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Discovery of a Novel Class of Potent HCV NS4B Inhibitors: SAR Studies on Piperazinone Derivatives

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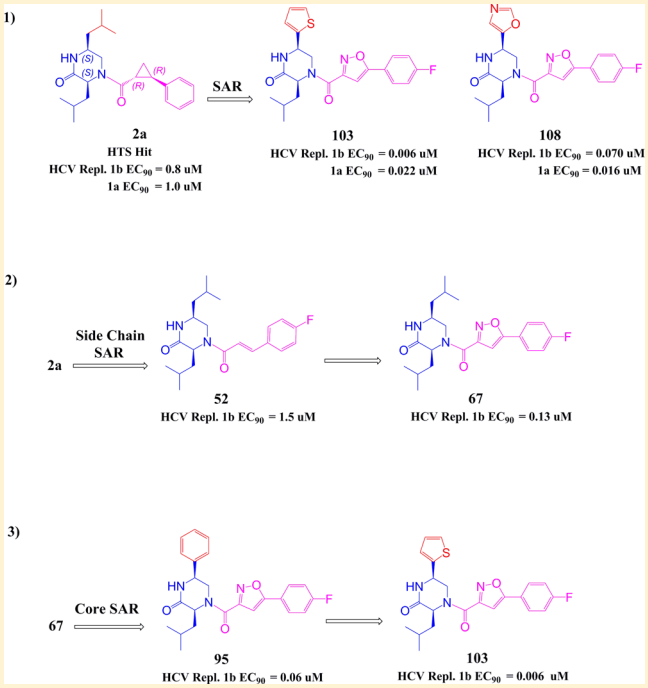
ABSTRACT: HTS screening identified compound **2a** (piperazinone derivative) as a low micromolar HCV genotype 1 (GT-1) inhibitor. Resistance mapping studies suggested that this piperazinone chemotype targets the HCV nonstructural protein NS4B. Extensive SAR studies were performed around **2a** and the amide function and the *C*-3/*C*-6 cis stereochemistry of the piperazinone core were essential for HCV activity. A 10-fold increase in GT-1 potency was observed when the chiral phenylcyclopropyl amide side chain of **2a** was replaced with *p*-fluorophenylisoxazole-carbonyl moiety (**67**). Replacing the *C*-6 nonpolar hydrophobic moiety of **67** with a phenyl moiety (**95**) did not diminish the GT-1 potency. A heterocyclic thiophene moiety (**103**) and an isoxazole moiety (**108**) were incorporated as isosteric replacements for the *C*-6 phenyl moiety (**95**), resulting in significant improvement in GT-1b and 1a potency. However, the piperazonone class of compounds lacks GT-2 activity and, consequently, were not pursued further into development.



INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health problem that affects more than 200 million individuals worldwide and an estimated 4.5 million people in the United States.[1] Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or hepatocellular carcinoma.[2] Recently approved standard of care (SOC) for genotype 1 patients involves the combination of a NS3/4A protease inhibitor, pegylated α-interferon (IFN), and the oral nucleoside ribavirin. The sustained virologic response (SVR or undetectable HCV RNA in serum post treatment) rate for most-difficult-to-treat genotype-1 HCV patients is about 70% with approved drugs boceprevir and telaprevir in combination with ribavirin and IFN.[3] The current SOC provides limited clinical benefit for HCV genotype 2, 3, 4, 5, and 6 infected patients.[4] Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved oral drugs or drug combination therapies that effectively combat chronic HCV infection, are pan-genotypic, have a high barrier to resistance, and are well tolerated.

The HCV virion is an enveloped positive-strand RNA virus of the family flaviviridae with a single oligoribonucleotide genomic sequence of about 9600 bases, which encodes a polyprotein of about 3010 amino acids.[5] The protein products of the HCV gene consist of the structural proteins C, E1, and E2 and the nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The nonstructural (NS) proteins are believed to provide the catalytic machinery for viral replication. A number of molecular targets have been pursued in an effort to identify direct acting antivirals as anti-HCV therapeutics.[6] These include, but are not limited to, the NS3/4A[7] protease, NS5A,[8] and the NS5B[9] polymerase. Agents targeting these nonstructural proteins have proven to be clinically effective at reducing HCV viral load in infected patients. However, the emergence of resistant virus, and the observation that combining direct acting antivirals appears to be necessary to

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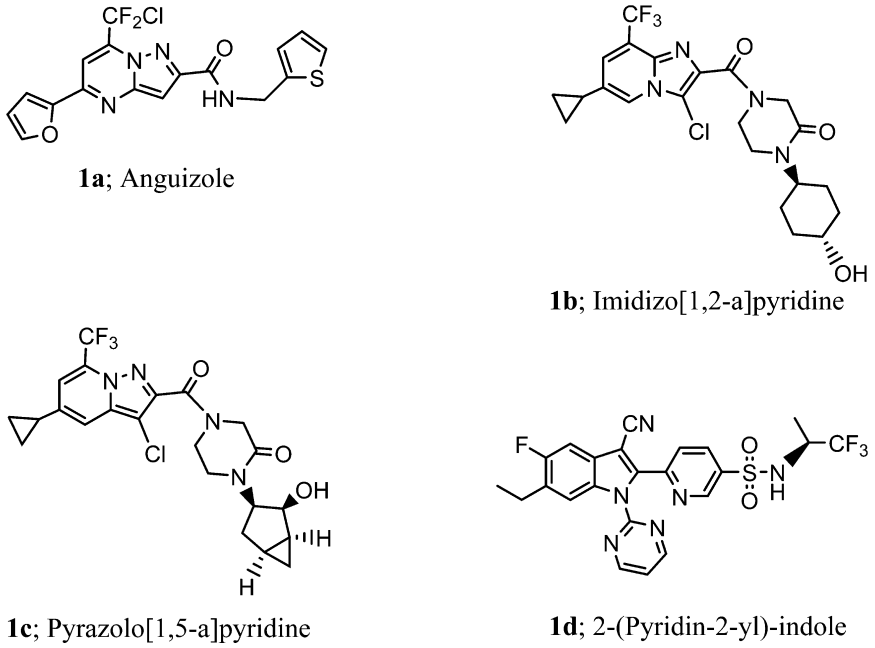
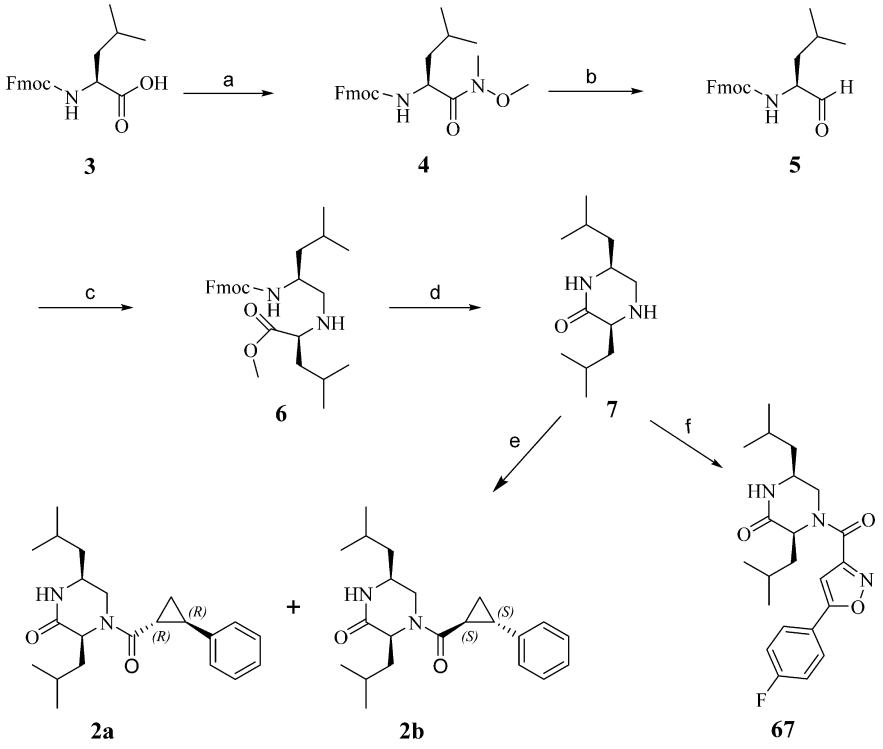


Figure 1. HCV NS4B inhibitors with sub-μM GT1b potency.

Scheme 1. Synthesis of Piperazinone Core 7 and Compounds 2a, 2b, and 67[a]



[a]Reagents and conditions: (a) MeNHOMe·HCl, TBTU, DIPEA, CH_3CN, 0 °C to RT (98%); (b) LiAlH4, THF, −78 °C (98%); (c) NH_2-Leu-OMe, $NaBH(OAc)_3$, DCE (90%); (d) 20% Et_2NH in DCM, DCM (95%); (e) (±)-(1*R*,2*R*)-2-phenylcyclopropanecarboxylic acid, TBTU, DIPEA, DCM (90%); (f) 5-(4-fluorophenyl)isoxazole-3-carboxylic acid, TBTU, DIPEA, DCM (91%).

achieve sustained viral suppression in the absence of IFN, has necessitated the search for novel and safe direct acting antivirals that can be combined with other anti-HCV agents in development.[10]

One of the HCV auxiliary NS proteins is NS4B. This protein is a relatively poorly characterized 27 kDa protein with at least four predicted transmembrane (TM) domains.[11] It is believed that as a consequence of polyprotein processing by the NS3/4A protease, the N- and C-terminal parts of NS4B are oriented toward the cytosolic side of the endoplasmic reticulum (ER) membrane. Furthermore, it is believed that HCV NS4B associates with a number of additional NS proteins and permits formation of the so-called "membranous web" structure that facilitates HCV replication.[11] Because NS4B plays a key role in

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HCV replication, disrupting NS4B function represents an attractive new anti-HCV strategy.

Efforts to find inhibitors of HCV that act on the NS4B protein utilized either the purified NS4B protein binding assay or the HCV GT1 replicon luciferase reporter-based in vitro assay and resulted in the identificatioin of low μM inhibitors.[12a] The first reported potent sub-μM (HCV 1b) NS4B inhibitor was anguizole[12b] (**1a**) (Figure 1), which showed potency in both GT1b and GT1a replicon assays (EC_{50}) of 0.31 and 0.56 μM, respectively. Lead optimization studies based on the anguzole core resulted in a novel imidazo[1,2-*a*]pyridines[12c] (**1b**) series (Figure 1) with low nM HCV GT1a and 1b replicon activity. Further PK profile optimization studies on **1b** resulted in pyrazolo[1,5-*a*]pyridine[12d] analogue **1c** (Figure 1) with improved viral resistance profile when compared to compound **1b**. Recently, a new potent HCV GT1b Inhibitor (**1d**) based on the 2-(pyridine-2-yl)-indole[12e−g] (1d) core with 1.7 nM (EC_{50})1b replicon activity was also disclosed (Figure 1). Anguizole and compounds **1b**, **1c**, and **1d** all showed similar resistance profiles at amino acid residues (H94, F98, V105) of TM1 domain. Resistant profile data of **1a**−**1d** appears to indicate that each of these molecules are interacting at the same region of NS4B, however, due to the lack of cocrystal data, their specific mode of binding and identification of key molecular interactions with the NS4B protein have not been determined. Although the current clinical status of compounds **1c** and **1d** is not available, it is known that anguizole (**1a**) was evaluated in a phase 1 clinical trial, but no further data has been reported. Herein, we report the identification and SAR development of a novel class of highly potent HCV NS4B inhibitors that is structurally distinct from those that have been previously disclosed.

A high-throughput screen (HTS) was carried out on 277000 compounds using a HCV 1b replicon luciferase reporter-based assay resulted in identification of the active compound **2a**. Compound **2a** contained a piperazinone core and a cyclopropylphenyl side chain (Figure 1). The activity of **2a** was confirmed after resynthesis (Scheme 1) from commercially available L-leucine derivatives. Compound **2a** (*trans-R,R*) exhibited HCV replicon genotype 1b (GT-1b) potency of 0.8 μM and genotype 1a (GT-1a) potency of 1.0 μM. *trans-S,S* isomer **2b** did not show any HCV replicon activity (GT-1b or GT-1a) when tested up to a 20 μM concentration. Compound **2a** was inactive against HIV and inactive as a HCV NS5B inhibitor. Cytotoxicity assessment against a panel of cell lines (Huh7, HepG2, BxPC3, and CEM) showed that **2a**'s replicon activity could not be attributed to a cytotoxic effect (CC_{50} range = 40−76 μM). Compound **2a** with its unique diisobutylpiperazin-2-one core was further investigated as a novel compound class targeting HCV.

Because **2a** did not inhibit HCV polymerase (data not shown), resistant replicons were generated in an effort to determine the viral target for this class of compounds. The generation of a resistant replicon resulted in the identification of a replicon with decreased sensitivity to **2a**. Genotypic and phenotypic analyses suggested that the NS4B protein (NS4B) was the target for **2a**. When known inhibitors of NS3/4A and NS5A were tested against the compound **2a**-resistant replicon, no significant shift in HCV EC_{50} values was observed. Three separate rounds of resistance selection generated high frequency mutations at residues 90 and 98 of NS4B. High frequency mutations found in NS4B were constructed in the GT-1b ET:PVI replicon by site-directed mutagenesis, and the sensitivity was determined using the luciferase reporter assay. Compound **2a** and **95** showed a 17-fold reduction and >20-fold reduction in activity against these mutant replicons, respectively, thus indicating that NS4B was the target for this class of molecules. The known NS4B inhibitor anguizole showed ∼10-fold reduction in activity against residue 94 and 98 mutant replicons. Despite submicromolar potency of **2a** and **95** against GT-1a and GT-1b replicons, no appreciable activity was observed using a GT-2a replicon. JFH-1 GT-2a WT naturally contains a Leu at position 98. When GT-2a L98 was mutated to Phe, some inhibition was recovered for compound **2a** and **95**.

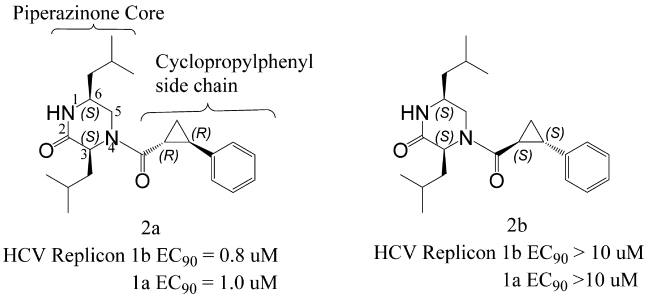

Figure 2. HTS hit compound (**2a**) and its inactive isomer (**2b**).

SYNTHESIS OF HCV NS4B INHIBITORS

The general synthesis of piperazinone core **7** (Scheme 1) was achieved by employing a modified literature procedure.[13] The aldehyde **5** was prepared via Weinreb amide intermediate **4**, which was in turn synthesized from commercially available Fmoc-L-leucine **3**. Reductive amination of methyl ester of L-leucine with aldehyde **5** furnished **6**, which on subsequent internal cyclization afforded the core 3,6-disiobutylpiperazinone **7**. Compound **7** was coupled to commercially available (±)-(*R,R*)-phenyl-cyclopropane carboxylic acid followed by chiral column (SFC) separation to furnish active inhibitor **2a** and its inactive isomer **2b** (Scheme 1). TBTU mediated coupling of **7** with 5-(4-fluorophenyl)isoxazole-3-carboxylic acid furnished inhibitor **67** (Scheme 1).

Synthesis of core **12** bearing a furanyl moiety began by treating furanyl-2-boronic acid (**8**) with glyoxylic acid monohydrate and bis(4-methoxyphenyl)methanamine in dichloromethane using the Petasis[14] multicomponent coupling reaction to afford the acid **9** (Scheme 2). The acid compound **9** was converted to the corresponding Weinreb amide compound **10**. Weinreb amide **10** was reduced with $LiAlH_4$ and then underwent a reductive amination with the methyl ester of L-leucine to furnish the corresponding ester **11**. Ester **11** was cyclized using aqueous acetic acid followed by SFC (chiral) separation providing the cores **12** and **13**. Compound **12** was then coupled to the 5-(4-fluorophenyl)isoxazole-3-carboxylic acid to furnish the inhibitor **107**.

Synthesis of oxazole core **16** began with coupling of commercially available FMOC-L-Ser-O^{t}Bu and the hydrochloride salt of L-leucine methyl ester by the method described for compound **7** (Scheme 1) to afford intermediate **14** (Scheme 3). Acid hydrolysis followed by the nitrogen protection with a Boc group furnished alcohol **15**. Oxidation of hydroxy group in **15** with Dess−Martin's reagent followed by treatment with tosylmethyl isocyanide and SFC (chiral) separation furnished the desired oxazole core **16**. Oxazole core **16** was subsequently treated with HCl to deprotect the Boc

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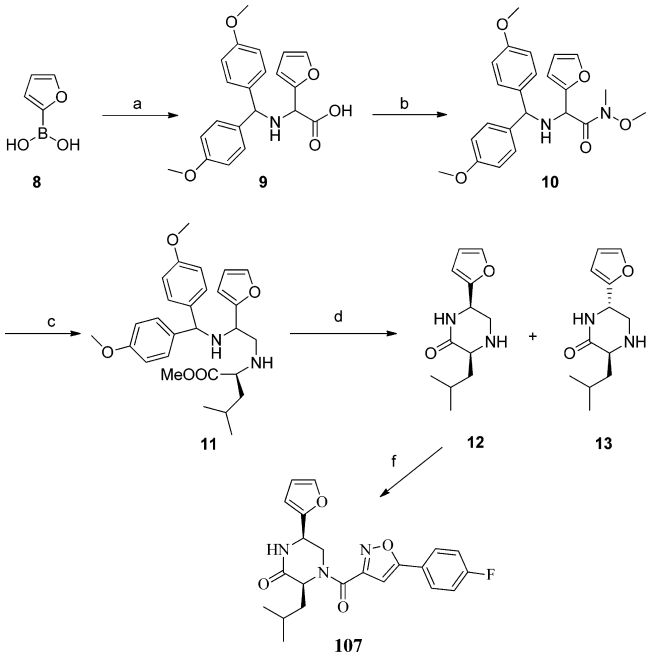
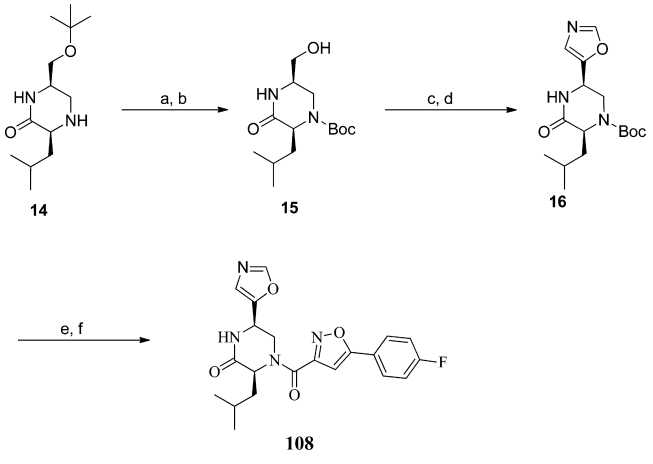
Scheme 2. Synthesis of Furanyl Derivative 107[a]



[a]Reagents and conditions: (a) glyoxylic acid monohydrate, bis(4-methoxyphenyl)methanamine, DCM (94%); (b) MeNHOMe·HCl, TBTU, DIPEA, CH_3CN (93%); (c) $LiAlH_4$,THF, −78 °C, then NH_2-Leu-OMe, $NaBH(OAc)_3$, DCM (49%); (d) 70% aqueous AcOH, reflux (75%); (e) 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid, TBTU, DIPEA, DCM (50%).

Scheme 3. Synthesis of Oxazole Derivative 108[a]



[a]Reagents and conditions: (a) HCl in dioxane, 70 °C; (b) $(BOC)_2O$, DIPEA, DCM, DMF (2 steps 87%); (c) DMP, DCM, 0 °C to RT; (d) tosylmethyl isocyanide, K_2CO_3, MeOH, 70 °C (2 steps 15%); (e) HCl in dioxane, DCM, 0 °C to RT; (f) HATU, DIPEA, DMF at RT (2 steps, 70%).

group and coupled with 5-(4-fluorophenyl)isoxazole-3-carboxylic acid to furnish inhibitor **108**.

Synthesis of piperazinone core derivative **109** bearing a oxazole moiety began with Fmoc protection of (*S*)-2-amino-but-3-en-1-ol (**17**) to give Fmoc protected alcohol **18** (Scheme 4). Oxidation of **18** with Dess−Martin's reagent followed by reductive amination with (*S*)-2-amino-4-methylpentanoic acid methyl ester furnished **19**. Compound **19** was converted to the piperazinone core **20** via the reaction sequence outlined in Scheme 1. Piperazinone **20** was coupled with 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid giving **21**, and subsequent oxidation of the vinyl goup with OsO_4 afforded the acid **22**. Acid **22** was treated with 2,2-dimethoxy-ethylamine to give the acetal product **23**, which was cyclized to furnish the inhibitor **109**.

Synthesis of pyrrolidine **26** began with a Petasis[14] multicomponent coupling reaction utilizing (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (**24**) followed by treatment with *N*-methylmethoxyamine to afford the corresponding Weinreb amide derivative **25** (Scheme 5). Amide **25** was subjected to reductive amination, deprotection, and acylation as described for compound **107** (Scheme 2) to furnish inhibitor **111** (trans diastereomers).

RESULTS AND DISCUSSION

A study was first undertaken to understand which structural elements of the piperazinone core were critical to its HCV inhibitory activity. This was accomplished by preparing analogues of key structural motifs found in **2a** (Table 1). Compounds derived from combination of core **2a** with *trans-S,S*, side chain or core **2b** with *trans-R,R* side chain were shown to be inactive, indicating that the stereochemistry of the core as well as the side chain were essential for activity. Alkylation of the *N*-1 amide nitrogen (**27**) resulted in a 10-fold loss in GT-1b potency. Removal of the core carbonyl group was also shown to be detrimental to GT-1b activity as exemplified by compound **28**. Together, these results seemed to imply that the piperazinone *N*-1 nitrogen and *C*-2 carbonyl groups might be providing an important hydrogen bond donor and acceptor interaction with the NS4B protein. The cis stereochemical relationship between *C*-3 and *C*-6 of the piperazinone core was also shown to be important because changing the stereochemistry at *C*-3 or *C*-6 to the *R*-stereochemistry as shown in compound **29** and **30** resulted in complete loss of activity. Molecular modeling of **2a** indicated that the lowest energy conformers of **2a** have the *i*-Bu at *C*-3 above plane and the *i*-Bu at *C*-6 in plane with the piperazinone ring. In the case of **29** and **30**, the *i*-Bu substituents at *C*-3 and *C*-6 are in opposite orientation to each other and also out of plane to the piperazinone ring. On the basis of these molecular modeling and activity results, we concluded that the cis stereochemical relationship between *C*-3 and *C*-6 substituents of the piperazinone core confers the preferred orientation for tight binding.

Futher investigation of the *C*-3 and *C*-6 core substitutents showed that substituting an ethyl moiety for the *i*-Bu at *C*-3 (**31**) or *C*-6 (**32**) resulted in substantial loss of activity. Replacing the *i*-Bu moiety with a *sec*-Bu (**33**) group at *C*-6 also led to a loss of GT-1b potency. The only modification to **2a** that maintained GT-1b activity was replacement of *n*-Pr (**34**) for *i*-Bu at *C*-6. The observed results for modifications at *C*-3 and *C*-6 suggest that these positions are sensitive to the nature of the hydrophobic groups. On the basis of the piperazinone core SAR, it is apparent that the amide function and the cis stereochemistry (*S,S*) of the piperazinone core are essential for GT1b activity.

