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Design, parallel synthesis, and crystal structures of biphenyl antithrombotics as selective inhibitors of tissue factor FVIIa complex. Part 1: Exploration of S2 pocket pharmacophores

Pravin L. Kotian [a,*], Raman Krishnan [a], Scott Rowland [b], Yahya El-Kattan [a], Surendra K. Saini [c], Ramanda Upshaw [a], Shanta Bantia [a], Shane Arnold [d], Y. Sudhakar Babu [a,*], Pooran Chand [a,*]

[a] BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Birmingham, AL 35244, USA
[b] Presently at Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, USA
[c] Presently at Southern Research Institute, 2000 Ninth Avenue South, Birmingham, AL 35205, USA
[d] 2333 Farley Terrace, Birmingham, AL 35226, USA

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ABSTRACT

Factor VIIa (FVIIa), a serine protease enzyme, coupled with tissue factor (TF) plays an important role in a number of thrombosis-related disorders. Inhibition of TF·FVIIa occurs early in the coagulation cascade and might provide some safety advantages over other related enzymes. We report here a novel series of substituted biphenyl derivatives that are highly potent and selective TF·FVIIa inhibitors. Parallel synthesis coupled with structure-based drug design allowed us to explore the S2 pocket of the enzyme active site. A number of compounds with IC_{50} value of <10 nM were synthesized. The X-ray crystal structures of some of these compounds complexed with TF·FVIIa were determined and results were applied to design the next round of inhibitors. All the potent inhibitors were tested for inhibition against a panel of related enzymes and selectivity of 17,600 over thrombin, 450 over trypsin, 685 over FXa, and 76 over plasmin was achieved. Two groups, vinyl **36b** and 2-furan **36ab**, were identified as the optimum binding substituents on the phenyl ring in the S2 pocket. Compounds with these two substituents are the most potent compounds in this series with good selectivity over related serine proteases. These compounds will be further explored for structure–activity relationship.

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1. Introduction

Coagulation cascade involves the activation of a number of serine proteases leading to clot formation.[1] Factor VIIa (FVIIa) is a serine protease which plays an important role in the coagulation cascade. In recent years, the role of tissue factor/FVIIa complex (TF·FVIIa) has been studied in thrombosis-related disorders (the number one cause of mortality in developed countries), such as unstable angina, acute myocardial infarction, myocardial ischemia-reperfusion injury, venous thrombosis, sepsis, and glomerulonephritis.[2–9] The blood-coagulation cascade is divided into extrinsic and intrinsic coagulation pathways. FVIIa in complex with tissue factor (TF) initiates the extrinsic coagulation pathway. This complex activates factors IX to IXa and X to Xa, and factor Xa activates prothrombin to thrombin, which cleaves fibrinogen to fibrin, ultimately resulting in the formation of a fibrin clot.[1] It is also known that the excessive inhibition of the coagulation cascade at the final stages can lead to bleeding complications; therefore, the inhibition of TF·FVIIa complex which is the main trigger of thrombotic events could be advantageous.[10,11] The experiments in animal models from different research groups have established the proof-of-concept and have shown that specific inhibition of the TF·FVIIa complex results in an antithrombotic effect without enhancing bleeding propensity.[9,12,13] Different types of TF·FVIIa inhibitors have been reported. These range from proteins to small peptides and peptidomimetic organic small molecules. Active site inhibited FVIIa, TF mutants, NAP (nematode anticoagulant proteins), and antibodies to TF have been studied. Also, peptides derived from phage display have been shown to inhibit TF·FVIIa.[14]

While there were numerous publications of small molecule inhibitors for thrombin and FXa, there were only a few reports of FVIIa inhibitors at the onset of our drug design efforts. A recent review article by Lazarus has described most of the inhibitors reported until 2004, which includes different scaffolds having an amidine moiety attached to phenyl, indole, benzimidazole, or naphthalene ring systems.[15] A number of recent reports after 2004 have also described potent small molecules inhibitors.[16] Recently, a selective, slow binding inhibitor of FVIIa which binds

* Corresponding authors. Tel.: +1 205 444 4600; fax: +1 205 444 4640.
E-mail addresses: pkotian@biocryst.com (P.L. Kotian), babu@biocryst.com (Y.S. Babu), pchand@biocryst.com (P. Chand).

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to a nonstandard active site conformation of the enzyme and attenuates thrombosis formation in vivo has been described by Olivero, et al.[17] A few reports have appeared on biphenyl derivatives also.[18] Recently, various groups have attempted to replace the highly basic amidine moiety with nonamidine groups.[19a,b] Based on the favorable pharmacological properties of TF·FVIIa inhibition, we have initiated a program to develop potent and selective inhibitors of TF·FVIIa complex for the treatment of coagulation disorders.

