3N, pyridine, rt, 16 h. ii) *p*-toluensulfonic acid, toluen, diphenylether, ethylhydrazinecarboxylate, 110 °C, 5 h. iii) *tert*-butyl 4-bromopiperidine-1-carboxylate, NaH, DMF, 166 °C, 48 h. iv) TFA, DCM, rt, 16 h. v) 4-chlorothieno[2,3-*d*]pyrimidin-2-amine, Et_3N, DCM, rt, 16 h.

Because the double bond of the phthalazinone can be sensitive to epoxidation and allylic oxidation in Phase I metabolism, the most promising compounds were also modified by removing this double bond by hydrogenation in the presence of Pd(C) (Scheme 7).

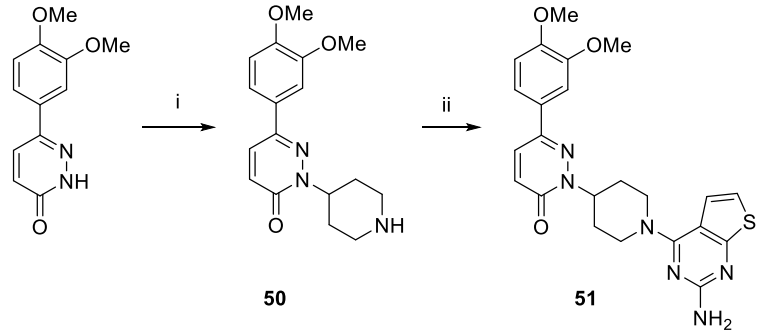


Comp.	R	R_2	Yield (%)
44	OMe		12

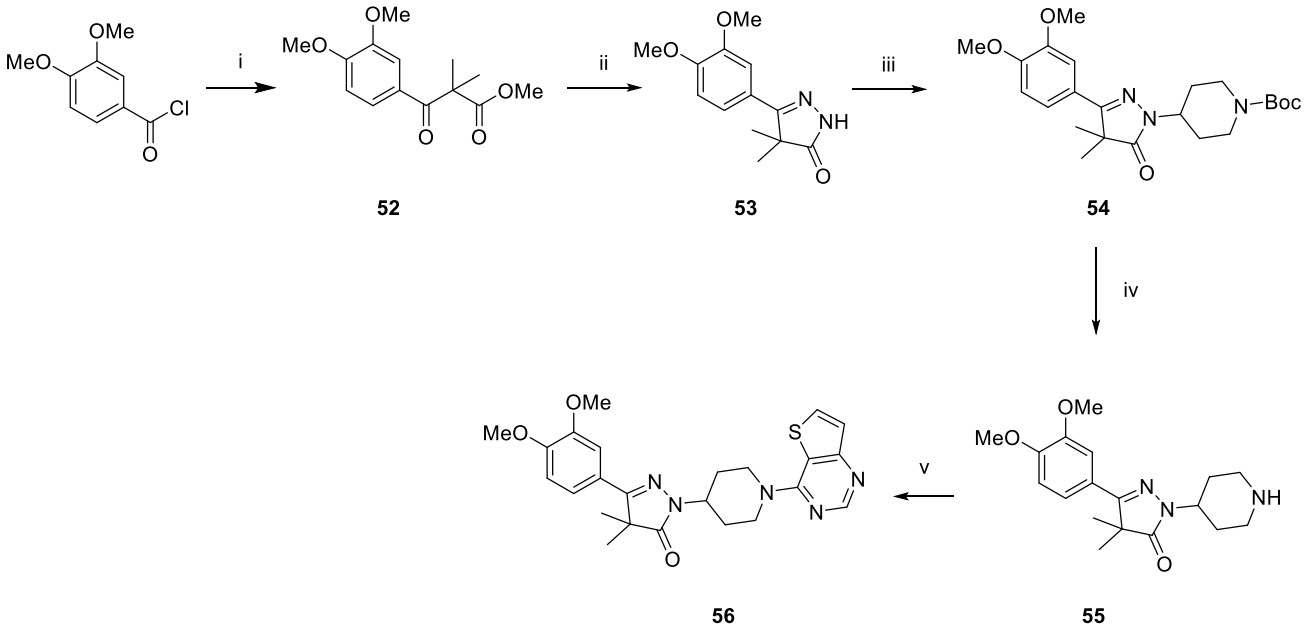
45	OMe		80
46	F		77
47	F		95
48	OMe		56
49	$OCHF_2$		79

Scheme 7. Reaction conditions: i) H_2, Pd(C), methanol, r.t, 1 – 72 h.

In order to decrease the molecular weight, two other scaffolds were also explored. A pyridazinone was synthesized as described in scheme 8. The already described pyrazolone core was obtained using the synthesis described in scheme 9.[26]

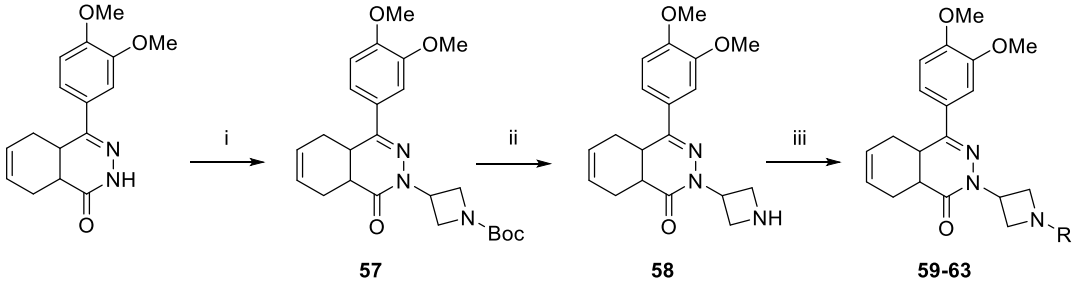


Scheme 8. Reaction conditions: i) *tert*-butyl-4-bromopiperidine-1-carboxylate, NaH, DMF, 153 °C, 2 days. ii) 4-chlorothieno[2,3-*d*]pyrimidin-2-amine, K_2CO_3, Et_3N, DMF, 130 °C, 16 h.

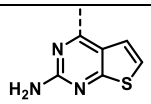
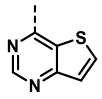
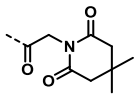
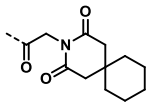
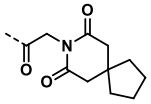


Scheme 9. Reaction conditions: i) methyl isobutyrate, LDA, THF, −45 °C, 30 min, then added to 3,4-dimethoxybenzoyl chloride, from −55 °C to rt, 16 h; ii) N_2H_4, EtOH, 80 °C, 16 h; iii) *tert*-butyl-4-bromopiperidine-1-carboxylate, NaH, DMF, 153 °C, 16 h; iv) TFA, DCM, rt, 3 h; v) 4-chlorothieno[3,2-*d*]pyrimidine, NaH, DMF, 153 °C, 16 h.

The last modification was the replacement of the piperidine ring by an azetidine ring using a very similar synthetic pathway (Scheme 10).



Comp.	R	Yield (%)[a]

59		3
60		4
61		58
62		58
63		80

[a] Calculated yield for the last step of the reaction.

Scheme 10. Reaction conditions: i) *tert*-butyl 3-bromoazetidine-1-carboxylate, NaH, DMF, 153 °C, 16 h ii) TFA, DCM, rt, 16 h; iii) heteroaryl chloride, NaH, DMF, 153 °C, 4 – 16 h or 2-chloroacetyl chloride, Et_3N, DCM, 0 °C, 30 min followed by 2,6-dione derivative, K_2CO_3, 100 °C, 3 – 16 h.

In vitro evaluation of the compounds against TbrPDEB1, hPDE4, *T. brucei*

All obtained compounds were evaluated against a parasite panel including *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania Infantum*, *Plasmodium falciparum*, and against a human cell line (MRC5) and *peritoneal macrophages* from *mouse* (PMM) for cytotoxicity determination (Fig. 4 and Table 1, full panel in supplementary information).

Furthermore, most of the compounds were evaluated on the purified catalytic domain of TbrPDEB1 enzyme. As a measure of the selectivity, inhibition of the catalytic domain of the human PDE4 for compounds with pKi > 6 on TbrPDEB1 was determined.

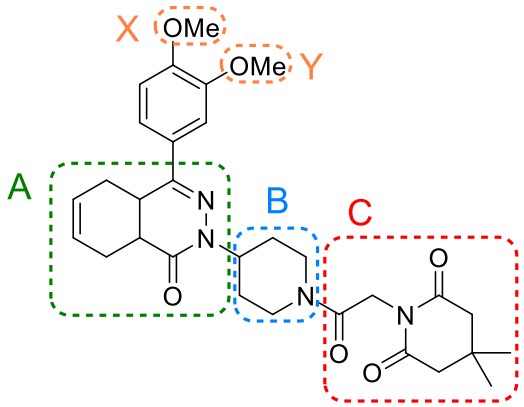
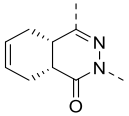
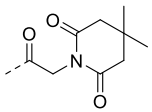
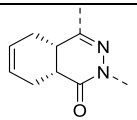
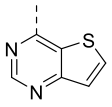
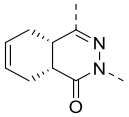
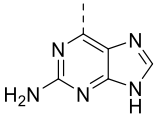
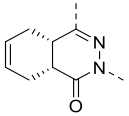
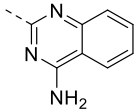
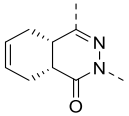
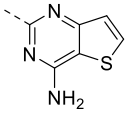
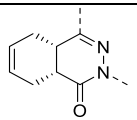
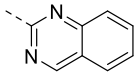
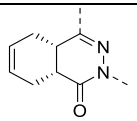
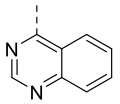
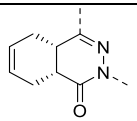
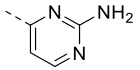
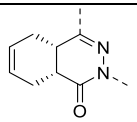
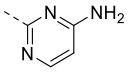
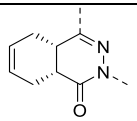
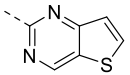
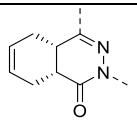
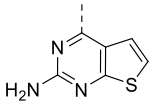
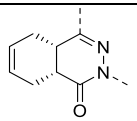
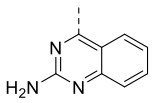
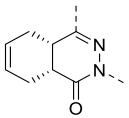
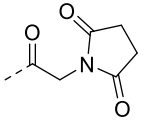
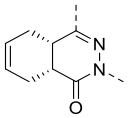
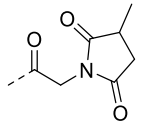
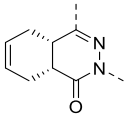
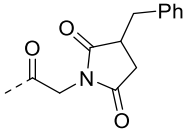
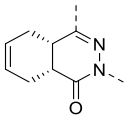
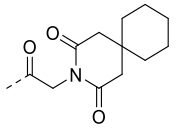
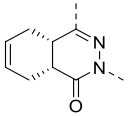
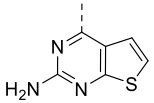
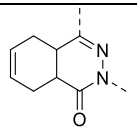
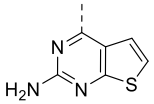
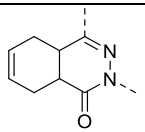
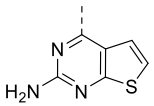


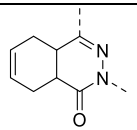
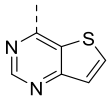
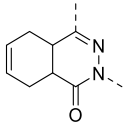
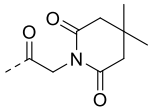
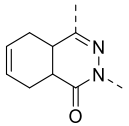
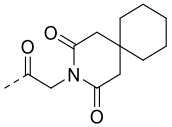
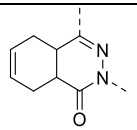
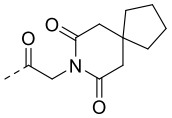
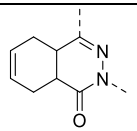
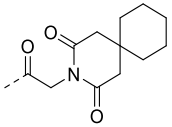
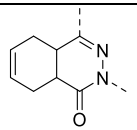
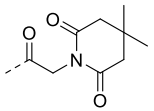
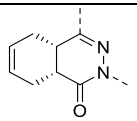
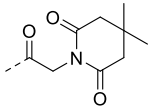
Figure 4. Scheme of the general structure and the parts that have been modified.

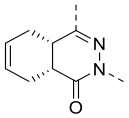
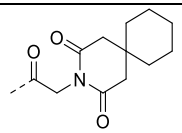
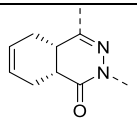
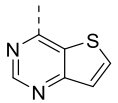
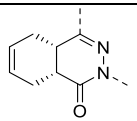
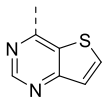
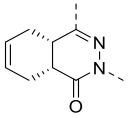
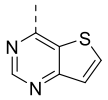
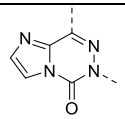
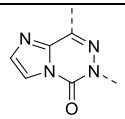
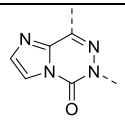
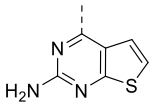
Table 1. Synthesized analogues of compound **1** and their evaluation against *T.brucei*, MRC5, TbrPDEB1 and hPDE4.

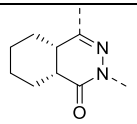
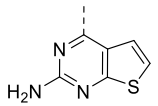
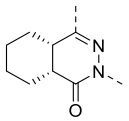
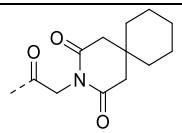
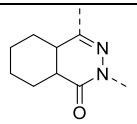
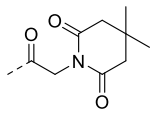
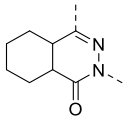
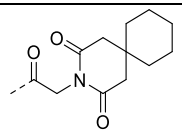
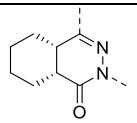
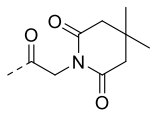
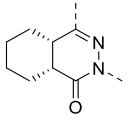
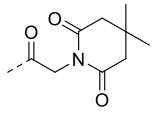
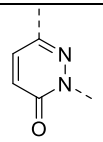
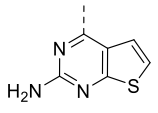
Comp.	X	Y	A	B	R	pIC$_{50}$ (*T.bruc*)[a]	pIC$_{50}$ (MRC5)[a,b]	pKi (TbrPDEB1-CD)[a]	pKi (hPDE4-CD)[a]
1	OMe	OMe				6.35	4.35	7.36	9.91
2	OMe	OMe				6.43	5.14	7.18	9.63
3	OMe	OMe				5.77	4.75	6.98	9.06
4	OMe	OMe				6.06	5.13	6.19	8.61
5	OMe	OMe				5.75	4.19	6.47	9.17

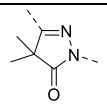
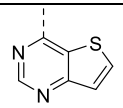
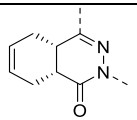
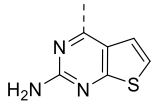
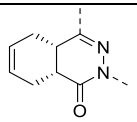
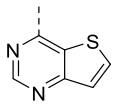
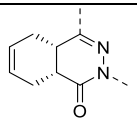
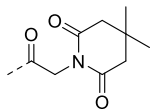
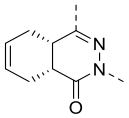
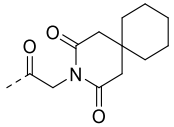
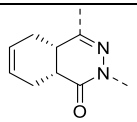
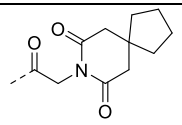
6	OMe	OMe				5.77	4.19	6.19	8.61
7	OMe	OMe				6.58	5.52	7.41	9.65
8	OMe	OMe				5.44	5.41	6.59	9.29
9	OMe	OMe				5.73	4.76	6.96	9.50
10	OMe	OMe				5.64	4.19	6.16	8.59
11	OMe	OMe				6.33	4.19	7.37	9.45
12	OMe	OMe				6.09	5.11	7.61	9.81