A series of replacements for the cyclopropyl moiety were investigated (Table 2). Replacement of the cyclopropyl amide moiety of **2a** with propiolamide (**35**) produced a 3-fold loss in activity. Cinnamide (*trans-E*) **36** showed 2-fold loss in activity, while the *cis*-(*Z*)-cinnamide (**37**) showed a complete loss of activity. These findings indicated that the trans geometry of the

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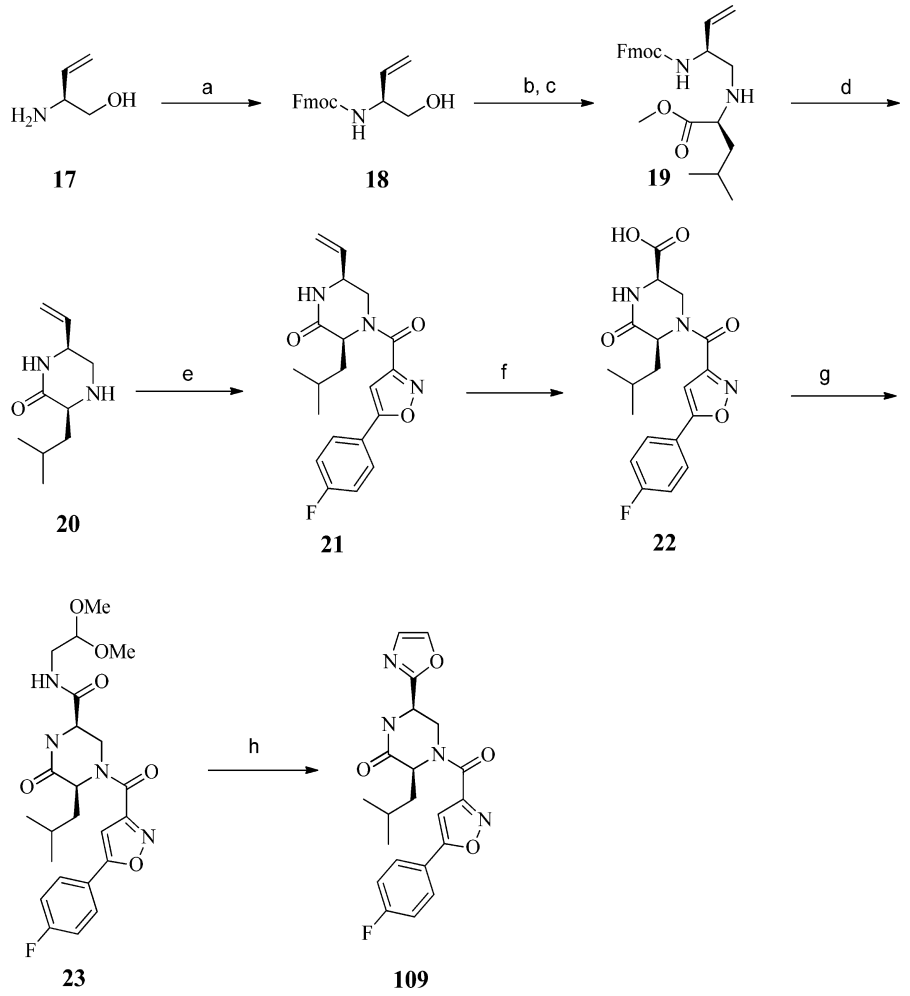
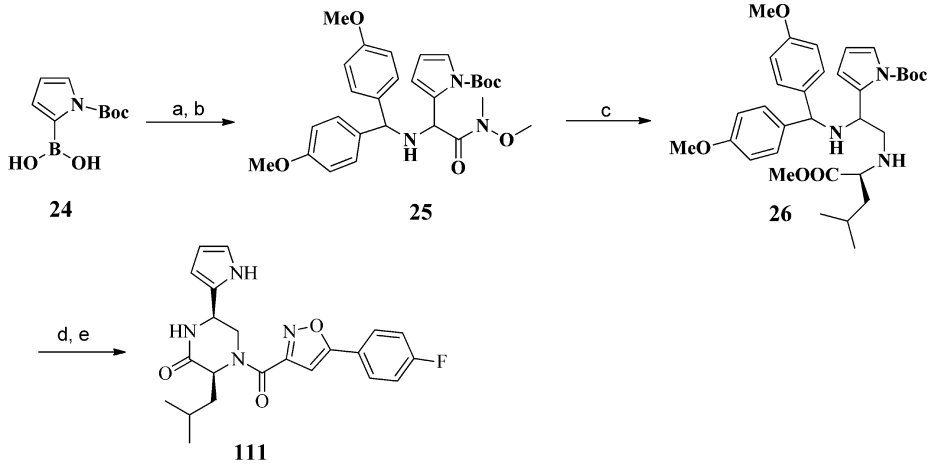
Scheme 4. Syntheses of Inhibitors 21, 22, and 109[a]



[a]Reagents and conditions: (a) Fmoc-Cl, aq dioxane, 0 °C to RT (quantitative); (b) DMP, DCM; (c) (*S*)-2-amino-4-methylpentanoic acid methyl ester HCl salt, $NaBH(OAc)_3$, THF (2 steps 42%); (d) 40% Et_2NH in EtOH, 60 °C (42%); (e) 5-(4-fluorophenyl)isoxazole-3-carboxylic acid, HOBT hydrate, EDC, DIPEA, CH_3CN (30%); (f) OsO_4, oxone, DMF (60%); (g) 2,2-dimethoxy-ethylamine, HOBT hydrate, EDC, DIPEA, CH_3CN (50%); (h), TFA, DCM (75%).

Scheme 5. Synthesis of Pyrrolidine Derivative 111[a]



[a]Reagents and conditions: (a) glyoxylic acid monohydrate, bis(4-methoxyphenyl)methanamine, DCM; (b) MeNHOMe·HCl, TBTU, DIPEA, CH_3CN (2 steps 98%); (c) $LiAlH_4$,THF, −78 °C, then NH_2-Leu-OMe, $NaBH(OAc)_3$, DCM, (67%); (d) 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid, TBTU, DIPEA, CH_3CN; (e) 70% aq AcOH, reflux (2 steps 12%).

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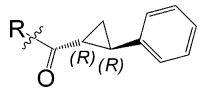
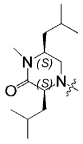
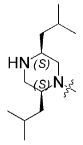
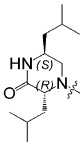
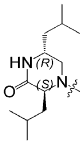
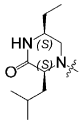
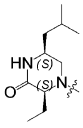
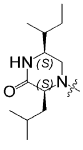
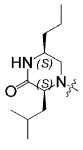
Table 1. SAR of Piperazinone Core

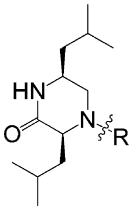


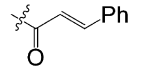
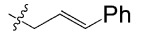
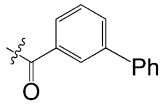
Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)	CC_{90}[a] (μM)
27		8.8	>10
28		>10	>10
29		>10	>10
30		>10	>10
31		5.3	>10
32		>10	>10
33		>10	>10
34		0.9	>10

[a]For description of the assays, see ref 15. Replicon data is the average of n = 2 with ±0.2 mM SD.

side chain is important for potency. Further analysis indicated that the side chain carbonyl group may not be required for potency as shown by the *N*-alkylated analogue **38**. Removal of side chain phenyl group (**39**) led to complete loss of activity, indicating that the phenyl group is essential and might be

Table 2. SAR of Cyclopropyl Amide Side Chain Replacements



Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
35		3.2
36		2.3
37		>10
38		3.3
39		>10
40		>10

[a]For description of the assay, see ref 15. Replicon data is the average of n = 2 with ±0.2 μM SD.

involved in $\pi-\pi$ interactions with the target protein. Replacement of cyclopropyl moiety with a bulky pheny moiety (**40**) led to significant loss in GT-1b activity. On the basis of the observed SAR, we decided to utilize the *trans*-cinnamide side chain for further SAR studies.

In an effort to improve the potency of the nonchiral cinnamide side chain, investigation of phenyl group substituent effects was undertaken. Several analogues[16] were prepared by coupling the piperazinone core (**7**) with commercially available cinnamic acid derivatives (Scheme 1). For selected compounds, GT1b inhibitory activity is shown in Table 3. Methyl (**41**), trifluoromethyl (**42**), and methoxy (**43**) phenyl substituents were not tolerated, as indicated by the significant decrease in GT1b activity. Loss of activity was also observed for compounds having a nitro (**44**), dimethylamino (**45**), methyl sulfone (**46**), or carboxylate (**47**) phenyl substituent. These results indicate that strong electron withdrawing and polar functionalities were not tolerated on the phenyl moiety of the cinnamide side chain. Improvement in potency was seen for *p*-chloro (**49**) and *p*-fluoro (**52**) phenyl-substituted cinnamide derivatives, however, these derivatives only showed comparable potency to **2a**. The *p*-Br, *m*-Cl, and *o*-Cl analogues **48**, **50**, and **51** showed 4-fold loss in activity. Interestingly, the disubstituted compounds **53** and **54** did demonstrate GT-1b potency similar to the mono substituted analogue **52**. For the cinnamide series, the SAR showed that substituents at the *para*-position

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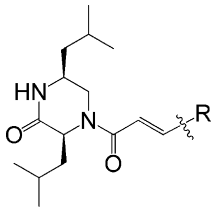
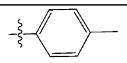
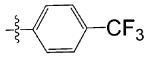
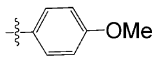
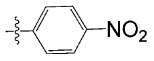
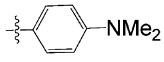
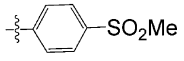
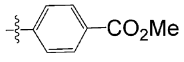
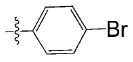
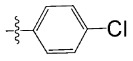
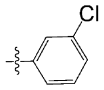
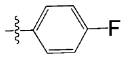
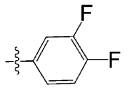
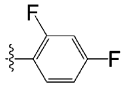
Table 3. SAR of the *trans*-Cinnamide Side Chain Analogues



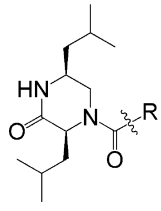
Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
41		5.3
42		8.1
43		8.7
44		>10
45		>10
46		>10
47		>10
48		7.8
49		1.6
50		8.1
51		9.7
52		1.5
53		1.5
54		1.5

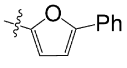
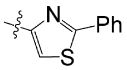
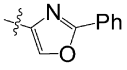
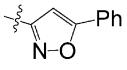
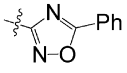
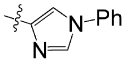
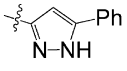
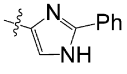
[a]For description of the assay, see ref 15. Replicon data is the average of n = 2 determinations with ±0.2 μM SD.

consistently produced compounds with improved potency relative to compounds having a *meta*- or *ortho*-substituted phenyl moiety and that fluoro and chloro are optimal substituents. The replicon potency of compounds **49**, **52**, and **53** which lack the chiral cyclopropyl unit was shown to be comparable to that observed for compound **2a** and, consequently, served as the basis for further lead optimization by exploring cinnamide double-bond replacements.

To further improve the potency relative to compounds having the cinnamide side chain (**52**), we investigated amide side chains having 5-membered heterocyclic ring systems in place of the cinnamide double bond. The hope was that such a replacement might provide additional electrostatic interactions while still maintaining a favorable geometry. A commercially available diverse set of heterocyclic acid derivatives capable of forming H-bond acceptor and/or donor interactions were coupled to the piperazinone core[16] by the method shown in Scheme 1, and the activity of selected compounds is shown in Table 4. Compound **56** exhibiting H-acceptor characteristics

Table 4. HCV Replicon Activity of Heterocyclic Amide Side Chain



Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
55		>5.8
56		>7.9
57		>10
58		1.1
59		1.4
60		>10
61		>10
62		>10

[a]For description of the assay, see ref 15. Replicon data is the average of n = 2 determinations with ±0.2 μM SD.

led to a several-fold decrease in GT-1b activity. Similarly, the oxazole **57** and imidazole **60** did not show GT-1b activity up to 10 μM. Pyrazole **61** and imidazole **62** having H-donor ability were also shown to be inactive up to 10 μM. However, the isoxazole **58** and oxadiazole **59** showed a 2-fold increase in GT-1b activity. We surmise that both **67** and **68** provide favorable

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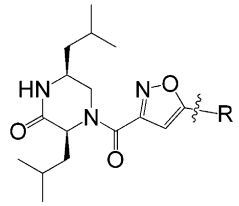
substituent orientation and electronic character to result in the observed 2-fold increase in GT-1b potency when compared to the corresponding cinnamide **36**. Replacing the side chain phenyl group of **58** with other heterocyclic moieties that can provide similar $\pi-\pi$ interaction with protein did not pan out. Isoxazole **58** became the basis for further lead optimization by studying substituent effects on the phenyl moiety.

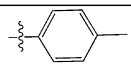
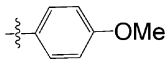
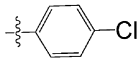
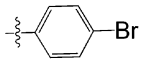
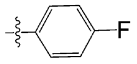
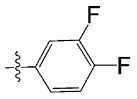
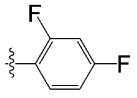
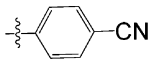
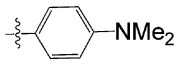
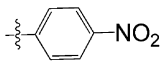
The next step in our attempt to improve the GT-1b potency beyond that of **58** investigated the effect of substituents on the phenyl moiety while maintaining intact the piperazinone core and isoxazole amide side chain (Table 5). Synthesis of these compounds was achieved by the method in Scheme 1. As expected (see Table 3), the *para*-substituted methyl (**63**), methoxy (**64**), cyano (**70**), and nitro (**72**) derivatives showed no improvement in GT-1b activity, while the GT-1b potency of *para*-F (**67**), *para*-Cl (**65**), and *para*-Br (**66**) derivatives increased by 8-fold, 4-fold, and 2-fold, respectively. The 3,4- and 2,4-disubstituted compounds (**68** and **69**) showed a 5-fold decrease in potency when compared to the *para*-F substituted compound (**67**). After optimizing the GT-1b potency of the piperazinone side chain, we decided to focus on modification of the core in an attempt to further improve the GT-1b potency.

Piperazinone core *C*-6 substituent effects were probed using hydrophobic moieties (acyclic, branched, and cyclic) to study space constraints and substituents having H-acceptor and/or H-donor moieties to evaluate potential electrostatic interactions (Table 6).[16] The neopentyl (**75**), *sec*-butyl (**76**), and *tert*-butyl (**84**) derivatives produced a significant reduction in GT-1b activity compared to **67**. The *n*-propyl analogue **73** retained potency, while the ethyl analogue **82** and *n*-Bu analogue **80** showed a 6−4-fold decrease in potency, respectively. The propenyl compound **74** exhibited 2-fold improvement in GT-1b potency, yet the allyl moiety (**77**) and vinyl moiety (**21**) containing compounds showed 3-fold and 8-fold decrease in GT-1b potency, respectively. The cyclopropyl methyl compound **78** showed 3-fold decrease in potency, and the cyclopropyl analogue **85** showed 8-fold loss of potency. The *i*-propyl analogue **83** retains the GT-1b potency of parent compound **67**. The thio-ether analogues **79** and **81** showed 2- and 3-fold decrease in GT-1b potency, respectively. Among the cycloalkyl analogues, cyclobutyl derivative **86** showed 6-fold loss of potency, but the cyclopentyl analogue **87** proved to be a slightly more potent inhibitor than **67**. Among the 6-membered cycloalkyl analogues, cyclohexyl compound **88** proved to be slightly less potent, while the tetrahydropyran analogue **93** showed a 4-fold loss of potency, and thio-pyran **94** showed 8-fold decrease in potency. The phenyl analogue **95** showed potency similar to that seen for **74** and **87** but demonstrated better metabolic stability[17] and was chosen for further SAR optimization.

The substituent effects on the *C*-6 phenyl group of **95** were investigated but did not lead to any improvement in potency (Table 7). A nearly 10-fold decrease in GT-1b potency was observed when a methyl (**96**) or fluoro (**99**) substituent was introduced at the 4-position of the phenyl moiety. Significant loss of potency also occurred with introduction of chloro (**98**) and trifluoromethyl (**102**) substitution, indicating that the substitution at the 4-position of the phenyl moiety was not well tolerated. In the case of fluoro substituted compounds, substitution at the *meta* position (**100**) resulted in 4-fold loss of potency, while the *ortho*-F compound (**101**) showed only a modest loss of potency. Because we were not able to improve the GT-1b potency of inhibitor **95** through the addition of

Table 5. Effect of Phenyl Ring Substitution on the GT-1b Potency of Isoxazole Derivatives



Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
63		1.54
64		1.35
65		0.29
66		0.47
67		0.13
68		0.65
69		0.68
70		1.53
71		>10
72		2.60

[a]For description of the assay, see ref 15. Replicon data is the average of $n = 2$ determinations with ±0.20 μM SD.

substituents on the phenyl moiety, our next step was to investigate heterocyclic moieties as replacements for the *C*-6 phenyl group.

We envisioned that introduction of a heterocyclic moiety at the C-6 position of the piperazinone ring should result in retention of any favorable $(\pi-\pi)$ interactions provided by a *C*-6 phenyl group and may introduce additional interactions that could further improve the GT1 potency. Consequently, we synthesized a series of *C*-6 heterocyclic derivatives (**103**−**113**)

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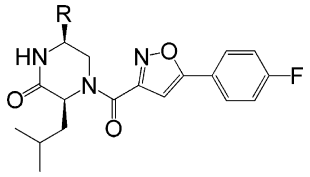
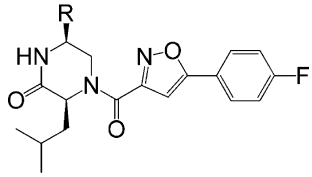
Table 6. Piperazinone *C*-6 Substituent SAR

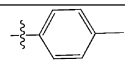
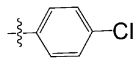
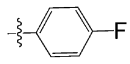
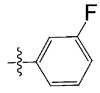
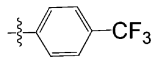


Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
21		1.05
73		0.10
74		0.06
75		2.00
76		>10
77		3.04
78		0.45
79		0.24
80		0.53
81		0.39
82		0.71
83		0.17
84		1.80
85		1.10
86		0.69
87		0.09
88		0.19
89		7.25
90		>10
91		>10
92		2.74
93		0.47
94		0.87
95		0.06

[a]For description of the assay, see ref 15. Replicon data is the average of $n = 2$ determinations with ±0.20 μM SD and for **95** it was ±0.02 μM SD.

Table 7. Substitution Effects on the GT-1b Potency of Piperazinone *C*-6 Phenyl Analogues



Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
96		0.76
97		1.42
98		1.53
99		0.65
100		0.27
101		0.10
102		2.75

[a]For description of the assay, see ref 15. Replicon data is the average of $n = 2$ determinations with ±0.20 μM SD.

using synthetic methods depicted in Schemes 2−5 (Table 8). The 2-thiophene compound **103** showed a dramatic 10-fold increase in potency, while the 3-thiophene compound **104** showed a less dramatic 3-fold increase the GT1b potency when compared to the corresponding phenyl analogue **95**. The 3-methyl-thiophene compound **105** was 110-fold less active, and 5-methyl-thiophene **106** was 30-fold less active than the unsubstituted 2-thiophene analogue **103**. The furan derivative **107** was 3-fold less active than **95** and 30-fold less active than the 2-thiophene derivative **103**. The 2-oxazole **109** and 2-thiazole **110** were inactive, but the 4-oxazole **108** retained the activity of the phenyl derivative **95**. The results for the oxazoles **108** and **109** suggested that the position of the nitrogen atom in the heterocyclic ring was critical to the GT-1b potency. The unsubstituted 2-pyrrole compound **111** showed a 11-fold loss of activity, but the 3-pyrrole compound **112** showed similar potency to the phenyl analogue **98**.

An attempt to further optimize the potency of 3-thiophene compound (**104**) led to the 2-chloro thiophene derivative (**113**) that showed a 6-fold improvement in the GT-1b potency (Table 8). Further evaluation of inhibitors **103** and **108** in both GT-1a and 1b replicons showed that the GT-1b potency was superior to GT-1a potency (Table 9). The thiophene derivative **103** exhibited a preferable metabolic stability, yet the oxazole **108** had superior solubility characteristics and none of these compounds showed any CYP (1A2, 2C9, 2D6, and 3A4) inhibition up to 10 μM. Neither **103** nor **108** demonstrated any appreciable GT-2 activity when tested in a replicon assay. Unfortunately, due to their lack of broad genotype coverage, the piperazinone class of NS4B inhibitors did not progress further into preclinical development.

CONCLUSION

HCV infection is a disease that afflicts a large patient population with negative long-term consequences. Research

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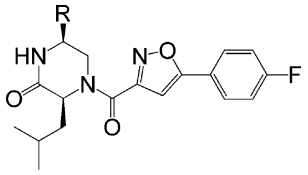
Table 8. Optimization of GT-1b Potency with *C*-6 Heterocyclic Moieties



Compound	R	HCV Replicon[a] GT1b EC_{90} (μM)
103		0.006
104		0.026
105		0.680
106		0.180
107		0.180
108		0.070
109		>10
110		>10
111		0.680
112		0.063
113		0.004

[a]For description of the assay, see ref 15. Replicon data is the average of $n = 2$ determinations with ±0.025 μM SD, and for **113** it was ±0.002 μM SD.

has shown that the combination of potent direct acting antivirals can provide a cure for this debilitating and often fatal disease. Therefore, novel direct acting antiviral agents acting by new mechanisms of action have the potential to become part of future therapeutic regimens. With this desire in mind, an HTS campaign was undertaken that led to the identification of piperazinone **2a** as a novel and specific HCV inhibitor. Compound **2a** was shown to be an effective inhibitor of HCV genotype 1b and 1a replicons, with a 50-fold activity/toxicity window in whole cells and excellent selectivity over other viruses. Resistance mapping studies aimed at determining which HCV nonstructural protein was the target for piperazinone **2a** revealed that mutations at residues 90 and 98 in the TM1 domain of NS4B resulted in a significant reduction in the HCV inhibitory activity of compound **2a**, thus supporting NS4B was the primary target. An extensive chemistry-driven SAR development effort looking at multiple positions of the piperazinone core and amide side chain was performed on compound **2a**. Although we did not have structural information to guide the inhibitor design, we were able to effectively develop a robust SAR picture that led to potent HCV GT1 inhibitors. Initial studies kept the piperazinone core constant, explored the side chain cyclopropyl replacements as well as the substituents on the side chain phenyl moiety to optimize compound **2a** potency. These studies resulted in the *p*-fluorophenylisoxazole amide as the optimized side chain as shown in compound **67**. The core *C*-6 SAR investigation demonstrated that the *i*-Bu moiety could be replaced with a phenyl moiety and resulted in molecule **95** with double-digit nanomolar GT-1b potency. Further optimization of the lead compounds GT-1b potency led to the single-digit nanomolar GT-1b and low double-digit nanomolar GT-1a HCV inhibitors **103** and **108** having acceptable metabolic stability and solubility parameters. Through this effort, we have discovered a useful novel prototype inhibitor of HCV NS4B that has good genotype 1b and 1a potency with suitable stability, solubility parameters, and no CYP liabilities but lacked broad genotype coverage suitable for further development.

