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Rational Drug Design of Topically Administered Caspase 1 Inhibitors for the Treatment of Inflammatory Acne

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Dedicated to Professor E. J. Corey on the occasion of his 90th birthday

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ABSTRACT: The use of an interleukin beta antibody is currently being investigated in the clinic for the treatment of acne, a dermatological disorder affecting 650M persons globally. Inhibiting the protease responsible for the cleavage of inactive pro-IL1β into active IL-1β, caspase-1, could be an alternative small molecule approach. This report describes the discovery of uracil **20**, a potent (38 nM in THP1 cells assay) caspase-1 inhibitor for the topical treatment of inflammatory acne. The uracil series was designed according to a published caspase-1 pharmacophore model involving a reactive warhead in P1 for covalent reversible inhibition and an aryl moiety in P4 for selectivity against the apoptotic caspases. Reversibility was assessed in an enzymatic dilution assay or by using different substrate concentrations. In addition to classical structure-activity-relationship exploration, topical administration challenges such as phototoxicity, organic and aqueous solubility, chemical stability in solution and skin metabolic stability are discussed and successfully resolved.

INTRODUCTION

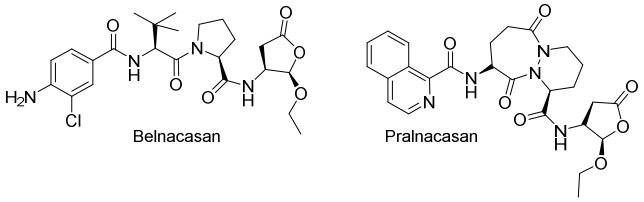
Acne is a dermatological disorder affecting 40-50 million Americans and 650 million globally.[1] Lesions typically appear in the face, upper back and chest area and can lead to permanent scarring as well as low self-esteem. Current treatment consists of the use teratogenic retinoids such as Roaccutane™. Therefore, drugs with novel mechanisms of action and improved safety profiles would benefit patients.[2]

The pathogenesis of acne can be divided in different steps: 1) (over)production of sebum by the sebaceous gland; 2) colonization of the hair follicle by P. Acnes; 3) hyperkeratinisation of the follicle; and 4) the release of inflammatory mediators into the skin.[3] Pro-inflammatory cytokines, including TNFα, IL-1α and IL-1β are considered to be involved for follicular hyperkeratinisation and the inflammatory lesions characteristic of acne. This cytokine usually exists in its pro-form

which is cleaved to its active form by the aspartic cysteine protease caspase-1 upon inflammatory stimulus.[4] Recently, French and co-workers have demonstrated that the active form of IL-1β is abundant in human acne lesions.[5] Moreover, they demonstrated that the production induced by P. Acnes of active IL-1β in monocytic lineage and in mice is dependant of the NLRP3/Caspase-1 inflammasome by P. Acnes exposed cells. This data indicates that IL-1β as a novel potential therapeutic target in acne, and therefore, we postulate that inhibiting caspase-1, could have a beneficial effect in inflammatory acne.

Most dermatological diseases such as acne are non-life threatening, and as such, treatments are subject to a very low tolerance to risk. Thus, a common strategy employed is ensuring the concentration of drug in the target organ, skin, is significantly higher than that in blood. Because the free drug hypothesis precludes accomplishing this via the systemic route,[6] a local topical administration of drugs showing high systemic clearance is often favored (soft drug[7]). Furthermore, with dermatological diseases which affect only a small portion of the body surface area (BSA), this can also lead to dose minimization and therefore to a reduced risk. This could be of particular interest in the case of targets which are typically treated with covalent drugs.[8]

Caspase-1 is a well-documented target for which a few clinical candidates have already been evaluated, including Vertex' belnacasan and pralnacasan.[9] However, previously disclosed inhibitors have been optimized for the systemic rather than the topical route and are not necessarily appropriate for use in dermatology.[10] We have therefore initiated a chemistry program to identify a caspase-1 inhibitor better suited to the topical treatment of inflammatory acne.

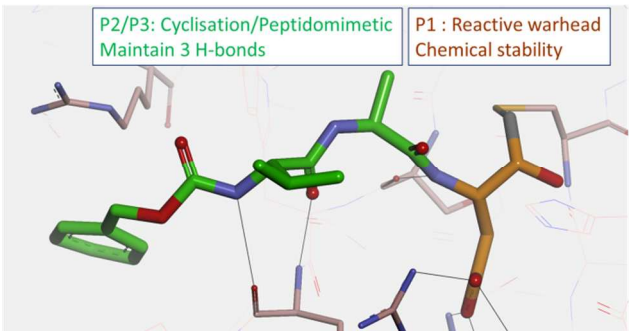


RESULTS AND DISCUSSION

Hit Generation and Reversibility Issues

Most published structures of caspase-1 inhibitors follow a few general rules derived from the elucidation of the protein's tertiary structure by crystallography, and from the key binding elements of the tetrapeptide Ac-YVAD-CHO, an early tool compound.[11] As shown in Figure 1a, a reactive warhead and (L)-aspartic acid mimic in P1 is key for potency and selectivity vs other cysteine proteases, key H-bond donors and acceptors in P2 and P3 and an aromatic residue in P4 for selectivity vs the apoptotic caspases.[11] Consequently, about a dozen different new scaffolds were designed in accordance to these guidelines and were evaluated in an enzymatic assay using two different concentrations (K_M and $20K_M$) of substrate, a fluorescent pro-IL-1β analog (Figure 1b). The aim was for these peptidomimetic scaffolds to present a chemical topology that would allow the formation of the same H-bonds with caspase-1 in P2 and P3 as depicted in Figure 1a. Namely, the two NHs flanking R_1 and R_4 and, between these, one acceptor (carbonyl or pyridine). In addition, the outgoing vectors for R_1 and R_4 should be consistent with those from ZVAD-CHO to provide the other binding elements: the covalent bond and salt bridge in P1 and the π-stacking in P4. To standardize this phase, each synthesized scaffold was combined at the very least with the ubiquitous aspartyl aldehyde warhead in P1 and a 2-naphthyl (similar to pralnacasan's 1-isoquinolyl) group in P4.

a)



b)

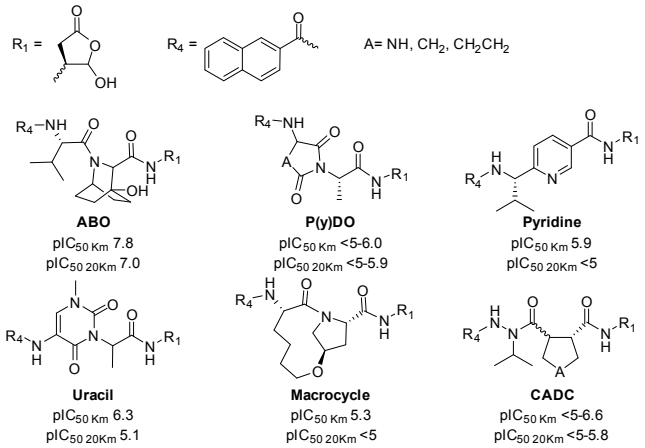
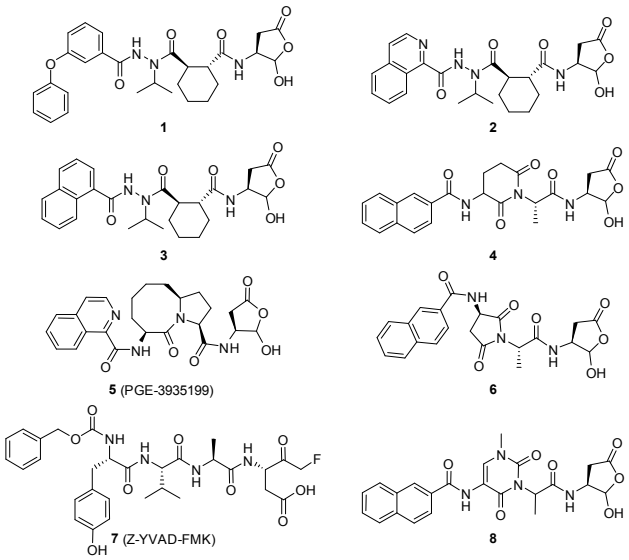


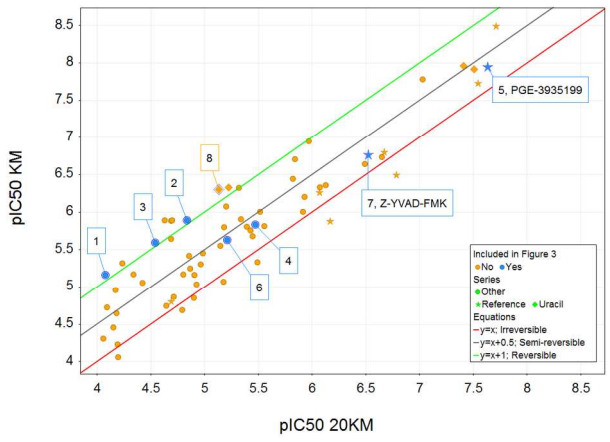
Figure 1. (a) ZVAD-CHO bound to caspase-1 shows the key binding elements that need to be reproduced in novel scaffolds: covalent bond with CYS285, ionic interactions with ARG179, GLN283 and ARG341, H-bonds with SER339's carbonyl and ARG341's carbonyl and NH ; (b) Some examples of novel scaffolds designed to reproduce key binding elements with caspase-1 and tested for inhibitory activity

In principle, the IC_{50}s of competitive inhibitors following Michaelis-Menten kinetics should be right shifted about 10-fold at the higher substrate concentration ($K_i = IC_{50}/(S/K_M + 1)$).[12] By testing at two different substrate concentrations, we were able to get a first crude idea of the binding kinetics of our inhibitors. Indeed, since our inhibitors contained a reactive warhead which after an initial pre-complexation step makes a covalent-reversible bond with the protein's

catalytic cysteine,[13] we anticipated that some of our inhibitors would not reach equilibrium under our assay conditions (Figure 2).



(a)



(b)

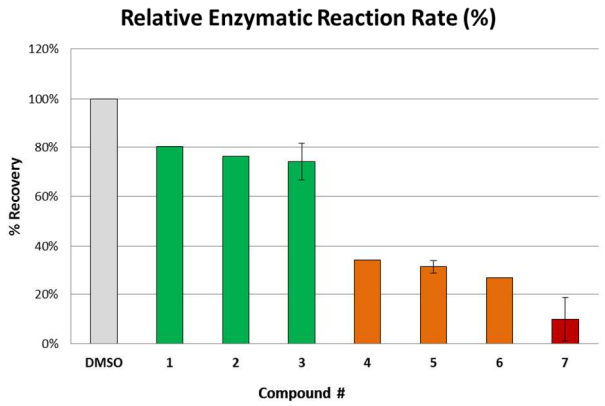


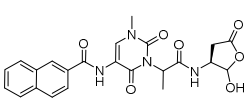
Figure 2. (a) Correlation between the pIC_{50} measured at K_M vs $20K_M$ substrate concentration for early compounds. For competitive inhibition following Michaelis-Menten kinetics, a one log unit difference in pIC_{50} values would be expected (green line). (b) Reversibility profiles as evaluated by the recovery of enzymatic reaction rate in a kinetic dilution experiment. Comparing results from both graphs suggest that reversibility of the inhibition – under the condition of the assay – can be quickly evaluated using the ratio of IC_{50} values: fully reversible inhibitors (green line/bars) have a 10-fold difference whereas pseudo-irreversible inhibitors (red line/bars) have equal IC_{50}s irrespective of the substrate concentration. Under this assumption, uracil compound **8** is fully reversible.

For a few selected compounds displaying various profiles, a kinetic experiment was done to assess the reversibility of the inhibitors (Figure 2b).[14] In this assay, the enzyme is pre-incubated with inhibitors at 10x their IC_{50} values before being diluted a 100 fold with the substrate. Recovery of the enzymatic activity is then monitored and the extent of reaction speed recovery (from the slope of the curve) indicates whether the inhibitor is displaying a fully reversible profile, a pseudo-irreversible (irreversible in the timescale of the assay) or partially-reversible profile.[12] Since the results fully corroborated the ratio of the IC_{50}s with substrate concentrations

at $20K_M$ vs K_M, this higher throughput method was thereafter used to monitor the reversibility of the covalent bond formation with the catalytic cysteine.[15] Consequently, this methodology gave us confidence that we could properly rank our compounds and conduct a normal SAR.[16]

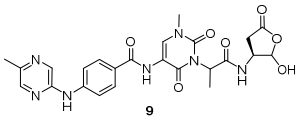
From Hit to Lead

This article focuses on the work around the uracil hit **8**, but other scaffolds were also investigated in parallel.[17] Analogs with varying substituents in the P2 to P4 subpockets were prepared in a few SAR iteration cycles with most of the focus on R4, identifying lead compound **10** ($pIC_{50\ Km}$ 8.3; $pIC_{50\ 20Km}$ 7.5) bearing Sunesis' quinoxaline in P4 and which was isolated as a yellow solid.[18] Conducting a retrospective binding efficiency index (BEI)[19] analysis as proposed by Hajduck showed that although the BEI slowly crept up during the hit to lead (H2L) phase for the new "concept compounds", a linear relationship between Mw and pIC_{50} could be found with a slope of 48 amu/pIC_{50} unit (Figure 3).[20] These findings are in line with Hajduk's retroanalysis across 15 Abbott projects, and points towards the same conclusion: when adding new atoms on a hit or lead, if only the optimal ones are retained, then the molecular weight and potency of the starting point (hit) should be projectable to those of the end point (candidate). This analysis thus strengthened our belief the uracil series could be optimized to deliver a candidate.



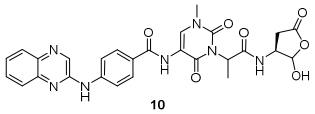
8

$pIC50_{Km}$ 6.3 ; Mw 466 ; BEI 13.5



9

$pIC50_{Km}$ 7.4 ; Mw 524 ; BEI 14.2



10

$pIC50_{Km}$ 8.3 ; Mw 560 ; BEI 14.8

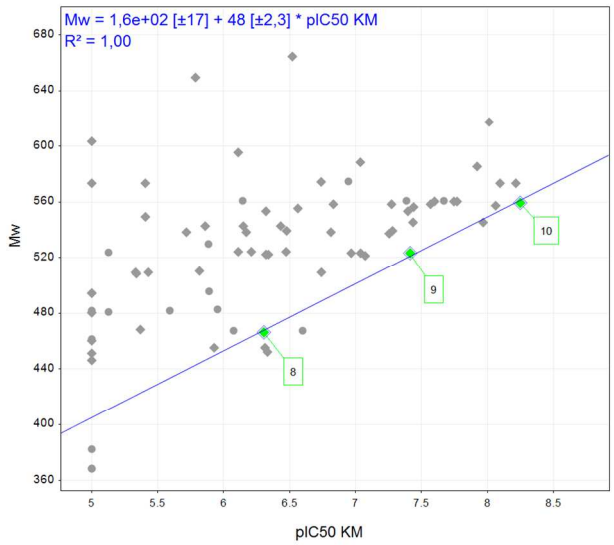


Figure 3. Binding efficiency index plot of the H2L phase of the uracil series. Starting from hit **8**, a 10-fold improvement in potency can be achieved by the addition of 48 amu. Potency gains that come at the cost of larger molecular weight contributions should be rejected.

However, the lead uracil compounds were subject to a number of liabilities specific to topical drug development, namely color of the drug substance, solvent solubility, phototoxicity and chemical stability in solution. These were, therefore, the parameters chosen as the main focus of the lead optimization phase.

Lead Optimisation

Solvent Solubility. In the case of orally administered drugs, good aqueous solubility (>20-400 μM for compounds with medium permeabilities) is necessary to achieve good absorption in the gut.[21] As per Yalkowsky's General Solubility Equation (GSE), this can be achieved either by decreasing the melting point (approximation of the crystalline lattice energy) or the LogD of compounds.[22] However, in the case of topically administered drugs, it is often necessary to achieve orders of higher magnitude solubilities (>20 mM) for formulation purposes.[23] This is

usually achieved by the use of non-aqueous solvents such as ethanol, which means that the main driver for solubility will be its crystallinity/Mp (the LogD component in the GSE serves to "account for the difference between an ideal solution and an aqueous solution"; it is, therefore, specific to aqueous solubility).[22,24] Considerable efforts were therefore invested in getting meaningful solubility measurements: semi-standardized crystallization processes were elaborated, crystallinity and melting points was assessed by DSCs with large enthalpies (>50 J/g) and thermodynamic solubilities were measured in a routine assay directly from crystalline material after 24 h in a 30/70 mixture of PG/EtOH. In the case of the uracil series, compounds with high solubility had lower melting points and higher (not lower!) ChromLogD (Figure 4).[25]

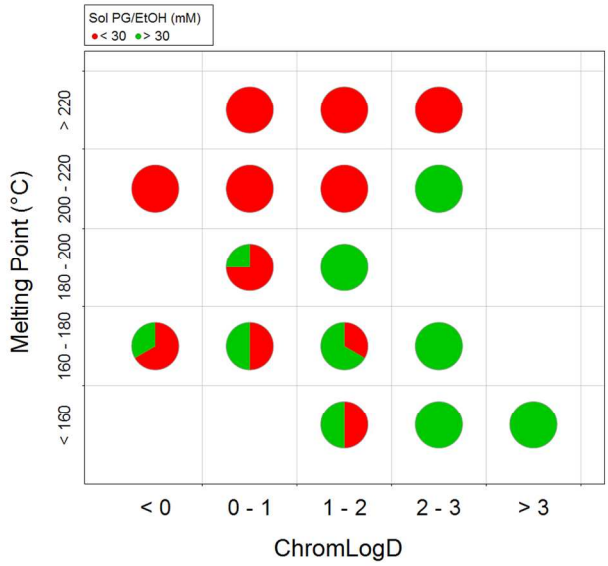
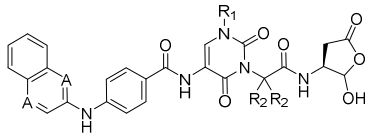


Figure 4. Solubility in PG/EtOH is a function of the melting point in the uracil series with no positive impact from lowering ChromLogD.

We surmised we might disrupt the crystalline lattice of our uracils by increasing their sp^3 fraction,[26] and more specifically, by increasing the substitution pattern of the carbon atoms

directly connected to the uracil core. This strategy proved fruitful as the isopropyl substituted uracil **11** showed a 10-fold improvement on PG/EtOH solubility while maintaining aqueous solubility vs **10** (Table 1).[27] Inversely, removing the methyl substituent at R_2 resulted in a compound with decreased solubilities (**12** vs **11**) despite the small drop in lipophilicity. Finally, switching the quinoxaline ring for a naphthyl ring also led to significant improvements in both types of solubility (**12** vs **13**). The fact that the aqueous solubility increased 6-fold, in spite of the ΔChromLogD, confirms the importance of this structural elements on crystallinity and solubility.[28] Moreover, this switch also resulted in colorless compounds as opposed to the yellow quinoxaline analogs.[29] Unfortunately, the LipE took a double hit as the potency decreased at the same time the ChromLogD increased. This behavior was generally observed across many matched pairs.

Table 1. Optimisation of PG/EtOH 30/70 solubility. Replacing the quinoxaline for a naphthalene group (**13**) and steric perturbation of the H-bond donors/acceptors (**14**) give rise to solubility improvements.



Cpd	A	R1	R2	Chrom LogD	$Sol_{PG/-EtOH}$ (mM)	Sol_{Aq} (μM)	IC_{50} K_M (nM)	IC_{50} THP1 (nM)	LipE K_M	LipE THP1
10	N,N	Me	H,Me	1.9	0.89	96	5.6	48	6.4	5.5
11	N,N	*i*Pr	H,Me	2.4	11	86	5.6	50	5.9	4.9
12	N,N	*i*Pr	H,H	2.3	5.2	8.3	3	53	6.2	5
13	CH,CH	*i*Pr	H,H	3.2	36	52	21	140	4.5	3.7
14	N,N	*i*Pr	-CH_2CH_2-	2.4	25	87	4.7	27	6.0	5.2

Next we thought the addition of a second substituent alpha to the aliphatic amide might further improve solubility as well as stereochemical stability. Furthermore, inspection of the X-Ray structure of **10** in Caspase-1 revealed that the pocket formed by Val338 and Trp340 might accommodate a small substituent and we hoped we could improve potency at the same time Indeed, the overlay of the docking of cyclopropyl **14** over the X-ray structure of **10** shows that the cyclopropyl group fits nicely in the shallow S2 lipophilic pocket with no perturbation of the surrounding side chain, while the 3 essential backbone H-bonds of the ligand are also maintained (Figure 5). Cyclopropane **14** was therefore synthesized and we were rewarded with small but reproducible improvements in solubility, cellular potency and LipE vs **11**. Figure 6 shows the potency boost was specific to the cyclopropyl moiety and reproducible across many matched pairs.

