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Optimization of the first small-molecule relaxin/insulin-like family peptide receptor (RXFP1) agonists: Activation results in an antifibrotic gene expression profile

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Abstract

A dose responsive quantitative high throughput screen (qHTS) of >350,000 compounds against a human relaxin/insulin-like family peptide receptor (RXFP1) transfected HEK293 cell line identified 2-acetamido-N-phenylbenzamides **1** and **3** with modest agonist activity. An extensive structure-activity study has been undertaken to optimize the potency, efficacy, and physical properties of the series, resulting in the identification of compound **65** (ML-290), which has excellent in vivo PK properties with high levels of systemic exposure. This series, exemplified by **65**, has produced first-in-class small-molecule agonists of RXFP1 and is a potent activator of antifibrotic genes.

Graphical Abstract

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SUPPORTING INFORMATION

Mouse PK data, crystal structure of compound **65** and X-ray data, carbon, proton and fluorine NMR spectra of selected compounds including compound **65**, maximum tolerated dose study for compound **65**

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Keywords

Relaxin; G-protein coupled receptor; antifibrotic; 2-acetamido-N-phenylbenzamide

INTRODUCTION

The small-peptide hormone relaxin was discovered in 1926 and is primarily associated with pregnancy, due to its effects to relax pubic ligaments and soften the cervix to facilitate parturition.^{1,2} Since then it has been shown that blood concentrations of relaxin rise during the first trimester of pregnancy, stimulating cardiovascular and renal adjustments to accommodate the increased nutritional demands of the growing fetus and the elevated requirements for renal clearance of metabolic waste.³ Relaxin production increases cardiac output, arterial compliance, renal blood flow and a decrease in systemic vascular resistance during pregnancy.⁴⁻⁶ Both clinical and non-clinical studies using this hormone reinforce these cardiovascular effects in both males and females, which suggest the pharmacological utility of relaxin as a modulator of cardiovascular and renal functions in humans.^{7,8}

The target of relaxin is a group of G-protein coupled receptors, relaxin/insulin-like family peptide receptor (RXFP).^{9,10} Some of the physiological effects of relaxin are mediated by its interaction with RXFP1, which modulates several signal transduction pathways.^{11,12} Relaxin activation of RXFP1 up-regulates the endothelin system which leads to vasodilation. Activation also promotes extracellular matrix remodeling through regulation of collagen deposition, proliferation, cell invasiveness, and perhaps most importantly, overall tissue homeostasis. It also moderates inflammation by reducing levels of inflammatory cytokines such as TNF- α and TGF- β , as well as the induction of angiogenesis via the activation of transcription of vascular endothelial growth factor (VEGF).¹³

Understanding the biological effects of RXFP1 activation by relaxin has led to the evaluation of relaxin as a pharmacologic agent for the treatment of patients with acute heart failure (AHF), preeclampsia, and hypertensive disease.^{6,14} A recent phase 3 clinical trial utilizing recombinant human relaxin-2 (serelaxin) met its primary endpoint of improving dyspnea through the fifth day in patients admitted for acute heart failure.^{15,16} We have previously reported the identification and evaluation of a series of small-molecule human relaxin agonists.^{17,18} Optimized compounds from this series are potent and highly selective activators of RXFP1 with similar efficacy to the natural hormone in functional assays.^{19,20} Using quantitative high-throughput screening, over 350,000 compounds from our Molecular Libraries Small Molecule Repository (MLSMR) were screened in an effort to identify small-

molecule agonists of RXFP1.²¹ This small molecule library was created by the NIH to combine chemical resources from both academic and private institutions to increase chemical space diversity of compounds for a large variety of screening campaigns. In this disclosure we present the syntheses, SAR studies, and compound optimization which led to the identification of preclinical candidate **65**.

RESULTS AND DISCUSSION

The ability of these compounds to increase cyclic adenosine monophosphate (cAMP) levels in a human RXFP1 transfected HEK293 cell line was measured, and two compounds (**1** and **3**), both having a unifying molecular motif of 2-acetamido-N-phenylbenzamide were discovered (Figure 1).¹⁷

The activity is reported through two parameters: EC₅₀ (concentration necessary to reach 50% of maximum cAMP signal) and maximum response (efficacy indicated as the maximum observed increase in cAMP response), which were used to perform SAR analysis. Efficacies were normalized to forskolin, which had an EC₅₀ of 47 nM and a defined maximum response of 100%.²²

Initial measurements of aqueous kinetic solubility of **1** and **3** (1.6 µg/mL, 3.7 µM and 0.9 µg/mL, 2.2 µM respectively) show poor solubility for these compounds. However, in assessing the stability of compounds **1** and **3** in mouse liver microsomes, compound **3** demonstrated very good stability after 60 minutes (70% parent remaining) versus **1** (2% parent remaining), so these data and the inherent problems associated with furans in mammalian metabolism led us to focus on the optimization of the cyclohexyl series based on compound **3**.²³ The general synthetic scheme to these is represented in Scheme 1.

The first series of compounds **3–22** (Table 1) were prepared in a straightforward manner from **2** (see Experimental Standard Procedures), with the first amide formation performed by reaction with cyclohexylcarbonyl chloride followed by subsequent AlMe₃ mediated second amide formation with the appropriate aniline or amine. This direct amidation using an aniline and an ester proved to be robust and broad in scope. Compounds **18–20** were prepared from bromo-compound **13** using standard Suzuki conditions.

Table 1 discloses the initial results of our SAR studies. It is clear upon viewing the data that the 3-position of the pendant R-phenyl group provides the best agonism activity. Most of the compounds have single digit micromolar activity, except for **7** (37.4 µM), **18** and **20** (both inactive). Interestingly, the 3-trifluoromethylbiphenyl derivative **19** is active (EC₅₀ = 2.65 mM), leading us to believe that the trifluoromethyl moiety in this part of the molecule could be important by promoting the activation of RXFP1. This is further supported by compounds **16** and **17**, the latter trifluoromethylsulfonyl compound being the most potent in this set, having an EC₅₀ of 1.06 µM with an 87% of maximal activity. It is also worth noting that tert-butyl compound **9** maintains activity, and that less polar substituents may be preferred over more polar ones such as the 3-methoxy derivative **14**.

The unsubstituted phenyl compound **5** is minimally active as is 4-trifluoromethylsulfonyl phenyl compound **4**. The inactivity of compound **6** might indicate that substituents at the

ortho position impair agonism. Thus, either no substitution or 2- and 4-phenyl substitutions are deleterious to RXFP1 activation, with the 3-substituted phenyl compounds generally being more active. Drawing parallels to compounds **1** and **3**, we combined the cyclohexyl and furanyl chemotypes, exemplified by compound **22**, which retained some activity but did not encourage further investigation.

We then explored the replacement of the cyclohexyl group with various saturated alkyl rings, alkyl groups and differentially substituted aryl rings, while holding the 3-trifluoromethylphenyl eastern hemisphere region of the molecule constant (Scheme 2). Readily available BOC-protected anthranilic acid **23** was coupled to 3-trifluoromethylaniline and deprotected to afford **24a** (which is also commercially available), and then converted to the amides **25–44** by reacting with the appropriate acid chloride, or coupled with the appropriate carboxylic acid in the case of the cycloalkyl isosteres **27–30** (Table 2). The ethers **43–44** required an additional synthetic step using CuI catalyzed coupling of either 2-methoxyethan-1-ol or dimethylaminoethan-1-ol with iodo-compound **24b**. It is evident from the data in Table 2 that the ring size of R is important. The cycloalkyl analogues **28–30** maintain good potency while the smaller cyclopropyl adduct **27** experiences a significant (> 4-fold) reduction in activity from the parent compound **3**.

Compounds **27–30** clearly demonstrated a correlation between activity and ring size (**3** < **4** < **5** < **6** > **7**, EC₅₀ = 8.38, 2.97, 2.36, 1.88, and 2.11 μM, respectively), with the 6-membered ring being the optimal size for activity. The branched and linear alkanes **25** and **26** are tolerated but not ideal for agonism activity. Again, as in compound **8**, substitution at the 4-position substantially reduces the activity of compound **33** relative to 2-substitution (compound **36**). The phenyl example **34** retains good activity while the 3-pyridyl **32** is an unfavorable substitution. A clear SAR profile begins to manifest as seen in the activity of micromolar to submicromolar 2-alkyl ethers **36**, **39–41** and **43**, with the 2-ethoxy compound **43** having the best activity at EC₅₀ = 265 nM. These data were very encouraging as we demonstrated the additive effects of the 3-trifluorophenyl eastern hemisphere and the 2-alkoxyphenyl western hemisphere.

Imparting more solubility to this series of compounds would be desirable. Our next objective was to assess whether the central phenyl ring could tolerate derivatization in the form of pyridine analogues and retain, if not improve, potency and especially the solubility.

The four possible central core pyridine isomers were synthesized using readily available and inexpensive aminopyridines **45–48** (Scheme 3). From our SAR data the 3-sulfonylphenyl was chosen as a constant for the eastern region and 2-ethoxyphenyl was chosen for the western hemisphere. The resultant aminopyridines **49–52** were then assayed and the results are shown in Table 3. There is a substantial loss in activity of all but 3-pyridyl compound **51**, which exhibits approximately a 3-fold reduction in activity from compound **40**.

The modification of the central benzamide linker was evaluated next, where the western region sulfonamide **53**, N-methylated amides **54** and **55**, amine **57**, thiophene surrogate **59**, and 1,3-benzamide **61** were prepared using the standard chemistry detailed in this manuscript (Scheme 4), and then evaluated in the cAMP assay (Table 4). As our binding

model predicts (vide infra), the 1,3 benzamide linked compound **61** was inactive, as were the N-methylated version **54** and sulfonamide **53**. Methylated amide **55** retained weak activity, and surprisingly, so did the ethylmethoxyaniline **57**. The thiophene isostere **59** had the best activity ($EC_{50} = 2.35 \mu M$).

A clear picture had emerged of the optimal SAR requirements for the 2-acetamido-N-phenylbenzamide series to effect RXFP1 activation. From the SAR data we determined that the 2-alkoxyphenyl western hemisphere and 3-trifluoromethylsulfonyl eastern hemisphere groups offered the best opportunity for discovering the most potent compounds, and gratifyingly, they did. Compounds **62–67** were synthesized according to the procedure in Scheme 1. The assay results of these compounds are shown in Table 5. The larger (**65–67**) or more hydrophobic alkyl groups (such as **64**) confer the best activity, e.g., **62** or **63** ($EC_{50} = 0.188 \mu M$) as compared to compound **67** ($EC_{50} = 0.047 \mu M$). That the 3-trifluoromethylsulfonyl is a superior pharmacophore for activity to 3-trifluoromethyl on the eastern region phenyl is confirmed comparing **36** to **62** ($EC_{50} = 0.334 \mu M$ versus $EC_{50} = 0.188 \mu M$), **40** to **63** ($EC_{50} = 0.265 \mu M$ versus $EC_{50} = 0.188 \mu M$), and especially the obvious contrast between **41** and **65** ($EC_{50} = 0.471 \mu M$ versus $EC_{50} = 0.094 \mu M$).

We have previously reported the structural elucidation and insight into the activation of the activation of human relaxin family peptide receptor by **65**.¹⁸ Exhaustive site-directed mutagenesis in conjunction with computational modeling analysis allowed us to develop a predictive model of our small molecule agonists' mode of binding. (Figure 2). Figure 2 shows the binding model of compound **65** (ML-290) to the RXFP1 receptor. The intramolecular hydrogen bonding interaction of the amide carbonyl to the threonine 660 is essential for favorable conformational bias within the active site. The inactivity of compound **54** and to a lesser extent the reduced activity of **55** (vide supra) clearly demonstrate that the replacement of the N-amido hydrogen with a methyl group results in the disruption of the required active site conformation for binding to RXFP1, as well as altering the critical T660 H-bonding interaction to one of the sulfonyl oxygens.

The microsome stability, PBS solubility, and toxicity in both rat and mice were investigated for compounds **62–67**. Rat and mouse microsome stability are excellent for these compounds (Table 5). An improvement in PBS solubility was also observed for compounds **62** and **64–67**. It can be argued that the sulfonyl group imparts greater solubility to these compounds as compared to the more lipophilic trifluoromethyl moiety.

As we previously reported, **65** was chosen as our lead compound for further PK analysis because of favorable profiles which included: (1) selectivity against HEK293 cells transfected with the RXFP2 receptor (2) decreased adenosine triphosphate (ATP) cytotoxicity, and (3) especially its ability to activate the transcription of the known relaxin target gene VEGF in human monocyte leukemia cells (THP1).^{9, 17}

The pharmacokinetics of IV and PO delivery of **65** in mice was analyzed (Table 6). Compound **65** has an excellent half-life in plasma by both routes of administration and in heart by IV dosing, with the exposure in heart being about six times greater than plasma by IV administration. This is important as organ fibrosis (heart, liver, lung, etc) is an important

indication of therapeutic intervention. While the plasma clearance is high, the high volume of distribution probably imparts the half-life observed. Importantly, no toxicity or abnormal behavior were detected. We have recently performed a maximum tolerated PO administration) dose study on C57/Bl6 mice (5 day with 5 day post-dosing observation), with no mortality observed at 90, 300, and 3000 mg/kg (see supplementary material). No obvious abnormalities were noted post-necropsy in any of the in vivo mammalian studies.