14	OMe	OMe				5.73	4.19	7.68	10.21
15	OMe	OMe				5.74	4.19	7.12	9.77
16	OMe	OMe				4.19	4.19	7.01	9.50
17	OMe	OMe				6.32	4.5	7.51	9.81
24	Cl	OMe				5.7	4.19	6.08	7.52
25	H	F				5.68	4.19	<5.03	nd
26	F	F				5.72	4.19	<5.08	nd

27	F	F				4.88	4.42	<5.25	nd
28	H	F				4.19	4.53	7.11	9.92
29	H	F				4.99	4.19	<5.02	nd
30	H	F				4.49	4.39	<4.78	nd
31	F	F				4.48	4.19	<5.34	nd
32	F	F				4.32	4.51	<5,01	nd
35	$OCHF_2$	$OCHF_2$				6	4.19	6.39	9.52

36	$OCHF_2$	$OCHF_2$				5.12	4.19	6.11	8.79
37	OH	OH				5.29	4.6	6.05	7.20
38	$OCHF_2$	$OCHF_2$				5.17	4.19	<5.67	nd
39	$OCHF_2$	OH				5.47	4.19	<5.78	nd
41	OMe	OMe		H	H	4.19	4,19	<4.50	nd
42	OMe	OMe			H	4.49	4,19	<4.50	nd
43	OMe	OMe				4.19	4,19	<4.74	nd

44	OMe	OMe				6.02	4.54	7.03	9.18
45	OMe	OMe				5.82	5.07	<5.01	nd
46	F	F				4.34	4.37	<4.65	nd
47	F	F				5.12	4.19	<5.16	nd
48	OMe	OMe				5.71	4.25	7.07	9.79
49	$OCHF_2$	$OCHF_2$				5.19	4.19	<5.80	nd
51	OMe	OMe				5.11	4.95	<5.43	nd

56	OMe	OMe				4.49	4.6	<5.69	nd
59	OMe	OMe				5.11	4.19	6.56	8.93
60	OMe	OMe				4.82	4.19	6.54	9.25
61	OMe	OMe				5.74	4.19	6.99	9.41
62	OMe	OMe				5.94	4.19	7.04	9.07
63	OMe	OMe				5.6	4.19	6.77	9.17

[a]*Each value is the mean of at least two independent determinations.* [b]*Cytotoxicity measurement using human lung fibroblast MRC-5 SV2cells.*

Structure-activity relationship (SAR)

In general, the compounds that were active in the phenotypic panel against *T. brucei* also presented potency on the isolated catalytic domain TbrPDEB1 enzyme. This suggests that the compounds are working through PDE inhibition. There are also some compounds that do not present activity in the phenotypic panel but present potency on the enzyme (compounds **16** and **28**), this might possibly be because of a lack of membrane permeability.

Among all the modifications that have been introduced, we observe that the preferred core scaffold is the saturated or unsaturated phthalazinone (compound **11** *vs* **43** or **51**). As linker between the phthalazinone and the imide or heterocycle, a piperidine is clearly favored over an azetidine with a 3-10 fold loss in potency against both *T. brucei* and TbrPDEB1 (compound **1** *vs* **61**, **12** *vs* **59** or **17** *vs* **62**).

Regarding the substituents in the phenyl ring, the methoxy groups are the most favourable ones (Compound **1** *vs* **32** or **35**). When the OMe moiety is substituted by Cl, the potency on PDEB1 decreases about 20 folds (compound **11** *vs* **24**). When the substitution is H,F or F,F, compounds are inactive on PDEB1 except compound **28**; however, this compound did not show activity on the antiparasitic panel. On the other hand, $OCHF_2$ substitution, maintains antiparasitic activity (compound **35** *vs* **1**) although a 1 fold loss of activity on PDEB1 is observed. Furthermore, when the chain is longer (compound **36** *vs* **17**) the potency on both PDEB1 and on the parasitic panel decreases when the $OCHF_2$ is present. About the heterocycle ring attached to the piperidinyl, the thieno-pyrimidine-2-amino (Compound **11**) maintains the activity on the phenotypic panel and presents a lower pIC_{50} against the human cell line MRC5 comparing with the hit compound **1**, it is also as potent on PDEB1 as compound **1**. When a free amino substituent is adedd to the quinazoline ring (compound **7** *vs* **12**, and **4** *vs* **6**) the activity on PDEB1 increases or is maintained while the activity on the phenotypic panel decreases.

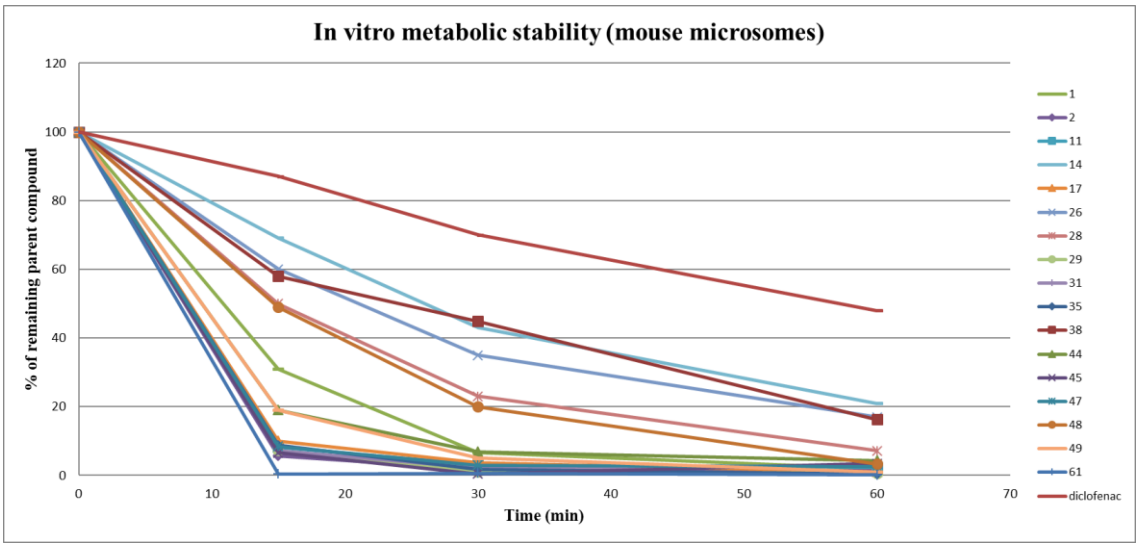
Regarding the imide moiety, when the 6-member ring is changed by a five member ring (compound **1** *vs* **15**) the phenotypic activity decreases while the potency on PDEB1 is kept. Also, when another six-member ring is fused the activity is higher on PDEB1 and it is maintained on the phenotypic panel. This behavior is only valid when the substitution on the phenyl ring is OMe, OMe (compound **17** *vs* **31** or **1** *vs* **32**).

Metabolic stability

Metabolism plays an important role in the development of a drug candidate; it impacts on pharmacokinetic parameters such as oral bioavailability, clearance and half-life. This will affect the efficacy and toxicology of the drug due to changes in the concentrations within the plasma and tissues of the body.[27]

To evaluate Phase I and Phase II metabolism of the compounds, mouse and human microsomes were used. Compounds with structural differences have been selected to be studied in mouse and microsomes. With the mouse microsomes results, some of those compounds with promising stability or important structural changes were selected to be evaluated in human microsomes. In Figure 5, mouse and human microsomal stabilities at different time points are represented, full data with standard deviation is shown in the Supplementary Information.

A


In vitro metabolic stability (mouse microsomes)
% of remaining parent compound
Time (min)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
1
2
11
14
17
26
28
29
31
35
38
44
45
47
48
49
61
diclofenac

B

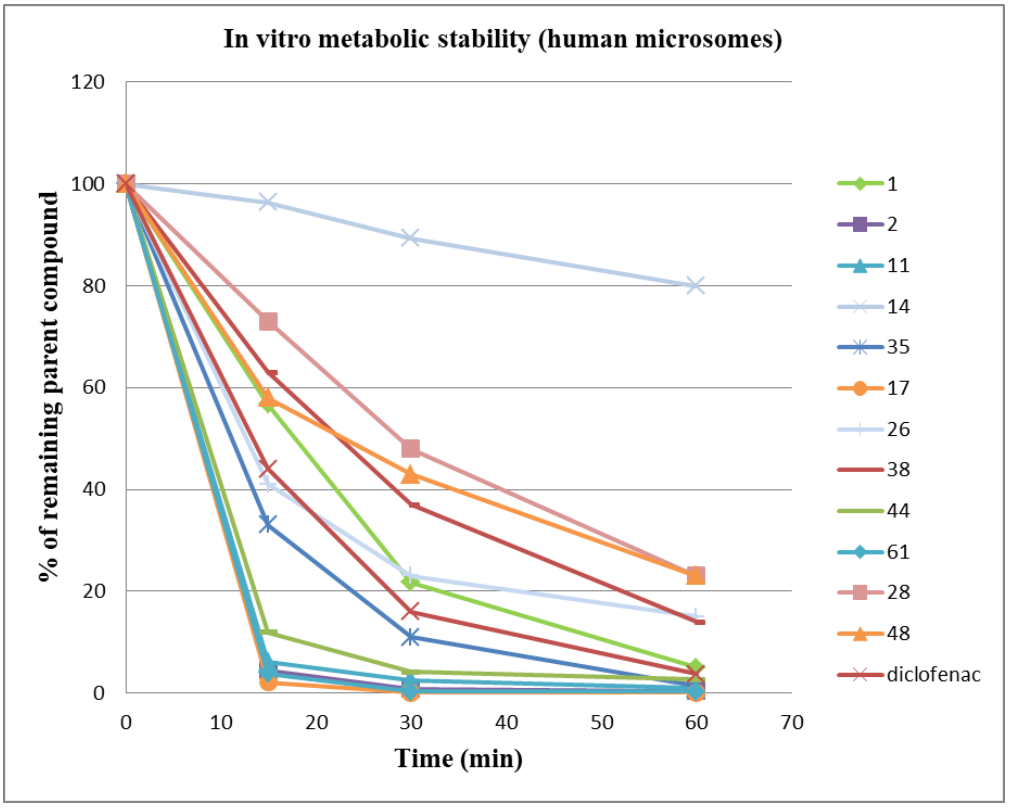

In vitro metabolic stability (human microsomes)
% of remaining parent compound
Time (min)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
1
2
11
14
35
17
26
38
44
61
28
48
diclofenac

Figure 5. **A**) Mouse microsomal stability of selected compounds, **B**) Human microsomal stability of selected compounds.

From these results, we can conclude that the different regions of the molecules contribute to differences in stability. In general, the saturated phthalazinone core is more stable than the unsaturated one (compounds **48** *vs* **1**, **47** *vs* **31** and **49** *vs* **35**), the piperidinyl also increases the stability comparing it to the azetidine residue (compound **17** *vs* **62**). Regarding the substituents of the phenyl ring, generally, the substitution of the methoxy group by difluoromethoxy moieties or fluorine atoms also enhances the stability (compound **5** *vs* **25**, **26**, **27**, **38**, and **39**), suggesting that the catechol moiety suffers from oxidative *O*-demethylation.

Compound **14** presented a good metabolic stability profile in mouse (43% after 30 min) and human microsomes (89% after 30 min), for this reason, derivative **14** (pIC_{50}(Tbr) = 5,73; pK_i (TbrPDEB1) = 7,68) will be selected for an *in vivo* proof of concept in the acute mouse model of *T. brucei*.

X-ray analysis of selected inhibitors

To get a better understanding of their mode of interaction at atomic level, crystal structures of selected derivatives (Figure 6) bound to TbrPDEB1 and hPDE4D catalytic domains were determined by X-ray. We have chosen inhibitors which have shown good potency in phenotypic assays or those with significant structural modifications, together with compound **14** as the most stable compound and the selected one for further studies. Since the selected inhibitors fail to demonstrate any selectivity over human off-target enzyme hPDE4D, we hoped structural findings may provide us design ideas to help improve these and other chemically similar inhibitors.

Structures of TbrPDEB1 bound to inhibitors **1**, **11**, **12**, **14** and **35** were obtained in a resolution range between 1.6 to 2.5 Å (Supplementary information). In all cases, (4a*S*,8a*R*)-enantiomer form of the inhibitors were observed in the crystal structures and they display almost identical binding modes to the parasitic enzyme (Figure 7). The phthalazinone core occupies the area near the metal site while the substituted phenyl group engages the hydrophobic clamp, also known as the aromatic clamp in TbrPDEB1[7], residues Val840 and Phe877. In addition to the aromatic stacking, the ring substitutes further engage TbrPDEB1 via hydrogen bond interactions involving Gln874, a residue strictly conserved across phosphodiesterase family. The different tail groups orient themselves towards a space lined by helix-15 and the M-loop[7] and points slightly away from the parasite specific P-pocket (Figure 7). They are largely stabilised by hydrophobic interactions from residues located on helix-15 and the M-loop, specially by Met861 and, up to some extent, Phe880.

We have also determined structures of the hPDE4D catalytic domain in complex with the same set of inhibitors (Supplementary information). Their overall binding mode in hPDE4D is highly similar to that in TbrPDEB1 with strict conservation of key hydrophobic clamping (with Phe372 and Ile336; hPDE4D numbering) and H-bond interactions (with Gln369; hPDE4D numbering) in the two enzymes (Supplementary information). Despite the overall similarity, two important observations can be deduced from the determined inhibitor-bound structures in TbrPDEB1 and hPDE4D, which may have implications in future design works; first, the tail, stabilising hydrophobic stacking from Phe880 in TbrPDEB1, is absent in hPDE4D with the substitution of Phe880 by Tyr375 in the equivalent position. Secondly, the tail is localized close to the parasite specific P-pocket in TbrPDEB1. In TbrPDEB1, interaction with Phe880 may be improved by the introduction of aromatic moieties to the existing heterocyclic tail while maintaining the current vector direction, which may result in improved selectivity. On the other hand, targeting the tail towards the parasite specific P-pocket may also result in improved

selectivity over hPDE4D. The latter approach has successfully been demonstrated in a recent work where probing the P-pocket resulted in several folds selectivity and led to the discovery of first TbrPDEB1 selective inhibitors[12]. As demonstrated by the crystal structures, currently none of our compounds are able to probe the P-pocket, explaining their lack of selectivity for TbrPDEB1, however, localisation of their tail part close to the P-pocket is certainly encouraging and with a careful design approach successful targeting of the P-pocket may well be achieved.

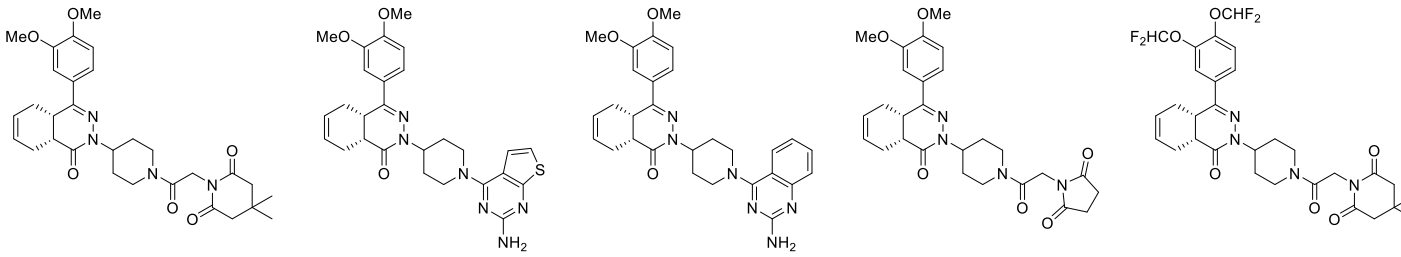


Figure 6. Structure of the compounds studied by X-ray.

