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Discovery of pyrazinone based compounds that potently inhibit the drug-resistant enzyme variant R155K of the hepatitis C virus NS3 protease

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ABSTRACT

Herein, we present the design and synthesis of 2(1*H*)-pyrazinone based HCV NS3 protease inhibitors with variations in the C-terminus. Biochemical evaluation was performed using genotype 1a, both the wild-type and the drug resistant enzyme variant, R155K. Surprisingly, compounds without an acidic sulfonamide retained good inhibition, challenging our previous molecular docking model. Moreover, selected compounds in this series showed nanomolar potency against R155K NS3 protease; which generally confer resistance to all HCV NS3 protease inhibitors approved or in clinical trials. These results further strengthen the potential of this novel substance class, being very different to the approved drugs and clinical candidates, in the development of inhibitors less sensitive to drug resistance.

1. Introduction

Hepatitis C virus (HCV) is regarded a global health problem with around 130–150 million people chronically infected worldwide and approximately 350,000–500,000 related deaths each year.[1,2] 75–85% of those who get infected will develop a chronic infection which could lead to development of cirrhosis, end-stage liver disease and hepatocellular carcinoma if left untreated.[3] The medical treatment has for many years relied on the broad-spectrum antiviral agent ribavirin in combination with pegylated interferon-α (pegIFNα).[4] This therapy is associated with serious adverse effects, and the sustained virological response (SVR) is highly dependent on the genotype of the virus.[4] The SVR is especially low for genotype (Gt) 1 infection (40–50%) and higher for Gt 2 and 3 (80%).[4,5] During the past decade several drug discovery programs have focused on the development of direct-acting antivirals (DAAs) especially directed towards HCV Gt 1 infection.[6] DAAs interfering with various stages of the HCV life cycle, for instance the NS3 protease, the NS5A phosphoprotein and the NS5B polymerase have been developed.[7] In 2011, the first DAAs, the electrophilic HCV NS3 protease inhibitors, boceprevir[8] (Victrelis™) and telaprevir[9] (Incivek™, **1**) were approved for the treatment of HCV Gt 1 infection in combination with ribavirin and pegIFNα. These were followed by two novel drugs being approved in 2013; the product based NS3 protease inhibitor simeprevir[10] (Olysio™, **2**), and the NS5B polymerase inhibitor sofosbuvir[11] (Sovaldi™, **3**), as outlined in Figure 1, both in combination with pegIFNα and ribavirin. These drugs showed less side effects and improved pharmacokinetic properties which enabled attractive dosing regimens.[12,13] Of particular note, the first all oral treatments, without the need for ribavirin or pegIFNα injections, were recently approved: the combination pill, Harvoni™ (ledipasvir[14], NS5A inhibitor/Sofosbuvir **3**), a combination of simeprevir (**2**) and sofosbuvir (**3**) and Viekira Pak™, a combination of three DAAs and ritonavir, i.e., paritaprevir[15] (**4**) (NS3 protease inhibitor), ombitasvir[16] (NS5A inhibitor) and dasabuvir[17] (non-nucleoside NS5B polymerase inhibitor). Thus, the treatment options have been improved drastically for HCV infected patients in recent years. Moreover, there are still many drugs and combinations in the pipeline, including for example several NS3 protease inhibitors[18,19] (e.g., Danoprevir (**5**), Fig. 1).

Despite recent success in drug development within the field, the majority of people infected with HCV is still undiagnosed, which is a problem that remains.[3,13,20,21] Moreover, the high costs of the anti-HCV drugs will limit the access to effective HCV therapies for people living in resource-poor countries.[6,7] Another concern

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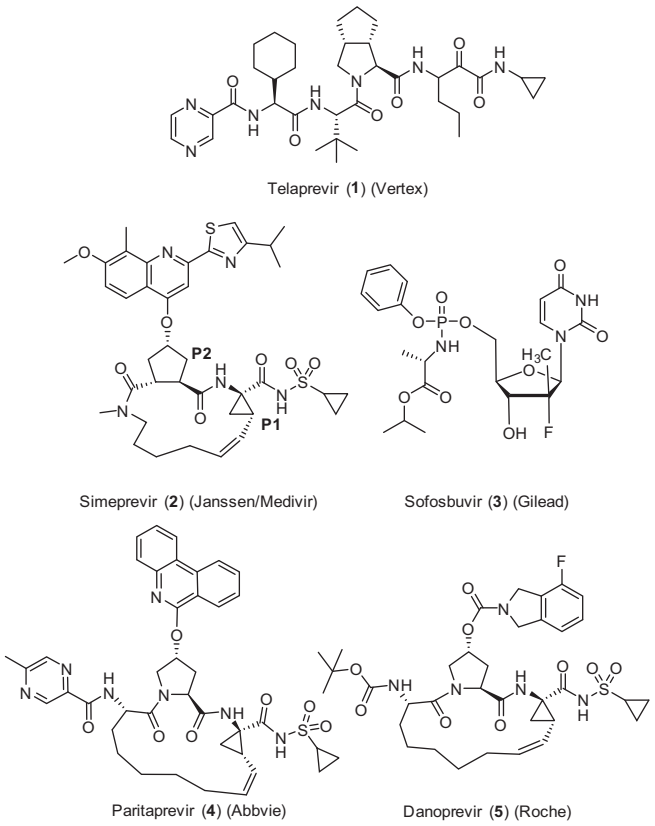


Figure 1. Examples of approved drugs for HCV treatment: the HCV NS3/4a protease inhibitors **1**, **2**, **4**, **5** and the NS5B polymerase inhibitor **3**.

with the highly potent antivirals and an error prone viral polymerase, such as the one in HCV, is the apparent risk for development of drug-resistant enzyme variants.[22] Mutations in the genome corresponding to amino acid positions R155, A156 and D168 in the NS3 protease are common in Gt 1 infected patients and, e.g., R155K/T/Q confer resistance to all NS3 protease inhibitors approved and in clinical trials.[23]

Most of the advanced NS3 protease inhibitors share structural similarities, such as a P2-proline/cyclopentyl in combination with a P1-vinylcyclopropyl/ethylcyclopropyl. In addition they all contain an acyl sulfonamide as a carboxylic acid bioisostere in the C-terminal (P1P1′) and a bulky P2-substituent (**2**, **4** and **5**, Fig. 1). Most of these structural fragments are associated with the cross-resistance observed for the compounds.[24,25] We have previously reported on novel achiral compounds based on a 2(1*H*)-pyrazinone, which inhibit the wild-type NS3 protease as well as drug-resistant enzyme variants (e.g., **6**, Fig. 2).[26] These inhibitors were based on the 2(1*H*)-pyrazinone scaffold encompassing an aromatic P1P1′ substituent in combination with various R^6-substituents (P2) which were presumed to interact with the S2 pocket in the NS3 protease. The improved potency gained by changing a *tert*-butyl carbamate with a *tert*-butyl urea as a P3-capping group was postulated to be related to an internal hydrogen bond between the urea and the 4-*N* on the pyrazinone, stabilizing the bound conformation of the inhibitor.[26]

We herein present synthesis and further development of pyrazinone-based HCV NS3 protease inhibitors as well as biochemical evaluation against Gt 1a, both wild-type and the resistant enzyme variant R155K. Different variations in the R′ (P1P1′) region (Fig. 3), including various regioisomers, substituents, and reversed acyl sulfonamides were studied.

2. Chemistry

A diverse set of R′-substituents were synthesized (Fig. 3), as described in Section 2.1. These building blocks were subsequently combined with P3-pyrazinones with varied R^6 moieties yielding the final inhibitors, as described in Sections 2.2 and 2.3.

2.1. Synthesis of R′-building blocks

The R′-building blocks **12**–**14** (Scheme 1) encompassing a bromo-, fluoro- or trifluoromethyl substituent, were prepared starting from the corresponding commercially available aminobenzoic acids (**7**–**9**) which were *N*-Boc protected using di-*tert*-butyl dicarbonate in the presence of 1 M NaOH in dioxane. The resulting benzoic acids (**12**–**14**) were coupled with 4-(trifluoromethyl) benzene sulfonamide using carbonyl-diimidazole (CDI) and 1,8-diazabicycloundecene (DBU) in dry THF yielding **20**, **23**–**24** (74–80%). The same coupling procedure, but with various regioisomers of the trifluoromethyl- or methylbenzene sulfonamide, was followed in the preparation of the analogs **15**–**19** and **27** (Scheme 1) starting from **10**–**11** and **26** (19–74% yield). The formation of the spirocyclic analog **30** (Scheme 1) began with hydrolysis of the ethyl ester **29**[27,28] by LiOH in THF/H_2O at room temperature producing the carboxylic acid **30** in 93% yield. CDI coupling with 4-(trifluoromethyl) benzene sulfonamide gave the Boc protected building block **31** in excellent yield (89%). Compounds **21** and **22** (Scheme 1) were prepared from **20** in a microwave (MW) assisted Suzuki coupling, using 2,4,6-trivinylcycloboroxane pyridine complex[29] and 5-pyrimidine boronic acid (66% and 48%, respectively). The methylated acyl sulfonamide **25** (Scheme 1) was prepared by treating **15**[30] with methyl iodide and cesium carbonate in dry DMF (47% yield). In order to deprotect the free amine of **15**–**25** and **31** for subsequent amide coupling, the *N*-Boc moieties were removed using 4 M HCl in dioxane yielding the hydrochloride salts of the corresponding amines, while the nitro group in compound **27** was reduced by catalytic hydrogenation to give **28** in 92% yield.

The synthesis of reversed acyl sulfonamides **38**–**40** started with the corresponding nitro benzene sulfonamides **32**–**34**, which were coupled with 4-trifluoromethyl benzoyl chloride, giving intermediates **35**–**37** in moderate yields (45–58%) as depicted in Scheme 2.

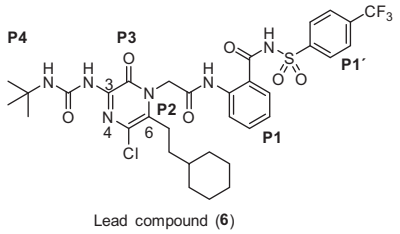


Figure 2. The lead compound (**6**).[26] 2(1*H*)-Pyrazinone based HCV NS3 protease inhibitor, comprising a P3-pyrazinone scaffold, an P1P1′ aromatic acyl sulfonamide and *tert*-butyl urea as a hypothetical P3-capping group.

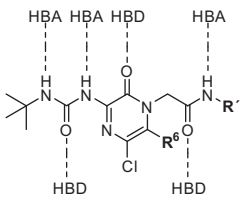
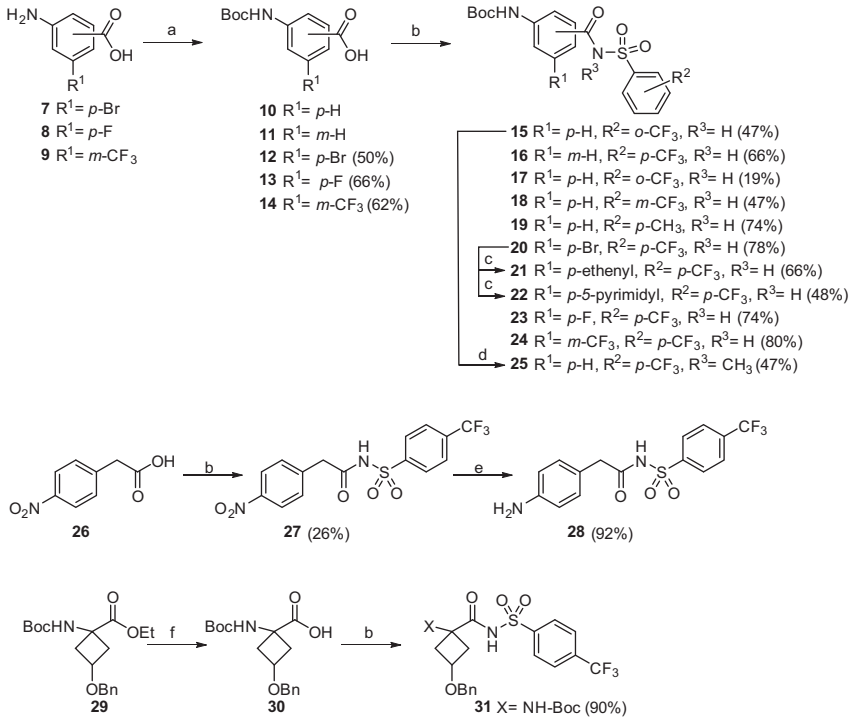


Figure 3. A schematic figure of 2(1*H*)-pyrazinone based HCV NS3 protease inhibitors. Possible hydrogen bond interactions (β-strand) between the peptide backbone and the protease (HBA and HBD) are illustrated.



Scheme 1. Reagents and conditions: (a) Boc_2O, NaOH, dioxane; (b) CDI, THF, 2-CF_3-benzene sulfonamide, 3-CF_3-benzene sulfonamide, 4-CF_3-benzene sulfonamide or 4-CH_3-benzene sulfonamide, DBU, rt; (c) 2,4,6-trivinylcycloboroxane pyridine complex or 5-pyrimidine boronic acid, $Pd(OAc)_2$, $HP(tBu)_3BF_4$, K_2CO_3, H_2O, DME, MW 100 °C, 15 min; (d) Cs_2CO_3, MeI, DMF, 65 °C; (e) Pd/C (10%), H_2, EtOAc, 1 atm, rt, (f) LiOH, H_2O, THF, rt, quant.

The nitro group was next reduced by catalytic hydrogenation, producing the building blocks **38**–**40** in 65–87% yields.

2.2. Amide coupling of R′- substituents to 2(1*H*)-pyrazinone: final inhibitors 6, 46–67

The preparation of final inhibitors **6**, **46**–**67** (Scheme 3, Tables 1 and 2) started with hydrolysis[30] of the benzylester (**41**–**44**) followed by amide coupling using the hydrochloride salts of the R′-building blocks **15**–**25** and **31**, as well as the amines **28**, **38**–**40**, **45**[31] (the corresponding R′ in the final inhibitor **47**) and methyl 2-aminobenzoate, either by using $POCl_3$ (4-89% over 2 steps) or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (11–68% over 2 steps) for activation of the carboxylic acid, as described in Scheme 3.

2.3. Removal of protecting groups: final inhibitors 68–70

The final inhibitors **68**–**70** (Tables 1 and 2) were accomplished as described in Scheme 4. The methylester in **57** as well as the benzyl ester in **44** were hydrolyzed by K_2CO_3 in CH_3CN/H_2O and heated in the microwave reactor at 100 °C for 15 min[30] to give the carboxylic acid derivatives **68** and **69** in 75% and 98% yield, respectively. Compound **66** was dissolved in MeOH and treated with Pd/C under hydrogen gas, at 1 atm and room temperature which yielded compound **70** in 59%. A shorter reaction time was explored to avoid removal of the chlorine, but these attempts were not successful.

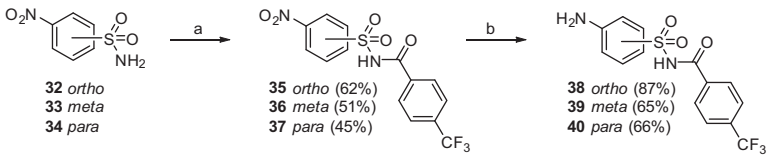
3. Biochemical evaluation

Compounds **6**, **46**–**70** were biochemically evaluated in an in vitro assay using the full-length NS3 1a and the central part of the NS4A as a cofactor (Tables 1 and 2).[32] Moreover, a selected set of inhibitors (**6**, **47**, **53**–**54**, **57**–**61**, **64**–**65** and **68**) were evaluated on the R155K mutant form of the protease (NS3 R155K). The arginine (R) to lysine (K) mutation was introduced by PCR at position 155, and cloned in a similar way as has been described previously.[26,33] The protease activity of the full-length HCV NS3 protein (protease-helicase/NTPase) was measured using a FRET-assay as previously described.[32,34] In short, 1 nM enzyme was incubated for 10 min at 30 °C in 50 mM HEPES, pH 7.5, 10 mM DTT, 40% glycerol, 0.1% *n*-octyl-β-D-glucoside, 3.3% DMSO with 25 μM of the peptide cofactor 2K-NS4A (KKGSVVIVGRIVLSGK) and inhibitor. The reaction was started by the addition of 0.5 μM substrate (Ac-DED(Edans)EEAbuψ-[COO]ASK(Dabcyl)-NH2) obtained from AnaSpec Inc. (San Jose, USA). The non-linear regression analysis was made using Grafit 5.0.8 (Erithacus Software Limited).