Previously described antifibrotic effects of relaxin in various organs were associated with changes in transcriptional activity of key genes involved in extracellular matrix production and turnover.²⁴ In fibrotic liver, hepatic stellate cells (HSCs) become activated and differentiate into profibrogenic myofibroblast-like cells. Importantly, RXFP1 gene expression is significantly upregulated in activated HSCs.²⁵ It has been shown that relaxin treatment of activated HSCs leads to a significant decrease in profibrotic genes such as smooth muscle actin (ACTA2), transforming growth factor beta-1 (TGFB1), connective tissue growth factor (CTGF), and several collagens, but at the same time stimulates expression of matrix metalloproteinase 1 (MMP1) and peroxisome proliferator-activated receptor gamma (PPARG).^{26,27}

Therefore, we have tested the effect of ML290 on primary human HSC gene expression (Figure 3). Cells were cultured on plastic, which induces and maintains activation of HSCs.²⁸ Upon treatment with 5 μ M of ML290, we observed a clear anti-fibrotic, relaxin-like gene expression pattern at 24 and 72 hr. Similar results have been recently reported by another group in the HSC LX-2 cell line using ML-290.²⁹

CONCLUSION

Optimization of two lead compounds discovered by an extensive screening campaign of over 350,000 compounds at the National Center for the Advancement of Translational Science resulted in a series of potent, stable, selective and well-tolerated compounds which exhibit excellent systemic exposure in plasma and especially heart.

To summarize the SAR of the compounds presented here, the core aromatic ring must be ortho substituted to maintain activity. Replacing the central phenyl ring with either a thiophene or pyridine is tolerated although a decrease in activity is observed. The western hemisphere amide is critical for the activity.

For the western region, phenyl, pyridine and cycloalkanes of varying ring size are acceptable, but a six membered ring is optimal. An electron-donating substituent on the aromatic ring is tolerated at both the *ortho*- and *meta*-positions, with *ortho*-alkoxy having the best activity. At the eastern phenyl position, a *meta*-substitution is optimal and the trifluoromethylsulfonyl is best as a critical hydrogen bond interaction is formed between the sulfonyl oxygens and threonine 660.

Thus, the first small-molecule preclinical candidate agonist for the relaxin hormone receptor has been optimized through a medicinal chemistry approach and the resulting SAR is supported by a later-stage binding model obtained by iterative site-directed mutagenesis.

EXPERIMENTAL SECTION

Biology

Materials and Methods—Primary human hepatic stellate cells (HSCs) were grown on plastic in hepatic stellate growth media provided by the manufacturer (Zen-Bio Inc., Research Triangle Park, NC). Cells were seeded overnight in 35 mm poly-L-lysine-coated dishes (Corning, Durham, NC) at a density of 5×10^4 cells per dish and treated in triplicates with either 5 μ M of ML290 in DMSO (ML290 treatment) or DMSO (control) for 24 or 72 hr. Total RNA was extracted from cells samples with RNeasy kit (Qiagen, Germantown, MD). Quality and concentration of RNA was analyzed with a Nanovue (GE Healthcare Biosciences, Pittsburgh, PA). cDNA was synthesized using a Verso cDNA kit (Thermo Scientific, Fair Lawn, NJ). Quantitative PCR (qPCR) was performed using GoTaq qPCR Master Mix (Promega, Madison, WI), and gene specific primers in a RealPlex² Mastercycler (Eppendorf, Westbury, NY) according to the manufacturer's instructions. Values were normalized to *GAPDH* gene expression. The relative fold change in mRNA level was calculated by the comparative C_t (2^{-C_t}) method. Results were analyzed using GraphPad Prism software (GraphPad Software, La Jolla, CA) for statistical significance using Student's *t* test.

Measurements of cAMP concentration—cAMP assay was performed using HTRF cAMP HiRange kit (CisBio, Bedford, MA, USA). The THP1 and HEK293 cells (ATCC, Manassas, VA, USA) stably or transiently transfected with human RXFP1, RXFP2 or AVPR1B receptor were stimulated with relaxin, the compounds or forskolin for 30 min at 37 °C, 5% CO₂, after which, 8 ml per well of each HTRF detection reagent (diluted according to assay kit directions in HTRF lysis buffer) was added.^{30,31} The plates were incubated for 30 min at room temperature, and the signal was read on a ViewLux (PerkinElmer, Waltham, MA, USA) or a FLUOstar Omega (BMG Labtech, Cary, NC, USA) plate readers. Nonlinear regressions to the Hill equation were performed using Prism software (GraphPad Software, San Diego, CA, USA).

Modulation of HSC gene expression—Primary human hepatic stellate cells (HSCs) were grown on plastic in hepatic stellate growth media provided by the manufacturer (Zen-Bio Inc., Research Triangle Park, NC). Cells were seeded overnight in 35 mm poly-L-lysine-coated dishes (Corning, Durham, NC) at a density of 5×10^4 cells per dish and treated in triplicates with either 5 μ M of ML290 in DMSO (ML290 treatment) or DMSO (control) for 24 or 72 hr. Total RNA was extracted from cells samples with RNeasy kit (Qiagen, Germantown, MD). Quality and concentration of RNA was analyzed with a Nanovue (GE Healthcare Biosciences, Pittsburgh, PA). cDNA was synthesized using a Verso cDNA kit (Thermo Scientific, Fair Lawn, NJ). Quantitative PCR (qPCR) was performed using GoTaq qPCR Master Mix (Promega, Madison, WI), and gene specific primers in a RealPlex² Mastercycler (Eppendorf, Westbury, NY) according to the manufacturer's instructions. Values were normalized to *GAPDH* gene expression. The relative fold change in mRNA level was calculated by the comparative C_t (2^{-C_t}) method. Results were analyzed using GraphPad Prism software (GraphPad Software, La Jolla, CA) for statistical significance using Student's *t* test. The sequences of the primers are shown in Table 7.

Chemistry

General Methods—All air or moisture sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents such as dichloromethane, N, N-dimethylformamide (DMF), acetonitrile, methanol and tetrahydrofuran were purchased from Sigma-Aldrich (St. Louis, Mo.). Preparative purification was performed on a Waters semi-preparative HPLC system (Waters Corp., Milford, Mass.). The column used was a Phenomenex Luna C18 (5 micron, 30 × 75 mm; Phenomenex, Inc., Torrance, Calif.) at a flow rate of 45.0 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection at 220 nm. Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, Calif.). Method 1 (t_1): A 7-minute gradient of 4% to 100% acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8-minute run time at a flow rate of 1.0 mL/min. Method 2 (t_2): A 3-minute gradient of 4% to 100% acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5-minute run time at a flow rate of 1.0 mL/min. A Phenomenex Luna C18 column (3 micron, 3 × 75 mm) was used at a temperature of 50 °C. Purity determination was performed using an Agilent diode array detector for both Method 1 and Method 2. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in positive detection mode. ^1H NMR spectra were recorded on Varian 400 MHz spectrometers (Agilent Technologies, Santa Clara, Calif.). Chemical shifts are reported in ppm with undeuterated solvent ($\text{DMSO}-d_6$ at 2.49 ppm) as internal standard for $\text{DMSO}-d_6$ solutions. All of the analogs tested in the biological assays have a purity of greater than 95% based on both analytical methods. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight (TOP) LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with Agilent Masshunter software (Version B. 02).

General Protocol A: A solution of methyl or ethyl benzoate (0.191 mmol) and amine (0.383 mmol) in toluene (2.00 mL) was treated at room temperature with AlMe_3 (0.192 mL, 2.0 M in toluene, 0.384 mmol). The reaction mixture was stirred overnight at 100° C and then quenched with 100 μL of water. The mixture was concentrated, redissolved in 2.00 mL of DMSO, filtered and purified via C18 reverse phase HPLC to give the final product.

General Protocol B: A solution of carboxylic acid (0.178 mmol) in DMF (2.00 mL) was treated at room temperature with 2-amino-N-(3-(trifluoromethyl)phenyl)benzamide (25.0 mg, 0.089 mmol) followed by EDC (17.1 mg 0.089 mmol) and DMAP (10.9 mg, 0.089 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was purified via C18 reverse phase HPLC to give the final product.

General Protocol C: A solution of 2-amino-N-(3-(trifluoromethyl)phenyl)benzamide (50.0 mg, 0.178 mmol) in dichloromethane (2.00 mL) and TEA (0.075 mL, 0.535 mmol) was treated at room temperature with carbonyl chloride (0.357 mmol). The reaction mixture was

stirred at room temperature for 2 h. The mixture was concentrated, re-dissolved in 2.00 mL of DMSO, filtered and purified via C18 reverse phase HPLC to give the final product.

General Protocol D: A solution of carboxylic acid (0.357 mmol) in DMF (2.00 mL) was added DIPEA (0.093 mL, 0.535 mmol) and HATU (136 mg, 0.357 mmol). The reaction mixture was stirred at room temperature for 5 min, followed by 2-amino-N-(3-(trifluoromethyl)-phenyl)benzamide (50.0 mg, 0.178 mmol). The reaction mixture was stirred overnight at room temperature, filtered and purified via C18 reverse phase HPLC to give the final product.

General Protocol E: A mixture of 2-bromo-N-(3-(trifluoromethyl)phenyl)benzamide (50.0 mg, 0.145 mmol, 1.0 equiv.) or N-(3-bromophenyl)-2-(cyclohexanecarboxamido)benzamide (100 mg, 0.249 mmol, 1.0 equiv.), boronic acid or pinacol ester (2.0 equiv.) and Pd(PPh₃)₄ (0.05 eq) in DMF (1.50 mL) and 2.0 N Na₂CO₃ (0.50 mL) aqueous solution was heated in μ W at 100° C for 30 min to 1 h. The reaction was cooled to room temperature, and a small portion of Si-THIOL was added to remove palladium. The reaction mixture was filtered and purified via C18 reverse phase HPLC to give the final product.

General Protocol F: 2-Iodo-N-(3-(trifluoromethyl) phenyl)benzamide (100 mg, 0.256 mmol), amine (0.767 mmol), and CuCl (7.59 mg, 0.077 mmol) in DMF (1.00 mL) was stirred at room temperature for 15–30 min. The reaction was treated with a small portion of Si-THIOL to remove palladium, filtered and purified via C18 reverse phase HPLC to give the final product.

General Protocol G: A solution of thio-compound (0.255 mmol) in dichloromethane (3.00 mL) was treated at room temperature with MCPBA (220 mg, 1.27 mmol). The reaction mixture was stirred over-night at room temperature. 10% Aqueous NaHSO₃ solution was added to quench excess MCPBA and the mixture was stirred at room temperature for 15 min. The reaction mixture was partitioned between dichloromethane and water. The organic layer was separated, dried (Na₂SO₄), filtered, concentrated, and purified via C18 reverse phase HPLC to give the final product.

General Protocol H: A tube was charged with CuI (0.1 equiv.), 1,10-phenanthroline (0.2 equiv.), Cs₂CO₃ (2.0 equiv.), iodo substrate (1.0 equiv.) and alcohol (2.0 equiv.) in toluene (2.00 mL) under N₂. The tube was sealed and the reaction mixture was stirred at 110 ° C for 24 h. The resulting mixture was cooled to room temperature and treated with a small portion of Si-THIOL to remove copper. The mixture was concentrated, dissolved in 2.00 mL of DMSO, filtered and purified via C18 reverse phase HPLC to give the final product.

3-(Cyclohexanecarboxamido)-N-(3-(trifluoromethyl)phenyl)-benzamide (3)—
The title compound was prepared according to general protocol A. Yield: 35%; LC-MS Retention Time: t₁ (Method 1) = 6.516 min; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.53 (s, 1H), 10.00 (s, 1H), 8.21 (t, *J* = 2.1 Hz, 1H), 8.14 (t, *J* = 2.0 Hz, 1H), 8.03 (ddd, *J* = 8.2, 1.2, 1.0 Hz, 1H), 7.81 (ddd, *J* = 8.1, 2.2, 1.1 Hz, 1H), 7.55–7.64 (m, 2H), 7.37–7.49 (m, 2H), 2.24–2.41 (m, 1H), 1.69–1.92 (m, 4H), 1.57–1.69 (m, 1H), 1.08–1.50 (m, 5H); ¹⁹F NMR (376 MHz, DMSO- *d*₆) δ ppm –61.29 (s, 3F); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 174.40, 167.65,

140.12, 138.19, 132.20, 130.29, 129.90, 129.58, 129.19, 125.92, 125.07, 124.52, 123.62, 122.48, 120.58, 120.54, 117.14, 117.10, 45.47, 29.45, 25.82, 25.50; HRMS (ESI) m/z (M+H) calcd. for $C_{21}H_{22}F_3N_2O_2$, 391.1628; found 391.1637.