2. Structure-based drug design

Since the catalytic domain of FVIIa is a trypsin-like serine protease,a wealth of structural data is available as complexes between small molecule inhibitors and their target enzymes such as thrombin, FXa, and trypsin. Based on these data and numerous crystal structures of FVIIa,[15] several crucial interactions were identified that would be important for both potency toward FVIIa and selectivity over other related serine proteases. Since the present inhibitors were designed to bind at the active site of FVIIa (Fig. 1), it was necessary to eschew the structural features that are common to the closely related enzymes such as FXa, thrombin, and trypsin and to focus the design effort toward utilizing the structural features that are unique to the FVIIa binding site. Crystal structures of the ternary complexes between soluble tissue factor (sTF), FVIIa, and the reported compounds were determined wherever deemed necessary to assist and guide the synthetic efforts. The numbering of the FVIIa and other serine protease residues used is based on topological equivalences with chymotrypsin.[19c]

Structure 9 shown in Scheme 1 represents the core of the designed inhibitors. Almost all the reported FVIIa active site inhibitors use a positively charged benzamidine moiety to anchor the inhibitor in the S1 pocket of the enzyme (Fig. 1). We added a phenyl ring (Ring A) with a planar peptide group to the benzamidine moiety occupying the S1 pocket. This addition was inferred after studying the DPhe-Phe-Arg chloromethyl ketone[19c] and 5L15 -a BPTI (bovine pancreatic trypsin inhibitor) mutant protein-inhibited sTF·FVIIa[20] crystal structures, where the relatively large S2 apolar site is occupied by a hydrophobic phenylalanine side chain and a disulfide bridge, respectively. Another phenyl ring (Ring B) was added to this scaffold to help us probe the S′ sites downstream to the active site as well. The carboxylic group was added to capture the positively charged Lys192 residue, a unique feature of FVIIa, as other related proteases have a structurally equivalent glutamine or glutamic acid residue at this position. Hence, we expected the negative charge on this ring to enhance the selectivity over thrombin **9**. With these two rings in place, manual and automated docking studies plus the structural work with **21** and a few other compounds led us to conclude that our core group of Rings A, B, and C bound exactly the same in the active site of FVIIa irrespective of any other additional substituents on them. As expected, the benzamidine group acted as an ideal arginine surrogate and formed the anchor point for all the inhibitors reported in this paper. The positively charged amidino group of the benzamidine moiety formed a symmetric salt bridge with the side chain carboxylate of Asp189 and was stabilized further by hydrogen bonds with the main chain of Gly219 and the side chain of Ser190 in the S1 site of sTF·FVIIa. However, the carboxylate group on Ring B bound on the inside of the active site interacting with the catalytic His57 instead of Lys192 residue whose side chain lies exposed to the solvent. All the inhibitors reported herein have an N-isobutylamide group in the R position that extends into the S′ sites of FVIIa downstream to the catalytic triad, filling in the shallow hydrophobic cavity lined by residues Leu41, Gln143, and Gln140 adopting a conformation that allows for maximum van der Waals interactions.[18]

The main differences between FVIIa and related enzymes are in their S2 pocket. The S2 pocket of FVIIa is relatively open and has a negative potential due to the presence of Asp60. Among the coagulation proteases, only FVIIa has a negatively charged residue at position 60. The side chain of Asp60 makes a hydrophilic pocket with the side chain oxygen atom of Tyr94 and the main chain carbonyl oxygen atom of Thr98 in the S2 site which is occupied by a water molecule in the crystal structure of sTF·FVIIa (Fig. 2). Armed with these facts, we decided to probe the S2 site with different substituents in search of a selective and potent inhibitor.

All the synthesized compounds were screened for activity against TF·FVIIa and most of them for related serine proteases such as thrombin, FXa, trypsin, and plasmin. The inhibition in each case is reported as the IC_{50}, and the selectivity is reported as their IC_{50} ratios Tables 1–4. Although chemistry and structure-based drug design efforts were done simultaneously, we report in this paper only the progress in structure–activity relationships around the S2 pocket. Further work will be presented in forthcoming papers.