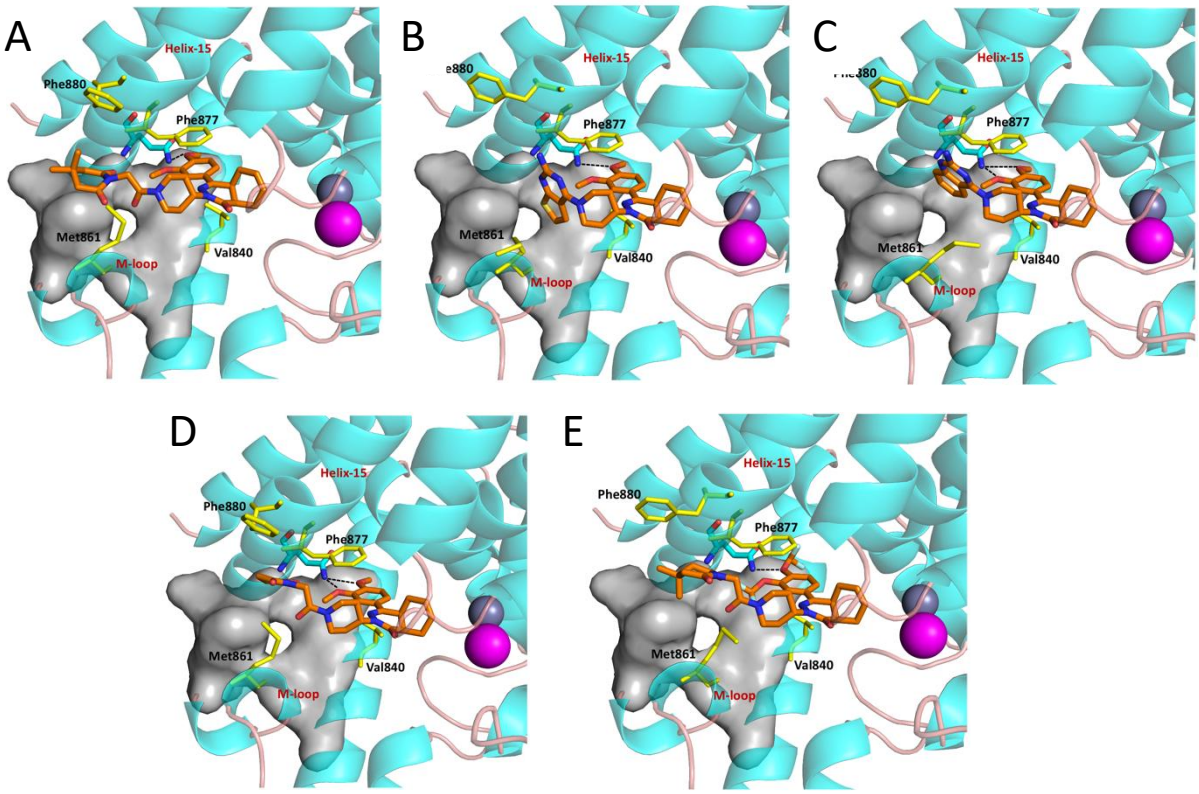


Figure 7. Crystal structure of TbrPDEB1 in complex with selected inhibitors: compound **1** (panel A), compound **11** (panel B), compound **12** (panel C), compound **14** (panel D) and compound **35** (panel E). The inhibitors are shown in orange sticks. The P-pocket formed by residues Ala837, Thr841, Tyr845, Asn867, Met868, Glu869 and Leu870 is represented as surface, key residues involved in hydrophobic interactions, including the hydrophobic clamp residues Phe877 and Val840, are shown in yellow lines, conserved Gln874 is shown in green stick, the two metal ions magnesium and zinc of the catalytic center are shown in magenta and grey spheres respectively and the hydrogen bond interactions are indicated by dashed black lines. Helix-15 and the M-loop are labelled.

DISCUSSION AND CONCLUSIONS

Compound **1**, with a submicromolar range of activity against *T. brucei* showed a lack of activity in the acute *in vivo* mouse model. This prompted us to further modify this derivative in order

to improve the metabolic stability and the selectivity between TbrPDEB1 and hPDE4. The binding mode of a selection of compounds was also studied to understand the future modifications that can be performed in the molecule in order to reach the P-pocket.

Different derivatives with good pIC_{50} were obtained, when the piperidinyl substituent is an imide moiety. The nature of the phenyl substituents do not affect nor the activity nor the microsomal stability; the unsatured phtalazinone yields more potent compounds but also more unstable and the piperidinyl linker is more favoured than the azetidinyl. On the other hand, when the piperidinyl substituent is a heterocycle, substitution of the OMe moieties by F or $OCHF_2$ yields better activity values and more stable compounds; the addition of a free amino group to the heterocycle gives more stable compounds while the potency is maintained. And as for the previous case, the piperidinyl linker is favoured over the azetidine one. Compound **14**, presented a good microsomal stability in mouse and human microsomes (mouse microsomal stability: 43% after 30 min; human microsomal stability: 89% after 30 min) and will be studied in the future in an acute *in vivo T. brucei* mouse model.

EXPERIMENTAL PART

Chemistry. Reagents were purchased from commercial sources and without further purification. The products were purified with flash chromatography on IsoleraOne flash purification system from Biotage. Compounds were detected with UV light (254 nm). ^{1}H NMR spectra were obtained on a 400 MHz Bruker Avance DRX-400 and 400 MHz Bruker Avance III nanobay spectrometer with ultrashield. Typical spectral parameters: special width 16 ppm, pulse width 9 µs (57 °), data size 32 K. ^{13}C NMR experiments were carried out on the Bruker 400 MHz Bruker Avance DRX-400 and 400 MHz Bruker Avance III nanobay spectrometer with ultrashield operating at 100 MHz. The acquisition parameters: special width 16 ppm, pulse

width 9 μs (57 °), data size 32 K. Chemical shifts are reported in values (ppm) relative to internal Me_4Si, and *J* values are reported in Hz. The Ultra Performance liquid chromatography (UPLC), used to quantify the purity of the products was an ACQUITY UPLC H-Class System with an TUV detector Waters coupled to a MS detector Waters QDa. An Acquity UPLC BEH C18 1.7μm (2.1 x 50 mm) column was used and as eluent a mixture of 0.1% formic acid (FA) in water, 0.1% FA in acetonitrile, water, acetonitrile. Final compounds were analyzed by high resolution mass. 10 μL of 10-5 M solution of each sample was injected using the CapLC system (Waters, Manchester, UK) and electrosprayed using a standard electrospray source. Samples were injected with an interval of 5 min. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The MS was calibrated prior to use with a 0.2% H_3PO_4 solution. The spectra were lock mass corrected using the know mass of the nearest H_3PO_4 cluster. All the compounds were obtained as amorphous solids. All the final compounds presented a purity at least 95% determined by UPLC and HNMR

General synthetic procedure for compounds 1 - 12, **14 - 17**, **25 - 32**, **35**, **36**, **43**, **55**, **59 - 63**: A solution of amine derivative (1 equiv), and a base, specified in every reaction, in DMF was stirred at a fixed temperature during 1 h. After that, the alkyl reagent (1 equiv) was added and the mixture was kept during a fixed time at a fixed temperature specified in each case. Ethyl acetate (50 mL) and a solution of HCl 0.1M (50 mL) were added, the organic phase was washed with a satured solution of $NaHCO_3$ (3 x 50 mL) and a satured solution of NaCl (3 x 50 mL).

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**1**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-

1(2*H*)-one (500 mg, 1.1 mmol), 4,4-dimethylpiperidine-2,6-dione (202.0 mg, 1.8 mmol), potassium carbonate (493 mg, 3.57 mmol) and DMF (10 mL). Reaction conditions: 18 h at rt after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 150.1 mg, 26%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.46 (d, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.82 – 5.76 (m, 1H), 5.68 (d, *J* = 9.4 Hz, 1H), 4.93 – 4.84 (m, 1H), 4.71 – 4.56 (m, 3H), 3.97 (s, 3H), 3.96 – 3.86 (m, 4H), 3.36 – 3.31 (m, 1H), 3.31 – 3.20 (m, 1H), 3.00 (d, *J* = 18.0 Hz, 1H), 2.80 – 2.68 (m, 2H), 2.56 (s, 4H), 2.27 – 2.07 (m, 3H), 2.08 – 1.66 (m, 4H), 1.19 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 172.0, 167.0, 167.0, 164.9, 154.6, 154.4, 151.0, 149.5, 127.7, 127.6, 126.2, 126.0, 124.1, 124.0, 119.4, 119.3, 110.7, 108.4, 108.3, 56.3, 56.3, 56.1, 52.3, 46.2, 44.3, 42.0, 40.5, 34.9, 34.8, 31.2, 31.1, 30.5, 29.7, 29.6, 27.9, 23.5, 23.4, 22.5. UPLC: purity > 99%. m/z (ES) 551.3 [M + 1]. HRMS: Calc. 550.2870 Found. 551.2861.

(4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**2**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (800 mg, 2,2 mmol), K_2CO_3 (449 mg, 3.3 mmol), triethylamine (0.4 mL, 2.8 mmol), 7-Chlorothieno[3,2-*d*]pyrimidine (443 mg, 2,60 mmol) and DMF (8 mL). Reaction conditions: 30 min at 120 °C before the alkyl reagent and 1 h at 120 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 756.6 mg, 69%. ^{1}H NMR (600 MHz, CDCl3) δ: 8.59 (s, 1H), 7.75 (d, *J* = 5.6 Hz, 1H), 7.48 (d, *J* = 5.6 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 5.83 – 5.78 (m, 1H), 5.71 – 5.66 (m, 1H), 5.07 – 4.97 (m, 2H), 4.96 – 4.90 (m, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.36 – 3.25 (m, 3H), 3.02 (d, *J* = 16.0 Hz, 1H), 2.78 (t, *J* = 5.9 Hz, 1H), 2.32 – 2.14 (m, 3H), 2.07 – 1.96 (m, 3H), 1.92 –1.86 (m, 1H). ^{13}C NMR (150 MHz, CDCl3) δ: 166.9, 157.9, 154.3, 153.7, 150.9, 149.2, 131.7, 127.5, 126.0, 124.9, 123.9,

119.2, 114.4, 110.6, 108.2, 56.0, 55.8, 52.2, 45.9, 34.8, 31.1, 30.0, 29.3, 23.3, 22.3. UPLC: purity > 99%. m/z (ES) 504.2 [M + 1]. HRMS: Calc. 503.2069, found. 504.2071.

(4a*S*,8a*R*)-2-(1-(2-Amino-7*H*-purin-6-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**3**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (350 mg, 0.9 mmol), K_2CO_3 (154.5 mg, 1.1 mmol), triethylamine (0.4 mL, 2.6 mmol), 6-chloro-9H-purin-2-amine (145.8 mg, 0.9 mmol) and DMF (2 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 66.1 mg, 15%. ^{1}H NMR (400 MHz, MeOD-*d*$_4$) δ: 7.68 (s, 1H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.33 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 5.83 - 5.63 (m, 2H), 5.47 - 5.34 (m, 2H), 5.00 - 4.91 (m, 1H), 3.83 (s, 3H), 3.66 (s, 3H), 3.52 - 3.41 (m, 1H), 3.14 (td, *J* = 13.2, 5.8 Hz, 2H), 2.89 (d, *J* = 18.1 Hz, 1H), 2.82 (t, *J* = 5.9 Hz, 1H), 2.37 - 2.09 (m, 4H), 2.07 - 1.67 (m, 5H). ^{13}C NMR (100 MHz, MeOD-*d*$_4$) δ: 169.0, 161.3, 156.6, 155.7, 154.3, 152.4, 150.5, 136.5, 128.8, 126.9, 125.1, 120.8, 115.4, 112.1, 109.6, 56.3, 56.2, 54.2, 45.9, 36.0, 31.9, 30.8, 30.2 (2C), 24.2, 23.3. UPLC: purity > 99%. m/z (ES) 503.3 [M + 1]. HRMS: Calc: 502.24 Found: 503.2532 [M + 1].

(4a*S*,8a*R*)-2-(1-(4-Aminoquinazolin-2-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**4**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (300 mg, 0.7 mmol), triethylamine (0.3 mL, 2.2 mmol), potassium carbonate (132.7 mg, 0.9 mmol), 2-chloroquinazolin-4-amine (132.1 mg, 0.7 mmol) and DMF (3 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 4 h at 153 °C after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents. Yield: 96.4 mg, 26%. ^{1}H NMR (400 MHz,

$CDCl_3$) δ: 7.55 (d, *J* = 8.1 Hz, 1H), 7.53 - 7.45 (m, 2H), 7.36 (d, *J* = 2.0 Hz, 1H), 7.21 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.02 (ddd, *J* = 8.1, 6.0, 2.1 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 5.85 - 5.52 (m, 4H), 5.07 - 4.83 (m, 3H), 3.85 (s, 3H), 3.69 (s, 3H), 3.29 (dt, *J* = 11.5, 5.8 Hz, 1H), 3.10 - 2.83 (m, 3H), 2.75 (t, *J* = 5.8 Hz, 1H), 2.25 - 1.66 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8, 161.8, 158.8, 153.9, 152.3, 150.6, 149.1, 133.2, 127.7, 126.0, 125.5, 124.0, 122.1, 121.2, 119.1, 110.4, 109.7, 108.1, 55.9, 55.7, 53.2, 43.7, 43.6, 34.7, 30.9, 29.8, 29.2, 23.3, 22.4. UPLC: purity > 99%. m/z (ES) 513 [M + 1]. HRMS: Calc: 512.25 Found: 513.2623 [M + 1].

(4a*S*,8a*R*)-2-(1-(4-Aminothieno[3,2-*d*]pyrimidin-2-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**5**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (350 mg, 0.9 mmol), triethylamine (0.4 mL, 2.6 mmol), potassium carbonate (154.5 mg, 1.1 mmol), 2-chlorothieno[3,2-*d*]pyrimidin-4-amine (159.9 mg, 0.9 mmol) and DMF (3 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 18 h at 153 °C after the addition of the agent. Purification: IsoleraOne using hexane (0.5% triethylamine)/ethyl acetate (0.5% triethylamine) as eluents. Yield: 42.8mg, 5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.64 (d, *J* = 5.1 Hz, 1H), 7.36 (d, *J* = 1.8 Hz, 1H), 7.29 - 7.23 (m, 2H), 6.85 (d, *J* = 8.5 Hz, 1H), 5.88 - 5.64 (m, 2H), 5.08 - 4.87 (m, 3H), 3.89 (s, 3H), 3.83 (s, 3H), 3.31 (dt, *J* = 11.5, 5.8 Hz, 1H), 3.14 (d, *J* = 11.5 Hz, 2H), 3.06 - 2.94 (m, 1H), 2.79 (t, *J* = 5.8 Hz, 1H), 2.33 - 2.11 (m, 3H), 2.11 - 1.78 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.1, 158.4, 154.7, 150.9, 149.3, 134.8, 127.7, 126.1, 124.1, 120.7, 119.4, 110.7, 108.6, 107.0, 56.2, 56.1, 52.6, 45.3, 34.9, 31.4, 29.8, 29.1, 23.4, 22.5. UPLC: purity > 99%. m/z (ES) 519 [M + 1]. HRMS: Calc: 518.21 Found: 519.2183 [M + 1]

(4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-2-(1-(quinazolin-2-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**6**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (250 mg, 0.6 mmol), triethylamine (0.3 mL, 2.2 mmol), potassium carbonate (109 mg, 0.7 mmol), 2-chloroquinazoline (108 mg, 0.6 mmol) and DMF (3 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 4 h at 153 °C after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents. Yield: 182.7 mg, 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.22 (d, *J* = 0.7 Hz, 1H), 7.84 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.73 (ddd, *J* = 8.5, 6.9, 1.5 Hz, 1H), 7.51 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.38 - 7.20 (m, 3H), 6.93 (d, *J* = 8.5 Hz, 1H), 5.79 – 5.56 (m, 2H), 5.00 - 4.79 (m, 3H), 3.74 (s, 3H), 3.47 (s, 3H), 3.46 – 3.39 (m, 1H), 3.16 - 3.03 (m, 2H), 2.87 - 2.71 (m, 2H), 2.24 - 1.64 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.3, 162.0, 158.8, 153.7, 151.6, 150.3, 148.6, 134.4, 127.8, 127.2, 125.8, 125.0, 124.1, 122.5, 119.2, 119.1, 111.3, 108.2, 55.5, 54.9, 52.1, 43.3, 33.9, 29.9, 29.2, 28.6, 22.7, 22.0. UPLC: purity > 99%. m/z (ES) 498 [M + 1]. HRMS: Calc: 497.24 Found: 498.2515 [M + 1].