The identification and optimization of the piperazinone class of NS4B inhibitors did however introduce a new structural class of NS4B inhibitors into the repertoire of HCV replication inhibitors. Each of the previously identified NS4B inhibitors **1a**–**d** incorporates a critical 6,5-bicyclic ring system containing similar patterns of substitution. In fact, compounds **1b** and **1c** are based on the original anguizole motif where each are substituted similarly and include an essential amide side chain. Even though indole inhibitor **1d** does not display an amide side chain directly attached to the 5-membered ring of the bicycle, it shares with **1b** and **1c** a common amide-type functionality on its side chain at a similar distance from the 5-membered ring point of attachment. The clear structural similarities among inhibitors **1a**–**d** and the existence of identical resistance profiles supports the hypothesis that they all share a common binding site on the NS4B protein. In stark contrast to the known NS4B inhibitors **1a**–**d**, the piperazinone class of inhibitors exemplified by lead **2a** and optimized compound **103** do not incorporate a bicyclic nucleus, however, this class does incorporate an amide side chain. Any attempts to incorporate structural features, such as bicyclic systems, similar to those noted in **1a**–**d**, resulted in loss of activity. In addition, efforts to draw structural comparisons between the piperazinones **2a** and **103** with inhibitors **1a**–**d** did not lead to any understanding of common topographical motifs. However, irrespective of the apparent lack of significant structural similarity between the piperazinone class of NS4B inhibitors and the previously reported inhibitors **1a**–**d**, they all share a F98L resistant phenotype and lose substantial activity when assayed against the GT2a replicon where the wild-type phenotype is L98. It can therefore be surmised that because of this common resistant amino acid substitution and activity loss against the GT2a replicon, there must be some overlap in binding sites between the structurally similar inhibitors **1a**–**d** and the piperazinone class of NS4B inhibitors. Therefore, it may be reasonable to speculate that overlapping binding sites occur in the region of the NS4B

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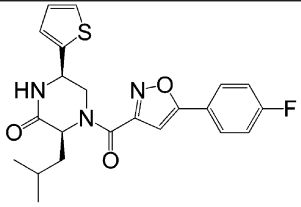
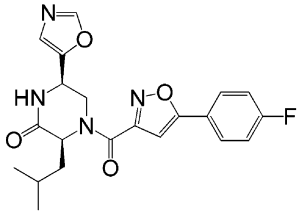
Table 9. Lead Compounds Profiling Data[a]

Compound	R	HCV Replicon 1b EC_{90} (μM)	HCV Replicon[a] 1a EC_{90} (μM)	MetStab HLM Half Life (min)	Solubility (μg/ml) pH 7.4	Solubility (μg/ml) pH4.0
103		0.006	0.022	120	0.2	0.2
108		0.070	0.016	40	151	147

[a]For description of the assays, see ref 15. GT 1a Replicon data is the average of n = 2 determinations with ±0.010 μM SD.

protein where the amide side chains of **1a−d** and that of the piperazinones bind. However, definitive proof of this common binding site will only occur when cocrytal structures of these different inhibitors with the NS4B protein are obtained. The possibility exists that with the identification of the structurally novel and potent piperazinone class of NS4B inhibitors in conjunction with the information reported for the other classes of NS4B inhibitors, further work may lead to the identification of NS4B inhibitors that can overcome the genotype coverage limitations that currently plague the search for clinically viable NS4B inhibitors.

EXPERIMENTAL SECTION

General Methods. Unless specified otherwise, starting materials were available from commercial sources. Dry solvents and reagents were of commercial quality and were used as purchased. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Advance II 400 MHz and Varian AS 400 MHz spectrometers at room temperature with tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and peak multiplicity are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Purities of the final compounds were determined by HPLC and UPLC-MS and were ≥95% purity. HPLC conditions to assess ≥95% purity of final compounds were as follows: Shimadzu HPLC 20AB Sepax HP-C18 4.6 mm × 50 mm (5 μm); flow rate, 3.0 mL/min; acquisition time, 6 min; wavelength, UV 230 nm; oven temperature, 40 °C. UPLC-MS conditions to assess ≥95% purity for final compounds were as follows: Waters Acquity UPLCMS UPLC BEH C18 2.1 mm × 50 mm (1.7 μm); flow rate, 0.8 mL/min; wavelength, UV 254 nm; oven temperature, 50 °C. Chiral HPLC was conducted on Shimadzu HPLC 20A. The preparative HPLC system includes two sets of Gilson 306 pumps, a Gilson 156 UV/vis detector, and a Gilson 215 injector and fraction collector, with Unipoint control software. A YMC 25 mm × 30 mm × 2 mm column was used. The mobile phase was HPLC grade water (A) and HPLC grade acetonitrile (B) system. SFC separation conditions were as follows: Berger Multi-Gram SFC from Mettler Toledo Co, Ltd.; column, AD 250 mm × 30 mm (5 μm), 38 °C; mobile phase, supercritical CO_2 (A), MeOH (B), A/B = 75/25 at 60 mL/min; nozzle pressure (100 Bar), nozzle temperature (60 °C); evaporator temperature (20 °C); trimmer temperature (25 °C); wavelength (220 nm). LC/MS was conducted on Shimadzu LCMS 2010EV using electrospray positive [ES + ve to give MH^+] equipped with a Shim-pack XR-ODS 2.2 μm column (3.0 mm × 30 mm, 3.0 mm i.d.), eluting with 0.0375% TFA in water (solvent A) and 0.01875% TFA in acetonitrile (solvent B). Unless specified otherwise, Column chromatography was performed on Intelli Flash 280 (AnaLogix) using silica flash columns.

General Procedures for the Synthesis of Piperazinone Core. *(3S,6S)-3,6-Diisobutylpiperazin-2-one (7). A. (S)-(9H-Fluoren-9-yl)-methyl-(1-methoxy-(methyl)-amino)-4-methyl-1-oxopentan-2-yl)-carbamate (4).* A mixture of FMOC-Leu-OH (**3**, 0.5 g, 1.41 mmol), TBTU (0.68 g, 2.12 mmol), and *N,O*-dimethylhydroxylamine hydrochloride (0.207 mg, 2.12 mmol) in MeCN (5 mL) were stirred at 0 °C under nitrogen for 10 min. DIPEA (0.736 mL, 4.23 mmol) was added dropwise (5 min) and stirred for 30 min. The reaction mixture was allowed to warm to rt and stirred for 2.5 h. The reaction mixture concentrated in vacuo, and the residue was dissolved in EtOAc (100 mL) and washed sequentially with 1 M aqueous HCl (15 mL × 2), 1 M aqueous $NaHCO_3$ (15 mL × 2), and brine (20 mL). The solution was dried over Na_2SO_4 and concentrated in vacuo to dryness to afford **4** (574 mg, 98% yield), which was used for the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.76−7.58 (m, 4 H), 7.41−7.29 (m, 4 H), 5.42 (d, J = 9.2 Hz, 1 H), 4.82−4.80 (m, 1 H), 4.37−4.32 (m, 2 H), 4.23−4.20 (t, J = 14.0 Hz, 1 H), 3.79 (s, 3 H), 3.21 (s, 3 H), 1.74−1.70 (m, 1 H), 1.52−1.48 (m, 1 H), 0.99−0.94 (m, 6 H). MS (ESI): m/z 413.2 (M + 1).

B. (S)-(9H-Fluoren-9-yl)methyl (4-methyl-1-oxopentan-2-yl)-carbamate (5). To a solution of **4** (0.4 g, 0.97 mmol) in dry THF (10 mL) at −78 °C was added $LiAlH_4$ (80 mg, 1.94 mmol), and the

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reaction was stirred under argon for 30 min. The reaction was quenched with 6% aqueous HCl (10 mL, pH 4−5) at −78 °C, and the solution was allowed to warm to 0 °C and stirred for 5 min. This mixture was partitioned between EtOAc/brine (1:2, 100 mL) and extracted with EtOAc (50 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **5** (320 mg, 98% yield), which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1 H), 7.77−7.59 (m, 4 H), 7.42−7.30 (m, 4 H), 5.18 (d, J = 7.2 Hz, 1 H), 4.45 (d, J = 6.8 Hz, 2 H), 4.34−4.32 (m, 1 H), 4.24−4.21 (t, J = 6.8 Hz, 1 H), 1.75−1.68 (m, 2 H), 1.48−1.39 (m, 1 H), 0.98−0.96 (m, 6 H). MS (ESI): m/z 438.1 (M + 1).

*C. (S)-Methyl 2-(((S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)-amino)-4-methylpentyl)-amino)-4-methylpentanoate (**6**).* To a well dried mixture of aldehyde **5** (290 mg, 0.86 mmol) and Leu-OMe hydrochloride (160 mg, 0.86 mmol) were added DCM (3 mL) and $NaBH(OAc)_3$ (260 mg, 1.2 mmol) and stirred at rt under nitrogen for 6 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL) and was extracted with EtOAc (50 mL) and dried over Na_2SO_4. The solvent was concentrated in vacuo and the crude product purified by column chromatography (0−25% EtOAc in hexanes) to afford **6** (360 mg, 90% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77−7.59 (m, 4 H), 7.41−7.29 (m, 4 H), 4.82 (d, J = 5.6 Hz, 1 H), 4.40 (d, J = 7.2 Hz, 2 H), 4.24 (t, J = 6.8 Hz, 1 H), 3.71 (s, 3 H), 3.27−3.24 (m, 1 H), 2.71−2.42 (m, 2 H), 1.76−1.60 (m, 2 H), 1.46−1.30 (m, 4 H), 0.92−0.87 (m, 12 H). MS (ESI): m/z 467.2 (M + 1).

*D. (3S,6S)-3,6-Diisobutylpiperazin-2-one (**7**).* To a solution of **6** (120 mg, 0.26 mmol) in DCM (1.6 mL) was added diethyl amine (0.4 mL, 3.86 mmol 20% in DCM) at rt under nitrogen and stirred overnight. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (0−25% EtOAc in hexanes) to afford **7** (52 mg, 95% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (s, 1 H), 3.47−3.39 (m, 2 H), 3.02−2.98 (m, 1 H), 2.79−2.74 (m, 1 H), 1.80−1.54 (m, 4 H), 1.45−1.31 (m, 2 H), 0.96−0.90 (m, 12 H). MS (ESI): m/z 213.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**2a**) and (3S,6S)-3,6-Diisobutyl-4-((1S,2S)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**2b**).* To a well dried (±)-(1*R*,2*R*)-2-phenylcyclopropanecarboxylic acid (53.5 mg, 0.33 mmol), TBTU (127.5 mg, 0.396 mmol), and **7** (70 mg, 0.33 mmol) was added MeCN (3 mL) followed by DIPEA (0.17 mL, 0.99 mmol) dropwise (5 min) and stirred at rt for 3 h. The reaction mixture was concentrated in vacuo, and the crude residue was dissolved in EtOAc (25 mL) and washed sequentially with 1 M aqueous HCl (15 mL × 2), 1 M aqueous $NaHCO_3$ (15 mL × 2), and brine (20 mL). The solution was dried over Na_2SO_4 and concentrated in vacuo, and the crude product was purified by column chromatography (0−60% EtOAc in hexanes) to afford a mixture of products **2a** and **2b** (105.8 mg, 90% yield). The product mixture (**2a** and **2b**) was separated by SFC (chiral) column to furnish **2a** (30 mg, 25% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (s, 1 H), 3.23−3.26 (m, 1 H), 3.07 (dd, J = 9.6 Hz, 3.6 Hz,1 H), 2.80 (dd, J = 12.8 Hz, 4.4 Hz, 1 H), 2.58 (dd, J = 13.2 Hz, 5.2 Hz, 1 H), 2.36 (s, 1 H), 1.74−1.78 (m, 1 H), 1.59−1.65 (m, 1 H),1.28−1.48 (m, 4 H), 0.80−0.89 (m, 12 H). MS (ESI): m/z 213.1 (M + 1). Compound **2b** (29 mg, 25% yield, white solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (s, 1 H), 3.23−3.26 (m, 1 H), 3.07 (dd, J = 9.6 Hz, 3.6 Hz,1 H), 2.80 (dd, J = 12.8 Hz, 4.4 Hz, 1 H), 2.58 (dd, J = 13.2 Hz, 5.2 Hz, 1 H), 2.36 (s, 1 H), 1.74−1.78 (m, 1 H), 1.59−1.65(m, 1 H),1.28−1.48 (m, 4 H), 0.80−0.89 (m, 12 H). MS (ESI): m/z 213.1 (M + 1).

*(3S,6R)-6-(Furan-2-yl)-3-isobutylpiperazin-2-one (**12**) and (3S,6S)-6-(Furan-2-yl)-3-isobutylpipera-zin-2-one (**13**). A. 2-((Bis(4-methoxyphenyl)methyl)amino)-2-(furan-2-yl)acetic Acid (**9**).* To a stirred solution of glyoxylic acid monohydrate (920 mg, 10.11 mmol) in DCM (70 mL) were added bis(4-methoxyphenyl)methanamine (2.46 g, 10.11 mmol) and 2-furanboronic acid (1.13 g, 10.11 mmol), and the reaction mixture was stirred at rt for 2 min to form clear solution. The solution was purged with argon (2 min), and the sealed reaction mixture was stirred for overnight. The reaction mixture was concentrated in vacuo to afford compound **9** (3.5 g, 94% yield) as pale-yellow foam. The crude product was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1 H), 7.27−7.24 (m, 4 H), 6.81−6.79 (m, 4 H), 6.28 (br s, 1 H), 6.24 (br s, 1 H), 4.80 (s, 1 H), 4.44 (s, 1 H) 3.76 (s, 3 H), 3.75 (s, 3 H) 3.70 (s, 1 H). MS (ESI): m/z 368.0 (M + 1).

*B. 2-((Bis(4-methoxyphenyl)methyl)amino)-2-(furan-2-yl)-N-methoxy-N-methyl-acetamide (**10**).* To a mixture of **9** (0.5 g, 1.36 mmol), TBTU (0.66 g, 2.04 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (0.2 g, 2.04 mmol) in dry MeCN (6 mL), DIPEA (710 uL, 4.08 mmol) was added and stirred at rt for 4 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (0−40% EtOAc in hexanes) to afford compound **10** (520 mg, 93% yield) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1 H), 7.34−7.25 (m, 4 H), 6.85−6.79 (m, 4 H), 6.33−6.32 (m, 1 H), 6.22 (d, J = 3.2 Hz, 1 H), 4.83 (s, 1 H), 4.68 (s, 1 H) 3.77 (s, 3 H), 3.75 (s, 3 H) 3.28 (s, 3 H), 3.20 (s, 3 H). MS (ESI): m/z 411.1 (M +1).

*C. (2S)-Methyl 2-((2-((Bis(4-methoxyphenyl)methyl)amino)-2-(furan-2-yl)ethyl)amino)-4-methyl-pentanoate (**11**).* To a solution of compound **10** (0.2 g, 1.22 mmol) in dry THF (3 mL) at −78 °C, $LiAlH_4$ (40 mg, 0.97 mmol) was added and stirred at −78 °C under argon for 3 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (2 mL) by dropwise addition. This mixture was partitioned between EtOAc and brine (1:1, 50 mL) and extracted with EtOAc (50 mL). The organic layers were combined and washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude aldehyde intermediate was used for the next step without further purification. To a solution of aldehyde (0.4 g, 1.14 mmol) and HCl salt of Leu-OMe (210 mg, 1.14 mmol) in DCM (8 mL), $NaBH(OAc)_3$ (340 mg, 1.59 mmol) was added and the mixture was stirred at rt under argon for 6 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (30 mL), and the aqueous solution was extracted with EtOAc (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (0−40% EtOAc in hexanes) to afford compound **11** (0.27 g, 49% yield) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.36 (m, 1 H), 7.30−7.17 (m, 4 H), 6.85−6.76 (m, 4 H), 6.32−6.31 (m, 1 H), 6.16−6.11 (m, 1 H), 4.64 (d, J = 5.2 Hz, 1 H), 3.78−3.67 (m, 7 H) 3.28−3.20 (m, 1 H), 2.94−2.88 (m, 1 H) 2.75−2.66 (m, 1 H), 1.96 (s, 1 H), 1.70−1.64 (m, 1 H), 1.48−1.40 (m, 2 H), 0.94−0.86 (m, 6 H). MS (ESI): m/z 481.1 (M + 1).

*D. (3S,6R)-6-(Furan-2-yl)-3-isobutylpiperazin-2-one (**12**) and (3S,6S)-6-(Furan-2-yl)-3-isobutylpiperazin-2-one (**13**).* Compound **11** (0.4 g, 0.84 mmol) was dissolved in 70% aqueous AcOH (5 mL) and heated under reflux for 3 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (20−60% EtOAc in hexanes) to afford compounds **12** (85 mg, 40% yield) and **13** (75 mg, 35% yield). Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.38 (m, 1H), 6.40 (s, 1H), 6.45−6.34 (m, 1H), 6.26−6.25 (m, 1H), 4.63−4.61 (m, 1H), 3.46−3.43 (m, 1H), 3.24−3.23 (m, 2H), 1.91 (s, 1H), 1.89−1.79 (m, 2H), 1.53−1.47 (m, 1H) 0.95−0.82 (m, 6H). MS (ESI): m/z 223.0 (M + 1). Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.37 (m, 1H), 6.34−6.33 (m, 1H), 6.26−6.25 (m, 1H), 6.07 (s, 1H), 4.68 (m, 1H), 3.75 (d, J = 7.2 Hz, 1H), 3.47−3.43 (m, 1H), 3.40−3.35 (m, 2H), 3.06−3.01 (m, 1H), 1.89−1.78 (m, 2H), 1.60−1.54 (m, 1H) 0.97−0.92 (m, 6H). MS (ESI): m/z 223.0 (M + 1).

*(3S,6R)-6-(tert-Butoxymethyl)-3-isobutylpiperazin-2-one (**14**).* Synthesized from FMOC-L-Ser-O^{t}Bu (42.0 g, 0.114 mol) and HCl salt of Leu-OMe (21.0 g, 0.115 mol) by the method described for the compound **7** (Scheme 1) to afford **14** (4.2 g, 5.7% yield) as a colorless gum. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38−7.37 (m, 1 H), 3.35−3.39 (m, 1 H), 3.21−3.32 (m, 2 H), 3.03−3.06 (m, 1 H), 2.77−2.84 (m, 2 H), 1.72−174 (m, 1 H), 1.56 −1.57 (m, 1 H), 1.34−1.33 (m, 1 H), 1.121−1.126 (m, 9 H) and 0.82−0.88 (m, 6 H). MS (ESI): m/z 243.2 (M + 1).

*(2S,5R)-tert-Butyl 5-(Hydroxymethyl)-2-isobutyl-3-oxopiperazine-1-carboxylate (**15**).* Compound **14** (2.00 g, 8.25 mmol) and HCl in dioxane (4 N, 20 mL) were stirred at 70 °C for 1.5 h. The reaction

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mixture was concentrated in vacuo, and the crude compound was dissolved in DMF/DCM (2:1, 30 mL) and DIPEA (3.5 mL) was added followed by BOC-anhydride (1.60 mL, 6.96 mmol) in DCM (5 mL). After 5 h stirring, the reaction mixture was concentrated in vacuo, and the oily residue was partioned in EtOAc (15 mL) and water (15 mL). The organic layer was separated and washed with brine, dried ($MgSO_4$), concentrated in vacuo, and purified by column chromatography (0−90% EtOAc in hexanes) to give **15** (2.06 g, 87% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 7.69 (s, 1 H), 4.88 (t, J = 5.6 Hz, 1 H), 4.40−4.22 (m, 1 H), 4.11−3.90 (m, 1 H), 3.44−3.24 (m, 2 H), 2.90−2.76 (m, 2 H), 1.65−1.56 (m, 2 H), 1.51−1.46 (m, 1 H), 1.41 (s, 9 H), 0.91 (m, 6 H). MS (ESI): m/z 287.2 (M + 1).

*(2S,5R)-tert-Butyl 2-Isobutyl-5-(oxazol-5-yl)-3-oxopiperazine-1-carboxylate (**16**).* To a solution of the alcohol **15** (0.50 g, 1.75 mmol) in DCM (25 mL) at 0 °C was added Dess−Martin reagent (0.965 g, 4.55 mmol) and water (0.5 mL). Reaction mixture was warmed to rt and stirred for 6 h. Then, *i*-PrOH (0.3 mL) was added and the mixture was stirred for 15 min followed by saturated aqueous sodium thiosulfate (10 mL) and saturated aqueous NaHCO3 (10 mL) were added. Organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 5 mL). Combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to give the aldehyde product. To the crude aldehyde in MeOH (20 mL) was added tosylmethyl isocyanide (480 mg, 2.46 mmol) and K_2CO_3 (483 mg, 3.50 mmol) and was stirred at 70 °C for 1 h. The reaction mixture was concentrated in vacuo, and EtOAc (30 mL) and water (20 mL) were added. Organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 2). Combined organic layers were washed with brine, dried ($MgSO_4$), concentrated in vacuo, and purified by column chromatography (0−95% EtOAc in hexanes) to give **16** (78 mg, 14% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.90 (s, 1 H), 7.08 (s, 1 H), 5.93 (br s, 1 H), 4.88−4.84 (m, 1 H), 4.69−4.64 (m, 1 H), 4.50−4.44 (m, 1 H), 3.18 (t, J = 12.4 Hz, 1 H), 1.78−1.62 (m, 3 H), 1.02 (d, J = 5.6 Hz, 3 H), 0.97 (d, J = 6.0 Hz, 3 H). MS (ESI): m/z 324.1 (M + 1).

*(2R,5S)-N-(2,2-Dimethoxyethyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-5-isobutyl-6-oxopiperazine-2-carboxamide (**23**). A. (S)-(9H-Fluoren-9-yl)methyl (1-Hydroxybut-3-en-2-yl)carbamate (**18**).* To a mixture of **17** (HCl salt, 1g, 8.1 mmol) and a solution of K_2CO_3 (3.4 g, 24.3 mmol) in water (50 mL) was added a solution of Fmoc-Cl (2.3g, 8.91 mmol) in dioxane (50 mL) at 0 °C and stirred at rt for 3 h. The reaction mixture was extracted with DCM (100 mL × 3) and washed with saturated aqueous NH_4Cl (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the residue was purified by column chromatography (0−30% EtOAC in hexanes) to give **18** (2.5 g, quantitative) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.6 Hz, 2 H), 7.60 (d, J = 7.6 Hz, 2 H), 7.41 (t, J = 7.6 Hz, 2 H), 7.32 (t, J = 7.6 Hz, 2 H), 5.89−5.74 (m, 1 H), 5.25 (d, J = 12.4 Hz, 2 H), 5.12 (s, 1 H), 4.45 (d, J = 6.8 Hz, 2 H), 4.40−4.26 (m, 1 H), 4.22 (t, J = 6.8 Hz, 1 H), 3.78−3.61(m, 2 H), 1.93(s, 1 H). MS (ESI): m/z 310.0 (M + 1).

*B. (S)-Methyl 2-((((S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)-amino)but-3-en-1-yl)amino)-4-methyl-pentanoate (**19**).* To a solution of **18** (1.85 g, 6 mmol) in anhydrous DCM (30 mL) was added Dess−Martin's reagent (5.33 g, 12.6 mmol) and stirred at rt for 1 h. The reaction mixture was diluted with ether (21 mL), and a solution of sodium thiosulfate (10.43 g, 66 mmol) in saturated aqueous $NaHCO_3$ (20 mL) was added. The reaction mixture was stirred for 10 min, the layers were separated, and the aqueous layer was extracted with ether (50 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL) and then dried over Na_2SO_4 and concentrated in vacuo to furnish the crude aldehyde (1.8g, quantitative), which was used for the next step without further purification. MS (ESI): m/z 308.0 (M + 1). To a solution of the crude aldehyde (1.842 g, 6 mmol) in THF (20 mL) was added HCl salt of Leu-OMe (1.2 g, 6.6 mmol) and $NaBH(OAc)_3$ (1.9 g, 9 mmol) and stirred at rt overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with EtOAc (100 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo, and the residue was purified by column chromatography (10−50% EtOAC in hexanes) to give **19** (1.1 g, 42% yield) as a pale-brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25(s, 1 H), 7.78−7.75 (m, 2 H), 7.62−7.60 (m, 2 H), 7.41(t, J = 7.6 Hz, 2 H), 7.43−7.38 (m, 2 H), 7.34−7.29 (m, 2 H), 5.81−5.74 (m, 1 H), 5.24−5.17 (m, 2 H), 4.46−4.39 (m, 3 H), 4.27−4.21 (m, 2 H), 3.71 (s, 3 H), 3.30 (t, J = 7 Hz, 1 H), 1.87−1.67 (m, 3 H), 0.98−0.87 (m, 6 H). MS (ESI): m/z 437.3 (M + 1).