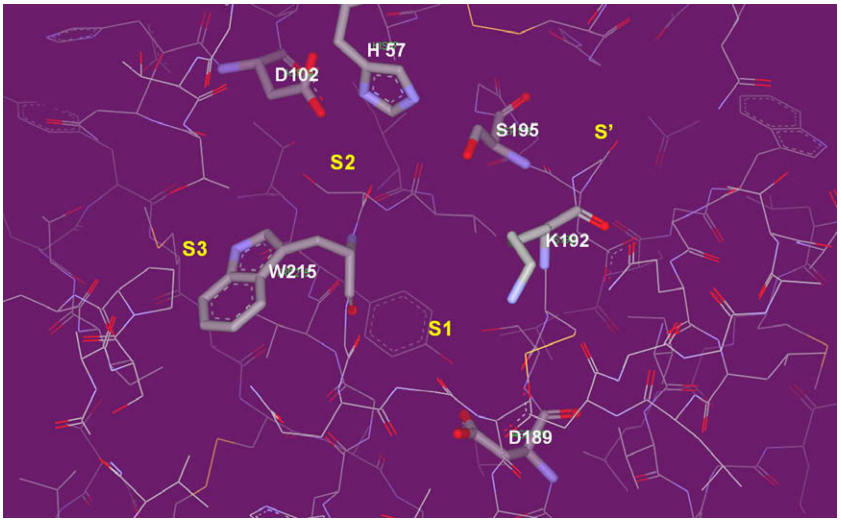
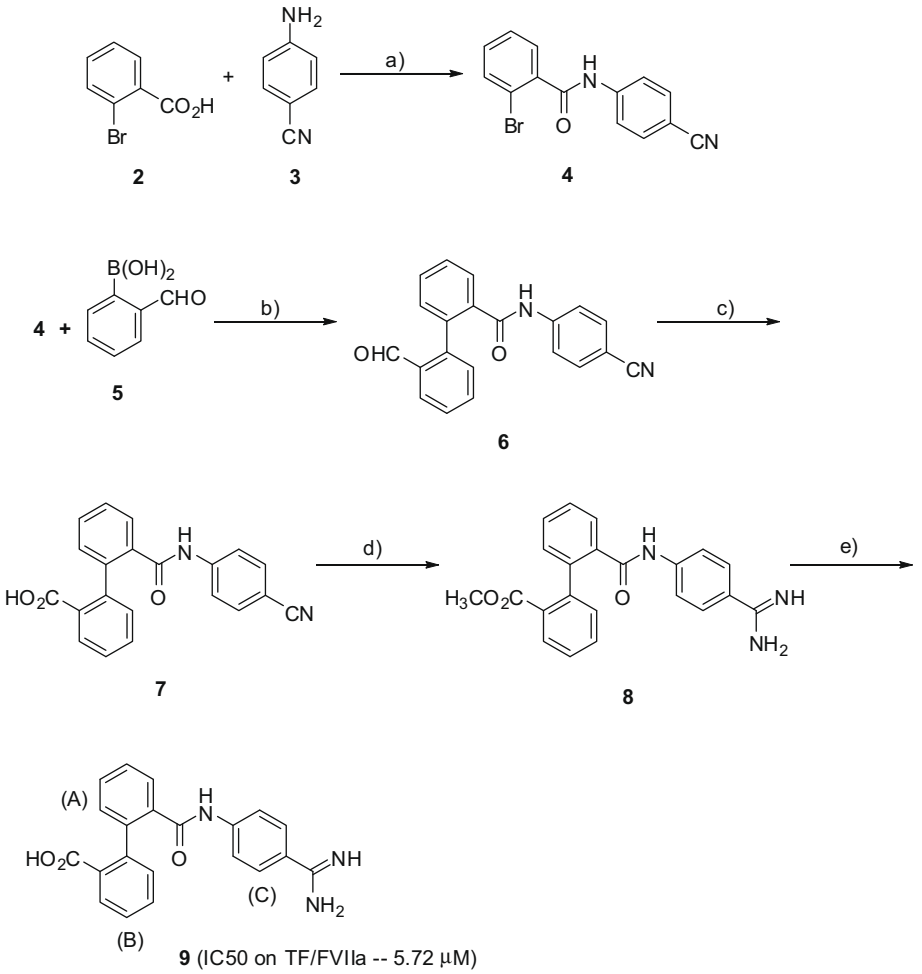


Figure 1. Binding site of the BCX inhibitors in the serine protease catalytic domain sTF·FVIIa complex. The catalytic triad of Serine 195, Aspartic Acid 102, and Histidine 57 is shown in sticks, with binding pockets S1, S2, S3, and S′ shown in yellow. Lysine 192, Tryptophan 215, and Asp189 have key interactions with the bound inhibitor.



Scheme 1. Reagents: (a) $SOCl_2$, CH_2Cl_2; (b) $Pd[(C_6H_5)_3P]_4$, K_3PO_4, KBr, dioxane; (c) $NaClO_2$, *t*-BuOH, CH_3CN, 2-methyl-2-butene, water; (d) (i) MeOH/HCl; (ii) MeOH/NH_3; and (e) NaOH, water.