(4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-2-(1-(quinazolin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**7**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (300 mg, 0.7 mmol), triethylamine (0.1 mL, 0.9 mmol), potassium carbonate (122.7 mg, 0.8 mmol), 4-chloroquinazoline (95.7 mg, 0.7 mmol) and DMF (5 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using DCM/methanol as eluents. Yield: 298.3 mg, 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.79 (ddd, *J* = 16.8, 8.4, 0.8 Hz, 2H), 7.61 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.34 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 7.21 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 5.74 – 5.55 (m, 2H), 4.96 – 4.86 (m, 1H), 4.39 (dd, *J* = 17.8, 14.6

Hz, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.32 – 3.11 (m, 3H), 2.99 – 2.86 (m, 1H), 2.71 (t, *J* = 5.8 Hz, 1H), 2.34 (qd, *J* = 12.6, 4.1 Hz, 1H), 2.19 – 2.04 (m, 3H), 2.02 – 1.72 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.6, 164.4, 153.9, 153.7, 151.5, 150.6, 149.0, 132.2, 128.4, 127.4, 125.8, 125.1, 124.8, 123.7, 119.0, 116.5, 110.4, 108.1, 55.7, 55.7, 52.3, 49.1, 49.1, 34.5, 30.8, 29.8, 29.1, 23.1, 22.1. UPLC: purity > 99%. m/z (ES) 498 [M + 1], HRMS: Calc: 467.24 Found: 498.2515 [M + 1].

(4a*S*,8a*R*)-2-(1-(2-Aminopyrimidin-4-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**8**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (300 mg, 0.7 mmol), triethylamine (0.1 mL, 0.9 mmol), potassium carbonate (122.7 mg, 0.8 mmol), 4-chloropyrimidin-2-amine (95.7 mg, 0.7 mmol) and DMF (5 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 3 h at 153 °C after the addition of the agent. Purification: IsoleraOne using DCM/methanol as eluents. Yield: 164 mg, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 6.2 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.30 - 7.22 (m, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 5.98 (d, *J* = 6.2 Hz, 1H), 5.83 – 5.62 (m, 2H), 4.97 - 4.80 (m, 3H), 4.45 (t, *J* = 15.5 Hz, 2H), 3.89 (s, 3H), 3.85 (s, 3H), 3.31 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.05 - 2.84 (m, 3H), 2.76 (t, *J* = 5.8 Hz, 1H), 2.27 - 1.67 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9, 162.6, 162.5, 156.32, 154.1, 150.9, 149.3, 127.6, 126.0, 124.0, 119.2, 110.6, 108.3, 94.4, 56.0, 55.9, 52.6, 43.5, 43.4, 34.8, 31.04, 29.48, 28.81, 23.34, 22.39. UPLC: purity > 99%. m/z (ES) 463 [M + 1], HRMS: Calc: 462.24 Found: 463.2463 [M + 1].

(4a*S*,8a*R*)-2-(1-(4-Aminopyrimidin-2-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**9**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (300 mg, 0.7 mmol),

triethylamine (0.1 mL, 0.9 mmol), potassium carbonate (122.7 mg, 0.8 mmol), 4-chloropyrimidin-2-amine (95.7 mg, 0.7 mmol) and DMF (5 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using DCM/methanol as eluents. Yield: 123.4 mg, 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 5.6 Hz, 1H), 7.40 (d, *J* = 2.0 Hz, 1H), 7.23 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 5.72 (d, *J* = 5.6 Hz, 1H), 5.81 - 5.63 (m, 2H), 4.92 - 4.67 (m, 5H), 3.88 (s, 3H), 3.84 (s, 3H), 3.29 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.04 - 2.82 (m, 3H), 2.75 (t, *J* = 5.8 Hz, 1H), 2.30 - 2.04 (m, 4H), 1.94 – 1.77 (m, 2H), 1.73 – 1.63 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8, 163.4, 161.8, 156.7, 153.9, 150.3, 149.2, 127.8, 126.0, 124.0, 119.1, 110.5, 108.3, 94.6, 56.0, 55.9, 53.2, 43.4, 43.3, 34.8, 31.0, 29.8, 29.1, 23.3, 22.4. UPLC: purity > 99%. m/z (ES) 463 [M + 1], HRMS: Calc: 462.24 Found: 463.2477 [M + 1].

(4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-2-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**10**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (200 mg, 0.5 mmol), triethylamine (0.2 mL, 1.5 mmol), potassium carbonate (89 mg, 0.6 mmol), 2-chlorothieno[3,2-*d*]pyrimidine (84 mg, 0.5 mmol) and DMF (1 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 170.5 mg, 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.85 (s, 1H), 7.83 (d, *J* = 5.4 Hz, 1H), 7.39 (d, *J* = 2.0 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 5.94 - 5.58 (m, 2H), 5.15 - 4.83 (m, 3H), 3.89 (s, 3H), 3.76 (s, 3H), 3.32 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.14 - 2.97 (m, 3H), 2.79 (t, *J* = 5.9 Hz, 1H), 2.31 - 2.13 (m, 3H), 2.13 - 1.76 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9, 163.5, 160.0, 158.8, 154.0, 152.5, 150.9, 149.3, 127.9, 126.1, 124.1, 123.2, 120.4, 119.2, 110.6,

108.4, 56.1, 55.9, 53.1, 44.4, 44.3, 34.9, 31.2, 29.9, 29.2, 23.5, 22.5. UPLC: purity > 99%. m/z (ES) 504.3 [M + 1]. HRMS: Calc: 503.20 Found: 504.2086 [M + 1].

(4a*S*,8a*R*)-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**11**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (400 mg, 1.0 mmol), triethylamine (0.2 mL, 1.5 mmol), potassium carbonate (177 mg, 1.2 mmol), 4-chlorothieno[2,3-*d*]pyrimidin-2-amine (183 mg, 0.985 mmol) and DMF (2 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 3 h at 153 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 243.4 mg, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (d, *J* = 2.0 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.18 (d, *J* = 6.1 Hz, 1H), 6.87 - 6.81 (m, 2H), 5.84 - 5.64 (m, 2H), 4.97 (tt, *J* = 11.4, 4.3 Hz, 1H), 4.83 (s, 2H), 4.72 - 4.58 (m, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 3.32 (dt, *J* = 5.8, 4.1 Hz, 1H), 3.18 (dtd, *J* = 15.6, 13.2, 2.6 Hz, 2H), 3.01 (dd, *J* = 17.7, 2.6 Hz, 1H), 2.78 (t, *J* = 5.8 Hz, 1H), 2.33 - 1.71 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 166.2, 159.0, 159.5, 154.2, 150.9, 149.3, 127.6, 126.0, 123.9, 120.9, 119.2, 116.1, 110.6, 110.4, 108.3, 56.0, 55.6, 52.6, 46.5, 34.8, 31.1, 29.9, 29.3, 23.4, 22.4. UPLC: purity > 99% m/z (ES) 519.2 [M + 1]. HRMS: Calc: 518.21 Found: 519.2178 [M + 1].

(4a*S*,8a*R*)-2-(1-(2-Aminoquinazolin-4-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**12**). Reagents: (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one hydrochloride (320 mg, 0.8 mmol), potassium carbonate (142 mg, 1.0 mmol), triethylamine (0.16 mL, 1.2 mmol), 4-chloroquinazolin-2-amine (141.5 mg, 0.8 mmol) and DMF anhydrous (3 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 2 h at 153 °C after the addition of the

agent. Purification: IsoleraOne using heptane/ethyl acetate followed by a second purification by IsoleraOne using DCM/methanol as eluents. Yield: 100 mg, 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 8.1 Hz, 1H), 7.65 - 7.53 (m, 2H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.32 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.19 (ddd, *J* = 8.2, 6.6, 1.5 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 5.90 - 5.65 (m, 3H), 5.07 - 4.95 (m, 1H), 4.61 - 4.46 (m, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.41 - 3.21 (m, 4H), 3.09 - 2.98 (m, 1H), 2.81 (t, *J* = 5.5 Hz, 1H), 2.38 (ddd, *J* = 16.9, 13.6, 5.0 Hz, 1H), 2.31 - 2.13 (m, 4H), 2.03 - 1.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 166.1, 158.3, 154.4, 151.0, 149.4, 133.3, 127.7, 126.1, 125.6, 124.0, 121.8, 119.3, 112.1, 110.8, 108.4, 56.1, 56.1, 52.6, 49.3, 49.3, 34.9, 31.2, 30.1, 29.5, 23.5, 22.5. UPLC: purity > 99% m/z (ES) 513 [M + 1]. HRMS: Calc: 512.25 Found: 513.2614 [M + 1].

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)pyrrolidine-2,5-dione (**14**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (500 mg, 1.1 mmol), pyrrolidine-2,5-dione (178.2 mg, 1.8 mmol), potassium carbonate (495.8 mg, 3.57 mmol) and DMF (10 mL). Reaction conditions: 18 h at rt after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents. Yield: 280.63 mg, 50%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.43 (dd, *J* = 6.1, 1.7 Hz, 1H), 7.32 – 7.29 (m, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.84 – 5.76 (m, 1H), 5.68 (d, *J* = 7.0 Hz, 1H), 4.88 (tt, *J* = 10.9, 5.4 Hz, 1H), 4.70 – 4.59 (m, 1H), 4.42 – 4.29 (m, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.91 – 3.82 (m, 1H), 3.38 – 3.31 (m, 1H), 3.32 – 3.20 (m, 1H), 3.01 (d, *J* = 18.5 Hz, 1H), 2.81 (s, 4H), 2.80 – 2.71 (m, 2H), 2.27 – 2.08 (m, 3H), 2.08 – 1.98 (m, 1H), 1.98 – 1.87 (m, 1H), 1.86 – 1.69 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 177.0, 167.1, 167.0, 163.3, 163.3, 154.7, 154.5, 151.1, 151.1, 149.5, 127.6, 127.6, 126.2, 126.0, 124.1, 124.0, 119.4, 119.3, 110.8, 110.7, 108.4, 108.3, 56.4, 56.3, 56.1, 52.2, 44.2, 42.1, 39.8, 39.8, 34.9, 34.8, 31.3, 31.2, 30.4, 29.7, 29.5, 28.9, 28.4, 23.5,

23.4, 22.5. UPLC: purity > 99%, m/z (ES) 509.2 [M + 1]. HRMS: Calc: 508.2400 Found: 509.2389 [M + 1].

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-methylpyrrolidine-2,5-dione (**15**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (500 mg, 1.1 mmol), 3-methylpyrrolidine-2,5-dione (202.0 mg, 1.8 mmol), potassium carbonate (493 mg, 3.57 mmol) and DMF (10 mL). Reaction conditions: 18 h at rt after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 150.1 mg, 26%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48 - 7.40 (m, 1H), 7.31 (dd, *J* = 8.5, 1.7 Hz, 1H), 6.90 (dd, *J* = 8.5, 3.7 Hz, 1H), 5.86 - 5.64 (m, 2H), 4.90 (ddd, *J* = 15.6, 7.7, 4.0 Hz, 1H), 4.73 - 4.57 (m, 1H), 4.43 - 4.25 (m, 2H), 4.03 - 3.80 (m, 7H), 3.31 (ddd, *J* = 38.4, 17.8, 9.6 Hz, 2H), 3.07 - 2.91 (m, 3H), 2.86 - 2.69 (m, 2H), 2.44 (t, *J* = 11.8 Hz, 1H), 2.31 - 1.59 (m, 7H), 1.40 (t, *J* = 8.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 180.4, 176.3, 167.1, 163.4, 154.7, 151.1, 149.5, 127.6, 126.2, 124.1, 119.4, 110.8, 108.4, 56.3, 52.2, 44.2, 42.1, 39.7, 36.6, 35.1, 34.9, 31.3, 30.4, 29.7, 28.9, 23.4, 22.5, 16.9. UPLC: purity > 99%, m/z (ES) 523.0 [M + 1]. HRMS: Calc: 522.25 Found: 523.2589 [M + 1].

3-Benzyl-1-(2-(1-((4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-4-yl)-2-oxoethyl)pyrrolidine-2,5-dione (**16**). Reagents: (4a*S*,8a*R*)-2-(4-(2-chloroacetyl)piperidin-1-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (135 mg, 0.3 mmol), 3-benzylpyrrolidine-2,5-dione (57 mg, 0.3 mmol), potassium carbonate (84 mg, 0.6 mmol) and DMF (2 mL). Reaction conditions: 18 h at 60 °C after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 35 mg, 19%. ^{1}H

NMR (400 MHz, $CDCl_3$) δ: 7.45 - 7.19 (m, 7H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.81 – 5.67 (m, 2H), 4.98 - 4.60 (m, 4H), 3.95 (s, 3H), 3.94 (s, 3H), 3.79 - 3.61 (m, 1H), 3.42 - 3.09 (m, 3H), 3.09 - 2.91 (m, 3H), 2.89 - 2.67 (m, 3H), 2.59 (dd, *J* = 6.3, 2.7 Hz, 1H), 2.30 - 1.95 (m, 4H), 1.95 - 1.68 (m, 3H). UPLC: purity > 99%, m/z (ES) 599.0 [M + 1]. HRMS: Calc: 598.3 Found: 599.2896 [M + 1].