*C. (3S,6S)-3-Isobutyl-6-vinylpiperazin-2-one (**20**).* To a solution of **19** (0.55 g, 1.26 mmol) in EtOH (6 mL) was added diethylamine (4 mL) at rt and heated at 60 °C overnight. The reaction mixture concentrated in vacuo, and the residue was purified by column chromatography (10−40% EtOAC in hexanes) to give **20** (92 mg, 42% yield) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 5.93−5.85 (m, 1 H), 5.26−5.21 (m, 2 H), 4.03−3.95 (m, 1 H), 3.34−3.29 (m, 1 H), 3.02 (dd, J = 13.4 Hz, 4.8 Hz, 1 H), 2.85 (dd, J = 13.4 Hz, 4.8 Hz, 1 H), 1.88−1.80 (m, 1 H), 1.72−1.64 (m, 1 H), 1.55−1.48 (m, 1 H), 0.95(d, J = 6.4 Hz, 3 H), 0.92 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 183.0 (M + 1).

*D. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-vinylpiperazin-2-one (**21**).* A mixture of **20** (61.9 mg, 0.34 mmol), 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (84.4 mg, 0.41 mmol), HOBT hydrate (65.1 mg, 0.425 mmol), EDC (78.2 mg, 0.41 mmol), and DIPEA (0.12 mL, 0.68 mmol) in MeCN (5 mL) were stirred at rt overnight. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (0−60% EtOAC in hexanes) to give **21** (37.5 mg, 30% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.21−7.15 (m, 2 H), 6.87 (s, 1 H), 6.20 (s, 1 H), 5.76−5.68 (m, 1 H), 5.43 (d, J = 17.2 Hz, 1 H), 5.32 (d, J = 10.0 Hz, 1 H), 5.36−5.27 (m, 1 H), 4.88−4.82 (m, 1 H), 4.28−4.21 (m, 1 H), 3.21(dd, J = 14.0 Hz, 11.0 Hz, 1H), 1.92−1.67 (m, 3 H), 1.07 (d, J = 6.4 Hz, 3 H), 0.98 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 372.1 (M + 1).

*E. (2R,5S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-5-isobutyl-6-oxopipera-zine-2-carboxylic Acid (**22**).* To a solution of **21** (92.9 mg, 0.25 mmol) in DMF (5 mL) was added OsO_4 (2.5% in *t*-BuOH, 0.03 mL, 0.0025 mmol) and stirred at rt for 5 min ,and then oxone (614.8 mg, 1 mmol) was added and stirred at rt for 3 h. Na_2SO_3 (189 mg, 1.5 mmol) was added to the reaction mixture and stirred for 1 h, and then EtOAc (20 mL) and 1N aqueous HCl (20 mL) were added. The organic layer was separated, washed with 1N aqueous HCl (10 mL × 2) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (0−10% MeOH in DCM) to give **22** (58.4 mg, 60% yield) as a brown solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.95−7.91 (m, 2 H), 7.30−7.25 (m, 2 H), 7.05(s, 1 H), 5.16 (dd, J = 10.0 Hz, 4.0 Hz, 1 H), 4.78−4.70 (m, 1 H), 4.18−4.10 (m, 1 H), 3.49−3.43 (m, 1 H), 1.89−1.68 (m, 3 H), 1.06 (d, J = 6.0 Hz, 3 H), 0.99 (d, J = 6.0 Hz, 3 H). MS (ESI): m/z 390.0 (M + 1).

*F. (2R,5S)-N-(2,2-Dimethoxyethyl)-4-(5-(4-fluorophenyl)-isoxazole-3-carbonyl)-5-isobutyl-6-oxopiperazine-2-carboxamide (**23**).* Compound **22** (96 mg, 0.25 mmol) and 2,2-dimethoxy-ethylamine (0.03 mL, 0.3 mmol) were coupled (96 mg, 0.25 mmol) according to the method described for the preparation of compound **21** to give **23** (60 mg, 50% yield) as a pale-yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.77 (m, 2 H), 7.22−7.17 (m, 2 H), 6.91 (s, 1 H), 6.60 (s, 1 H), 6.32 (t, J = 5.6 Hz, 1 H), 5.29−5.26 (m, 1 H), 5.11 (dd, J = 14.0 Hz, 4.0 Hz, 1 H), 4.43−4.38 (m, 3 H), 3.50−3.36 (m, 8 H), 1.93−1.65 (m, 3 H), 1.08 (d, J = 6.4 Hz, 3 H), 0.98 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 477.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-1-methyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**27**).* To a mixture of **2a** (100 mg, 0.281 mmol) and BMAP (0.5 mL, 1.800 mmol) in DCM (25 mL) were added iodomethane (2700 mg, 19.005 mmol) stirred at rt for 36 h. The reaction mixture was washed with water (15 mL × 2) and dried over $MgSO_4$, and the organic layers were concentrated in vacuo. The residue was purified by pre-HPLC under basic conditions to afford **27** (12 mg, 12% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 −7.21 (m, 3 H), 6.96−7.00 (m, 2 H), 4.50−4.77 (m, 2 H), 3.00−3.50 (m, 1 H), 2.63−2.80 (m, 1 H), 2.30−2.43 (m, 1 H),

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1.81−1.96 (m, 1 H), 1.07−1.75 (m, 10 H), 0.70−0.97 (m, 9 H), 0.47−0.55 (m, 3 H). MS (ESI): *m/z* 371.2 (M + 1).

*((2S,5S)-2,5-Diisobutylpiperazin-1-yl)((1R,2R)-2-phenylcyclopropyl)methanone (**28**).* To a solution of compound **7** (300 mg, 1.41 mmol) in THF (30 mL) was added $LiAlH_4$ (107 mg, 2.82 mmol) at −40 °C and the mixture was stirred for 6 h. The reaction mixture was quenched with water, filtered, extracted withed EtOAc (50 mL), dried over Na_2SO_4, and concentrated in vacuo to give the decarbonylated compound (200 mg, 72% yield), which was used for the next reaction without further purification. The decarbonylated compound (200 mg, 1.01 mmol) was coupled to (1*R*,2*R*)-2-phenylcyclopropanecarboxylic acid (164 mg, 1.01 mmol) according to the method described for compound **2a** to give compound **28** (110 mg, 28% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24−7.30 (m, 2 H), 7.16−7.22 (m, 1 H), 7.06−7.10 (m, 2 H), 4.54−4.58 (m, 1 H), 3.84−3.88 (m, 1 H), 3.03−3.14 (m, 3 H), 2.78−2.86 (m, 1 H), 2.36−2.45 (m, 1 H), 1.87−1.95 (m, 1 H), 1.71−1.74 (m, 2 H), 1.27−1.51 (m, 6 H), 0.91−0.96 (m, 6 H), 0.63−0.82 (m, 6 H). MS (ESI): *m/z* 343.1 (M + 1).

*(3R,6S)-3,6-Diisobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**29**).* Compound **29** was synthesized from Fmoc-Leu and D-Leu-OMe according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25−7.29 (m, 2 H), 7.19−7.21 (m, 1 H), 7.05−7.08 (m, 2 H), 6.48−6.63 (m, 1 H), 5.17−5.21 (m, 0.5 H), 4.54−4.57 (m, 0.5 H), 4.39−4.42 (m, 0.5 H), 3.79−3.82 (m, 0.5 H), 3.65−3.69 (m, 0.5 H), 3.41−3.50 (m, 1 H), 3.22−3.27 (m, 0.5 H), 2.43−2.50 (m, 3 H), 1.89−1.92 (m, 1 H), 1.63−1.75 (m, 4 H), 1.33−1.49 (m, 3 H), 0.90−1.02 (m, 6 H), 0.58−0.80 (m, 6 H). MS (ESI): *m/z* 357.1 (M + 1).

*(3S,6R)-3,6-Diisobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**30**).* Compound **30** was synthesized from Fmoc-D-leu and Leu-OMe according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26−7.30 (m, 2 H), 7.20−7.23 (m, 1 H), 7.06−7.12 (m, 2 H), 6.44−6.60 (m, 0.5 H), 5.13−5.17 (m, 0.5 H), 4.53−4.56 (m, 0.5 H), 4.39−4.42 (m, 0.5 H), 3.82−3.85 (m, 0.5 H), 3.61−3.66 (m, 0.5 H), 3.48−3.49 (m, 1 H), 3.19−3.23 (m, 0.5 H), 2.55−2.57 (m, 1 H), 1.94−1.97 (m, 0.5 H), 1.83−1.91 (m, 2.5 H), 1.63−1.71 (m, 4 H), 1.30−1.52 (m, 3 H), 0.92−1.01 (m, 12 H). MS (ESI): *m/z* 357.1 (M + 1).

*(3S,6S)-6-Ethyl-3-isobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**31**).* Compound **31** was synthesized from Fmoc-Ala and Leu-OMe according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.05 (m, 5 H), 5.97 (s, 1 H), 5.22−4.71 (m, 1 H), 4.58−4.06 (m, 1 H), 3.50−3.39 (m, 1 H), 3.12−2.60 (m, 1 H), 2.56−2.51 (m, 1 H), 1.95−1.91 (m, 1 H), 1.86−1.72 (m, 2 H), 1.70−1.58 9m, 2 H), 1.56−1.45 (m, 2 H), 1.33−1.28 (m, 1 H), 1.02−0.93 (m, 9 H). MS (ESI): *m/z* 329.20 (M − 1).

*(3S,6S)-3-Ethyl-6-isobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**32**).* Compound **32** was synthesized from Leu-OMe and Fmoc-Ala according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10−7.40 (m, 5 H), 5.90 (s, 1 H), 5.05−5.15 (m, 0.3 H), 4.70−4.80 (m, 0.7 H), 4.45−4.55 (m, 0.7 H), 4.05−4.15 (m, 0.3 H), 3.50−3.65 (m, 1 H), 2.45−2.55 (m, 2 H), 0.90−2.20 (m, 17 H). MS(ESI): *m/z* 328.9 (M + 1)].

*(3S,6S)-6-((S)-sec-Butyl)-3-isobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)piperazin-2-one (**33**).* Compound **33** was synthesized from (2*S*,3*R*)-2((((9*H*-fluoren-9-yl)methoxy)-carbonyl)amino)-3-methylpentanoic acid and Leu-OMe according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05−7.30 (m, 5 H), 5.90−6.05 (m, 1H), 4.40−4.65 (m, 1 H), 4.05−4.15 (m, 0.5 H), 3.55−3.65 (m, 0.5 H), 3.15−3.25 (m, 2 H), 2.50−2.65 (m, 1 H), 0.85−1.95 (m, 20 H). MS (ESI): *m/z* 357.1 (M + 1).

*(3S,6S)-3-Isobutyl-4-((1R,2R)-2-phenylcyclopropanecarbonyl)-6-propylpiperazin-2-one (**34**).* Compound **34** was synthesized from (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pentanoic acid and Leu-OMe according to the reaction sequence outlined in Scheme 1 for compound **2a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.05 (m, 5 H), 6.29 (m, 1 H), 5.21−4.70 (m, 1 H), 4.57−4.04 (m, 1 H), 3.56−3.48 (m, 1 H), 3.12−2.60 (m, 1 H), 2.56−2.51 (m, 1 H), 2.12−2.05 (m, 1 H), 1.94−1.58 (m, 5 H), 1.49−1.28 (m, 4 H), 1.01−0.92 (m, 9 H). MS (ESI): *m/z* 343.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(3-phenylpropioloyl)piperazin-2-one (**35**).* Compound **7** (400 mg, 1.88 mmol) was coupled with 3-phenylpropiolic acid (275 mg, 1.88 mmol) according to the procedure described for the preparation of compound **2a** (Scheme 1) to give **35** (195 mg, 30% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51−7.55 (m, 2 H), 7.34−7.45 (m, 3 H), 6.10−6.14 (m, 1 H), 5.08−5.18 (m, 0.5 H), 4.92−5.15 (m, 0.5 H), 4.47−4.70 (m, 1 H), 3.59−3.67 (m, 1 H), 3.11−3.14 (m, 0.5 H), 2.67−2.73 (m, 0.5 H), 1.78−1.86 (m, 2 H), 1.65−1.73 (m, 2 H), 1.34−1.40 (m, 2 H), 0.90−1.06 (m, 12 H). MS (ESI): *m/z* 341.1 (M + 1).

*(3S,6S)-4-Cinnamoyl-3,6-diisobutylpiperazin-2-one (**36**).* Compound **7** (200 mg, 0.941 mmol) was coupled with cinnamic acid (139 mg, 0.939 mmol) according to the procedure described for the preparation of compound **2a** (Scheme 1) to give **36** (39 mg, 13% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72−7.76 (m, 1 H), 7.46−7.53 (m, 2 H), 7.35−7.42 (m, 3 H), 6.81−6.87 (m, 1 H), 5.82 (s, 1 H), 4.57−4.82 (m, 2 H), 3.59−3.64 (m, 1 H), 2.66−3.16 (m, 1 H), 1.69−1.89 (m, 4 H), 1.32−1.42 (m, 2 H), 1.05−1.10 (m, 3 H), 0.90−1.03 (m, 9H). MS (ESI): *m/z* 343.1 (M + 1) and 365.1 (M + 23).

*(3S,6S)-3,6-Diisobutyl-4-((Z)-3-phenylacryloyl)piperazin-2-one (**37**).* Compound **35** (140 mg, 0.41 mmol) in MeOH (15 mL) was added Lindlar-Pd (30 mg), and the mixture was stirred at rt under H_2 (40 psi) for 24 h. The reaction mixture was filtered, concentrated in vacuo, and purified by prep HPLC under basic conditions to afford **37** (41 mg, 29% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23−7.36 (m, 5 H), 6.67−6.74 (m, 1 H), 6.02(d, *J* = 2.4 Hz, 1 H), 5.46−5.72 (m, 1 H), 4.65−5.19 (m, 1 H), 3.85−4.26 (m, 1 H), 2.85−3.41 (m, 1 H), 2.50−2.80 (m, 1 H), 1.45−1.72 (m, 4 H), 1.21−1.25 (m, 1 H), 1.05−1.11 (m, 1 H), 0.81−0.90 (m, 9 H), 0.70−0.78 (m, 3 H). MS (ESI): *m/z* 343.3 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-phenylallyl)piperazin-2-one(**38**).* To a solution of compound **7** (150 mg, 0.706 mmol) in DMF (10 mL) was added (*E*)-(3-chloroprop-en-1-yl)benzene (210 mg, 0.85 mmol) and K_2CO_3 (97 mg, 0.706 mmol) and stirred at 80 °C for 12 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (15 mL × 3), and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by prep HPLC under acidic conditions to give **38** (63 mg, 27% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (s, 1 H), 7.37−7.39 (m, 2 H), 7.27−7.30 (m, 2 H), 7.20−7.21 (m, 1 H), 6.51−6.55 (m, 1 H), 6.22 (m, 1 H), 3.45−3.49 (m, 1 H), 2.87 (m, 1 H), 2.65−2.67 (m, 2 H), 1.80−1.78 (m, 1 H), 1.58−1.60 (m, 2 H), 1.34−1.37 (m, 2 H), 1.19 (s, 3 H) 0.78−0.84 (m, 12 H). MS (ESI): *m/z* 329.3 (M + 1).

*(3S,6S)-4-(Cyclopropanecarbonyl)-3,6-diisobutylpiperazin-2-one (**39**).* Compound **7** (200 mg, 0.941 mmol), cyclopropanecarboxylic acid (86 mg, 1.0 mmol), HATU (360 mg, 0.947 mmol), and DIPEA (129 mg, 1.000 mmol) in DCM (15 mL) were stirred at rt for 2 h. The reaction mixture was washed with water (15 mL × 2), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by prep HPLC under basic conditions to afford **39** (128 mg, 49% yield) as a colorless syrup. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63−6.91 (m, 1 H), 4.17−5.21 (m, 2 H), 3.52−3.65 (m, 1 H), 2.57−3.15 (m, 1 H), 1.59−1.89 (m, 5 H), 1.32−1.41 (m, 2 H), 0.91−1.06 (m, 14 H), 0.82−0.88 (m, 2 H). MS (ESI): *m/z* 281.2 (M + 1).

*(3S,6S)-4-([1,1′-Biphenyl]-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**40**).* Compound **7** (100 mg, 0.47 mmol) was coupled with [1,1′-biphenyl]-3-carboxylic acid (112 mg, 0.564 mmol) according to the procedure described for the preparation of compound **2a** (Scheme 1) to give **40** (37 mg, 20% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, *J* = 8.0 Hz, 1 H), 7.67−7.56 (m, 4 H), 7.45−7.36 (m, 4 H), 5.20 (d, *J* = 8.4 Hz, 1 H), 3.79−3.73 (m, 1 H), 3.62−3.52 (m, 1 H), 3.20−3.14 (m, 1 H), 1.92−1.70 (m, 3 H), 1.45−1.19 (m, 4

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H), 1.10−0.96 (m, 6 H), 0.79−0.65 (m, 5 H). MS (ESI): *m/z* 393.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-(p-tolyl)acryloyl)piperazin-2-one (**41**).* Compound **7** (60 mg, 0.29 mmol) and 4-methylcinnamic acid (50 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **41** (95 mg, 95% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, J = 14.0 Hz, 4.5 Hz, 1 H), 7.41 (t, J = 6.5 Hz, 1 H), 7.19 (d, J = 8.0 Hz, 1 H), 6.82−6.76 (dd, J = 15.0 Hz, 7.2 Hz, 1 H) (s, 1H), 5.93 (br s, 1 H), 5.35 (br s, 1 H), 4.81 (dd, J = 12.5 Hz, 6.5 Hz, 1 H), 4.57 (br s, 1 H), 3.60 (m, 1 H), 3.14 (m, 1 H), 2.67 (m, 1 H), 2.37 (m, 1 H), 1.75 (m, 4 H), 1.36 (m, 2 H), 1.06−0 (m, 12 H). MS (ESI): *m/z* 357.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-(4-(trifluoromethyl)phenyl)-acryloyl)piperazin-2-one (**42**).* Compound **7** (60 mg, 0.29 mmol) and 4-trifluorocinnamic acid (65 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **42** (44 mg, 38% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77−7.58 (m, 5 H), 6.91 (dd, J = 8.0 Hz, 7.2 Hz, 1 H), 5.87 (s, 1 H), 5.33 (d, J = 9.6 Hz, 1 H), 4.81 (d, J = 13.6 Hz, 1 H), 4.56−4.53 (m, 1 H), 4.00 (d, J = 12.4 Hz, 1 H), 3.62 (m, 2 H), 2.70 (m, 1 H), 1.90−1.69 (m, 2 H), 1.44−0.93 (m, 14 H). MS (ESI): *m/z* 411.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-(4-methoxyphenyl)acryloyl)-piperazin-2-one (**43**).* Compound **7** (60 mg, 0.29 mmol) and (*E*)-3-(4-methoxyphenyl) acrylic acid (60 mg, 0.34 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **43** (84 mg, 96% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 1 H), 7.46−7.43 (br s, 2 H), 6.92−6.90 (br s, 2 H), 6.69 (m, 1 H), 5.93 (br s, 1 H), 4.79 (m, 1 H), 4.56 (m, 1 H), 3.83 (s, 3 H) 3.66−3.52 (m, 1 H), 2.67 (m, 1 H), 1.87−1.60 (m, 5 H), 1.38−1.28 (m, 2 H), 1.05−0.93 (m, 12 H). MS (ESI): *m/z* 373.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-(4-nitrophenyl)acryloyl)piperazin-2-one (**44**).* Compound **7** (99.8 mg, 0.47 mmol) and (*E*)-3-(4-nitrophenyl) acrylic acid (109 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **44** (82 mg, 45% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.4 Hz, 2 H), 7.73 (d, J = 15.2 Hz, 1 H), 7.63 (d, J = 8.4 Hz, 2 H), 6.95 (d, J = 15.2 Hz, 1 H), 6.50 (s, 1 H), 4.77 (d, J = 13.2 Hz, 1 H), 4.55−4.49 (m, 1 H), 3.64−3.52 (m, 1 H), 2.72−2.66 (m, 1 H), 1.91−1.58 (m, 4 H), 1.42−1.32 (m, 2 H), 1.04−0.89 (m, 12 H). MS (ESI): *m/z* 388.1 (M + 1).

*(3S,6S)-4-((E)-3-(4-(Dimethylamino)phenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**45**).* Compound **7** (60 mg, 0.28 mmol) and (*E*)-3-[4-(dimethylamino)phenyl]acrylic acid (60 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give the product **45** (100 mg, 96% yield) as an yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 16.0 Hz, 1 H), 7.41−7.39 (br s, 2 H), 6.68−6.66 (br s, 2 H), 6.64−6.58 (m, 1 H), 5.83 (br s, 1 H), 4.83−4.80 (m, 1 H), 4.61−4.59 (m,1 H), 3.66−3.57 (m, 1 H), 3.01 (s, 6 H); 2.68−2.62 (m,1 H), 1.86−1.66 (m, 4 H), 1.38−1.32 (m, 2 H), 1.05−0.92 (m, 12 H). MS (ESI): *m/z* 387.4 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-((E)-3-(4-(methylsulfonyl)phenyl)-acryloyl)piperazin-2-one (**46**).* Compound **7** (99.8 mg, 0.47 mmol) and (*E*)-3-(4-(methylsulfonyl)phenyl)acrylic acid (127.6 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give the product **46** (151 mg, 76% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 2 H), 7.73 (d, J = 15.6 Hz, 1 H), 7.66 (d, J = 8.4 Hz, 2 H), 6.94 (d, J = 15.6 Hz, 1 H), 6.33 (s, 1 H), 4.78 (d, J = 13.2 Hz, 3.8 Hz, 1 H), 4.55−4.51 (m, 1 H), 3.65−3.52 (m, 1 H), 3.06 (s, 3 H), 2.69 (d, J = 13.2 Hz, 11.2 Hz, 1 H), 1.91−1.62 (m, 4 H), 1.43−1.33 (m, 2 H), 1.06−0.91 (m, 12 H). MS (ESI): *m/z* 421.4 (M + 1).

*Methyl 4-((E)-3-((2S,5S)-2,5-Diisobutyl-3-oxopiperazin-1-yl)-3-oxoprop-1-en-1-yl)benzoate (**47**).* Compound **7** (55 mg, 0.26 mmol) and (*E*)-3-[4-(methoxycarbonyl)phenyl] acrylic acid (60 mg, 0.29 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **47** (94 mg, 90% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.0 Hz, 2 H), 7.72 (dd, J = 15.2 Hz, 8.0 Hz, 1 H), 7.55 (t, J = 8.0 Hz, 2 H), 6.91 (dd, J = 15.2 Hz, 8.0 Hz, 1 H), 6.30 (brs, 1 H), 5.30 (dd, J = 8.0 Hz, 4.0 Hz, 1 H), 4.78 (dd, J = 13.2 Hz, 4.0 Hz, 1 H), 4.53 (t, J = 8.0 Hz, 1 H), 3.91 (s, 1 H), 3.65−3.55 (m, 1 H), 2.67 (t, J = 12.0 Hz,1 H), 1.89−1.63 (m, 4 H), 1.38−1.34 (m, 2 H), 1.04−0.93 (m, 12 H). MS (ESI): *m/z* 401.5 (M + 1).

*(3S,6S)-4-((E)-3-(4-Bromophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**48**).* Compound **7** (60 mg, 0.29 mmol) and 4-bromocinnamic acid (55 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **48** (70 mg, 65% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80−8.60 (m, 2 H), 7.85−7.70 (m, 2 H), 7.4 (br s, 1 H), 6.95 (br s, 1 H), 5.95 (s, 1 H), 5.38 (br s, 1 H), 5.06−4.99 (m, 1 H), 4.80 (br s, 1 H), 4.55 (br s, 1 H), 4.05 (m, 1 H), 3.10 (m, 1 H), 2.70 (m, 1 H), 1.90−1.10 (m, 5 H), 1.05−0.85 (m, 12 H). MS (ESI): *m/z* 377.2 (M + 1).