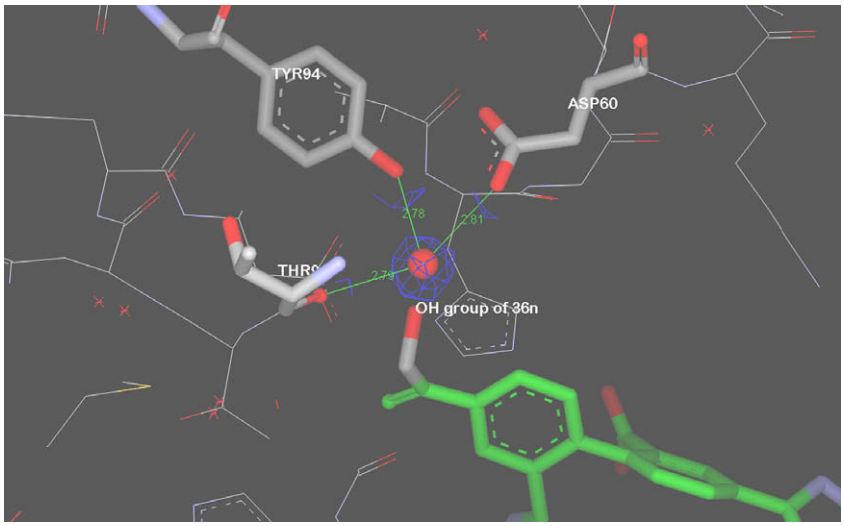
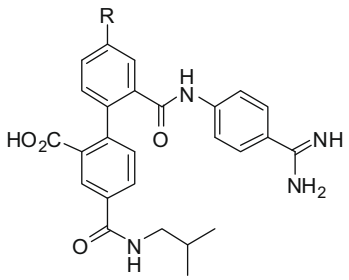
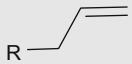
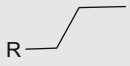
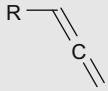
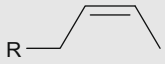
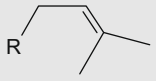
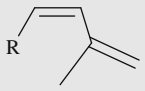


Figure 2. Negative charge in S2 site of FVIIa; with the conserved water molecule interacting with residues Tyr94, Thr98, and Asp60. The partial structure of **36n** (modeled atoms in gray/red) shown at the bottom of the picture. The hydroxyl group falls short of duplicating the water–protein interactions.

Table 1



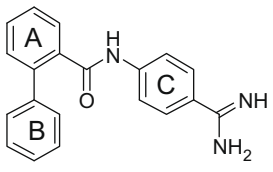
General Structure for 36a-36aq

Compound number	R	FVIIa*	Trypsin*	Thrombin*	FXa*	Plasmin*	Try/FVIIa	Thr/Fviia	Fxa/Fviia	Pla/FVIIa
21	R-H	0.120	0.17	64.00	103.00	ND	1.5	533.3	858.3	–
36a**	R-OH	0.067	0.39	50.00	50.00	ND	5.7	746.3	746.3	–
24	R-OCH_3	0.03	0.82	14.87	6.63	14.367	25.3	460.4	205.4	444.8
36b**		0.009	0.47	9.40	1.65	ND	52.2	1044.4	183.3	–
36c		0.097	5.99	12.30	50.00	>5.0	61.8	126.8	515.5	–
36d		0.042	2.42	19.17	1.90	2.470	57.6	456.3	45.2	58.8
36e		0.092	41.38	39.57	46.05	>5.0	449.8	430.1	500.5	–
36f		0.025	2.87	50.24	8.14	1.000	114.8	2009.6	325.6	40.0
36g**		0.013	0.53	17.90	1.17	0.070	40.8	1376.9	90.0	5.4
36h		0.009	0.56	27.96	1.50	ND	59.8	2974.5	159.6	–
36i		0.034	1.66	50.00	5.22	0.127	48.8	1470.6	153.5	3.7
36j		0.011	1.34	4.88	7.60	0.684	118.8	431.9	672.5	60.5
36k		0.045	0.95	12.98	26.96	0.308	21.1	288.4	599.1	6.8
36l		0.130	ND	ND	0.31	ND	–	–	2.4	–

* IC_{50} (nM).
** Compounds were complexed with TF·FVIIa and their crystal structures determined using single crystal X-ray diffraction.
ND = Not determined.

3. Chemistry

The core structure used to orient the key functionalities within the enzyme active site required three phenyl rings (designated A, B, and C in **1**).

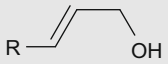
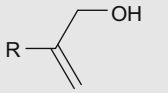
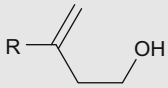
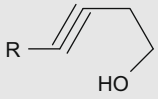
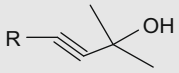
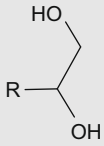
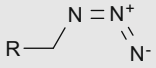
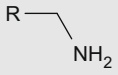


1

Three key functionalities augmenting the binding within the active site were identified: (a) the benzamidino group (Ring C) forming a salt bridge anchoring the inhibitor in the S1 pocket, (b) the carboxyl group on Ring B (shown in **9**, Scheme 1) for potential interaction with the positively charged Lys192 residue in the pocket, and (c) the isobutylcarboxamide group also on Ring B (shown in **21**, Scheme 3) filling the need for hydrophobic bonding. These three groups proved to be crucial for maintaining inhibitory potency.

In order to confirm the contribution to inhibitory potency of the Ring B substituents, specifically the carboxylic acid group, we synthesized **9**. We also synthesized the lead compound **21** bearing all the key substituents.