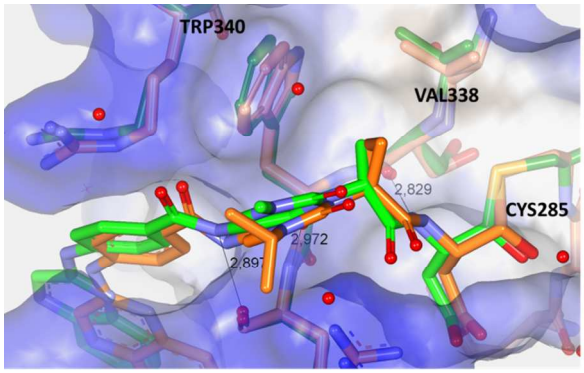
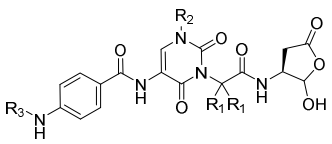


Figure 5. Docking studies of **14** (brown) vs X-Ray structure of **10** (green; PDB ID 6F6R) suggest the cyclopropyl moiety in S2 is optimally fits a small lipophilic pocket.



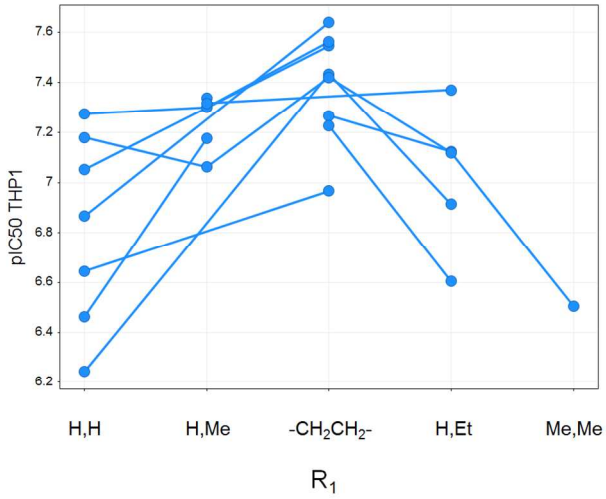


Figure 6. Molecular matched pairs on the R1 substituents (connected dots) vs celluar potency: cyclopropyl unit gives a consistent increase in potency vs non-substituted, mono-methyl, mono-ethyl and gem-dimethyl substituents.

Phototoxicity. Following its topical application, a drug substance is typically found in much higher concentration in skin than what occurs after oral administration. Furthermore, following UV irradiation by the sun, some benign compounds may become irritant or even toxic. This phenomenon is called phototoxicity. Because skin irritation is often times incompatible with the desired therapeutic effect, phototoxicity can be a liability and needs to be screened early on in the development. Photocytotoxicity potential is assessed by evaluating cell viability after 24h exposure with caspase-1 inhibitors in the presence and absence of UV light. A phototoxicity irritation factor (PIF) value is determined by the ratio of LC_{50} values and compounds are classified as follows: PIF<2 is non phototoxic, 2<PIF<5 has low phototoxic potential and PIF>5 is phototoxic.[30] A good practice when evaluating phototoxicity SPR is to keep an eye on the specific LC_{50} values for more subtle effects (provided in supplementary material, Table 1S). In

the case of the present discussion, we find that simply taking into account the overall conclusion suffices.

When we initially switched our focus from the quinoxaline to naphthalene substituents in P4 pocket, we observed a higher proportion of phototoxic products. For example, **13** was phototoxic whereas **12** was not. A more detailed analysis revealed a clear dependency on lipophilicity (Figure 7) which gave an upper limit of ChromLogD of about 3.5 to 4 for the design of new inhibitors.[31] Because of their better suited physical properties and despite their inherent higher lipophilicity, the naphthalene subseries was pursued since a design window remained and other strategies to mitigate the phototoxicity risk, for instance breaking conjugation between π-systems, remained (see below).

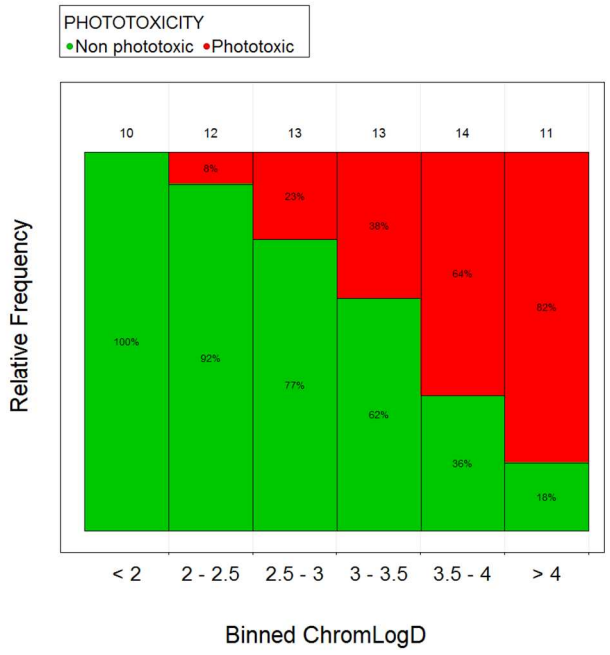
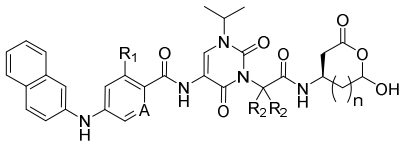


Figure 7. Phototoxicity in the uracil series is highly dependent on ChromLogD: 100% of the compounds with ChromLogD lower than 2 were photosafe vs only 18% when it was higher than 4.

For example, structural elements such as the addition of an ortho-methyl on the phenyl ring of phototoxic **13** resulted in an inhibitor with only a low risk of phototoxicity (**15**) along with an improvement in solubility and potency (Table 2). The likely explanation is that the ortho-substituent twists the acyl moiety out of plane and breaks the conjugation with the phenyl ring, an important factor in light absorption.[32]

Table 2. Final Optimisation: cyclopropane **20** combines all the beneficial chemical features which translate into a compound with the required profile in terms of solubility, potency, safety and stability.



Cpd	A	R1	R2	n	Chrom LogD	$Sol_{PG/-EtOH}$[a]	Sol_{Aq}[b]	IC_{50} THP1[c]	Photo-toxicity	3-Mo Stab[d]	Skin S9 CL_{int}[e]
13	CH	H	H,H	0	3.2	36	52	140	Phototoxic	82	<0.82
15	CH	Me	H,H	0	3.5	51	72	66	Low phototox potential	85	(3.9)
16	CH	Me	H,Et	0	4	n/a	40	76	n/a	72	(4.9)
17	N	Me	H,Et	0	4.5	49	2	200	Phototoxic	100	2 (7.7)
18	CH	Me	H,Et	1	3.8	n/a	42	210	n/a	84	2.9 (13)
19	CH	H	$-CH_2CH_2-$	0	3.5	31	66	23	Low phototox potential	99	<0.82 (2.1)
20	CH	Me	$-CH_2CH_2-$	0	3.7	43	70	38	Non phototox	100	0.92 (4.6)
21	CH	Me	$-CH_2CH_2-$	1	3.4	n/a	63	360	Non	88	3.5

phototox	(84d)/

[a] Solubility in mM [b] Solubility in µM [c] Potency in nM [d] In percent of recovered parent compound. [e] S9 CL_{int} in µL/min/mg of protein. For numbers in parentheses, CL_{int} values measured in NHEK cells in µL/min/10^6 cells.

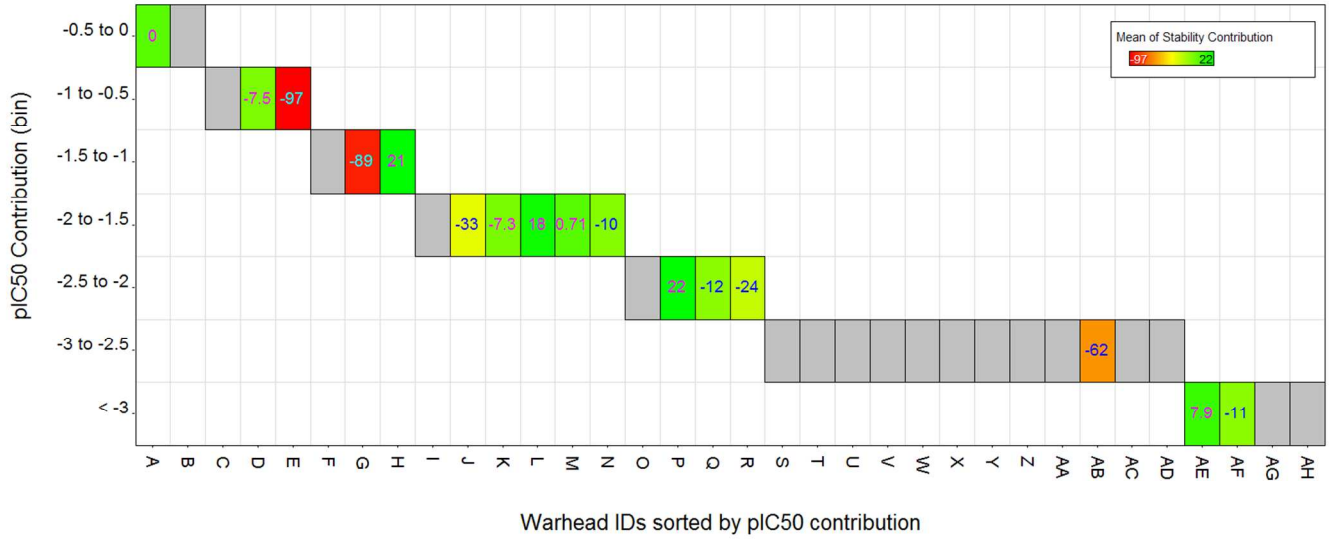
Chemical and Skin Metabolic[33] Stability. The chemical stability of drug substances is paramount in topical delivery medicinal chemistry projects as APIs are not formulated in solid forms, but rather dissolved in various excipients and need to have a shelf life stability of at least a year. From the start of the project, this parameter was judged to be at high risk due to the electrophilic nature of the aspartyl aldehyde warhead and was, therefore, constantly monitored.

To this effect, stability of compounds with the aspartyl aldehyde warhead was evaluated in 96% ethanol at 23 °C for 12 weeks,[34] but SPR information could be used after 4 weeks by projecting stability results to 12 weeks using the appropriate (best fit) kinetic order equations.[35,36] It was found that a significant proportion of our inhibitors were not stable at 23 °C for more than 3 months. Consequently, significant efforts went into the synthesis of novel and hopefully more stable electrophilic warheads.[37]

These novel warheads should feature an electrophilic moiety susceptible to thiolate attack by caspase-1's CYS285 as well as an acidic residue for the ionic interactions with caspase-1's ARG179, GLN283 and ARG341. Herein lays the challenge: to be reactive, yet stable in solution despite the combination of an electrophile with an acid. Different acids, electrophiles, linker length and masking groups were evaluated. The results, however, were quite disappointing as Free-Wilson analysis of the contributions of many warheads on chemical stability and potency showed that most were much weaker binders in the enzymatic assay (2 log units loss) and/or did not offer improvements in stability (Figure 8).[38] Most of the remaining warheads were shifted in

the cellular assay to even lower potencies,[39] leaving the homo-aspartyl aldehyde warhead as the sole candidate for further exploration.[40]

a)



b)

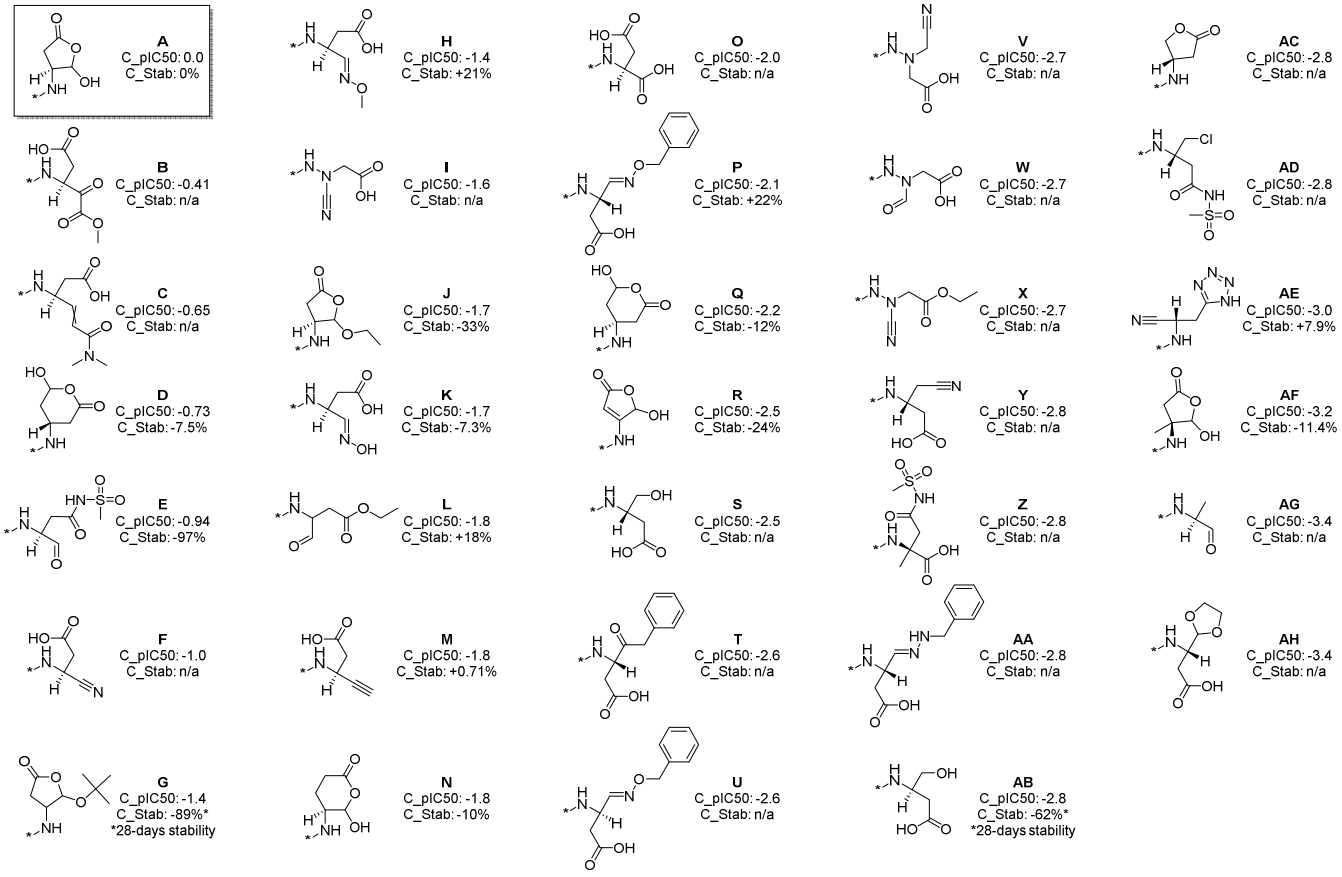


Figure 8. (a) Stability[a] vs potency[b] of various electrophilic warheads. [a] Numbers in boxes represent the Free-Wilson contribution on chemical stability in ethanol at 23°C after three months as compared to the standard aspartyl aldehyde warhead **A**. For example, molecular matched pairs with warhead **J** give, on average, analogs with 33% less parent product after three months. Grey boxes: no stability measurements were made. [b] The y-coordinate of boxes represent the binned Free-Wilson contribution on enzymatic pIC_{50} (at K_M concentration of substrate) as compared to the standard aspartyl aldehyde warhead **A**. For example, molecular matched pairs with warheads **F** through **H** give, on average, analogs that are 10 to 30 fold less potent compounds (-1 to -1.5 log unit). (b) Structure of warheads used in Free-Wilson plot. Aspartylaldehyde warhead **A** is the optimal warhead when taking into account both potency and chemical stability.

Indeed, homo-aspartyl aldehyde **18** showed a modest increase in 3-month stability vs the related aspartyl aldehyde **16** (84% vs 72%) with minimal drop in potency (IC_{50} THP1 210 nM vs 76 nM), however skin metabolism was also almost three-fold higher (13 vs 4.9 μL/min/mg protein in normal human epidermal keratinocytes). Furthermore, whereas the increase in the skin S9 fraction or NHEK clearance was observed across many molecular match pairs, the increase in chemical stability was not (Table 2).

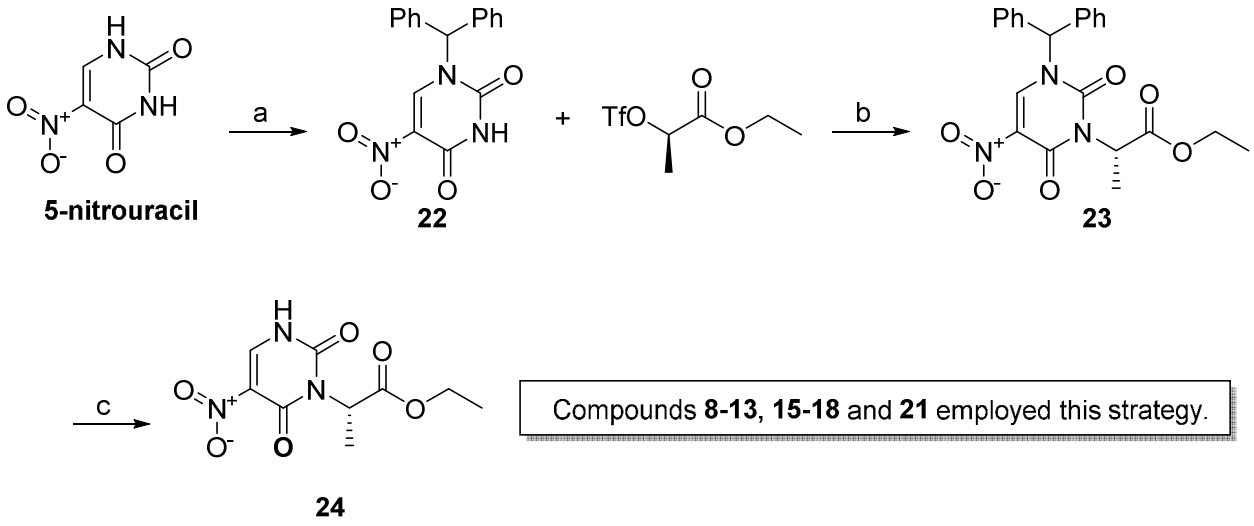
Surprisingly, we observed that pyridine **17** was much more chemically stable than the analogous phenyl **16** (100% vs 72%). The role of this pyridine in the compound's stability was unclear as the only degradation products observed for this chemical series involved the warhead. However, the pyridine analogs also displayed increased skin clearance and lipophilicity[41] along with lower potency. This approach was therefore not pursued.

Fortunately, cyclopropane **19** displayed a good mix of solubility, potency, chemical and skin metabolic stability,[42] albeit with a low phototoxicity potential. As described previously, addition of the ortho-methyl to break π-conjugation gave **20**, a non-phototoxic compound that retained all the qualities of **19**. This compound was therefore selected for further evaluation.

Synthesis

The general strategy towards our novel P2/P3 peptidomimetic uracil fragment relied upon selective *N*-3-protection of 5-nitrouracil with the benzhydryl group followed by *N*-1 alkylation to prepare **23** in acceptable yield.[43] Subsequent deprotection of the benzhydryl group under hydrolytic conditions[43b] gave access to **24** for exploration from the *N*-3 vector (Scheme 1).