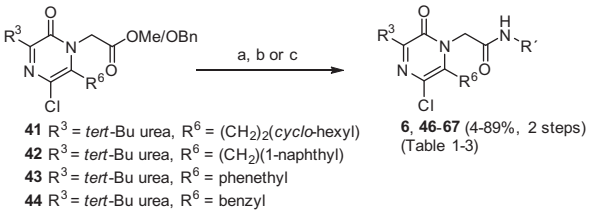
The inhibition measurements were corrected for the compounds' concentration-dependent auto-fluorescent intensities, which were determined for each compound by measuring fluorescence intensities at each tested concentrations. The percentage of correction was calculated by dividing the fluorescence intensity in presence of compound, to fluorescence intensity in the absence of compound and applied to the enzyme's measured initial velocities. The presence of 15 μM DABCYL did not affect the auto-fluorescence of the compounds meaning that no auto-fluorescence quenching could be observed. Inhibition of viral replication (EC_{50}) was determined in a subgenomic HCV replicon assay[35] for compounds **54**, **57** and **68** (Table 3).

4. Modeling

Five structurally different but equipotent inhibitors (**6**, **46**, **47**, **57**, and **68**) were studied in a series of molecular modeling experiments to identify a common binding mode. Initially, the previously proposed binding mode **A**[26] was evaluated by constrained



Scheme 2. Reagents and conditions: (a) 4-pyrrolidino pyridine, pyridine, 4-(CF_3)-benzoyl chloride, toluene, rt, N_2-atm; (b) Pd/C (10%), H_2, EtOAc, 1 atm, rt.



Scheme 3. Reagents and conditions: (a) K_2CO_3, CH_3CN/H_2O, MW 100–120 °C, 15–20 min. (b) (i) **15**–**25** and **31** in 4 M HCl in dioxane, rt, quant. (ii) $POCl_3$, pyridine, −15 °C/rt *or* HATU, DIEA, DCM, 45 °C; (c) **28**, **38**–**40**, **45**[31] and methyl 2-aminobenzoate, $POCl_3$, pyridine, −15 °C/rt, or HATU, DIEA, DCM, 45 °C.

molecular mechanics conformational searches. As illustrated in Figure 4 by compound **6** (orange) with the macrocyclic ligand of 4A92 superimposed (green), the acidic acyl sulfonamide moiety in **6** interacts with the catalytic site (red) of the protease. In addition, the interaction is stabilized by a series of hydrogen bond interactions (Fig. 5) between the ligand and the backbone of βE_2 (R155, A157, and C159).[36] The aryl of the anilide is placed in the hydrophobic S1 pocket whereas the cyclohexyl and *tert*-butyl groups and the aryl on the sulfone are placed in more water exposed regions of the protease. The OPLS3 energy of this pose was 20 kJ/mol higher in energy compared to poses where the anilide has moved out from the S1 pocket and the hydrogen bonds to the anionic group are lost. Thus, the ligand becomes rather strained in Pose **A**. Compounds **46**, **47**, and **57** could not be modeled into this binding mode.

Alternative binding modes were generated by induced fit docking of the five compounds using hydrogen bond constraints to βE_2 (A157 and C159). The obtained poses were clustered based on pairwise RMSD values based on the maximum common substructure, and clusters containing the highest number of different ligands were considered further. This generated three new binding modes of which only one (**B**) formed interactions to the backbone of βE_2 (A157 and C159, see Fig. 5 and Supporting information). Furthermore, both NH groups of the urea interacts with the carbonyl of C159, and the cyclohexyl group is placed in S4 extending slightly to what is known as the allosteric site of the protease usually hosting hydrophobic extensions from P2. The *tert*-butyl group of the urea is pointing towards the C-terminal of helix α18 and is placed in S6.[37]

A third binding mode (**C**, Fig. 6) was constructed by rotating the initially proposed pose **A** 180° around the NH bond of the pyrazine amine (Figs. 5 and 6). This move maintains the key interactions to A157 and C159 and places the anilide moiety towards the exit at the end of the C-terminal of helix α18, and bulk water, with the *ortho*, *meta*, and *para* positions being solvent exposed. The cyclohexylethyl moiety is now inserted in between F438 of the Phe-loop and F531 and W532 of helix α14. Finally, the urea is directed towards the catalytic site and is placed in S3.

Based on the presumptions that the *ortho*, *meta*, and *para* positions of the anilide should be water exposed, that the acid is not required, and that hydrogen bonds to the backbone of βE_2 should be present, several alternative binding modes were discarded (e.g., binding mode **D** and **E**, Supporting information).

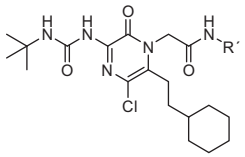
5. Discussion

Our previous SAR studies of this class of NS3 protease inhibitors have focused on the P2 substituent, located at the R^6-position of the pyrazinone.[26] Additionally, a few commonly used α-amino acid based C-terminal building blocks in HCV NS3 protease inhibitors, i.e., an electrophilic α-keto amide, a carboxylic acid and an acyl sulfonamide were evaluated and compared to an aryl acyl sulfonamide substituent (same as in lead compound **6**, Fig. 2), of which the latter was identified as the most attractive moiety based on favorable potency and a less peptide-like character.[26,30] Thus, we felt prompted to study the different optimization possibilities of the new aromatic R′-substituent, having two aryl moieties which synthetically allow for introduction of various substituents and evaluation of different regioisomers.[26,30,35] Hence, inhibitors **6**, **46**–**70** in Tables 1 and 2 were synthesized and evaluated towards the full-length NS3 1a. Moreover, a set of selected inhibitors in the series were evaluated against the drug resistant variant R155K.

To our surprise, compared to the lead compound **6** (K_i = 140 nM), altering the R′-regioisomer into the *meta* **46** (K_i = 170 nM) and *para* **47** (K_i = 260 nM) derivatives did not affect the inhibitory potencies, despite the significant geometrical changes (Table 1). In analogy, it was allowed to move the electron withdrawing trifluoromethyl group to the *ortho* (**48**) and *meta* (**49**) positions with preserved or slightly higher K_i-values (130 and 230 nM, respectively), as well as replacing the CF_3 moiety with an electron donating methyl group in **50** (K_i = 120 nM). These results indicate that no specific interactions exist between the aromatic substituent on the anilide and the enzyme, and that the CF_3-groups possibility to increase acidity of the acyl sulfonamide hydrogen do not improve the potency, which has shown to be the case for other types of NS3 protease inhibitors.[35]

In order to investigate steric and electronic effects by various substituents on the anilide, compounds **51** (K_i = 150 nM), **52** (K_i = 120 nM), **53** (K_i = 120 nM), **54** (K_i = 110 nM) and **55** (K_i = 100 nM) were evaluated (Table 1). Again, it was found that large differences in size and electronic properties had minor influence on the inhibitory potencies. For example, the bulky pyrimidine moiety in **53** (120 nM) was tolerated. The vinyl moiety (**52**, K_i = 120 nM) as well as the electron withdrawing groups in **51** (K_i = 150 nM), **54** (K_i = 110 nM) and **55** (K_i = 100 nM) did not increase the potency, partly questioning the importance of an acidic acyl sulfonamide. Considering the steric bulk tolerable in the position *para* to the carboxylic acid in **53**, the previously proposed binding mode **A** can again be questioned. To investigate the importance of the acid further, the methylated acyl sulfonamide **56** (K_i = 260 nM) was evaluated. Thus, removal of the acidic hydrogen resulted in an almost equipotent inhibitor (**56**) to **6** (K_i = 140 nM), in disparity to previous observations of HCV NS3 protease inhibitors developed by us.[38] Altogether, the flat SAR shown by the R′ modifications discussed above indicated no important contributions from this part. This finding was further supported by comparable and low K_i-values for both the truncated inhibitors having a carboxylic acid in *ortho* position **68** (K_i = 120 nM) and the corresponding methylester **57** (K_i = 120 μM).

Table 1
Inhibitory potencies (K_i) of inhibtors **6**, **46**–**58**, **68**–**69**



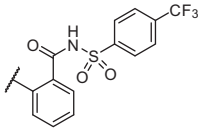
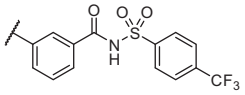
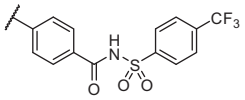
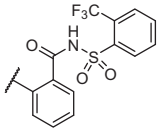
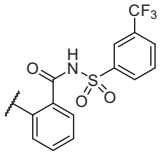
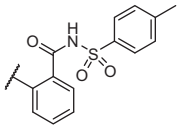
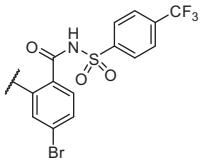
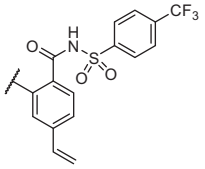
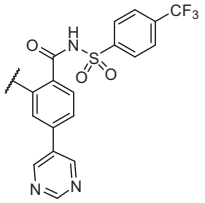
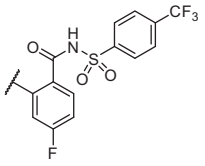
Compound	R′	Full-length NS3 1a $K_i \pm$ SD (nM)	Full-length NS3 1a R155K $K_i \pm$ SD (nM)
6[a]		140 ± 20	30 ± 2
46		170 ± 30	nd
47		260 ± 60	60 ± 8
48		130 ± 20	nd
49		230 ± 50	nd
50		120 ± 40	nd
51		150 ± 30	nd
52		120 ± 20	nd
53		120 ± 20	30 ± 4
54		110 ± 20	20 ± 2

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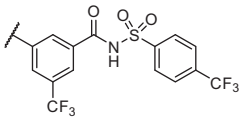
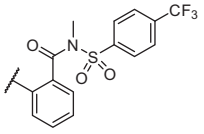
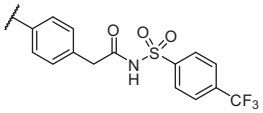
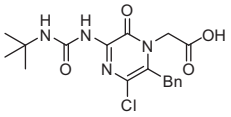
Table 1 (*continued*)

Compound	R′	Full-length NS3 1a K_i ± SD (nM)	Full-length NS3 1a R155K K_i ± SD (nM)
55		100 ± 20	nd
56		260 ± 150	nd
57		120 ± 30	15 ± 2
68		120 ± 30	17 ± 4
58		140 ± 30	24 ± 3
69		>10,000	nd

[a] Previously reported in Gising et al.[26]; SD, standard deviation; nd, not determined.

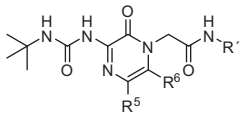
We hypothesized that increased flexibility of the R′-substituent could facilitate further interactions with the enzyme. Therefore, compound **58** (K_i = 140 nM) with a methylene linker was tried (Table 1). However, in comparison to the carboxylic acid and ester **68**/**57** (K_i-values = 120 nM) no additional beneficial effect could be seen. Overall, this led us to questioning the importance of the R′-part, and hence a compound without an R′-moiety was evaluated, which resulted in a drastic decrease in activity (**69**, K_i >10 μM). The drop in activity observed by replacing the anilide with the corresponding carboxylic acid either show that the R′-aryl is required for the binding, or that an acidic group is not tolerated in this part. It could also suggest that the hydrogen bond donor of the acetamide linker could be involved in polar interactions with the protein.

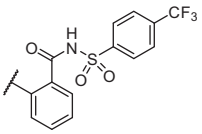
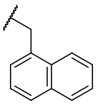
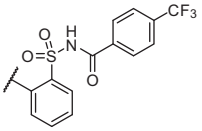
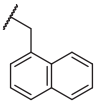
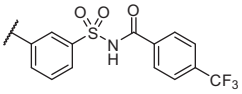
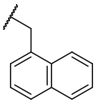
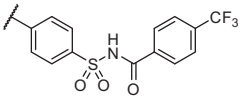
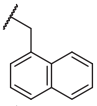
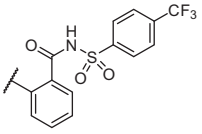
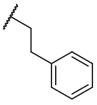
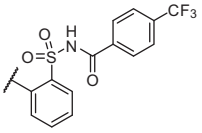
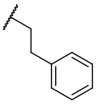
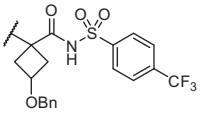
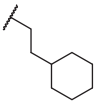
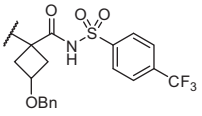
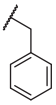
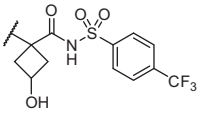
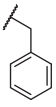
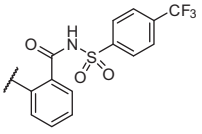
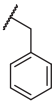
An interesting property of aryl acyl sulfonamides is the possibility to reverse the acyl sulfonamide functionality with retained or slightly altered hydrogen bond pattern of the oxygens of the sulfone and the carbonyl. Therefore, compounds **59**–**64** were prepared and evaluated as presented in Table 2. The varied regioisomers of reversed acyl sulfonamides (**60**, K_i = 180 nM, **61**, K_i = 190 nM and **62**, K_i = 170 nM) showed slightly lower K_i-values compared with the original acyl sulfonamide (**59**, K_i = 310 nM). A better R^6-group (phenethyl) was combined with the reversed acyl sulfonamide (**64**, K_i = 170 nM) showing similar potency as the original acyl sulfonamide (**63**, K_i = 220 nM). These results indicated that a distinct binding to active site and/or the prime side are lacking even in these compounds.

During the course of this investigation we decided to look into non-aryl moiety alternatives to the aryl R′-substituent, which could be advantageous for drug properties.[39,40] We speculated that a spirocyclobutyl group could be useful,[41] with the inherent opportunity to introduce different functionalities to the ring structure in forthcoming SAR studies.[27,28,42] Thus, **65** and **66** (Table 2) were evaluated showing slightly improved potencies (**65**, K_i = 70 nM vs **6**, K_i = 140 nM and **66**, K_i = 150 nM vs **67**, K_i = 270 nM). The lower inhibitory potency for **70** (K_i = 450 nM) indicated either a beneficial interaction from the benzyl group or that a hydroxyl moiety in that position is suboptimal. However, it cannot be ruled out that the lower potency is related to the removal of the C5-Cl in **66**, since we were not able to solely remove the benzyl group. Again, the flat SAR in this region of the inhibitors is indicative of a minor interaction with the enzyme target. However, the relatively low K_i-values for the inhibitors in this series, compared with **1** (15 nM) and the hexapeptide analogue of the N-terminal cleavage product[43] (N-1725, 91 nM), makes these inhibitors interesting for further evaluation.