*(3S,6S)-4-((E)-3-(4-Chlorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**49**).* Compound **7** (60 mg, 0.29 mmol) and 4-chlorocinnamic acid (55 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **49** (70 mg, 65% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80−8.60 (m, 2 H), 7.85−7.70 (m, 2 H), 7.4 (br s, 1 H), 6.95 (br s, 1 H), 5.95 (s, 1 H), 5.38 (br s, 1 H), 5.06−4.99 (m, 1 H), 4.80 (d, 1 H), 4.55 (br s, 1 H), 4.05 (m, 1 H), 3.10 (m, 1 H), 2.70 (m, 1 H), 1.90−1.10 (m, 5 H), 1.05−0.85 (m, 12 H). MS (ESI): *m/z* 377.2 (M + 1).

*(3S,6S)-4-((E)-3-(3-Chlorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**50**).* Compound **7** (60 mg, 0.29 mmol) and 3-chlorocinnamic acid (60 mg, 0.33 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **50** (88 mg, 98% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, J = 15.6 Hz, 5.6 Hz, 1 H), 7.50 (d, J = 15.6 Hz, 1 H); 7.38−7.29 (m, 2 H), 6. 83 (dd, J = 15.6 Hz, 5.6 Hz, 1 H), 5.95 (br s, 1 H), 5.33 (dd, J = 9.6 Hz, 4.0 Hz, 1 H), 4.79 (dd, J = 13.2 Hz, 4.0 Hz, 1H), 4.56−4.53 (m, 1 H), 3.65−3.56 (m, 2 H), 2.68 (dd, J = 13.2 Hz, 11.2 Hz, 1 H), 1.88−1.63 (m, 4 H), 1.39−1.34 (m, 2 H), 1.05−0.93 (m, 12 H). MS (ESI): *m/z* 379 (M + 1).

*(3S,6S)-4-((E)-3-(2-Chlorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**51**).* Compound **7** (60 mg, 0.29 mmol) and 2-chlorocinnamic acid (60 mg, 0.33 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **51** (50 mg, 56% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 15.6 Hz, 1 H), 7.50 (d, J = 15.6 Hz, 1 H); 7.35 (dd, J = 8.4 Hz, 2.0 Hz, 1 H), 7.20−7.16 (m, 1 H), 6.99 (d, J = 15.6 Hz, 1 H), 5.89 (br s, 1 H), 5.33 (dd, J = 8.0 Hz, 4.0 Hz, 1 H), 4.79 (dd, J = 13.2 Hz, 4.0 Hz, 1 H), 4.46 (dd, J = 8.0 Hz, 4.0 Hz, 1 H), 3.69−3.56 (m, 2 H), 2.71 (dd, J = 13.2 Hz, 11.2 Hz, 1 H), 1.84−1.64 (m, 4 H), 1.39−1.32 (m, 2 H), 1.07−0.92 (m, 12 H). MS (ESI): *m/z* 379 (M + 1).

*(3S,6S)-4-((E)-3-(4-Fluorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**52**).* Compound **7** (53 mg, 0.25 mmol) and 4-fluorocinnamic acid (50 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **52** (80 mg, 94% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71−7.57 (m, 3 H), 7.19−7.11 (m, 3 H), 5.19 (dd, J = 10.5 Hz, 3.2 Hz, 1 H) 4.72 (m, 1 H), 4.36 (dd, J = 14.4 Hz, 3.2 Hz, 1 H), 3.58−3.52 (m, 1 H), 3.19 (dd, J = 12.0 Hz, 10.8 Hz, 1 H), 2.79−2.74 (m, 1 H), 2.37 (m, 1 H), 1.85−1.60 (m, 4 H), 1.41 (t, J = 7.2 Hz, 2 H), 1.03−0.94 (m, 12 H). MS (ESI): *m/z* 361.5 (M + 1).

*(3S,6S)-4-((E)-3-(3,4-Difluorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**53**).* Compound **7** (53 mg, 0.25 mmol) and 3,4-difluorocinnamic acid (60 mg, 0.33 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **53** (90 mg, 97% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66−7.60 (m, 1 H), 7.35−7.13 (m, 3 H), 6. 73 (dd, J = 15.2 Hz, 4.8 Hz, 1 H), 6.23 (d, J = 15.2, 1 H), 5.31 (dd, J = 9.6 Hz, 3.6 Hz, 1 H), 4.78 (dd, J = 13.2 Hz, 4.0 Hz, 1 H), 4.53 (dd, J = 8.8 Hz, 4 Hz, 1 H), 3.64−3.55 (m, 1 H), 3.59 (m, 1 H), 3.15 (dd, J = 14.0 Hz, 11.2 Hz, 1 H), 2.68 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 1.87−1.65 (m, 4 H), 1.38−1.34 (m, 2 H), 1.04−0.92 (m, 12 H). MS (ESI): *m/z* 379.1 (M + 1).

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*(3S,6S)-4-((E)-3-(2,4-Difluorophenyl)acryloyl)-3,6-diisobutylpiperazin-2-one (**54**).* Compound **7** (60 mg, 0.29 mmol) and 2,4-difluorocinnamic acid (60 mg, 0.33 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **54** (104 mg, 95% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66−7.73 (m, 1 H), 7.41−7.47 (m, 1 H), 6.81−6.97 (m, 3 H), 6.43 (d, J = 13.8 Hz, 1 H), 5.29 (dd, J = 10.0 Hz, 4.0 Hz, 1 H), 4.01 (d, J = 11.2 Hz, 1 H), 3.57−3.60 (m, 1 H), 3.08−3.14 (m, 1 H), 1.72−1.86 (m, 4 H), 1.34−1.38 (m, 2 H), 0.91−1.02 (m, 12 H). MS (ESI): m/z 379.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-phenylfuran-2-carbonyl)piperazin-2-one (**55**).* Compound **7** (100 mg, 0.47 mmol) and 5-phenylfuran-2-carboxylic acid (106 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **55** (52 mg, 29% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 7.6 Hz, 2 H), 7.44−7.39 (m, 2 H), 7.36−7.33 (m, 1 H), 7.21(d, J = 3.6 Hz, 1 H), 6.75 (d, J = 3.6 Hz, 1 H), 6.04 (br s, 1 H), 5.35−5.26 (s, 1 H), 4.77 (dd, J = 20.0 Hz, 4 Hz, 1H), 3.82−3.73 (m, 1 H), 3.25−3.12 (m, 1 H), 1.92−1.65 (m, 4 H), 1.41−1.37 (m, 2 H), 1.00−0.89 (m, 12 H). MS (ESI): m/z 383.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(2-phenylthiazole-4-carbonyl)piperazin-2-one (**56**).* Compound **7** (100 mg, 0.47 mmol) and 2-phenylthiazole-4-carboxylic acid (116 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **56** (58 mg, 31% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1 H), 7.94−7.89 (m, 2 H), 7.45−7.43 (m, 3 H), 6.48 (s, 1 H), 5.31−5.29 (m, 1 H), 5.13 (d, J = 14.0 Hz, 1 H), 3.92−3.84 (m, 1 H), 3.08 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 1.90−1.66 (m, 4 H), 1.41−1.24 (m, 2 H), 1.09−0.82 (m, 12 H). MS (ESI): m/z 400.1 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(2-phenyloxazole-4-carbonyl)piperazin-2-one (**57**).* Compound **7** (99.8 mg, 0.47 mmol) and 2-phenyloxazole-4-carboxylic acid (106.7 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **57** (55 mg, 30% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (s, 1 H), 8.08−8.03 (m, 2 H), 7.56−7.50 (m, 3 H), 5.39 (dd, J = 14.2 Hz, 3.6 Hz, 1 H), 5.16−5.13 (m, 1 H), 3.84−3.74 (m, 1 H), 3.18 (dd, J = 14.0 Hz, 11.2 Hz, 1 H), 1.92−1.66 (m, 4 H), 1.50−1.38 (m, 2 H), 1.08−0.92 (m, 12 H). MS (ESI): m/z 384.4 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-phenylisoxazole-3-carbonyl)piperazin-2-one (**58**).* Compound **7** (100 mg, 0.47 mmol) and 5-phenylisoxazole-3-carboxylic acid (107 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **58** (120 mg, 67% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.51−7.45 (m, 3 H), 6.92 (s, 1 H), 6.36 (s, 1 H), 5.29−5.26 (m, 1 H), 4.87 (dd, J = 14.2 Hz, 4.0 Hz, 1 H), 3.78−3.67 (m, 1 H), 3.11 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 1.92−1.82 (m, 1 H), 1.81−1.67 (m, 3 H), 1.42−1.34 (m, 2 H), 1.08 (d, J = 6.4 Hz, 3 H), 0.99−0.93 (m, 9 H). MS (ESI): m/z 384.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-phenyl-1,2,4-oxadiazole-3-carbonyl)piperazin-2-one (**59**).* Compound **7** (50 mg, 0.24 mmol) and 5-phenyl-1,2,4-oxadiazole-3-carboxylic acid (54 mg, 0.28 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **59** (30 mg, 33% yield) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21−8.18 (m, 2 H), 7.67−7.54 (m, 3 H), 5.93 (s, 1 H), 5.32−5.29 (m, 1 H), 4.89−4.82 (m, 1 H), 3.87−3.74 (m, 1 H), 3.14 (dd, J = 13.8 Hz, 11.0 Hz, 1 H), 1.94−1.63 (m, 4 H), 1.43−1.32 (m, 2 H), 1.11−0.89 (m, 12 H). MS (ESI): m/z 385.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(1-phenyl-1H-imidazole-4-carbonyl)piperazin-2-one (**60**).* Compound **7** (100 mg, 0.47 mmol) and 1-phenyl-1*H*-imidazole-4-carboxylic acid (106 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **60** (83 mg, 46% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1 H), 7.77 (s, 1 H), 7.53−7.49 (m, 2 H), 7.43−7.39 (m, 3 H), 5.94 (s, 1 H), 5.84−5.76 (m, 1 H), 5.36−5.32 (m, 1 H), 3.76−3.64 (m, 1 H), 3.10−3.04 (m, 1 H), 1.87−1.71 (m, 4 H), 1.42−1.27 (m, 2 H), 1.08−0.93 (m, 12 H). MS (ESI): m/z 383.3 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-phenyl-1H-pyrazole-3-carbonyl)piperazin-2-one (**61**).* Compound **7** (100 mg, 0.47 mmol) and 5-phenyl-1*H*-pyrazole-3-carboxylic acid (106 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **61** (33 mg, 18% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.7 (br s, 1 H), 7.69−7.64 (m, 2 H), 7.45−7.35 (m, 3 H), 6.94 (s, 1 H), 6.05 (s, 1 H), 5.38−5.31 (m, 1 H), 4.88−4.79 (m, 1 H), 3.76−3.63 (m, 1 H), 3.15−3.05 (m, 1 H), 1.92−1.55 (m, 4 H), 1.43−1.28 (m, 2 H), 1.12−0.78 (m, 12 H). MS (ESI): m/z 383.4 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(2-phenyl-1H-imidazole-4-carbonyl)piperazin-2-one (**62**).* Compound **7** (100 mg, 0.47 mmol) and 2-phenyl-1*H*-imidazole-4-carboxylic acid (106 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **62** (45 mg, 25% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (d, J = 6.8 Hz, 2 H), 7.74 (s, 1 H), 7.48−7.39 (m, 3 H), 5.86 (d, J = 12.0 Hz, 1 H), 5.20 (d, J = 9.2 Hz, 1 H), 3.78−3.62 (m, 1 H), 3.17−3.11 (m, 1 H), 1.89−1.73 (m, 4 H), 1.44−1.39 (m, 2 H), 1.07−0.91 (m, 12 H). MS (ESI): m/z 383.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-(p-tolyl)isoxazole-3-carbonyl)piperazin-2-one (**63**).* Compound **7** (100 mg, 0.47 mmol) and 5-(*p*-tolyl)isoxazole-3-carboxylic acid (114 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **63** (107 mg, 57% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.0 Hz, 2 H), 7.27 (d, J = 8.0 Hz, 2 H), 6.85 (s, 1 H), 6.51 (s, 1H), 5.26 (dd, J = 9.4 Hz, 4 Hz, 1 H), 4.86 (dd, J = 14.2 Hz, 4.0 Hz, 1 H), 3.77−3.67 (m, 1 H), 3.10 (dd, J = 14.2 Hz, 11.0 Hz, 1 H), 2.40 (s, 3 H), 1.91−1.67 (m, 4 H), 1.41−1.33 (m, 2 H), 1.08−0.91 (m, 12 H). MS (ESI): m/z 398.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-(4-methoxyphenyl)isoxazole-3-carbonyl)piperazin-2-one (**64**).* Compound **7** (100 mg, 0.47 mmol) and 5-(4-methoxyphenyl)isoxazole-3-carboxylic acid (123 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **64** (106 mg, 55% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2 H), 6.98 (d, J = 8.8 Hz, 2 H), 6.78 (s, 1 H), 6.51 (s, 1 H), 5.26 (dd, J = 9.4 Hz, 4.4 Hz, 1 H), 4.85 (dd, J = 14.0 Hz, 4.4 Hz, 1 H), 3.85 (s, 3 H), 3.77−3.66 (m, 1 H), 3.09 (dd, J = 13.8 Hz, 10.8 Hz, 1 H), 1.91−1.67 (m, 4 H), 1.41−1.33 (m, 2 H), 1.07−0.90 (m, 12 H). MS (ESI): m/z 414.1 (M + 1).

*(3S,6S)-4-(5-(4-Chlorophenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**65**).* Compound **7** (100 mg, 0.47 mmol) and 5-(4-chlorophenyl)isoxazole-3-carboxylic acid (126 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **65** (109 mg, 56% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.4 Hz, 2 H), 7.46 (d, J = 8.4 Hz, 2 H), 6.91 (s, 1 H), 6.49 (s, 1 H), 5.26 (dd, J = 9.4 Hz, 4.0 Hz, 1 H), 4.85 (dd, J = 14.2 Hz, 4.0 Hz, 1 H), 3.77−3.67 (m, 1 H), 3.10 (dd, J = 14.2 Hz, 11.0 Hz, 1 H), 1.91−1.67 (m, 4 H), 1.41−1.34 (m, 2 H), 1.07−0.91 (m, 12 H). MS (ESI): m/z 418.4 (M + 1).

*(3S,6S)-4-(5-(4-Bromophenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**66**).* Compound **7** (100 mg, 0.47 mmol) and 5-(4-bromophenyl)isoxazole-3-carboxylic acid (151 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **66** (130 mg, 60% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66−7.60 (m, 4 H), 6.92 (s, 1 H), 6.54 (s, 1 H), 5.27−5.24 (m, 1 H), 4.86−4.76 (m, 1 H), 3.77−3.66 (m, 1 H), 3.10 (dd, J = 13.6 Hz, 11.2 Hz, 1 H), 1.91−1.69 (m, 4 H), 1.41−1.34 (m, 2 H), 1.07−0.91 (m, 12 H). MS (ESI): m/z 462.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**67**).* Compound **7** (100 mg, 0.47 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (117 mg, 0.564 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **67** (172 mg, 91% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96−7.92 (m, 2 H), 7.30−7.26 (m, 2 H), 7.06 (s, 1 H), 5.16−5.12 (m, 1 H), 4.53 (dd, J = 14.2 Hz, 4.4 Hz, 1 H), 3.79−3.71 (m, 1 H), 3.21 (dd, J = 14.2 Hz, 11.0 Hz, 1 H), 1.92−1.62 (m, 4 H), 1.48−1.32 (m, 2 H), 1.12−0.88 (m, 12 H). MS (ESI): m/z 402.2 (M + 1).

*(3S,6S)-4-(5-(3,4-Difluorophenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**68**).* Compound **7** (45 mg, 0.21 mmol) and 5-(3,4-difluorophenyl)isoxazole-3-carboxylic acid (56 mg, 0.25 mmol) were coupled according to the procedure described for the preparation

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of compound **2a** to give **68** (57 mg, 65% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.65−7.60 (m, 1 H), 7.55−7.53 (m, 1 H), 7.33−7.26 (m, 1 H), 6.89 (s, 1 H), 5.93 (s, 1 H), 5.28 (dd, J = 9.8 Hz, 3.8 Hz, 1H), 4.86 (dd, J = 14.0 Hz, 3.8 Hz, 1 H), 3.79−3.69 (m, 1 H), 3.12 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 1.92−1.85 (m, 1 H), 1.79−1.65 (m, 3 H), 1.42−1.32 (m, 2 H), 1.08−0.92 (m, 12 H). MS (ESI): m/z 420.2 (M + 1).

*(3S,6S)-4-(5-(2,4-Difluorophenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**69**).* Compound 7 (50 mg, 0.24 mmol) and 5-(2,4-difluorophenyl)isoxazole-3-carboxylic acid (53 mg, 0.24 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **69** (72 mg, 73% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00−7.93 (m, 1 H), 7.07−6.95 (m, 3 H), 5.88 (s, 1 H), 5.30−5.22 (m, 1 H), 4.81−4.76 (m, 1 H), 3.78−3.70 (m, 1 H), 3.15−2.79 (m, 1 H), 1.89−1.61 (m, 4 H), 1.42−1.31 (m, 2 H) 1.09−0.76 (m, 12 H). MS (ESI): m/z 420.1 (M + 1).

*4-(3-((2S,5S)-2,5-Diisobutyl-3-oxopiperazine-1-carbonyl)isoxazol-5-yl)benzonitrile (**70**).* Compound 7 (100 mg, 0.47 mmol) and 5-(4-cyanophenyl)isoxazole-3-carboxylic acid (121 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **70** (61 mg, 32% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.4 Hz, 2 H), 7.79 (d, J = 8.4 Hz, 2 H), 7.07 (s, 1 H), 6.32 (s, 1 H), 5.30−5.24 (m, 1 H), 4.87−4.83 (m, 1 H), 3.79−3.67 (m, 1 H), 3.12 (dd, J = 13.6 Hz, 11.2 Hz, 1 H), 1.92−1.67 (m, 4 H), 1.42−1.35 (m, 2 H), 1.08−0.90 (m, 12 H). MS (ESI): m/z 409.2 (M + 1).

*(3S,6S)-4-(5-(4-(Dimethylamino)phenyl)isoxazole-3-carbonyl)-3,6-diisobutylpiperazin-2-one (**71**).* Compound 7 (100 mg, 0.47 mmol) and 5-(4-(dimethylamino)phenyl)isoxazole-3-carboxylic acid (131 mg, 0.56 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **71** (52 mg, 26% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 8.8 Hz, 2 H), 6.72 (d, J = 8.8 Hz, 2 H), 6.67 (s, 1 H), 6.43 (s, 1 H), 5.29−5.25 (m, 1 H), 4.87−4.81 (m, 1 H), 3.78−3.65 (m, 1 H), 3.08 (dd, J = 13.6 Hz, 10.8 Hz, 1 H), 3.03 (s, 6 H), 1.90−1.67 (m, 4 H), 1.41−1.32 (m, 2 H), 1.08−0.90 (m, 12 H). MS (ESI): m/z 427.2 (M + 1).

*(3S,6S)-3,6-Diisobutyl-4-(5-(4-nitrophenyl)isoxazole-3-carbonyl)-piperazin-2-one (**72**).* Compound 7 (60 mg, 0.28 mmol) and 5-(4-nitrophenyl)-isoxazole-3-carboxylic acid (60 mg, 0.28 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **72** (90 mg, 74% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37−7.34 (m, 2 H), 7.99−7.96 (m, 2 H), 7.12 (s, 1 H), 6.20 (s, 1 H), 5.29−5.25 (m, 1 H), 4.88−4.76 (m, 1 H), 3.79−3.69 (m, 1 H), 3.18 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 1.91−1.69 (m, 4 H), 1.42−1.35 (m, 2 H) and 1.08−0.92 (m, 12 H). MS (ESI): m/z 429.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-propylpiperazin-2-one (**73**). A. (3S,6S)-3-Isobutyl-6-propyl-piperazin-2-one.* Synthesized from Fmoc-L-norvaline (19.82 g, 58 mmol) and HCl salt of Leu-OMe (4.7 g, 26.25 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-3-isobutyl-6-propyl-piperazin-2-one (3.4 g, 25% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (s, 1 H), 3.06 (dd, J = 14.0 Hz, 4.0 Hz, 1 H), 2.78 (dd, J = 12.8 Hz, 4.0 Hz, 1 H), 2.59 (dd, J = 12.8 Hz, 5.2 Hz, 1 H), 2.49 (dd, J = 3.6 Hz, 2.0 Hz, 1 H), 2.35 (s, 1 H), 1.73−1.80 (m, 1 H), 1.36−1.51 (m, 6 H), 0.80−0.90 (m, 9 H). MS (ESI): m/z 198.9 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-propylpiperazin-2-one (**73**).* (3S,6S)-3-Isobutyl-6-propylpiperazin-2-one (50 mg, 0.25 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (53 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **73** (93 mg, 95% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.21−7.17 (m, 2 H), 6.86 (s, 1 H), 6.08 (s, 1 H), 5.29−5.28 (m, 1 H), 4.89−4.78 (m, 1 H), 3.71−3.63 (m, 1 H), 3.17−2.83 (m, 1 H), 1.921.65 (m, 3 H), 1.58−1.37 (m, 4 H), 1.08−0.77 (m, 9 H). MS (ESI): m/z 388.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-((E)-prop-1-en-1-yl)pipe-razin-2-one (**74**). A. (3S,6S)-3-Isobutyl-6-propenyl-piperazin-2-one.* Synthesized from 2-amino-pent-3-enoic acid (11.93 g, 104 mmol) and HCl salt of Leu-OMe (7.8 g, 42.95 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-3-Isobutyl-6-propenyl-piperazin-2-one (1.623 g, 8% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (s, 1 H), 5.43−5.57(m, 2 H), 3.74 (s, 1 H), 3.07 (dd, J = 10.8 Hz, 3.2 Hz, 1 H), 2.82 (dd, J = 12.8 Hz, 4.8 Hz, 1 H), 2.63 (dd, J = 12.8 Hz, 5.6 Hz, 1 H), 1.74−1.82 (m, 1 H), 1.64 (d, J = 7.2 Hz, 3 H), 1.47−1.54 (m, 1 H), 1.35−1.42 (m, 1 H), 0.82−0.88 (m, 7 H). MS (ESI): m/z 197.2 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-((E)-prop-1-en-1-yl)pipera-zin-2-one (**74**).* (3S,6S)-3-Isobutyl-6-propenyl-piperazin-2-one (40 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **74** (32 mg, 41% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.76 (m, 2 H), 7.20−7.15 (m, 2 H), 6.86 (s, 1 H), 5.92−5.81 (m, 2 H), 5,34−5.25 (m, 2 H), 4.80−4.72 (m, 1 H), 4.20−4.13 (m, 1 H), 3.19 (dd, J = 14.0 Hz, 11.2 Hz, 1 H), 1.92−1.68 (m, 6 H) and 1.08−0.77 (m, 6 H). MS (ESI): m/z 386.2 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-neopentylpiperazin-2-one (**75**). A. (3S,6S)-6-(2,2-Dimethyl-propyl)-3-isobutyl-piperazin-2-one.* Synthesized from (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4,4-dimethylpentanoic acid (15.0 g, 40 mmol) and HCl salt of Leu-OMe (3.5 g, 19.35 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(2,2-dimethyl-propyl)-3-isobutyl-piperazin-2-one (1.6 g, 35% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (s, 1 H), 3.30 (dd, J = 5.6 Hz, 4.0 Hz, 1 H), 3.07 (dd, J = 9.6 Hz, 4.0 Hz, 1 H), 2.83 (dd, J = 12.8 Hz, 4.0 Hz, 1 H), 2.56 (dd, J = 13.2 Hz, 6.0 Hz, 1 H), 1.90 (s, 1 H), 1.74−1.79 (m, 1 H), 1.33−1.49 (m, 4 H), 0.80−0.90 (m, 15 H). MS (ESI): m/z 227.0 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-neopentylpiperazin-2-one (**75**).* (3S,6S)-6-(2,2-Dimethyl-propyl)-3-isobutyl-piperazin-2-one (56 mg, 0.25 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (53 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to give **75** (94 mg, 90% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86−7.82 (m, 2 H), 7.29−7.26 (m, 2 H), 7.06 (s, 1 H), 5.16−5.12 (m, 1 H), 4.53 (dd, J = 14.2 Hz, 4.4 Hz, 1 H), 3.79−3.71 (m, 1 H), 3.21 (dd, J = 14.2 Hz, 11.0 Hz, 1 H), 1.92−1.62 (m, 4 H), 1.48−1.32 (m, 2 H), 1.12−0.88 (m, 15 H). MS (ESI): m/z 416.1 (M + 1).