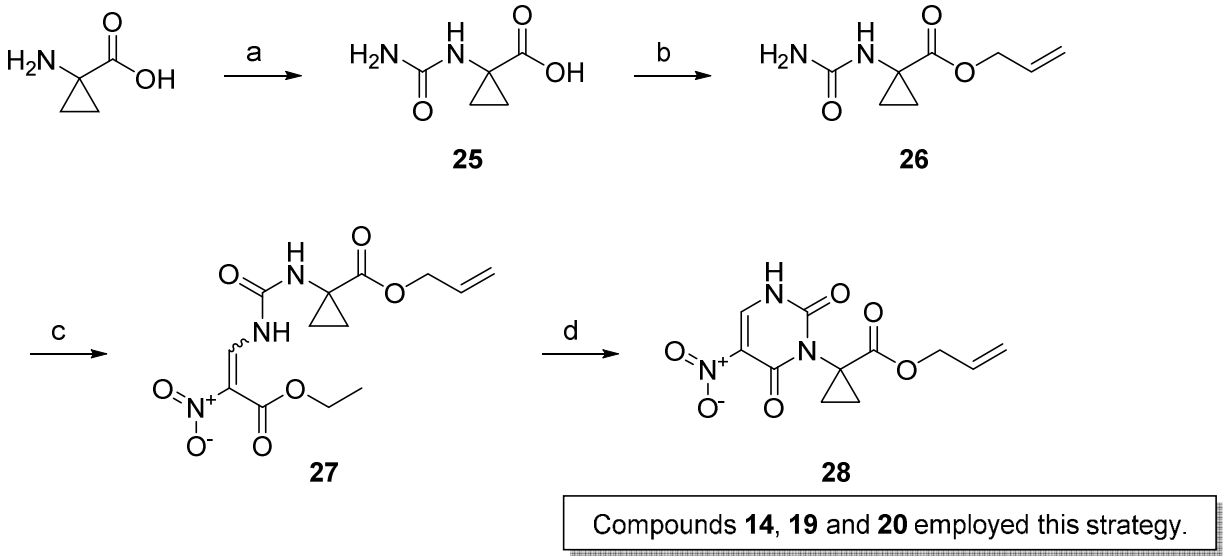
Scheme 1. General synthesis strategy employed for our uracil-based P2/P3 peptidomimetic fragments building blocks exemplified by the synthesis of **24**.



Reagents and conditions: (a) Ph_2CHBr, *N,O*-Bis(trimethylsilyl)acetamide, cat. I_2, acetonitrile, reflux, yield 85%; (b) K_2CO_3, acetonitrile, 23 °C, yield 64%; (c) TFA-TfOH (1:1), 23 °C, yield 98%.

For analogs bearing a quaternary C centres at N-1, we were obliged to develop a novel 3MCR (multi-component reaction) to prepare the *gem* dimethyl and cyclopropyl analogues (Scheme 2).[44] Briefly, aminocarbonylation of the amino acid residues followed by protection of the carboxylic acid residue afforded primary urea precursors **26**. Condensation of **26** with ethyl nitroacetate in the presence of triethylorthoformate afforded a 6:4 *E/Z* ratio of ene-urea intermediates **27**. Finally cyclisation to the urea scaffolds was carried out in the presence of a two-fold excess of cesium carbonate to afford **28** (Scheme 2).

Scheme 2. Preparation of P2/P3 uracil peptidomimetic fragments building blocks with quaternary C centres at N-1.

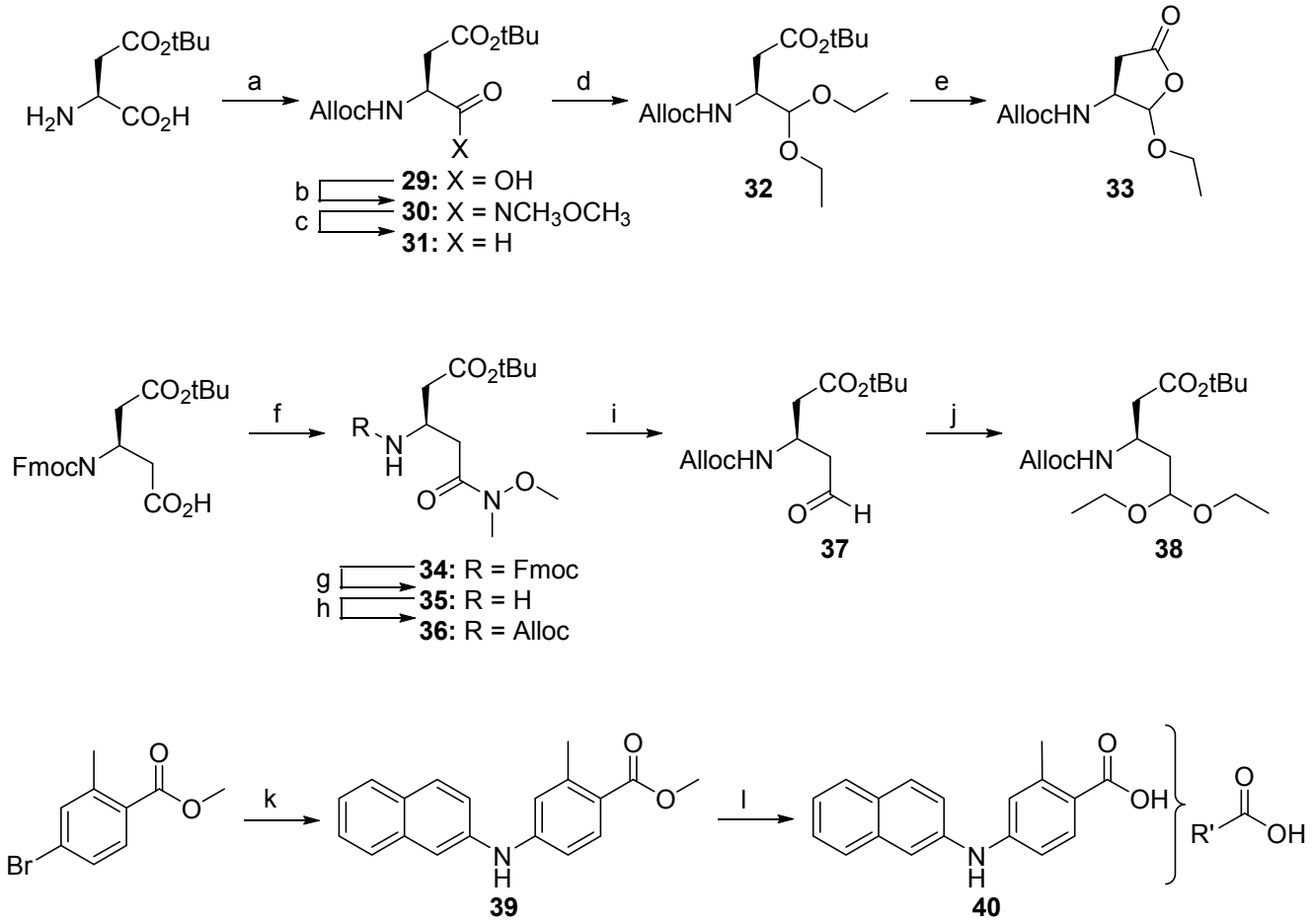


Reagents and conditions: (a) KOCN, water, reflux, yield 78%; (b) Allyl bromide, DIPEA, THF/H_2O (10:1), 60 °C, 18 h, yield 80%; (c) ethyl nitroacetate, triethyl orthoformate, toluene, reflux, yield 92%; (d) Cs_2CO_3, MeCN, reflux, yield 81%.

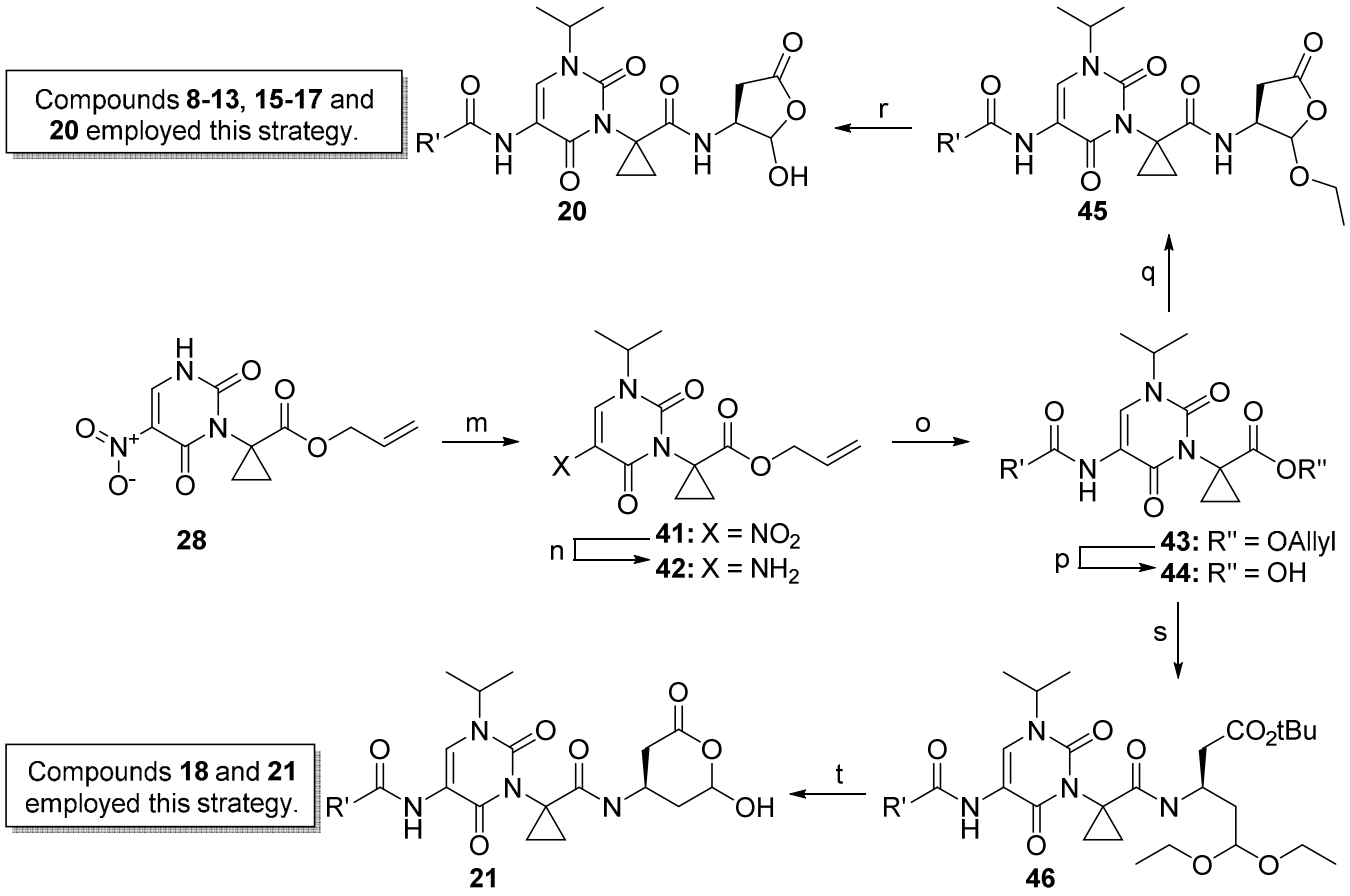
Intermediates **24** and **28** were then converted to caspase-1 inhibitors following one of the three strategies enumerated below. The most common approach was to begin by alkylation at *N*-3 to afford intermediate **41** (Scheme 3b). Then, installation of the P4 fragment was carried out by reduction of the nitro group under dissolving metal conditions[45] and subsequent amide bond formation using COMU (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate)[46] as the coupling agent and acid **40** (Scheme 3a) as the coupling partner to give **43**. After saponification of ester **43** to acid **44**,[47] final compound assembly was terminated by coupling the warheads **33** or **38** (Scheme 3a)[48] after *in situ* deprotection of the Alloc group with the pre-activated Pfp (pentafluorophenol) ester of **44** followed by hydrolysis of the acetals **45** or **46** to the hemi-acetals **20** or **21**, respectively.

Scheme 3. General synthesis strategy employed to our uracil-based caspase-1 inhibitors (a) P1 and P4 building blocks (b) and final assembly

a)



b)

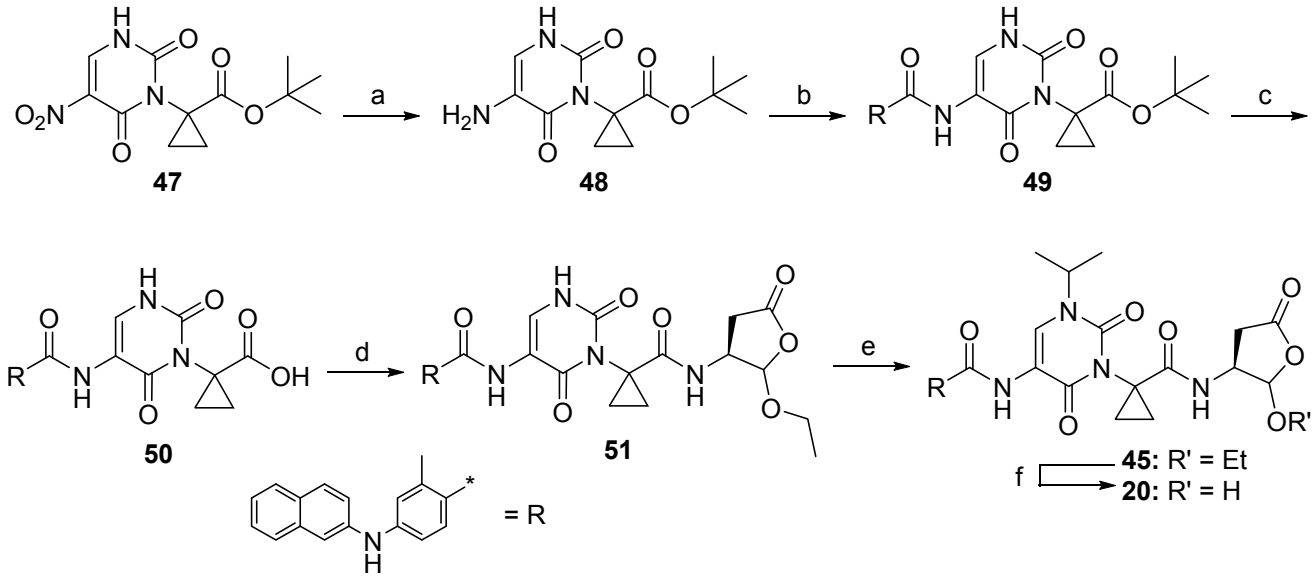


Reagents and conditions: (a) $NaHCO_3$, Allyl chloroformate, THF/H_2O (1:3), 23 °C, yield 80%; (b) MeONHMe·HCl, NMM, EDCI, CH_2Cl_2, 23 °C, yield 86%; (c) $LiAlH_4$, THF, -78 °C, yield 83%; (d) $(EtO)_3CH$, 3Å molecular sieves, *p*-TsOH, EtOH, 23 °C, yield 92%; (e) TFA, CH_2Cl_2, 23 °C, yield 71% (f) MeONHMe·HCl, NMM, EDCI, CH_2Cl_2, 23 °C, yield quantitative (g) DBU, CH_2Cl_2, 23 °C, yield 68%; (h) $NaHCO_3$, Allyl chloroformate, THF/H_2O (1:3), 23 °C, yield 88%; (i) $LiAlH_4$, THF, -78 °C, yield 95%; (j) $(EtO)_3CH$, 3 Å molecular sieves, *p*-TsOH, EtOH, 23 °C, yield 81%; (k) 2-aminonaphthalene, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, CH_2Cl_2, μwave 120 °C, yield 89%; (l) $LiOH_{(aq)}$, MeOH, 50 °C, yield 92% (m) 2-iodopropane, K_2CO_3, DMF, 23 °C, yield 46%; (n) Fe(s), NH_4Cl, THF/EtOH/H_2O (1:1:1), 70 °C, yield 98%; (o) **40**, COMU, NMM, DMF, 23 °C, yield 90%; (p) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2, 23 °C, yield 60%; (q) *i.*

HATU, CH_2Cl_2/DMF (1:1), 23 °C; *ii.* **33**, $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2/DMF (1.4:1), 23 °C, yield quantitative; (r) $HCl_{(aq)}$, acetonitrile, 23 °C, yield 65%; (s) *i.* HATU, CH_2Cl_2/DMF (0.7:1), 23 °C; *ii.* **38**, $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2/DMF (1.4:1), 23 °C, yield 95%; (t) TFA, CH_2Cl_2, 0-23 °C, yield 80%.

The second strategy was developed to permit *N*-3 alkylation at the end of the sequence in order to probe SAR at this position. Reduction of **47** – obtained using the methodology developed for **28** – by hydrogenation afforded amine **48** in poor yield (Scheme 4). Coupling of the P4 fragment using COMU followed by deprotection of the *tert*-butyl ester afforded **50**. The pre-cyclised hemiacetal warhead **33** was next deprotected and coupled *in situ* using T3P as the coupling agent to afford **51**.[49] Finally, exploration at *N*-3 was best achieved through *Mitsunobu* alkylation chemistry and hydrolysis of the acetal pro-drug to the hemiacetal drug molecules was achieved using aqueous HCl to afford **20**.

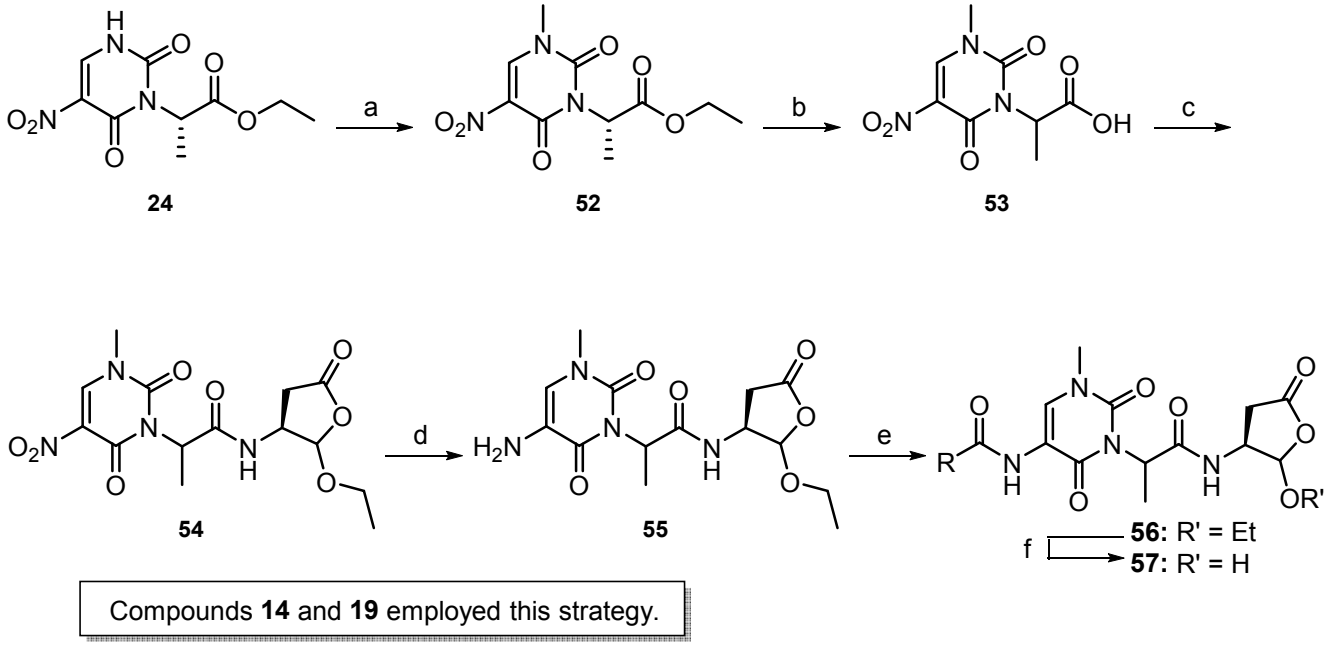
Scheme 4. Strategy employed for *N*-3 alkylation exploration based on a late-stage *Mitsunobu* alkylation.