3-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**17**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (300 mg, 0.7 mmol) and potassium carbonate (296 mg, 2.1 mmol), 3-azaspiro[5.5]undecane-2,4-dione (194 mg, 1.1 mmol) and DMF (2 mL). Reaction conditions: 1 h at 153 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 187.7 mg, 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (s, 1H), 7.32 – 7.28 (m, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 5.85 – 5.65 (m, 2H), 4.95 – 4.83 (m, 1H), 4.73 – 4.56 (m, 3H), 3.99 (s, 2H), 3.94 (s, 3H), 3.39 – 3.18 (m, 2H), 3.07 – 2.97 (m, 1H), 2.83 – 2.69 (m, 2H), 2.63 (s, 4H), 2.31 – 1.64 (m, 7H), 1.63 – 1.48 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.9, 167.0, 164.9, 154.5, 150.9, 149.5, 127.7, 126.2, 124.1, 119.3, 110.6, 108.3, 56.2, 52.3, 44.3, 43.9, 42.0, 40.5, 36.2, 36.1, 34.7, 31.1, 30.4, 29.6, 29.0, 25.8, 23.4, 22.5, 21.6. UPLC: purity > 99%, m/z (ES) 591.3 [M + 1]. HRMS: Calc: 590.30 Found: 591.3182 [M + 1].

cis-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(3-chloro-4-methoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**24**). Reagents: *cis*-4-(3-chloro-4-methoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (40,3 mg, 0,1 mmol), potassium carbonate (22,34 mg, 0,162 mmol), 4-chloro-2-aminothieno[2,3-*d*]pyrimidine (20 mg, 0,1 mmol) and DMF (3 mL). Reaction conditions: 1 h at 120 °C without the alkyl agent and 16 h

at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 45 mg, 80%. 1H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, *J* = 2.2 Hz, 1H), 7.66 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.20 (d, *J* = 6.1 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 1H), 6.87 (d, *J* = 6.1 Hz, 1H), 5.84 - 5.64 (m, 2H), 5.01 - 4.91 (m, 1H), 4.86 (s, 2H), 4.76 - 4.61 (m, 2H), 3.93 (s, 3H), 2.25 - 3.32 (m, 1H), 3.24 - 3.11 (m, 2H), 3.01 (d, *J* = 18.0 Hz, 1H), 2.78 (t, *J* = 5.8 Hz, 1H), 2.29 - 2.12 (m, 3H), 2.09 - 1.76 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 165.8, 158.9, 157.3, 156.4, 153.4, 128.2, 127.8, 126.1, 125.7, 123.9, 123.1, 121.2, 117.2, 112.0, 110.6, 56.5, 52.4, 46.7, 46.6, 34.8, 31.2, 30.1, 29.4, 23.2, 22.4. UPLC: purity ≥ 99% m/z (ES) 523.2 [M + 1]. HRMS: Calc: 522.16 Found: 523.1689 [M + 1].

cis-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(4-fluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**25**). Reagents: *cis*-4-(4-fluorophenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (176 mg, 0.5 mmol), potassium carbonate (112 mg, 0.8 mmol), 4-chloro-2-aminothieno[2,3-*d*]pyrimidine (100 mg, 0.5 mmol) and DMF (3 mL). Reaction conditions: 1 h at 120 °C without the alkyl agent and 16 h at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 40 mg, 16%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.93 - 7.85 (m, 2H), 7.32 (d, *J* = 6.2 Hz, 1H), 7.28 - 7.21 (m, 2H), 7.01 (d, *J* = 6.1 Hz, 1H), 6.22 (s, 2H), 5.60 - 5.76 (m, 2H), 4.78 - 4.88 (m, 1H), 4.52 - 4.65 (m, 2H), 3.43 - 3.50 (m, 1H), 3.20 - 3.09 (m, 2H), 2.90 (t, *J* = 5.8 Hz, 1H), 2.76 (d, *J* = 17.8 Hz, 1H), 2.24 - 1.98 (m, 3H), 1.92 - 1.69 (m, 4H). ^{13}C NMR (100 MHz, DMSO- d_6) δ: 171.7, 166.5, 162.96 (d, *J* = 247.4 Hz), 159.9, 158.8, 153.2, 131.20 (d, *J* = 3.0 Hz), 128.20 (d, *J* = 8.6 Hz), 125.8, 124.0, 121.4, 115.70 (d, *J* = 21.6 Hz), 114.9, 108.6, 52.0, 45.2, 33.8, 30.0, 29.8, 29.6,

22.4, 21.9. UPLC: purity > 99%, m/z (ES) 477 [M + 1]. HRMS: Calc: 476.18 Found: 477.1873 [M + 1].

cis-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(3,4-difluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**26**). Reagents: *cis*-4-(3,4-difluorophenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (130 mg, 0.4 mmol), potassium carbonate (78 mg, 0.6 mmol), 4-chloro-2-aminothieno[2,3-*d*]pyrimidine (70 mg, 0.4 mmol) and DMF (3 mL). Reaction conditions: 1 h at 120 °C without the alkyl agent and 4 h at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 60 mg, 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 - 7.66 (m, 1H), 7.52 - 7.45 (m, 1H), 7.23 - 7.14 (m, 2H), 6.88 (d, *J* = 6.1 Hz, 1H), 5.84 - 5.64 (m, 2H), 5.02 - 4.89 (m, 1H), 4.83 (s, 2H), 4.75 - 4.62 (m, 2H), 3.24 - 3.31 (m, 1H), 3.10 - 3.23 (m, 2H), 2.97 - 3.07 (m, 1H), 2.80 (t, *J* = 5.9 Hz, 1H), 2.29 - 2.13 (m, 3H), 2.10 - 1.78 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.4, 166.9, 159.2, 158.2, 152.5 (t, *J* = 2.1 Hz), 151.5 (dd, *J* = 252.8, 12.8 Hz), 150.7 (dd, *J* = 248.9, 12.9 Hz), 132.0 (dd, *J* = 5.6, 3.7 Hz), 126.1, 123.7, 122.2 (dd, *J* = 6.5, 3.5 Hz), 121.0, 117.7 (d, *J* = 17.7 Hz), 116.9, 114.9 (d, *J* = 18.6 Hz), 110.6, 52.7, 46.6, 46.5, 34.8, 31.3, 30.2, 29.5, 23.1, 22.3. UPLC: purity > 99%, m/z (ES) 495.2 [M + 1]. HRMS: Calc: 494.17 Found: 495.1773 [M + 1].

(4a*R*,8a*S*)-4-(3,4-Difluorophenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**27**). Reagents: (4a*R*,8a*S*)-4-(3,4-difluorophenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (300 mg, 0,9 mmol), potassium carbonate (180 mg, 1,3 mmol), triethylamine (114 mg, 1,1 mmol), 7-chlorothieno[3,2-*d*]pyrimidine (148.3 mg, 0.9 mmol) and DMF (2 mL). Reaction conditions: 30 min at 120 °C without the alkyl agent and 3 h at 120 °C after the addition of the agent. Purification: IsoleraOne

using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 90.8 mg, 22%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.77 (dd, *J* = 10.4, 5.6 Hz, 1H), 7.60 (ddd, *J* = 11.6, 7.6, 2.2 Hz, 1H), 7.54 (d, *J* = 5.5 Hz, 1H), 7.47 (ddd, *J* = 8.7, 4.1, 1.6 Hz, 1H), 7.18 (dt, *J* = 9.7, 8.4 Hz, 1H), 5.88 - 5.64 (m, 2H), 5.12 - 4.92 (m, 3H), 3.41 - 3.20 (m, 3H), 3.02 (dd, *J* = 13.3, 7.1 Hz, 1H), 2.81 (t, *J* = 5.8 Hz, 1H), 2.32 - 2.13 (m, 3H), 2.12 - 1.84 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9, 160.7, 157.9, 153.7, 152.5, 150.7 (d, *J* = 236.5 Hz), 131.9, 126.1, 125.0, 123.7, 122.2 (dd, *J* = 6.4, 3.5 Hz), 117.7 (d, *J* = 17.8 Hz), 114.9 (d, *J* = 18.5 Hz), 114.4, 52.7, 45.8, 45.7, 34.8, 31.3, 30.4, 29.6, 23.6, 22.3. UPLC: purity > 99%, m/z (ES) 480.1 [M + 1]. HRMS: Calc: 479.16 Found: 480.1670 [M + 1].

cis-1-(2-(4-(4-(4-Fluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**28**). Reagents: *cis*-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(4-fluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (200 mg, 0.5 mmol), potassium carbonate (205 mg, 1.5 mmol), 4,4-dimethylpiperidine-2,6-dione (105 mg, 0.7 mmol) and DMF (3 mL). Reaction conditions: 3 h at 100 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 65 mg, 26%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 - 7.72 (m, 2H), 7.09 - 7.01 (m, 2H), 5.81 - 5.53 (m, 2H), 4.88 - 4.73 (m, 1H), 4.71 - 4.49 (m, 3H), 3.94 - 3.75 (m, 1H), 3.35 - 3.09 (m, 2H), 3.02 - 2.87 (m, 1H), 2.78 - 2.58 (m, 2H), 2.52 (s, 4H), 2.24 - 1.59 (m, 7H), 1.13 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.4, 167.0, 164.8, 163.9 (d, *J* = 250.7 Hz), 153.6, 130.9, 128.0 (dd, *J* = 8.1 Hz), 126.1, 123.9, 115.9 (d, *J* = 21.8 Hz), 77.2, 52.3, 46.2, 44.2, 41.8, 40.6, 34.9, 31.3, 30.4, 29.6, 29.5, 28.9, 27.9, 23.2, 22.4. UPLC: purity > 99%, m/z (ES) 509.4 [M + 1]. HRMS: Calc: 508.25. Found: 509.2587 [M + 1].

cis-3-(2-(4-(4-(4-Fluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**29**). Reagents: *cis*-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(4-fluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (200 mg, 0.5 mmol), potassium carbonate (205 mg, 1.5 mmol), 3-azaspiro[5.5]undecane-2,4-dione (135 mg, 0.7 mmol) and DMF (3 mL). Reaction conditions: 3 h at 100 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 70 mg, 26%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 - 7.80 (m, 2H), 7.17 - 7.08 (m, 2H), 5.86 - 5.61 (m, 2H), 4.97 - 4.47 (m, 5H), 4.01 - 3.81 (m, 1H), 3.29 (m, 2H), 3.00 (d, *J* = 18.2 Hz, 1H), 2.84 - 2.57 (m, 6H), 2.31 - 1.36 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1, 166.9, 164.8, 163.9 (d, *J* = 250.6 Hz), 153.5, 130.9, 128.0 (d, *J* = 8.4 Hz), 126.1, 123.9, 115.9 (d, *J* = 21.8 Hz), 52.3, 43.9, 41.8, 40.5, 36.2, 34.8, 32.1, 31.3, 29.0, 25.9, 25.8, 23.1, 22.4, 21.7. UPLC: purity > 99%, m/z (ES) 549.5 [M + 1]. HRMS: Calc: 548.28 Found: 549.2877 [M + 1].

cis-8-(2-(4-(4-(4-Fluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-8-azaspiro[4.5]decane-7,9-dione (**30**). Reagents: *cis*-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(4-fluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (200 mg, 0.5 mmol), potassium carbonate (205 mg, 1.5 mmol), 8-azaspiro[4.5]decane-7,9-dione (124 mg, 0.7 mmol) and DMF (3 mL). Reaction conditions: 3 h at 100 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 60 mg, 23%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 - 7.73 (m, 2H), 7.09 - 7.01 (m, 2H), 5.78 - 5.55 (m, 2H), 4.81 (tt, *J* = 11.4, 3.9 Hz, 1H), 4.69 - 4.44 (m, 3H), 3.95 - 3.74 (m, 1H), 3.33 - 3.08 (m, 2H), 2.94 (d, *J* = 18.3 Hz, 1H), 2.76 - 2.48 (m, 6H), 2.23 - 1.36 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.4, 167.0, 164.8, 163.9 (d, *J* = 250.7 Hz), 153.6, 130.9, 128.0 (dd, *J* = 8.1 Hz), 126.1, 123.9, 115.9 (d, *J* = 21.8 Hz), 77.2, 52.3, 44.5, 44.2, 41.8, 40.6, 39.7, 37.8, 34.9,

31.3, 30.4, 29.6, 28.9, 24.2, 23.2, 22.4. UPLC: purity > 99%, m/z (ES) 535.6 [M + 1]. HRMS: Calc: 534.26 Found: 535.2721 [M + 1].

cis-3-(2-(4-(4-(3,4-Difluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**31**). Reagents: *cis*-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-difluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (169,3 mg, 0,4 mmol), 3,3-pentamethyleneglutarimide (87 mg, 0,5 mmol), potassium carbonate (222 mg, 1,6 mmol), and DMF (5 ml). Reaction conditions: 1 h at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 99.3 mg, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 - 7.66 (m, 1H), 7.61 - 7.54 (m, 1H), 7.30 - 7.20 (m, 1H), 5.89 - 5.64 (m, 2H), 4.94 - 4.86 (m, 1H), 4.78 - 4.54 (m, 3H), 4.04 - 3.86 (m, 1H), 3.37 - 3.19 (m, 2H), 3.06 - 3.00 (m, 1H), 2.85 - 2.60 (m, 6H), 2.32 - 1.65 (m, 7H), 1.66 - 1.40 (m, 10H). ^{13}C NMR (100 MHz, CDCl3) δ 172.1, 166.9, 165.0 (d, *J* = 4.4 Hz), 132.0, 126.1 (d, *J* = 12.7 Hz), 123.7 (d, *J* = 10.4 Hz), 122.3, 117.7 (d, *J* = 17.8 Hz), 115.1 (dd, *J* = 19.8, 2.8 Hz), 52.5, 52.4, 44.3, 43.9, 41.9, 40.5, 40.4, 36.2, 34.8, 34.8, 32.1, 31.2, 31.1, 30.4, 29.6, 28.97, 25.9, 23.2, 23.0, 22.3, 21.7. UPLC: purity > 99%, m/z (ES) 567.3 [M + 1]. HRMS: Calc: 566.27 Found: 567.2805 [M + 1].

cis-1-(2-(4-(4-(3,4-Difluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**32**). Reagents: *cis*-2-(1-(2-chloroacetyl)piperidin-4-yl)-4-(3,4-difluorophenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (400 mg, 0,9 mmol), 4,4-dimethylpiperidine-2,6-dione (161 mg, 1,1 mmol), potassium carbonate (524 mg, 3.8 mmol) and DMF (5 ml). Reaction conditions: 16 h at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate/methanol as eluents. Yield: 22.6 mg, 5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (dd, *J* = 11.3, 7.9 Hz, 1H), 7.55 (ddd,

J = 8.7, 4.0, 1.7 Hz, 1H), 7.21 (dt, *J* = 9.7, 8.5 Hz, 1H), 5.83 - 5.62 (m, 2H), 4.96 - 4.79 (m, 1H), 4.76 - 4.51 (m, 3H), 3.91 (t, *J* = 16.5 Hz, 1H), 3.35 - 3.14 (m, 2H), 2.99 (d, *J* = 18.0 Hz, 1H), 2.84 - 2.62 (m, 2H), 2.58 (s, 4H), 2.29 - 1.61 (m, 7H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1, 166.9, 165.0, 152.3 (dd, *J* = 78.9, 12.8 Hz), 149.8 (dd, *J* = 75.2, 12.8 Hz), 131.9 (d, *J* = 4.0 Hz), 126.1, 123.8, 122.3 (d, *J* = 3.7 Hz), 117.6 (d, *J* = 17.6 Hz), 115.1 (d, *J* = 16.5 Hz), 53.6, 52.4, 52.4, 46.2, 41.9, 40.4, 34.8, 34.7, 31.2, 31.1, 30.4, 29.5, 29.0, 27.9, 23.1, 23.0, 22.3. UPLC: purity > 99%, m/z (ES) 527.2 [M + 1]. HRMS: Calc: 526.24 Found: 527.2470 [M + 1].

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Bis(difluoromethoxy)phenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**35**). Reagents: (4a*S*,8a*R*)-4-(3,4-bis(difluoromethoxy)phenyl)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (170 mg, 0,3 mmol), 3,3-dimethylglutarimide (93 mg, 0,7 mmol), potassium carbonate (181 mg, 1,3 mmol) and DMF (2 ml). Reaction conditions: 16 h at 120 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 20 mg, 10%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72 (dt, *J* = 6.5, 3.2 Hz, 1H), 7.69 (d, *J* = 1.9 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 6.72 (t, *J* = 73.4 Hz, 2H), 6.60 (t, *J* = 73.2 Hz, 2H), 5.90 - 5.61 (m, 2H), 4.99 - 4.84 (m, 1H), 4.66 (s, 2H), 4.07 - 3.84 (m, 1H), 3.32 (dt, *J* = 11.6, 6.9 Hz, 1H), 3.03 (d, *J* = 18.5 Hz, 1H), 2.80 (dd, *J* = 14.5, 8.8 Hz, 1H), 2.61 (s, 4H), 2.34 - 1.68 (m, 8H), 1.22 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1 (2C), 166.9, 165.0, 152.3, 143.4, 142.6, 133.6, 126.1, 124.2, 123.7, 122.3, 119.5, 115.9 (t, *J* = 263.0 Hz), 115.7 (t, *J* = 263.0 Hz), 52.4, 46.2 (2C), 44.3, 42.0, 40.4, 34.8, 32.0, 31.2, 29.5 (2C), 27.9 (2C), 23.1, 22.3. UPLC: purity > 99%, m/z (ES) 623.1 [M + 1]. HRMS: Calc: 622.24 Found: 623.2495 [M + 1].