*(3S,6S)-6-((R)-sec-Butyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**76**). A. (3S,6S)-6-((R)-sec-Butyl)-3-isobutylpiperazin-2-one.* Synthesized from (2*S*,3*R*)-2-(Fmoc)amino-3-methylpentanoic acid (14.5 g, 40 mmol) and HCl salt of Leu-OMe (7.8 g, 42.95 mmol) by the method described for the preparation of compound 7 (Scheme 1) to afford (3S,6S)-6-((*R*)-*sec*-butyl)-3-isobutylpiperazin-2-one (4.26 g, 50% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (s, 1 H), 3.10−3.43 (m, 3 H), 2.68−2.94 (m, 1 H), 1.05−1.94 (m, 6 H), 0.89−0.96 (m, 12 H). MS (ESI): m/z 213.1 (M + 1).

*B. (3S,6S)-6-((R)-sec-Butyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**76**).* (3S,6S)-6-((*R*)-*sec*-Butyl)-3-isobutylpiperazin-2-one (50 mg, 0.24 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (53 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **76** (79 mg, 83% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83−7.76 (m, 2 H), 7.22−7.16 (m, 2 H), 6.86 (s, 1 H), 6.24 (s, 1 H), 5.33−5.25 (m, 1 H), 5.00−4.70 (m, 1 H), 3.76−3.36 (m, 1 H), 3.34−3.11 (m, 1H), 1.91−1.71 (m, 3 H), 1.48−1.16 (m, 2 H), 1.05−0.71 (m, 12 H). MS (ESI): m/z 402.20 (M + 1).

*(3S,6S)-6-Allyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**77**). A. (3S,6S)-6-Allyl-3-isobutylpiperazin-2-one.* Synthesized from (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)-carbonyl)-amino)pent-4-enoic acid (12.5 g, 37 mmmol) and HCl salt of Leu-OMe (7.8 g, 42.95 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-allyl-3-isobutylpiperazin-2-one (2.6 g, 36% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 (s, 1 H), 5.66−5.74 (m, 1 H), 5.15−5.17 (m, 1 H), 5.12−5.15 (m, 1 H), 3.38−3.43 (m, 2 H), 2.97−3.01 (m, 1 H), 2.79−2.84 (m, 1 H), 2.28−2.33 (m, 1 H), 2.16−2.20 (m, 1 H), 1.70−1.79

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(m, 2 H), 1.66−1.69 (m, 1 H), 1.53−1.59 (m, 1 H), 0.90−0.96 (m, 6 H). MS (ESI): m/z 197.2 (M + 1).

*B. (3S,6S)-6-Allyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**77**).* (3S,6S)-6-Allyl-3-isobutylpiperazin-2-one (40 mg, 0.20 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **77** (60 mg, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.76 (m, 2 H), 7.22−7.16 (m, 2 H), 6.86 (m, 1 H), 5.99 (br s, 1 H), 5.76−5.67 (m, 1 H), 5.32−5.20 (m, 2 H), 4.88−4.78 (m, 1 H), 3.76−3.65 (m, 1 H), 3.18 (dd, J = 14.0 Hz, 11.2 Hz, 0.5 H), 2.89 (dd, J = 14.0 Hz, 11.2 Hz, 0.5 H), 2.42−2.36 (m, 1 H), 2.16−2.05 (m, 1 H), 1.92−1.63 (m, 4 H), 1.08−0.77 (m, 6 H). MS (ESI): m/z 386.1 (M + 1).

*(3S,6S)-6-(Cyclopropylmethyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-pipe-razin-2-one (**78**). A. (3S,6S)-6-(Cyclopropylmethyl)-3-isobutylpiperazin-2-one.* Synthesized from Fmoc-cyclopropylalanine (23.6 g, 67.3 mmol) and HCl salt of Leu-OMe (6.6 g, 37.0 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(cyclopropylmethyl)-3-isobutylpiperazin-2-one (1.6 g, 11.5% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (s, 1 H), 3.24−3.27 (m, 1 H), 3.05−3.08 (dd, J = 9.6 Hz, 4.0 Hz, 1 H), 2.82−2.86 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 2.69−2.74 (dd, J = 12.8 Hz, 5.2 Hz, 1 H), 1.72−1.78 (m, 1 H), 1.39−1.49 (m, 2 H), 1.31−1.36 (m, 2 H), 0.81−0.89 (m, 6 H), 0.62−0.67 (m, 1 H), 0.38−0.40 (m, 2 H), 0.01−0.08 (m, 2 H). MS (ESI): m/z 210.9 (M + 1).

*B. (3S,6S)-6-(Cyclopropylmethyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpipera-zin-2-one (**78**).* (3S,6S)-6-(Cyclopropylmethyl)-3-isobutylpiperazin-2-one (50 mg, 0.24 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (53 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **78** (53 mg, 54% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79−7.61 (m, 2 H), 7.19−7.15 (m, 2 H), 6.86 (s, 1 H), 6.47 (s, 1 H), 5.29−5.25 (m, 1 H), 4.93−4.88 (m, 1 H), 3.78−3.74 (m, 1 H), 3.21 (dd, J = 14.0 Hz, 11.0 Hz, 1 H), 1.88−1.52 (m, 4 H), 1.30−1.22 (m, 1 H), 1.08−0.96 (m, 6 H), 0.74−0.52 (m, 3 H), 0.21−0.08 (m, 2 H). MS (ESI): m/z 400.1 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-((methylthio)methyl)-piperazin-2-one (**79**). A. (3S,6R)-3-Isobutyl-6-((methylthio)methyl)piperazin-2-one.* Synthesized from (*R*)-2-(Fmoc)amino-3-(methylthio)propanoic acid (23.9 g, 67 mmol) and HCl salt of Leu-OMe (6.6 g, 37.0 mmol) by the method described for the preparation of compound **7** to afford (3S,6R)-3-isobutyl-6-((methylthio)methyl)piperazin-2-one (1.7 g, 12% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (s, 1 H), 3.31−3.33 (m, 1 H), 3.06−3.09 (m, 1 H), 2.84−2.89 (m, 2 H), 2.68−2.71 (m, 1 H), 2.53−2.54 (m,1 H), 2.05−2.06 (m, 3 H), 1.74−1.78 (m, 1 H), 1.51−1.54 (m, 1 H), 1.36−1.39 (m, 1 H), 0.82−0.88 (m, 6 H). MS (ESI): m/z 217.14 (M +1).

*B. (3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-((methylthio)methyl)-piperazin-2-one (**78**).* (3S,6R)-3-Isobutyl-6-((methylthio)methyl)piperazin-2-one (50 mg, 0.23 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (50 mg, 0.24 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **79** (30 mg, 30% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.82−7.77 (m, 2 H), 7.25−7.17 (m, 2 H), 6.88 (s, 0.5 H), 6.78 (s, 0.5 H), 6.52 (br s, 1 H), 5.39−5.28 (m, 1 H), 5.04−4.82 (m, 1 H), 4.50−4.39 (m, 1 H), 3.85- 3.74 (m, 1 H), 3.42−2.36 (m, 2 H), 2.15 (s, 2 H), 2.13 (s, 1 H), 1.94−1.86 (m, 1 H), 1.81−1.72 (m, 2 H), 1.10−0.78 (m, 6 H). MS (ESI): m/z 406.2 (M + 1).

*(3S,6S)-6-Butyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**80**). A. (3S,6S)-3-Isobutyl-6-butyl-piperazin-2-one.* Synthesized from Fmoc-(*S*)-2-aminohexanoic acid (9.91 g, 28.5 mmol) and HCl salt of Leu-OMe (4.7 g, 26.25 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-3-isobutyl-6-butyl-piperazin-2-one (3.4 g, 25% yield) as a white solid. MS (ESI): m/z 213.3 (M + 1).

*B. (3S,6S)-6-Butyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**80**).* (3S,6S)-3-Isobutyl-6-butyl-piperazin-2-one (40 mg, 0.19 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (50 mg, 0.24 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **80** (60 mg, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.76 (m, 2 H), 7.20−7.15 (m, 2 H), 6.86 (s, 1 H), 6.43 (br s, 1 H), 5.28−5.25 (m, 1 H), 4.87−4.77 (m, 1 H), 3.69−3.59 (m, 1 H), 3.13 (dd, J = 14.0 Hz, 11.2 Hz, 0.5H), 2.84 (dd, J = 14.0 Hz, 11.2 Hz, 0.5H), 2.80−2.74 (m, 2 H), 1.92−1.68 (m, 4 H), 1.58−1.24 (m, 7 H), 1.07−0.76 (m, 9 H). MS (ESI): m/z 402.2 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(2-(methylthio)ethyl)-piperazin-2-one (**81**). A. (3S,6S)-3-Isobutyl-6-(2-(methylthio)ethyl)piperazin-2-one.* Synthesized from (*S*)-2-(Fmoc)amino-4-(methylthio)butanoic acid (24.9 g, 67 mmol) and HCl salt of Leu-OMe (6.6 g, 37.0 mmol) by the method described for the preparation of compound **7** to afford (3S,6S)-3-isobutyl-6-(2-(methylthio)ethyl)piperazin-2-one (5.0 g, 49% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.14 (s, 1 H), 3.54 (s, 1 H), 3.39−3.42 (m, 1 H), 3.03−3.07 (m, 1 H), 2.83−2.87 (m, 1 H), 2.78 (s, 1 H), 2.53−2.58 (m, 2 H), 2.09−2.15 (m, 3 H), 1.86−1.90 (m, 1 H), 1.71−1.82 (m, 2 H), 1.54−1.57 (m, 1 H), 0.90−0.96 (m, 6 H). MS (ESI): m/z 231.1 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(2-(methylthio)ethyl)-piperazin-2-one (**81**).* (3S,6S)-3-Isobutyl-6-(2-(methylthio)ethyl)piperazin-2-one (50 mg, 0.22 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **81** (71 mg, 78% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.76 (m, 2 H), 7.22−7.16 (m, 2 H), 6.87 (s, 1 H), 6.41 (s, 1 H), 5.33−5.26 (m, 1 H), 4.93−4.79 (m, 1 H), 3.86−3.77 (m, 1 H), 3.20−2.91 (m, 1 H), 2.63−2.58 (m, 2 H), 2.13 (s, 3 H), 1.92−1.70 (m, 5 H), 1.08−0.78 (m, 6 H). MS (ESI): m/z 420.10 (M + 1).

*(3S,6S)-6-Ethyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**82**). A. (3S,6S)-3-Isobutyl-6-ethylpiperazin-2-one.* Synthesized from (*S*)-2-(Fmoc)amino-butanoic acid (21.8 g, 67 mmol) and HCl salt of Leu-OMe (6.6 g, 37.0 mmol) by the method described for the compound **7** to afford (3S,6S)-3-isobutyl-6-ethylpiperazin-2-one (2.1 g, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.03 (s, 1 H), 3.21−3.37 (m, 2 H), 2.91−2.97 (m, 1 H), 2.72−2.76 (m, 1 H), 1.63−1.67 (m, 1 H), 1.44−1.52 (m, 3 H), 0.85−0.91 (m, 9 H). MS (ESI): m/z 185.1 (M + 1).

*B. (3S,6S)-6-Ethyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**82**).* (3S,6S)-3-Isobutyl-6-ethylpiperazin-2-one (50 mg, 0.27 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (56 mg, 0.27 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **82** (50 mg, 50% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.76 (m, 2 H), 7.26−7.16 (m, 2 H), 6.86 (s, 1 H), 5.97 (s, 1 H), 5.32−5.27 (m, 1 H), 4.91−4.80 (m, 1 H), 3.64−3.55 (m, 1 H), 3.18−2.83 (m, 1 H), 1.92−1.69 (m, 2 H), 1.63−1.48 (m, 3 H), 1.09−0.77 (m, 9 H). MS (ESI): m/z 374.10 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-isopropylpiperazin-2-one (**83**). A. (3S,6S)-3-Isobutyl-6-isopropylpiperazin-2-one.* Synthesized from Fmoc-L-valine (27.4 g, 80 mmol) and HCL salt of Leu-OMe (3.4 g, 23 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford the (3S,6S)-3-isobutyl-6-isopropylpiperazin-2-one (2.5 g, 22% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (s, 1 H), 3.40 (dd, J = 10.4 Hz, 4.0 Hz, 1H), 3.09−3.06 (m, 1 H), 2.94−2.86 (m, 2 H), 1.77−1.72 (m, 2 H), 1.59−1.55 (dd, J = 10.4 Hz, 4.4 Hz, 1 H), 1.31−1.23 (m, 1 H), 0.95−0.90 (m, 12 H). MS (ESI): m/z 199.0 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-isopropylpiperazin-2-one (**83**).* (3S,6S)-3-Isobutyl-6-isopropylpiperazin-2-one (50 mg, 0.25 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (52 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **83** (43 mg, 44% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.76 (m, 2 H), 7.21−7.16 (m, 2 H), 6.87 (s, 1 H), 5.97 (s, 1 H), 5.31−5.27 (m, 1 H), 4.92−4.77 (m, 1 H), 3.53−3.44 (m, 1 H), 3.23−2.90 (m, 1 H), 1.90−1.69 (m, 4 H), 1.08−0.77 (m, 12 H). MS (ESI): m/z 388.1 (M + 1).

*(3S,6S)-6-(tert-Butyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**84**). A. (3S,6S)-6-(tert-Butyl)-3-isobutyl-*

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piperazin-2-one. Synthesized from Fmoc-L-*tert*-leucine (23.0 g, 16 mmol) and HCL salt of Leu-OMe (5.0 g, 27.0 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3*S*,6*S*)-6-(*tert*-butyl)-3-isobutylpiperazin-2-one (1.0 g, 16% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (s, 1 H), 3.05−3.09 (m, 2 H), 2.97−2.99 (m, 1 H), 2.66−2.69 (m, 1 H), 2.60−2.64 (m, 1 H), 1.76−1.89 (m, 1 H), 1.38−1.44 (m, 2 H), 0.86−0.89 (m, 15 H). MS (ESI): *m*/*z* 213.0 (M + 1).

*B. (3S,6S)-6-(tert-Butyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**84**).* (3*S*,6*S*)-3-Isobutyl-6-isopropylpiperazin-2-one (50 mg, 0.24 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (50 mg, 0.24 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **84** (78 mg, 82% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.21−7.16 (m, 2 H), 6.87 (s, 1 H), 5.85 (s, 1 H), 5.30−5.27 (m, 1 H), 4.95−4.79 (m, 1 H), 3.48−3.37 (m, 1 H), 3.25−2.92 (m, 1 H), 1.90−1.81 (m, 1 H), 1.77−1.71 (m, 2 H), 1.09−0.79 (m, 15 H). MS (ESI): *m*/*z* 402.10 (M − 1).

*(3S,6S)-6-Cyclopropyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**85**). A. (3S,6S)-6-Cyclopropyl-3-isobutylpiperazin-2-one.* Synthesized from Fmoc-L-cyclopropylglycine (13.78 g, 43 mmol) and HCl salt of Leu-OMe (6.0 g, 32.9 mmol) by the method described for the compound **7** (Scheme 1) to afford (3*S*,6*S*)-6-cyclopropyl-3-isobutylpiperazin-2-one (1.7 g, 20% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.575 (s, 1 H), 3.43 (t, *J* = 4.0 Hz, 1 H), 2.81−2.82 (m, 1 H), 2.78−2.79 (m, 2 H), 2.38−2.39 (m, 2 H), 1.72−1.80 (m, 1 H), 1.49−1.52 (m, 1 H), 1.45−1.47 (m, 1 H), 0.95−1.05 (m, 1 H), 0.87 (d, *J* = 3.2 Hz, 3 H), 0.82 (d, *J* = 3.2 Hz, 3 H), 0.42−0.45 (m, 1 H), 0.32−0.36 (m, 1 H), 0.24−0.26 (m, 1 H), 0.08−0.12 (m, 1 H). MS (ESI): *m*/*z* 197.1 (M + 1).

*B. (3S,6S)-6-Cyclopropyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**85**).* (3*S*,6*S*)-6-Cyclopropyl-3-isobutylpiperazin-2-one (50 mg, 0.25 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (52 mg, 0.25 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **85** (78 mg, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.20−7.16 (m, 2 H), 6.85 (s, 1 H), 6.05 (s, 1 H), 5.29−5.24 (m, 1 H), 4.94−4.86 (m, 1 H), 3.35−3.02 (m, 1 H), 2.88−2.77 (m, 1 H), 1.92−1.88 (m, 1 H), 1.83−1.71 (m, 2 H), 1.09−0.92 (m, 6 H), 0.79−0.75 (m, 2 H), 0.64−0.60 (m, 2 H), 0.43−0.27 (m, 1 H). MS (ESI): *m*/*z* 386.1 (M + 1).

*(3S,6S)-6-Cyclobutyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**86**). A. (3S,6S)-6-Cyclobutyl-3-isobutylpiperazin-2-one.* Synthesized from Fmoc-(*S*)-amino-cyclobutyl-acetic acid (11.3 g, 32.19 mmol) and HCl salt of Leu-OMe (3.67 g, 20.3 mmol) by the method described for the compound **7** (Scheme 1) to afford (3*S*,6*S*)-6-cyclobutyl-3-isobutylpiperazin-2-one (1.55 g, 26% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1 H), 3.35−3.38 (m, 1 H), 3.24−3.26 (m, 1 H), 2.89−2.94 (dd, *J* = 13.2 Hz, 4.4 Hz, 1 H), 2.68−2.73 (m, 1 H), 2.41−2.45 (m, 1 H), 1.90−2.04 (m, 3 H), 1.63−1.81 (m, 5 H), 1.51−1.54 (m, 2 H), 0.85−0.94 (m, 6 H). MS (ESI): *m*/*z* 211.1 (M + 1).

*B. (3S,6S)-6-Cyclobutyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**86**).* (3*S*,6*S*)-6-Cyclobutyl-3-isobutylpiperazin-2-one (42 mg, 0.20 mmol) and 5-(4-fluorophenyl)isoxa-zole-3-carboxylic acid (42 mg, 0.20 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **98** (55 mg, 69% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.26−7.16 (m, 2 H), 6.87 (s, 1 H), 5.91 (s, 1 H), 5.30−5.25 (m, 1 H), 4.84−5.73 (m, 1 H), 3.69−3.55 (m, 1 H), 3.09 (dd, *J* = 14.0 Hz, 11.2 Hz, 0.5 H), 2.73 (dd, *J* = 14.0 Hz, 11.2 Hz, 0.5 H), 2.38−2.29 (m, 1 H), 2.14−1.66 (m, 9 H), 1.08−0.76 (m, 6 H). MS (ESI): *m*/*z* 400.0 (M + 1).

*(3S,6S)-6-Cyclopentyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**87**). A. (3S,6S)-6-Cyclopentyl-3-isobutylpiperazin-2-one.* Synthesized from Fmoc-L-cyclopentyl-glycine (10.23 g, 28 mmol) and HCl salt of Leu-OMe (6.7 g, 36.8 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3*S*,6*S*)-6-cyclopentyl-3-isobutyl-piperazin-2-one (1.1 g, 16% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (s, 1 H), 3.08 (dd, *J* = 10.0 Hz, 3.6 Hz, 1 H), 2.93 (dd, *J* = 7.6 Hz, 3.6 Hz, 1 H), 2.77 (dd, *J* = 12.8 Hz, 4.0 Hz, 1 H), 2.68 (dd, *J* = 12.8 Hz, 4.8 Hz, 1 H), 1.97 (s, 1 H), 1.38−1.55 (m, 9 H), 1.08−1.22 (m, 3 H), 0.80−0.90 (m, 6 H). MS (ESI): *m*/*z* 225.1 (M + 1).

*B. (3S,6S)-6-Cyclopentyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**87**).* (3*S*,6*S*)-6-Cyclopentyl-3-isobutylpiperazin-2-one (50 mg, 0.22 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (46 mg, 0.22 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **87** (56 mg, 61% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.76 (m, 2 H), 7.21−7.16 (m, 2 H), 6.87 (s, 1 H), 5.94 (s, 1 H), 5.29−5.26 (m, 1 H), 4.93−4.79 (m, 1 H), 3.53−3.41 (m, 1 H), 3.19−2.86 (m, 1 H), 1.91−1.58 (m, 10 H), 1.32−1.23 (m, 2 H), 1.09−0.77 (m, 6 H). MS (ESI): *m*/*z* 414.1 (M + 1).

*(3S,6S)-6-Cyclohexyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**88**). A. (3S,6S)-6-Cyclohexyl-3-isobutylpiperazin-2-one.* Synthesized from Fmoc-L-cyclohexylglycine (22.78 g, 60 mmol) and HCl salt of Leu-OMe (6.0 g, 32.8 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford the product (3*S*,6*S*)-6-cyclohexyl-3-isobutylpiperazin-2-one (1.2 g, 8% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (s, 1 H), 3.08 (dd, *J* = 10.0 Hz, 4.4 Hz, 1 H), 2.95 (d, *J* = 5.6 Hz, 1 H), 2.76 (dd, *J* = 13.6 Hz, 6.0 Hz, 1 H), 2.68 (dd, *J* = 13.2 Hz, 4.4 Hz, 1 H), 1.59−1.77 (m, 6 H), 1.38−1.45 (m, 3 H), 1.09−1.16 (m, 3 H), 0.89−0.95 (m, 5 H), 0.72−0.88 (m, 3 H). MS (ESI): *m*/*z* 239.0 (M + 1).