Reagents and conditions: (a) H_2, Pd-C, EtOAc, 23 °C, yield 32%; (b) **40**, COMU, NMM, DMF, 80 °C, yield 79%; (c) 4M HCl in dioxane, 23 °C, yield 78%; (d) *i.* DIPEA, T3P, DMF, 23 °C; *ii.* **33**, $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2/DMF (1:1), 23 °C, yield 73%; (e) *i*-PrOH, DIAD, PPh_3, CH_2Cl_2, 0 °C to 23 °C, yield 11%; (f) 1M HCl (aq), MeCN, 23 °C, 65%.

Finally, we also developed an efficient library route to probe the P4 region (Scheme 5). Alkylation of **24** followed by saponification of ester **52** afforded the acid **53**. Unfortunately, **53** underwent partial epimerization during the saponification step but we decided to continue with this route to build up our SAR knowledge of this key selectivity pocket. Coupling of **53** with the pre-cyclised warhead **33** *via* the Pfp ester methodology followed by reduction afforded the key library synthon **55**. Final amide coupling through *Schotten-Baumann* conditions or using COMU followed by hydrolysis of the warhead acetal **56** afforded hemiacetal final compounds **57** in moderate to good yields.

Scheme 5. Strategy employed for the P4 region exploration based on a late-stage amide coupling reaction.

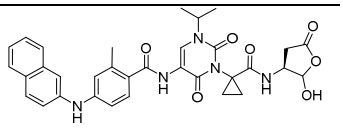


Reagents and conditions: (a) Me-I, K_2CO_3, DMF, 23 °C, yield 88%; (b) LiOH, THF/MeOH (10:1), 50 °C, yield 92% (S/R, 86:14); (c) *i.* Pfp-OH, EDCI, CH_2Cl_2/DMF (4:1), 23 °C; *ii.* **33**, $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2/DMF (2:1), 23 °C, yield 60%; (d) H_2, Pd-C, EtOAc, 23 °C, yield 80%; (e) RCOCl, DIEA, THF or RCOOH, HATU, NMM, THF, 23 °C or RCOOH, COMU, NMM, DMF, 80 °C, yield 34-95%; (f) AcOH/H_2O (1:1), 40 °C or 1M $HCl_{(aq)}$, acetonitrile, 23 °C, yield >70%.

Pharmacological profiling

Our lead compound cyclopropyl analog **20** was evaluated in a caspase selectivity panel and we were pleased by the results indicating complete (>1000 fold) selectivity vs the apoptotic caspases 3, 6, 7 and 8. In addition, selectivity vs inflammatory caspase 5 was 300-fold.

Table 3. Cellular potency and biochemical selectivity vs apoptotic and inflammatory caspases of uracil **20** shows the compound is potent and selective.

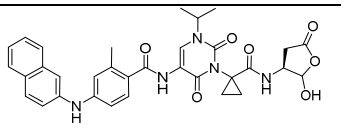
	 **20**
IC_{50} Caspase-1[a]	9.8
IC_{50} Caspase-3[a]	>9999
IC_{50} Caspase-5[a]	2980
IC_{50} Caspase-6[a]	>9999
IC_{50} Caspase-7[a]	>9999
IC_{50} Caspase-8[a]	>9999
IC_{50} THP1[b]	38
IC_{50} Macrophage[b]	160

[a] Biochemical IC_{50} evaluated in nM at K_M concentration of substrate. [b] Cellular IC_{50} evaluated in nM.

Ex-Vivo and In Vitro Pharmacokinetics

Next we wanted to evaluate the pharmacokinetic profile of **20**. Topical drugs should provide high tissue exposure in the target skin layer, the dermis in this case, with minimal plasma exposure to provide a high safety margin. A high skin flux is needed to achieve high exposure in the deeper skin layers such as the dermis (in our experience, at the very least 1 μmol/cm^2/h, but 10-100 μmol/cm^2/h is a safer range), but since this is vascularized, high hepatic clearance is also needed to keep plasmatic exposure low.[50] Skin exposure was measured at micromolar levels in dermis after a single topical application both in mice PK and in a cryogenically preserved human skin explant (Table 4). Furthermore, the maximum systemic exposure in mice following topical administration was only 1 nM and the intrinsic clearance in human hepatocytes is 9.8 μL/min/10^6 cells, thus minimizing risk of adverse effects.

Table 4. Skin penetration profile of **20**: >3000-fold tissue selectivity between dermis and plasma.

	 **20**
Flux[a]	4.3
Mouse dermis C_{max}[b]	4.2
Mouse plasma C_{max}[b]	0.0013
Human dermis concentration[c]	4.5
Human hepatocyte clearance[d]	9.8

[a] Flux in μmol/cm^2/h calculated using Fick's law and Potts and Guy K_p.[51] [b] C_{max} in μM after topical (0.1 μmol/cm^2 applied using a 10 mM PG/EtOH 3/7 solution) mouse PK. [c] Concentration in μM after topical (0.2 μmol/cm^2 applied using a 40 mM PG/EtOH 3/7 solution) on a cryogenically preserved human skin explant. [d] Intrinsic clearance in μL/min/10^6 cells.

CONCLUSION

Acne is a skin disease affecting the quality of life of 650 million people globally.[1] Inhibition of cysteine-aspartic protease Caspase-1 was identified as a potential new approach to treat inflammatory acne due to its role in the formation of IL-1β. Structure-based drug design was therefore used to identify several novel scaffolds with an electrophilic warhead. The uracil series was thus identified and optimized to deliver **20** as a potent, soluble and stable inhibitor of caspase-1. Key structural improvements included a) the addition of cyclopropyl substituent in P2, which boosted cellular potency, solubility and chemical stability, and b) the use of an o-tolyl derivative in P4 to break π-conjugation and eliminate the risk of phototoxicity. Further profiling of **20** revealed it was selective vs apoptotic caspases, was able to expose the mouse and human

dermis at micromolar levels after topical administration and yet did not give rise to a systemic exposure.

EXPERIMENTAL SECTION

Caspase-1 Enzymatic Assay

Human recombinant Caspase-1, assay buffer (50mM HEPES pH=7.4, 100mM NaCl, 0.1% CHAPS, 1mM EDTA, 10% Glycerol, 10mM DTT) and pro-fluorescent peptidic substrate (AC-WEHD-AMC) were purchased from Enzo life sciences. 4µL of 200µM or 10µM final concentration of substrate (corresponding to its K_M) was dispensed in a 384-well microplate together with 4µL of appropriate dilutions of the evaluated compounds (10 000-0.04nM, 1% DMSO). 2µL of enzyme was then added at a final concentration allowing for the conversion of less than 20% of substrate after 20 minutes at room temperature. Free AMC was measured by fluorescence detection (λex=340nm, λem=450nm). Percent inhibition was calculated from the averaged values of the uninhibited and the uncatalyzed controls, and IC_{50} values were determined by fitting the concentration-response data with a four-parameter logistic equation.

Caspase Panel Enzymatic Assays

Human recombinant Caspase proteins (ie Caspase-3, Caspase-5, Caspase-6, Caspase-7 and Caspase-8), assay buffer (50mM HEPES pH=7.4, 100mM NaCl, 0.1% CHAPS, 1mM EDTA, 10% Glycerol, 10mM DTT) and pro-fluorescent peptidic substrates (AC-WEHD-AMC for Caspase-1 / -5, Ac-DEVD-AMC for Caspase-3 / -7, Ac-VEID-AMC for Caspase-6 and Ac-IETD-AMC for Caspase-8) were purchased from Enzo life sciences. 4µL of Caspase-specific

substrate (with a final concentration corresponding to its K_M) was dispensed in a 384-well microplate together with 4µL of appropriate dilutions of the evaluated compounds (10 000-0.04nM, 1% DMSO). 2µL of enzyme was then added at a final concentration allowing for the conversion of less than 20% of substrate after 20 minutes (or 2 hours depending on enzymatic activity) at room temperature. Free AMC was measured by fluorescence detection (λex=340nm, λem=450nm). Percent inhibition was calculated from the averaged values of the uninhibited and the uncatalyzed controls, and IC_{50} values were determined by fitting the concentration-response data with a four-parameter logistic equation.

Jump Dilution Method

A simple test to discriminate between reversible and pseudo-irreversible inhibition is provided by the jump dilution method previously described by Copeland.[12] The overall procedure and the reagents were the same as the one used for Caspase-1 enzymatic assay. 12µL of 100X concentrated enzyme solution were pre-incubated with 8µL of the evaluated inhibitor at a concentration corresponding to its IC_{90} (1% DMSO) for 1 hour. 10µL of the latter solution was then diluted with 990µL of substrate (diluted to the concentration corresponding to the K_M) to start the enzymatic reaction. 10µL of the reaction mixture were immediately dispensed in a 384-well microplate and the free AMC production was continuously recorded for 2 hours. The slope of the progress curve (*ie*, a plot of activity as a function of time) was calculated and normalized to the DMSO control. For a rapidly reversible compound, the resulting progress curve is expected to be linear and to display a slope value that is approximately 90% that of the solvent control. For a pseudo-irreversible inhibitor, the slope should be only approximately 10% that of the control.

IL-1 beta release on monocytic cell line (THP1)

This assay is designed to measure the inhibition of the IL-1 beta secretion from PMA differenciated monocytic cells (THP1) after activation of the two signals leading to the formation of the inflammasome (LPS activation of the pro-IL-1 beta synthesis and ATP activation of the pro-Caspase-1). IL-1 beta levels were measured using HTRF® Human IL-1 beta kit (Cisbio # 62IL1PEC) which is a rapid and quantitative sandwich immunoassay based on TR-FRET technology.

THP1 cells were seeded in 96-well plates (10,000 cells/well) in culture medium (RPMI 10% FBS 400ng/mL PMA). After 24 hours of incubation at 37°C, serial dilutions of tested inhibitors (10000nM-0.04nM, 0.1% DMSO) and 1µg/mL of LPS (Lipopolysaccharides from Escherichia coli) and 5mM ATP were added to the cells. After overnight incubation at 37°C, 10µL of cell supernatant were mixed with 5µL of each tagged anti-IL-1 beta specific antibody as recommended by the supplier. After 2 hours of incubation at room temperature, the HTRF® signals were measured using a microplate reader (wavelengths: excitation=337nm, first emission=620nm, second emission=665nm).

The ratios of the measured signals (665/620) were normalized using the average of positive controls (ATP/LPS-activated with no inhibitor) and negative controls (not activated). IC_{50} values were determined by fitting the concentration-response data with a four-parameter logistic equation.

IL-1 beta release on human monocyte-derived macrophages[52]

Cryopreserved human peripheral blood monocytes (STEMCELL Technologies) were seeded in 48-well plates (300,000 cells/well) in culture medium (RPMI, 10% heat inactivated FBS) and 100ng/mL recombinant human GM-CSF to induce M1 macrophages polarization.

After 10 days of culture at 37°C, monocyte-derived macrophages were stimulated with LPS (10µg/mL) and INFy (20ng/mL) to activate the formation of the inflammasome and treated by serial dilutions of tested compounds (10000nM-0.04nM, 0.1% DMSO). IL-1 beta levels were measured after 24 hours using HTRF® Human IL-1 beta kit (Cisbio # 62IL1PEC) which is a rapid and quantitative sandwich immunoassay based on TR-FRET technology.

General methods

^{1}H NMR spectra were recorded on a BRUKER Biospin AVANCE 400 spectrometer unless otherwise stated. Chemical shifts are reported as δ values downfield from internal TMS in appropriate organic solutions. The abbreviations used for explaining the multiplicities were as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass (ESI HRMS) was recorded on a Thermofisher Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer.

The relative purity ≥95% and the mass of the products were confirmed by LC/MS (220 nm to 420 nm) on a Waters acquity uplc photodiode array detector system using the following conditions: Column, BEH C18 50*2.1 mm; 1.8µm ; Solvent A, water 0.1% formic acid or water ammonium carbonate 2 g/l; Solvent B, CH3CN; flow rate, 0.8 ml/min; run time, 2.2 min; gradient, from 5 to 95% solvent B; mass detector, Waters SQ detector. All compounds were purified by LC/MS on a waters Autopurification system using the following conditions : Column, Xbridge C18 150*30mm, 5µm; Solvent A, water 0.1% formic acid or water ammonium

carbonate 2 g/l; Solvent B, CH3CN; flow rate, 50 ml/min; run time, 10 or 15 min; with adapted isocratic elution mode; mass detector, Waters ZQ detector.

All experimental activities involving animals were carried out in accordance with Galderma / Nestlé Skin Health animal welfare protocols which are consistent with The American Chemical Society Publications rules and ethical guidelines and were approved by the Galderma animal welfare ethics committee.

_N_-(3-(1-(((3_S_)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-naphthamide (8)

To a stirred solution of *N*-(3-(1-(((3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-naphthamide (70.0 mg, 0.14 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in MeCN (2.00 ml) at r.t., was added HCl (aq) (1.0 M, 2.00 ml, 2.00 mmol, 14 eq.) and the reaction mixture is stirred for 4 h at r.t. A saturated solution of $NaHCO_3$ was added adjusting the pH to 7. The product was extracted in AcOEt (3 x 30 ml). The organic phases were combined, dried over $MgSO_4$ and concentrated to afford a beige solid. The solid was triturated with diethyl ether, recuperated by filtration and dried to a constant weight under vacuum to afford *N*-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-naphthamide (50 mg, 74%) as an off-white solid: LCMS (t_R = 1.02 min., purity = 100%, MS ES^+ *m/z* 467 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.51 (d, *J* = 8.2 Hz, 1H), 8.58 (s, 1H), 8.39 (d, *J* = 6.7 Hz, 1H), 8.23 (s, 1H), 7.91 – 8.15 (m, 5H), 7.51 – 7.76 (m, 2H), 5.53 (s, 1H), 5.32 (q, *J* = 6.7 Hz, 1H), 4.17 (s, 1H), 3.40 (d, *J* = 1.7 Hz, 3H), 2.27 – 2.49 (m, 2H), 1.46 (dd, *J* = 6.9, 3.1 Hz, 3H); ^{13}C NMR (101

MHz, DMSO-d_6) δ 169.2, 165.55, 159.62, 149.67, 134.43, 132.13, 130.97, 129.08, 128.21, 128.06, 127.71, 126.96, 124.19, 112.11, 50.05, 46.21, 36.83, 13.86.

_N_-(3-(1-(((3_S_)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-((5-methylpyrazin-2-yl)amino)benzamide (9)

To a stirred solution of *N*-{3-[(*S*)-1 ((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl]-1-methyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(5-methyl-pyrazin-2-ylamino)-benzamide (80.0 mg, 0.15 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in formic acid (1.60 ml, 42.4 mmol; 20.0 V) was added water (1.60 ml). The reaction mixture was stirred for 5 h at r.t., concentrated to dryness and the residue was suspended in EtOAc (20 ml) and the suspension was stirred at r.t. overnight. The precipitate was collected by filtration and dried to a constant weight at 40 °C in a vacuum oven to afford *N*-(3-(1-(((3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-((5-methylpyrazin-2-yl)amino)benzamide (56.0 mg, 74%) as a pale orange solid: LCMS (t_R = 0.80 min., purity = 85%, MS ES^+ *m/z 524.16* $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.06 (d, *J* = 7.5 Hz, 1H), 8.34 (d, *J* = 6.8 Hz, 1H), 8.23 (s, 1H), 8.11 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.78 (d, *J* = 8.7 Hz, 2H), 5.30 (d, *J* = 7.1 Hz, 1H), 4.13 (s, 1H), 3.38 (s, 3H), 2.3- 2.7 (m, 2H), 2.39 (s, 3H), 1.43 (d, *J* = 6.9 Hz, 3H).

_N_-(3-(1-(((3_S_)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(quinoxalin-2-ylamino)benzamide (10)

To a stirred solution of formic acid (15.0 ml, 398 mmol) and water (15.0 ml) was added *N*-{3-[1-((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl]-1-methyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (750 mg, 1.28 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3). The reaction mixture was stirred at room temperature overnight, concentrated to dryness and dissolved in EtOAc. The solution was triturated with heptane and the precipitate was collected by filtration and dried to a constant weight to afford *N*-{3-[(S)-1-((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl]-1-methyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (620 mg, 87%) as a pale yellow solid: LCMS (t_R = 0.94 min., purity = 100%, MS ES$^+$ *m/z* 560.14 (M+H)$^+$; ^{1}H NMR (DMSO-d_6) δ 10.30 (s, 1H), 9.17 (d, *J* = 8.9 Hz, 1H), 8.63 (s, 1H), 8.36 (d, *J* = 7.4 Hz, 1H), 8.16 – 8.26 (m, 1H), 8.13 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 8.6 Hz, 2H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 5.32 (m, 1H), 4.25 (m, 1H), 3.39 (d, *J* = 1.7 Hz, 3H), 2,3-2,6 (m, 2H), 1.45 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.89, 159.68, 149.62, 149.36, 143.79, 140.64, 140.37, 137.21, 136.50, 136.24, 136.10, 130.30, 128.70, 128.59, 126.74, 126.47, 125.64, 117.81, 112.29, 109.92, 54.98, 48.67, 38.47, 36.81, 13.88; HRMS (ESI) calculated for $C_{27}H_{25}N_7O_7$ [M+H]+ 560.18154, found 560.18829.

***N*-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxopropan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(quinoxalin-2-ylamino)benzamide (11)**

To a stirred solution of *N*-{3-[(S)-1-((S)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (155 mg, 0.25 mmol, 1.00 eq.) obtained following the procedures described for **24**

(Scheme 1) and **20** (Scheme 3) in formic acid (3.10 ml) at r.t., was added water (3.10 ml). The reaction mixture was stirred at r.t. for 5 h. The volatiles were removed under vacuum and the crude product was triturated in a mixture of AcOEt and heptane to afford a yellow solid. The solid was suspended in AcOEt and stirred overnight at r.t., collected by filtration, washed with cold AcOEt and dried to a constant weight in a vacuum oven at 40 °C to afford *N*-{3-[(*S*)-1-((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (100 mg, 67%) as a pale yellow solid: LCMS (t_R = 1.03 min., purity = 100%, MS ES$^+$ *m/z* 588.12 (M+H)$^+$; ^{1}H NMR (DMSO-d_6) δ 10.33 (s, 1H), 9.23 (d, *J* = 8.6 Hz, 1H), 8.64 (s, 1H), 8.38 (d, *J* = 9.3 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 2H), 8.00 (dd, *J* = 8.8, 1.6 Hz, 2H), 7.91 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.83 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.71 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 1H), 7.54 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 5.33 (q, *J* = 6.8 Hz, 1H), 4.68 – 4.84 (m, 1H), 4.17 (s, 1H), 1.45 (d, *J* = 6.9 Hz, 3H), 1.27 – 1.39 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.89; 159.68, 149.62, 149.36, 143.79, 140.64, 140.37, 137.21, 136.50, 136.24, 136.10, 130.30, 128.70, 128.59, 126.74, 126.47, 125.64, 117.81, 112.29, 109.92, 54.98, 48.67, 38.47, 36.81, 13.88; HRMS (ESI) calculated for $C_{27}H_{25}N_5O_7$ [M+H]+ 560.1815, found 560.1882.

***N*-(3-(2-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-2-oxoethyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(quinoxalin-2-ylamino)benzamide (12)**

To a stirred solution *N*-{3-[((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-methyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (58.0 mg, 0.10 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in MeCN (1.16 ml) at r.t., was added 2N HCl (aq) (0.48 ml, 0.96 mmol, 10.0 eq.) and the reaction mixture was stirred at r.t. for 7 h. The precipitate was collected by filtration,

washed with diethyl ether and dried to a constant weight in a vacuum oven at 45 °C to afford *N*-{3-[((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-methyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (50.0 mg, 87%) as an orange crystalline solid : LCMS (t_R = 1.00 min., purity = 96.5%, MS ES+ m/z 574.22 (M+H)+; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 9.30 (d, *J* = 7.5 Hz, 1H), 8.72 (d, *J* = 6.8 Hz, 1H), 8.64 (s, 1H), 8.31 (d, *J* = 2.6 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 2H), 7.90 (dd, *J* = 8.2-1.2 Hz, 1H), 7.82 (dd, *J* = 8.3-1.0 Hz, 1H), 7.70 (td, *J* = 7.0, 1.4 Hz, 1H), 7.52 (td, *J* = 7.0, 1.4 Hz, 1H), 5.48 (s, 1H), 4.77 (q, *J* = 6.8 Hz, 1H), 4.49-4.61 (m, 3H), 2.98-3.05 (m, 1H), 2.45-2.48 (m, 1H), 2.32-2.37 (m, 1H), 1.33 (d, *J* = 6.8 Hz, 6H).