2-(Cyclohexanecarboxamido)-N-(4-(trifluoromethyl)phenyl)benzamide (4)—The

title compound was prepared according to general protocol A. Yield: 62%; LC-MS Retention Time: t_1 (Method 1) = 6.943 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.65 (s, 1H), 10.29 (s, 1H), 8.09 (dd, J = 8.4, 1.0 Hz, 1H), 7.91 (d, J = 8.6 Hz, 2H), 7.74 (dd, J = 7.8, 1.4 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.51 (ddd, J = 8.4, 7.3, 1.6 Hz, 1H), 7.21 (td, J = 7.6, 1.2 Hz, 1H), 2.18–2.35 (m, 1H), 1.80 (dd, J = 12.7, 2.9 Hz, 2H), 1.68 (dt, J = 12.2, 3.4 Hz, 2H), 1.59 (d, J = 11.9 Hz, 1H), 1.01–1.43 (m, 5H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –60.26 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 173.94, 167.32, 142.64, 141.12, 137.79, 134.12, 131.86, 131.13, 128.90, 125.96, 123.64, 123.51, 122.03, 120.34, 45.10, 29.02, 25.41, 25.13. HRMS (ESI) m/z (M+H) calcd. For $C_{21}H_{22}F_3N_2O_2$, 391.1628; found 391.1628.

2-(Cyclohexanecarboxamido)-N-phenylbenzamide (5)—The title compound was prepared according to general protocol A. Yield: 68% LC-MS Retention Time: t_1 (Method 1) 6.326 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.54 (s, 1H), 10.37 (s, 1H), 8.23 (dd, J = 8.2, 1.0 Hz, 1H), 7.77 (dd, J = 7.9, 1.5 Hz, 1H), 7.63–7.71 (m, 2H), 7.49 (ddd, J = 8.4, 7.2, 1.6 Hz, 1H), 7.28–7.41 (m, 2H), 7.19 (td, J = 7.6, 1.3 Hz, 1H), 7.07–7.14 (m, 1H), 2.17–2.34 (m, 1H), 1.76–1.88 (m, 2H), 1.69 (ddd, J = 12.5, 3.4, 3.2 Hz, 2H), 1.59 (d, J = 12.1 Hz, 1H), 1.03–1.43 (m, 5H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.98, 170.13, 141.77, 141.35, 134.88, 131.88, 131.73, 127.14, 126.60, 125.99, 124.48, 123.91, 48.42, 43.00, 42.86, 42.72, 42.58, 42.44, 42.30, 42.16, 32.14, 28.48, 28.20; HRMS (ESI) m/z (M+H) calcd. For $C_{20}H_{23}N_2O_2$, 323.1754; found 323.1758.

2-(Cyclohexanecarboxamido)-N-o-tolylbenzamide (6)—The title compound was prepared according to general protocol A. Yield: 38%; LC-MS Retention Time: t_1 (Method 1) = 6.401 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 11.00 (s, 1H), 10.09 (s, 1H), 8.38 (dd, J = 8.4, 1.2 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.51 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 7.25–7.35 (m, 2H), 7.10–7.25 (m, 3H), 2.21 (s, 3H), 2.16–2.28 (m, 1H), 1.77–1.91 (m, 2H), 1.69 (dt, J = 12.4, 3.5 Hz, 2H), 1.53–1.65 (m, 1H), 0.99–1.44 (m, 5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.86, 167.36, 139.15, 135.79, 134.00, 132.11, 130.36, 128.60, 126.87, 126.43, 126.11, 122.63, 121.31, 120.78, 45.61, 29.06, 25.36, 25.06, 17.81; HRMS (ESI) m/z (M+H) calcd. for $C_{21}H_{25}N_2O_2$, 337.1911; found 337.1912.

2-(Cyclohexanecarboxamido)-N-m-tolylbenzamide (7)—The title compound was prepared according to general protocol A. Yield: 55%; LC-MS Retention Time: t_1 (Method 1) = 6.632 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.54 (s, 1H), 10.30 (s, 1H), 8.24 (dd, J = 8.3, 1.1 Hz, 1H), 7.76 (dd, J = 7.8, 1.4 Hz, 1H), 7.38–7.54 (m, 3H), 7.10–7.31 (m, 2H), 6.93 (dddd, J = 7.5, 1.5, 1.1, 0.8 Hz, 1H), 2.29 (s, 3H), 2.19–2.35 (m, 1H), 1.82 (dd, J = 12.8, 2.6 Hz, 2H), 1.69 (dt, J = 12.4, 3.4 Hz, 2H), 1.59 (d, J = 12.3 Hz, 1H), 1.04–1.42 (m, 5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.88, 167.00, 138.53, 138.30, 137.75, 131.75, 128.74,

128.45, 124.76, 123.40, 122.83, 121.39, 121.32, 118.09, 45.33, 29.05, 25.38, 25.07, 21.19; HRMS (ESI) m/z (M+H) calcd. for $C_{21}H_{25}N_2O_2$, 337.1911; found 337.1908.

2-(Cyclohexanecarboxamido)-N-p-tolylbenzamide (8)—The title compound was prepared according to general protocol A. Yield: 49%; LC-MS Retention Time: t_1 (Method 1) = 6.636 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.61 (s, 1H), 10.30 (s, 1H), 8.26 (dd, J = 8.2, 0.8 Hz, 1H), 7.77 (dd, J = 7.8, 1.4 Hz, 1H), 7.52–7.59 (m, 2H), 7.48 (ddd, J = 8.4, 7.2, 1.6 Hz, 1H), 7.18 (dd, J = 7.8, 1.2 Hz, 1H), 7.11–7.17 (m, 2H), 2.26 (s, 3H), 2.16–2.36 (m, 1H), 1.82 (dd, J = 13.0, 2.1 Hz, 2H), 1.69 (ddd, J = 12.2, 3.3, 3.1 Hz, 2H), 1.51–1.64 (m, 1H), 1.02–1.47 (m, 5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 174.29, 167.36, 138.85, 136.50, 133.57, 132.20, 129.45, 129.14, 123.60, 123.23, 121.67, 121.37, 45.84, 29.50, 25.83, 25.54, 20.98; HRMS (ESI) m/z (M+H) calcd. for $C_{21}H_{25}N_2O_2$, 337.1911; found 337.1916.

N-(3-(tert-Butyl)phenyl)-2-(cyclohexanecarboxamido)benzamide (9)—The title compound was prepared according to general protocol A. Yield: 80%; LC-MS Retention Time: t_1 (Method 1) = 7.324 min; 1H NMR (400 MHz, DMSO- d_6) δ 10.51 (s, 1H), 10.30 (s, 1H), 8.20 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.54–7.45 (m, 1H), 7.27 (dd, J = 8.3, 7.6 Hz, 1H), 7.24–7.11 (m, 2H), 2.30–2.20 (m, 1H), 1.91–1.79 (m, 2H), 1.69 (d, J = 12.7 Hz, 3H), 1.60 (d, J = 13.3 Hz, 1H), 1.43–1.32 (m, 4H), 1.27 (s, 9H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.82, 167.22, 140.35, 137.77, 132.75, 131.81, 130.31, 128.73, 124.21, 123.55, 123.04, 121.80, 120.03, 118.95, 45.10, 29.00, 25.37, 25.05, 45.46, 33.89, 30.55, 29.44, 25.82, 25.51; HRMS (ESI) m/z (M+H) calcd. For $C_{24}H_{31}N_2O_2$, 379.2380; found 379.2387.

2-(Cyclohexanecarboxamido)-N-(3-nitrophenyl)benzamide (10)—The title compound was prepared according to general protocol A. Yield: 30%; LC-MS Retention Time: t_1 (Method 1) = 6.392 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.76 (s, 1H), 10.26 (s, 1H), 8.69 (t, J = 2.2 Hz, 1H), 8.01–8.15 (m, 2H), 7.95 (ddd, J = 8.3, 2.3, 1.0 Hz, 1H), 7.75 (dd, J = 7.8, 1.6 Hz, 1H), 7.64 (t, J = 8.2 Hz, 1H), 7.46–7.56 (m, 1H), 7.22 (td, J = 7.6, 1.2 Hz, 1H), 2.19–2.35 (m, 1H), 1.79 (dd, J = 12.5, 2.5 Hz, 2H), 1.68 (dt, J = 3.1 Hz, 2H), 1.58 (d, J = 12.5 Hz, 1H), 1.03–1.43 (m, 5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 174.40, 167.74, 148.30, 140.58, 138.17, 132.30, 130.49, 129.23, 126.87, 125.04, 123.66, 122.55, 118.72, 115.03, 45.46, 29.44, 25.82, 25.51; HRMS (ESI) m/z (M+H) calcd. for $C_{20}H_{22}N_3O_4$, 368.1605; found 368.1616.

2-(Cyclohexanecarboxamido)-N-(3-fluorophenyl)benzamide (11)—The title compound was prepared according to general protocol A. Yield: 73%; LC-MS Retention Time: t_1 (Method 1) = 6.529 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.51 (s, 1H), 10.33 (s, 1H), 8.12 (dd, J = 8.3, 1.1 Hz, 1H), 7.73 (dd, J = 7.7, 1.5 Hz, 1H), 7.64 (dt, J = 11.7, 2.3 Hz, 1H), 7.43–7.55 (m, 2H), 7.37 (td, J = 8.2, 6.7 Hz, 1H), 7.20 (td, J = 7.5, 1.2 Hz, 1H), 6.82–6.98 (m, 1H), 2.21–2.35 (m, 1H), 1.81 (d, J = 11.5 Hz, 2H), 1.69 (dt, J = 12.4, 3.3 Hz, 2H), 1.59 (d, J = 11.7 Hz, 1H), 1.02–1.43 (m, 5H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –112.20–112.29 (m, 1F); HRMS (ESI) m/z (M+H) calcd. for $C_{20}H_{22}FN_2O_2$, 341.1660; found 341.1660.

N-(3-Chlorophenyl)-2-(cyclohexanecarboxamido)benzamide (12)—The title compound was prepared according to general protocol A. Yield: 40%; LC-Retention Time: t_1 (Method 1) = 6.898 min; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 10.48 (s, 1H), 10.31 (s, 1H), 8.11 (d, $J = 7.8$ Hz, 1H), 7.85 (t, $J = 2.0$ Hz, 1H), 7.71 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.59 (dd, $J = 8.0, 1.8$ Hz, 1H), 7.43–7.54 (m, 1H), 7.35 (t, $J = 8.1$ Hz, 1H), 7.06–7.24 (m, 2H), 2.26 (tt, $J = 11.3, 3.6$ Hz, 1H), 1.79 (d, $J = 14.1$ Hz, 2H), 1.68 (ddd, $J = 12.7, 3.0, 2.7$ Hz, 2H), 1.52–1.62 (m, 1H), 1.03–1.41 (m, 5H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 173.90, 167.10, 140.30, 137.91, 132.85, 131.77, 130.27, 128.73, 124.17, 123.55, 123.04, 121.80, 120.03, 118.95, 45.10, 29.00, 25.37, 25.05; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{20}\text{H}_{22}\text{ClN}_2\text{O}_2$, 357.1364; found 357.1366.

N-(3-Bromophenyl)-2-(cyclohexanecarboxamido)benzamide (13)—The title compound was prepared according to general protocol A. Yield: 90%; LC-MS Retention Time: t_1 (Method 1) = 6.904 min; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 10.47 (s, 1H), 10.31 (s, 1H), 8.11 (dd, $J = 8.4, 1.0$ Hz, 1H), 7.94–8.04 (m, 1H), 7.72 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.64 (ddd, $J = 6.7, 2.3, 2.2$ Hz, 1H), 7.49 (ddd, $J = 8.4, 7.2, 1.6$ Hz, 1H), 7.24–7.35 (m, 2H), 7.19 (td, $J = 7.6, 1.2$ Hz, 1H), 2.21–2.37 (m, 1H), 1.80 (d, $J = 11.5$ Hz, 2H), 1.69 (dt, $J = 12.2, 3.2$ Hz, 2H), 1.59 (d, $J = 12.5$ Hz, 1H), 1.07–1.42 (m, 5H); ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 177.00, 170.14, 143.70, 141.05, 134.85, 133.69, 131.84, 129.67, 129.53, 127.55, 126.17, 125.07, 124.93, 122.38, 48.30, 32.24, 28.61, 28.29; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{20}\text{H}_{22}\text{BrN}_2\text{O}_2$, 401.0859; found 401.0859.

2-(Cyclohexanecarboxamido)-N-(3-methoxyphenyl)benzamide (14)—The title compound was prepared according to general protocol A. Yield: 47%; LC-MS Retention Time: t_1 (Method 1) = 6.344 min; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 10.47 (s, 1H), 10.33 (s, 1H), 8.20 (dd, $J = 8.4, 1.0$ Hz, 1H), 7.74 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.49 (ddd, $J = 8.4, 7.2, 1.6$ Hz, 1H), 7.33 (t, $J = 2.2$ Hz, 1H), 7.13–7.30 (m, 3H), 6.69 (ddd, $J = 7.8, 2.5, 1.4$ Hz, 1H), 3.73 (s, 3H), 2.26 (tt, $J = 11.3, 3.6$ Hz, 1H), 1.82 (dd, $J = 12.5, 2.7$ Hz, 2H), 1.69 (ddd, $J = 12.4, 3.1, 2.8$ Hz, 2H), 1.51–1.64 (m, 1H), 1.02–1.41 (m, 5H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 174.31, 167.47, 159.82, 140.28, 138.58, 132.17, 129.84, 129.16, 124.20, 123.34, 121.95, 113.48, 109.81, 107.12, 55.49, 45.72, 29.48, 25.83, 25.53; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_3$, 353.1860; found 353.1856.