3-(2-(4-((4a*S*,8a*R*)-4-(3,4-Bis(difluoromethoxy)phenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**36**). Reagents: (4a*S*,8a*R*)-4-(3,4-bis(difluoromethoxy)phenyl)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (243 mg, 0,2 mmol), potassium carbonate (130 mg, 0,9 mmol), 3,3-pentamethyleneglutarimide (51,0 mg, 0,3 mmol) and DMF (2 ml). Reaction conditions: 16 h at 100 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 9.7 mg, 6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.64 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.59 (s, 1H), 7.26 (d, *J* = 8.6 Hz, 1H), 6.83 - 6.29 (m, 2H), 5.81 - 5.54 (m, 2H), 4.82 (tt, *J* = 11.4, 4.0 Hz, 1H), 4.69 - 4.45 (m, 3H), 3.86 (dd, *J* = 30.0, 14.1 Hz, 1H), 3.30 - 3.08 (m, 2H), 2.93 (d, *J* = 18.4 Hz, 1H), 2.78 - 2.61 (m, 2H), 2.57 (s, 4H), 2.27 - 1.28 (m, 17H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 166.8, 164.8, 152.2, 143.9, 142.5, 133.5, 126.0, 124.1, 123.6, 122.2, 119.5, 115.7 (t, *J* = 262.8 Hz), 115.6 (t, *J* = 262.8 Hz), 52.4, 52.3, 44.2, 43.8, 41.8, 40.4, 40.3, 36.1, 34.7, 32.0, 31.2, 30.3, 29.6, 28.9, 25.8, 23.1, 23.0, 22.2, 21.6. UPLC: purity = 95%, m/z (ES) 663.1 [M + 1]. HRMS: Calc: 662.3 Found: 663.2806 [M + 1].

(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-(3,4-dimethoxyphenyl)imidazo[1,2-*d*][1,2,4]triazin-2-one (**43**). Reagents: (3,4-dimethoxyphenyl)-(piperidin-4-yl)imidazo[1,2-*d*][1,2,4]triazin-2-one (100 mg, 0,3 mmol), triethylamine (42,7 mg, 0,4 mmol), potassium carbonate (50.6 mg, 0.4 mmol), 4-chloro-2-aminothieno[2,3-*d*]pyrimidine (52,2 mg, 0,3 mmol) and DMF (5 mL). Reaction conditions: 90 min at 130 °C without the alkyl agent and 3 h at 130 °C after adding the agent. Purification: IsoleraOne using water/methanol. Yield: 29,7 mg, 21 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (s, 1H), 7.99 - 7.92 (m, 2H), 7.71 (d, *J* = 1.3 Hz, 1H), 7.31 (d, *J* = 5.8 Hz, 1H), 7.14 (d, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 5.34 - 5.22 (m, 1H), 5.03 - 4.91 (m, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 3.60 - 3.47 (m, 2H), 2.46 - 2.15 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.8, 153.7, 151.4, 149.0, 144.3, 138.5, 134.7, 124.7, 121.7, 121.6,

121.3, 119.1, 114.8, 114.3, 111.9, 111.7, 111.2, 110.9, 110.7, 56.3, 56.2, 55.0, 46.8, 30.4. UPLC: purity > 99%, m/z (ES) 505 [M + 1]. HRMS: Calc: 504.17 Found: 505.1776 [M + 1].

2-(1-(2-Aminothieno-[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-6-(3,4-dimethoxyphenyl)pyridazin-3(2*H*)-one (**51**). Reagents: 6-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)pyridazin-3(2*H*)-one (200 mg, 0,6 mmol), triethylamine (0,1 ml, 0,9 mmol), potassium carbonate (114 mg, 0.8 mmol), 4-chlorothieno[2,3-*d*]pyrimidin-2-amine (118 mg, 0,6 mmol) and DMF (3 mL). Reaction conditions: 90 min at 130 °C without the alkyl agent and 16 h at 130 °C after adding the agent. Purification: IsoleraOne using water/methanol and after that a second purification using hexane/ethyl acetate as eluents. Yield: 31.6 mg, 11%. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 7.99 (d, J = 9.3 Hz, 1H), 7.60 (d, J = 4.7 Hz, 1H), 7.42 (s, 2H), 7.34 (d, J = 4.7 Hz, 1H), 7.06 (d, J = 9.3 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 5.38 (s, 2H), 4.97 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.59 (s, 2H), 2.21 (s, 4H). ^{13}C NMR (100 MHz, CDCl3) δ: 159.4, 157.8, 153.6, 150.7, 149.5, 144.8, 130.1, 130.0, 127.5, 121.7, 119.3, 111.5, 110.7, 109.2, 56.7, 56.3, 54.2, 47.1, 30.6. UPLC: purity > 99%, m/z (ES) 465 [M + 1]. HRMS: Calc: 464.16 Found: 465.1709 [M + 1].

3-(3,4-Dimethoxyphenyl)-4,4-dimethyl-1-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-*1H*-pyrazol-5(4*H*)-one (**56**). Reagents: 3-(3,4-dimethoxyphenyl)-4,4-dimethyl-1-(piperidin-4-yl)-1*H*-pyrazol-5(4*H*)-one 2,2,2-trifluoroacetate (223 mg, 0.5 mmol), sodium hydride (24 mg, 1 mmol), 4-chlorothieno[3,2-*d*]pyrimidine (128 mg, 0.75 mmol) and DMF anhydrous (2 mL). Reaction conditions: 1 h at 153 °C before the alkyl reagent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using hexane/ethyl acetate as eluents. Yield: 61.4 mg, 26%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.63 (s, 1H), 7.82 (d, J = 5.6 Hz, 1H), 7.59 (d, J = 5.4 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.27 (dd, J = 8.4, 2.1 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 5.04 (d,

J = 13.7 Hz, 2H), 4.52 (tt, *J* = 11.3, 4.3 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.36 (t, *J* = 12.0 Hz, 2H), 2.23 (ddd, *J* = 16.2, 12.7, 4.1 Hz, 2H), 2.06 (dd, *J* = 12.7, 2.9 Hz, 2H), 1.51 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.3, 162.1, 158.8, 157.8, 152.9, 151.0, 149.4, 132.7, 124.4, 123.7, 119.7, 114.4, 110.6, 108.8, 56.2, 56.1, 50.5, 48.9, 45.8, 30.4, 23.1. UPLC: purity ≥ 99% m/z (ES) 466 [M + 1]. HRMS: Calc: 465.18 Found: 466.1912 [M + 1].

(4a*S*,8a*R*)-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(*2H*)-one (**59**). Reagents: (4a*S*,8a*R*)-2-(azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(*2H*)-one 2,2,2-trifluoroacetate (446 mg, 1.0 mmol), sodium hydride (47 mg, 1.9 mmol), 4-chlorothieno[2,3-*d*]pyrimidin-2-amine (218 mg, 1.2 mmol) and DMF anhydrous (2 mL). Reaction conditions: 1 h at 153 °C without the alkyl agent and 4 h at 153 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 12 mg, 3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (d, *J* = 1.9 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.08 (d, *J* = 6.0 Hz, 1H), 6.85 (d, *J* = 5.9 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 5.92 - 5.64 (m, 3H), 4.98 (s, 2H), 4.90 - 4.76 (m, 1H), 4.72 - 4.55 (m, 2H), 4.55 - 4.44 (m, 1H), 3.90 (s, 3H), 3.55 (s, 3H), 3.44 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.02 (d, *J* = 18.6 Hz, 1H), 2.83 (t, *J* = 5.7 Hz, 1H), 2.33 - 2.14 (m, 2H), 2.15 - 1.96 (m, 1H). UPLC: purity > 99%, m/z (ES) 491.2 [M + 1]. HRMS: Calc: 490.18 Found: 491.1872 [M + 1].

(4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)azetidin-3-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**60**). Reagents: (4a*S*,8a*R*)-2-(azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one 2,2,2-trifluoroacetate (243,7 mg, 0.5 mmol), sodium hydride (25,7 mg, 1.1 mmol), 4-chlorothieno[3,2-*d*]pyrimidine (110 mg, 0.6

mmol) and DMF anhydrous (2 mL). Reaction conditions: 1 h at 153 °C without the alkyl agent and 16 h at 153 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents, followed by a second purification by IsoleraOne using water/methanol as eluents. Yield: 10 mg, 4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.78 (d, *J* = 5.4 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.34 (d, *J* = 2.0 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 5.88 (ddd, *J* = 15.9, 8.0, 5.3 Hz, 1H), 5.84 - 5.68 (m, 2H), 4.94 (dd, *J* = 8.7, 5.1 Hz, 1H), 4.73 (dd, *J* = 16.8, 8.4 Hz, 2H), 4.60 (dd, *J* = 9.1, 5.3 Hz, 1H), 3.89 (s, 3H), 3.51 - 3.39 (m, 4H), 3.02 (d, *J* = 18.1 Hz, 1H), 2.84 (t, *J* = 5.7 Hz, 1H), 2.33 - 2.17 (m, 2H), 2.13 - 1.98 (m, 1H). UPLC: purity > 99%, m/z (ES) 476.2 [M + 1]. HRMS: Calc: 475.17 Found: 476.1756 [M + 1].

1-(2-(3-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)azetidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**61**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (249 mg, 0,6 mmol), potassium carbonate (247 mg, 1,8 mmol), 4,4-dimethylpiperidine-2,6-dione (126 mg, 0,9 mmol) and DMF (4 mL). Reaction conditions: 16 h at 100 °C after the addition of the agent. Purification: IsoleraOne using water/methanol as eluents. Yield: 180 mg, 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (s, 1H), 7.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 5.63 - 5.83 (m, 3H), 4.65 - 4.13 (m, 6H), 3.93 (s, 6H), 3.37 - 3.46 (m, 1H), 2.98 (d, *J* = 15.3 Hz, 1H), 2.77 - 2.84 (m, 1H), 2.54 (s, 4H), 2.31 - 1.90 (m, 3H), 1.16 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 167.9, 167.8, 166.5, 155.9, 155.6, 151.3, 149.6, 127.1, 127.0, 125.9, 125.8, 124.0, 124.0, 119.6, 119.5, 110.6, 110.6, 108.3, 108.2, 56.2, 56.1, 55.2, 54.9, 53.6, 53.4, 46.1, 44.9, 44.7, 38.8, 35.0, 34.9, 31.3, 29.5, 27.8, 23.7, 23.5, 22.3. UPLC: purity > 99%, m/z (ES) 523.3 [M + 1]. HRMS: Calc: 522.6 Found: 523.2557 [M + 1].

3-(2-(3-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)azetidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**62**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (245 mg, 0,6 mmol), 3-azaspiro[5.5]undecane-2,4-dione (159 mg, 0,9 mmol), potassium carbonate (243 mg, 1,8 mmol) and DMF (4 ml). Reaction conditions: 4 h at 100 °C after the addition of the agent. Purification: IsoleraOne using water/AcCN as eluents. Yield: 190 mg, 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 - 7.53 (m, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.82 - 5.62 (m, 3H), 4.66 - 4.18 (m, 6H), 3.94 (s, 3H), 3.92 (s, 3H), 3.37 - 3.46 (m, 1H), 2.98 (d, *J* = 17.0 Hz, 1H), 2.77 - 2.84 (m, 1H), 2.59 (d, *J* = 3.0 Hz, 4H), 1.94 - 2.30 (m, 3H), 1.49 (s, 8H), 1.43 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 167.9, 167.8, 166.4, 155.9, 155.5, 151.3, 149.6, 127.1, 127.0, 126.0, 125.8, 124.0, 123.9, 119.6, 119.5, 110.6, 110.6, 108.3, 108.2, 56.2, 56.1, 55.2, 54.9, 53.6, 53.4, 44.9, 44.7, 43.8, 38.8, 36.1, 35.0, 34.9, 32.1, 31.4, 31.3, 25.8, 23.7, 23.5, 22.3, 21.6. UPLC: purity > 99%, m/z (ES) 563.3 [M + 1]. HRMS: Calc: 562.28 Found: 563.2870 [M + 1].

8-(2-(3-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)azetidin-1-yl)-2-oxoethyl)-8-azaspiro[4.5]decane-7,9-dione (**63**). Reagents: (4a*S*,8a*R*)-2-(1-(2-chloroacetyl)azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (132 mg, 0.3 mmol), 8-azaspiro[4.5]decane-7,9-dione (80 mg, 0,5 mmol), potassium carbonate (131 mg, 0,9 mmol) and DMF (1 ml). Reaction conditions: 3 h at 100 °C after the addition of the agent. Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 139 mg, 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 1.9 Hz, 1H), 7.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 5.84 – 5.62 (m, 3H), 4.68 – 4.16 (m, 6H), 3.98 – 3.88 (m, 6H), 3.42 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.04 – 2.92 (m, 1H), 2.80 (t, *J* = 5.7 Hz, 1H), 2.63 (s, 4H), 2.32 – 2.12 (m, 2H), 2.12 – 1.93 (m, 1H), 1.77 – 1.49 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1,

167.9, 166.4, 151.3, 149.6, 127.1, 125.9, 124.0, 119.6, 110.7, 108.4, 56.3, 56.1, 53.6, 44.9, 44.5, 39.6, 38.9, 37.7, 35.0, 31.4, 24.2, 23.6, 22.3. UPLC: purity > 99%, m/z (ES) 549.3 [M + 1].

(4a*S*,8a*R*)-4-(3,4-Dihydroxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**37**). (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (2.5 g, 4,9 mmol) was dissolved in anhydrous DCM (120 mL) under inert atmosphere. Tribromoborane (2,4 ml, 24,6 mmol) was added very slowly at -40 °C. The mixture was kept at this temperature during 1 h and after that it was kept at rt for 2 h more. After that, the mixture was poured onto ice-water and extracted with DCM (2 x 100 mL). The crude was purified by IsoleraOne using methanol/water as eluents obtaining the final compound (2.34 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.54 (s, 1H), 7.75 (d, *J* = 5.6 Hz, 1H), 7.43 (d, *J* = 5.6 Hz, 1H), 7.33 (d, *J* = 2.1 Hz, 1H), 7.17 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.83 - 5.63 (m, 2H), 5.11 - 4.83 (m, 3H), 3.41 - 3.25 (m, 3H), 3.00 (d, *J* = 17.5 Hz, 1H), 2.76 (t, *J* = 5.8 Hz, 1H), 2.31 - 2.11 (m, 3H), 2.06 - 1.83 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.2, 157.6, 155.1, 152.1, 152.0, 147.2, 145.1, 133.4, 126.7, 126.0, 124.1, 122.9, 118.6, 115.1, 114.3, 112.5, 51.4, 46.1, 45.9, 34.9, 31.2, 30.1, 29.4, 23.4, 22.5. UPLC: purity > 99%, m/z (ES) 476.3 [M + 1]. HRMS: Calc: 475.17 Found: 476.1756 [M + 1].

General synthetic procedure for compounds 38, **39**: The dimethoxi derivative (1 equiv) was dissolved in AcCN/water (10 mL:10 mL) and the solution was cooled down to -40 °C. Potassium hydroxide (40 equiv) was added and the mixture was kept at that temperature during 30 min. After that time, bromodifluoromethyldiethylphosphonate (10 equiv) was added and the reaction was stirred at -40 °C and at rt during a specified time for every reaction. Diethyl ether was added and the organic phase was purified by IsoleraOne using different eluents, described in each reaction.

(4a*S*,8a*R*)-4-(3,4-Bis(difluoromethoxy)phenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**38**). Reagents: (4a*S*,8a*R*)-4-(3,4-dihydroxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (719 mg, 1,5 mmol), potassium hydroxide (3 g, 60,5 mmol) and bromodifluoromethyldiethylphosphonate (2,7 ml, 15,1 mmol). Reaction conditions: 2 h at -40 °C and 16 h at rt Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 13.6 mg, 2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 7.83 (d, *J* = 5.5 Hz, 1H), 7.65 - 7.59 (m, 3H), 7.31 - 7.24 (m, 1H), 6.53 (td, *J* = 73.2, 6.7 Hz, 2H), 5.87 - 5.63 (m, 2H), 5.11 - 4.92 (m, 3H), 3.43 – 3.21 (m, 3H), 3.08 – 2.92 (m, 1H), 2.82 (t, *J* = 5.8 Hz, 1H), 2.32 – 2.11 (m, 3H), 2.11 - 1.86 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 157.9, 152.6, 143.6, 142.3, 133.5, 133.0, 126.1, 124.2, 123.9, 123.6, 122.2, 120.3, 115.7 (t, *J* = 263.8 Hz), 115.6 (t, *J* = 263.8 Hz), 77.2, 52.5, 46.1, 34.8, 31.3, 30.3, 29.6, 23.2, 22.3. UPLC: purity > 99%, m/z (ES) 576.2 [M + 1].