***N*-(3-(2-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-2-oxoethyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(naphthalen-2-ylamino)benzamide (13)**

To a stirred solution *N*-(3-(2-(((3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-yl)amino)-2-oxoethyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(naphthalen-2-ylamino)benzamide (0.024 mmol) which can be obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) was dissolved in MeCN (1.65 ml) and HCl (aq) (2.0 M, 1.65 ml) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was poured into a mixture of ice and a saturated solution of $NaHCO_3$ (aq) and diluted with EtOAc (20 ml). The pH was decreased to 5 with AcOH and the product extracted with EtOAc (3 x 20 ml). The organic phases were combined, washed with water (2 x 10 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered Prep LCMS (formic acid buffer) to afford *N*-{3-[((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-methyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(naphthalen-2-ylamino)-benzamide/S-SCM-001646.001 (65.0 mg, 45%) as a beige solid: LCMS (t_R = 1.22 min., purity = 97%, MS ES^+ *m/z* 572.08 $(M+H)^+$; ^{1}H

NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.91 (s, 1H), 8.30 (s, 1H), 8.00 – 7.72 (m, 6H), 7.64 (d, m, 1H), 7.44 (m, 1H), 7.39 – 7.29 (m, 2H), 7.30 – 7.16 (m, 2H), 4.77 (m, 1H), 4.52 (m, 2H), 4.08 (m, 1H), 3.81 (s, 0H), 2.71 – 2.55 (m, 1H), 2.45 – 2.24 (m, 1H), 1.33 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.14, 159.37, 158.07, 152.00, 149.49, 147.01, 139.63, 134.19, 132.11, 129.31, 129.06, 128.96, 127.53, 126.64, 126.45, 125.70, 123.86, 123.72, 122.87, 120.82, 102.50, 97.23, 65.74, 48.43, 43.33, 33.21, 20.77; HRMS (ESI) calculated for $C_{30}H_{29}N_5O_7$ $[M+H]^+$ 572.2067, found 572.2135.

***N*-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(quinoxalin-2-ylamino)benzamide (14)**

To a stirred solution of *N*-{3-[1-((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-cyclopropyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(quinoxalin-2-ylamino)-benzamide (191 mg, 0.22 mmol, 1.00 eq.) obtained following the procedures described for **28** (Scheme 2) and **57** (Scheme 5) in MeCN (2.71 ml) at r.t., was added HCl (aq) (2.00 M, 1.08 ml, 2.16 mmol, 10.0 eq.) and the reaction mixture was stirred at r.t. for 18 h. The volatiles were eliminated under a flow of nitrogen and the residue was treated with a saturated aqueous solution of $NaHCO_3$ (pH 8) then diluted with EtOAc. The organic phase was discarded and the pH of the aqueous phase was adjusted to 5 with AcOH. The product was extracted in EtOAc (3 x 20 ml), the organic phases were combined, washed with water (3 x 5 ml) dried over $MgSO_4$ and concentrated. The product crystallised during evaporation and was recuperated by filtration, washed with cold EtOAc and dried to a constant weight un a vacuum oven at 40 °C to afford *N*-(3-(1-(((3S)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(quinoxalin-2-ylamino)benzamide (76 mg, 58%) as a

pale yellow solid: LCMS (t_R = 1.03 min., purity = 100%, MS ES$^+$ *m/z* 600.10 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.2 (s, 1H), 8.62 (s, 1H), 8.40 (s, 1H), 8.11-8.14 (d, *J* = 8.9 Hz, 2H), 7.99–8.01 (d, *J* = 8.8 Hz, 2H), 7.89-7.92 (dd, *J* = 8.2-1.2 Hz, 1H), 7.80–7.83 (dd, *J* = 8.3-1.0 Hz, 1H), 7.68–7.73 (td, *J* = 7.0-1.4 Hz, 2H), 7.51-7.55 (td, *J* = 7.0-1.4 Hz, 1H), 5.40 (m, 1H), 4.74-4.81 (m, 1H), 4.13 (m, 1H), 2.52-2.53 (m, 1H), 2.32-2.37 (m, 1H), 1.66-1.68 (s, 2H), 1.30-1.35 (m, 6H), 1.18 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.77, 160.33, 149.96, 149.34, 143.77, 140.61, 140.35, 137.20, 135.26, 132.83, 132.09, 130.28, 128.66, 128.57, 126.71, 126.52, 125.62, 117.80, 113.45, 99.57, 59.79, 48.03, 37.29, 32.70, 20.81, 14.14; HRMS (ESI) calculated for $C_{30}H_{29}N_7O_7$ $[M+H]^+$ 600.2128, found 600.2195.

***N*-(3-(2-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-2-oxoethyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (15)**

To a stirred solution of *N*-{3-[((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-methyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-2-methyl-4-(naphthalen-2-ylamino)-benzamide (280 mg, 0.46 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in MeCN (5.60 ml) at r.t., was added 2N HCl (aq) (2.28 ml, 4.56 mmol, 10.0 eq.) and the solution was stirred at r.t. for 4 h. The volatiles were eliminated through sparging with nitrogen and the reaction mixture was suspended in a saturated solution of $NaHCO_3$(aq) and extracted with EtOAc (5 ml). The organic layer discarded and the pH of the aqueous phase was lowered to 5-5.5 with 1N AcOH (aq). The product was extracted with EtOAc (3 x 10 ml). The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was recrystallized from a EtOAc heptane mixture to afford *N*-(3-(2-(((3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl)amino)-2-oxoethyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (180 mg, 67%) as an

off-white solid: LCMS (t_R = 1.16 min., purity = 97.0%, MS ES+ m/z 586.13 (M+H)+; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.69 (s, 1H), 8.33 (s, 1H), 7.71-7.84 (m, 3H), 7.53-7.59 (m, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.26-7.37 (m, 2H), 7.02-7.09 (m, 1H), 7.02 (s, 1H), 5.48 (s, 1H), 4.76 (q, J = 6.7 Hz, 1H), 4.50 (s, 2H), 4.12 (s, 1H), 2.98 (s, 1H), 2.43 (s, 3H), 2.37 (m, 1H), 1.32 (d, J = 6.9 Hz, 6H).

***N*-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (16)**

To a stirred solution of *N*-{3-[1-((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-propyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-2-methyl-4-(naphthalen-2-ylamino)-benzamide (210 mg, 0.33 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in MeCN (4.2 ml), was added HCl (aq) (2M, 1.64 ml, 3/27 mmol, 10.0 eq.) and the reaction mixture was stirred for 2 h. The volatiles were eliminated using nitrogen purging. The concentrate was treated with a saturated solution of $NaHCO_3$ (aq) and diluted with EtOAc (10 ml). The phases were separated and the aqueous phase was retained and the pH was lowered to 5 through the addition of AcOH. The product was extracted with EtOAc (3 x 50 ml). The organic phases were combined, washed with water (3 x 10 ml), dried over $MgSO_4$ and concentrated to dryness, triturated with a mixture of EtOAc/ Et_2O and the solid was collected and dried to a constant weight to afford *N*-{3-[1-((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-propyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-2-methyl-4-(naphthalen-2-ylamino)-benzamide (75.0 mg, 35%) as a beige solid: LCMS (t_R = 1.23 min., purity = 93%, MS ES^+ *m/z* 614.16 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.92 (d, J = 7.1 Hz, 1H), 8.71 (s, 1H), 8.44 (d, J = 6.2 Hz, 1H), 8.10-8.30 (br s, 1H), 7.70-7.90 (m, 3H), 7.58

(s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.43 (t, *J* = 7,12Hz, 1H), 7.30-7.40 (m, 2H), 7.00-7.10 (m, 2H), 5.20 (m, 1H), 4.75 (m, 1H), 4.15 (m, 1H), 2.45 (s, 3H), 2.30-2.50 (m, 2H), 1.85-2.25 (br m, 2H), 1.25-1.40 (m, 6H), 0.78 (t, *J* = 6.6 Hz, 3H).

***N*-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methyl-5-(naphthalen-2-ylamino)picolinamide (17)**

To a stirred solution of *N*-(3-(1-(((3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methyl-5-(naphthalen-2-ylamino)picolinamide (120 mg, 0.19 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **20** (Scheme 3) in MeCN (2.40 ml) at r.t., was added 2 M HCl(aq) (934 µl, 1.87 mmol, 10.0 eq.) and the reaction mixture was stirred for 3 h at r.t., neutralized with a saturated solution of $NaHCO_3$(aq) and EtOAc (5 ml) was added. The phases were separated and the organic phase was discarded. The pH was lowered to 5 with 1N AcOH (aq) and the product extract with AcOEt (3 x 5 ml). The organic phases were combined, washed with water (5 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was triturated in EtOAc and the solid was recuperated by filtration and dried to a constant weight in a vacuum oven at 40 °C to afford *N*-(3-(1-(((3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methyl-5-(naphthalen-2-ylamino)picolinamide (95.0 mg, 82%) as a yellow crystalline solid: LCMS (t_R = 1.29 min., purity = 99%, MS ES+ m/z 615.19 (M+H)+; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 9.18 (s, 1H), 8.70 (s, 1H), 8.37 (d, *J* = 2.6 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.85 (dd, *J* = 8.1, 3.9 Hz, 3H), 7.72 (d, *J* = 2.3 Hz, 2H), 7.44 – 7.51 (m, 2H), 7.29 – 7.43 (m, 2H), 5.50 (s, 1H), 5.22 (s, 1H), 4.77 (p, *J* = 6.7 Hz, 1H), 4.12 (s, 1H), 2.89 (m, 1H), 2.68 (s, 3H), 2.07 – 2.27 (m,

1H), 1.83 (s, 1H), 1.36 (d, J = 6.8 Hz, 3H), 1.31 (d, J = 6.8 Hz, 3H), 0.77 (td, J = 7.5, 2.5 Hz, 3H).

_N_-(3-(1-(((4_R_)-2-Hydroxy-6-oxotetrahydro-2_H_-pyran-4-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (18)

To a stirred solution of (*R*)-5,5-diethoxy-3-(2-{3-isopropyl-5-[2-methyl-4-(naphthalen-2-ylamino)-benzoylamino]-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl}-butyrylamino)-pentanoic acid *tert*-butyl ester (440 mg, 0.58 mmol, 1.00 eq.) obtained following the procedures described for **24** (Scheme 1) and **21** (Scheme 3) in DCM (13.2 ml) was added dropwise TFA (13.2 ml, 0.17 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. LCMS confirmed the presence of the cyclized OEt warhead intermediate. The reaction mixture was carefully added to a mixture of ice and a saturated solution of $NaHCO_3$ (aq). The pH of the reaction mixture was lowered to 5 with AcOH and the product extracted with DCM (3 x 10 ml). The organic extracts were combined, washed with water (2 x 5 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, gradient of 2-10% MeOH in DCM) to afford *N*-(3-(1-(((4*R*)-2-hydroxy-6-oxotetrahydro-2*H*-pyran-4-yl)amino)-1-oxobutan-2-yl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (300 mg, 81%) as a beige solid: LCMS (t_R = 1.23 min., purity = 100%, MS ES^+ *m/z* 628.20 $(M+H)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.72 (m, 1H), 8.76 (d, J = 6.8 Hz, 1H), 8.09 (s, 1H), 7.81 (t, J = 7.8 Hz, 2H), 7.73 (d, J = 8.2 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H); 7.43 – 7.52 (m, 2H), 7.38 (t, J = 7.5 Hz, 1H), 7.31 (m, 2H), 6.96 (m, 2H), 6.75 (d, J = 8.8 Hz, 1H), 5.40 (m, 1H), 4.92 (m, 1H), 4.67 (m, 1H), 2.65 – 3.02 (m, 4H), 2.53 (s, 3H), 2.17 (m, 2H), 1.35 – 1.48 (m, 6H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz,

DMSO-d_6) δ 168.00, 167.93, 167.41, 161.93, 159.43, 145.40, 140.14, 138.40, 134.28, 133.56, 130.98, 129.60, 129.02, 128.73, 127.53, 126.43, 126.11, 123.49, 122.03, 120.62, 118.20, 113.40, 112.80, 111.54, 59.82, 56.29, 54.99, 48.67, 47.37, 20.95, 20.63, 14.16, 10.73; HRMS (ESI) calculated for $C_{34}H_{37}N_5O_7$ $[M+H]^+$ 628.26930, found 628.27612.

_N_-(3-(1-(((3*S*)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4-(naphthalen-2-ylamino)benzamide (19)

To a stirred solution of *N*-{3-[1-((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-cyclopropyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(naphthalen-2-ylamino)-benzamide (170 mg, 0.20 mmol, 1.00 eq.) obtained following the procedures described for **28** (Scheme 2) and **57** (Scheme 5) in MeCN (2.48 ml) at r.t., was added HCl (aq) (2.00 M, 0.99 ml, 10.0 eq.) and the reaction mixture was agitated for 4 hours at r.t. The volatiles were eliminated under a flow of nitrogen and the residue was treated with a saturated aqueous solution of $NaHCO_3$ (pH 8) then diluted with EtOAc. The organic phase was discarded and the pH of the aqueous phase was adjusted to 5 with AcOH. The product was extracted in EtOAc (3 x 20 ml), the organic phases were combined, washed with water (3 x 5 ml) dried over $MgSO_4$ and concentrated to afford a gum. The gum was crystallised from by dissolution in mixture of toluene and EtOAc at 60 °C and the solution was allowed to cool slowly to r.t. and stirred overnight. The solid formed was recuperated by filtration washed with cold EtOAc and dried to a constant weight in a vacuum oven at 40 °C to afford *N*-{3-[1-((*S*)-2-hydroxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-cyclopropyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-4-(naphthalen-2-ylamino)-benzamide (60.0 mg, 50%) as an off-white solid: LCMS (t_R = 1.16 min., purity = 100%, MS ES^+ *m/z* 598.12 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H),

8.92 (s, 1H), 8.39 (s, 1H), 7.85-7.87 (t, 2H), 7.77–7.82 (q, 2H), 7.63–7.64 (d, J = 2.0 Hz, 1H), 7.42-7.46 (td, J = 8.0-1.1 Hz, 1H), 7.32-7.38 (m, 2H), 7.22-7.27 (m, 2H), 7.13-7.19 (m, 2H), 5.4 (m, 1H), 4.74-4.76 (m, 1H), 4.10 (s, 1H), 2.30 (s, 2H), 1.65-1.67 (m, 2H), 1.29-1.34 (m, 6H), 1.16-1.19 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 186.48, 178.94, 164.68, 160.34, 147.08, 139.59, 137.99, 149.89, 134.17, 129.23, 129.07, 128.98, 128.95, 128.25, 127.53, 126.63, 126.46, 125.36, 123.79, 120.83, 123.74, 114.92, 113.66, 112.43, 65.94, 48.00, 47.03, 39.06, 38.01, 37.28, 21.09, 20.93; HRMS (ESI) calculated for $C_{32}H_{31}N_5O_7$ [M+H]+ 598.2223, found 598.22986.

_N_-(3-(1-(((3_S_)-2-Hydroxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (20)

To a stirred solution of **45** (1.72 g, 2.69 mmol, 1.00 eq.) in MeCN (34.40 ml) was added HCl (aq) (2M, 13.44 ml, 26.89 mmol, 10.00 eq.) and the reaction mixture was stirred at room temperature for 5 hours. The volatiles were eliminated under a flow of nitrogen and the residue was treated with a saturated aqueous solution of $NaHCO_3$ (pH 8) then diluted with EtOAc. The organic phase was discarded and the pH of the aqueous phase was adjusted to 5 with AcOH. The product was extracted in EtOAc (3 x 20 ml), the organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The resulting beige solid was crystallised from a mixture of EtOAc / toluene and the solid was recuperated by filtration and dried to a constant weight un a vacuum oven at 40 °C to afford *N*-(3-(1-(((3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (1.07 g, 65%) as an off-white solid: LCMS (t_R = 1.19 min., purity = 100%, MS ES$^+$ *m/z* 612.21 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 9.29-9.31 (d, J = 7.5 Hz, 1H), 8.72-8.73 (d, J = 6.8 Hz, 1H), 8.64 (s, 1H), 8.31 (d, J = 2.6 Hz,

1H), 8.12–8.14 (d, *J* = 8.8 Hz, 2H), 7.99-8.01 (d, *J* = 8.8 Hz, 2H), 7.89-7.91 (dd, *J* = 8.2-1.2 Hz, 1H), 7.81–7.84 (dd, *J* = 8.3-1.0, 1H), 7.68–7.72 (td, *J* = 7.0-1.4 Hz, 1H), 7.51-7.55 (td, *J* = 7.0-1.4 Hz, 1H), 5.48 (s, 1H), 4.75-4.80 (q, *J* = 6.8 Hz, 1H), 4.49-4.61 (m, 3H), 2.98-3.05 (m, 1H), 2.45-2.48 (m, 1H), 2.32-2.37 (m, 1H), 1.32-1.34 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 167.31, 160.22 , 149.87 , 145.41 , 140.15 , 138.33 , 134.28 , 129.53 , 129.02 , 128.75 , 128.48 , 127.53 , 126.53, 126.44, 126.14 , 123.50 , 120.61 , 118.24 , 113.99 , 112.86 , 111.55 , 48.09 , 39.07 , 37.32 , 20.98 , 20.84 , 20.60 , 19.38. HRMS (ESI) calculated for $C_{33}H_{34}N_5O_7$ $[M+H]^+$ 612.2458, found 612.2452.

_N_-(3-(1-(((4_R_)-2-Hydroxy-6-oxotetrahydro-2_H_-pyran-4-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)-benzamide (21)

To a stirred solution of **46** (200 mg, 0.26 mmol, 1.00 eq.) in DCM (6.00 ml) at 0 °C, was added TFA (6.00 ml, 78.4 mmol) dropwise over 2 minutes. The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was carefully added to a mixture of ice and a saturated solution of $NaHCO_3$ (aq). The pH of the reaction mixture was brought up to 8 with 1M NaOH (aq) then lowered to 5 with AcOH and the product extracted with DCM (2 x 10 ml). The organic extracts were combined, washed with water (2 x 2 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, gradient of 2-10% MeOH in DCM) to afford a yellow solid. The solid was triturated with heptane and stirred overnight, recuperated by filtration and dried to a constant weight to afford *N*-(3-(1-(((4*S*)-2-hydroxy-6-oxotetrahydro-2*H*-pyran-4-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)-benzamide (134 mg, 80%) as an

off-white solid: LCMS (t_R = 1.22 min., purity = 100%, MS ES$^+$ *m/z* 626.25 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.84 (s, 1H), 8.72 (s, 1H), 8.41 (s, 1H), 7.99 (s, 1H), 7.71 – 7.88 (m, 3H), 7.58 (d, *J* = 2.4 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.43 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H), 7.26 – 7.37 (m, 2H), 6.94 – 7.03 (m, 2H), 4.76 (s, 1H), 4.46 (s, 1H), 2.30-2.50 (m, 2H), 2.44 (s, 3H), 2.38 (d, *J* = 10.9 Hz, 2H), 1.61 (m, 2H), 1.28-1.38 (m, 6H), 1.11 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.04, 167.23, 160.07, 149.74, 148.66, 145.39, 140.14, 138.32, 135.06, 134.26, 129.48, 128.99, 128.72, 128.33, 127.51, 126.51, 126.40, 126.10, 123.46, 120.60, 118.24, 113.92, 112.85, 111.52, 48.08, 42.61, 37.37, 31.30, 28.41, 22.14, 20.59, 14.00; HRMS (ESI) calculated for $C_{34}H_{35}N_5O_7$ $[M+H]^+$ 626.2536, found 626.2605.