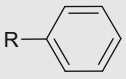
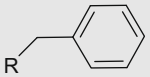
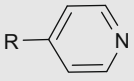
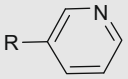
Table 2

Compound number	R	FVIIa*	Trypsin*	Thrombin*	FXa*	Plasmin*	Try/FVIIa	Thr/Fviia	Fxa/Fviia	Pla/FVIIa
36m**		0.052	1.77	22.73	6.62	ND	34.0	437.1	127.3	–
36n		0.012	3.54	39.70	7.10	0.810	295.0	3,308.3	591.7	67.5
36o		0.029	2.19	56.22	11.27	0.358	75.5	1,938.6	388.6	12.3
36p		0.320	ND	ND	ND	ND	–	–	–	–
36q		0.500	0.40	17.90	59.00	ND	0.8	35.8	118.0	–
36r		0.926	ND	ND	ND	ND	–	–	–	–
36s		1.610	22.14	50.00	50.00	>5.0	13.8	31.1	31.1	–
36t		0.060	ND	ND	ND	ND	–	–	–	–
36u		1.220	ND	ND	ND	ND	–	–	–	–

* IC_{50} (nM).
** Compounds were complexed with TF·FVIIa and their crystal structures determined using single crystal X-ray diffraction.
ND = Not determined.

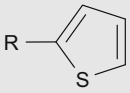
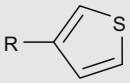
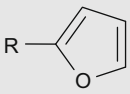
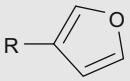
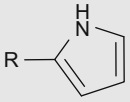
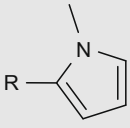
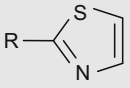
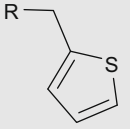
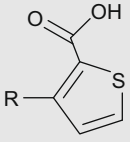
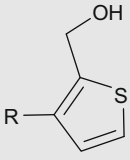
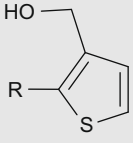
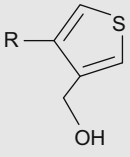
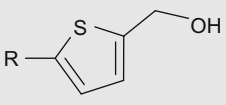
Synthetic routes to the test analog **9** are shown in Scheme 1. Some of the procedures of Scheme 1 later proved applicable in the syntheses of candidate inhibitors with structures having various substituents in the Ring A and the key functional groups fixed in Rings B and C. General procedures used in these syntheses are represented in Section 6 by illustrative procedures, and specific applications in the present work are identified in the appropriate synthetic schemes.

Table 3

Compound number	R	FVIIa*	Trypsin*	Thrombin*	FXa*	Plasmin*	Try/FVIIa	Thr/Fviia	Fxa/Fviia	Pla/FVIIa
36v		0.098	0.42	11.82	0.35	ND	4.3	120.6	3.6	–
36w**		0.021	0.57	8.51	1.75	0.656	26.9	399.5	82.2	30.8
36x		0.231	1.21	35.39	19.41	ND	5.3	153.2	84.0	–
36y		0.546	3.02	50.00	36.49	ND	5.5	91.6	66.8	–

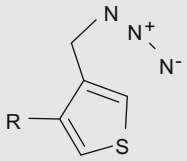
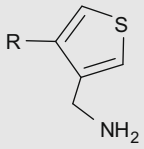
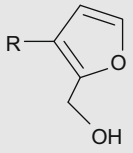
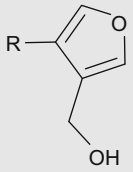
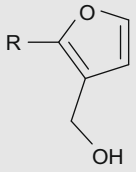
* IC_{50} (nM).
** Compounds were complexed with TF·FVIIa and their crystal structures determined using single crystal X-ray diffraction.
ND = Not determined.

Table 4

Compound number	R	FVIIa*	Trypsin*	Thrombin*	FXa*	Plasmin*	Try/FVIIa	Thr/Fviia	Fxa/Fviia	Pla/FVIIa
36z		0.010	0.24	8.02	0.15	ND	16.0	534.7	10.0	—
36aa**		0.010	0.38	14.99	0.04	0.035	37.7	1499.0	4.0	3.5
36ab		0.009	0.37	14.67	1.30	0.163	41.4	1630.0	144.4	18.1
36ac		0.011	0.47	16.07	0.12	0.252	42.6	1460.9	10.9	22.9
36ad		0.014	0.43	19.88	0.05	ND	30.7	1420.0	3.4	—
36ae		0.076	0.41	86.86	0.05	ND	5.4	1142.9	0.7	—
36af		0.104	1.47	70.40	0.23	0.494	14.1	676.9	2.2	4.8
36ag		0.011	1.51	42.12	8.15	0.913	126.6	3539.5	685.2	76.7
36ah		0.190	ND	ND	ND	ND	—	—	—	—
36ai		0.0360	3.04	50.00	0.45	0.408	84.4	1388.9	12.5	11.3
36aj**		0.330	4.39	50.00	0.64	ND	13.3	151.5	1.9	—
36ak**		0.009	0.51	63.10	0.13	0.060	56.7	7011.1	14.4	6.7
36al		0.650	ND	ND	ND	ND	—	—	—	—