Thirteen inhibitors, selected from Tables 1 and 2, were evaluated against the known drug-resistant enzyme variant R155K.[23] R155 is positioned in the S2 pocket and is part of a stabilizing salt bridge with D168. An amino acid substitution, e.g., R155K, disrupts the salt bridge, which could lead to drug-resistance for inhibitors with interactions in this area, i.e., inhibitors containing extended P2-substituents.[44] We were very pleased to see that this series in general gave lower K_i-values against the drug resistant variant as compared with the wild-type, with inhibitory potencies between 15 and 90 nM. However, due to the difference in kinetic parameters for the wild-type and mutant variant (see Supporting information), it is not directly translatable into improved potency. The retained potency of this class of inhibitors against the R155K NS3 enzyme compared with inhibitors **1**, **2**, **4**, and **5** (Fig. 1) is indicative of a reduced interaction with the extended S2 site. Inhibitor **60**, containing a reversed acyl sulfonamide in R′, was slightly more potent against the R155K enzyme (30 nM) compared with **59** containing the original R′-moiety (90 nM). Above all, the most intriguing finding was the potency of the R′-truncated inhibitors **57** (K_i = 15 nM) and **68** (K_i = 17 nM) (Table 2), and especially in

Table 2
Inhibitory potencies (K_i) of inhibitors **59**–**67**, **70**, and reference compounds

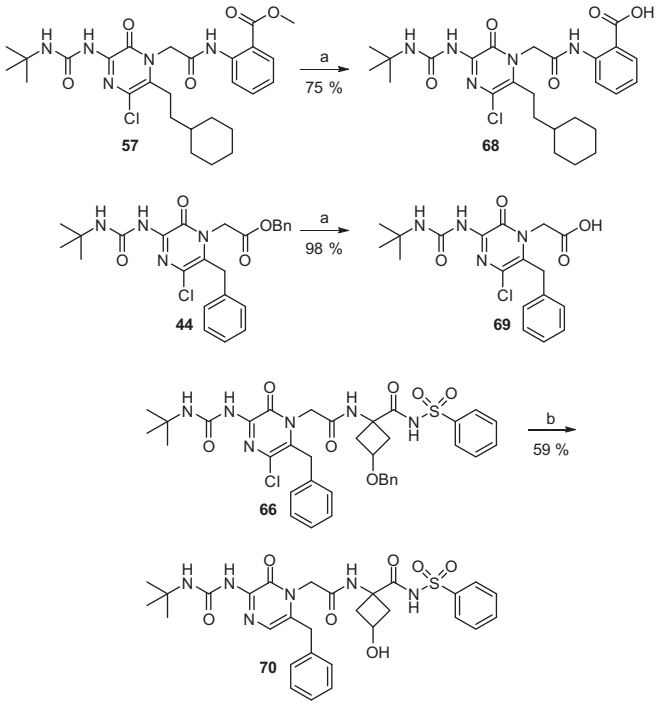


Compound	R′	R^5	R^6	Full-length NS3 1a K_i ± SD (nM)	Full-length NS3 1a R155K K_i ± SD (nM)
59[a]		Cl		310 ± 110	90 ± 10
60		Cl		180 ± 30	30 ± 4
61		Cl		190 ± 36	60 ± 4
62		Cl		170 ± 40	nd
63[a]		Cl		220 ± 60	nd
64		Cl		170 ± 90	40 ± 10
65		Cl		70 ± 10	20 ± 3
66		Cl		150 ± 30	nd
70		H		450 ± 70	nd
67[a]		Cl		270 ± 80	70 ± 7
1				15[b]	82[a]
N-1725[c]				91 ± 21	nd
5				nd	9 ± 1

[a] Previously reported in Gising et al.[26]
[b] Previously reported in Dahl et al.[33]
[c] Ac-Asp-D-Gla-Leu-Ile-Cha-Cys-OH, SD, standard deviation; nd, not determined.

comparison to the clinical inhibitors **1** (82 nM) and **5** (9 nM). These inhibitors were found to be the most active among the evaluated compounds against the R155K enzyme variant.

The flat SAR of the R′-part of the inhibitors challenged our design and previous docking model (Fig. 2 and pose **A** in Figs. 4 and 5). As the pyrazinone inhibitors have not yet been



Scheme 4. Reagents and conditions: (a) K_2CO_3, CH_3CN/H_2O, MW 100 °C, 15 min; (b) Pd/C (10%), H_2, MeOH, 1 atm, rt, 59%.

co-crystalized with the NS3 protein, and that they are very different to the more well-known inhibitors (Fig. 1) an impartial docking study was required in this stage, resulting in a number of alternative models (e.g., **B** and **C**, Figs. 5 and 6 and Supporting information). Except for the *ortho* substituted inhibitors (e.g., **6**), which could be docked into model **A** (Fig. 4) close to the binding poses proved by other advanced inhibitors (e.g., **5**),[45] none of the alternative suggested binding poses occupy the S1–S1′ region. Based on the presumption that the equipotency of the differently substituted compounds is a result of a common productive pose of the shared scaffold (i.e., hydrogen bond interactions between the inhibitors and the backbone of βE2; R155, A157 and C159), the originally proposed pose **A** could be considered less probable due to restrictions in the S1 binding pocket, hosting the aniline moiety (or only valid for the *ortho* analogues). Binding mode **B** (Fig. 5) fulfill the criteria for a flat SAR not too much affected by the substitution regiochemistry at the anilide. However, no significant hydrophobic interactions are made with the target protein. Therefore, the binding mode is not fully compatible with the need for a cyclohexyl ethyl group or other hydrophobic moieties in this region of the molecule.[26] Out of the remaining alternatives, pose **C** is making the best use of the hydrophobic cyclohexyl moiety, embedding this in between the Phe-loop and helix α14 of the helicase (Fig. 6). This pocket is created by a conformational change in the F438 χ_1 angle from gauche to anti and in the F531 χ_1 angle from anti to gauche. If this pose is valid, it could be expected that the inhibitory potency would be dependent on the use of full-length NS3 in the assay, which has been the case in this study. Until a co-crystal structure of this series is produced, compound analogue evaluation to challenge the different hypothetical poses remains the best option. However, one should also bear in mind that the NS3 protease with its comparatively shallow pockets constitute a difficult target for docking studies. Indeed, this is reflected by the many alternative potential binding poses derived in this study.

Table 3
EC_{50} values of inhibitors **54**, **57** and **68** and the reference compounds **1** and **5**

Compound	EC_{50}[a] (μM)	CC_{50}[a] (μM)
54	7.3	38
57	22	>100
68	49	>100
1	0.65	>1
5	0.011	>1

[a] Evaluated in a HCV replicon assay.[35], mean value of two experiments.

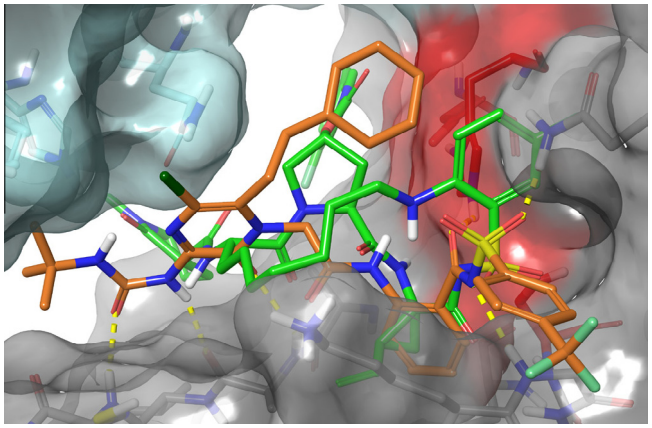


Figure 4. The previously proposed binding mode **A** of **6** (orange) superpositioned with the ligand of 4A92 (green) with the protease shown in gray; the helicase in turquoise; the catalytic triad is highlighted in red. Hydrogen bonds to βE_2 are indicated by yellow dashes.

As discussed above, an advantage of this class of inhibitors is the preserved potency against the R155K enzyme. This is supported by both the alternative binding modes **B** and **C**, suggesting no interactions with the S2 pocket. Thus, inhibitor binding should be less affected by amino acid substitutions in this region.

Three compounds in the series (**54**, **57** and **68**) were evaluated in a cell based replicon assay (Table 3).[35] They all showed detectable inhibitory potencies, with a slight preference for the inhibitor containing an acyl sulfonamide (**54**, 7.3 μM) compared with the truncated analogues, i.e., **57** (22 μM) and **68** (49 μM). Possibly the increased lipophilic character of **54** compared with **57** and **68** could influence cell-permeability positively. The potency range was somewhat expected since a drop in potency going from binding studies to cell-based replicon studies is often seen for HCV protease inhibitors.[46] This is true also for the reference inhibitor **1** (K_i = 15 nM, EC_{50} = 0.65 μM).

6. Conclusion

Dissimilar to the so-called product-based inhibitors[47,48] originating from peptides containing a crucial C-terminal carboxylic acid and in later design, a bioisosteric acyl sulfonamide (**2**, **4** and **5**, Fig. 1) this series had a different starting point; that is the β-sheet mimicking 2(1*H*)-pyrazinone (Fig. 3). Results herein demonstrate that an acidic C-terminal is not decisive for the binding of these inhibitors. Thus, the SAR studies indicate that the aryl sulfonamide probably protrudes out in the solvent. This allowed design of smaller inhibitors, which not only retained potency for the wild-type but were particularly effective against the drug resistant R155K enzyme variant (**57** K_i = 15 nM and **68** K_i = 17 nM). Based on molecular modeling experiments it was suggested that this class of inhibitors probably binds to the HCV NS3 protease in a different manner as compared to the well-known, advanced inhibitors approved or in clinical studies. Therefore, the pyrazinone based HCV NS3 protease inhibitors could be useful for developing coming generation drug candidates that should be active against drug-resistant variants and less prone to resistance development.

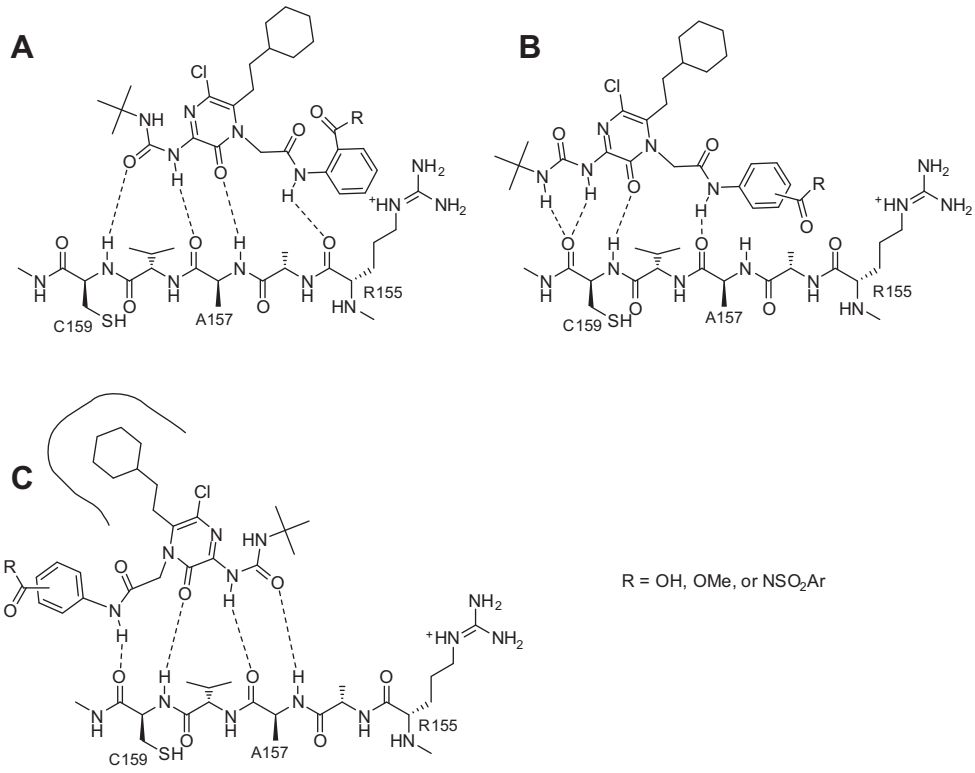


Figure 5. A schematic 2D illustration of the three poses (**A**–**C**) considered. Hydrogen bond interactions to the backbone of βE_2 (R155, A157, and C159) are shown by dashed lines. For pose **C** the hydrophobic pocket is depicted by a solid line.

7. Experimental section

7.1. Chemistry

NMR spectra were recorded on a Varian Mercury Plus at 25 °C for ^{1}H at 399.9 MHz and for ^{13}C NMR at 100.5 MHz. All microwave-assisted syntheses were carried out in a Smith synthesizer performed in sealed vials dedicated for microwave processing. Column flash chromatography was performed using silica gel 60 (particle size 0.040–0.063 mm, Sigma-Aldrich). Thin-layer chromatography was performed with aluminum sheets coated with silica gel 60-F_{254} (0.2 mm E. Merck), using UV-light for visualization. Analytical HPLC-UV/MS was performed on a Dionex UltiMate 3000 HPLC system with a Bruker amaZon SL ion trap mass spectrometer and detection by UV (DAD) and MS (ESI+), using a Phenomenex Kinetex C18 column (50 × 3.0 mm, 2.6 μM particle size, 100 Å pore size) and a flow rate of 1.5 mL/min. A gradient of H_2O/CH_3CN/0.05% HCOOH was used. Preparative RP-HPLC was performed by UV-triggered (254 nM) fraction collection with a Gilson HPLC system using an Agilent PrepHT Zorbax SB C8 column (150 × 21.2 mm, 5 μM particle size) and a H_2O/CH_3CN/0.05% HCOOH gradient at a flow rate of 10 mL/min. High resolution molecular mass (HRMS) was determined on a Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. All products were >95% pure according to HPLC-UV at 254 nM. Optical rotation was obtained on a Rudolph Research Analytical Autopol II Automatic Polarimeter and the concentration *c* is given as g/100 ml in the specified solvent. Reactants and reagents, including **7**–**9**, **10**–**11**, **26**, **32**–**34** that were commercially available were used without further purification. Compounds **6**,[26] **15**,[49] **29**,[27,28] **41**–**44**,[26] **45**,[31] **59**,[26] **63**[26] and **67**[26] are known compounds. Compounds **12**–**14**, **16**–**25**, **27**–**28**, **30**–**31**, **35**–**40**, **46**–**58**, **60**–**62**, **64**–**66** and **68**–**70** are all new compounds and have been fully characterized.

7.1.1. General procedure for preparation of compounds 12–14. Method A

The aminobenzoic acid was dissolved in dioxane followed by addition of 1 M NaOH aq di-*tert*-butyl dicarbonate was dissolved in dioxane and the resulting solution was added, dropwise, to the reaction mixture, which was allowed to stir at room temperature. The progress of the reaction was analysed by analytical LC/MS and found to be slow. After 24 h extra di-*tert*-butyl dicarbonate was added in order to complete the reaction. After another 24–48 h stirring the reaction was worked up. After evaporation of parts of the solvent, 0.1 M NaOH aq was added and the reaction mixture was washed with ether. The product precipitated from the alkaline water phase by dropwise addition of 1 M HCl to pH around 6. The difference of pK_a-values of the product and the starting material was used to obtain good separation.

7.1.2. General procedure for synthesis of compounds 15–20, 23–24, 27 and 30. Method B

All solid chemicals used were dried in vacuum over P_2O_5 overnight. The acid derivative and CDI were dissolved in dry THF under N_2 atmosphere and the mixture was allowed to stir at 66–68 °C for 2 h. The sulfonamide and DBU dissolved in THF were added to the reaction mixture and stirring was continued at room temperature (4 h-overnight).

Method B1: The solvent was removed in vacuo, water was added and pH was adjusted to 2 by addition of 1 M HCl aq. The aqueous phase was extracted with EtOAc (2 × 40 ml), dried with $MgSO_4$, filtered and evaporated in vacuo. For most of the compounds, a silica gel column was first run, followed by purification on aluminum oxide.

Method B2: The solvent was removed in vacuo. The crude material was dissolved in EtOAc or DCM (25 ml), washed with 5% citric acid aq (2 × 15 ml) and brine (15 ml), dried with $MgSO_4$, filtered and evaporated in vacuo. Purification by silica column chromatog-

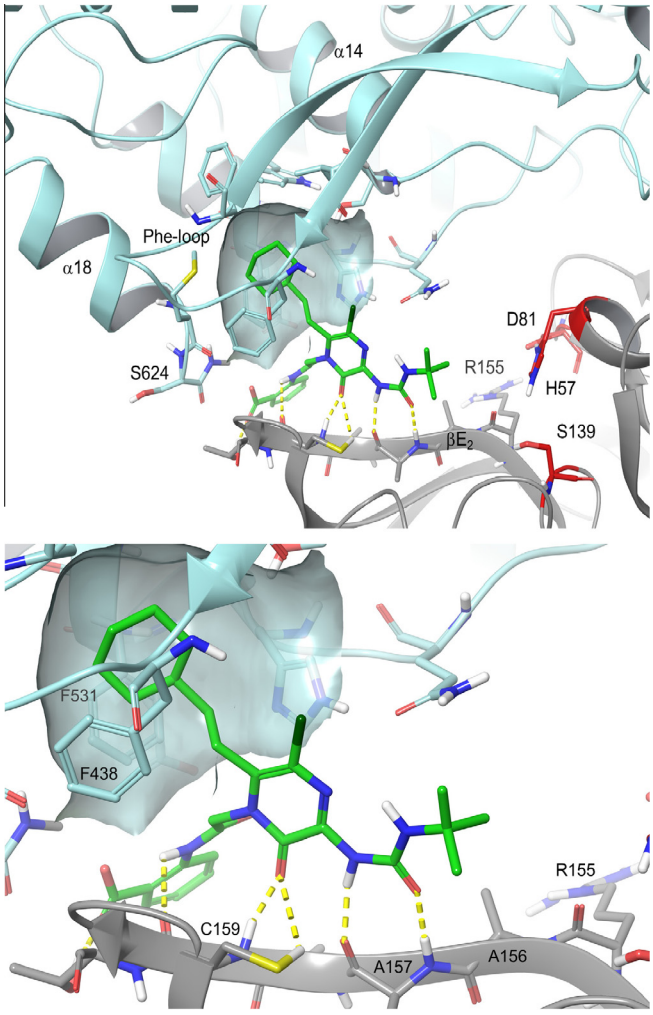


Figure 6. Binding mode **C** illustrated by compound **68** (green). The protease is shown in gray; the helicase in turquoise; in the top view, the catalytic triad is highlighted in red. The hydrophobic pocket in between the Phe-loop and helix α14 is shown as a turquoise surface.

raphy gave the product as a DBU salt. The material was washed with 0.1 N $NaHSO_4$ aq to give the pure product.