1-Benzhydryl-5-nitropyrimidine-2,4(1*H*,3*H*)-dione (22)

N,O-Bis(trimethylsilyl)acetamide (97.5 ml, 0.40 mol, 2.50 eq.) was added dropwise to a stirred suspension of 5-nitrouracil (25.0 g, 0.16 mol, 1.00 eq.) in acetonitrile (500 ml). The reaction mixture is stirred until a a clear solution was obtained then bromodiphenylmethane (62.1 g, 0.24 mol, 1.50 eq.) and iodine (4.04 g, 0.02 mol, 0.10 eq.) were added. The reaction mixture was heated at reflux for 3 h 30 min., allowed to cool to room temperature and the reaction mixture was stirred overnight. The product partially precipitated (20 g) and was collected by filtration and washed with cold MeCN (3 x 10 ml). The filtrate was concentred to dryness and the residue was dissolved in EtOAc (500 ml) and washed with water (3 x 50 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was triturated in EtOAc and the resulting precipitate was collected by filtration combined with the previous precipitate and dried to constant weight in a vacuum oven to afford 1-benzhydryl-5-nitropyrimidine-2,4(1*H*,3*H*)-dione (43.5 g, 84.5%) as a beige solid: LCMS (t_R = 0.87 min., purity = 100%, MS ES$^+$ *m/z* 322.0 (M-H)$^-$; ^{1}H NMR (400

MHz, DMSO-d_6) δ 12.12 (s, 1H), 8.32 (s, 1H), 7.51 – 7.29 (m, 7H), 7.29 – 7.14 (m, 4H), 6.88 (s, 1H).

Ethyl (*S*)-2-(3-benzhydryl-5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)propanoate (23)

To a stirred suspension of **22** (14.8 g, 45.9 mmol, 1.00 eq.) and potassium carbonate (6.66 g, 48.2 mmol, 1.05 eq.) in MeCN (115 ml) à r.t., was added (*R*)-2-trifluoromethanesulfonyloxypropionic acid ethyl ester (9.00 ml, 48.2 mmol, 1.05 eq.) over 10 minutes and the reaction mixture was agitated at room temperature for a further 2 hours. The reaction mixture was diluted with EtOAc (500 ml) and washed with water (3 x 50 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by chromatography (silica gel, gradient of 0-30%) EtOAc in heptane) to afford (*S*)-2-(3-benzhydryl-5-nitro-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-yl)-propionic acid ethyl ester (12.50 g, 64.31 %): LCMS (t_R = 1.14 min., purity = 100%, MS ES^+ *m/z* 424.0 $(M+H)^+$, 446 $(M+Na+)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 7.56 – 7.24 (m, 11H), 7.00 (s, 1H), 5.43 (q, *J* = 6.9 Hz, 1H), 4.01 – 3.83 (m, 2H), 1.44 (d, *J* = 6.9 Hz, 3H), 0.92 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.82, 153.41, 148.61, 145.80, 136.56, 136.18, 129.18, 129.12, 128.98, 128.94, 128.60, 128.40, 125.10, 65.59, 60.85, 50.01, 13.63, 13.50; HRMS (ESI) calculated for $C_{22}H_{21}N_3O_6$ [M+H]+ 424.14303; found, 424.1498, 446.1319 $[M+Na]^+$.

Ethyl (*S*)-2-(5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)propanoate (24)

23 (12.4 g, 29.3 mmol, 1.00 eq.) was dissolved in a mixture trifluoroacetic acid (124 ml) and methanesulfonic acid (124 ml) and stirred for 2 days at r.t. The reaction mixture was added to a mixture of NaOH (1N) and ice until pH of 6 was achieved. The reaction mixture was extracted with EtOAc (200 ml) and washed with water (3 x 20 ml), dried over $MgSO_4$ and concentrated to

dryness to afford (S)-2-(5-nitro-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-propionic acid ethyl ester (7.40 g, 98%) as a yellow residue: LCMS (t_R = 0.82 min., purity = 100%, MS ES^+ *m/z* 256.1 $(M-H)^-$; ^1H NMR (400 MHz, DMSO-d_6) δ 13.2-12.5 (br s, 1H), 8.97 (s, 1H), 5.38 (q, *J* = 6.9 Hz, 1H), 4.29 – 3.88 (m, 2H), 1.44 (d, *J* = 6.9 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H).

1-Ureidocyclopropane-1-carboxylic acid (25)

To a stirred suspension of the 1-amino-cyclopropanecarboxylic acid (120 g, 1.18 mol) (1 eq) in water (500 ml) was added potassium isocyanate (150 g, 1.85 mol) and the reaction mixture was heated to reflux for 3 hours, cooled to 0-5 °C and the pH was adjusted to 2 with the addition of concentrated HCl [CARE: vigorous evolution of carbon dioxide]. The resulting white precipitate was collected by filtration, washed with ice cold water (250 ml), acetone (3 x 250 ml) and dried to a constant weight to afford the desired urea intermediate (134 g, 78%) as a white solid without the need for further purification: LCMS (t_R = 0.22 min., MS ES^+ *m/z* 144.94 $(M+H)^+$, 166.99 $(M+Na)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 6.61 (s, 1H), 5.55 (s, 2H), 1.26 (q, *J* = 4.2 Hz, 2H), 0.93 (q, *J* = 4.2 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.80, 158.71, 51.96, 17.47.

Allyl 1-ureidocyclopropane-1-carboxylate (26)

To a stirred suspension of **25** (10 g, 69 mmol) and *N,N*-diisopropylethylamine (14.3 ml, 83 mmol) in THF-H_2O (10:1; 110 mL), was added allyl bromide (7.2 mL, 83 mmol). The reaction mixture was heated to 60 °C, stirred overnight, and concentrated to about 35 mL. A few drops of NH_4Cl were added followed by water to dissolve solids. The aqueous phase is extracted with EtOAc (100 ml). The aqueous phase is further acidified to pH 2-3 with HCl 37% and again extracted with EtOAc (3 x 100 mL). The combined organic layers are dried (MgSO4) and concentrated to dryness to afford the title compound (10.2 g, 79.6% yield) as a white powder containing 6% wt DIPEA salts: MS ES+ m/z 184.98 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ

6.66 (s, 1H), 5.88 (ddt, *J* = 17.3, 10.3, 5.0 Hz, 1H), 5.58 (s, 2H), 5.39 – 5.11 (m, 2H), 4.53 (dt, *J* = 5.0, 1.7 Hz, 2H), 1.33 (q, *J* = 4.3 Hz, 2H), 1.02 (q, *J* = 4.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.97, 158.73, 132.65, 117.10, 64.66, 33.49, 17.51; HRMS (ESI) calculated for $C_8H_{12}N_2O_3$ [M+H]+ 185.0848, found 185.0921.

Allyl (*E/Z*) 1-(3-(3-ethoxy-2-nitro-3-oxoprop-1-en-1-yl)ureido)cyclopropane-1-carboxylate (27)

To a stirred solution of **26** (14.2 g, 0.07 mol) in toluene (140 ml) at r.t., was added ethyl nitroacetate (11.8 ml, 0.11 mol) and triethyl orthoformate (17.7 ml, 0.11 mol). The resulting suspension was heated at 95 °C to reflux for 16 h. The solution was concentrated to dryness triturated with Et_2O-heptane (1:1, 100 ml) and the resulting precipitate was collected by filtration, washed with Et_2O and dried to constant weight to afford allyl (*E/Z*) 1-(3-(3-ethoxy-2-nitro-3-oxoprop-1-en-1-yl)ureido)cyclopropane-1-carboxylate (22.3 g, 92%) as yellow solid in a E/Z ratio of ~7/3: MS ES$^+$ m/z 344.14 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (d, *J* = 13.4 Hz, 0.7 H), 10.26 (d, *J* = 13.1 Hz, 0.3 H), 8.88 (d, *J* = 13.1 Hz, 0.3H), 8.80 (s, 0.3H), 8.64 (s, 0.7H), 8.51 (d, *J* = 13.4 Hz, 0.7 H), 4.33 (q, *J* = 7.1 Hz, 0.6H), 4.23 (q, *J* = 7.1 Hz, 1.4H), 1.38 (s, 9H), 1.33 – 1.18 (overlapping t, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.24, 159.93, 152.08, 141.43, 132.42, 119.58, 117.53, 65.24, 61.21, 33.60, 17.11, 14.07; HRMS (ESI) calculated for $C_{13}H_{17}N_3O_7$ $[M+H]^+$ 328.1066, found 328.1137.

Allyl 1-(5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (28)

To a stirred solution of **27** (1.0 g, 3.06 mmol) in acetonitrile (10 ml) at r.t., was added Cs_2CO_3 (2.19 g; 6.72 mmol) and the reaction mixture was heated at reflux for 1 h, cooled to r.t., concentrated to dryness and the residue was dissolved in water (10 mL). The aqueous solution

was washed with TBME (10 ml), cooled and the pH reduced to 2 with concentrated HCl (aq) [CARE: Vigorous evolution of CO_2(g)] and the product was extracted with CH_2Cl_2 (3 x 25 mL). The organic phase was dried ($MgSO_4$) and concentrated to dryness to afford allyl 1-(5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (0.70 g, 81%) without the need for further purification: MS ES^+ m/z 282.02 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H), 8.97 (s, 1H), 5.85 (m, 1H), 5.28 – 5.13 (m, 2H), 4.58 (m, 2H), 1.83 – 1.72 (m, 2H), 1.41 (m, 2H).

(*S*)-2-(((Allyloxy)carbonyl)amino)-4-(tert-butoxy)-4-oxobutanoic acid (29)

To a cooled (4 °C) solution of L-aspartic acid 4-*tert*-butyl ester (25.0 g, 132.1 mmol) in THF/H_2O (80 mL/240 mL) were added sodium bicarbonate (44.4 g, 528.5 mmol) and allyl chloroformate (25.28 mL, 237.8 mmol). After 2 h 30 of stirring at room temperature, the medium was extracted with EtOAc (three times). The aqueous layer was acidified with aqueous HCl (6 N) until obtaining pH 2. The aqueous layer was extracted (EtOAc, three times). The combined organic layer was dried over sodium sulphate, filtered and concentrated in vacuo to afford the title compound (29.2 g, 80%, as pale yellow oil): MS m/z (ES^-) 272.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87-5.96 (m, 1H). 5.74 (bd, *J* = 8.5 Hz, 1H), 5.31 (m, 1H), 5.22 (m, 1H), 4.55-4.65 (m, 3H), 2.97 (dd, *J* = 17.0 Hz and 4.0 Hz, 1H), 2.76 (dd, *J* = 17.0 Hz and 5.0 Hz, 1H), 1.44 (s, 9H).

***tert*-Butyl (*S*)-3-(((allyloxy)carbonyl)amino)-4-(methoxy(methyl)amino)-4-oxo-butanoate (30)**

To a cooled (4 °C) solution of **29** (29.1 g, 106.4 mmol) in DCM (600 mL) were added N,O-dimethylhydroxylamine hydrochloride (12.4 g, 127.8 mmol), 4-methylmorpholine (14.05 mL, 127.8 mmol) and 1,(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (24.5 g, 127.8

mmol). After 3h of stirring at room temperature, the medium was washed with aqueous HCl (0.2 N), with water, with brine, dried over sodium sulphate, filtered and concentrated in vacuo to afford the title compound (29.2 g, 86%, as yellow oil): MS, m/z (ES+) 317.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85-5.93 (m, 1H). 5.59 (bd, *J* = 8.5 Hz, 1H), 5.30 (m, 1H), 5.19 (m, 1H), 5.00 (m, 1H), 4.56 (m, 2H), 3.78 (s, 3H), 3.21 (s, 3H), 2.70 (dd, *J* = 15.0 Hz and 5.5 Hz, 1H), 2.54 (dd, *J* = 15.0 Hz and 6.5 Hz, 1H), 1.43 (s, 9H).

***tert*-Butyl (*S*)-3-(((allyloxy)carbonyl)amino)-4-oxobutanoate (31)**

A solution of lithium aluminium hydride (2 N in THF, 5.93 mL, 11.8 mmol) was added dropwise to a cooled (-78 °C) solution of **30** (5.0 g, 15.8 mmol) in anhydrous THF (65 mL). The mixture was stirred at -78 °C for 2h, then aqueous HCl (1 N) was slowly added to the medium and the temperature was allowed to warm to 0°C. The mixture was diluted with EtOAc. The aqueous layer was extracted (EtOAc, two times). The combined organic layer was washed with aqueous HCl (0.5 N), with brine, dried over sodium sulphate, filtered and concentrated in vacuo to afford the title compound (3.4 g, 83%, as pale pink oil): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.66 (s, 1H), 5.83 (br d, 1H), 5.82-5.99 (m, 1H), 5.33 (m, 1H), 5.24 (m, 1H), 4.61 (m, 2H), 4.36 (m, 1H), 2.96 (dd, *J* = 17.2 Hz and 4.5 Hz, 1H), 2.76 (dd, *J* = 17.2 Hz and 5.1 Hz, 1H), 1.43 (s, 9H).

***tert*-Butyl (*S*)-3-(((allyloxy)carbonyl)amino)-4,4-diethoxybutanoate (32)**

To a freshly prepared solution of **31** (3.40 g, 13.23 mmol) in absolute ethanol (11 mL) were added, under argon atmosphere, triethyl orthoformate (5.62 mL, 33.09 mmol), p-toluenesulfonic acid (45 mg, 0.26 mmol) and 3Å molecular sieves. After stirring at room temperature overnight, the mixture was filtered over a pad of celite and rinsed with EtOH. The filtrate was concentrated under reduced pressure and co-evaporated with toluene (three times). Purification of the residue by flash chromatography on silica gel (cHx-EtOAc 9/1 to 8/2) afforded the title compound (4.05

g, 92%, colorless oil): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83-6.00 (m, 1H), 5.30 (d, *J* = 17.4 Hz, 1H), 5.23 (bd, *J* = 8.5 Hz, 1H), 5.21 (m, 1H), 4.56 (m, 2H), 4.49 (m, 1H), 4.11-4.22 (m, 1H), 3.65-3.78 (m, 2H), 3.48-3.61 (m, 2H), 2.56 (dd, *J* = 15.6 Hz and 5.7 Hz, 1H), 2.45 (dd, *J* = 15.6 Hz and 7.2 Hz, 1H), 1.44 (s, 9H), 1.21 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.12, 155.44, 133.78, 116.66, 102.57, 79.66, 64.16, 63.39, 62.22, 50.44, 36.09, 27.69, 15.15; $[\alpha]_D^{20}$ = -18.3° (*c* = 16 g/L, MeCN); HRMS (ESI) calculated for $C_{16}H_{29}NO_6$ $[M+H]^+$ 331.1995, found 354.1886 $[M+Na]^+$.

Allyl ((3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-yl)carbamate (33)

To a cooled (4 °C) solution of **32** (5.0 g, 15.1 mmol) in DCM (20 mL) was added trifluoroacetic acid (11.6 mL, 151 mmol). After 30 min of stirring at room temperature, the mixture was concentrated under vacuum, then co-evaporated with EtOAc. Purification of the residue by flash chromatography on silica gel (cHx-EtOAc 95/5 to 6/4) afforded the title compound (2.45 g, 71%, as pink oil, two diastereoisomers: 1/1.2): m/z (ES+): 229.9; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85-5.97 (m, 1H) 5.44 (d, *J* = 5.0 Hz, 0.5 H), 5.40 (bs, 0.5 H), 5.22-5.36 (m, 2.5H), 5.00 (br s, 0.5 H), 4.57-4.62 (m, 2H), 4.52-4.57 (br s, 0.5H), 4.20 (br t, *J* = 6.5 Hz, 0.5H), 3.83-3.95 (m, 1H), 3.62-3.69 (m, 1H), 3.01 (dd, *J* = 18.0 Hz and 7.5 Hz, 0.5H), 2.84 (dd, *J* = 17.0 Hz and 8.5 Hz, 0.5H), 2.46 (dd, *J* = 17.0 Hz and 10.5 Hz, 0.5H), 2.39 (dd, *J* = 18.0 Hz and 1.4 Hz, 0.5H), 1.26 (t, *J* = 7.0 Hz, 1.5H), 1.24 (t, *J* = 7.0 Hz, 1.5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.71,173.78, 155.73, 155.56, 133.58, 117.00, 107.23, 102.14, 65.16, 64.60, 52.85, 49.47, 32.89, 31.30, 14.90; HRMS (ESI) calculated for $C_{10}H_{15}NO_5$ [M+H]+ 229.0950, found 230.1023.

***tert*-butyl (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-(methoxy(methyl)amino)-5-oxopentanoate (34)**

To a cooled solution of Fmoc-β-homoaspartic acid (OtBu) (20.0 g, 47.0 mmol) in DCM (268 mL) were added N,O-dimethylhydroxylamine hydrochloride (5.50 g, 56.4 mmol), 4-methylmorpholine (6.2 mL, 56.4 mmol) and 1,(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.8 g, 56.4 mmol). After 2h of stirring at room temperature, the medium was washed with aqueous HCl (1 N, two times), with brine, dried over sodium sulphate**,** filtered and concentrated *in vacuo*. Purification of the residue by chromatography on silica gel (cHx-EtOAc 8/2 to 6/4) afforded the title compound (22.6 g, quantitative): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.57-7.60 (m, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 (td, *J* = 7.5 Hz and 1.0 Hz, 2H), 5.95 (d, *J* = 7.8 Hz, 1H), 4.35-4.40 (m, 3H), 4.21 (m, 1H), 3.70 (s, 3H), 3.21 (s, 3H), 2.94-2.97 (m, 1H), 2.68-2.73 (m, 2H), 2.61 (dd, *J* = 15.7 Hz and 7.0 Hz, 1H), 1.44 (s, 9H).

***tert*-Butyl (*R*)-3-amino-5-(methoxy(methyl)amino)-5-oxopentanoate (35)**

To a cooled (0 °C) solution of **34** (22.6 g, 48.2 mmmol) in DCM (377 mL) was added dropwise DBU (7.92 mL, 5.0 mmol). After 1 h 30 of stirring at room temperature, the medium was concentrated under vacuum. Purification of the residue by chromatography on silica gel (DCM - MeOH/NH_3 (7 N): 97/3 to 95/5) afforded the title compound (8.11g, 68%) as a yellow oil): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.63-3.70 (m, 1H), 3.68 (s, 3H), 3.17 (s, 3H), 2.60-2.64 (m, 1H), 2.52-2.57 (m, 1H), 2.45 (dd, *J* = 15.8 Hz and 5.0 Hz, 1H), 2.39 (dd, *J* = 15.8 Hz and 8.0 Hz, 1H), 1.45 (s, 9H).

***tert*-Butyl (*R*)-3-(((allyloxy)carbonyl)amino)-5-(methoxy(methyl)amino)-5-oxo-pentanoate (36)**

To a cooled (4 °C) solution of **35** (8.11 g, 32.9 mmol) in THF/H_2O (20 mL/60 mL) were added sodium bicarbonate (41.1 g, 131.6 mmol) and allyl chloroformate (6.30 mL, 59.2 mmol). After 1 h 30 of stirring at room temperature, the medium was extracted with EtOAc (three times). The

combined organic layer was dried over sodium sulphate, filtered and concentrated in vacuo. Purification of the residue by chromatography on silica gel (cHx-EtOAc 8/2 to 6/4) afforded the title compound (9.58 g, 88%, as colorless oil): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83-5.93 (m, 2H), 5.29 (d, *J* = 17.3 Hz, 1H), 5.18 (m, 1H), 4.54 (m, 2H), 4.32-4.36 (m, 1H), 3.67 (s, 3H), 3.17 (s, 3H), 2.86-2.91 (m, 1H), 2.65-2.72 (m, 2H), 2.59 (dd, *J* = 15.8 Hz and 6.9 Hz, 1H), 1.44 (s, 9H).