*B. (3S,6S)-6-Cyclohexyl-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**88**).* (3*S*,6*S*)-6-Cyclohexyl-3-isobutylpiperazin-2-one (50 mg, 0.24 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (43 mg, 0.21 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **88** (46 mg, 51% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.76 (m, 2 H), 7.21−7.16 (m, 2 H), 6.86 (s, 1 H), 5.86 (s, 1 H), 5.30−5.27 (m, 1 H), 4.92−4.78 (m, 1 H), 3.53−3.44 (m, 1 H), 3.26−2.93 (m, 1 H), 1.88−1.70 (m, 8 H), 1.45−1.37 (m, 1 H), 1.29−1.11 (m, 5 H), 1.09−0.77 (m, 6 H). MS (ESI): *m*/*z* 428.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(3,3,3-trifluoro-2-(trifluoromethyl)propyl)piperazin-2-one (**89**). A. (3S,6S)-3-Isobutyl-6-(3,3,3-trifluoro-2-(trifluoromethyl)-propyl)-piperazin-2-one.* Synthesized from (*S*)-2-((((9*H*-fluoren-9-yl)-methoxy)carbonyl)amino)-5,5,5-trifluoro-4-(trifluoro-methyl)-pentanoic acid (9.22 g, 20 mmol) and HCl salt of Leu-OMe (3.0 g, 16.4 mmol) by the method described for the compound 7 (Scheme 1) to afford (3*S*,6*S*)-3-isobutyl-6-(3,3,3-trifluoro-2-(trifluoromethyl)-propyl)piperazin-2-one (321 mg, 5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 1 H), 3.87−3.89 (m, 0.5 H), 3.72−3.75 (m, 0.5 H), 3.52−3.56 (m, 1 H), 2.89−2.92 (m, 1 H), 3.11−3.15 (m, 1 H), 2.86−2.90 (m, 1 H), 2.28−2.35 (m, 1 H), 1.97−2.01 (m, 1 H), 1.66−1.80 (m, 2 H), 1.49−1.52 (m, 1 H), 0.85−0.89 (m, 6 H). MS (ESI): *m*/*z* 321.1 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(3,3,3-trifluoro-2-(trifluoromethyl)propyl)piperazin-2-one (**89**).* (3*S*,6*S*)-3-Isobutyl-6-(3,3,3-trifluoro-2-(trifluoromethyl)propyl)-piperazin-2-one (40 m g, 0.14 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (30 mg, 0.14 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish compound **89** (40 mg, 48% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.79 (m, 2 H), 7.22−7.17 (m, 2 H), 6.91 (s, 1 H), 5.39 (t, *J* = 6.0 Hz, 0.5 H), 5.28 (dd, *J* = 7.2 Hz, 4.0 Hz, 1 H), 5.06 (d, *J* = 13.2 Hz, 1 H), 4.89 (d, *J* = 7.2 Hz, 0.5 H), 3.99−3.85 (m, 1 H), 3.40−3.34 (m, 1 H), 3.17 (dd, *J* = 14.8 Hz, 10.2 Hz, 1 H), 2.07−1.83 (m, 3 H), 1.77−1.71 (m, 2 H), 1.08 (d, *J* = 6.4 Hz, 2 H), 0.99 (d, *J* = 6.4 Hz, 2 H), 0.94 (d, *J* = 6.0 Hz, 1 H), 0.81 (d, *J* = 6.0 Hz, 1 H). MS (ESI): *m*/*z* 510.2 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-6-(hydroxymethyl)-3-isobutylpipera-zin-2-one (**90**).* Compound **14** (2.00 g, 8.25 mmol) and HCl in dioxane (4 N, 20 mL) were stirred at 70 °C for 1.5 h and then concentrated under vacuo to give the crude (3*S*,6*R*)-6-(hydroxymethyl)-3-isobutylpiperazin-2-one, which was used for the next step without further purification. Crude compound of (3*S*,6*R*)-6-(hydroxymethyl)-3-isobutylpiperazin-2-one (0.20 g, 0.90 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (0.19 g, 0.90 mmol)

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were coupled according to the procedure described for the preparation of compound **2a** to furnish **90** (0.294 g, 87% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03−7.99 (m, 2 H), 7.93 (s, 0.3 H), 7.90 (s, 0.7 H), 7.45−7.41 (m, 2 H), 7.37 (s, 1 H), 5.01 (m, 0.3 H), 4.92−4.89 (m, 1.7 H), 4.69−4.60 (m, 0.3 H), 4.21 (dd, J = 14.4 Hz, 4.4 Hz, 0.7 H), 3.53−3.48 (m, 1 H), 3.46−3.41 (m, 1 H), 3.31−3.27 (m, 1.7 H), 3.05 (m, 0.3 H), 1.81 (t, J = 9.6 Hz, 1 H), 1.68−1.61 (m, 2 H), 0.98 (d, J = 6.0 Hz, 2.4 H), 0.94 (d, J = 6.0 Hz, 2.4 H), 0.81 (d, J = 6.4 Hz, 0.6 H), 0.61 (d, J = 6.4 Hz, 0.6 H). MS (ESI): m/z 376.2 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(methoxymethyl)pipera-zin-2-one (**91**).* To a soluton of alcohol **90** (50 mg, 0.13 mmol) in MeCN (3 mL) under argon was added MeI (83 uL, 1.33 mmol) and Ag_2O (154 mg, 0.66 mmol) and stirred at rt for 3 days. The reaction mixture was filtered through a short pad of Celite, concentrated in vacuo, and purified by column chromatography (0−80% EtOAc in hexanes) to give **91** (19 mg, 37% yield) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06−8.00 (m, 3 H), 7.45−7.40 (m, 2 H), 7.38 (s, 1 H), 4.90 (m, 1 H), 4.70−4.56 (m, 1 H), 4.21 (m, 1 H), 3.68 (m, 1 H), 3.44−3.37 (m, 1 H), 3.23 (s, 2 H), 3.18−3.05 (m, 1 H), 2.69 (s, 1 H), 1.81 (t, J = 10.0 Hz, 1 H), 1.69−1.59 (m, 2 H), 0.98 (d, J = 5.6 Hz, 3 H), 0.94 (d, J = 6.0 Hz, 3 H). MS (ESI): m/z 390.2 (M +1).

*(3S,6S)-6-((Dimethylamino)methyl)-4-(5-(4-fluorophenyl)-isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**92**).* To a solution of the alcohol **90** (0.20 g, 0.53 mmol) in DMF (3 mL) was added Et_3N (0.23 mL, 1.65 mmol) and MsCl (70 uL, 0.90 mmol) and stirred at rt for 16 h and then concentrated in vacuo. The residue was purified by column chromatography (0−75% EtOAc in hexanes) to give the crude mesylate product (0.132 g, 55% yield), which was used for the next step without further purification. MS (ESI): m/z 454.2 (M + 1). To a solution of the mesylate (30 mg, 0.07 mmol) in DMF (2 mL) was added dimethyamine in THF (2 M, 0.17 mL, 0.34 mmol) and heated at 60 °C for 16 h. The reaction mixture was concentrated in vacuo and purified by column chromatography (0−90% EtOAc in hexanes) to give **92** (19 mg, 71% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (m, 2 H), 7.76 (s, 1 H), 7.68 (s, 3 H), 7.45−7.38 (m, 3 H), 4.91−4.89 (m, 1 H), 4.70−4.64 (m, 1 H), 4.25−4.18 (m, 1 H), 3.74−3.64 (m, 1 H), 3.22 (m, 1 H), 2.36−2.25 (m, 2 H), 2.09 (s, 6 H), 1.84 (t, J = 10.8 Hz, 1 H), 1.67−1.63 (m, 1 H), 0.98 (d, J = 5.6 Hz, 2.5 H), 0.94 (d, J = 6.0 Hz, 2.5 H), 0.81 (d, J = 6.4 Hz, 0.5 H), 0.61 (d, J = 6.4 Hz, 0.5 H). MS (ESI): m/z 403.2 (M + 1).

*(3S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(tetrahydro-2H-pyran-4-yl)-piperazin-2-one (**93**). A. (3S)-3-Isobutyl-6-(tetrahydro-2H-pyran-4-yl)piperazin-2-one.* Synthesized from Fmoc-2-amino-2-(tetrahydro-2*H*-pyran-4-yl)acetic acid (11.98 g, 31.4 mmol) and hydrochloride salt of Leu-OMe (3.0 g, 16.4 mmol) by the method described for the compound **7** to afford (3*S*)-3-isobutyl-6-(tetrahydro-2*H*-pyran-4-yl)piperazin-2-one (0.50 g, 6.70% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12−6.35 (m, 1 H), 3.94−4.09 (m, 2 H), 2.94−3.38 (m, 5 H), 2.00−2.01 (m, 1 H), 1.20−1.86 (m, 9 H), 0.87−0.93 (m, 6 H). MS (ESI): m/z 241.17 (M + 1).

*B. (3S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(tetrahydro-2H-pyran-4-yl)-piperazin-2-one (**93**).* (3*S*)-3-Isobutyl-6-(tetrahydro-2*H*-pyran-4-yl)piperazin-2-one (36 mg, 0.15 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (35 mg, 0.17 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **93** (10 mg, 15% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.21−7.10 (m, 2 H), 6.88 (s, 1 H), 6.17 (br s, 1 H), 5.34−5.27 (m, 1 H), 4.99 and 4.82 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 4.06−4.00 (m, 2 H), 3.59−3.47 (m, 1 H), 3.43−3.34 (m, 2 H), 3.23 and 2.95 (dd, J = 13.6 Hz, 11.2 Hz, 1 H), 1.90−1.42 (m, 8 H) and 1.08−0.78 (m, 6 H). MS (ESI): m/z 430.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(tetrahydro-2H-thiopyran-4-yl)piperazin-2-one (**94**). A. (3S,6S)-3-Isobutyl-6-(tetrahydro-2H-thiopyran-4-yl)piperazin-2-one.* Synthesized from Fmoc-2-amino-2-(tetrahydro-2*H*-thiopyran-4-yl)acetic acid (12 g, 31 mmol) and HCl salt of Leu-OMe (3.0 g, 16.4 mmol) by the method described for the preparation of compound **7** to afford (3*S*,6*S*)-3-isobutyl-6-(tetrahydro-2*H*-thiopyran-4-yl)piperazin-2-one (0.77 g, 37% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 1 H), 3.31−3.37 (m, 2 H), 3.10−3.14 (m, 1 H), 2.62−2.74 (m, 5 H), 1.90−2.03 (m, 3 H), 1.84−1.88 (m, 1 H), 1.71−1.80 (m, 1 H), 1.37−1.54 (m, 4 H), 0.87−0.95 (m, 6 H). MS (ESI): m/z 257.17 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(tetrahydro-2H-thiopyran-4-yl)piperazin-2-one (**94**).* (3*S*,6*S*)-3-Isobutyl-6-(tetrahydro-2*H*-thiopyran-4-yl)piperazin-2-one (50 mg, 0.27 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (44 mg, 0.27 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish compound **94** (37 mg, 42% yield) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.77 (m, 2 H), 7.22−7.16 (m, 2 H), 6.87 (s, 1 H), 6.32 (s, 1 H), 5.32−5.26 (m, 1 H), 4.92−4.71 (m, 1 H), 3.65−3.54 (m, 1 H), 3.29−2.95 (m, 1 H), 2.73−2.66 (m, 4 H), 2.09−2.06 (m, 2 H), 1.90−1.82 (m, 1 H), 1.76−1.66 (m, 2 H), 1.60−1.54 (m, 2 H), 1.08−0.0.78 (m, 6 H). MS (ESI): m/z 445.97 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-phenylpiperazin-2-one (**95**). A. (3S,6S)-3-Isobutyl-6-phenylpiperazin-2-one.* Synthesized from Fmoc-L-2-phenylglycine (23.41 g, 63 mmol) and HCl salt of Leu-OMe (8 g, 56 mmol) by the method described for the compound **7** (Scheme 1) to afford the (3*S*,6*S*)-3-isobutyl-6-phenylpiperazin-2-one (2.0 g, 9% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35−7.40 (m, 2 H), 7.24−7.32 (m, 2 H), δ 6.12 (s, 1 H), 4.62 (q, J = 7.2 Hz, 1 H), 3.49 (dd, J = 10.4 Hz, 3.2 Hz, 1 H), 3.22−3.27 (m, 1 H), 2.98 (dd, J = 13.6 Hz, 5.2 Hz, 1 H), 1.68−1.87 (m, 2 H), 1.54−1.61 (m, 1 H), 0.94 (d, J = 3.2 Hz, 3 H), 0.91 (d, J = 3.2 Hz, 3 H). MS (ESI): m/z 233.2 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-phenylpiperazin-2-one (**95**).* (3*S*,6*S*)-3-Isobutyl-6-phenylpiperazin-2-one (50 mg, 0.22 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **95** (69 mg, 76% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.44−7.36 (m, 5 H), 7.22−7.16 (m, 2 H), 6.89 (s, 1 H), 6.04 (s, 1 H), 5.43−5.36 (m, 1 H), 4.97−4.88 (m, 2 H), 3.37−3.06 (m, 1 H), 2.04−1.94 (m, 1 H), 1.93−1.84 (m, 1 H), 1.80−1.70 (m, 1 H), 1.11−0.81 (m, 6 H). MS (ESI): m/z 422.1 (M +1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(p-tolyl)piperazin-2-one (**96**). A. (3S,6S)-3-Isobutyl-6-p-tolyl-piperazin-2-one.* Synthesized from (*S*)-2-amino-2-(*p*-tolyl)acetic acid (5.0g, 30 mmol) and HCl salt of Leu-OMe (4.37 g, 24.3 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3*S*,6*S*)-3-Isobutyl-6-*p*-tolyl-piperazin-2-one (1.44 g, 19% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (s, 1 H), 7.15 (s, 4 H), 4.41−4.44 (m, 1 H), 3.17−3.20 (m, 1 H), 3.00−3.03 (m, 1 H), 2.68−2.72 (m, 1 H), 2.31 (s, 1 H), 2.28 (s, 3 H), 1.72−1.80 (m, 1 H), 1.51−1.62 (m, 1 H), 1.44−1.49 (m, 1 H), 0.89 (d, J = 5.6 Hz, 3 H), 0.85 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 247.1 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(p-tolyl)piperazin-2-one (**96**).* (3*S*,6*S*)-3-Isobutyl-6-*p*-tolyl-piperazin-2-one (50 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (41 mg, 0.20 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **96** (77 mg, 88% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.28−7.18 (m, 6 H), 6.89 (s, 0.7 H), 6.81 (s, 0.3 H), 6.01 (br s, 0.7 H), 5.97 (br s, 0.3 H), 5.45−5.36 (m, 1 H), 4.94−4.73 (m, 2 H), 3.33 (dd, J = 14.0 Hz, 10.4 Hz, 1 H), 2.38 (s, 1 H), 2.37 (s, 2 H), 2.05−1.68 (m, 3 H), 1.17−0.81 (m, 6 H). MS (ESI): m/z 436.2 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(o-tolyl)piperazin-2-one (**97**). A. (3S,6S)-3-Isobutyl-6-o-tolyl-piperazin-2-one.* Synthesized from (*S*)-amino-*o*-tolyl-acetic acid (4 g, 24.2 mmol) and HCl salt of Leu-OMe (3.3 g, 22.9 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3*S*,6*S*)-3-Isobutyl-6-*o*-tolyl-piperazin-2-one (1.2 g, 20% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (s, 1 H), 7.18 (dd, J = 4.0 Hz, 2.0 Hz, 2 H), 7.11 (dd, J = 4.8 Hz, 3.2 Hz, 2 H), 4.66 (m, 1 H), 3.17 (dd, J = 10.0 Hz, 3.6 Hz, 1 H), 3.01 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 2.63 (dd, J = 13.2 Hz, 5.6 Hz, 1 H), 2.29−2.31 (m, 1 H), 2.24 (s,

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3 H), 1.71−1.77 (m, 1 H), 1.43−1.62 (m, 2 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H). MS (ESI): m/z 247.1 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(o-tolyl)piperazin-2-one (**97**).* (3S,6S)-3-Isobutyl-6-(*o*-tolyl)-piperazin-2-one (50 mg, 0.2 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (41 mg, 0.2 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **97** (77 mg, 87% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.77 (m, 2 H), 7.42−7.39 (m, 1 H), 7.30−7.17 (m, 5 H), 6.89 (s, 1 H), 5.90 (s, 1 H), 5.46−5.37 (m, 1 H), 5.17−5.02 (m, 1 H), 4.98−4.87 (m, 1 H), 3.30−2.96 (m, 1 H), 2.45 (s, 3 H), 2.04−1.71 (m, 3 H), 1.12−0.83 (m, 6 H). MS (ESI): m/z 436.1 (M + 1).

*(3S,6S)-6-(4-Chlorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**98**). A. (3S,6S)-6-(4-Chloro-phenyl)-3-isobutyl-piperazin-2-one.* Synthesized from (*S*)-2-amino-2-(4-chlorophenyl)acetic acid (10.0 g, 53.88 mmol) and HCl salt of Leu-OMe (13.9 g, 76.51 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(4-chlorophenyl)-3-isobutyl-piperazin-2-one (445 mg, 3% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.37 (m, 2 H), 7.20−7.25 (m, 2 H), 5.94 (s, 1 H), 4.60 (dd, J = 7.8 Hz, 4.8 Hz, 1 H), 3.51(dd, J = 10.4 Hz, 3.2 Hz, 1 H), 3.26 (dd, J = 13.6 Hz, 4.8 Hz,1 H), 2.94−2.99 (m, 1 H), 1.75−1.88 (m, 3 H), 1.55−1.61 (m, 1 H), 0.94 (dd, J = 12.8 Hz, 6.4 Hz, 6H). MS (ESI): m/z 267.0 (M + 1).

*B. (3S,6S)-6-(4-Chlorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**98**).* (3S,6S)-6-(4-Chloro-phenyl)-3-isobutyl-piperazin-2-one (53 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (41 mg, 0.20 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **98** (73 mg, 85% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.42−7.34 (m, 4 H), 7.20 (m, 2 H), 6.90 (s, 1 H), 5.93 (br s, 1 H), 5.39 (dd, J = 14.4 Hz, 4.4 Hz, 1 H), 4.99−4.76 (m, 2 H), 3.30 (dd, J = 14.0 Hz, 10.8 Hz, 1 H), 2.01−1.70 (m, 3 H), 1.02 (d, J = 6.4 Hz, 2 H), 1.00 (d, J = 6.8 Hz, 2 H), 0.97−0.82 (m, 2 H). MS (ESI): m/z 456.2 (M + 1).

*(3S,6S)-6-(4-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**99**). A. (3S,6S)-6-(4-Fluorophenyl)-3-isobutyl-piperazin-2-one.* Synthesized from (*S*)-amino-(4-fluorophenyl)-acetic acid (10 g, 60 mmol) and HCl salt of Leu-OMe (1.56 g, 10.67 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(4-fluorophenyl)-3-isobutyl-piperazin-2-one (420 mg, 3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1 H), 7.37 (dd, J = 6.4 Hz, 2.8 Hz, 2 H),7.23 (dd, J = 11.2 Hz, 9.2 Hz, 2 H), 4.54 (d, J = 2.4 Hz, 1 H), 3.26 (dd, J = 10 Hz, 3.6 Hz, 1 H), 3.10 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 2.79 (dd, J = 12.8 Hz, 4.8 Hz, 1 H), 2.45 (s, 1 H), 1.80−1.84 (m, 1 H), 1.53−1.70 (m, 2 H), 0.95 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 251.1 (M +1).

*B. (3S,6S)-6-(4-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**99**).* (3S,6S)-6-(4-Fluorophenyl)-3-isobutyl-piperazin-2-one (50 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (41 mg, 0.20 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **99** (68 mg, 78% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.40−7.37 (m, 2 H), 7.22−7.18 (m, 2 H), 7.14−7.10 (m, 2 H), 6.90 (s, 1 H), 6.06 (br s, 1 H), 5.39−5.36 (m, 1 H), 4.98−4.76 (m, 2 H), 3.31 (dd, J = 13.6 Hz, 10.0 Hz, 1 H), 2.01−1.92 (m, 1 H), 1.89−1.73 (m, 2 H), 1.11 (d, J = 6.4 Hz, 2 H), 1.00 (d, J = 6.8 Hz, 2 H), 0.96−0.82 (m, 2 H). MS (ESI): m/z 440.2 (M +1).

*(3S,6S)-6-(3-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutylpiperazin-2-one (**100**). A. (3S,6S)-6-(3-Fluorophenyl)-3-isobutyl-piperazin-2-one.* Synthesized from (*S*)-amino-(3-fluorophenyl)-acetic acid (9 g, 53 mmol) and HCl salt of Leu-OMe (6.9 g, 38 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(3-fluorophenyl)-3-isobutyl-piperazin-2-one (478 mg, 4% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.38 (m, 1 H), 6.99−7.07 (m, 2 H), 6.10 (s, 1 H), 4.62 (dd, J = 7.6 Hz, 4.4 Hz, 1 H), 3.50 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 3.00 (dd, J = 13.2 Hz, 4.8 Hz, 1 H), 1.76−1.88 (m, 2 H), 1.55−1.60 (m, 2 H), 0.95 (dd, J = 12.8 Hz, 6.4 Hz, 6 H). MS (ESI): m/z 251.0 (M + 1).

*B. (3S,6S)-6-(3-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**100**).* (3S,6S)-6-(3-Fluorophenyl)-3-isobutyl-piperazin-2-one (50 mg, 0.2 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (41 mg, 0.2 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **100** (67 mg, 76% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.78 (m, 2 H), 7.44−7.37 (m, 1 H), 7.26−7.18 (m, 3 H), 7.13−7.05 (m, 2 H), 6.90−6.81 (m, 1 H), 6.12−6.10 (m, 1 H), 5.45−5.35 (m, 1 H), 5.01−4.77 (m, 2 H), 3.35−3.04 (m, 1 H), 1.99−1.92 (m, 1 H), 1.89−1.08 (m, 1 H), 1.78−1.71 (m, 1 H), 1.11−0.81 (m, 6 H). MS (ESI): m/z 440.0 (M + 1).

*(3S,6S)-6-(2-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**101**). A. (3S,6S)-6-(3-Fluorophenyl)-3-isobutyl-piperazin-2-one.* Synthesized from (*S*)-amino-(2-fluorophenyl)-acetic acid (5.0 g, 29.58 mmol) and HCl salt of Leu-OMe (7.8 g, 42.95 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-6-(2-fluorophenyl)-3-isobutyl-piperazin-2-one (660 mg, 9% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.36 (m, 2 H), 7.17−7.21 (m, 1 H), 7.04−7.09 (m, 1 H), 6.14 (s, 1 H), 4.96 (dd, J = 7.8 Hz, 4.0 Hz, 1 H), 3.50 (dd, J = 10.8 Hz, 3.6 Hz, 1 H), 3.32 (dd, J = 10.8 Hz, 3.6 Hz, 1 H), 3.07 (dd, J = 13.2 Hz, 3.6 Hz, 1 H), 1.85−1.91 (m, 1 H), 1.75−1.81 (m, 1 H), 1.51−1.58 (m, 2 H), 0.91−0.96 (m, 6 H). MS (ESI): m/z 251.1 (M + 1).

*B. (3S,6S)-6-(2-Fluorophenyl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**101**).* (3S,6S)-6-(2-Fluorophenyl)-3-isobutyl-piperazin-2-one (50 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (41 mg, 0.20 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish **101** (69 mg, 86% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.83−7.78 (m, 2 H), 7.43−7.34 (m, 2 H), 7.25−7.09 (m, 4 H), 6.89 (s, 1 H), 5.90 (br s, 1 H), 5.41 (dd, J = 10.0 Hz, 4.8 Hz, 1 H), 5.17−5.02 (m, 2 H), 3.49 (dd, J = 14.4 Hz, 11.2 Hz, 1 H), 2.01−1.86 (m, 2 H), 1.82−1.70 (m, 1 H), 1.11 (d, J = 6.4 Hz, 3 H), 1.01 (d, J = 6.8 Hz, 3 H). MS (ESI): m/z 440.2 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(4-(trifluoromethyl)-phenyl)piperazin-2-one (**102**). A. (3S,6S)-3-Isobutyl-6-(4-trifluoromethyl-phenyl)-piperazin-2-one.* Synthesized from (*S*)-amino-(4-trifluoromethyl-phenyl)-acetic acid (3.0 g, 13.70 mmol) and HCl salt of Leu-OMe (3.5 g, 19.20 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6S)-3-isobutyl-6-(4-trifluoromethyl-phenyl)-piperazin-2-one (217 mg, 5% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2 H), 7.42 (d, J = 8.0 Hz, 2 H), 6.07 (s, 1 H), 4.70−4.71 (m, 1 H), 3.54 (dd, J = 10.4 Hz, 3.6 Hz, 1 H), 3.33 (dd, J = 13.2 Hz, 4.8 Hz, 1 H), 3.02 (dd, J = 13.2 Hz, 4.4 Hz, 1 H), 1.75−1.88 (m, 3 H), 1.58−1.62 (m, 1 H), 0.97 (d, J = 6.4 Hz, 3 H), 0.94 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 301.0 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(4-(trifluoromethyl)-phenyl)piperazin-2-one (**102**).* (3S,6S)-3-Isobutyl-6-(4-trifluoromethyl-phenyl)-piperazin-2-one (50 mg, 0.17 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (35 mg, 0.17 mmol) were coupled according to the procedure described for the preparation of compound **2a** to furnish compound **102** (43 mg, 53% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69−7.67 (m, 2 H), 7.51−7.47 (m, 2 H), 7.32−7.20 (m, 3 H), 7.12−7.08 (m, 1 H), 6.03 (s, 1 H), 5.35−4.80 (m, 1 H), 4.78−4.72 (m, 1 H), 4.69−4.17 (m, 1 H), 3.37−2.81 (m, 1 H), 2.60−2.55 (m, 1 H), 1.98−1.91 (m, 2 H), 1.88−1.82 (m, 1 H) 1.79−1.60 (m, 2 H), 1.40−1.33 (m, 1 H), 1.04−0.91 (m, 6 H). MS (ESI): m/z 445.1 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(thiophen-2-yl)piperazin-2-one (**103**). A. (3S,6R)-3-Isobutyl-6-thiophen-2-yl-piperazin-2-one.* Synthesized from (*R*)-amino-(thiophen-2-yl)-acetic acid (5.0 g, 31.85 mmol) and HCl salt of Leu-OMe (38.6 g, 47.78 mmol) by the method described for the preparation of compound **7** (Scheme 1) to afford (3S,6R)-3-isobutyl-6-thiophen-2-yl-piperazin-2-one (610 mg, 8.0% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26−7.28 (m, 1 H), 6.97−7.01 (m, 2 H), 5.96 (s, 1 H), 4.88 (dd, J = 9.2 Hz, 4.4 Hz, 1 H), 3.49 (dd, J = 10.0 Hz, 3.6 Hz, 1

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H), 3.36−3.41 (m, 1 H), 2.93−2.99 (m, 1 H), 1.88−1.94 (m, 1 H), 1.75−1.84 (m, 1 H), 1.63−1.71 (m, 1 H), 1.54−1.60 (m, 1 H), 0.94−0.99 (m, 6 H). MS (ESI): m/z 239.1 (M + 1).