2-(Cyclohexanecarboxamido)-N-(3-(methylthio)phenyl)benzamide (15)—The title compound was prepared according to general protocol A. Yield: 36%; LC-MS Retention Time: t_1 (Method 1) = 6.687 min; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 10.44 (s, 1H), 10.37 (s, 1H), 8.18 (d, $J = 8.2$ Hz, 1H), 7.75 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.63 (t, $J = 2.1$ Hz, 1H), 7.44–7.55 (m, 2H), 7.29 (t, $J = 7.9$ Hz, 1H), 7.20 (td, $J = 7.6, 1.3$ Hz, 1H), 7.01 (ddd, $J = 7.8, 2.0, 1.0$ Hz, 1H), 2.46 (s, 3H), 2.19–2.35 (m, 1H), 1.76–1.90 (m, 2H), 1.65–1.76 (m, 2H), 1.52–1.65 (m, 1H), 1.03–1.47 (m, 5H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 173.89, 167.05, 139.25, 138.41, 138.08, 131.74, 129.12, 128.70, 123.86, 122.95, 121.60, 121.35, 117.88, 117.23, 45.23, 29.04, 25.38, 25.08, 14.67; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_2\text{S}$, 369.1631; found 369.1625.

2-(Cyclohexanecarboxamido)-N-(3-(trifluoromethyl)phenyl)benzamide (16)—

The title compound was prepared according to general protocol A. Yield: 50%; LC-MS Retention Time: t_1 (Method 1) = 7.349 min; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.58 (s, 1H), 10.28 (s, 1H), 8.12 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.90 (ddd, J = 8.3, 2.2, 1.1 Hz, 1H), 7.72 (dd, J = 7.8, 1.6 Hz, 1H), 7.50 (q, J = 7.2, 6.5 Hz, 2H), 7.43 (dt, J = 7.9, 1.3 Hz, 1H), 7.21 (td, J = 7.6, 1.2 Hz, 1H), 2.26 (tt, J = 11.5, 3.6 Hz, 1H), 1.82 – 1.75 (m, 2H), 1.67 (dt, J = 12.9, 3.5 Hz, 2H), 1.58 (dt, J = 13.0, 3.4 Hz, 1H), 1.37 – 1.17 (m, 5H), 1.11 (dddd, J = 16.1, 12.4, 7.9, 3.5 Hz, 1H), 1.02 (s, 2H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 177.05, 170.21, 143.28, 140.76, 134.83, 134.25, 133.79, 133.33, 131.88, 131.75, 130.35, 127.94, 126.33, 126.18, 125.19, 48.41, 32.09, 28.48, 28.18; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_2\text{S}$, 423.1349; found 423.1360.

2-(Cyclohexanecarboxamido)-N-(3((trifluoromethyl)sulfonyl)phenyl)benzamide (17)—

The title compound was prepared according to general protocol G. Yield: 80%; LC-MS Retention Time: t_1 (Method 1) = 6.964 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.83 (s, 1H), 10.16 (s, 1H), 8.57 (t, J = 1.6 Hz, 1H), 8.26 (ddd, J = 5.7, 3.7, 2.2 Hz, 1H), 7.93 (dd, J = 8.3, 1.3 Hz, 1H), 7.76–7.86 (m, 2H), 7.72 (dd, J = 7.8, 1.6 Hz, 1H), 7.52 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H), 7.23 (td, J = 7.6, 1.2 Hz, 1H), 2.16–2.36 (m, 1H), 1.73–1.87 (m, 2H), 1.62–1.73 (m, 2H), 1.50–1.62 (m, 1H), 0.82–1.46 (m, 5H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –78.42 (s, 3F); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 174.04, 167.31, 141.09, 137.28, 131.77, 131.30, 129.82, 128.79, 128.47, 125.80, 125.24, 123.52, 122.71, 122.61, 120.99, 120.55, 118.38, 116.22, 44.85, 29.00, 25.42, 25.11; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_4\text{S}$, 455.1247; found 455.1253.

N-([1,1'-Biphenyl]-3-yl)-2-(cyclohexanecarboxamido) benzamide (18)—The title compound was pre-pared according to general protocol A. Yield: 39%; LC-MS Retention Time: t_1 (Method 1) = 7.181 min; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.51 (d, J = 13.2 Hz, 2H), 8.22 (dd, J = 8.4, 1.1 Hz, 1H), 8.00 (t, J = 2.0 Hz, 1H), 7.82 (dd, J = 7.9, 1.6 Hz, 1H), 7.73 (dt, J = 8.0, 1.6 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.56 – 7.36 (m, 6H), 7.23 (td, J = 7.6, 1.2 Hz, 1H), 2.29 (tt, J = 11.5, 3.6 Hz, 1H), 1.88 – 1.81 (m, 2H), 1.71 (dp, J = 10.7, 3.5 Hz, 2H), 1.64 – 1.57 (m, 1H), 1.37 (qd, J = 12.5, 3.3 Hz, 2H), 1.26 (qt, J = 12.6, 3.3 Hz, 2H), 1.15 (qt, J = 12.5, 3.5 Hz, 1H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 177.02, 170.21, 143.70, 143.15, 142.36, 141.23, 134.87, 132.36, 132.11, 131.85, 130.73, 129.72, 126.97, 126.08, 125.49, 124.68, 122.92, 122.25, 48.36, 32.16, 28.48, 28.18; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{26}\text{H}_{27}\text{N}_2\text{O}_2$, 399.2067; found 399.2071.

2-(Cyclohexanecarboxamido)-N-(2'-(trifluoromethyl)-[1, 1'-biphenyl]-3-yl)benzamide (19)—

The title compound was prepared according to general protocol E. Yield: 25%; LC-MS Retention Time: t_1 (Method 1) = 7.417 min; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.50 (s, 1H), 10.41 (s, 1H), 8.16 (dd, J = 8.2, 1.1 Hz, 1H), 7.85 (dd, J = 7.9, 1.2 Hz, 1H), 7.82 – 7.71 (m, 4H), 7.63 (td, J = 7.6, 1.1 Hz, 1H), 7.51 (ddd, J = 8.6, 7.4, 1.6 Hz, 1H), 7.43 (t, J = 7.9 Hz, 2H), 7.22 (td, J = 7.6, 1.2 Hz, 1H), 7.07 (dd, J = 7.5, 1.6 Hz, 1H), 2.28 (tt, J = 11.4, 3.5 Hz, 1H), 1.82 (dd, J = 12.9, 3.5 Hz, 2H), 1.69 (dp, J = 10.6, 3.2 Hz, 2H), 1.60 (dtt, J = 12.7, 3.4, 1.5 Hz, 1H), 1.35 (qd, J = 12.5, 3.2 Hz, 2H), 1.25 (qt, J = 12.6, 3.3 Hz, 2H), 1.14 (qt, J = 12.5, 3.5 Hz, 1H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm

–55.34 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 173.96, 167.09, 140.54, 139.69, 138.49, 137.97, 132.37, 132.08, 131.72, 128.81, 128.29, 128.20, 126.92, 126.73, 126.10, 125.10, 124.27, 123.29, 123.09, 121.78, 120.66, 119.82, 45.20, 29.05, 25.40, 25.10; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{27}\text{H}_{26}\text{F}_3\text{N}_2\text{O}_2$, 467.1941; found 467.1931.

2-(Cyclohexanecarboxamido)-N-(3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)benzamide (20)—

A mixture of N-(3-bromophenyl)-2-(cyclohexanecarboxamido)benzamide (50.0 mg, 0.125 mmol), 3-(trifluoromethyl)phenylboronic acid (35.5 mg, 0.187 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (7.2 mg, 6.23 μmol) in DMF (1.50 mL) and 2.0 N Na_2CO_3 (0.50 mL) aqueous solution was heated in μW at 100 °C for 30 min. The reaction was cooled to room temperature, a small portion of Si-THIOL was added to remove palladium. The mixture was filtered and purified via C18 reverse phase HPLC to give the 35 mg (70% yield) of product. LC-MS Retention Time: t_1 (Method 1) = 7.438 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 10.50 (br s, 1H), 10.49 (s, 1H), 8.14–8.24 (m, 1H), 8.01–8.08 (m, 1H), 7.94–8.01 (m, 1H), 7.91 (s, 1H), 7.78–7.84 (m, 2H), 7.70–7.77 (m, 2H), 7.41–7.56 (m, 3H), 7.22 (td, J = 7.6, 1.3 Hz, 1H), 2.21–2.35 (m, 1H), 1.83 (d, J = 5.8 Hz, 2H), 1.64–1.74 (m, 2H), 1.52–1.63 (m, 1H), 1.08–1.46 (m, 5H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.11 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 173.12, 167.66, 142.23, 139.78, 139.54, 137.09, 135.78, 132.21, 131.29, 129.51, 129.48, 127.76, 127.72, 126.89, 126.84, 124.47, 124.22, 123.65, 122.98, 120.51, 119.86, 43.09, 29.23, 25.11, 24.84; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{27}\text{H}_{26}\text{F}_3\text{N}_2\text{O}_2$, 467.1941; found 467.1943.

2-(Cyclohexanecarboxamido)-N-(3-(trifluoromethyl)benzyl)benzamide (21)—

The title compound was prepared according to general protocol A. Yield: 71%; LC-MS Retention Time: t_1 (Method 1) = 6.781 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 11.13 (s, 1H), 9.34 (t, J = 6.1 Hz, 1H), 8.37 (dd, J = 8.4, 1.4 Hz, 1H), 7.76 (dd, J = 7.9, 1.7 Hz, 1H), 7.66–7.70 (m, 1H), 7.58–7.66 (m, 2H), 7.53–7.58 (m, 1H), 7.47 (ddd, J = 8.5, 7.2, 1.7 Hz, 1H), 7.13 (td, J = 7.6, 1.3 Hz, 1H), 4.54 (d, J = 5.9 Hz, 2H), 2.20 (tt, J = 1.12, 3.5 Hz, 1H), 1.75–1.87 (m, 2H), 1.68 (dt, J = 12.3, 3.2 Hz, 2H), 1.53–1.64 (m, 1H), 1.02–1.42 (m, 5H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.02 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.76, 168.56, 140.60, 139.05, 132.02, 131.43, 129.40, 129.17, 128.86, 128.01, 123.82, 123.78, 123.64, 123.60, 122.54, 120.71, 120.59, 45.69, 42.25, 29.03, 25.35, 25.07; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{24}\text{F}_3\text{N}_2\text{O}_2$, 405.1784; found 405.1790.

2-(Cyclohexanecarboxamido)-N-(furan-2-yl-methyl)benzamide (22)—

The title compound was prepared according to general protocol A. Yield: 42%; LC-MS Retention Time: t_1 (Method 1) = 6.799 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 12.07 (s, 1H), 10.69 (s, 1H), 9.29 (t, J = 5.8 Hz, 1H), 8.41 (dd, J = 8.3, 1.3 Hz, 1H), 8.22 (dd, J = 8.3, 1.3 Hz, 1H), 7.80 (dd, J = 8.0, 1.6 Hz, 1H), 7.73 (dd, J = 7.8, 1.6 Hz, 1H), 7.45–7.64 (m, 3H), 7.13–7.29 (m, 2H), 6.36 (dd, J = 3.2, 1.9 Hz, 1H), 6.22–6.33 (m, 1H), 4.45 (d, J = 5.5 Hz, 2H), 2.18–2.36 (m, 1H), 1.76–1.91 (m, 2H), 1.64–1.76 (m, 2H), 1.50–1.64 (m, 1H), 1.05–1.44 (m, 5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 174.40, 168.70, 166.89, 152.14, 142.57, 139.06, 138.97, 132.64, 132.60, 128.73, 128.13, 123.82, 122.10, 121.79, 121.73, 110.92,

107.51, 45.81, 36.52, 29.41, 25.82, 25.53; HRMS (ESI) m/z (M+H) calcd. for $C_{26}H_{28}N_3O_4$, 446.2074; found 446.2080.

2-Iodo-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (24b)—

The title compound was prepared according to general protocol C. Yield: 89%; LC-MS Retention Time: t_1 (Method 1) = 6.879 min; 1H NMR (400 MHz, DMSO- d_6) δ 10.79 (s, 1H), 10.74 (s, 1H), 8.19–8.07 (m, 2H), 8.02–7.90 (m, 2H), 7.83 (dd, J = 7.8, 1.5 Hz, 1H), 7.67–7.41 (m, 5H), 7.34 (td, J = 7.6, 1.2 Hz, 1H), 7.23 (ddd, J = 7.9, 7.1, 2.0 Hz, 1H); ^{19}F NMR (376 MHz, DMSO- d_6) δ –61.29 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.10, 166.99, 142.26, 139.72, 139.42, 136.99, 131.84, 131.43, 129.83, 129.44, 129.13, 128.93, 128.24, 128.00, 125.64, 124.16, 123.99, 122.75, 122.45, 120.09, 116.59, 116.55, 93.28; HRMS (ESI) m/z (M+H) calcd. for $C_{21}H_{15}F_3N_2O_2$, 511.0125; found 511.0122.