***tert*-Butyl (*R*)-3-(((allyloxy)carbonyl)amino)-5-oxopentanoate (37)**

A solution of lithium aluminium hydride (2 N in THF, 5.90 mL, 11.8 mmol) was added dropwise to a cooled (-78 °C) solution of **36** (6.0 g, 18.1 mmol) in anhydrous THF (80 mL). The mixture was stirred at -78 °C for 2 h 30, then aqueous HCl (1 N) was slowly added to the medium, and the temperature was allowed to warm to 0°C. The mixture was diluted with EtOAc. The aqueous layer was extracted (EtOAc, two times). The combined organic layer was washed with aqueous HCl (1 N), with brine, dried over sodium sulphate, filtered and concentrated in vacuo to afford the title compound (4.7 g, 95%, as pale yellow oil): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (t, *J* = 1.4 Hz, 1H), 5.86-5.94 (m, 1H), 5.48 (br s, 1H), 5.29 (m, 1H), 5.20 (m, 1H), 4.54 (d, *J* = 4.9 Hz, 2H), 4.37-4.40 (m, 1H), 2.83 (dd, *J* = 17.5 Hz and 5.5 Hz, 1H), 2.74 (ddd, *J* = 17.5 Hz, 6.0 Hz and 1.4 Hz, 1H), 2.58 (d, *J* = 5.8 Hz, 2H), 1.44 (s, 9H).

***tert*-Butyl (*R*)-3-(((allyloxy)carbonyl)amino)-5,5-diethoxypentanoate (38)**

To a freshly prepared solution of **37** (4.7 g, 17.3 mmol) in absolute ethanol (20 mL) were added, under Argon atmosphere, triethyl orthoformate (11.53 mL, 69.3 mmol), p-toluenesulfonic acid (90 mg, 0.52 mmol) and 3Å molecular sieves. After stirring at room temperature overnight, the mixture was filtered over a pad of celite and rinsed with EtOH. The filtrate was concentrated under reduced pressure and co-evaporated with toluene (three times). Purification of the residue by flash chromatography on silica gel (cHx-EtOAc 9/1 to 8/2) afforded the title compound (4.85

g, 81%, as pale yellow oil): ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.13 (d, J = 8.8 Hz, 1H), 5.85-5.92 (m, 1H), 5.24-5.27 (m, 1H), 5.14-5.17 (m, 1H), 4.44-4.47 (m, 3H), 3.84-3.90 (m, 1H), 3.50-3.59 (m, 2H), 3.37-3.43 (m, 2H), 2.28-2.36 (m, 2H), 1.65 (m, 2H), 1.37 (s, 9H), 1.09 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.97, 155.13, 133.83, 116.63, 100.06, 79.73, 64.09, 61.11, 60.48, 45.22, 41.12, 38.26, 27.67, 15.29; $[\alpha]_D^{20}$= +13.4° (c = 10 g/L, EtOH); HRMS (ESI) calculated for $C_{17}H_{31}NO_6$ [M+H]+ 345.2151, found 368.2083 $[M+Na]^+$.

Methyl 2-methyl-4-(naphthalen-2-ylamino)benzoate (39)

A stirred solution of 2-aminonaphthalene (2.38 g, 16.63 mmol, 1.00 eq.), 4-bromo-2-methyl-benzoic acid methyl ester (4.00 g, 17.5 mmol, 1.05 eq.), palladium(II) acetate (373 mg; 1.66 mmol, 0.10 eq.), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (1.92 g, 3.33 mmol, 0.20 eq.) and cesium carbonate (7.04 g, 21.6 mmol, 1.30 eq.) in 1,4-dioxanne (38.10 ml) was degased and purged with nitrogen then heated at 120 °C under microwave irradiation for 30 minutes. The reaction was concentrated to dryness and partitioned between DCM (50 ml) and water (5 ml). The organic phase was retained and the aqueous phase was washed with DCM (3 x 5 ml). The organic phases were combined, washed with water (10 ml), dried over $MgSO_4$ and concentrated to dryness to afford a pale yellow residue. The residue was triturated with a mixture of DCM and heptane to afford a solid that was collected by filtration and dried to a constant weight to afford 2-methyl-4-(naphthalen-2-ylamino)-benzoic acid methyl ester (4.30 g, 89%) as a pale brown solid: LCMS (t_R = 1.35 min., purity = 100%, MS ES^+ m/z 292.01 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.93 – 7.75 (m, 5H), 7.63 (d, J = 2.2 Hz, 1H), 7.44 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 7.40 – 7.29 (m, 2H), 7.05 (dd, J = 8.6, 2.4 Hz, 1H), 7.00 (d, J = 2.3 Hz, 1H), 3.78 (s, 3H), 2.50 (s, 3H expected under DMSO signal).

2-Methyl-4-(naphthalen-2-ylamino)benzoic acid (40)

To a stirred solution of **39** (4.00 g, 13.7 mmol, 1.00 eq.) in a mixture of THF (80.0 ml) and MeOH (20.0 ml), was added lithium hydroxide (1.0 M, 41.2 ml, 41.2 mmol, 3.00 eq.). The reaction mixture was stirred at 50 °C overnight, concentrated to a minimum volume and the residue was partitioned between DCM (30 ml) and water (5 ml). The pH of the aqueous phase was adjusted to 1 by the addition of HCl (aq) (1.0 M) resulting in a thick precipitate that was collected by filtration and dried to a constant weight to afford 2-methyl-4-(naphthalen-2-ylamino)benzoic acid (3.50 g, 92%) as a beige solid: LCMS (t_R = 1.20 min., purity = 100%, MS ES^+ *m/z* 278.02 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 12.19 (br s, 1H), 8.82 (s, 1H), 7.98 – 7.72 (m, 4H), 7.61 (d, *J* = 2.3 Hz, 1H), 7.50 – 7.25 (m, 3H), 7.10 – 6.90 (m, 2H), 2.51 (s, 3H hidden by DMSO signal); ^{13}C NMR (400MHz, DMSO-d_6) δ 168.07, 146.95, 141.82, 139.60, 134.19, 132.88, 129.01, 127.51, 128.96, 126.66, 126.43, 123.72, 120.93, 120.18, 117.95, 112.68, 112.31, 40.07, 22.25; HRMS (ESI) calculated for $C_{18}H_{15}NO_2$ $[M+H]^+$ 278.1102, found 278.1172.

Allyl 1-(3-isopropyl-5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (41)

To a stirred suspension of **28** (4.58 g, 16.3 mmol, 1.00 eq.) and K_2CO_3 (3.38 g, 0.02 mol, 1.50 eq.) in DMF (91.6 ml) at r.t., was added 2-iodopropane (8.15 ml; 81.5 mmol; 5.00 eq.). The reaction mixture was slowly added to water and the product was extracted in EtOAc (3 x 100 ml). The organic phases were combined, washed with water (3 x 50 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, gradient 0-20% EtOAc in heptane) to afford 1-(3-isopropyl-5-nitro-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-cyclopropanecarboxylic acid allyl ester (2.41 g, 46%) as a white solid: LCMS (t_R = 1.03 min., purity = 100%, ES^+ m/z 324.06 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H),

5.97 – 5.69 (m, 1H), 5.31 – 5.05 (m, 2H), 4.79 – 4.68 (m, 1H), 4.58 (ddt, *J* = 8.5, 4.9, 1.7 Hz, 2H), 1.91 – 1.59 (m, 2H), 1.50 – 1.10 (m, 8H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 170.51, 165.59, 149.39, 141.42, 132.32, 124.69, 116.63, 64.86, 47.04, 39.05, 35.46, 20.92, 20.48, 19.53, 19.33.

Allyl 1-(5-amino-3-isopropyl-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (42)

A stirred suspension of **41** (2.41 g, 7.45 mmol, 1.00 eq.), ammonium chloride (0.20 g, 3.73 mmol, 0.50 eq.), iron (1.67 g, 29.8 mmol, 4.00 eq.) in a mixture of EtOH (14.5 ml), THF (14.5 ml) and water (14.5 ml) was heated at 70°C for 1 h. The reaction mixture was cooled to r.t., filtered through a pad of Celite® and the pad of Celite® was washed with EtOH (5 x 5 ml). The filtrate was concentrated to dryness to afford 1-(5-amino-3-isopropyl-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-cyclopropanecarboxylic acid allyl ester (2.31 g, 98%) as an orange gum: LCMS (t_R = 0.85 min., purity = 100%, MS ES$^+$ *m/z* 294.11 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.97 (s, 1H), 5.83 (ddt, *J* = 17.3, 10.7, 4.7 Hz, 1H), 5.28 – 5.03 (m, 2H), 4.69 (hept, *J* = 6.8 Hz, 1H), 4.63 – 4.44 (m, 2H), 4.28 – 3.89 (br s, 2H), 1.71 (m, 2H), 1.26 – 1.08 (m, 8H); ^{13}C NMR (400MHz, DMSO-d_6) δ 170.51, 160.39, 149.39, 132.32, 122.42, 116.63, 115.19, 64.86, 47.04, 39.05, 35.46, 20.92, 20.48, 19.53, 19.33; HRMS (ESI) calculated for $C_{14}H_{19}N_3O_4$ $[M+H]^+$ 293.1375, found 294.1448.

Allyl 1-(3-isopropyl-5-(2-methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydro-pyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (43)

To a stirred solution of **40** (520 mg, 1.88 mmol, 1.10 eq.) and *N*-methylmorpholine (375 µl, 3.41 mmol; 2.00 eq.) in DMF (10.0 ml) at r.t., was added COMU ({[1-Cyano-1-ethoxycarbonyl-meth-(*Z*)-ylideneaminooxy]-morpholin-4-yl-methylene}-dimethylammonium

hexafluorophosphate) (876 mg, 2.05 mmol, 1.20 eq.) and the reaction mixture was stirred at r.t. for 30 minutes. **42** (500 mg, 1.70 mmol, 1.00 eq.) in DMF (5.00 ml) was added and the reaction mixture was heated at 50 °C for 48 h. The reaction mixture was treated with a saturated solution of $NaHCO_3$(aq) and the product extracted with EtOAc (3 x 30 ml). The organic phases were combined and washed with water (3 x 10 ml), dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography (silica gel, gradient of 0-100% EtOAc in heptane) to afford 1-{3-Isopropyl-5-[2-methyl-4-(naphthalen-2-ylamino)-benzoylamino]-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl}-cyclopropanecarboxylic acid allyl ester (850 mg, 90%) as a pale yellow solid: LCMS (t_R = 1.43 min., purity = 100%, MS ES^+ *m/z* 533.22 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.69 (s, 1H), 8.30 (s, 1H), 7.93 – 7.68 (m, 4H), 7.57 (d, *J* = 2.2 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.42 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H), 7.37 – 7.28 (m, 2H), 7.10 – 6.99 (m, 2H), 5.85 (ddt, *J* = 17.3, 10.7, 4.8 Hz, 1H), 5.33 – 5.01 (m, 2H), 4.77 (h, *J* = 6.7 Hz, 1H), 4.70 – 4.43 (m, 2H), 2.43 (s, 3H), 1.84 – 1.64 (m, 2H), 1.55 – 1.20 (m, 8H); ^{13}C NMR (DMSO-d_6) δ 170.24, 159.94, 149.89, 145.30, 140.18, 138.35, 134.26, 132.27, 131.18, 129.61, 128.98, 128.70, 127.50, 126.50, 126.40, 126.19, 123.44, 120.58, 118.16, 116.88, 113.03, 112.76, 111.48, 65.04, 54.95, 48.38, 35.67, 20.93, 20.58, 19.48, 19.22; HRMS (ESI) calculated for $C_{32}H_{32}N_4O_5$ $[M+H]^+$ 553.2372, found, 553.2443.

1-(3-Isopropyl-5-(2-methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydro-pyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylic acid (44)

A stirred solution of **43** (840 mg, 1.52 mmol, 1.00 eq.), 1,3-dimethylbarbituric acid (380 mg, 2.43 mmol, 1.60 eq.) in DCM (42 ml) was degassed and purged with nitrogen and tetrakis(triphenylphosphine)palladium(0) (70.3 mg, 0.06 mmol, 0.04 eq.) was added . The reaction mixture was stirred at r.t. for 45 minutes, concentrated and purified by flash

chromatography (silica gel, gradient of 0-10% MeOH in DCM) to afford 1-{3-isopropyl-5-[2-methyl-4-(naphthalen-2-ylamino)-benzoylamino]-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-yl}-cyclopropane-carboxylic acid (470 mg, 60%) as a beige solid: LCMS (t_R = 1.28 min., purity = 100%, MS ES^+ *m/z* 513.28 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.6 (br s, 1H), 8.93 (s, 1H), 8.69 (s, 1H), 8.28 (s, 1H), 7.92 – 7.70 (m, 3H), 7.57 (d, *J* = 2.2 Hz, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.42 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H), 7.37 – 7.28 (m, 2H), 7.09 – 7.00 (m, 2H), 4.96 – 4.49 (m, 1H), 2.44 (s, 3H), 1.67 (m, 2H), 1.45 – 1.20 (m, 8H).

_N_-(3-(1-(((3*S*)-2-Ethoxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (45)

Step 1: To a stirred solution of **44**, (1.39 g, 2.71 mmol, 1.00 eq.) and *N*-methyl morpholine (358 µl, 3.25 mmol, 1.20 eq.) in a mixture of DCM (13.90 ml) and DMF (13.90 ml), HATU (1.24 g, 3.25 mmol, 1.20 eq.) was added and the reaction mixture was stirred at r.t. for 40 minutes.

Step 2: In parallel, a solution of **33**, (870 mg, 3.80 mmol, 1.40 eq.) and 1,3-dimethylbarbituric acid (593 mg, 3.80 mmol, 1.40 eq.) in a mixture of DCM (9.73 ml) and DMF (6.95 ml) at r.t. was degassed and purged with nitrogen then tetrakis(triphenylphosphine)palladium(0) (125 mg, 0.11 mmol; 0.04 eq.) was added. The reaction mixture was stirred at room temperature for 1 h.

Step 3: The product, (4*S*)-4-amino-5-ethoxydihydrofuran-2(3*H*)-one, from the Alloc deprotection step was added to the pre-activated ester for the first step and the reaction mixture was stirred at room temperature overnight. The reaction mixture was treated with a saturated solution of $NaHCO_3$(aq) and the product extracted with EtOAc (2 x 50 ml). The organic phases

were combined and washed with water (3 x 10 ml), dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography (silica gel, gradient of 0-5% MeOH in DCM) to afford *N*-{3-[1-((*S*)-2-ethoxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-cyclopropyl]-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}-2-methyl-4-(naphthalen-2-ylamino)-benzamide (1.80 g, quant.) as an orange solid (mixture of diastereoisomers): LCMS (t_R = 1.34 min., purity = 100%, MS ES^+ *m/z* 640.20 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ ^1H NMR (400 MHz, DMSO-d_6) δ 9.03 – 8.75 (m, 1H), 8.71 (s, 1H), 8.42 (s, 1H), 7.87 – 7.72 (m, 3H), 7.68 – 7.53 (m, 3H), 7.52 – 7.39 (m, 1H), 7.37 – 7.28 (m, 1H), 7.12 – 7.01 (m, 1H), 5.50 (d, *J* = 6.0 Hz, 0.5H), 5.22 (d, J = 6.0 Hz, 0.5H), 4.77 (m, 0.5H), 4.29 – 4.07 (m, 0.5H), 3.83 – 3.65 (m, 0.5H), 3.61 (overlapping signals, 1H), 2.55 (m, 1H), 2.44 (s, 3H), 2.41 – 2.29 (m, 1H), 1.67 (m, 2H), 1.33-1.16 (overlapping signals, 11H).

***tert*-Butyl (*R*)-5,5-Diethoxy-3-(1-(3-isopropyl-5-(2-methyl-4-(naphthalen-2-ylamino)-benzamido)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxamido)-pentanoate (46)**

Step 1: To a stirred solution **44** (100 mg, 0.20 mmol, 1.00 eq.) and *N*-methylmorpholine (25.7 µl, 0.23 mmol, 1.20 eq.) in a mixture of DCM (700 µl) and DMF (1.00 ml), was added HATU (89.0 mg, 0.23 mmol, 1.20 eq.) and the reaction mixture was stirred at room temperature for 30 minutes.

Step 2: In a separate flask, a solution of **38** (101 mg, 0.29 mmol, 1.50 eq.) and 1,3-dimethylbarbituric acid (45.7 mg, 0.29 mmol, 1.50 eq.) in a mixture of DCM (700 µl) and DMF (500 µl) was degassed and purged with nitrogen then tetrakis(triphenylphosphine)palladium(0) (9.02 mg, 0.01 mmol, 0.04 eq.) was added. The reaction mixture was stirred at r.t. for 30 minutes.

Step 3: The solution from the Alloc deprotection containing *tert*-butyl (*R*)-3-amino-5,5-diethoxypentanoate was added dropwise to the activated ester formed in Step 1 and the resulting solution was stirred for 2 h at r.t. The reaction mixture was diluted with EtOAc (30 ml) and washed with a saturated aqueous solution of $NaHCO_3$. The aqueous phase was washed with EtOAc (2 x 10 ml). The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, gradient of 0-100% EtOAc in heptane) to afford *tert*-butyl (*R*)-5,5-diethoxy-3-(1-(3-isopropyl-5-(2-methyl-4-(naphthalen-2-ylamino)-benzamido)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxamido)-pentanoate (140 mg, 95%) as a yellow solid: LCMS (t_R = 1.44 min., purity = 100%, MS ES^- *m/z* 754.48 $(M-H)^-$; ^1H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, *J* = 6.3 Hz, 1H), 8.72 (s, 1H), 8.42 (d, *J* = 6.3 Hz, 1H), 7.89 – 7.71 (m, 3H), 7.69 – 7.55 (m, 2H), 7.53 – 7.38 (m, 2H), 7.38 – 7.28 (m, 2H), 7.13 – 6.99 (m, 2H), 4.77 (p, *J* = 6.7 Hz, 1H), 4.46 (dd, *J* = 8.1, 3.1 Hz, 1H), 3.49 (overlapping m, 4H), 2.65 – 2.53 (m, 1H), 2.44 (s, 3H), 2.39 – 2.17 (m, 1H), 1.81 – 1.50 (m, 2H), 1.47 – 1.18 (overlapping signals, 17H), 1.10 (m, 6H).

tert-Butyl 1-(5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (47)

To a stirred solution of 1-[3-((*E*)-2-ethoxycarbonyl-2-nitro-vinyl)-ureido]-cyclopropanecarboxylic acid *tert*-butyl ester (22.3 g, 0.06 mol, 1.00 eq.) obtained following the procedures described for **27** in MeCN (223 ml), was added cesium carbonate (42.3 g, 0.13 mol, 2.00 eq.) at r.t. The resulting bright yellow suspension was heated at 120 °C for 1 h 20 minutes, cooled to r.t. and the precipitate was removed by filtration and discarded. The filtrate was concentrated to dryness and the residue was partitioned between DCM (200 ml) and water (20 ml). The pH was adjusted to 3 with a saturated aqueous solution of $NaHSO_4$ and the organic

phase was retained. The aqueous phase was washed with DCM (3 x 20 ml) and the organic phases were combined, washed with water (2 x 20 ml), dried over $MgSO_4$ and concentrated to dryness to afford *tert*-butyl 1-(5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (19.2 g, 99%) as a yellow solid: LCMS (t_R = 0.86 min., purity = 100%, MS ES^- *m/z* 296.26 $(M-H)^-$; ^1H NMR (DMSO-d_6) δ 12.56 (s, 1H), 8.97 (s, 1H), 1.82 – 1.53 (m, 1H), 1.34 (s, 9H), 1.33 – 1.23 (m, 2H); 13C NMR (400 MHz, DMSO-d_6) δ 169.11, 155.02, 149.96, 146.58, 124.93, 81.26, 35.67, 27.51, 18.47, 18.23; HRMS (ESI) calculated for $C_{12}H_{15}N_3O_6$ [M-H]- 296.0960, found 296.0884.

***tert*-Butyl 1-(5-amino-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (48)**

To a stirred solution of **47** (18.1 g, 61 mmol, 1.00 eq.) in AcOEt (181 ml) was added 10% Pd-C (3.89 g, 1.83 mmol, 0.03 eq.). The stirred suspension was degassed and put under 1.5 bars of hydrogen for 22 h. The reaction mixture was filtered through a pad of Celite® and concentrated to dryness. The residue was purified by flash chromatography (silica gel, gradient of 2-5% MeOH in DCM) to afford *tert*-butyl 1-(5-amino-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (5.23 g, 32%) as a beige solid: LCMS (t_R = 0.69 min., purity = 100%, MS ES^+ *m/z* 266.17 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 10.20 (br s, 1H), 6.63 (d, *J* = 4.4 Hz, 1H), 3.98 (s, 2H), 1.75 – 1.45 (m, 2H), 1.35 (overlapping signals, 11H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.81, 161.51, 150.01, 121.61, 115.69, 80.65, 35.33, 27.69, 27.56, 18.48; HRMS (ESI) calculated for $C_{12}H_{17}N_3O_4$ $[M-H]^-$ 266.1219, found 266.1144.