7.1.3. General procedure for synthesis of compounds 35–37. Method C

Nitrobenzenesulfonamide, 4-pyrrolidino pyridine and pyridine were mixed and put on an ice bath under N_2 atmosphere. 4-(Trifluoromethyl)benzoyl chloride was dissolved in toluene and added through a syringe to the reaction mixture. The reaction was allowed to stir at room temperature. The reaction was quenched with water, and the solvents were evaporated to yield a yellow oil. Purification by silica column flash chromatography and/or extractions gave the product.

7.1.4. General procedure for boc deprotection of compounds 15–25, and 31. Method D

The R′-building block was dissolved in 4 M HCl in dioxane and stirred at room temperature. When the reaction was complete (LC–MS analysis) the solvent was evaporated in vacuo. The resulting products as hydrochloride salts were dried under vacuum and used in the subsequent coupling reactions without further purification.

7.1.5. General procedure for synthesis of compounds 28 and 38–40. Method E

The R′-building block was dissolved in EtOAc. Pd/C (10%) was added and the reaction mixture was put under a hydrogen atmosphere, at 1 atm, and was allowed to stir at room temperature for 2–2.5 h. The reaction mixture was filtered through a celite plug and the solvent was evaporated to give the product without further purification.

7.1.6. General procedure for preparation of the final inhibitors 6, 46–67. Method F

A 2–5 ml microwave vial was charged with the benzyl/methyl ester of the pyrazinone (**41**–**44**) K_2CO_3, CH_3CN, and H_2O. The vial was capped under air and irradiated by MW to 100–120 °C for 15–20 min.[30] 1 M HCl was added and the mixture was extracted with EtOAc. The organic layers were dried over $MgSO_4$ and evaporated.

Method F1: The crude acid and the amine (free or as the hydrochloride salt) were dissolved in pyridine and cooled to −15 °C under N_2. $POCl_3$ was added and the reaction mixture was stirred at −15 °C to room temperature for 0.5–2 days. In most of the cases, extra $POCl_3$ (reaction on ice/aceton bath) and/or amine was added to consume the pyrazinone-carboxylic acid. H_2O (20 ml) was added and the pH adjusted to 1 using 6.0 M HCl aq followed by extraction with EtOAc (2–3 × 20–30 ml). The organic layer was dried over $MgSO_4$ and evaporated. Purification by silica column flash chromatography gave the product.

Method F2: To the crude acid, the amine (hydrochloride salt) and HATU dissolved in dry DCM, DIEA was added and pH controlled to be 10 or higher. The reaction mixture was allowed to stir at 45 °C for 2–4.5 h. After addition of DCM and washing with 0.1 M $NaHSO_4$ aq the organic layer was evaporated. Purification on silica column gave the final compound.

7.1.7. Compound 12. 4-Bromo-2-((*tert*-butoxycarbonyl)amino) benzoic acid

12 was prepared according to general method A. **7** (2.16 g, 0.01 mol), 1 M NaOH (15 ml, 0.015 mol), dioxane (15 ml), di-*tert*-butyl dicarbonate (3.27 g, 0.015 mol) in dioxane (15 ml). Stirred at room temperature overnight. Di-*tert*-butyl dicarbonate (1.09 g, 0.005 mol) and 1 M NaOH (5 ml) were added. Stirred at room temperature overnight and then worked up. The product precipitated at pH 6.4 (1.586 g, 50%). ^{1}H NMR ($CDCl_3$) δ 10.02 (s, 1H), 8.74 (d, J = 1.8 Hz, 1H), 7.95 (d, J = 8.6 Hz, 1H), 7.18 (dd, J = 8.6, 1.8 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 172.5, 152.6, 143.9, 133.1, 131.2, 124.9, 122.0, 111.9, 81.6, 28.4. MS calcd for $C_{12}H_{14}BrNO_4$ [M+H$^+$]: 316.0 found: 316.1.

7.1.8. Compound 13. 2-((*tert*-Butoxycarbonyl)amino)-4-fluorobenzoic acid

13 was prepared according to general method A using **8** (776 mg 0.005 mol), 1 M NaOH (7.5 ml, 0.0075 mol), dioxane (7.5 ml), di-*tert*-butyl dicarbonate (1.636 g, 0.0075 mol) in dioxane (7.5 ml). Stirred at room temperature overnight and then another portion of di-*tert*-butyl dicarbonate (546 mg, 0.0025 mol) was added. Stirred at room temperature for two days followed by work up. The product precipitated at pH 4.3, (793 mg, 66%). ^{1}H NMR ($CDCl_3$) δ 10.14 (s, 1H), 8.29 (dd, J = 12.2, 2.6 Hz, 1H), 8.13 (dd, J = 9.0, 6.6 Hz, 1H), 6.73 (ddd, J = 9.0, 7.4, 2.6 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 172.4, 167.4 (d, J = 254.3 Hz), 152.6, 145.5 (d, J = 13.5 Hz), 134.6 (d, J = 11.2 Hz), 109.5 (d, J = 2.5 Hz), 109.1 (d, J = 22.7 Hz), 106.2 (d, J = 28.7 Hz), 81.6, 28.4. MS calcd for $C_{12}H_{14}FNO_4$ [M+H$^+$]: 256.1 found: 256.1.

7.1.9. Compound 14. 3-((*tert*-Butoxycarbonyl)amino)-5-(trifluoromethyl)benzoic acid

14 was prepared according to general method A using **9** (250 mg, 0.001 mol), 1 M NaOH (1.5 ml, 0.0015 mol), dioxane (1.5 ml), di-*tert*-butyl dicarbonate (0.327 g, 0.0015 mol) in dioxane (1.5 ml). The reaction mixture was allowed to stir for two days. Di-*tert*-butyl dicarbonate (0.109 g, 0.5 mmol) dissolved in dioxane (0.5 ml) was added and the reaction mixture stirred for another one day and worked up. The product precipitated at pH 6, (188 mg, 62%). ^{1}H NMR (CD_3OD) δ 8.25 (s, 1H), 8.07 (s, 1H), 7.86 (s, 1H), 1.54 (s, 9H). ^{13}C NMR (CD_3OD) δ 168.2, 154.8, 142.2, 134.0, 132.4 (q, J = 32.0 Hz), 125.1 (q, J = 273.4 Hz), 123.6, 120.6 (q, J = 4.1 Hz), 119.4 (q, J = 3.6 Hz), 81.7, 28.6. MS calcd for $C_{13}H_{14}F_3NO_4$ [M+H$^+$]: 306.1 found: 306.2.

7.1.10. Compound 16. *tert*-Butyl(3-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared according to method B1 described in general procedure. **11** (0.593 g, 0.0025 mol), CDI (0.811 g, 0.005 mol), THF (60 ml), 4-(Trifluoromethyl)benzenesulfonamide (1.16 g, 0.005 mol), DBU (0.75 ml, 0.005 mol). Stirred at room temperature overnight. Purification on silica gel (DCM/MeOH 9:1) and aluminum oxide (DCM/HCOOH 100:0 to 99:1) gave **16** in 66% yield (0.734 g). ^{1}H NMR (CD_3OD) δ 8.26 (dm, J = 8.3 Hz, 2H), 7.91 (dm, J = 8.3 Hz, 2H), 7.86 (m, 1H), 7.60 (m, 1H), 7.46 (ddd, J = 7.8, 1.7, 1.1 Hz, 1H), 7.35 (m, 1H), 1.51 (s, 9H). ^{13}C NMR (CD_3OD) δ 168.9, 164.6, 155.2, 145.5, 141.1, 135.6 (d, J = 32.8 Hz), 134.7, 130.1, 130.1, 127.0 (d, J = 3.8 Hz), 124.9 (d, J = 272.1 Hz), 124.1, 123.4, 81.3, 28.6. HRMS calcd for $C_{19}H_{19}F_3N_2O_5S$ [M+H$^+$]: 445.1045; found 445.1049.

7.1.11. Compound 17. *tert*-Butyl(2-(((2-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared according to general method B1. **10** (1.0 g, 0.004 mol), CDI (1.387 g, 0.008 mol), THF (60 ml), 2-(trifluoromethyl)benzenesulfonamide (1.9 g, 0.008 mol), DBU (1.3 ml, 0.008 mol). Stirred at room temperature overnight. Purification on silica gel (DCM/MeOH 9:1) and aluminium oxide (DCM/HCOOH 100:0 to 99:1) gave **17** in 19% yield (0.350 g). ^{1}H NMR (CD_3OD) δ 8.42 (dd, J = 8.0, 1.3 Hz, 1H), 8.14 (dd, J = 8.4, 1.2 Hz, 1H), 8.05 (dd, J = 7.9, 1.8 Hz, 1H), 7.83 (dm, J = 7.6 Hz, 1H), 7.77-7.58 (m, 2H), 7.33 (ddd, J = 8.4, 7.3, 1.7 Hz, 1H), 6.94 (ddd, J = 7.9, 7.2, 1.3 Hz, 1H), 1.47 (s, 9H). ^{13}C NMR (CD_3OD) δ 174.4, 154.7, 143.2, 141.6, 133.1, 133.0, 132.9, 132.3, 128.9 (q, J = 6.3 Hz), 128.8 (q, J = 6.3 Hz), 128.5 (q, J = 33.2 Hz), 124.6 (q, J = 273.7 Hz), 124.0, 122.1, 119.6, 80.8, 28.6. HRMS calcd for $C_{19}H_{19}F_3N_2O_5S$ [M+H$^+$]: 445.1045; found 445.1037.

7.1.12. Compound 18. *tert*-Butyl(2-(((3-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared following general method B1. **10** (1.0 g, 0.004 mol), CDI (1.387 g, 0.008 mol), THF (60 ml), 3-(trifluoromethyl)benzenesulfonamide (1.9 g, 0.008 mol), DBU (1.3 ml, 0.008 mol). Stirred at room temperature overnight. Purification on silica gel (DCM/MeOH 97:3 to 93:3 followed by acetone/*i*-hexane 1:4 to 2:3) gave **18** in 47% yield (0.833 g). ^{1}H NMR ($(CD_3)_2SO$) δ 8.16–8.05 (m, 3H), 8.00 (dd, J = 7.8, 1.7 Hz, 1H), 7.83 (dm, J = 7.6 Hz, 1H), 7.69 (m, 1H), 7.31 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 6.91 (ddd, J = 7.8, 7.2, 1.1 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR ($(CD_3)_2SO$) δ 171.8, 152.4, 146.7, 140.4, 131.3, 130.9, 130.9, 129.4, 128.6 (q, J = 31.8 Hz), 127.0 (q, J = 3.9 Hz), 124.0 (d, J = 272.7 Hz), 123.5 (q, J = 4.0 Hz), 123.0, 120.6, 117.6, 79.1, 28.0. HRMS calcd for $C_{19}H_{19}F_3N_2O_5S$ [M+H$^+$]: 445.1045; found 445.1042.

7.1.13. Compound 19. *tert*-Butyl(2-(tosylcarbamoyl)phenyl)carbamate

Prepared following general method B1. **10** (1.0 g, 0.004 mol), CDI (1.367 g, 0.008 mol), THF (60 ml), 4-methylbenzenesulfonamide (1.44 g, 0.008 mol), DBU (1.3 ml, 0.008 mol). Stirred at room temperature overnight. Purification on silica gel (DCM/MeOH 9:1) and aluminium oxide (DCM/HCOOH 100:0 to 99:1) gave **19** in 74% yield (0.149 g). ^{1}H NMR (CD_3OD) δ 8.10 (dd, J = 8.4, 1.2 Hz, 1H), 7.92 (dm, J = 8.3 Hz, 2H), 7.81 (m, 1H), 7.41 (ddd, J = 8.4, 7.3, 1.6 Hz, 1H), 7.35 (dm, J = 8.3 Hz, 2H), 7.01 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 2.42 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD) δ 154.5, 144.7, 141.4, 140.0, 133.8, 131.0, 130.2, 128.9, 127.2, 122.7, 122.2, 120.4, 81.3, 28.6, 21.5.

7.1.14. Compound 20. *tert*-Butyl(5-bromo-2-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared according to general method B2. **12** (316 mg, 1.0 mmol), CDI (324 mg, 2.0 mmol) in THF (20 ml), 66 °C. 4-(Trifluoromethyl)benzenesulfonamide (450 mg, 2.0 mmol), dissolved in THF (2 ml), DBU (450 µl, 3.0 mmol). Stirred at room temperature for 4 h. Purification on silica column (DCM/MeOH 9:1 to 11:3). In order to remove the DBU salt, the product was dissolved in EtOAc and washed with 0.1 N $NaHSO_4$. The organic layer was evaporated to give the title compound in 78% yield (409 mg). ^{1}H NMR (CD_3OD) δ 8.40 (d, J = 1.9 Hz, 1H), 8.28 (d, J = 8.4 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.6 Hz, 1H), 7.23 (dd, J = 8.5, 2.0 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (CD_3OD) δ 168.5, 153.6, 144.1, 142.8, 136.1 (q, J = 32.8 Hz), 131.6, 130.2, 129.8, 127.4 (q, J = 190.0 Hz), 127.1 (q, J = 3.9 Hz), 125.8, 123.6, 117.5, 82.2, 28.4. MS calcd for $C_{19}H_{18}BrF_3N_2O_5S$ [M+H$^+$]: 523.0 found: 523.1.

7.1.15. Compound 21. *tert*-Butyl(2-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)-5-vinylphenyl)carbamate

20 (83.7 mg, 0.160 mmol), 2,4,6-trivinylcycloboroxane pyridine complex (71.2 mg, 0.16 mmol), $Pd(OAc)_2$ (3.9 mg, 0.016 mmol), HP(tBu)$_3BF_4$ (9.3 mg, 0.0.032 mmol), K_2CO_3 (133 mg, 0.960 mmol), H_2O (1 ml) and DME (3 ml) were added to a microwave vial, under N_2 atmosphere, and irradiated with MW to 100 °C for 15 min. The crude material was filtered and the solvents were evaporated in vacuo. 4 ml H_2O was added and acidified to pH∼1 using 6 M HCl. EtOAc (8 ml) was added and extracted with the aqueous phase. The organic layer was separated and evaporated. Purification on a silica column (DCM/MeOH 93:7) gave the title compound in 66% yield (49.6 mg). ^{1}H NMR (CD_3OD) δ 8.27 (d, J = 1.8 Hz, 1H), 8.15 (d, J = 8.2 Hz, 2H), 8.04 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 8.2 Hz, 2H), 7.00 (dd, J = 1.8, 8.3 Hz, 1H), 6.69 (dd, J = 11.0, 17.7 Hz, 1H), 5.83 (d, J = 17.7 Hz, 1H), 5.31 (d, J = 11.0 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (CD_3OD) δ 174.4, 154.6, 148.5, 142.9, 142.0, 137.8, 134.2 (q, J = 33.5 Hz), 132.8, 128.5, 126.7 (q, J = 3.6 Hz), 125.1 (q, J = 272.5 Hz), 122.8, 119.9, 117.3, 116.3, 81.1, 28.6. MS calcd for $C_{21}H_{21}F_3N_2O_5S$ [M+H$^+$]: 471.1 found: 471.2.

7.1.16. Compound 22. *tert*-Butyl(5-(pyrimidin-5-yl)-2-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

20 (78.5 mg, 0.15 mmol), pyrimidine boronic acid (55.4 mg, 0.45 mmol), $Pd(OAc)_2$ (3.56 mg, 0.015 mmol), HP(tBu)$_3BF_4$ (8.7 mg, 0,03 mmol), K_2CO_3 (124 mg, 0,9 mmol), H_2O (0.95 ml) and DME (2.85 ml) were added to a microwave vial, under N_2 atmosphere, and was heated to 100 °C for 2 h. The crude material was filtered and the solvents were evaporated in vacuo. 4 ml H_2O was added and acidified to pH <2 using 1 M HCl. EtOAc (5 ml) was added and extracted with the aqueous phase. The organic layer was separated and evaporated. Purification on a silica column (*i*-hexane/EtOAc/HCOOH 60:40:2) gave the title compound in 48% yield (37 mg). ^{1}H NMR (CD_3OD) δ 9.15 (s, 1H), 9.05 (s, 2H), 8.49 (d, J = 1.7 Hz, 1H), 8.28 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.36 (dd, J = 1.8, 8.3 Hz, 1H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD) δ 170.0, 158.5, 156.2, 154.1, 145.3, 142.5, 140.0, 135.5 (q, J = 31.7 Hz), 134.8, 131.9, 130.0, 126.9 (q, J = 3.8 Hz),

124.7 (q, J = 270.5 Hz), 121.0, 120.4, 118.8, 81.9, 28.5; MS calcd for $C_{23}H_{21}F_3N_4O_5S$ [M+H$^+$]: 523.1 found: 523.1.