2-(2-Ethylbutanamido)-N-(3-(trifluoromethyl)phenyl)benzamide (25)—

The title compound was pre-pared according to general protocol C. Yield: 12%; LC-MS Retention Time: t_1 (Method 1) = 6.751 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.60 (s, 1H), 10.25 (s, 1H), 8.15 (t, J = 2.2 Hz, 1H), 7.98 (dd, J = 8.2, 1.4 Hz, 1H), 7.93 (ddd, J = 8.2, 1.2, 1.0 Hz, 1H), 7.72 (dd, J = 7.7, 1.7 Hz, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.50 (ddd, J = 8.4, 7.1, 1.7 Hz, 1H), 7.38–7.47 (m, 1H), 7.23 (td, J = 7.6, 1.3 Hz, 1H), 2.15 (tt, J = 8.8, 5.4 Hz, 1H), 1.30–1.62 (m, 4H), 0.74–0.86 (m, 6H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.23 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 176.94, 170.24, 142.91, 140.15, 134.62, 132.92, 132.47, 132.26, 131.68, 129.09, 128.15, 126.93, 126.67, 126.34, 125.82, 123.11, 123.09, 123.06, 119.56, 119.53, 119.50, 53.43, 28.26, 14.82; HRMS (ESI) m/z (M+H) calcd. for $C_{20}H_{22}F_3N_2O_2$, 379.1628; found 379.1634.

2-Pentanamido-N-(3-(trifluoromethyl)phenyl)benzamide (26)—

The title compound was prepared according to general protocol C. Yield: 22%; LC-MS Retention Time: t_1 (Method 1) = 6.546 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.61 (s, 1H), 10.19 (s, 1H), 8.17 (t, J = 2.2 Hz, 1H), 7.98 (dd, J = 8.1, 1.1 Hz, 1H), 7.92 (ddd, J = 8.2, 2.0, 0.8 Hz, 1H), 7.71 (dd, J = 7.7, 1.7 Hz, 1H), 7.57 (t, J = 8.1 Hz, 1H), 7.47–7.54 (m, 1H), 7.39–7.47 (m, 1H), 7.21 (td, J = 7.6, 1.3 Hz, 1H), 2.28 (t, J = 7.4 Hz, 2H), 1.43–1.60 (m, 2H), 1.18–1.34 (m, 2H), 0.80 (t, J = 7.6 Hz, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.21 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 171.63, 167.56, 140.24, 137.76, 132.01, 130.23, 129.90, 129.59, 129.08, 125.93, 124.35, 123.79, 122.82, 120.49, 116.95, 116.91, 36.83, 27.59, 22.10, 14.08; HRMS (ESI) m/z (M+H) calcd. for $C_{19}H_{20}F_3N_2O_2$, 365.1471; found 365.1476.

2-(Cyclopropanecarboxamido)-N-(3-(trifluoromethyl)phenyl)benzamide (27)—

The title compound was prepared according to general protocol B. Yield: 51%; LC-MS Retention Time: t_1 (Method 1) = 6.131 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 10.61 (s, 1H), 10.46 (s, 1H), 8.17 (s, 1H), 7.95–8.08 (m, 1H), 7.91 (dd, J = 7.9, 1.3 Hz, 1H), 7.71 (dd, J = 7.9, 1.5 Hz, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.46–7.53 (m, 1H), 7.40–7.46 (m, 1H), 7.20 (tt, J = 7.6, 0.8 Hz, 1H), 1.61–1.81 (m, 1H), 0.65–0.79 (m, 4H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.28 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.12, 167.57, 140.23, 137.84, 132.08, 130.26, 129.90, 129.13, 125.54, 124.42, 123.69, 122.71, 120.53, 117.02, 116.98, 15.48, 7.85; HRMS (ESI) m/z (M+H) calcd. for $C_{18}H_{16}F_3N_2O_2$, 349.1158; found 349.1160.

2-(Cyclobutanecarboxamido)-N-(3-(trifluoromethyl)phenyl)benzamide (28)—

The title compound was prepared according to general protocol B. Yield: 53%; LC-MS Retention Time: t_1 (Method 1) = 6.412 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.61 (s, 1H), 10.22 (s, 1H), 8.15 (t, $J = 2.2$ Hz, 1H), 8.09 (dt, $J = 8.1, 0.6$ Hz, 1H), 7.93 (ddd, $J = 8.2, 1.2, 1.0$ Hz, 1H), 7.75 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.58 (t, $J = 7.8$ Hz, 1H), 7.51 (ddd, $J = 8.5, 7.2, 1.6$ Hz, 1H), 7.40–7.48 (m, 1H), 7.21 (td, $J = 7.6, 1.3$ Hz, 1H), 3.15–3.26 (m, 1H), 2.01–2.24 (m, 4H), 1.80–1.97 (m, 1H), 1.64–1.80 (m, 1H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.18 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 172.88, 167.20, 139.68, 137.72, 131.81, 129.83, 129.45, 128.76, 124.03, 123.15, 121.87, 116.61, 39.73, 24.62, 17.51; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{19}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_2$, 363.1315; found 363.1317.

2-(Cyclopentanecarboxamido)-N-(3-(trifluoromethyl)phenyl)benzamide (29)—

The title compound was prepared according to general protocol B. Yield: 76%; LC-MS Retention Time: t_1 (Method 1) = 6.780 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.60 (s, 1H), 10.29 (s, 1H), 8.15 (t, $J = 2.2$ Hz, 1H), 8.04 (d, $J = 7.8$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.73 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.57 (t, $J = 7.9$ Hz, 1H), 7.50 (ddd, $J = 8.5, 7.2, 1.6$ Hz, 1H), 7.44 (d, $J = 7.6$ Hz, 1H), 7.21 (td, $J = 7.6, 1.3$ Hz, 1H), 2.75 (quin, $J = 8.0$ Hz, 1H), 1.74–1.86 (m, 2H), 1.63–1.74 (m, 2H), 1.41–1.63 (m, 4H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.21 (s, 3F); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 174.82, 174.17, 167.21, 139.81, 139.79, 137.64, 131.74, 129.88, 129.63, 129.42, 129.21, 129.00, 128.77, 126.90, 125.09, 125.03, 123.98, 123.28, 122.15, 121.48, 120.14, 120.12, 120.09, 120.06, 116.61, 116.59, 116.56, 116.53, 45.67, 29.76, 25.53; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{20}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_2$, 377.1471; found 377.1481.

N-(2-((3-(Trifluoromethyl)phenyl)carbamoyl)phenyl)cycloheptanecarboxamide (30)—

The title compound was prepared according to general protocol B. Yield: 70%; LC-MS Retention Time: t_1 (Method 1) = 7.093 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.58 (s, 1H), 10.18 (s, 1H), 8.13 (s, 1H), 7.98 (dd, $J = 8.1, 0.9$ Hz, 1H), 7.93 (d, $J = 8.0$ Hz, 1H), 7.71 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.57 (t, $J = 7.8$ Hz, 1H), 7.49 (ddd, $J = 8.4, 7.2, 1.6$ Hz, 1H), 7.39–7.46 (m, 1H), 7.21 (td, $J = 7.6, 1.2$ Hz, 1H), 2.40–2.51 (m, 1H), 1.75–1.88 (m, 2H), 1.31–1.71 (m, 1H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.20 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 174.95, 167.10, 139.75, 137.55, 131.58, 129.80, 129.43, 128.66, 125.33, 123.94, 123.25, 122.31, 120.02, 46.58, 40.15, 27.92, 25.83; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{24}\text{F}_3\text{N}_2\text{O}_2$, 405.1784; found 405.1794.

2-(2-Phenylacetamido)-N-(3-(trifluoromethyl)phenyl)benzamide (31)—

The title compound was prepared according to general protocol C. Yield: 85%; LC-MS Retention Time: t_1 (Method 1) = 6.443 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.61 (s, 1H), 10.30 (s, 1H), 8.17 (t, $J = 2.1$ Hz, 1H), 8.00–8.07 (m, 1H), 7.83–7.91 (m, 1H), 7.71 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.57 (t, $J = 7.9$ Hz, 1H), 7.50 (ddd, $J = 8.5, 7.2, 1.6$ Hz, 1H), 7.41–7.47 (m, 1H), 7.11–7.34 (m, 6H), 3.66 (s, 2H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.15 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 169.27, 166.93, 139.70, 137.18, 135.28, 131.61, 129.77, 129.45, 129.35, 129.14, 128.55, 128.28, 126.61, 125.52, 125.26, 123.89, 123.51, 122.81, 122.21, 120.09, 116.42, 43.61; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_2$, 399.1315; found 399.1312.

N-(2-((3-(Trifluoromethyl)phenyl)carbamoyl)-phenyl)-nicotinamide (32)—The title compound was prepared according to general protocol C. Yield: 55%; LC-MS Retention Time: t_1 (Method 1) = 5.342 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 11.27 (s, 1H), 10.74 (s, 1H), 8.95–9.12 (m, 1H), 8.67–8.91 (m, 1H), 8.19–8.29 (m, 1H), 8.17 (dd, J = 8.3, 1.3 Hz, 1H), 8.10 (t, J = 2.1 Hz, 1H), 7.92–8.02 (m, 1H), 7.86 (dd, J = 7.8, 1.6 Hz, 1H), 7.52–7.69 (m, 3H), 7.38–7.48 (m, 1H), 7.33 (td, J = 7.6, 1.4 Hz, 1H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.20 (s, 3F); ^{13}C NMR (101 MHz $\text{DMSO}-d_6$) δ 167.23, 163.41, 152.11, 148.03, 139.63, 137.31, 135.43, 132.04, 130.28, 129.88, 129.47, 129.16, 129.03, 125.35, 124.19, 123.94, 122.71, 120.21, 116.75, 116.71; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_3\text{O}_2$, 386.1111; found 386.1116.

2-(4-Methoxybenzamido)-N-(3-((trifluoromethyl)phenyl)benzamide (33)—The title compound was prepared according to general protocol C. Yield: 64%; LC-MS RetentionTime: t_1 (Method 1) = 6.679 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 11.31 (s, 1H), 10.75 (s, 1H), 8.36 (dd, J = 8.3, 1.3 Hz, 1H), 8.08 (t, J = 2.2 Hz, 1H), 8.01 (ddd, J = 8.2, 1.2, 1.0 Hz, 1H), 7.78–7.92 (m, 3H), 7.54–7.64 (m, 2H), 7.42–7.49 (m, 1H), 7.27 (td, J = 7.6, 1.2 Hz, 1H), 7.00–7.12 (m, 2H), 3.81 (s, 3H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.25 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.69, 164.23, 162.19, 139.43, 138.62, 132.31, 129.89, 129.49, 129.17, 129.01, 126.52, 124.48, 123.17, 121.66, 120.41, 117.06, 117.02, 114.07, 55.46, 11.88; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_3$, 415.1264; found 415.1272.

2-(3-Methoxybenzamido)-N-(3-(trifluoromethyl)phenyl)benzamide (35)—The title compound was prepared according to general protocol C. Yield: 60%; LC-MS Retention Time: t_1 (Method 1) = 6.778 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 11.33 (s, 1H), 10.75 (s, 1H), 8.32 (dd, J = 8.3, 1.3 Hz, 1H), 8.14 (t, J = 2.1 Hz, 1H), 7.98 (dt, J = 8.1, 1.2 Hz, 1H), 7.89 (dd, J = 7.9, 1.7 Hz, 1H), 7.52–7.66 (m, 2H), 7.39–7.52 (m, 4H), 7.30 (td, 1.4 Hz, 1H), 7.13–7.25 (m, 1H), 3.80 (s, 3H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.31 (s, 3F); HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_3$, 415.1264; found 415.1270.

2-Methoxy-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (36)—The title compound was prepared according to general protocol C. Yield: 64%. LC-MS RetentionTime: t_1 (Method 1) = 6.734 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 11.54 (s, 1H), 10.84 (s, 1H), 8.52–8.63 (m, 1H), 8.32 (t, J = 2.2 Hz, 1H), 7.94–8.06 (m, 2H), 7.79 (dd, J = 7.7, 1.7 Hz, 1H), 7.51–7.68 (m, 3H), 7.43–7.51 (m, 1H), 7.24–7.30 (m, 1H), 7.17–7.23 (m, 1H), 6.99–7.13 (m, 1H), 3.97 (s, 3H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.40 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.35, 163.02, 157.16, 139.69, 137.66, 133.68, 131.80, 131.52, 129.99, 129.57, 129.26, 128.57, 124.41, 123.72, 123.13, 121.99, 121.32, 120.86, 120.78, 116.30, 116.25, 112.26, 55.59; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{13}\text{F}_3\text{N}_2\text{O}_3$, 415.1264; found 415.1266.

2-Methyl-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (37)—The title compound was prepared according to general protocol C. Yield: 46%; LC-MS Retention Time: t_1 (Method 1) = 6.769 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.74 (s, 1H), 10.70 (s, 1H), 8.08–8.19 (m, 2H), 7.95 (dt, J = 8.2, 1.2 Hz, 1H), 7.79 (dd, J = 7.8, 1.8

Hz, 1H), 7.48–7.65 (m, 3H), 7.33–7.47 (m, 2H), 7.22–7.33 (m, 3H), 2.37 (s, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.30 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 168.70, 167.24, 139.85, 139.02, 137.32, 136.46, 135.88, 131.81, 131.74, 130.91, 130.60, 130.20, 130.10, 129.62, 129.42, 129.21, 129.05, 128.88, 127.06, 125.86, 123.94, 122.57, 116.50, 21.30; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_2$, 399.1315; found 399.1324.