***tert*-Butyl 1-(5-(2-methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydro-pyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (49)**

To a stirred solution of **40** (2.59 g, 9.35 mmol, 1.00 eq.) and *N*-methylmorpholine (1.23 ml, 11.2 mmol, 1.20 eq.) in DMF (25.0 ml) at r.t., was added COMU {[1-Cyano-1-ethoxycarbonyl-meth-(Z)-ylideneaminooxy]-morpholin-4-yl-methylene}-dimethyl-ammonium; hexafluoro phosphate (4.81 g, 11.2 mmol, 1.20 eq.) and the reaction mixture was stirred for 15 min. The resulting solution was added to **48** (2.50 g, 9.35 mmol, 1.00 eq.) in DMF (50 ml) and the reaction mixture was heated at 80 °C for 6 h then at r.t. overnight.

To the resulting orange solution, was added a saturated aqueous solution of $NaHCO_3$ (20 ml) followed by water (500 ml). The precipitate was recuperated by filtration, suspended in EtOAc (20 ml) and re-filtered to afford *tert*-butyl 1-(5-(2-methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydro-pyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylate (3.88 g, 79%) as a pale yellow solid: LCMS (t_R = 1.34 min., purity = 97%, MS ES^+ *m/z* 527.33 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.20 (s, 1H), 8.00 (s, 1H), 7.88 – 7.66 (m, 3H), 7.54 (m, 1H), 7.48 – 7.22 (m, 3H), 7.12 – 6.94 (m, 2H), 2.41 (s, 3H), 1.64 – 1.50 (m, 2H), 1.33 (s, 9H), 1.11 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.53, 160.93, 150.17, 145.24, 140.21, 138.24, 134.27, 129.48, 128.99, 128.69, 127.51, 126.50, 126.40, 126.30, 123.43, 120.57, 118.22, 112.84, 112.48, 111.40, 80.95, 35.53, 27.55, 20.50, 18.34; HRMS (ESI) calculated for $C_{30}H_{30}N_4O_5$ [M+H]+ 527.2216; found, 527.2286.

1-(5-(2-Methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylic acid (50)

49 (2.00 g, 3.80 mmol, 1.00 eq.) was suspended in a 4.0 M solution of HCl in 1,4-dioxane (40.0 ml) and stirred at r.t. for 5 h. The volatiles were removed under nitrogen and the remaining residue was suspended in EtOAc and evaporated to dryness. The gum obtained was triturated with EtOAc, stirred at r.t. overnight and the resulting solid was collected by filtration and dried

to a constant weight in a vacuum oven to afford 1-(5-(2-methyl-4-(naphthalen-2-ylamino)benzamido)-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)cyclopropane-1-carboxylic acid (1.40 g, 78%) as a pale yellow solid that was used without further purification: LCMS (t_R = 1.14 min., purity = 95%, MS ES$^+$ *m/z* 471.21 (M+H)$^+$; ^{1}H NMR (DMSO-d_6) δ 12.6 (br s, 1H) 8.55 (s, 1H), 8.19 (s, 1H), 8.02 (s, 1H), 7.85 – 7.64 (m, 3H), 7.55 (m, 1H), 7.48 – 7.22 (m, 3H), 7.14 – 6.98 (m, 2H), 2.45 (s, 3H), 1.62 – 1.44 (m, 2H), 1.13 (s, 2H).

_N_-(3-(1-(((3*S*)-2-Ethoxy-5-oxotetrahydrofuran-3-yl)carbamoyl)cyclopropyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (51)

Step 1: To a stirred solution of **50** (1.95 g, 4.14 mmol, 1.00 eq.) and DIPEA (1.43 ml, 8.29 mmol, 2.00 eq.) in DMF (40 ml), was added 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide 50% in DMF (3.63 ml, 6.22 mmol, 1.50 eq.) and the reaction mixture was stirred at r.t. for 1 h.

Step 2: In parallel, a solution of **33**, (1.52 g, 6.63 mmol, 1.60 eq.) and 1,3-dimethylbarbituric acid (1.04 g, 6.63 mmol, 1.60 eq.) in a mixture of DCM (19.5 ml) and DMF (19.5 ml) at r.t. was degassed and purged with nitrogen then tetrakis(triphenylphosphine)palladium(0) (192 mg, 0.17 mmol; 0.04 eq.) was added. The reaction mixture was stirred at room temperature for 1 h.

Step 3: The product, (4*S*)-4-amino-5-ethoxydihydrofuran-2(3*H*)-one, from the Alloc deprotection step was added to the pre-activated ester from the first step and the reaction mixture was stirred at room temperature overnight. The reaction mixture was treated with a saturated solution of $NaHCO_3$(aq) and the product extracted with EtOAc (2 x 100 ml). The organic phases were combined and washed with water (3 x 20 ml), dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography (silica gel, gradient of 0-5% MeOH in DCM) to afford *N*-(3-(1-(((3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-

yl)carbamoyl)cyclopropyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methyl-4-(naphthalen-2-ylamino)benzamide (1.82 g, 73%) as an off-white solid (mixture of diastereoisomers): LCMS (t_R = 1.18 min., purity = 98%, MS ES$^+$ *m/z* 598.27 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 6.89 – 8.95 (m, 14H), 5.50 (d, *J* = 5.3 Hz, 0.4H), 5.21 (d, *J* = 5.3 Hz, 0.6H), 4.54 – 4.69 (m, 0.4H), 4.13 – 4.28 (m, 0.6H), 3.51 – 3.81 (m, 2H), 2.42 (s, 3H), 2.31 – 3.07 (m, 2H), 1.64 (m, 2H), 1.10 – 1.30 (m, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.12, 174.04, 170.09, 161.23, 150.22, 145.32, 143.74, 141.87, 140.14, 138.21, 134.26, 129.41, 128.99, 128.71, 127.51, 126.51, 126.40, 123.46, 120.58, 118.24, 113.53, 112.88, 111.47, 107.43, 102.15, 65.47, 64.60, 51.81, 36.37, 32.68, 20.51, 14.88; HRMS (ESI) calculated for $C_{32}H_{31}N_5O_7$ [M+H]+ 598.2223; found, 598.2297.

(*S*)-2-(3-Methyl-5-nitro-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-propionic acid ethyl ester (52)

To a stirred suspension of **24** (7.54 g, 29.3 mmol, 1.00 eq.) and potassium carbonate (4.46 g, 0.03 mol, 1.10 eq.) in DMF (75.4 ml), was added iodomethane (2.01 ml, 0.03 mol, 1.10 eq.) and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was partitioned between EtOAc (200 ml) and water (20 ml). The organic phase was retained and the aqueous phase was extracted with EtOAc (3 x 50 ml). The organic phases were combined, washed with water (3 x 50 ml), dried over $MgSO_4$ and concentrated to dryness to afford a yellow residue that was purified by flash chromatography (silica gel, gradient of 30-60% EtOAc in heptane) to afford (*S*)-2-(3-methyl-5-nitro-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-propionic acid ethyl ester as a white foam: LCMS (t_R = 0.87 min., purity = 100%, MS ES$^+$ *m/z* 272.1 (M+H)$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 5.42 (q, *J* = 6.4 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.50 (s, 3H), 1.44 (d, *J* = 6.4 H, 3H), 1.14 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.95,

153.81, 150.55, 149.30, 124.13, 60.94, 50.02, 13.97, 13.61; HRMS (ESI) calculated for $C_{10}H_{13}N_3O_6$ $[M+H]^+$ 272.0804, found 272.0877.

(*S*)-2-(3-Methyl-5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)propanoic acid (53)

To a stirred solution of **52** (2.43 g, 8.96 mmol, 1.00 eq.) in a mixture of THF (50 ml) and MeOH (5 ml), was added lithium hydroxide monohydrate (1.00 M, 18 mL, 18 mmol, 2.00 eq.). The reaction mixture was heated at 50 °C for 1 h 30. The reaction mixture was partitioned between EtOAc (20 ml) and water (20 ml). The aqueous phase was retained and the pH adjusted to 1-2 with HCl(aq) and the aqueous phase was extracted with EtOAc (3 x 50 ml). The organic phases were combined, washed with water (3 x 10 ml), dried over $MgSO_4$ and concentrated to dryness to afford 2-(3-methyl-5-nitro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)propanoic acid (2.0 g, 92%) as a pale yellow solid: LCMS (t_R = 0.37 min., purity = 100%, MS ES^- *m/z* 242.1 $(M-H)^-$; ^1H NMR (400 MHz, DMSO-d_6) δ 12.4 (br s, 1H), 9.46 (s, 1H) 5.42 (q, *J* = 6.3 Hz, 1H), 3.48 (s, 3H), 1.44 (d, *J* = 6.3 H, 3H); HRMS (ESI) calculated for $C_8H_9N_3O_6$ [M+H]+ 244.04913, found 244.0563.

***N*-((3*S*)-2-Ethoxy-5-oxotetrahydrofuran-3-yl)-2-(3-methyl-5-nitro-2,6-dioxo-3,6-dihydro-pyrimidin-1(2*H*)-yl)propanamide (54)**

Step 1: To a stirred solution of **53** (2.00 g, 8.22 mmol, 1.00 eq.) and pentafluorophenol (1.67 g, 9.05 mmol, 1.10 eq.) in a mixture of DCM (40.0 ml) and DMF (10.0 ml), was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.73 g, 9.05 mmol, 1.10 eq.) and the reaction mixture was agitated at r.t. for 1 h.

Step 2: A stirred solution of **33** (2.55 g, 10.7 mmol, 1.30 eq.) and 1,3-dimethylbarbituric acid (835 mg, 5.35 mmol, 0.65 eq.) in a mixture of DCM (40.0 ml) and DMF (20.0 ml) was degassed and purged with nitrogen then tetrakis(triphenylphosphine)palladium(0) (190 mg, 0.16 mmol,

0.02 eq) was added and the reaction mixture was stirred for 1 h. The resulting solution containing (4*S*)-4-amino-5-ethoxydihydrofuran-2(3*H*)-one was added dropwise to the activated ester formed in Step 1 over 5 minutes. The reaction mixture was stirred overnight, diluted with DCM (50 ml) and washed with water (5 ml), a saturated solution of $NaHCO_3$(aq) (5 ml) and water (3 x 5 ml), dried over $MgSO_4$ and concentrated to dryness to afford the crude product as a pale orange oil. The crude product was purified by flash chromatography (silica gel, gradient of 0-3% MeOH in DCM) to afford (*S)-N*-((S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-2-(3-methyl-5-nitro-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl)-propionamide (1.83 g, 60%) as a beige solid and as a complex mixture of diastereoisomers: LCMS (t_R = 0.76 min., purity = 100%, MS ES^+ *m/z* 371.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 0.6H), 9.40 (s, 0.4H), 5.55 (d, *J* = 5.2 Hz, 0.4H), 5.44 (d, *J* = 5.2 Hz, 0.2H), 5.34 (d, *J* = 1.2 Hz, 0.4H), 5.30 – 5.10 (m, 1H), 4.59 (m, 1H), 4.23 – 3.94 (m, 1H), 3.81 – 3.54 (overlapping m, 2H), 3.48 (s, 3H), 2.97 (m, 1H), 2.70 – 2.55 (m, 1H), 2.33 (m, 1H), 1.44-1.33 (overlapping d, *J* = 6.3 H, 3H), 1.22 – 1.04 (overlapping m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.50, 153.89, 150.45, 149.35, 124.18, 49.96, 37.77, 13.70; HRMS (ESI) calculated for $C_8H_9N_3O_6$ $[M+H]^+$ 244.0491, found 244.0563.

2-(5-Amino-3-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)-*N*-((3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-yl)propanamide (55)

To a stirred solution of **54** (2.18 g, 5.89 mmol, 1.00 eq.) in AcOEt (22 ml) at r.t., was added 10% Pd-C (440 mg, 0.21 mmol, 0.04 eq.) and the resulting suspension was degassed, put under 3 bars of hydrogen and stirred overnight. The reaction mixture was filtered through a pad of Celite® and concentrated to dryness to afford a beige foam. The foam was triturated with diethyl ether and the solid collected by filtration and dried to a constant weight under vacuum to afford 2-(5-amino-3-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)-*N*-((3*S*)-2-ethoxy-5-

oxotetrahydrofuran-3-yl)propanamide (1.61 g, 80%) as a very complex diastereoisomeric mixture that was used without further purification and stored at -19 °C: LCMS (t_R = 0.48 min., 0.54 min, purity = 100%, MS ES^+ *m/z* 341.2 $(M+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ (complex mixture of diastereoisomers) 8.27 – 7.88 (m, 1H), 6.89 (m, *J* = 7.1 Hz, 1H), 5.65 – 5.35 (m, 1H), 5.33 – 5.24 (m, 1H), 5.22 – 4.51 (m, 1H), 4.22 (m, 2H), 3.91 – 3.45 (m, 2H), 3.37 (s, 3H), 3.01 – 2.60 (m, 2H), 1.51 – 1.37 (m, 3H), 1.27 – 1.11 (m, 3H).

General library procedure for P4 pocket exploration

Ethyl acetal 56

To a stirred solution of **55** (1 eq) and DIPEA (1.20 eq) in THF (5 vols) was added either the heteroarylcarbonyl chloride (1.2 eq.) or the pre-activated ester (prepared by adding HATU (1.2 eq.) to a stirred solution of heteroarylcarboxylic acid (1.2 eq.) in THF (5 vols) and *N*-methylmorpholine (1.2 eq.) at r.t. and agitating for 30 minutes prior to addition to the amine). The reaction mixture was stirred for 1-16 h, partitioned between water and EtOAc (20 ml). The organic layer was retained and the aqueous layer washed with EtOAc (2 x 5 ml). The organic phases were combined, washed with water (3 x 5 ml), dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (silica gel, gradient 0-5% MeOH in DCM) to afford the desired compounds usually as solids.

Hemi-acetal 57

Hydrolysis of the ethyl acetal prodrugs was carried out in AcOH-H_2O (1:1, 5 vols) at 40 °C overnight. The volatiles were removed by nitrogen sparging and the residues were triturated with diethyl ether to afford the desired hemiacetal drug molecules usually as solids in sufficient purity for *in vitro* evaluation.

ASSOCIATED CONTENT

Supporting Information

The following files are available free of charge.

- Additional chemical and biological figures and tables (PDF)
- Additional experimental information (PDF)
- Molecular formula strings (CSV)

This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

X-ray crystal structures have been deposited in the Protein Data Bank as follows: compound **6** bound to human Caspase-1, PDB code 6F6R. Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare the following competing financial interest(s): All authors were Nestlé Skin Health R&D, Edelris or Synchrotron Soleil full-time employees at the time this work was carried.

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ABBREVIATIONS

Ac-YVAD-CHO, acetyl-tyrosine-valine-alanine-aspartyl aldehyde peptide; µM, micromolar; AcOH, acetic acid; Alloc , allyloxycarbonyl; amu, atomic mass unit; API, active pharmaceutical ingredient; BEI, binding efficiency index; BSA, body surface area; $CDCl_3$, deuterated chloroform; ChromLogD, chromotographic octanol/water partition coefficient; cHx, cyclohexane; CL_{int}, intrinsic clearance; COMU, 1-cyano-2-ethoxy-2-oxo-ethyliden-aminooxy)-dimethylamino-morpholino-carbenium hexa-fluorophosphate; Cs_2CO_3, cesium carbonate; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; DSC, differential scanning calorimetry; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; Fmoc, fluorenylmethyloxycarbonyl; gem, geminal; GSE, general solubility equation; H2L, hit to lead; H_2O, water; HCl, hydrochloric acid; HPLC, high performance liquid chromatography; IC_{50}, concentration for 50% inhibition; IC_{50}, inhibitory concentration for 50% of cells/enzymes; IL, interleukin; KOCN, potassium cyanate; LC50, lethal concentration for 50% of cells; LCMS, liquid chromatography-mass spectrometry; LipE, lipophilic efficiency; MeCN, acetonitrile; MeOH, methanol; MgSO4, magnesium sulfate; Mo, month; Mw, molecular weight; NHEK, normal human epidermal keratinocytes; NLRP3, NOD-like receptor family, pyrin domain containing 3 ; nM, nanomolar; Pfp , pentafluorophenol; PG, propylene glycol; PIF, phototoxicity irritation factor; PK, pharmacokinetic; SAR, structure-activity relationship; Sol, solubility; Stab, stability; SPR, property-activity relationship; T3P, propylphosphonic anhydride; THF, tetrahydrofuran; TLC, thin layer chromatography; TM,

trademark; TMS, tetramethylsilane; TNF, tumor necrosis factor; UP-LCMS, ultra-perfromance liquid chromatography-mass spectrometry ;

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[27] Aqueous solubilities are determined using a semi-thermodynamic protocol where a mixture obtained from a 10 mM DMSO solution is diluted in pH 7.4 buffered water and is stirred for 24h.

[28] This match pair was an extreme case in our dataset. The average $\Delta pSol_{aq}$ over 4 naphthalene vs quinoxaline matched pairs was 0.08 and the average ΔChromLogD was 1.1. None of matched pairs showed a significantly more aqueous soluble quinoxaline.

29 Drug substance color can be a commercial liability in topical products. For this reason, colorless compounds are highly favored.

30 Photocytotoxicity potential was assessed in a keratinocytes NRU assay adapted from the 3T3 NRU assay described in the OECD guideline 432. Cells were irradiated with UV daily simulation at 6 J/cm^2 and cells viability was determined after 24h exposure with chemicals. The sensitivity of the 3T3 NRU-PT is high and if a compound is negative in this assay it would have a very low probability of being phototoxic in humans. However, a positive result in the 3T3 NRU-PT should not be regarded as indicative of a likely clinical phototoxic risk, but rather a flag for follow-up assessment. For more details, see: OECD, *Test No. 432: In Vitro 3T3 NRU Phototoxicity Test*, OECD Publishing, Paris, 2004.

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35 Average absolute error between extrapolation and measured value was 6.9% with a median of 3.5%.

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40 Stability SPR was not always additive. For this reason, caution was used when constituting the dataset and interpreting the analysis.

41 Albeit counterintuitive at first, picolinamide were in our hands generally more lipophilic than their benzamide counterpart in part due to the intramolecular H-bond between the pyridine lone pair and the carboxamide NH. In the case of our uracils, this can then lead to a higher risk of phototoxicity even in the case of the ortho-substituted **17** since conjugation of the π systems is then restored. For discussions on the role of IMHB in medicinal chemistry, see for example (a) Kuhn, B.; Mohr, P.; Stahl, M. Intramolecular hydrogen bonding in medicinal chemistry. *J. Med. Chem.* **2010**, *53*, 2601-2611. (b) Tardia, P.; Stefanachi, A.; Niso, M.; Stolfa, D. A.; Mangiatordi, G. F.; Alberga, D.; Nicolotti, O.; Lattanzi, G.; Carotti, A.; Leonetti, F.; Perrone, R.; Berardi, F.; Azzariti, A.; Colabufo, N. A.; Cellamare, S. Trimethoxybenzanilide-Based P-glycoprotein

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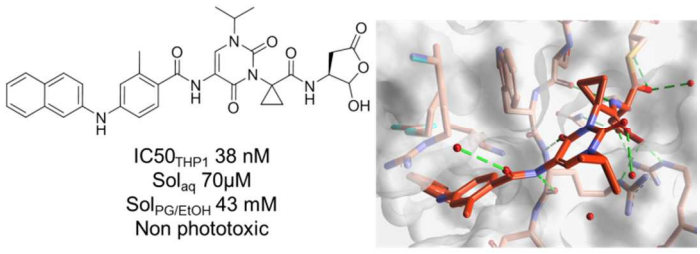

Table of Contents graphic

Keywords: acne, aspartylaldehyde warhead, caspase-1, covalent reversible, phototoxicity, skin, solubility, topical by design