(*continued on next page*)

Table 4 (*continued*)

Compound number	R	FVIIa*	Trypsin*	Thrombin*	FXa*	Plasmin*	Try/FVIIa	Thr/Fviia	Fxa/Fviia	Pla/FVIIa
36am		0.009	1.18	156.80	0.05	0.242	132.9	17618.0	5.7	27.2
36an		0.033	1.77	110.48	0.01	0.229	54.2	3378.6	0.3	7.0
36ao		0.008	0.74	>50	0.02	0.457	92.5	—	2.5	57.1
36ap		0.020	2.29	>50	0.23	0.554	114.5	—	11.5	27.7
36aq*		0.024	0.77	50.00	0.15	1.066	32.1	2083.3	6.3	44.4

* IC_{50} (nM).
** Compounds were complexed with TF·FVIIa and their crystal structures determined using single crystal X-ray diffraction.
ND = Not determined.

Synthesis of **9** (Scheme 1), whose Ring B is without the isobutylcarboxamide group started from 2-bromobenzoic acid **2**, which was coupled via its aroyl chloride (formed using thionyl chloride) with 4-aminobenzonitrile **3** to give the amide **4**. Suzuki coupling of **4** with commercially available 2-formylphenylboronic acid **5** gave **6** which was oxidized with sodium chlorite to give carboxylic acid **7**. Treatment of compound **7** with methanolic HCl effected dual conversion of the carboxyl group to the methyl ester and the nitrile to the imidate. Treatment of the imidate with ammonia then gave amidine **8**. Basic hydrolysis of the ester group then led to target **9.**

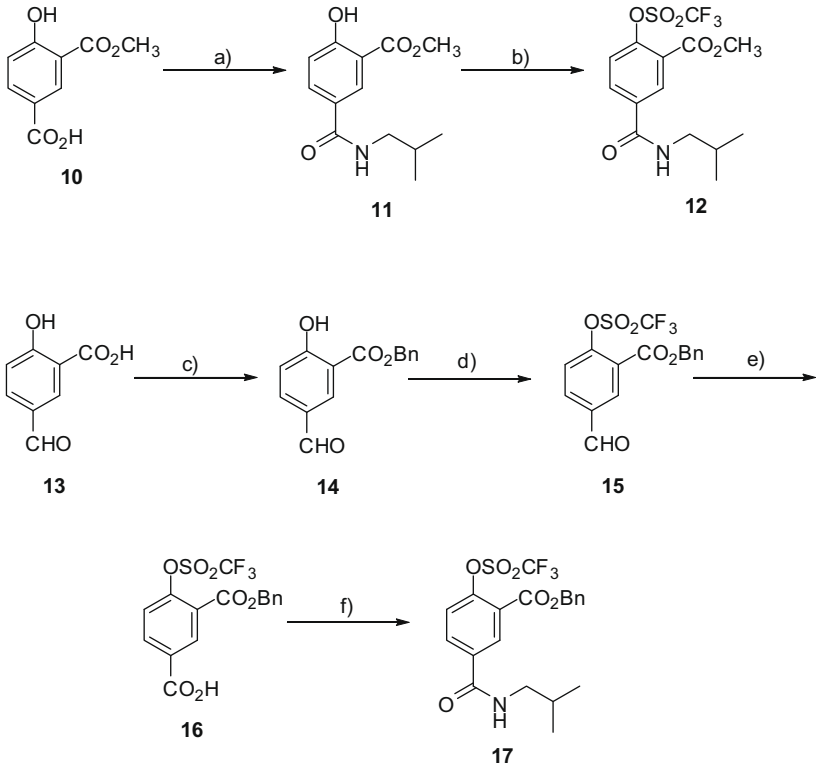
The synthesis of **21** is outlined in Schemes 2 and 3. In Scheme 2, **10**[21] was converted via its aroyl chloride (from thionyl chloride) to the isobutylcarboxamide **11**, which was converted by a standard method to its triflate **12** by reacting with trifluoromethanesulfonic anhydride. The benzyl ester **17** (corresponding to methyl ester **12**) was prepared from commercially available 3-formylsalicylic acid **13**. Treatment of **13** with benzyl bromide in DMF containing $NaHCO_3$ afforded benzyl ester **14**, which was converted to the triflate **15** using trifluoromethanesulfonic anhydride. Oxidation of the formyl group with sodium chlorite gave carboxylic acid **16** which was coupled with isobutylamine through the reaction of oxalyl chloride to give **17**.

Suzuki coupling of the triflate of **17** with 2-formylphenylboronic acid **5** gave **18** (Scheme 3). Mild oxidation of the formyl group of **18** with sodium chlorite gave the carboxylic acid **19**. Coupling of **19** with benzamidine gave the amide **20**, which upon hydrogenolysis, gave **21**.