2-Chloro-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (38)—

The title compound was prepared according to general protocol C. Yield: 81%; LC-MS Retention Time: t_1 (Method 1) = 6.765 min; ^1H NMR (600 MHz, DMSO- d_6) δ 10.82 (s, 1H), 10.71 (s, 1H), 8.12 (d, J = 2.0 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.95 – 7.92 (m, 1H), 7.78 (dd, J = 7.8, 1.6 Hz, 1H), 7.62 – 7.51 (m, 3H), 7.48 (td, J = 7.7, 1.7 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.32 (td, J = 7.6, 1.2 Hz, 1H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.28 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 166.97, 164.62, 139.81, 136.68, 136.22, 131.80, 131.58, 130.01, 129.98, 129.89, 129.40, 129.19, 129.03, 128.94, 127.40, 126.26, 125.05, 124.38, 123.93, 122.76, 120.10, 120.07, 116.50, 116.47; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{15}\text{ClF}_3\text{N}_2\text{O}_2$, 419.0769; found 419.0769.

2-(Trifluoromethoxy)-N-(2-((3-

(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (39)—The title compound was prepared according to general protocol C. Yield: 91%; LC-MS Retention Time: t_1 (Method 1) = 6.983 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 10.97 (s, 1H), 10.74 (s, 1H), 8.09–8.26 (m, 2H), 7.91–8.03 (m, 1H), 7.83 (dd, J = 7.8, 1.6 Hz, 1H), 7.77 (dd, J = 7.6, 2.0 Hz, 1H), 7.39–7.70 (m, 6H), 7.33 (td, J = 7.6, 1.3 Hz, 1H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –56.63 (s, 3F), –61.33 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.96, 162.80, 144.79, 139.64, 137.01, 132.53, 131.98, 130.14, 130.00, 129.83, 129.46, 128.90, 127.89, 125.23, 124.15, 124.00, 122.35, 121.83, 120.18, 116.60; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{15}\text{F}_6\text{N}_2\text{O}_3$, 468.0981; found 468.0982.

2-Ethoxy-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl) benzamide (40)—

The title compound was prepared according to general protocol C. Yield: 47%; LC-MS Retention Time: t_1 (Method 1) = 6.998 min; ^1H NMR (400 MHz, DMSO- d_6) δ 11.33 (s, 1H), 10.82 (s, 1H), 8.51 (dd, J = 8.4, 1.2 Hz, 1H), 8.28 (t, J = 2.1 Hz, 1H), 7.89–8.07 (m, 2H), 7.80 (dd, J = 7.8, 1.6 Hz, 1H), 7.55–7.65 (m, 2H), 7.38–7.55 (m, 2H), 7.27 (td, J = 7.6, 1.3 Hz, 1H), 7.19 (dd, J = 8.4, 1.0 Hz, 1H), 7.01–7.12 (m, 1H), 4.30 (q, J = 6.9 Hz, 2H), 1.30 (t, J = 6.9 Hz, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.40 (s, 3F); ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.66, 163.81, 156.43, 140.11, 138.08, 133.89, 132.28, 131.95, 130.39, 129.98, 129.66, 129.11, 124.75, 124.19, 123.65, 122.64, 122.57, 121.13, 120.74, 120.70, 116.73, 116.69, 113.48, 64.55, 40.60, 40.40, 40.19, 39.98, 39.77, 39.56, 39.35, 14.27; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_3$, 429.1421; found 429.1425.

2-Isopropoxy-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)-benzamide

(41)—The title compound was prepared according to general protocol H. Yield: 9%; LC-MS Retention Time: t_1 (Method 1) = 7.126 min; ^1H NMR (600 MHz, DMSO- d_6) δ 11.18 (s, 1H), 10.87 (s, 1H), 8.50 (dd, J = 8.3, 1.1 Hz, 1H), 8.36 (t, J = 1.9 Hz, 1H), 7.91 (ddd, J = 11.7, 7.5, 1.7 Hz, 2H), 7.82 (dd, J = 7.8, 1.6 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.54 – 7.44 (m,

2H), 7.29 (td, $J = 7.6, 1.2$ Hz, 1H), 7.22 – 7.17 (m, 1H), 7.05 (ddd, $J = 7.9, 7.3, 1.0$ Hz, 1H), 4.82 (p, $J = 6.1$ Hz, 1H), 1.34 (d, $J = 6.0$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 170.14, 166.71, 158.40, 142.76, 140.69, 136.44, 134.96, 134.68, 133.00, 132.55, 132.34, 131.84, 127.27, 126.83, 126.37, 125.83, 125.56, 123.54, 123.37, 123.35, 119.39, 119.36, 117.02, 74.24, 24.10; HRMS (ESI) m/z (M+H) calcd. For $\text{C}_{24}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_3$, 443.1577; found 443.1599.

2,6-Dimethoxy-N-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (42)

—The title compound was prepared according to general protocol C. Yield: 88%; LC-MS Retention Time: t_1 (Method 1) = 6.400 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 10.68 (s, 1H), 10.62 (s, 1H), 8.43 (d, $J = 8.2$ Hz, 1H), 8.08 (s, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.85 (dd, $J = 7.7, 1.1$ Hz, 1H), 7.52–7.64 (m, 2H), 7.45 (d, $J = 7.6$ Hz, 1H), 7.34 (t, $J = 8.4$ Hz, 1H), 7.27 (td, $J = 7.6, 1.1$ Hz, 1H), 6.71 (d, $J = 8.4$ Hz, 2H), 3.69 (s, 6H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.29 (s, 3F); $^1\text{HRMS}$ (ESI) m/z (M+H) calcd. for $\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_4$, 445.1370; found 445.1380.

2-(2-Methoxyethoxy)-N-(2-((3-

(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (43)—The title compound was prepared according to general protocol H. Yield: 16%; LC-MS Retention Time: t_1 (Method 1) = 6.625 min; ^1H NMR (600 MHz, DMSO- d_6) δ 11.25 (s, 1H), 10.84 (s, 1H), 8.45 (dd, $J = 8.4, 1.1$ Hz, 1H), 8.23 (d, $J = 1.9$ Hz, 1H), 7.94 (ddd, $J = 22.5, 8.1, 2.1$ Hz, 2H), 7.82 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.65 – 7.57 (m, 2H), 7.53 (ddd, $J = 8.8, 7.2, 1.8$ Hz, 1H), 7.51 – 7.46 (m, 1H), 7.29 (td, $J = 7.5, 1.2$ Hz, 1H), 7.25 (dd, $J = 8.4, 1.0$ Hz, 1H), 7.09 (td, $J = 7.6, 1.0$ Hz, 1H), 4.37 – 4.31 (m, 2H), 3.69 – 3.63 (m, 2H), 3.12 (s, 3H). ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –61.36 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 167.18, 163.39, 156.25, 139.72, 138.97, 137.58, 133.39, 131.90, 131.37, 129.99, 129.49, 129.28, 128.83, 124.55, 123.85, 123.39, 122.40, 122.35, 121.07, 120.29, 116.39, 113.62, 69.79, 67.95, 58.06; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{24}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_4$, 459.1526; found 459.1527.

2-(2-(Dimethylamino)ethoxy)-N-(2-((trifluoromethyl)phenyl)carbamoyl)-phenyl)benzamide (44)

—The title compound was prepared according to general protocol H. Yield: 4%; LC-MS Retention Time: t_1 (Method 1) = 4.705 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 11.11 (s, 1H), 10.84 (s, 1H), 8.41 (dd, $J = 8.5, 1.3$ Hz, 1H), 8.07–8.20 (m, 1H), 7.96 (dd, $J = 8.0, 1.2$ Hz, 1H), 7.87 (dd, $J = 7.7, 1.9$ Hz, 1H), 7.84 (dd, $J = 7.8, 1.8$ Hz, 1H), 7.57–7.65 (m, 2H), 7.52–7.57 (m, 1H), 7.45–7.52 (m, 1H), 7.24–7.33 (m, 2H), 7.13 (td, $J = 7.5, 1.0$ Hz, 1H), 4.55 (t, $J = 5.1$ Hz, 2H), 3.49–3.74 (m, 2H), 2.78 (s, 6H); HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{25}\text{H}_{25}\text{F}_3\text{N}_3\text{O}_3$, 472.1843; found 472.1851.

2-(2-Ethoxybenzamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)nicotinamide (49)

—The title compound was prepared according to general protocol A. Yield: 45%; LC-MS Retention Time: t_1 (Method 1) = 5.472 min; ^1H NMR (400 MHz, DMSO- d_6) δ ppm 10.98 (s, 1H), 10.81 (s, 1H), 8.47–8.66 (m, 2H), 8.05–8.24 (m, 2H), 7.75–7.87 (m, 2H), 7.72 (dd, $J = 7.8, 2.0$ Hz, 1H), 7.51 (ddd, $J = 8.5, 7.1, 2.0$ Hz, 1H), 7.39 (dd, $J = 7.6, 4.9$ Hz, 1H), 7.20 (d, $J = 8.2$ Hz, 1H), 7.00 (t, $J = 7.4$ Hz, 1H), 4.27 (q, $J = 7.0$ Hz, 2H), 1.42 (t, $J = 7.0$ Hz, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –78.44 (s, 3F); ^{13}C NMR (151 MHz, DMSO-

d_6) δ 165.91, 163.44, 156.44, 150.26, 148.04, 141.24, 137.87, 133.55, 131.38, 130.94, 129.86, 128.00, 125.15, 124.02, 121.63, 120.76, 120.51, 120.43, 120.27, 118.35, 113.33, 64.71, 14.44; HRMS (ESI) m/z (M+H) calcd. for $C_{22}H_{19}F_3N_3O_5S$, 494.0992; found 494.1004.

3-(2-Ethoxybenzamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)isonicotinamide (50)—The title compound was prepared according to general protocol A. Yield: 38%; LC-MS Retention Time: t_1 (Method 1) = 5.766 min; HRMS (ESI) m/z (M+H) calcd. for $C_{22}H_{19}F_3N_3O_5S$, 494.0992; found 494.1000.

4-(2-Ethoxybenzamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)nicotinamide (51)—The title compound was prepared according to general protocol A. Yield: 26%; LC-MS Retention Time: t_1 (Method 1) = 5.507 min; 1H NMR (400 MHz, DMSO- d_6) δ ppm 11.71 (s, 1H), 11.25 (s, 1H), 9.00 (s, 1H), 8.77 (t, J = 2.2 Hz, 1H), 8.65–8.71 (m, 1H), 8.54–8.64 (m, 1H), 8.15–8.26 (m, 1H), 7.96 (dd, J = 7.8, 2.3 Hz, 1H), 7.78–7.93 (m, 2H), 7.56 (ddd, J = 8.6, 7.0, 2.0 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H), 4.35 (q, J = 6.9 Hz, 2H), 1.29 (t, J = 6.8 Hz, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm –78.37 (s, 3F); ^{13}C NMR (151 MHz, DMSO- d_6) δ 169.07, 167.39, 159.21, 156.20, 153.04, 147.91, 143.56, 137.33, 134.89, 134.57, 133.02, 131.82, 129.06, 124.40, 124.05, 123.94, 123.60, 121.44, 120.83, 117.83, 116.25, 67.26, 16.85; HRMS (ESI) m/z (M+H) calcd. for $C_{22}H_{19}F_3N_3O_5S$, 494.0992; found 494.1004.

2-(2-Ethoxybenzamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)-nicotinamide (52)—The title compound was prepared according to general protocol A. yield: 51%; LC-MS Retention Time: t_1 (Method 1) = 7.402 min; HRMS (ESI) m/z (M+Na) $^+$ calcd. for $C_{22}H_{18}F_3N_3NaO_5S$, 516.0811; found 516.0837.

2-(2-Ethoxyphenylsulfonamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)-benzamide (53)—The title compound was prepared according to general protocol A. Yield: 29%; LC-MS Retention Time: t_1 (Method 1) = 6.631 min; 1H NMR (400 MHz, DMSO- d_6) δ 11.00 (s, 1H), 10.37 (s, 1H), 8.64 (t, J = 1.9 Hz, 1H), 8.24 (dt, J = 7.8, 1.8 Hz, 1H), 7.96–7.84 (m, 2H), 7.82 (dd, J = 7.8, 1.7 Hz, 2H), 7.60–7.50 (m, 2H), 7.48 (ddd, J = 8.5, 7.3, 1.5 Hz, 1H), 7.18 (td, J = 7.6, 1.2 Hz, 1H), 7.12–7.00 (m, 2H), 3.95 (q, J = 7.0 Hz, 2H), 1.18 (t, J = 7.0, 3H); ^{19}F NMR (376 MHz, DMSO- d_6) δ –78.37; ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.47, 155.62, 140.29, 137.78, 135.61, 133.03, 131.39, 130.49, 129.95, 129.46, 128.91, 125.92, 125.37, 123.06, 121.39, 121.31, 121.05, 120.03, 118.75, 117.80, 113.56, 64.53, 13.68; HRMS (ESI) m/z (M+H) calcd. for $C_{22}H_{20}F_3N_2O_6S_2$, 529.0709; found 529.0716.