7.1.17. Compound 23. *tert*-Butyl(5-fluoro-2-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared according to the general procedure method B2. **13** (255 mg, 1.0 mmol), CDI (324 mg, 2.0 mmol) in THF (20 ml), 66 °C. 4-(Trifluoromethyl)benzenesulfonamide (450 mg, 2 mmol), dissolved in THF (2 ml), DBU (450 µl, 3 mmol). Stirred at room temperature for 4 h. Purification on silica column (DCM/MeOH 92:8). In order to transfer the DBU salt to the acid form, the product was dissolved in EtOAc and washed with 0.1 N $NaHSO_4$.The organic layer was evaporated to give the title compound in 74% yield (340 mg). ^{1}H NMR (CD_3OD) δ 8.28 (d, J = 8.2 Hz, 2H), 7.99 (dd, J = 12.1, 2.6 Hz, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.82 (dd, J = 9.0, 6.3 Hz, 1H), 6.83 (ddd, J = 8.9, 7.7, 2.6 Hz, 1H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD) δ 168.7, 167.4 (d, J = 251.4 Hz), 153.8, 144.8, 144.6 (d, J = 2.3 Hz), 144.6 (d, J = 2.7 Hz), rotamer, 136.0 (q, J = 33.1 Hz), 133.2 (d, J = 11.1 Hz), 130.4, 127.2 (q, J = 3.8 Hz), 124.8 (q, J = 271.6 Hz), 115.0 (d, J = 2.7 Hz), 109.9 (d, J = 22.9 Hz), 107.5 (d, J = 28.5 Hz), 82.3, 28.4. MS calcd for $C_{19}H_{18}F_4N_2O_5S$ [M+H$^+$]: 463.1 found: 463.1.

7.1.18. Compound 24. *tert*-Butyl(3-(trifluoromethyl)-5-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

Prepared following method B2 in general procedure. **14** (219 mg, 0.72 mmol), CDI (233 mg, 1.43 mmol) in THF (20 ml), 66 °C. 4-(Trifluoromethyl)benzenesulfonamide (322 mg, 1.43 mmol), dissolved in THF (2 ml), DBU (324 µl, 2.16 mmol). Stirred at room temperature for 4 h. After evaporation the crude material was dissolved in DCM and washed repeated times with 0.1 N $NaHSO_4$. The organic phase was evaporated and purified on silica column (DCM/MeOH 88:12) to give the title compound in a yield of 80% (294 mg).^{1}H NMR (CD_3OD) δ 8.15 (d, J = 8.9 Hz, 2H), 8.14 (s, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.75 (d, J = 8.9 Hz, 2H), 1.50 (s, 9H). ^{13}C NMR (CD_3OD) δ 173.1, 154.9, 148.9, 141.5, 140.3, 134.0 (q, J = 32.1 Hz), 131.8 (q, J = 32.3 Hz), 128.6, 126.6 (q, J = 3.8 Hz), 125.3 (q, J = 272.4 Hz), 125.1 (q, J = 272.4 Hz), 123.2, 120.4 (q, J = 3.9 Hz), 118.3, 81.4, 28.6. MS calcd for $C_{20}H_{18}F_6N_2O_5S$ [M+H$^+$]: 513.1 found: 513.2.

7.1.19. Compound 25. *tert*-Butyl(2-(methyl((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)carbamate

To a suspension of **15** (0.25 g, 0.563 mmol) and Cs_2CO_3 (0.275 g, 0.844 mmol) in dry DMF (15 ml), MeI (0.168 g, 0.001 mol) was added. The reaction mixture was allowed to stir at 65 °C for 60 h. The solvent was evaporated and purification by silica column chromatography (*i*-hexane/EtOAc 9:1, followed by a second purification using the same eluent) gave the product as a white solid in 47% yield (0.12 g). ^{1}H NMR (CD_3OD) δ 8.28 (dm, J = 8.2 Hz, 2H), 7.92 (dm, J = 8.2 Hz, 2H), 7.48–7.40 (m, 2H), 7.23–7.16 (m, 1H), 3.40 (s, 3H), 1.20 (s, 9H). ^{13}C NMR (CD_3OD) δ 172.0, 155.3, 144.1, 138.0, 135.9 (q, J = 32.9 Hz), 132.9, 130.7, 129.1, 127.9, 127.2 (q, J = 3.8 Hz), 125.3 (q, J = 272.2 Hz), 124.9, 124.1, 81.1, 37.3, 28.5. HRMS calcd for $C_{20}H_{21}F_3N_2O_5S$ [M+H$^+$]: 459.1202 found; 459.1203.

7.1.20. Compound 27. 2-(4-Nitrophenyl)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)acetamide

Prepared according to the general procedure, method B1. 2-(4-Nitrophenyl)acetic acid (0.5 g, 0.003 mol), CDI (0.671 g, 0.004 mol), THF (20 ml), 4-(Trifluoromethyl) benzenesulfonamide (0.746 g, 0.003 mol), DBU (0.622 ml, 0.004 mol). Stirred at rt overnight. Purification on silica gel (EtOAc/*i*-hexane 1:2 to 1:1 followed by DCM/MeOH 9:1) gave **27** in 26% yield (0.28 g). ^{1}H NMR (CD_3OD) δ 8.15 (dm, J = 8.2 Hz, 2H), 8.11 (d, J = 8.8 Hz, 2H), 7.86 (dm, J = 8.2 Hz, 2H), 7.40 (dm, J = 8.8 Hz, 2H), 3.70 (s, 2H). ^{13}C NMR (CD_3OD) δ 171.5, 148.5, 144.9, 142.7, 135.7 (q, J = 32.8 Hz), 131.6, 129.9, 127.8 (q, J = 3.2 Hz), 124.8 (q, J = 271.9 Hz), 124.4, 43.5. HRMS calcd for $C_{15}H_{11}F_3N_2O_5S$ [M+H$^+$]: 389.0419; found 389.0418.

7.1.21. Compound 28. 2-(4-Aminophenyl)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)acetamide

Prepared following method E in general procedure. **27** (0.08 g, 0.21 mmol) and Pd/C (10%) (22 mg) in EtOAc (10 ml). Filtration and evaporation gave **28** in 92% yield. ^{1}H NMR ($CDCl_3$) δ 8.12 (dm, J = 8.2 Hz, 2H), 7.77 (dm, J = 8.2 Hz, 2H), 6.90 (dm, J = 8.3 Hz, 2H), 6.62 (dm, J = 8.3 Hz, 1H), 3.47 (s, 2H). (EtOAc: 4.12 (q, J = 7.2 Hz), 2.05 (s), 1.26 (t, J = 7.1 Hz)). ^{13}C NMR ($CDCl_3$) δ 169.6, 146.4, 142.0, 135.6 (q, J = 33.2 Hz), 130.5, 129.1, 126.2 (q, J = 3.8 Hz), 125.9 (q, J = 258.3 Hz), 121.6, 115.9, 43.1.; (EtOAc: 60.6, 21.2, 14.3). HRMS calcd for $C_{34}H_{40}ClF_3N_6O_6S$ [M+H$^+$]: 753.2449; found 753.2455.

7.1.22. Compound 30. 3-(Benzyloxy)-1-(((*tert*-butoxycarbonyl)-amino)cyclobutane-1-carboxylic acid

7.1.22.1. Mixture of isomers (isomer A:B 1:1.2). LiOH (0.069 g, 2.88 mmol) dissolved in water (0.5 ml) was added to **29**[28] (*cis*/*trans*) (0.10 g, 0.286 mmol) dissolved in THF (6 ml). The reaction mixture was allowed to stir at rt overnight. 10 ml water was added and the mixture was acidified with 1 M HCl to pH 1, followed by extraction with EtOAc (2 × 10 ml). The organic layer was dried with $MgSO_4$, filtered and evaporated to yield the product (*cis* and *trans* isomers) as a yellow/brown oil, which was not further purified (0.092 g, quant.). ^{1}H NMR ($CDCl_3$) 7.38–7.27 (m, 5H+5H; isomer A+B), 4.46 (s, 2H, isomer B), 4.44 (s, 2H, isomer A), 4.30–4.22 (m, 1H), 4.14–4.22 (m, 2H), 3.05–2.91 (m, 2H), 2.70–2.57 (m, 4H), 2.35–2.23 (m, 2H), 1.43 (s, 9H+9H; isomer A+B). ^{13}C NMR ($CDCl_3$) δ 178.4, 177.1, 157.1, 155.2, 138.0, 137.7, 128.6, 128.6, 128.0, 128.0, 127.9, 127.9, 81.2, 80.9, 70.7, 70.6, 68.5, 68.4, 53.0, 51.9, 40.9, 39.0, 28.5, 28.4.

7.1.23. Compound 31. *tert*-Butyl(3-(benzyloxy)-1-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)cyclobutyl)carbamate

7.1.23.1. Mixture of isomers (isomer A:B 1:2). Prepared following method B1 described in general procedure. **30** (0.092 g, 0.286 mmol), CDI (0.094 g, 0.580 mmol), THF (6 ml), 4-trifluorobenzenesulfonamide (0.131 g, 0.582 mmol), DBU (0.086 ml, 0.578 mmol). Stirred at rt overnight. Purification on silica gel (pentane/EtOAc 2:1-1:2) gave **31** as yellow crystals containing the *cis* and *trans* isomers in 90% yield (0.136 g). ^{1}H NMR ($CDCl_3$) δ 8.22–8.13 (m, 2H+2H, isomer A+B), 7.82–7.75 (m, 2H+2H, isomer A+B), 7.38–7.27 (m, 5H+5H isomer A+B), 4.46 (s, 2H, isomer B), 4.38 (s, 2H, isomer A), 4.25–4.17 (m, 1H, isomer B), 4.02–3.91 (m, 1H, isomer A), 3.01–2.90 (m, 2H, isomer A), 2.78–2.65 (m, 2H, isomer B), 2.40–2.50 (m, 2H, isomer B), 2.06–1.99 (m, 2H, isomer A), 1.50–1.35 (m, 9H+9H; isomer A+B).^{13}C NMR ($CDCl_3$) (Isomer A+B) δ 170.5, 170.5, 155.8, 155.7, 142.2, 142.1, 137.6, 137.0, 135.55 (q, J = 33.4 Hz), 135.57 (q, J = 32.9 Hz), 129.2, 129.1, 128.74, 128.65, 128.3, 128.2, 128.1, 128.0, 126.2 (q, J = 3.8 Hz), 126.2 (q, J = 3.8 Hz), 123.28 (q, J = 272.1 Hz), 123.25 (q, J = 273.0 Hz), 82.6, 81.9, 71.03, 70.93, 68.1, 66.7, 55.3, 53.6, 40.1, 37.7, 28.3, 28.2. HRMS calcd for $C_{24}H_{27}F_3N_2O_6S$ [M+Na$^+$]: 551.1440 found 551.1432.

7.1.24. Compound 35. *N*-((2-Nitrophenyl)sulfonyl)-4-(trifluoromethyl)benzamide

Prepared according to the general procedure method C, using **32** (500 mg, 2.47 mmol), 4-pyrrolidino-pyridine (660 mg, 4.45 mmol), pyridine (10 ml) and benzoyl chloride (441 µl, 2.97 mmol) in 3.5 ml toluene. The reaction mixture was allowed to stir at rt for 3 h. Purification by silica column flash chromatography (DCM/MeOH/HCOOH 100:0:0 to 93:7:3). In order to remove pyridine, the material was dissolved in EtOAc (40 ml) and washed with 5%

citric acid (3 × 40 ml). The organic phase was dried ($NaSO_4$), filtered and evaporated to give the product in 62% yield (575 mg). ^{1}H NMR ($(CD_3)_2SO$) δ 8.10 (dd, J = 1.6, 0.7 Hz, 1H), 8.07 (dm, J = 8.1 Hz, 2H), 7.71 (dm, J = 8.1 Hz, 2H), 7.69-7.59 (m, 3H) (Contains EtOAc). ^{13}C NMR (CD_3OD) δ 173.5, 149.8, 142.7, 137.7, 133.4 (q, J = 32.1 Hz), 133.3, 132.1, 132.0, 130.4, 125.7 (q, J = 3.8 Hz), 125.6 (q, J = 271.4 Hz), 124.5. HRMS calcd for $C_{14}H_9F_3N_2O_5S$ [M+H$^+$]: 375.0263; found 375.0265.

7.1.25. Compound 36. *N*-((3-Nitrophenyl)sulfonyl)-4-(trifluoro methyl)benzamide

Prepared according to the general procedure method C, using **33** (1.03 g, 5.08 mmol), 4-pyrrolidino-pyridine (1.32 g, 8.91 mmol), pyridine (15 ml) and benzoyl chloride (882 µl, 5.93 mmol) in 2 ml toluene. The reaction mixture was allowed to stir at rt overnight. Purification by silica column flash chromatography (DCM/MeOH/HCOOH 100:0:0 to 93:7:3). To remove impurities the material was dissolved in EtOAc (50 ml) and washed with 5% citric acid (3 × 50 ml) and sat. $NaHCO_3$ (aq) (3 × 30 ml). The organic phase was dried ($NaSO_4$), filtered and evaporated to give the product in 51% yield (977 mg). ^{1}H NMR (CD_3OD) δ 8.84 (m, 1H), 8.40-8.32 (m, 2H), 8.14 (dm, J = 7.8 Hz, 2H), 7.73 (ddd, J = 8.3, 7.7, 0.5 Hz, 1H), 7.64 (dm, J = 7.8 Hz, 2H). ^{13}C NMR (CD_3OD) δ 173.6, 149.1, 147.4, 142.6, 134.2, 133.5 (q, J = 32.1 Hz), 130.8, 130.3, 126.6, 125.7 (q, J = 3.8 Hz), 125.5 (q, J = 271.4 Hz), 123.5; HRMS calcd for $C_{14}H_9F_3N_2O_5S$ [M+H$^+$]: 375.0263; found 375.0270.

7.1.26. Compound 37. *N*-((4-Nitrophenyl)sulfonyl)-4-(trifluoro methyl)benzamide

Prepared following method C, using **34** (1.02 g, 5.03 mmol), 4-pyrrolidino-pyridine (1.32 g, 8.91 mmol), pyridine (15 ml) and benzoyl chloride (882 µl, 5.93 mmol) in 2 ml toluene. The reaction mixture was allowed to stir at rt overnight. The solvent was evaporated and the material was dissolved in 50 ml EtOAc and washed with 5% citric acid (3 × 50 ml), sat. $NaHCO_3$ (aq) (3 × 40 ml) and 1 M NaOH (3 × 30 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. To remove the sulfonamide residues, the material was re-dissolved in 50 ml EtOAc and washed with 1 M NaOH (3 × 30 ml). The organic phase was dried, filtered and evaporated to give the product in 45% yield (445 mg). ^{1}H NMR ($(CD_3)_2SO$) δ 8.26 (dm, J = 9.0 Hz, 2H), 8.10–8.02 (m, 4H), 7.68 (dm, J = 8.1 Hz, 2H). (Contains EtOAc). ^{13}C NMR (CD_3OD) δ 173.1, 173.0, 150.5, 141.8, 133.5 (q, J = 32.4 Hz), 130.3, 129.6, 125.7 (q, J = 3.8 Hz), 125.3 (q, J = 271.7 Hz), 124.4. HRMS calcd for $C_{14}H_9F_3N_2O_5S$ [M+H$^+$]: 345.0512; found 345.0515.