*B. (3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(thiophen-2-yl)piperazin-2-one (**103**).* (3S,6R)-3-Isobutyl-6-thiophen-2-yl-piperazin-2-one (112 mg, 0.46 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (117.2 mg, 0.57 mmol) were coupled according to the method described for the preparation of compound **2a** to give **103** (100 mg, 50% yield) as an yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.77 (m, 2 H), 7.35−7.32 (m, 1 H), 7.21−7.12 (m, 3 H), 7.04−7.00 (m, 1 H), 6.89 (s, 1 H), 6.45(s, 1 H), 5.35−5.31 (m, 1 H), 5.18−5.15 (m, 1 H), 5.04−4.99 (m, 1 H), 3.51−3.45 (m, 1 H), 1.95−1.71 (m, 3 H), 1.09 (d, J = 6.4 Hz, 3 H), 1.00 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 428.0 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(thiophen-3-yl)piperazin-2-one (**104**). A. (3S,6S)-3-Isobutyl-6-thiophen-3-yl-piperazin-2-one.* Synthesized from (S)-2-amino-2-(thiophen-3-yl)acetic acid (4 g, 25 mmol) and HCl salt of Leu-OMe (3.73 g, 20.6 mmol) by the method described for the preparation of compound 7 (Scheme 1) to afford (3S,6S)-3-isobutyl-6-thiophen-3-yl-piperazin-2-one (1.1 g, 18% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.37 (m, 1 H), 7.17−7.18 (m, 1 H), 6.99−7.00 (m, 1 H), 6.12 (s, 1 H), 4.71−4.74 (m, 1 H), 3.48−3.51 (m, 1 H), 3.23−3.27 (m, 1 H), 3.03−3.07 (m, 1 H), 1.77−1.90 (m, 3 H), 1.52−1.59 (m, 1 H), 0.94−0.99 (m, 6 H). MS (ESI): m/z 239.1 (M + 1).

*B. (3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(thiophen-3-yl)piperazin-2-one (**104**).* (3S,6S)-3-Isobutyl-6-thiophen-3-yl-piperazin-2-one (48 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the method described for the preparation of compound **2a** to furnish **104** (45 mg, 52% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.77 (m, 2 H), 7.42−7.10 (m, 5 H), 6.89 (br s, 1 H), 6.04 (br s, 1 H), 5.44−5.34 (m, 1 H), 5.02−4.89 (m, 2 H), 3.41 (dd, J = 15.2 Hz, 12.0 Hz, 0.5 H), 3.13 (dd, J = 15.2 Hz, 12.0 Hz, 0.5 H), 2.02−1.72 (m, 3 H) and 1.11−0.80 (m, 6 H). MS (ESI): m/z 428.0 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(5-methylthiophen-2-yl)piperazin-2-one (**105**). A. (3S,6R)-3-Isobutyl-6-(5-methyl-thiophen-2-yl)-piperazin-2-one.* Synthesized from (R)-2-amino-(5-methyl-thiophen-2-yl)-acetic acid (9 g, 52.6 mmol) and HCl salt of Leu-OMe (6.37 g, 35.2 mmol) by the method described for the preparation of compound 7 (Scheme 1) to afford (3S,6R)-3-isobutyl-6-(5-methyl-thiophen-2-yl)-piperazin-2-one (0.57 g, 4% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.35 (m, 1 H), 6.77−6.78 (m, 1 H), 6.73−6.74 (m, 1 H), 6.60−6.61 (s, 1 H), 6.02 (s, 1 H), 5.79 (s, 2 H), 4.76−4.79 (m, 1 H), 3.45−3.48 (m, 1 H), 3.33−3.37 (m, 1 H), 2.90−2.95 (m, 1 H), 2.51 (s, 3 H), 2.45 (s, 3 H), 1.78−1.90 (m, 4 H), 1.52−1.58(m, 1 H), 0.94−0.99 (m, 6 H). MS (ESI): m/z 253.15 (M + 1).

*B. (3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(5-methylthiophen-2-yl)-piperazin-2-one (**105**).* (3S,6R)-3-Isobutyl-6-(5-methyl-thiophen-2-yl)-piperazin-2-one (50 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the method described for the preparation of compound **2a** to furnish **105** (40 mg, 46% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81−7.77 (m, 2 H), 7.21−7.16 (m, 2 H), 6.90−6.87 (m, 2 H), 6.66−6.64 (m, 1 H), 6.08 (br s, 1 H), 5.41−5.29 (m, 1 H), 5.06−4.91 (m, 2 H), 3.46 (dd, J = 14.0 Hz, 9.8 Hz, 1 H), 2.47 (s, 3 H), 1.98−1.70 (m, 3 H) and 1.10−0.80 (m, 6 H). MS (ESI): m/z 442.2 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(3-methylthiophen-2-yl)-piperazin-2-one (**106**). A. (3S,6R)-3-Isobutyl-6-(3-methyl-thiophen-2-yl)-piperazin-2-one.* Synthesized from (R)-2-amino-(3-methyl-thiophen-2-yl)acetic acid (9 g, 52.6 mmol) and HCl salt of Leu-OMe (3.6 g, 19.6 mmol) by the method described for the preparation of compound 7 (Scheme 1) to afford (3S,6R)-3-isobutyl-6-(3-methyl-thiophen-2-yl)-piperazin-2-one (268 mg, 2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17−7.18 (m, 1 H), 6.81−6.82 (m, 1 H), 5.86 (s, 1 H), 4.93−4.97 (m, 1 H), 3.35−3.52 (m, 1 H), 3.30−3.34 (m, 1 H), 2.92−2.98 (m, 1 H), 2.22 (s, 3 H), 1.92−1.98 (m, 1 H), 1.68−1.84 (m, 1 H), 1.53−1.59 (m, 1 H), 0.95−1.01 (m, 6 H). MS (ESI): m/z 253.1 (M + 1).

*B. (3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(3-methylthiophen-2-yl)-piperazin-2-one (**106**).* (3S,6R)-3-Isobutyl-6-(3-methyl-thiophen-2-yl)-piperazin-2-one (50 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the method described for the preparation of compound **2a** to furnish product **106** (40 mg, 46% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21−8.07 (m, 2 H), 7.26−7.19 (m, 2 H), 7.13−7.08 (m, 1 H), 6.89−6.79 (m, 1 H), 6.64−6.52 (m, 1 H), 6.08 (br s, 1 H), 5.50−5.14 (m, 1 H), 5.06−4.91 (m, 1 H), 4.80−4.74 (m, 1 H), 4.00−3.46 (m, 1 H), 1.95 (s, 3 H), 1.93−1.71 (m, 3 H), 1.16−0.66 (m, 6 H). MS (ESI): m/z 442.1 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-6-(furan-2-yl)-3-isobutylpiperazin-2-one (**107**).* Compound **12** (40 mg, 0.18 mmol) and 5-(4-fluorophenyl)isoxazole-3-carboxylic acid (55 mg, 0.3 mmol) were coupled according to the procedure described for the preparation of compound **2a** to afford **107** (17 mg, 24% yield) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72−7.69 (m, 2 H), 7.19−7.15 (m, 2 H), 7.11−7.10 (m, 1 H), 6.71−6.60 (m, 1 H), 6.30 (s, 1 H), 6.26−6.22 (m, 2 H), 5.45−5.40 (m, 1 H), 5.01−4.95 (m, 1 H), 4.76−4.64 (m, 1 H), 3.93−3.54 (m, 1 H), 1.90−1.86 (m, 2 H), 1.75−1.69 (m, 1 H) 1.05−0.74 (m, 6 H). MS (ESI): m/z 412.1 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(oxazol-5-yl)piperazin-2-one (**108**).* To a solution **16** (26 mg, 0.078 mmol) in DCM (1.5 mL) at 0 °C was added 4 N HCl in dioxane (0.5 mL) and then stirred at rt for 1 h. The reaction mixture was concentrated in vacuo to give the Boc deprotected compound, which was used for the next step without further purification. To a stirred solution of 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (16 mg, 0.078 mmol) and HATU (22.9 mg, 0.079 mmol) in DMF (1.5 mL), BOC deprotected compound in DMF (0.5 mL) and DIPEA (0.1 mL) was added and stirred for 1 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (0−95% EtOAc in hexanes) to furnish **108** (24.6 mg, 71% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1 H), 7.80−7.77 (m, 2 H), 7.21−7.16 (m, 2 H), 7.02 (br s, 1 H), 6.90 (s, 1 H), 6.86 (s, 0.5 H), 6.83 (s, 0.5 H), 5.52−5.44 (m, 0.5 H), 5.37−5.29 (m, 0.5 H), 5.18−4.88 (m, 2 H), 3.65−3.56 (m, 0.5 H), 3.38−3.29 (m, 0.5 H), 1.93−1.86 (m, 1 H), 1.76−1.71 (m, 1 H), 1.10−0.80 (m, 6 H). MS (ESI): m/z 413.2 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(oxazol-2-yl)piperazin-2-one (**109**).* To a solution of **23** (11.2 mg, 0.0235 mmol) in DCM (2 mL) was added TFA (0.6 mL) and stirred at rt for 5 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (10−60% EtOAC in hexanes) to give **109** (7.3 mg, 75% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.77 (m, 2 H), 7.21−7.17 (m, 2 H), 6.92 (s, 1 H), 6.83 (s, 1 H), 6.69 (d, J = 5.6 Hz, 1 H), 5.92 (d, J = 5.6 Hz, 1 H), 5.42−5.38 (m, 1 H), 5.34−5.28 (m, 1 H), 4.51 (dd, J = 11.0 Hz, 4.6 Hz, 1 H), 3.73 (dd, J = 15.0 Hz, 11.0 Hz, 1 H), 1.91−1.68 (m, 3 H), 1.09 (d, J = 6.4 Hz, 3 H), 1.00 (d, J = 6.4 Hz, 3 H). MS (ESI): m/z 413.0 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(thiazol-2-yl)piperazin-2-one (**110**).* To a solution of the alcohol **90** (0.30 g, 0.80 mmol) in DCM (10 mL) at 0 °C was added Dess−Martin's reagent (441 mg, 1.04 mmol) and water (0.05 mL). The reaction mixture was stirred at rt for 6 h and then *i*-PrOH (0.2 mL), saturated aqueous sodium thiosulfate (5 mL), and saturated aqueous $NaCO_3$ (5 mL) were added. The aqueous layer was extracted with EtOAc (5 mL × 3), the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo, and the crude aldehyde product was used for the next step without further purification. To a solution of crude aldehyde (0.20 g, 0.53 mmol) in EtOH (5 mL) was added HCl salt of 2-aminoethanethiol (60.5 mg, 0.533 mmol) and KOAc (62.8 mg, 0.640 mmol) and stirred at rt for 2 h. The reaction mixture was concentrated in vacuo, and EtOAc (4 mL) and water (3 mL) were added. The aqueous layer was extracted with EtOAc (5 mL × 3). The

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combined organic layers were dried over $MgSO_4$ and concentrated in vacuo, and the crude thiazolidine product was used for the next step without further purification. To a solution of the thiazolidine (60 mg, 0.14 mmol) in dioxane (2 mL) was added activated MnO_2 (100 mg) and stirred at 55 °C for 6 h. The reaction mixture was filtered, and the filtrate was concentrated and purified by column chromatography (0−80% EtOAc in hexanes) to give the product **110** (14 mg, 24% yield). ^{1}H NMR (DMSO-d_6) δ 9.91 (s, 1 H), 8.06 (m, 2 H), 7.53−7.37 (m, 4 H), 4.95 (t, J = 7.6 Hz, 1 H), 4.33−4.27 (m, 2 H), 3.83 (m, 1 H), 1.63 (m, 1 H), 1.52−1.48 (m, 2 H), 0.95 (d, J = 6.4 Hz, 6 H). MS (ESI): m/z 429.2 (M + 1).

*(3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(1H-pyrrol-2-yl)piperazin-2-one (**111**). A. tert-Butyl 2-(1-((Bis(4-methoxyphenyl)methyl)amino)-2-(methoxy-(methyl)-amino)-2-oxo-ethyl)-1H-pyrrole-1-carboxylate (**25**).* To a stirred solution of glyoxylic acid monohydrate (0.92 g, 10.11 mmol) in DCM (70 mL), bis(4-methoxyphenyl)methanamine (2.46 g, 10.11 mmol) and **24** (2.13 g, 10.11 mmol) were added and stirred at rt for 2 min to form a clear solution. The solution was purged with argon (2 min), and the sealed reaction mixture was stirred overnight. Reaction mixture was concentrated in vacuo to yield crude acid product (4.5 g, 95% yield) as pale-yellow foam. Crude acid product (4.5 g, 10 mmol), TBTU (4.87 g, 15 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (1.47 g, 15 mmol) were dissolved in dry MeCN (50 mL), and DIPEA (5.3 mL, 30.22 mmol) was added and stirred at rt for 12 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (5 to 40% EtOAc in hexanes) to afford **25** (5.1 g, 98% yield) as a pale-yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.25 (m, 4 H), 7.15−7.14 (m, 1 H), 6.82−6.76 (m, 4 H), 6.18 (s, 1 H), 6.07 (t, J = 3.2 Hz, 1 H), 5.72 (br s, 1 H), 4.82 (s, 1 H) 3.76 (s, 3 H), 3.74 (s, 3 H) 3.24 (s, 3 H), 3.13 (s, 3 H). 2.58 (br s, 1 H), 1.53 (s, 9 H). MS (ESI): m/z 510.1 (M + 1).

*B. tert-Butyl 2-(1-((Bis(4-methoxyphenyl)methyl)amino)-2-(((S)-1-methoxy-4-methyl-1-oxo-pentan-2-yl)amino)ethyl)-1H-pyrrole-1-carboxylate (**26**).* To a solution of **25** (1.5 g, 2.94 mmol) in dry THF (30 mL) at −78 °C, $LiAlH_4$ (220 mg, 5.89 mmol) was added and stirred under argon for 3 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (5 mL) by dropwise addition. The reaction mixture was partitioned between EtOAc and brine and extracted with EtOAc (20 mL × 2). The organic layers were combined, washed with brine, and dried over $MgSO_4$ and concentrated in vacuo to afford the crude aldehyde (unstable, slowly decomposes at rt), which was used for the next step without further purification. To a mixture of aldehyde compound (1.2 g, 2.66 mmol) and HCl salt of Leu-OMe (0.58 g, 2.66 mmol) in DCM (24 mL), $NaBH(OAc)_3$ (790 mg, 3.73 mmol) was added and stirred at rt under argon for 6 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (25 mL), extracted with EtOAc (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (0−40% EtOAc in hexanes) to afford **26** (1.54 g, 67% yield) as a pale-yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24−7.21 (m, 4 H), 6.80−6.78 (m, 5 H), 6.15−6.14 (m, 2 H), 4.72 (s, 1 H), 4.30 (br s, 1 H), 3.79−3.75 (m, 4 H), 3.64 (s, 3 H) 3.26−3.90 (m, 2 H), 2.68−2.65 (m, 1 H) 2.05 (br s, 1 H), 1.70−1.65 (m, 1 H), 1.48−1.41 (m, 11 H), 0.91−0.85 (m, 6 H). MS (ESI): m/z 580.1 (M + 1).

*C. (3S,6R)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(1H-pyrrol-2-yl)pipera-zin-2-one (**111**).* To a stirred solution of **26** (225 mg, 0.39 mmol), TBTU (161 mg, 0.5 mmol), and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (116 mg, 0.56 mmol) in anhydrous MeCN (6 mL), DIPEA (0.41 mL, 2.33 mmol) was added and stirred at rt for 24 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by column chromatography (0−50% EtOAc in hexanes) to afford crude amide compound (250 mg, 83% yield) as pale-yellow foam. The crude amide compound (250 mg, 0.32 mmol) was dissolved in 70% aqueous AcOH (6 mL) and heated under reflux (80 °C) for 24 h. The reaction mixture was concentrated under vacuo, and the crude product was purified by column chromatography (0−4% MeOH in CH_2Cl_2) to afford **111** (13 mg, 15% yield) and it is trans core isomer (3*S*,6*S*)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(1*H*-pyrrol-2-yl)-piperazin-2-one (12 mg, 14% yield). Compound **111** (brown solid): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23−9.09 (d, J = 53 Hz, 1 H), 7.81−7.76 (m, 2 H), 7.21−7.16 (m, 2 H), 6.94−6.79 (m, 3 H), 6.18−6.16 (m, 1 H), 5.43−5.29 (m, 1 H), 4.97−4.85 (m, 1 H), 3.77 (br s, 1 H), 3.51−3.16 (m, 1 H), 1.90−1.84 (m, 2 H), 1.74−1.60 (m, 1 H), 1.09−0.77 (m, 6 H). MS (ESI): m/z 411.1 (M + 1). (3*S*,6*S*)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(1*H*-pyrrol-2-yl)-piperazin-2-one (brown solid): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05−8.81 (m, 1 H), 7.79−7.65 (m, 2 H), 7.19−7.08 (m, 2 H), 7.08−6.61 (m, 3 H), 6.05−5.91 (m, 1 H), 5.45−5.29 (m, 1 H), 4.83−4.58 (m, 1 H), 3.93−3.48 (m, 2 H), 1.96−1.81 (m, 2 H), 1.78−1.67 (m, 1 H), 1.04−0.75 (m, 6 H). MS (ESI): m/z 411.1 (M + 1).

*(3S,6S)-4-(5-(4-Fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-6-(1H-pyrrol-3-yl)piperazin-2-one (**112**).* Synthesized from (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (2.13 g, 10.11 mmol) by the procedures described for the preparation of **26** (Scheme 5) to furnish **112** (15 mg, 17% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (br s, 1 H), 7.81−7.76 (m, 2 H), 7.20−7.16 (m, 2 H), 6.86−6.79 (m, 3 H), 6.24−6.21 (m, 1 H), 5.97 (br s, 1 H), 5.35−5.32 (m, 1 H), 4.89−4.75 (m, 2 H), 3.45−3.11 (m, 1 H), 1.99−1.71 (m, 3 H), 1.10−0.0.78 (m, 6 H). MS (ESI): m/z 409.07 (M − 1).

*(3S,6S)-6-(2-Chlorothiophen-3-yl)-4-(5-(4-fluorophenyl)isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**113**). A. (3S,6S)-6-(2-Chlorothiophen-3-yl)-3-isobutylpiperazin-2-one and (3S,6R)-6-(2-Chlorothiophen-3-yl)-3-isobutylpiperazin-2-one.* Synthesized from 2-chlorothiophen-3-ylboronic acid (4.15 g, 56.12 mmol) and (*S*)-methyl 2-amino-4-methylpentanoate hydrochloride (3.15 g, 17.39 mmol) by the method described for the preparation of compound **12** (Scheme 2) to afford (3*S*,6*S*)-6-(2-chlorothiophen-3-yl)-3-isobutylpiperazin-2-one (237 mg, 1.6% yield) and (3*S*,6*R*)-6-(2-chlorothiophen-3-yl)-3-isobutylpiperazin-2-one (385 mg, 2.6% yield). (3*S*,6*S*)-6-(2-Chlorothiophen-3-yl)-3-isobutylpiperazin-2-one (white solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.13 (d, J = 6.0 Hz, 1 H), 6.99 (d, J = 6.0 Hz, 1 H), 5.91 (s, 1 H), 4.78−4.75 (m, 1 H), 3.82−3.74 (m, 1 H), 3.54−3.50 (m, 1 H), 3.26−3.21 (m, 1 H), 3.10−3.06 (m, 1 H), 1.88−1.80 (m, 2 H), 1.64−1.54 (m, 1 H), 0.99−0.94 (m, 6 H). MS (ESI): m/z 272.83 (M + 1). (3*S*,6*R*)-6-(2-Chlorothiophen-3-yl)-3-isobutylpiperazin-2-one (white solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (d, J = 4.8 Hz, 1 H), 6.96 (d, J = 4.8 Hz, 1 H), 5.82 (s, 1 H), 4.86−4.82 (m, 1 H), 3.77−3.75 (m, 1 H), 3.52−3.48 (m, 1 H), 3.34−3.30 (m, 1 H), 2.89−2.84 (m, 1 H), 1.99−1.92 (m, 1 H), 1.86−1.78 (m, 1 H), 1.62−1.53 (m, 1 H), 1.00−0.94 (m, 6 H). MS (ESI): m/z 272.84 (M + 1).

*B. (3S,6S)-6-(2-Chlorothiophen-3-yl)-4-(5-(4-fluorophenyl)-isoxazole-3-carbonyl)-3-isobutyl-piperazin-2-one (**113**).* (3*S*,6*S*)-6-(2-Chlorothiophen-3-yl)-3-isobutylpiperazin-2-one (55 mg, 0.20 mmol) and 5-(4-fluorophenyl)-isoxazole-3-carboxylic acid (45 mg, 0.22 mmol) were coupled according to the method described for the preparation of compound **2a** to furnish **113** (42 mg, 45% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85−7.77 (m, 2 H), 7.21−7.16 (m, 3 H), 6.99−6.95 (m, 1 H), 6.88 (s, 1 H), 6.03 (br s, 1 H), 5.46 and 5.36 (dd, J = 10.0 Hz, 4.4 Hz, 1 H), 5.07−4.89 (m, 2 H), 3.43 and 3.16 (dd, J = 14.4 Hz, 11.2 Hz, 1 H), 1.99−1.70 (m, 3 H) and 1.10−0.81 (m, 6 H). MS (ESI): m/z 463.92 (M + 2).

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Notes

The authors declare the following competing financial interest(s): All of the authors except W.S. and S.N. were employees of Pharmasset where this work was completed. Gilead Sciences acquired Pharmasset in January 2012.
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■ ABBREVIATIONS USED

AcOH, acetic acid; BuOH, butanol; Boc, *tert*-butyloxycarbonyl; CyP, cytochrome P450; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMF, dimethylformamide; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; Fmoc, 9-fluorenylmethyloxycarbonyl; HATU, (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate); HLM, human liver microsomal; HOBt, *N*-hydroxybenzotriazole; Leu, L-leucine; $LiAlH_4$, lithium aluminum hydride; MeCN, acetonitrile; MeOH, methanol; Phe, phenylalanine; *i*-PrOH, 2-propanol; SFC, supercritical fluid chromatography; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; WT, wild type

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(17) HLM of compound **95** is 54 min (half-life) and for the compounds, **74** and **87** is 17 and 29 min, respectively.