(4a*S*,8a*R*)-4-(4-(Difluoromethoxy)-3-hydroxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**39**). Reagents: (4a*S*,8a*R*)-4-(3,4-dihydroxyphenyl)-2-(1-(thieno[3,2-*d*]pyrimidin-4-yl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (700 mg, 1,5 mmol), potassium hydroxide (3.3 g, 58,9 mmol) and bromodifluoromethyldiethylphosphonate (2.7 ml, 15.1 mmol). Reaction conditions: 45 min at -40 °C and 100 min at rt Purification: IsoleraOne using heptane/ethyl acetate as eluents. Yield: 18 mg, 2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.63 (s, 1H), 7.76 (d, *J* = 5.5 Hz, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 5.5 Hz, 1H), 7.19 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 6.66 (t, *J* = 74.2 Hz, 1H), 5.86 - 5.64 (m, 2H), 5.10 - 4.80 (m, 3H), 3.41 - 3.25 (m, 3H), 3.03 (d, *J* = 19.2 Hz, 2H), 2.80 (t, *J* = 5.8 Hz, 1H), 2.23 (ddd, *J* = 16.5, 12.1, 4.0 Hz, 3H), 2.11 - 1.83 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.1, 158.0, 153.7, 153.5, 153.4, 148.7, 140.1

(t, *J* = 2.6 Hz), 133.3, 132.3, 126.1, 124.4, 123.9, 121.0, 117.9, 116.7 (t, *J* = 261.9 Hz), 114.6, 114.2, 52.0, 45.8, 45.6, 34.8, 31.3, 30.2, 29.4, 23.3, 22.4. UPLC: purity > 99%, m/z (ES) 526.2 [M + 1].

(3,4-Dimethoxyphenyl)imidazo[1,2-*d*][1,2,4]triazin-2-one (**41**). Procedure was followed as previously described.[28] A mixture of (3,4-dimethoxyphenyl)(1*H*-imidazol-2-yl)methanone (200 mg, 0.2 mmol), ethyl hydrazinecarboxylate (161 mg, 0.3 mol), and toluenesulfonic acid (14.7 mg, 0.02 mmol) in 4.3 mL of mesitylene was refluxed for 2 h. While still hot, the clear brown solution was decanted from a dark brown gum. On cooling, the crude was purified by IsoleraOne using hexane and ethyl acetate as eluents obtaining the final compound (167.3 mg, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.49 (s, 1H), 8.19 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.97 (d, *J* = 1.4 Hz, 1H), 7.92 (d, *J* = 2.0 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.02 (d, *J* = 8.6 Hz, 1H), 4.00 (s, 3H), 3.97 (s, 3H). ^{13}C NMR (100 MHz, -d_6) δ: 150.4, 148.5, 144.6, 138.8, 137.3, 133.6, 125.0, 121.4, 114.3, 111.3, 110.5, 55.6, 55.5. UPLC: purity > 99%, m/z (ES) 273.2 [M + 1]. HRMS: Calc: 272.09 Found: 273.0988 [M + 1].

(3,4-Dimethoxyphenyl)-(piperidin-4-yl)imidazo[1,2-*d*][1,2,4]triazin-2-one (**42**). *Tert*-butyl 4-((3,4-dimethoxyphenyl)-2-oxoimidazo[1,2-*d*][1,2,4]triazinyl)piperidine-1-carboxylate (172,2 mg, 0,4 mmol) was dissolved in dichloromethane (5 mL). This solution was cooled down to 0°C. 2,2,2-Trifluoroacetic acid (0,3 ml, 3,8 mmol) was added and the reaction was kept at rt 16 h. The organic solvent was evaporated and the crude was purified by IsoleraOne using water/methanol as eluents obtaining the desired compound (100 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 - 7.79 (m, 3H), 7.59 - 7.44 (m, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 5.04 - 4.88 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.47 (d, *J* = 12.6 Hz, 2H), 3.02 (t, *J* = 11.7 Hz, 2H), 2.51 - 2.29 (m, 2H), 2.06 (d, *J* = 13.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 151.5, 149.1, 144.5, 139.4,

138.7, 134.7, 124.9, 122.2, 115.1, 111.5, 111.2, 56.2, 56.1, 53.3, 43.5, 27.5. UPLC: purity > 99%, m/z (ES) 356.2 [M + 1]. HRMS: Calc: 355.16. Found: 356.1723 [M + 1].

General synthetic procedure for compounds 44 - 49: Under inert atmosphere, alkene derivative (1 equiv) was dissolved in anhydrous methanol, after this, Pd(C) (4 catalyst spatulas) was added and the hydrogen atmosphere was connected to the flask leaving the reaction at room temperature for a specified time for each reaction. The crude was filtered over a celite pad and the crude was purified using the conditions described in every reaction.

(4a*S*,8a*R*)-2-(1-(2-Aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one (**44**). Reagents: (4a*S*,8a*R*)-2-(1-(2-aminothieno[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (100 mg, 0,2 mmol) and anhydrous methanol (10 mL). Reaction conditions: 3 days. Purification: IsoleraOne using hexane/ethyl acetate as eluents. Yield: 12.5 mg, 12%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (d, *J* = 2.0 Hz, 1H), 7.23 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.21 (d, *J* = 6.2 Hz, 1H), 6.88 (d, *J* = 6.1 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 5.02 (tt, *J* = 11.4, 4.3 Hz, 1H), 4.86 (s, 2H), 4.76 - 4.63 (m, 2H), 3.91 (s, 3H), 3.83 (s, 3H), 3.29 - 3.14 (m, 2H), 3.13 - 3.01 (m, 1H), 2.71 (d, *J* = 3.2 Hz, 1H), 2.58 (d, *J* = 8.9 Hz, 1H), 2.23 (ddd, *J* = 24.8, 12.9, 4.4 Hz, 1H), 2.13 - 1.99 (m, 1H), 1.97 - 1.74 (m, 3H), 1.68 (s, 2H), 1.47 - 1.34 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.1, 159.5, 159.1, 153.2, 150.8, 149.3, 127.7, 121.0, 119.2, 116.4, 110.6, 110.5, 108.5, 56.1, 56.0, 52.2, 46.7, 46.7, 36.9, 35.7, 30.0, 29.4, 25.8, 24.7, 24.1, 22.1. UPLC: purity > 99%, m/z (ES) 521.3 [M + 1]. HRMS: Calc: 520.23 Found: 521.2334 [M + 1].

3-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,6,7,8,8a-hexahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**45**). Reagents: 3-(2-(4-((4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (150 mg, 0,3 mmol) and anhydrous methanol (25 mL). Reaction conditions: 3 h. Purification: no further purification was needed. Yield: 120.3 mg, 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (s, 1H), 7.27 - 7.22 (m, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.89 (t, *J* = 10.8 Hz, 1H), 4.73 - 4.52 (m, 3H), 3.97 (s, 3H), 3.92 (s, 3H), 3.27 (t, *J* = 14.5 Hz, 1H), 3.07 (dt, *J* = 9.9, 5.1 Hz, 1H), 2.87 - 2.52 (m, 7H), 2.20 - 1.28 (m, 22H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 167.1, 164.9, 153.4, 150.9, 149.4, 127.7, 119.2, 110.6, 108.4, 56.3, 56.1, 52.0, 43.9, 40.5, 36.8, 36.2, 35.7, 32.1, 25.9, 25.8, 24.7, 24.1, 22.1, 21.7. UPLC: purity > 99%, m/z (ES) 593 [M + 1]. HRMS: Calc: 592.33 Found: 593.3339 [M + 1].

1-(2-(4-((4a*R*,8a*S*)-4-(3,4-Difluorophenyl)-1-oxo-4a,5,6,7,8,8a-hexahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**46**). Reagents: 1-(2-(4-((4a*R*,8a*S*)-4-(3,4-difluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (130 mg, 0,2 mmol) and anhydrous methanol (30 mL). Reaction conditions: 3 h. Purification: precipitated in heptane at -78 °C. Yield: 101 mg, 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (ddd, *J* = 11.6, 7.7, 2.2 Hz, 1H), 7.53 (d, *J* = 7.1 Hz, 1H), 7.20 (dt, *J* = 9.7, 8.5 Hz, 1H), 4.89 (t, *J* = 10.6 Hz, 1H), 4.75 - 4.54 (m, 3H), 3.92 (t, *J* = 12.4 Hz, 1H), 3.25 (dd, *J* = 19.5, 9.1 Hz, 1H), 3.01 (dt, *J* = 9.9, 5.0 Hz, 1H), 2.82 - 2.62 (m, 2H), 2.56 (d, *J* = 15.7 Hz, 5H), 2.19 - 1.55 (m, 8H), 1.54 - 1.23 (m, 4H), 1.18 (d, *J* = 11.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.3, 167.1 (d, *J* = 2.8 Hz), 165.2 (d, *J* = 4.8 Hz), 152.8 - 151.9 (m), 151.4 (d, *J* = 22.4 Hz), 150.3, 132.1, 122.5, 117.7 (d, *J* = 17.6 Hz), 115.2 (dd, *J* =

18.6, 5.0 Hz), 52.3 (d, *J* = 2.8 Hz), 46.3, 44.4, 42.1, 40.5 (d, *J* = 4.7 Hz), 36.9 (d, *J* = 6.1 Hz), 35.8 (d, *J* = 6.5 Hz), 30.5, 29.7, 29.6, 29.1, 28.1, 25.8, 24.6 (d, *J* = 11.7 Hz), 24.1, 22.1. UPLC: purity > 99%, m/z (ES) 529.3 [M + 1]. MP = 222 - 224 °C. HRMS: Calc: 528.25 Found: 529.2626 [M + 1].

3-(2-(4-((4a*R*,8a*S*)-4-(3,4-Difluorophenyl)-1-oxo-4a,5,6,7,8,8a-hexahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (**47**). Reagents: 3-(2-(4-((4a*R*,8a*S*)-4-(3,4-difluorophenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-3-azaspiro[5.5]undecane-2,4-dione (105 mg, 0,2 mmol) and anhydrous methanol (30 mL). Reaction conditions: 3 h. Purification: no further purification was needed. Yield: 100 mg, 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (ddd, *J* = 11.7, 7.7, 2.2 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.22 (dt, *J* = 9.8, 8.5 Hz, 1H), 4.90 (t, *J* = 10.9 Hz, 1H), 4.76 - 4.55 (m, 3H), 3.92 (t, *J* = 12.1 Hz, 1H), 3.27 (t, *J* = 14.0 Hz, 1H), 3.02 (dt, *J* = 10.0, 4.9 Hz, 1H), 2.85 - 2.47 (m, 6H), 2.21 - 1.21 (m, 23H). 13C NMR (100 MHz, $CDCl_3$) δ: 172.3, 167.1 (d, *J* = 2.8 Hz), 165.2 (d, *J* = 4.8 Hz), 151.5 (dd, *J* = 252.0, 12.7 Hz), 151.4 (d, *J* = 22.4 Hz), 151.1 (dd, *J* = 179.9, 12.7 Hz), 132.1, 122.5, 117.7 (d, *J* = 17.6 Hz), 115.2 (dd, *J* = 18.6, 5.0 Hz), 52.1, 44.3, 43.9, 41.9, 40.5, 40.4, 36.8, 36.2, 35.7, 35.6, 32.1, 30.4, 29.6, 29.0, 25.9, 25.7, 24.5, 24.4, 24.0, 22.0, 21.7. UPLC: purity > 99%, m/z (ES) 569.3 [M + 1]. MP = 222 - 224 °C.

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,6,7,8,8a-hexahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**48**). Reagents: 1-(2-(4-((4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (370 mg, 0,7 mmol) and anhydrous methanol (20 mL). Reaction conditions: 16 h. Purification: IsoleraOne using water/methanol as eluents. Yield: 208.4 mg, 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (dd, *J* =

6.0, 1.7 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.89 (ddd, *J* = 15.3, 7.8, 3.9 Hz, 1H), 4.72 - 4.54 (m, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.26 (td, *J* = 13.3, 5.1 Hz, 1H), 3.06 (dt, *J* = 10.0, 5.1 Hz, 1H), 2.74 (ddd, *J* = 10.5, 9.6, 4.9 Hz, 1H), 2.67 (s, 1H), 2.55 (s, 4H), 2.19 - 1.58 (m, 9H), 1.49 - 1.27 (m, 4H), 1.18 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 167.1, 164.8, 164.5, 153.2, 150.7, 149.3, 127.6, 119.2, 110.5, 108.3, 56.2, 56.0, 51.9, 46.1, 44.2, 41.9, 40.4, 36.8, 35.6, 30.4, 29.5, 29.4, 28.9, 27.8, 25.7, 24.7, 24.5, 24.0, 22.0. UPLC: purity > 99%, m/z (ES) 553.3 [M + 1]. HRMS: Calc: 552.29 Found: 553.3031 [M + 1].

1-(2-(4-((4a*S*,8a*R*)-4-(3,4-Bis(difluoromethoxy)phenyl)-1-oxo-4a,5,6,7,8,8a-hexahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (**49**). Reagents: 1-(2-(4-((4a*S*,8a*R*)-4-(3,4-bis(difluoromethoxy)phenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl)piperidin-1-yl)-2-oxoethyl)-4,4-dimethylpiperidine-2,6-dione (60 mg, 0,1 mmol) and anhydrous methanol (5 mL). Reaction conditions: 1 h. Purification: IsoleraOne using water/methanol as eluents. Yield: 47.7 mg, 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.65 (d, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.69 (t, *J* = 73.5 Hz, 2H), 6.77 - 6.37 (m, 2H), 4.92 (tt, *J* = 11.5, 4.1 Hz, 1H), 4.74 - 4.59 (m, 3H), 3.93 (t, *J* = 12.4 Hz, 1H), 3.27 (t, *J* = 12.0 Hz, 1H), 3.03 (dt, *J* = 10.2, 5.0 Hz, 1H), 2.83 - 2.66 (m, 2H), 2.59 (s, 4H), 2.21 - 1.55 (m, 8H), 1.55 - 1.29 (m, 4H), 1.21 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1, 167.0, 165.0, 164.9, 151.3, 143.3 (t, *J* = 2.9 Hz), 142.5 (t, *J* = 2.9 Hz), 133.7, 124.2, 122.3, 119.6, 115.9 (t, *J* = 262.5 Hz), 115.7 (t, *J* = 262.5 Hz), 77.2, 52.1, 46.2, 44.3, 42.0, 40.4, 36.8, 35.7, 30.4, 29.7, 29.7, 29.0, 27.9, 25.6, 24.5, 23.9, 22.0. UPLC: purity > 99%, m/z (ES) 625.3 [M + 1].