2-Methoxy-N-methyl-N-(2-((3-((trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (54)—The title compound was prepared according to general protocol A. Yield: 47%; LC-MS Retention Time: t_1 (Method 1) = 6.046 min; HRMS (ESI) m/z (M+H) calcd. for $C_{23}H_{20}F_3N_2O_3$, 429.1421; found 429.1438.

2-(2-Methoxybenzamido)-N-methyl-N-(3-(trifluoromethyl)phenyl) benzamide

(55)—The title compound was prepared according to general protocol A. Yield: 60%; LC-MS Retention Time: t_1 (Method 1) = 6.442 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.65 (d, $J=0.8$ Hz, 1H), 8.21 (dt, $J=8.4$, 0.6 Hz, 1H), 8.03 (dd, $J=7.8$, 1.8 Hz, 1H), 7.54–7.66 (m, 2H), 7.37–7.54 (m, 3H), 7.20–7.33 (m, 2H), 7.13 (ddd, $J=7.9$, 7.2, 1.0 Hz, 1H), 7.04–7.11 (m, 1H), 6.86–6.96 (m, 1H), 4.02 (s, 3H), 3.44 (s, 3H); HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_3$, 429.1241; found 429.1418.

2-((2-Methoxyethyl)amino)-N-(3-(trifluoromethyl)phenyl)-benzamide (57)—The title compound was prepared according to general protocol F. Yield: 19%; LC-MS Retention Time: t_1 (Method 1) = 6.254 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.33 (s, 1H), 8.14 (t, $J=2.2$ Hz, 1H), 7.88–8.05 (m, 1H), 7.70 (dd, $J=7.8$, 1.6 Hz, 1H), 7.54–7.63 (m, 1H), 7.44–7.52 (m, 1H), 7.37–7.44 (m, 1H), 7.34 (ddd, $J=8.6$, 7.0, 1.7 Hz, 1H), 6.72–6.84 (m, 1H), 6.65 (ddd, $J=8.0$, 7.1, 1.2 Hz, 1H), 3.53 (t, $J=5.5$ Hz, 2H), 3.28 (s, 3H), 3.24–3.33 (m, 2H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.23 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ ppm 168.36, 149.33, 139.94, 133.15, 129.75, 129.19, 129.08, 124.02, 119.73, 116.55, 116.51, 114.97, 114.37, 111.30, 70.34, 58.03, 41.90; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{17}\text{H}_{18}\text{F}_3\text{NO}_2$, 339.1315; found 339.1326.

2-(2-Ethoxybenzamido)-N-(3-((trifluoromethyl)sulfonyl)phenyl)thiophene-3-carboxamide (59)

—The title compound was prepared according to general protocol A. Yield: 33%; LC-MS Retention Time: t_1 (Method 1) = 7.341 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 13.25 (s, 1H), 10.45 (s, 1H), 8.90 (d, $J=2.2$ Hz, 1H), 8.30–8.20 (m, 1H), 8.08 (dd, $J=7.9$, 1.9 Hz, 1H), 7.90–7.80 (m, 2H), 7.75 (d, $J=6.0$ Hz, 1H), 7.60 (ddd, $J=8.9$, 7.3, 1.9 Hz, 1H), 7.31 (dd, $J=8.6$, 0.9 Hz, 1H), 7.18–7.08 (m, 2H), 4.51 (q, $J=7.0$ Hz, 2H), 1.47 (t, $J=7.0$ Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ ppm 164.07, 162.19, 156.99, 147.77, 141.20, 134.92, 132.43, 131.69, 130.33, 129.06, 125.79, 123.30, 121.47, 121.39, 119.79, 117.58, 115.83, 113.79, 64.98, 14.46; ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –78.43 (s, 3F); HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{21}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_5\text{S}_2$, 499.0604; found 499.0608.

2-Methoxy-N-(3-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (61)

—The title compound was prepared according to general protocol A. Yield: 68%; LC-MS Retention Time: t_1 (Method 1) = 6.471 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 10.58 (s, 1H), 10.30 (s, 1H), 8.27–8.35 (m, 1H), 8.23 (t, $J=2.1$ Hz, 1H), 8.04 (dt, $J=8.2$, 1.1 Hz, 1H), 7.88–7.99 (m, 1H), 7.67 (ddd, $J=8.0$, 1.4, 1.2 Hz, 1H), 7.63 (dd, $J=7.6$, 1.8 Hz, 1H), 7.56–7.62 (m, 1H), 7.47–7.56 (m, 2H), 7.41–7.47 (m, 1H), 7.14–7.23 (m, 1H), 7.06 (td, $J=7.4$, 1.0 Hz, 1H), 3.90 (s, 3H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –61.28 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ ppm 166.43, 165.23, 156.95, 140.40, 139.69, 135.69, 132.59, 130.32, 130.08, 129.95, 129.24, 125.95, 125.21, 124.18, 123.45, 122.99, 120.93, 119.66, 116.76, 116.72, 112.46; HRMS (ESI) m/z (M+H) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_3$, 415.1264; found 415.1275.

2-Methoxy-N-(2-((3-

((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (62)—The title

compound was prepared according to general protocol G. Yield: 25%; LC-MS Retention Time: t_1 (Method 1) = 6.810 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 11.58 (s, 1H), 11.09 (s, 1H), 8.82 (t, J = 2.1 Hz, 1H), 8.58 (dd, J = 8.5, 1.3 Hz, 1H), 8.23 (dt, J = 7.0, 2.2 Hz, 1H), 8.02 (dd, J = 7.7, 1.9 Hz, 1H), 7.76–7.99 (m, 3H), 7.51–7.63 (m, 2H), 7.27 (td, J = 7.5, 1.2 Hz, 1H), 7.22 (dd, J = 8.4, 1.2 Hz, 1H), 7.09 (ddd, J = 7.2, 1.2 Hz, 1H), 3.99 (s, 3H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm –78.39 (s, 3F); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.57, 163.01, 157.16, 140.75, 137.78, 133.69, 132.05, 131.51, 131.37, 129.94, 128.69, 128.43, 125.58, 123.90, 123.10, 122.05, 121.24, 120.84, 120.77, 112.26, 55.59; HRMS (ESI) m/z ($\text{M}+\text{H}$) calcd. for $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_5\text{S}$, 479.0883; found 479.0886.

2-Ethoxy-N-(2-((3-

((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (63)—The title compound was prepared according to general protocol A. Yield: 53%; LC-MS Retention Time: t_1 (Method 1) = 6.963 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.35 (s, 1H), 11.10 (s, 1H), 8.76 (t, J = 2.2 Hz, 1H), 8.53 (d, J = 8.2 Hz, 1H), 8.23 (dt, J = 7.1, 2.3 Hz, 1H), 7.96 (dd, J = 7.8, 2.0 Hz, 1H), 7.80–7.92 (m, 3H), 7.57–7.65 (m, 1H), 7.53 (ddd, J = 8.6, 7.0, 2.0 Hz, 1H), 7.30 (td, J = 7.6, 1.2 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.04–7.10 (m, 1H), 4.31 (q, J = 6.8 Hz, 2H), 1.31 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.44, 163.39, 156.00, 140.75, 137.73, 133.44, 132.10, 131.48, 131.34, 129.93, 128.81, 128.49, 125.58, 123.86, 123.21, 122.27, 122.08, 120.81, 120.67, 119.41 (q, J = 328 Hz), 113.00, 64.09, 13.80; ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ –78.40 (s, 3F); HRMS (m/z): [$\text{M}+\text{H}$] calcd. for $\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_5\text{S}$, 493.1040; found 493.1044.

2-Isopropoxy-N-(2-((3-

((trifluoromethylsulfonyl)phenyl)carbamoyl)phenyl)benzamide (64)—The title compound was prepared according to general protocol A. Yield: 44%; LC-MS Retention Time: t_1 (Method 1) = 6.804 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.10 (s, 1H), 11.05 (s, 1H), 8.64 (s, 1H), 8.44 (d, J = 8.2 Hz, 1H), 8.27 (dt, J = 7.1, 2.3 Hz, 1H), 7.79–7.92 (m, 4H), 7.60–7.69 (m, 1H), 7.52–7.60 (m, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 4.94 (q, J = 9.0 Hz, 2H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.46, 163.36, 154.24, 140.76, 137.68, 132.87, 132.27, 131.25, 130.85, 129.87, 128.99, 128.60, 125.45, 124.28, 123.83, 123.63 (q, J = 280 Hz), 123.49, 122.52, 121.97, 121.05, 119.42 (q, J = 329 Hz), 114.03, 65.00 (q, J = 35.0 Hz); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ 72.46 (t, J = 9.4 Hz, 3F), –78.48 (s, 3F); HRMS (m/z): [$\text{M}+\text{H}$] calcd. for $\text{C}_{23}\text{H}_{17}\text{F}_6\text{N}_2\text{O}_5\text{S}$, 547.0757; found 547.0762.

2-Isopropoxy-N-(2-((3-

((trifluoromethylsulfonyl)phenyl)carbamoyl)phenyl)benzamide (65)—The title compound was prepared according to general protocol A. Yield: 57%; LC-MS Retention Time: t_1 (Method 1) = 6.974 min; t_2 (Method 2) = 3.881 min; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.11 (s, 1H), 11.09 (s, 1H), 8.75 (s, 1H), 8.48 (d, J = 8.2 Hz, 1H), 8.20 (dt, J = 7.4, 2.2 Hz, 1H), 7.91 (dd, J = 7.8, 1.6 Hz, 1H), 7.77–7.89 (m, 3H), 7.57–7.66 (m, 1H), 7.51 (ddd, J = 8.6, 7.0, 2.0 Hz, 1H), 7.30 (td, J = 7.5, 1.0 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 4.75–4.85 (m, 1H), 1.32 (d, J = 5.9 Hz, 6H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.24, 163.63, 155.29, 140.75, 137.61, 133.30, 132.06, 131.52, 131.28,

129.97 (br.), 128.89, 128.51, 125.58, 123.93, 123.29, 122.79, 122.56, 120.82, 120.44, 119.40 (q, $J = 328$ Hz), 113.98, 71.19, 21.05; ^{19}F NMR (376 MHz, DMSO- d_6): δ -78.38 (s, 3F); HRMS (m/z): [M+H] calcd. for $\text{C}_{24}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_5\text{S}$, 507.1196; found 507.1221.

2-Propoxy-*N*-(2-((3-

((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (66)—The title compound was prepared according to general protocol A. Yield: 41%; LC-MS Retention Time: t_1 (Method 1) = 7.147 min; ^1H NMR (400 MHz, DMSO- d_6): δ 11.26 (s, 1H), 11.10 (s, 1H), 8.72 (s, 1H), 8.54 (d, $J = 8.6$ Hz, 1H), 8.24 (dt, $J = 7.1, 2.3$ Hz, 1H), 7.94 (dd, $J = 7.8, 2.0$ Hz, 1H), 7.78–7.91 (m, 3H), 7.56–7.67 (m, 1H), 7.52 (ddd, $J = 8.5, 7.1, 2.0$ Hz, 1H), 7.25–7.33 (m, 1H), 7.20 (d, $J = 8.2$ Hz, 1H), 7.07 (t, $J = 7.4$ Hz, 1H), 4.16 (t, $J = 7.0$ Hz, 2H), 1.71 (sxt, $J = 7.2$ Hz, 2H), 0.77 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 167.42, 163.49, 156.14, 140.78, 137.75, 133.37, 132.14, 131.39, 131.33, 129.95 (br.), 128.90, 128.48, 125.54, 123.80, 123.21, 122.25, 122.15, 120.88, 120.68, 119.43 (q, $J = 328$ Hz), 113.09, 69.82, 21.24, 9.74; ^{19}F NMR (376 MHz, DMSO- d_6): δ -78.43 (s, 3F); HRMS (m/z): [M+H] calcd. for $\text{C}_{24}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_5\text{S}$, 507.1196; found 507.1219.

2-Butoxy-*N*-(2-((3-

((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (67)—The title compound was prepared according to general protocol A. Yield: 49%; LC-MS Retention Time: t_1 (Method 1) = 7.325 min; t_2 (Method 2) = 3.971 min; ^1H NMR (400 MHz, DMSO- d_6): δ 11.27 (s, 1H), 11.10 (s, 1H), 8.71 (s, 1H), 8.55 (d, $J = 8.2$ Hz, 1H), 8.26 (dt, $J = 7.3, 2.0$ Hz, 1H), 7.93 (dd, $J = 7.8, 2.0$ Hz, 1H), 7.81–7.91 (m, 3H), 7.58–7.68 (m, 1H), 7.47–7.57 (m, 1H), 7.29 (t, $J = 7.4$ Hz, 1H), 7.22 (d, $J = 8.2$ Hz, 1H), 7.08 (t, $J = 7.4$ Hz, 1H), 4.19 (t, $J = 6.8$ Hz, 2H), 1.57–1.73 (m, 2H), 1.23 (sxt, $J = 7.5$ Hz, 2H), 0.70 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 167.41, 163.52, 156.13, 140.81, 137.81, 133.37, 132.20, 131.36, 131.34, 129.96 (br.), 128.94, 128.41, 125.54, 123.59, 123.18, 122.30, 122.06, 120.85, 120.69, 113.09, 68.19, 29.93, 18.34, 13.37; ^{19}F NMR (376 MHz, DMSO- d_6): δ -78.42 (s, 3F); HRMS (m/z): [M+H] calcd. for $\text{C}_{25}\text{H}_{24}\text{F}_3\text{N}_2\text{O}_5\text{S}$, 521.1353; found 521.1359.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

AcOH	acetic acid
aq	aqueous

CH₂Cl₂	dichloromethane
conc	concentrated
DIPEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	N,Ndimethylformamide
DMSO	dimethyl sulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et₂O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
H₂O	water
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HCl	hydrochloric acid
iPr₂NH	diisopropylamine
IV	intravenous
K₂CO₃	potassium carbonate
KOAc	potassium acetate
LCMS	liquid chromatography/mass spectrometry
M	molar
mM	millimolar
MeOH	methanol
MHz	megahertz
N₂	nitrogen
NaHCO₃	sodium bicarbonate
Na₂CO₃	sodium carbonate
NaOH	sodium hydroxide
NH₄Cl	ammonium chloride

PBS	phosphate-buffered saline
Pd(dppf)Cl₂·CH₂Cl₂	[1,1]-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with dichloromethane
Pd(PPh₃)₄	tetrakis(triphenylphosphine)palladium (0)
PO	by mouth
RXFP	relaxin/insulin-like family peptide receptor
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TGF	tumor growth factor
TNF	tumor necrosis factor
μW	microwave irradiation.