7.1.27. Compound 38. *N*-((2-Aminophenyl)sulfonyl)-4-(trifluoro methyl)benzamide

Prepared according to the general procedure method E. **35** (649 mg, 1.73 mmol) and Pd/C (10%) (650 mg) in EtOAc (15 ml), 2.5 h. Filtration and evaporation gave **38** as a colorless oil in 87% yield (521 mg). ^{1}H NMR ($(CD_3)_2SO$) δ 8.08 (dm, J = 8.2 Hz, 2H), 7.68 (dm, J = 8.2 Hz, 2H), 7.58 (dd, J = 7.9, 1.7 Hz, 1H), 7.05 (ddd, J = 8.1, 7.1, 1.7 Hz, 1H), 6.59 (dd, J = 8.1, 1.0 Hz, 1H), 6.49 (ddd, J = 7.9, 7.1, 1.0 Hz, 1H). ^{13}C NMR ($(CD_3)_2SO$) δ 168.3, 145.8, 143.3, 130.8, 129.8 (q, J = 31.1 Hz),129.0, 128.9, 127.3, 124.4 (q, J = 3.7 Hz), 124.4 (q, J = 272.1 Hz), 115.7, 114.1; HRMS calcd for $C_{14}H_{11}F_3N_2O_3S$ [M+H$^+$]: 345.0512; found 345.0519.

7.1.28. Compound 39. *N*-((3-Aminophenyl)sulfonyl)-4-(trifluo romethyl)benzamide

Prepared following method E. **36** (409 mg, 1.09 mmol) and Pd/C (10%) (400 mg) in EtOAc (15 ml), 2 h. Filtration and evaporation gave **39** as an off-white solid (244 mg, 65%). ^{1}H NMR ($(CD_3)_2SO$) δ 8.08 (dm, J = 8.2 Hz, 2H), 7.68 (dm, J = 8.2 Hz, 2H), 7.07 (m, 1H), 7.04–6.94 (m, 2H), 6.58 (ddd, J = 7.7, 2.3, 1.3 Hz, 1H), 5.32 (s, 2H). Contains EtOAc. ^{13}C NMR ($(CD_3)_2SO$) δ 167.9, 148.2, 146.4, 143.2, 129.8 (q, J = 31.1 Hz), 128.9, 128.0, 124.4 (q, J = 3.9 Hz), 124.4 (q, J = 272.0 Hz), 115.3, 114.0, 112.1. HRMS calcd for $C_{14}H_{11}F_3N_2O_3S$ [M+H$^+$]: 345.0512; found 345.0515.

7.1.29. Compound 40. *N*-((4-Aminophenyl)sulfonyl)-4-(trifluoro methyl)benzamide

Prepared according to the general procedure method E using **37** (412 mg, 1.10 mmol) and Pd/C (410 mg) in EtOAc (15 ml), 2 h. Filtration and evaporation gave **40** as a white powder in 66% yield (251 mg). ^{1}H NMR (CD_3OD) δ 8.10 (d, J = 8.2 Hz, 2H), 7.75–7.69 (m, 2H), 7.63 (d, J = 8.2 Hz, 2H), 6.70-6.62 (m, 2H). (Contains EtOAc). ^{13}C NMR (CD_3OD) δ 173.0, 170.0, 153.9, 140.7, 134.0 (q, J = 32.3 Hz), 130.8, 130.1, 126.1 (q, J = 3.8 Hz), 125.4 (q, J = 271.4 Hz), 114.0. HRMS calcd for $C_{14}H_{11}F_3N_2O_3S$ [M+H$^+$]: 345.0512; found 345.0518.

7.1.30. Compound 46. 3-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (58 mg, 0.115 mmol), K_2CO_3 (24 mg, 0.173 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (20 mg, 0.128 mmol), −15 °C, 5 min. The hydrochloride salt of the amine (**16** treated by method D) (66 mg, 0.109 mmol), rt, 3 h. *Extra additions of $POCl_3$:* after 1 h: $POCl_3$ (18 mg, 0.115 mmol), 2 h: $POCl_3$ (9 mg, 0.058 mmol). Purification by silica column flash chromatography. EtOAc/*i*-hexane/HCOOH 20:80:3 to 40:60:3, second column: DCM/MeOH 100:0 to 90:10, gave **46** in 36% yield, 31 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.16 (dm, J = 8.0 Hz, 2H), 8.12 (m, 1H), 7.78–7.75 (m, 3H), 7.70 (ddd, J = 8.1, 2.3, 1.1 Hz, 1H), 7.29 (m, 1H), 4.92 (s, 2H), 2.70 (m, 2H), 1.79–1.57 (m, 6H), 1.42 (s, 9H), 1.37–1.07 (m, 5H), 0.93 (m, 2H); ^{13}C NMR (CD_3OD) δ 174.0, 166.3, 153.9, 152.3, 148.7, 144.9, 139.0, 138.8, 134.1 (d, J = 32.4 Hz), 132.1, 129.6, 128.7, 126.6 (d, J = 3.9 Hz), 126.1, 125.1 (d, J = 271.8 Hz), 124.2, 123.0, 121.7, 52.2, 52.1, 38.9, 36.0, 34.1, 29.2, 28.2, 27.6, 27.3. HRMS calcd for $C_{33}H_{38}ClF_3N_6O_6S$ [M+H$^+$]: 739.2292; found 739.2299.

7.1.31. Compound 47. 4-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (40 mg, 0.080 mmol), K_2CO_3 (16 mg, 0.119 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1.0 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (12 mg, 0.08 mmol), −15 °C, 15 min. **45**[31] (66 mg, 0.109 mmol), rt, 1 h. *Extra addition of $POCl_3$:* after 30 min: $POCl_3$ (12 mg, 0,080 mmol). Purification by silica column flash chromatography. EtOAc/*i*-hexane/HCOOH 20:80:0.5 to 50:50:0.5, second column: DCM/MeOH 95:5 to 90:10, gave **47** in 43% yield, 23 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.21 (dm, J = 8.3 Hz, 2H), 7.91–7.86 (m, 2H), 7.84 (dm, J = 8.3 Hz, 2H), 7.67–7.58 (m, 2H), 4.93 (s, 2H), 2.72 (m, 2H), 1.83–1.57 (m, 5H), 1.52–1.44 (m, 2H), 1.43 (s, 9H), 1.38–1.10 (m, 4H), 0.94 (m, 2H). ^{13}C NMR ($(CD_3)_2SO$) δ 165.1, 165.0, 150.8, 150.7, 150.4, 150.4, 143.4, 142.5, 132.6 (q, J = 31.1 Hz), 130.5, 129.8, 128.6, 126.2 (q, J = 3.4 Hz), 123.5 (q, J = 272.9 Hz), 120.7, 118.2, 50.1, 48.6, 36.6, 34.5, 32.4, 28.6, 26.6, 26.0, 25.6. HRMS calcd for $C_{33}H_{38}ClF_3N_6O_6S$ [M+H$^+$]: 739.2292; found 739.2297.

7.1.32. Compound 48. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((2-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (49 mg, 0.097 mmol), K_2CO_3 (20 mg, 0.146 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (16 mg, 0.104 mmol), −15 °C, 15 min. The hydrochloride salt of the amine (**17** treated by method D) (55 mg, 0.144 mmol), rt, 2 days. *Extra additions of $POCl_3$:* after 16 h, 18 h and 20 h: $POCl_3$ (15 mg, 0.097 mmol). Purification by silica column flash chromatography. EtOAc/pentane/HCOOH 20:80:3 to 40:60:3, followed by a final purification by preparative HPLC gave **48** in 10% yield, 7 mg. ^{1}H NMR (CD_3OD) δ 8.49 (dd, J = 7.3, 1.7 Hz, 1H), 8.11 (dd, J = 8.2, 1.2 Hz, 1H), 7.95 (dd, J = 7.4, 1.8 Hz, 1H), 7.92-7.82 (m, 3H), 7.70 (dd, J = 8.0, 1.5 Hz, 1H), 7.55 (ddd, J = 8.2, 7.5, 1.5 Hz, 1H), 7.23 (m, 1H), 4.86 (s, 2H), 2.71 (m, 2H), 1.76–1.57 (m, 5H), 1.45 (s, 9H), 1.39 (m, 2H), 1.34–1.10 (m, 4H), 0.90 (m, 2H). ^{13}C NMR (CD_3OD) δ 166.4, 153.9, 152.5, 145.2, 139.2, 139.0, 135.2, 135.1, 134.7, 133.5, 131.7, 130.2, 129.3 (q, J = 6.3 Hz), 129.3 (q, J = 6.2 Hz), 129.0 (q, J = 33.1 Hz), 125.4, 124.3 (q, J = 273.3 Hz), 123.5, 123.2, 123.2, 52.1, 50.0, 38.5, 36.0, 4.1, 29.2, 28.1, 27.5, 27.2. HRMS calcd for $C_{33}H_{38}ClF_3N_6O_6S$ [M+H$^+$]: 739.2292; found 739.2303.

7.1.33. Compound 49. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((3-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (90 mg, 0.179 mmol), K_2CO_3 (37 mg, 0.269 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1.0 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (30 mg, 0.196 mmol), −15 °C, 15 min. The hydrochloride salt of the amine (**18** treated by method D) (103 mg, 0.271 mmol), rt, 2 days. *Extra additions of $POCl_3$:* after 2 h, 3 h and 20 h: $POCl_3$ (27 mg, 0.179 mmol). Purification by silica column flash chromatography three times (EtOAc/pentane 1:4 to 1:1, DCM/EtOAc/MeOH 80:20:2 to 80:20:5, EtOAc/pentane 3:7 to 4:1). Final purification by preparative HPLC gave **49** in 4% yield, 6 mg. ^{1}H NMR (CD_3OD) δ 8.39–8.32 (m, 2H), 8.03–7.97 (m, 2H), 7.84 (m, 1H), 7.76 (dm, J = 8.0 Hz, 1H), 7.55 (ddd, J = 8.1, 7.4, 1.4 Hz, 1H), 7.25 (m, 1H), 4.87 (s, 2H), 2.69 (m, 2H), 1.77–1.57 (m, 5H), 1.44 (s, 9H), 1.43–1.38 (m, 2H), 1.37–1.11 (m, 4H), 0.93 (m, 2H). HRMS calcd for $C_{33}H_{38}ClF_3N_6O_6S$[M+H$^+$]: 739.2292; found 739.2286.

7.1.34. Compound 50. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-tosylbenzamide

The title compound was prepared according to method F1. **41** (49 mg, 0.097 mmol), K_2CO_3 (20 mg, 0.146 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 20 min. 1 M HCl (30 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (16 mg, 0.104 mmol), −15 °C, 15 min. The hydrochloride salt of the amine (**19** treated by method D) (47 mg, 0.145 mmol), rt, 3 h. *Extra additions of $POCl_3$*: after 1 h and 2.75 h: $POCl_3$ (7.5 mg, 0.049 mmol). The reaction mixture was placed in the ultrasonic bath for 1 min. Purification by silica column flash chromatography (EtOAc/*i*-hexane/HCOOH 20:80:0.5 to 50:50:0.5; Second column: DCM/MeOH 97:3 to 90:10) gave **50** in 89% yield, 59 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.32 (dm, J = 8.3 Hz, 1H), 8.15 (dd, J = 7.9, 1.6 Hz, 1H), 7.87 (dm, J = 8.2 Hz, 2H), 7.38 (ddd, J = 8.6, 7.9, 1.7 Hz, 1H), 7.25 (dm, J = 8.2 Hz, 2H), 7.04 (m, 1H), 4.81 (s, 2H), 2.71 (m, 2H), 2.34 (s, 3H), 1.74–1.54 (m, 5H), 1.38 (s, 9H), 1.32–1.06 (m, 5H), 0.96–0.81 (m, 2H). ^{13}C NMR (CD_3OD) δ 174.9, 165.8, 153.6, 152.1, 144.7, 143.7, 141.8, 140.8, 133.5, 132.7, 132.3, 130.3, 127.5, 124.5, 124.2, 123.5, 121.4, 52.0, 51.1, 38.7, 36.0, 34.0, 29.3, 28.2, 27.6, 27.2, 21.5; HRMS calcd for $C_{33}H_{41}ClN_6O_6S$ [M+H$^+$]: 685.2575; found 685.2568.

7.1.35. Compound 51. 4-Bromo-2-(2-(3-(3-(*tert*-butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (50 mg, 0.100 mmol), K_2CO_3 (21 mg, 0.150 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (10 ml), EtOAc (2 × 10 ml). The crude acid was dissolved in pyridine (1 ml). The hydrochloride salt of the amine (**20** treated by method D) (63 mg, 0.150 mmol) was added followed by $POCl_3$ (17 mg, 0.110 mmol), −15 °C, 10 min, rt, 18 h. *Extra additions of $POCl_3$:* after 1 h: $POCl_3$ (17 mg, 0.110 mmol). H_2O (10 ml). Extracted with EtOAc (2 × 10 ml). Purification by silica column flash chromatography (EtOAc/*i*-hexane/HCOOH 20:80:3) followed by a second column (DCM/MeOH 95:5 to 90:10) gave **51** in 32% yield, 26 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.59 (s, 1H), 8.20 (d, J = 8.3 Hz, 2H), 7.90 (d, J = 7.4 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 1H), 4.95 (s, 2H), 2.74–2.66 (br s, 2H), 1.70–1.51 (m, 5H), 1.41 (s, 9H), 1.39–1.31 (m, 2H), 1.27–1.03 (m, 4H), 0.92–0.75 (m, 2H). ^{13}C NMR (CD_3OD) δ 174.0, 166.3, 153.84, 153.76 (rotamer), 152.2, 148.5, 144.8, 141.7, 134.1 (q, J = 32.5 Hz), 134.0, 132.3, 128.7, 127.5, 126.9, 126.7 (q, J = 3.9 Hz), 125.1 (q, J = 271.8 Hz), 123.6, 123.4, 123.0, 52.1, 52.0 (rotamer), 51.0, 38.7, 36.0, 34.0, 29.23, 29.21 (rotamer), 28.3, 27.5, 27.2; HRMS calcd for $C_{33}H_{37}BrClF_3N_6O_6S$ [M+H$^+$]: 817.1398; found 817.1401.

7.1.36. Compound 52. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)-4-vinylbenzamide

The title compound was prepared following general method F2. **41** (50 mg, 0.100 mmol), K_2CO_3 (21 mg, 0.150 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (10 ml), EtOAc (2 × 10 ml). The resulting acid, the hydrochloride salt of the amine (**21** treated by method D) (56 mg, 0,138 mmol) and HATU (46 mg, 0.120 mmol) in DCM (5 ml). DIEA (83 mg, 0.640 mmol). 45 °C for 2.5 h. DCM (15 ml), 0.1 M $NaHSO_4$ (10 ml). Purification on silica column (DCM/MeOH 93:7) followed by a second column (EtOAc/*i*-hexane/HCOOH 20:80:3 to 30:70:3) and a final purification (DCM/MeOH 93:7) to remove som trace impurities, yielding 51 mg of the title compound (67%). ^{1}H NMR (CD_3OD) δ 8.45 (s, 1H), 8.19 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 8.3 Hz, 1H), 6.66 (q, J = 11.1, 17.7 Hz, 1H), 5.80 (d, J = 17.6 Hz, 1H), 5.30 (d, J = 11.1 Hz, 1H), 4.94 (s, 2H), 2.72 (t, J = 8.2 Hz, 2H), 1.72–1.51 (m, 5H), 1.48–1.33 (m, 10H), 1.32–1.19 (m, 2H), 1.17–1.03 (m, 3H), 0.93–0.78 (m, 2H). ^{13}C NMR (CD_3OD) δ 175.1, 166.0, 153.7, 152.1, 148.5, 144.7, 143.1, 141.1, 137.4, 134.1 (q, J = 32.5 Hz), 133.1, 132.4, 128.4, 126.7 (q, J = 3.8 Hz), 124.9 (q, J = 271.3 Hz), 123.5, 123.3, 121.7, 118.8, 116.8, 52.0, 51.3, 38.7, 36.0, 34.0, 29.2, 28.3, 27.5, 27.2; HRMS calcd for $C_{35}H_{40}ClF_3N_6O_6S$ [M+H$^+$]: 765.2449; found 765.2446.