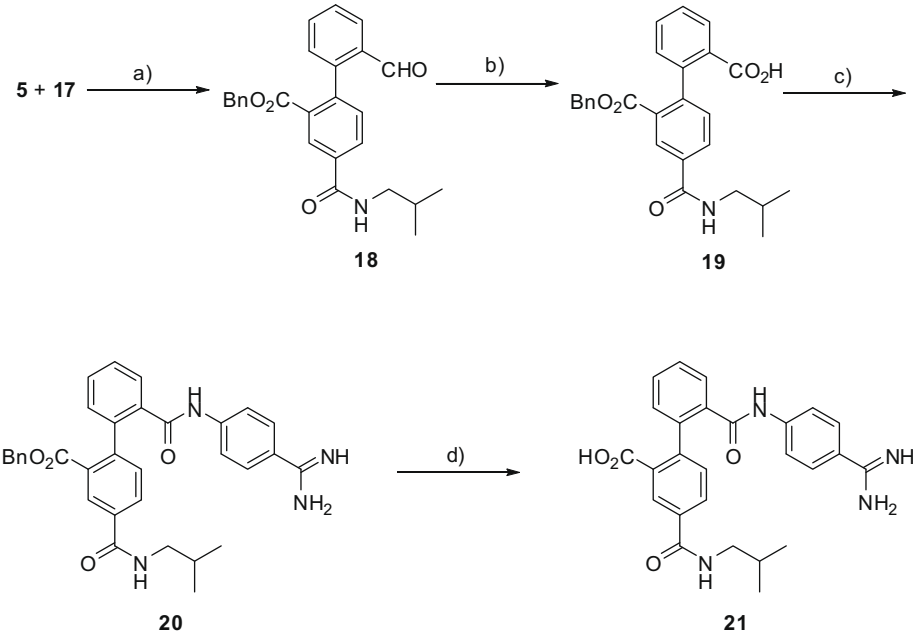
Comparison of the IC_{50} values against FVIIa for **9** and **21** shows the markedly improved potency of **21** relative to **9**. These findings encouraged us to synthesize a series of analogs of **21** bearing various substituents in Ring A while Rings B and C carried the key functions required for inhibition of FVIIa.

Synthesis of the analogous Ring A derivative **24** bearing a methoxy group is shown in Scheme 4. Suzuki coupling of triflate **12** with commercially available 2-formyl-4-methoxyphenylboronic acid **22** afforded **23** which upon sequential oxidation with sodium chlorite, coupling with benzamidine, and basic hydrolysis (as described for **21**) afforded **24**.

Outlined in Schemes 5 and 6 are the approaches used to synthesize a sizable library of derivatives for SAR studies bearing substituents in Ring A. The pivotal intermediates to the Ring A-substituted candidates were the benzyl ester triflate **31a** or the corresponding methyl ester triflate **31b** (Scheme 5). Suzuki coupling of benzyl ester **17** or methyl ester **12** with 2-formyl-4-benzyloxyphenylboronic acid **25**[22] gave aldehydes **26a,b**. Oxidation of aldehyde group on **26a,b** with sodium chlorite gave carboxylic acids **27a,b**. The free carboxylic acid on **27a,b** was converted to methoxyethylmethyl esters (MEM ester) **28a**,**b** using MEM chloride and sodium bicarbonate. Hydrogenolysis of **28b** using Palladium on Carbon in a hydrogen atmosphere gave phenol **30b**. However, similar hydrogenolysis of **28a** rendered hydrolysis of benzyl ester along with debenzylation of benzyl ether to furnish the corresponding phenolic carboxylic acid **29**. The benzyl group was selectively put back on carboxylic acid of **29** using benzyl bromide and sodium bicarbonate to afford **30a**.



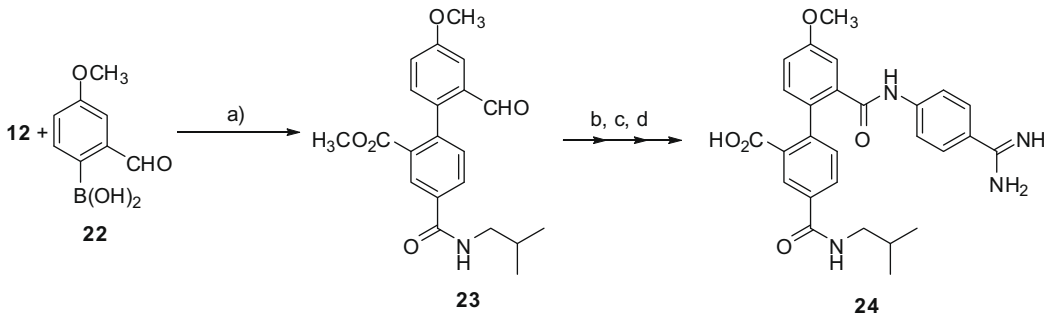
Scheme 2. Reagents: (a) (i) $SOCl_2$, CH_2Cl_2; (ii) isobutylamine, CH_2Cl_2; (b) $(SO_2CF_3)_2O$, pyridine, CH_2Cl_2; (c) $C_6H_5CH_2Br$, $NaHCO_3$, DMF; (d) $C_6H_5N(SO_2CF_3)_2$, Et_3N, DMF; (e) $NaClO_2$, *t*-BuOH, CH_3CN, 2-methyl-2-butene, water; and (f) (i) $(COCl)_2$, CH_2Cl_2; (ii) isobutylamine, CH_2Cl_2.