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Highlights

- A high throughput screening campaign identified the first small molecule relaxin peptide hormone receptor agonists
- An extensive medicinal chemistry effort produced an optimized, potent lead compound
- Pharmacokinetic studies show good compound bioavailability in mice with no observed toxicity
- The lead compound displays an antifibrotic gene expression profile in activated human hepatic stellate cells

Figure 1.
Initial RXFP1 hits from the Molecular Probe Center Network Library.

Figure 2.

Predicted model of ML290 bound in the TM binding site of hRXFP1. The receptor hRXFP1 is shown in ribbon (white) and key residues are shown in sticks (brown). In addition to an intramolecular H-bond, ML290 (sticks in cyan) forms a hydrogen-bond with the backbone N atom of residue T660 in ECL3 (magenta).¹⁸

Figure 3.

ML290 activates an antifibrotic gene expression profile in activated human hepatic stellate cells. HSCs were treated with 5 μ M of ML290 or DMSO for 24 and 72 hr. Gene expression was evaluated by quantitative RT-PCR, normalized to GAPDH gene expression and presented as the fold change vs DMSO-treated control. N=3, *p < 0.05, **p < 0.01.

Scheme 1.

Synthesis of the Western Cyclohexyl and Optimized Analogues

Reagents and Conditions: For **3–22**, **62–67**: (a) acid chloride, DCM, TEA, 0 °C to RT, 1–3 h; (b) substituted aniline or benzylamine, 2M AlMe₃ in toluene, 100 °C, 16 h; (c) For **17: 16**, DCM, MCPBA, RT, 12h; (d) For **18: 13**, phenylboronic acid, Pd(PPh₃)₄, 2M Na₂CO₃, DMF, microwave irradi., 100 °C, 1 h; For **19: 13**, 2-(trifluoromethylphenyl)boronic acid, Pd(PPh₃)₄, 2M Na₂CO₃, DMF, microwave irradi., 100 °C, 1 h; For **20: 13**, 3-(trifluoromethylphenyl)boronic acid, Pd(PPh₃)₄, 2M Na₂CO₃, DMF, μW., 100 °C, 1 h;

Scheme 2.**Synthesis of 3-Trifluoromethyl Analogues 25–44**

Reagents and Conditions: (a) 3-trifluoromethylaniline, DCM, DMAP, EDC, 24 h; (b) TFA, DCM, 0 °C; (c) For **24b**: 2-iodobenzoyl chloride, DCM, TEA, 0 °C, 1 h; (d) For **25–26, 31–42**: aryl acid chloride, DCM, TEA, RT, 2 h; For **27–30**: cycloalkyl carboxylic acid, DMF, EDC, DMAP, 12 h; (e) For **43: 24b**, 2-methoxyethan-1-ol, Cs₂CO₃, CuI, 1,10-phenanthroline, toluene, sealed tube, 110 °C, 24 h; For **44: 24b**, 2-dimethylaminoethan-1-ol, Cs₂CO₃, CuI, 1,10-phenanthroline, toluene, sealed tube, 110 °C, 24 h;

Scheme 3.

Synthesis of Pyridine Core Derivatives

Reagents and Conditions: (a) 2-ethoxybenzoylchloride, DCM, TEA, 0 °C-RT, 2 h, 11–94%;

(b) 3-trifluoromethylsulfonylaniline, 2M Al-Me₃ in toluene, 100 °C, 12 h, 56–75%;

Scheme 4.

Benzamide Linker Modifications: Sulfonamide, Amine, N-Methyl and 3-Acetamido-N-Phenylbenzamide Compounds

Reagents and Conditions:: (a) 2-ethoxyphenylsulfonylchloride, DCM, TEA, 0 °C-RT, 2 h; (b) 3-trifluoromethylsulfonylaniline, 2 M AlMe₃ in toluene, 100 °C, 1–16 h, 10–82%; (c) 2-methoxybenzoylchloride, DCM, TEA, RT; For **54**: (d) NaH, DMF, CH₃I, 0 °C, 1.5 h, then (b); For **55**: (b), then (d); (e) 3-trifluoromethylsulfonylaniline, DCM, TEA, 0 °C-RT; (f) 2-dimethylaminoethan-1-ol, Cs₂CO₃, CuI, 1,10-phenanthroline, toluene, sealed tube, 110 °C, 24 h;

Table 1

SAR of the Cyclohexyl-2-Acetamido-N-Phenylbenzamide Series

Cmpd	R	EC ₅₀ (μM) ^a	Max. Response ^b
3	3-trifluoromethylphenyl	1.88	92%
4	4-trifluoromethylphenyl	94.0	46%
5	phenyl	94.0	57%
6	2-methylphenyl	inactive	N/A
7	3-methylphenyl	37.4	65%
8	4-methylphenyl	187	32%
9	3-tert-butylphenyl	2.65	70%
10	3-nitrophenyl	5.93	93%
11	3-fluorophenyl	13.3	81%
12	3-chlorophenyl	3.34	89%
13	3-bromophenyl	2.65	91%
14	3-methoxyphenyl	37.4	74%
15	3-thiomethylphenyl	5.29	84%
16	3-trifluoromethylthiophenyl	1.88	90%
17	3-trifluoromethylsulfonylphenyl	1.06	87%
18	3-biphenyl	inactive	N/A
19		2.65	74%
20		inactive	N/A
21		inactive	N/A

Cmpd	R	EC ₅₀ (μM) ^a	Max. Response ^b
22	 <chem>O=C(NCc1ccoc1)c2ccccc2</chem>	9.40	45%

^aThe EC₅₀ is expressed in micromolar and is the concentration necessary to reach 50% of maximum cAMP signal.

^bThe maximum response is the efficacy indicated as the maximum observed increase in cAMP response.

Table 2

SAR of the Western Hemisphere N-3-Trifluorophenylbenzamide Series

Cmpd	R	EC ₅₀ (μM) ^a	Max. Response ^b
25	3-pentyl	5.29	59%
26	1-butyl	5.29	94%
27	cyclopropyl	8.38	79%
28	cyclobutyl	2.97	75%
29	cyclopentyl	2.36	88%
30	cycloheptyl	2.11	75%
31	benzyl	8.38	86%
32	3-pyridyl	5.93	95%
33	4-methoxyphenyl	9.40	71%
34	phenyl	1.32	64%
35	3-methoxyphenyl	1.88	94%
36	2-methoxyphenyl	0.334	99%
37	2-methylphenyl	4.20	90%
38	2-chlorophenyl	3.74	92%
39	2-trifluoromethoxyphenyl	1.18	96%
40	2-ethoxyphenyl	0.265	94%
41	2-isopropoxyphenyl	0.471	99%
42	2,6-dimethoxyphenyl	7.47	42%
43		0.747	98%
44		3.74	92%

^aThe EC₅₀ is expressed in micromolar and is the concentration necessary to reach 50% of maximum cAMP signal.^bThe maximum response is the efficacy indicated as the maximum observed increase in cAMP response.

Table 3

SAR of Pyridine Central Core Derivatives

Cmpd	AC ₅₀	Max. Response	PBS Solubility ^a	RLM Stability (t _{1/2} min)
49 (N1)	1.67	98%	<1 mM	19.85
50 (N2)	2.97	99%	<1 mM	ND
51 (N3)	0.747	97%	ND	>30
52 (N4)	4.71	73%	<1 mM	>30

^aPion's μ SOL assay for kinetic solubility determination was used. Saturation shake-flask solubility method was adapted to a 96-well microtiter plate format and a co-solvent method with n-propanol as the reference compound was utilized.

Table 4

SAR of Benzamide Linker Modifications

Cmpd	R ₁	R ₂	R ₃	R ₄	Linker	EC ₅₀ (μM) ^a	Max. ^b Response
53		H	H	SO ₂ CF ₃		inactive	N/A
54		CH ₃	H	CF ₃		inactive	N/A
55		H	CH ₃	CF ₃		29.7	77%
57		H	H	CF ₃		59.3	63%
59		H	H	SO ₂ CF ₃		2.36	95%

Cmpd	R ₁	R ₂	R ₃	R ₄	Linker	EC ₅₀ (μ M) ^a	Max. ^b Response
61		H	H	CF ₃		inactive	N/A

^aThe EC₅₀ is expressed in micromolar and is the concentration necessary to reach 50% of maximum cAMP signal.

^bThe maximum response is the efficacy indicated as the maximum observed increase in cAMP response.

Table 5

SAR of Optimized 2-Acetamido-N-Phenylbenzamide Compounds Including ML-290

Cmpd	R	EC ₅₀ (μM) ^a	Max. Response ^b	ATP toxicity EC ₅₀ (μM) ^c	PBS Solubility (μM) ^d	Stability (t _{1/2} min)	
						RLM	MLM ^e
62	OMe	0.188	99%	29.7	6.3	>30	ND
63	OEt	0.188	99%	18.8	<1.1	>30	1732
64	OCH ₂ CF ₃	0.067	97%	29.7	3.3	>30	100
65	OCH(CH ₃) ₂	0.094	98%	18.8	7.0	28.2	122
66	OCH ₂ CH ₂ CH ₃	0.052	98%	9.4	17.0	26.8	133
67	OBu	0.047	98%	59.3	5.3	19.5	178

^aThe EC₅₀ is expressed in micromolar and is the concentration necessary to reach 50% of maximum cAMP signal.^bThe maximum response is the efficacy indicated as the maximum observed increase in cAMP response.^cCytotoxicity assay to measure the effect of compounds on cell viability by measuring ATP levels (ATPLite; Promega, Madison, WI).^dKinetic solubility analysis in PBS solution performed by Analiza (www.analiza.com).^eRodent microsomal stability analysis performed by Pharmaron (www.pharmaron.com).

Table 6

Pharmacokinetic Parameters for Compound 65 in Male C57/Bl6 Mice

Route	Dosing Level (mg/kg)	CL obs ^a (mL/min/kg)	t _{1/2} ^b (h)	C ₀ ^c (µg/mL)	C _{max} ^d (µg/L)	AUC _{last} ^e (µg·h/mL)	AUC _{inf} ^f (µg·h/mL)	MRT _{last} ^g (h)	V _{ss} obs ^h (L/kg)	F ⁱ (%)
IV (plasma)	3	67.2	6.6	0.49	ND	0.70	0.74	4.4	24.5	ND
PO (plasma)	30	ND	5.5	ND	0.30	0.98	1.00	3.1	ND	14
IV (heart)	3	8.78	6.3	2.93	ND	5.19	5.69	5.5	4.2	ND
PO (heart)	30	ND	1.0	ND	1.03	2.63	2.68	2.2	ND	ND

^aTotal clearance after IV administration^bHalf-life^cConcentration^dMaximum serum concentration^eArea under the curve^fArea under the curve^gMean residence time^hVolume of distribution in steady stateⁱBioavailability

Table 7

Primer sequences used in qPCR.

Gene	Forward primer	Reverse primer
GAPDH ^a	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCCc
ACTA2 ^b	AAGCACAGAGCAAAAGAGGAAT	ATGTCGTCCCAGTTGGTGAT
COL1A1 ^c	AGACAGTGATTGAATACAAAACCA	GGAGTTTACAGGAAGCAGACA
CTGF ^d	CCCAGACCCAATATGATTAGAG	AGGCGTTGTCATTGGTAACC
MMP1 ^e	GCACTGAGAAAGAAGACAAAGG	CTAAGTCCACATCTTGCTCTTG
MMP2 ^f	CATACAGGATCATTGGCTACAC	TCACATCGCTCCAGACTTG
PPARGC1A ^g	ATAAATCACACGGCGCTCTT	TGAGAGGGCCAAGCAAAG
TGFB1 ^h	CACTCCCACTCCCTCTCTC	GTCCCCTGTGCCTTGATG

^aGlyceraldehyde-3-phosphate dehydrogenase^bAlpha-acti-2^cCollagen^dConnective tissue growth factor^eMatrix metalloproteinase 1^fMatrix metalloproteinase2^gPeroxisome proliferative activated receptor, gamma, coactivator 1 alpha^hVolume of distribution in steady stateⁱTransforming growth factor beta-1