7.1.37. Compound 53. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-4-(pyrimidin-5-yl)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared following general method F2. **41** (25 mg, 0.05 mmol), K_2CO_3 (10.4 mg, 0.075 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1.0 M HCl (10 ml), EtOAc (2 × 10 ml). The resulting acid, the hydrochloride salt of the amine (**22** treated by method D) (30.4 mg, 0.072 mmol) and HATU (23 mg, 0.060 mmol) in DCM (4 ml). DIEA (41 mg, 0.320 mmol). 45 °C for 2 h. DCM (5 ml), 0.1 M $NaHSO_4$

(5 ml). The organic layer was washed with brine (5 ml). Purfication on silica column (DCM/MeOH 93:7 to 90:10) gave the title compound in 54% yield (22 mg). ^{1}H NMR (CD_3OD) δ 9.10 (s, 1H), 8.99 (s, 2H), 8.74 (s, 1H), 8.26 (d, J = 8.3 Hz, 1H), 8.23 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.3 Hz, 1H), 5.01 (s, 2H), 2.71 (t, J = 8.4 Hz, 2H), 1.71–1.49 (m, 6H), 1.47–1.32 (m, 11H), 1.32–1.00 (m, 3H), 0.93–0.76 (m, 2H). ^{13}C NMR (CD_3OD) δ 174.0, 166.5, 158.4, 156.1, 153.4, 153.8 (rotamer), 152.3, 148.9, 144.9, 141.6, 138.5, 135.0, 133.9 (q, J = 33.1 Hz), 133.7, 132.2, 129.0, 126.5 (q, J = 3.9 Hz), 125.1 (q, J = 264.6 Hz), 124.9, 123.2, 122.2, 119.0, 52.1, 52.0 (rotamer), 50.8, 38.7, 36.0, 34.0, 29.2, 29.2 (rotamer), 28.3, 27.5, 27.2; HRMS calcd for $C_{37}H_{40}ClF_3N_8O_6S$ [M+H$^+$]: 817.2510; found 817.2513.

7.1.38. Compound 54. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-4-fluoro-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (69 mg, 0.140 mmol), K_2CO_3 (29 mg, 0.21 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (10 ml), EtOAc (2 × 10 ml). The crude acid was dissolved in pyridine (1 ml). The hydrochloride salt of the amine (**23** treated by method D) (76 mg, 0.210 mmol) was added followed by $POCl_3$ (24 mg, 0.154 mmol), −15 °C, 10 min, room temperature, 5 h. *Extra addition of $POCl_3$:* after 2.5 h: $POCl_3$ (24 mg, 0.154 mmol). H_2O (10 ml). Extracted with EtOAc (2 × 10 ml). Purification by silica column flash chromatography (EtOAc/*i*-hexane/HCOOH 20:80:3 to 40:60:3 followed by a second column (DCM/MeOH 95:5) gave **54** in 48% yield, 51 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.20 (d, J = 8.4 Hz, 2H), 8.15 (d, J = 11.7 Hz, 1H), 8.12-8.03 (br s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 6.65 (dd, J = 8.5, 8.5 Hz, 1H), 4.96 (s, 2H), 2.75–2.66 (br s, 2H), 1.69–1.51 (m, 5H), 1.40 (s, 9H), 1.38–1.30 (m, 3H), 1.26–1.03 (m, 3H), 0.90–0.76 (m, 2H). ^{13}C NMR (CD_3OD) δ 174.1, 166.3, 166.1 (d, J = 249.2 Hz), 153.7, 152.2, 148.5, 144.8, 142.8 (d, J = 12.2 Hz), 135.1 (d, J = 10.3 Hz), 134.1 (q, J = 32.5 Hz), 132.2, 128.6, 126.7 (q, J = 3.8 Hz), 125.0 (q, J = 271.9 Hz), 123.5, 120.2, 110.6 (d, J = 22.1 Hz), 107.7 (d, J = 28.0 Hz), 52.0, 51.2, 38.7, 36.0, 34.0, 29.2, 28.3, 27.5, 27.2; HRMS calcd for $C_{33}H_{37}ClF_4N_6O_6S$ [M+H$^+$]: 757.2198; found 757.2195.

7.1.39. Compound 55. 3-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-5-(trifluoromethyl)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (50 mg, 0.100 mmol), K_2CO_3 (21 mg, 0.150 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (10 ml), EtOAc (2 × 10 ml). The crude acid was dissolved in pyridine (1 ml). The hydrochloride salt of the amine (**24** treated by method D) (63 mg, 0.150 mmol) was added followed by $POCl_3$ (20 mg, 0.131 mmol), −15 °C, 10 min, rt, 4 h. *Extra additions of $POCl_3$:* after 2 h: $POCl_3$ (10 mg, 0.066 mmol). H_2O (10 ml). Extracted with EtOAc (2 × 15 ml). Purification by silica column flash chromatography (EtOAc/*i*-hexane/HCOOH 20:80:3 to 30:70:3) followed by a second column (DCM/MeOH 90:10) gave **55** in 22% yield, 19 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.26 (s, 1H), 8.16 (d, J = 8.2 Hz, 2H), 8.10 (s, 1H), 8.01 (s, 1H), 7.74 (d, J = 8.4 Hz, 2H), 4.94 (s, 2H), 2.75–2.68 (m, 2H), 1.77–1.56 (m, 5H), 1.47–1.07 (m, 6H), 1.42 (s, 9H), 0.98–0.85 (m, 2H). ^{13}C NMR (CD_3OD) δ 172.4, 166.7, 153.9, 152.4, 149.2, 145.0, 141.0, 139.9, 133.7 (q, J = 30.8 Hz), 132.1, 131.9 (q, J = 33.0 Hz), 129.1, 126.4 (q, J = 3.9 Hz), 125.2 (q, J = 263.8 Hz), 124.3 (q, J = 5.5 Hz), 123.0, 122.1 (q, J = 3.0 Hz), 119.6, 52.0, 38.8, 36.0, 34.1, 29.2, 28.2, 27.6, 27.2; HRMS calcd for $C_{34}H_{37}ClF6N_6O_6S$ [M+H$^+$]: 807.2166; found 807.2167.

7.1.40. Compound 56. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)-*N*-methyl-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide

The title compound was prepared according to method F1. **41** (40 mg, 0.080 mmol), K_2CO_3 (16 mg, 0.119 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (12 mg, 0.078 mmol), −15 °C, 15 min. The hydrochloride salt of the amine (**25** treated by method D) (43 mg, 0.109 mmol), rt, 1 h. *Extra additions of $POCl_3$:* after 30 min and 45 min: $POCl_3$ (12 mg, 0.080 mmol). The reaction mixture was placed in the ultrasonic bath for 1 min. Purification by silica column flash chromatography. EtOAc/*i*-hexane/HCOOH 20:80:0.5 to 50:50:0.5, second column: DCM/MeOH 95:5 to 90:10, gave **56** in 35% yield, 19 mg as white solid. ^{1}H NMR ($CDCl_3$) δ 8.09 (dm, J = 8.3 Hz, 2H), 7.80 (dm, J = 8.3, 2H), 7.72 (dd, J = 8.2, 1.2 Hz, 1H), 7.44 (ddd, J = 8.2, 7.6, 1.6 Hz, 1H), 7.34 (dd, J = 7.9, 1.6 Hz, 1 H), 7.16 (dm, J = 7.6 Hz, 1H), 4.72 (s, 2H), 3.33 (s, 3H), 2.76 (m, 2H), 1.89–1.57 (m, 5H), 1.41 (s, 9H), 1.39–1.12 (m, 6H), 1.05–0.84 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 170.5, 164.6, 152.3, 150.9, 146.0, 143.8, 142.0, 135.9, 135.4 (q, J = 33.0 Hz), 132.7, 130.3, 129.3, 128.7, 127.9, 126.2 (q, J = 4.0 Hz), 124.8, 123.5, 123.2 (q, J = 273.2 Hz), 51.3, 49.4, 37.6, 36.0, 35.4, 33.1, 29.0, 27.1, 26.6, 26.3. HRMS calcd for $C_{34}H_{40}ClF_3N_6O_6S$ [M+H$^+$]: 753.2449; found 753.2448.

7.1.41. Compound 57. Methyl 2-(2-(3-(3-(*tert*-butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido) benzoate

The title compound was prepared as described in general method F2: **41** (0.040 mg, 0.795 mmol) K_2CO_3 (0.016 g, 0.119 mmol), CH_3CN (2 ml), and H_2O (1 ml). 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (20 ml).

Methyl 2-aminobenzoate (0.018 g, 0.116 mmol), HATU (0.035 g, 0.93 mmol), DCM (2 ml), DIEA (0.041 ml, 0.232 ml). 45 °C, 3 h. DCM (15 ml), 0.1 M $NaHSO_4$ (20 ml). Purification on silica gel (*iso*-hexane/EtOAc 4:1) gave **57** in 57% yield (24 mg). ^{1}H NMR ($CDCl_3$) δ 11.42 (s, NH), 8.63 (s, NH), 8.59 (dd, J = 8.6, 1.2 Hz, 1H), 8.02 (dd, J = 8.2, 1.7 Hz, 1H), 7.93 (s, NH), 7.55 (ddd, J = 8.6, 7.3, 1.7 Hz, 1H), 7.13 (ddd, J = 8.2, 7.3, 1.2 Hz, 1H), 4.89 (s, 2H), 3.89 (s, 3H), 2.69 (m, 2H), 1.78–1.58 (m, 6H), 1.43 (s, 9H), 1.29–1.12 (m, 5H), 0.96 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 169.0, 164.1, 151.7, 151.1, 144.1, 140.5, 135.0, 131.0, 129.2, 123.6, 122.9, 120.5, 115.5, 52.7, 51.1, 49.1, 38.0, 35.4, 33.0, 29.0, 27.3, 26.5, 26.3. HRMS calcd for $C_{27}H_{36}ClN_5O_5$ [M+H$^+$]: 546.2405; found 546.2483.

7.1.42. Compound 58. 2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)-*N*-(4-(2-oxo-2-(4-(trifluoromethyl)phenylsulfonamido)ethyl)phenyl)acetamide

The title compound was prepared according to method F1. **41** (40 mg, 0.08 mmol), K_2CO_3 (16 mg, 0.119 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (12 mg, 0.078 mmol), −15 °C, 15 min. **28** (39 mg, 0.109 mmol), rt, 2 h. *Extra addition of $POCl_3$ and **28**:* after 1 h $POCl_3$ (12 mg, 0.080 mmol), **28** (10 mg, 0.028 mmol). Purification by silica column flash chromatography. EtOAc/*i*-hexane/HCOOH 20:80:0.5 to 50:50:0.5, second column: DCM/MeOH 100:0 to 95:5, gave **58** in 27% yield, 55 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.00 (dd, J = 8.1, 0.9 Hz, 2H), 7.75–7.69 (m, 2H), 7.40 (dd, J = 8.7, 2.2 Hz, 2H), 7.20–7.13 (m, 2H), 4.91 (s, 2H), 3.46 (s, 2H), 2.71 (m, 2H), 1.85–1.57 (m, 5H), 1.53–1.44 (m, 2H), 1.43 (s, 9H), 1.39–1.09 (m, 4H), 1.07–0.85 (m, 2H). ^{13}C NMR (CD_3OD) δ 166.2, 157.2, 153.9, 152.4, 147.7, 145.0, 137.8, 134.3 (q, J = 32.4 Hz), 133.6, 132.1, 130.9, 128.8, 126.6 (q, J = 3.8 Hz), 125.0 (q, J = 271.8 Hz), 123.0, 121.1, 52.1, 45.9, 43.0, 38.9, 35.9, 34.1,

29.2, 28.2, 27.6, 27.3; HRMS calcd for $C_{34}H_{40}ClF_3N_6O_6S$ [M+H$^+$]: 753.2449; found 753.2455.

7.1.43. Compound 60. *N*-((2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(naphthalen-1-ylmethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido) phenyl)sulfonyl)-4-(trifluoromethyl)benzamide

The title compound was prepared according to method F1. **42** (120 mg, 0.226 mmol), K_2CO_3 (47 mg, 0.339 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (25 ml), EtOAc (2 × 25 ml). The crude acid was dissolved in pyridine (2 ml). **38** (78 mg, 0.226 mmol) was added followed by $POCl_3$ (38 mg, 0.247 mmol), −15 °C to rt, 2 h. Extracted with EtOAc (3 × 30 ml). Purification by silica column flash chromatography (DCM/MeOH 100:0 to 90:10) gave **60** in 42% yield, 73 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.09 (dm, J = 8.2 Hz, 1H), 8.03 (dm, J = 8.3 Hz, 2H), 7.93 (dd, J = 8.0, 1.5 Hz, 1H), 7.87–7.82 (m, 2H), 7.72 (dm, J = 8.3 Hz, 1H), 7.53 (dm, J = 8.5 Hz, 2H), 7.50–7.45 (m, 2H), 7.40 (m, 1H), 7.27 (dd, J = 8.3, 7.1 Hz, 1H), 7.16 (m, 1H), 7.06 (m, 1H), 4.72 (s, 2H), 4.66 (s, 2H), 1.35 (s, 9H). ^{13}C NMR (CD_3OD) δ 173.5, 166.0, 153.7, 152.8, 149.8, 145.9, 141.8, 138.6, 136.9, 136.0, 135.3, 134.8, 133.6 (q, J = 32.0 Hz), 133.3, 132.7, 132.1, 130.5, 129.8, 129.0, 128.9, 127.8, 127.2, 126.6, 126.0, 125.8 (q, J = 3.9 Hz), 125.5 (q, J = 271.7 Hz), 125.2, 124.0, 52.0, 50.8, 33.5, 29.2; HRMS calcd for $C_{36}H_{32}ClF_3N_6O_6S$ [M+H$^+$]: 769.1823; found 769.1821.

7.1.44. Compound 61. *N*-((3-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(naphthalen-1-ylmethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido) phenyl)sulfonyl)-4-(trifluoromethyl)benzamide

The title compound was prepared according to method F1. **42** (156 mg, 0.293 mmol), K_2CO_3 (61 mg, 0.440 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (25 ml), EtOAc (2 × 25 ml). The crude acid was dissolved in pyridine (2 ml). **39** (101 mg, 0.293 mmol) was added followed by $POCl_3$ (50 mg, 0.323 mmol), −15 °C to rt, 3 h. *Extra additions of $POCl_3$ and **39**:* after 2 h: *$POCl_3$* (23 mg, 0.147 mmol), **39** (51 mg, 0.147 mmol). Extracted with EtOAc (3 × 30 ml). Purification by silica column flash chromatography (DCM/MeOH 100:0 to 90:10) gave **61** in 41% yield, 93 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.13 (dm, J = 8.1 Hz, 2H), 8.09 (m, 1H), 7.99 (m, 1H), 7.83 (m, 1H), 7.73–7.65 (m, 2H), 7.56 (dm, J = 8.1 Hz, 2H), 7.49–7.41 (m, 3H), 7.34–7.27 (m, 2H), 7.03 (dm, J = 7.1 Hz, 1H), 4.63 (s, 2H), 4.53 (s, 2H), 1.44 (s, 9H). ^{13}C NMR (CD_3OD) δ 172.8, 166.0, 153.9, 152.5, 150.0, 145.9, 145.2, 141.9, 139.3, 138.5, 135.4, 133.8 (q, J = 32.3 Hz), 132.6, 131.8, 130.7, 130.0, 130.0, 129.1, 127.8, 126.7, 125.8 (q, J = 3.7 Hz), 125.5 (q, J = 271.8 Hz), 125.5, 125.3, 123.8, 123.6, 123.3, 118.9, 61.5, 52.2, 33.1, 29.2; HRMS calcd for $C_{36}H_{32}ClF_3N_6O_6S$ [M+H$^+$]: 769.1823; found 769.1827.

7.1.45. Compound 62. *N*-((4-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(naphthalen-1-ylmethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido) phenyl)sulfonyl)-4-(trifluoromethyl)benzamide

The title compound was prepared according to method F1. **42** (60 mg, 0.113 mmol), K_2CO_3 (23 mg, 0.170 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The crude acid was dissolved in pyridine (2 ml), $POCl_3$ (19 mg, 0.0,124 mmol), −15 °C, 15 min. **40** (58 mg, 0.169 mmol), rt, 18 h. *Extra additions of $POCl_3$:* after 16 h: $POCl_3$ (9 mg, 0.057 mmol). Extracted with EtOAc (3 × 20 ml). Purification by silica column flash chromatography (EtOAc/*i*-hexane/HCOOH 20:80:3 to 50:50:3 followed by a second column (DCM/MeOH 100:0 to 95:5) gave **62** in 40% yield, 35 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.93 (s, 1H), 8.11–8.01 (m, 3H), 7.93 (dd, J = 9.1, 2.1 Hz, 2H), 7.84 (dd, J = 8.0, 1.6 Hz, 1H), 7.73 (m, 3H), 7.60–7.43 (m, 3H), 7.42–7.28 (m, 2H), 7.10 (m, 1H), 4.67 (s, 2H), 4.60 (s, 2H), 1.46 (s, 9H). ^{13}C NMR ($(CD_3)_2SO$) δ 167.8, 164.3, 150.9, 150.8, 144.6, 140.3 (q, J = 34.9 Hz), 133.4, 133.3, 131.4, 131.1, 128.9, 128.6, 128.0, 127.9, 127.6, 127.0, 126.6, 126.2, 125.7, 125.7, 124.8 (q, J = 268.6 Hz), 124.5 (q, J = 3.8 Hz), 124.0, 123.4, 123.0, 117.9, 55.9, 50.2, 29.6, 28.6. HRMS calcd for $C_{36}H_{32}ClF_3N_6O_6S$ [M+H$^+$]: 769.1823; found 769.1826.