Synthesis of intermediates:

(4a*S*,8a*R*)-2-(1-(2-Chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**13**)[29]. (4a*S*,8a*R*)-4-(3,4-dimethoxyphenyl)-2-(piperidin-4-yl)-

1,2,4a,5,8,8a-hexahydrophthalazine hydrochloride (300 mg, 0.7 mmol) was stirred with triethylamine (0.3 mL, 2.3 mmol), potassium carbonate (105.0 mg, 0.8 mmol) and THF (3 mL) during 1 h at rt After that, chloroacetilchloride (0.7 mL, 0.9 mmol) was added, and the reaction was kept at rt during 4 days. DichlorOMethane (50 mL) was added and the reaction was washed with brine (50 mL). The crude was purified by IsoleraOne using dichlorOMethane/MeOH as eluents obtaining the final product (259.2 mg, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.39 (d, J = 2.1 Hz, 2H), 7.01 (d, J = 9.0 Hz, 1H), 5.78 – 5.60 (m, 2H), 4.84 - 4.70 (m, 1H), 4.53 - 4.27 (m, 3H), 3.99 - 3.88 (m, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.46 (dt, J = 11.6, 5.8 Hz, 1H), 3.27 - 3.14 (m, 1H), 2.89 - 2.70 (m, 3H), 2.12 (dt, J = 23.7, 17.7 Hz, 2H), 1.90 - 1.58 (m, 5H). UPLC: purity > 99%, m/z (ES) 446.2 [M + 1].

cis-6-(3-Chloro-4-methoxybenzoyl)cyclohex-3-enecarboxylic acid (**18**). Procedure as previously reported[14]: 2-Chloroanisole (1.7 ml, 13.2 mmol) was added dropwise to a suspension of aluminum trichloride (2.1 g, 15.8 mmol) in DCM (20 ml) at 0 °C. After stirring at this temperature for 30 min cis-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (2 g, 13.2 mmol) was added and the reaction mixture was heated to reflux overnight. After that, the reaction mixture was poured onto ice and extracted with DCM. The organic phase was separated, dried over anh. Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (Hept/EtOAc) obtaining the desired compound (500 mg, 13%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.19 (s, 1H), 7.94 - 7.89 (m, 2H), 7.30 - 7.22 (m, 1H), 5.74 - 5.53 (m, 2H), 4.04 - 3.97 (m, 1H), 3.96 (d, J = 5.4 Hz, 3H), 2.89 (dt, J = 7.2, 3.9 Hz, 1H), 2.49 - 2.19 (m, 4H). UPLC: 1.61 min, m/z: 295 [M + 1] purity: 66%.

cis-6-(4-Fluorobenzoyl)cyclohex-3-enecarboxylic acid (**19**). To an ice-cooled solution of *cis*-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (1 g, 6.6 mmol) in THF (15 ml), (4-

fluorophenyl)magnesium bromide (6.6 ml, 6.6 mmol) was added dropwise and the reaction mixture was stirred 30 min at this temperature. Then it was allowed to warm up and stirred 16 h at rt The mixture was poured onto ice water and extracted with DCM (50 mL). Water layer was acidified with HCl and extracted again with DCM (3 x 50 mL). The organic phase was separated, dried over anh. N_2SO_4, filtered and evaporated (3 g, 92 %). UPLC: purity = 94%, m/z (ES) 249.1 [M + 1].

cis-6-(3,4-Difluorobenzoyl)cyclohex-3-enecarboxylic acid (**20**). To an ice-cooled solution of *cis*-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (2 g, 13.2 mmol) in THF (30 ml), (3,4-difluorophenyl)magnesium bromide (13.2 ml, 6.6 mmol) was added dropwise and the reaction mixture was stirred 30 min at this temperature. Then it was allowed to warm up and stirred 16 h at rt The mixture was poured onto ice water and extracted with DCM (50 mL). Water layer was acidified with HCl and extracted again with DCM (3 x 50 mL). The organic phase was separated, dried over anh. N_2SO_4, filtered and evaporated (3.1g, 89%). UPLC: purity > 99%, m/z (ES) 267.1 [M + 1].

cis-4-(3-Chloro-4-methoxyphenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**21**). To a suspension of *cis*-6-(3-chloro-4-methoxybenzoyl)cyclohex-3-enecarboxylic acid (310 mg, 1.1 mmol) and 4-hydrazinylpiperidine (303 mg, 2.6 mmol) in EtOH (4 ml) was added triethylamine (0.7 ml, 5.3 mmol) at room temperature and the reaction mixture was stirred overnight at 80 °C. The crude was purified by IsoleraOne using ethyl acetate/methanol as eluents. The unpure compound was used in the next step without further purification (200 mg, 51%). UPLC: purity = 76%. m/z (ES) 374.2 [M + 1].

cis-4-(4-Fluorophenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**22**). To a suspension of *cis*-6-(4-fluorobenzoyl)cyclohex-3-enecarboxylic acid (2 g, 8.1 mmol) and 4-hydrazinylpiperidine (2.3 g, 20.1 mmol) in EtOH (25 ml) was added triethylamine (5.6 ml, 40.3 mmol) at room temperature and the reaction mixture was stirred overnight at 80 °C. The crude was purified by IsoleraOne using heptane/ethyl acetate as eluents. The unpure compound was used in the next step without further purification (1.5 g, 57%). UPLC: purity = 66%. m/z (ES) 328.2 [M + 1]. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 – 7.76 (m, 2H), 7.12 – 6.97 (m, 2H), 5.83 – 5.49 (m, 2H), 4.80 (tt, *J* = 11.2, 3.9 Hz, 1H), 3.61 – 3.44 (m, 2H), 3.34 – 3.23 (m, 1H), 3.03 – 2.85 (m, 4H), 2.79 – 2.53 (m, 4H), 2.37 (ddd, *J* = 17.2, 13.4, 4.3 Hz, 2H), 2.23 – 2.05 (m, 3H), 1.97 – 1.77 (m, 3H).

cis-4-(3,4-Difluorophenyl)-2-(piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**23**). To a suspension of *cis*-6-(3,4-difluorobenzoyl)cyclohex-3-enecarboxylic acid (3 g, 11.3 mmol) and 4-hydrazinylpiperidine (3.2 mg, 28.2 mmol) in EtOH (4 ml) was added triethylamine (7.8 ml, 56.3 mmol) at room temperature and the reaction mixture was stirred overnight at 80 °C. The crude was purified by IsoleraOne using heptane/ethyl acetate as eluents. The unpure compound was used in the next step without further purification (1 g, 26%). UPLC: purity = 95%. m/z (ES) 346.2 [M + 1].

(4a*S*,8a*R*)-2-(1-(2-Chloroacetyl)piperidin-4-yl)-4-(3,4-dihydroxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**33**). (4a*S*,8a*R*)-2-(1-(2-Chloroacetyl)piperidin-4-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (1,5 g, 3,4 mmol) was dissolved in DCM anh (120 mL) and under Ar atmosphere, tribromoborane (1,6 ml, 16,8 mmol) was added carefully. The reaction was kept at -40 °C during 30 min and at room temperature for 30 min extra. The reaction was poured onto cold water and extracted with DCM (50 mL). The

crude was purified by IsoleraOne using DCM/methanol as eluents (907 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.38 (d, J = 6.3 Hz, 1H), 9.19 (d, J = 4.9 Hz, 1H), 7.29 (dd, J = 9.2, 2.0 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 5.75 – 5.58 (m, 2H), 4.84 – 3.84 (m, 6H), 3.25 – 3.10 (m, 2H), 2.86 – 2.64 (m, 3H), 2.22 – 1.55 (m, 6H). UPLC: purity > 99%. m/z (ES) 346.2 [M + 1].

(4a*S*,8a*R*)-4-(3,4-Bis(difluoromethoxy)phenyl)-2-(1-(2-chloroacetyl)piperidin-4-yl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**34**). (4a*S*,8a*R*)-2-(1-(2-Chloroacetyl)piperidin-4-yl)-4-(3,4-dihydroxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (549,4 mg, 1,3 mmol) was dissolved in water/AcCN (20 mL) and potassium hydroxide (2.9 g, 52,6 mmol) was added at -40°C. Bromodifluoromethyldiethylphosphonate (2,3 ml, 13,2 mmol) was added and the temperature was kept at -40°C. After 45 min, ethyl ether (50 mL) and water (50 mL) were added. The organic phase was dried oved sulfate magnesium and dried under vacuum. The crude was purified by IsoleraOne using heptane/ethyl acetate as eluents (185 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 – 7.55 (m, 2H), 7.27 (d, J = 8.6 Hz, 1H), 6.56 (t, J = 73.1 Hz, 2H), 5.84 – 5.57 (m, 2H), 4.86 (tt, J = 11.5, 4.1 Hz, 1H), 4.78 – 4.55 (m, 1H), 4.01 – 3.79 (m, 3H), 3.34 – 3.14 (m, 2H), 2.96 (d, J = 17.7 Hz, 1H), 2.81 – 2.64 (m, 2H), 2.27 – 1.63 (m, 7H). UPLC: purity > 99%. m/z (ES) 518.1 [M + 1].

(3,4-Dimethoxyphenyl)(1*H*-imidazol-2-yl)methanone (**40**). Procedure as previously described[25]: A solution of imidazol and triethylamine in pyridine at 0 °C is treated with 3,4-dimethoxybenzoyl chloride, stirred for 5 min, allowed to warm up to rt and kept at that temperature for 16 h.After that the 7.5 N solution of NaOH was added and the mixture was heated 2 h at 100 °C. Water and DCM were added and the organic phase was purified by IsoleraOne using DCM/MeOH as eluents. 1H NMR (400 MHz, DMSO-d_6) δ: 13.33 (s, 1H),

8.43 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.08 (d, *J* = 2.0 Hz, 1H), 7.47 (d, *J* = 1.5 Hz, 1H), 7.28 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 1H). UPLC: purity > 99%. m/z (ES) 233.2 [M + 1].

6-(3,4-Dimethoxyphenyl)-2-(piperidin-4-yl)pyridazin-3(2*H*)-one (**50**). 6-(3,4-Dimethoxyphenyl)-2,3-dihydropyridazin-3-one (1 g, 4,3 mmol) was solved in DMF anhydrous (5 mL) and sodiumhydride (0,1 g, 4,3 mmol) was added, the mixture was refluxed during 1 h. After that, *N*-boc-4-bromo-piperidine (2,3 g, 8,6 mmol) was added and the reaction was kept 48 h. Ethyl acetate (50 mL) and a solution of HCl 0.1M (50 mL) were added, the organic phase was washed with a satured solution of $NaHCO_3$ (3 x 50 mL) and a satured solution of NaCl (3 x 50 mL). The obtained compound was used in the next step without further purification or isolation (400 mg, 30%). UPLC: purity = 60%. m/z (ES) 316.3 [M + 1].

Methyl 3-(3,4-dimethoxyphenyl)-2,2-dimethyl-3-oxopropanoate (**52**).[30] Procedure as previously described.[26] Lithium diisopropylamide (1.2 g, 1.8 M in THF, 10.9 mmol) was added to a dry three-neck flask with 12.5 mL of anhydrous THF and cooled to −45 °C, and methyl isobutyrate (1.7 mL, 14.9 mmol) was added dropwise. The mixture was stirred for 30 min at −40 °C. 3,4-Dimethoxybenzoyl chloride (2 g, 9.9 mmol) was dissolved in dry THF (12.5 mL) and added dropwise during 30 min at −50 to – 40 °C. The reaction mixture was stirred for another 1 h before the cooling source was removed, and the stirring continued at room temperature overnight. The reaction mixture was acidified with 3 M HCl (aq) and diluted with EtOAc (10 mL), and the aqueous layer was extracted with EtOAc (2×10 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and reduced in vacuo. The crude product was purified with flash chromatography using hexane and ethyl

acetate as eluents togive the title compound (950 mg, 36%). UPLC: purity > 99%. m/z (ES) 267.2 [M + 1].

3-(3,4-Dimethoxyphenyl)-4,4-dimethyl-1*H*-pyrazol-5(4H)-one (**53**).[30] Procedure as previously described.[26] Methyl 3-(3,4-dimethoxyphenyl)-2,2-dimethyl-3-oxopropanoate (950 mg, 3.06 mmol) dissolved in ethanol (10 mL) was added hydrazine hydrate (357 mg, 7.1 mmol). The reaction mixture was stirred at 50 °C overnight. The reaction mixture was cooled on ice and the precipitate was filtered off over a glass filter, washed with cold ethanol, and dried at 40 °C under vacuum to give the title compound as a solid foam (460 mg, 52%). UPLC: purity > 99%. m/z (ES) 249.2 [M + 1].

Tert-butyl 4-(3-(3,4-dimethoxyphenyl)-4,4-dimethyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (**54**).[30] 3-(3,4-Dimethoxyphenyl)-4,4-dimethyl-1*H*-pyrazol-5(4*H*)-one (460 mg, 1.8 mmol) was dissolved in DMF anhydrous (3.5 mL) and sodium hydride (89 mg, 3.7 mmol) was added. The mixture was at 153 °C for 30 min and after that, *tert*-butyl 4-bromopiperidine-1-carboxylate (734 mg, 1.5 mmol) was added. The reaction was kept at 153 °C during 2 days. Ethyl acetate (50 mL) and a satured solution of NaCl (50 mL) were added and the crude was purified by IsoleraOne using hexane and ethyl acetate as eluents. The final compound was obtain with a purity of 60% and it was used in the next step without further purification (143.9 mg, 18%). UPLC: purity > 60%. m/z (ES) 376.2 [M + 1].

3-(3,4-Dimethoxyphenyl)-4,4-dimethyl-1-(piperidin-4-yl)-1*H*-pyrazol-5(4*H*)-one (**55**).[30] *Tert*-butyl 4-(3-(3,4-dimethoxyphenyl)-4,4-dimethyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (218.5 mg, 0.5 mmol) was dissolved in DCM at 0 °C. After that, 2,2,2-trifluoroacetic acid (577 mg, 5.0 mmol) was added and the mixture was kept at rt for 3 h.

The organic solvent was evaporated and the compound was used in the next step without further purification (109.4 mg, 99%). UPLC: purity > 55%. m/z (ES) 332.2 [M + 1].

(4a*S*,8a*R*)-2-(Azetidin-3-yl)-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (**57**). *Tert*-butyl-4-((3,4-dimethoxyphenyl)-2-oxoimidazo[1,2-*d*][1,2,4]triazin-1-yl)piperidine-1-carboxylate (733 mg, 1.7 mmol) was dissolved in DCM (3 mL) at 0 °C, after that 2,2,2-trifluoroacetic acid (1.3 mL, 17.6 mmol) was added and stirred at 0 °C for 30 min. The reaction was kept at room temperature overnight. The solvent was evaporated and the crude was used in the next step without further purification (243.7 mg, 43%). UPLC: purity = 70%, m/z (ES) 342.2 [M + 1].

ANCILLARY INFORMATION

Full panel of parasitology screens for the final compounds, antiprotozoal *in vitro* and *in vivo* assays such as cytotoxicity, *T. cruzi*, *L. infantum*, *P. falciparum*, microsomal stability assays and results. Protocols of X-ray experiments and activity assays are included.

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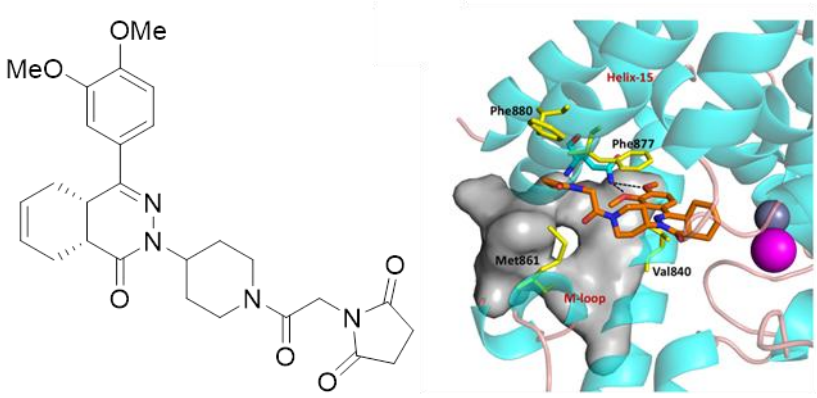
TOC Graphic



Compound 14

pIC_{50} (Tbruc.) = 5.73

pKi (TbrPDEB1-CD) = 7.68

%parent compound after 30 min in mouse = 43%

%parent compound after 30 min in human = 89%