7.1.46. Compound 64. *N*-((2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-2-oxo6-phenethylpyrazin-1(2*H*)-yl)acetamido)phenyl)sulfonyl)-4-(trifluoromethyl)benzamide

The title compound was prepared according to method F1. **43** (150 mg, 0.367 mmol), K_2CO_3 (61 mg, 0.440 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (25 ml), EtOAc (2 × 25 ml). The crude acid was dissolved in pyridine (2 ml). **38** (101 mg, 0.293 mmol) was added followed by $POCl_3$ (50 mg, 0.323 mmol), −15 °C, 3 h. Extracted with EtOAc (3 × 30 ml). Purification by silica column flash chromatography (DCM/MeOH 100:0 to 90:10) gave **64** in 61% yield, 95 mg as white solid. ^{1}H NMR (CD_3OD) δ 8.19 (dm, J = 8.4 Hz, 1H), 8.01 (dd, J = 8.1, 1.5 Hz, 1H), 7.97 (dm, J = 8.3 Hz, 2H), 7.70 (dm, J = 8.3 Hz, 2H), 7.60 (ddd, J = 8.4, 7.3, 1.5 Hz, 1H), 7.32 (m, 1H), 7.27–7.12 (m, 5H), 4.90 (s, 2H), 3.07 (dd, J = 9.3, 6.6 Hz, 2H), 2.92 (dd, J = 9.3, 6.6 Hz, 2H), 1.35 (s, 9H). ^{13}C NMR (CD_3OD) δ 166.3, 164.5, 153.8, 152.5, 145.4, 141.2, 137.1, 134.5, 134.3, 131.1, 130.9 (d, J = 33.6 Hz), 130.7, 130.3, 130.2, 129.7, 129.6, 127.6, 126.2 (d, J = 3.9 Hz) 126.1 (d, J = 276.9 Hz), 125.9, 124.7, 124.2, 52.0, 50.0, 34.6, 33.0, 29.1. HRMS calcd for $C_{33}H_{32}ClF_3N_6O_6S$ [M+H$^+$]: 733.1823; found 733.1826.

7.1.47. Compound 65. 3-(Benzyloxy)-1-(2-(3-(3-(*tert*-butyl) ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl) acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)cyclobutane-1-carboxamide. Mixture of isomers

The title compound was prepared following general method F2. **41** (71 mg, 0.141 mmol), K_2CO_3 (29 mg, 0.212 mmol), CH_3CN (3 ml), H_2O (1.5 ml). Irradiated by MW to 100 °C for 20 min. 1 M HCl (10 ml), EtOAc (20 ml). The resulting acid, the hydrochloride salt of the amine (**31** treated by method D) (97 mg, 0,210 mmol) and HATU (64 mg, 0.168 mmol) in DCM (1 ml). DIEA (0.112 ml, 0.643 mmol). Rt for 2 h. DCM (10 ml), 0.1 M $NaHSO_4$ (10 ml). Purified four times on silica column (DCM/MeOH 98:2–95:5)/*iso*-hexane/EtOAc/HCOOH 80:20:3–60:40:3. In order to remove residues of impurities, the product was finally purified on a preparative RP-HPLC, eluent: NH_4OAc in CH_3CN/H_2O, (0.013 g, 11%) as a mixture of *cis* and *trans* isomers in NH_4^+ salt form. ^{1}H NMR ($(CD_3)_2SO$) δ 8.42 (s, NH), 8.39 (s, NH), 8.33 (s, NH), 7.97 (m, 2H+2H; 2 isomers), 7.80 (m, 2H+ 2H; 2 isomers), 7.36 -7.20 (m, 5H+5H; 2 isomers), 4.71 (s, 2H; 1 isomer), 4.70 (s, 2H, 1 isomer), 4.35 (s, 2H, 1 isomer), 4.34 (s, 2H; 1 isomer), 4.07 (p, J = 7.3 Hz, 1H; 1 isomer), 3.89 (m, 1H; 1 isomer), 2.72–2.61 (m, 2H, 1 isomer), 2.57–2.49 (m, 2H+2H), 2.35–2.27 (m, 4H; 1 isomer), 2.14–2.03 (m, 2H) 1.75–1.54 (m, 10H), 1.34 (s, 9H+9H), 1.36–1.04 (m, 12H: 2 isomers), 0.95–0.81 (m, 2H+2H; 2 isomers). ^{13}C NMR ($(CD_3)_2SO$) δ 168.2, 167.8, 166.23, 166.16, 151.3 (2 carbons), 150.88, 150.86, 143.9, 143.8, 138.7, 138.6, 138.50, 138.47, 130.8, 130.7, 128.63(2 carbons), 128.58 (2 carbons), 128.1(2 carbons), 127.9, 127.8, 125.95, 125.95 (d, J = 4.3 Hz), 125.89, 125.85 (d, J = 4.6 Hz), 123.99 (d, J = 286.4 Hz) (2 carbons), 121.13 (2 carbons), 69.73, 69.69, 67.8, 67.7, 53.4, 53.3, 50.6 (2 carbons), 47.7, 47.6, 38.48, 38.46, 37.2, 37.1, 34.84, 34.79, 32.81, 32.75, 29.0 (2 carbons), 27.1, 27.0, 26.49, 26.46, 26.2, 26.1. HRMS (ES) m/z calcd for $C_{38}H_{46}ClF_3N_6O_7S$ [M+H$^+$]: 823.2868; found 823.2857.

7.1.48. Compound 66. 1-(2-(6-Benzyl-3-(3-(*tert*-butyl)ureido)-5-chloro-2-oxopyrazin-1(2*H*)-yl)acetamido)-3-(benzyloxy)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)cyclobutane-1-carboxamide

The title compound was prepared following general method F2. **44** (34 mg, 0.071 mmol), K_2CO_3 (15 mg, 0.107 mmol), CH_3CN (2 ml), H_2O (1 ml). Irradiated by MW to 100 °C for 15 min. 1 M HCl (20 ml), EtOAc (2 × 20 ml). The resulting acid, the hydrochloride salt of the amine (**31** treated by method D) (50 mg, 0,107 mmol) and HATU (33 mg, 0.086 mmol) in DCM (2 ml). DIEA (0.075 ml, 0.428 mmol). 45 °C for 3 h. DCM (15 ml), 0.1 M $NaHSO_4$ (20 ml). Purified on silica column (*iso*-hexane/EtOAc/HCOOH 80:20:0.5–0:50:0.5 followed by a second purification DCM/MeOH 98:2–9:1) to give the compound in 68% yield (0.039 g) as a mixture of *cis* and *trans* isomers. (Isomer A: 6 mg, Isomer B: 12 mg, Isomer A/B: 21 mg).

Isomer A: ^{1}H NMR (CD_3CN) δ 8.59 (s, NH), 8.11 (s, NH), 8.05 (m, 2H), 7.81 (m, 2H), 7.38–7.19 (m, 10H), 4.44 (s, 2H), 4.36 (s, 2H), 4.16 (s, 2H), 3.89 (p, J = 7.0 Hz, 1H), 2.73–2.65 (m, 2H), 1.97–1.89 (partly covered by solvent peak—confirmed by gCOSY experiment) (m, 2H), 1.42 (s, 9H). ^{13}C NMR (CD_3CN) δ 173.3, 166.6, 152.5, 152.3, 145.6, 143.8, 139.4, 136.3, 135.1 (q, J = 30.1 Hz), 129.98, 129.93, 129.7, 129.3, 128.9, 128.9, 128.8, 128.6, 128.4, 126.9 (q, J = 3.8 Hz), 123.2 (d, J = 274.1 Hz), 70.8, 67.7, 54.0, 51.7, 50.4, 40.9, 35.6, 29.0.

Isomer B (analysed as a mixture B/A 10:1): ^{1}H NMR (CD_3CN) δ 8.63 (s, NH), 8.15 (s, NH), 8.04 (m, 2H), 7.79 (m, 2H), 7.42–7.13 (m, 10H), 4.46 (s, 2H), 4.37 (s, 2H), 4.14 (s, 2H), 4.00 (p, J = 7.1 Hz, 1H), 1.42 (s, 10H). ^{13}C NMR (CD_3CN) δ 171.6, 167.3, 152.4, 152.4, 145.6, 143.5, 139.3, 136.3, 134.2 (q, J = 31.1 Hz), 130.0, 129.9, 129.3, 129.3, 128.9, 128.9, 128.8, 128.7, 128.3, 127.0 (q, J = 3.9 Hz), 123.7 (q, J = 280.6 Hz), 70.8, 68.3, 54.9, 51.7, 50.3, 39.0, 35.7, 29.1.

HRMS (ES) m/z calcd for $C_{37}H_{38}ClF_3N_6O_7S$ [M+H$^+$]: 803.2242; found 803.2237.

7.1.49. Compound 68. 2-(2-(3-(3-(*tert*-Butyl)ureido)-5-chloro-6-(2-cyclohexylethyl)-2-oxopyrazin-1(2*H*)-yl)acetamido)benzoic acid

57 (11 mg, 0.020 mmol) was dissolved in CH_3CN (1 ml). K_2CO_3 (0.004 g, 0.030 mmol) in H_2O (0.5 ml) was added and the reaction mixture was heated in the MW (100 °C, 15 min). 1 M HCl (20 ml) was added and the water phase was extracted two times with 20 ml EtOAc. The combined organic phases were dried ($MgSO_4$), filtered and evaporated. Purified on a silica gel column (DCM/MeOH 95:5) to yield the title compound as a white solid (8 mg, 75%). ^{1}H NMR (CD_3OD) δ 8.51 (dd, J = 8.4, 1.2 Hz, 1H), 8.08 (m, 1H), 7.51 (m, 1H), 7.15 (m, 1H), 4.96 (s, 2H), 2.75 (m, 2H), 1.82-1.49 (m, 5H), 1.43 (s, 9H), 1.48–1.09 (m, 6H), 0.92 (q, J = 11.3 Hz, 2H). ^{13}C NMR (CD_3OD) δ 169.7, 164.8, 151.6, 151.0, 147.6, 143.7, 134.8, 131.2, 130.2, 124.8, 123.0, 119.5, 110.0, 50.6, 48.9, 47.9, 37.3, 34.6, 32.6, 27.9, 26.8, 26.1, 25.8. HRMS (ES) m/z calcd for $C_{26}H_{34}ClN_5O_5$ [M+H$^+$]: 532.2327; found 532.2319.

7.1.50. Compound 69. 2-(6-Benzyl-3-(3-(*tert*-butyl)ureido)-5-chloro-2-oxopyrazin-1(2*H*)-yl)acetate

The title compound was prepared according to the first part of method F. **44** (0.040 g, 0.083 mmol), CH_3CN (2 ml), K_2CO_3 (0.017 g, 0.124 mmol), H_2O (1 ml). 100 °C, 15 min. 1 M HCl (10 ml), EtOAc (2 × 10 ml). H_2O (10 ml) was added and a solid was formed. The material was centrifugated for 2 min. The water layer was removed and the remaining solid was kept under vacuum overnight yielding the title compound in 98% yield (32 mg) with no further purification. ^{1}H NMR ($CDCl_3$) δ 7.37–7.26 (m, 3H), 7.16–7.09 (m, 2H), 4.58 (s, 2H), 4.06 (s, 2H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 168.32, 151.83, 150.84, 144.37, 134.89, 129.44, 127.76, 127.72, 127.04, 124.16, 51.23, 46.49, 35.31, 28.91. HRMS calcd for $C_{18}H_{21}ClN_4O_4$ [M+H$^+$]: 393.1330; found 393.1349.

7.1.51. Compound 70. 1-(2-(6-Benzyl-3-(3-(*tert*-butyl)ureido)-2-oxopyrazin-1(2*H*)-yl)acetamido)-3-hydroxy-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)cyclobutane-1-carboxamide

66 (0.010 g, 0.012 mmol) was dissolved in MeOH (2 ml). 10% Pd/C (0.05 g) was added and the reaction mixture was placed under H_2 (1 atm) and allowed to stir at rt for 6 h. The mixture was filtered through celite and the solvent was evaporated to give the title compound in 59% yield (5 mg). *Mixture of isomers (Isomer A:B 1.3:1.0)* ^{1}H NMR (CD_3CN) δ 8.11–8.00 (m, 2H+2H; isomer A+B), 7.86–7.75 (m, 2H+2H; isomer A+B), 7.38–7.18 (m, 5H+5H; isomer A+B), 6.83 (br s, 1H+1H; isomer A+B), 4.50 (s, 2H, one isomer), 4.48 (s, 2H; one isomer), 4.25–4.13 (m, 1H+1H; isomer A+B), 4.09 (s, 2H; one isomer), 4.05 (s, 2H; one isomer), 3.91–3.82 (m, 2H +2H; isomer A+B), 2.64–2.58 (m, 2H, one isomer), 2.50–2.29 (m, 1H+2H; isomer A+B), 1.38 (s, 9H), 1.25 (s, 9H). ^{13}C NMR (CD_3CN) δ 169.4, 169.0, 165.4, 164.5, 156.4, 155.9, 152.8, 152.0, 146.4, 145.8, 144.1, 142.9, 138.83, 138.79, 134.7 (d, J = 32.4 Hz), 134.4 (d, J = 32.0 Hz), 134.3, 134.2, 130.29, 130.27, 129.8, 129.7, 129.5, 129.5, 128.90 (d, J = 277.1 Hz), 128.87 (d, J = 274.1 Hz), 128.2, 128.1, 126.78 (d, J = 3.7 Hz), 126.79 (d, J = 3.7 Hz), 115.5, 115.2, 59.1, 59.0, 54.2, 54.1, 51.3, 50.2, 48.35 (2C), 44.7, 43.3, 38.7, 36.2, 29.2, 29.0. HRMS (ES) m/z calcd for $C_{30}H_{33}F_3N_6O_7S$ [M+H$^+$]: 679.2162; found 679.2175.

7.2. Computational methods

All calculations were performed within the Schrödinger Small Molecule Drug Discovery Suite (Schrödinger Release 2015-4). Crystal structures (4A92 and 4B74) of full-length NS3 were downloaded from the PDB and prepared using Protein Preparation Wizard.[50] To prepare structure 4B74 for docking, the C-terminal 625-631 was deleted and S624 was terminated by a *N*-methyl amine. Also, the allosteric ligand 1LH was deleted. The OPLS3 force field was used throughout this work,[51] in combination with the GB/SA continuum solvation model for water.[52] Grids for docking were prepared using Glide (Glide, version 6.9, Schrödinger, LLC, New York, NY, 2015) and subsequent docking in SP mode was performed using the same program.[53] Docking poses were used as starting points for subsequent molecular mechanics conformational searches in MacroModel (MacroModel, version 11.0, Schrödinger, LLC, New York, NY, 2015). During these searches, various flat bottomed distance constraints from the ligand to the protein backbone of βE2 (R155, A157, and C159) were used to keep the hydrogen bond pattern of the starting pose. The distance constraint used was 2.1 ± 0.3 Å using a force constant of 100 kcal mol^{-1} Å^{-2}. Torsions selected for the conformational searches included the rotatable bonds in the ligand investigated and side-chains of interacting amino acids. The number of steps in the Monte Carlo multiple minima (MCMM) were usually set to 1000 but in many cases, a single binding mode was investigated using several starting geometries and both crystal structures 4A92 and 4B74. Induced fit docking (Induced Fit Docking protocol; Glide version 6.9, Schrödinger, LLC, New York, NY, 2015; Prime version 4.2, Schrödinger, LLC, New York, NY, 2015) was performed on compounds **57** and **68** using 4A92.

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Supplementary data

Supplementary data (^{1}H, ^{13}C NMR, LC-UV/MS of target compounds, kinetic parameters of tested enzyme variants, binding curves corrected for auto-fluorescence and additional molecular modeling pictures of binding poses) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.03.066.

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