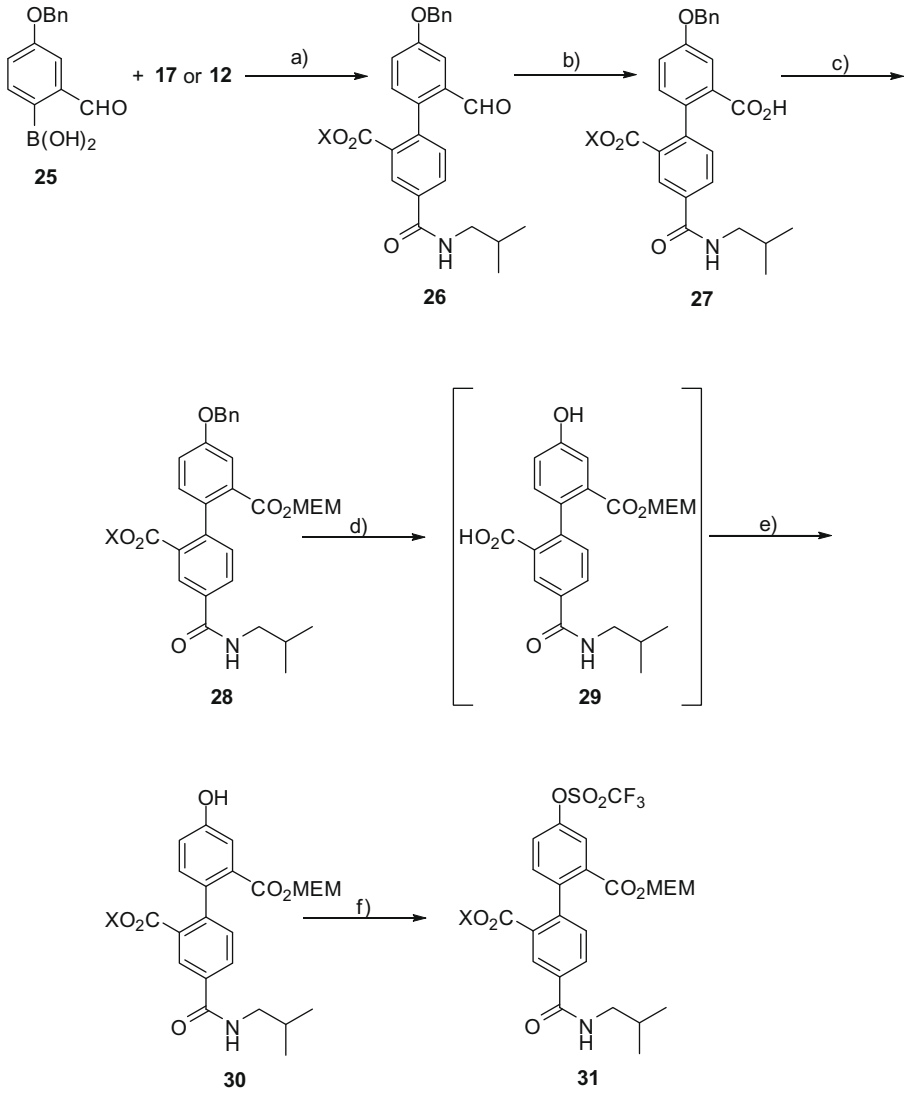
Scheme 3. Reagents: (a) $Pd[(C_6H_5)_3P]_4$, $NaHCO_3$, DME/H_2O; (b) $NaClO_2$, *t*-BuOH, CH_3CN, 2-methyl-2-butene, water; (c) 4-amino-benzamidine, DCC, pyridine; and (d) NaOH, water.

The hydroxyl group on **30a,b** was converted to the corresponding triflate using N-phenylbis(trifluoromethane sulfonamide) to give the required compounds **31a,b**.

With supplies of the versatile and reactive triflates **31a,b** available, we proceeded to prepare compounds substituted in Ring A using mainly three methods—Stille coupling, Suzuki coupling, or directly with substituted alkynes as outlined in Scheme 6. The appropriate R-y (**32**) was either commercially available or prepared through literature procedures using commercially available starting materials. The commercial source and the appropriate reference are given in Section 6. The coupling of triflate **31a** or **31b** with the appropriate **32** (tributylstannyl, trimethylstannyl, alkyne,

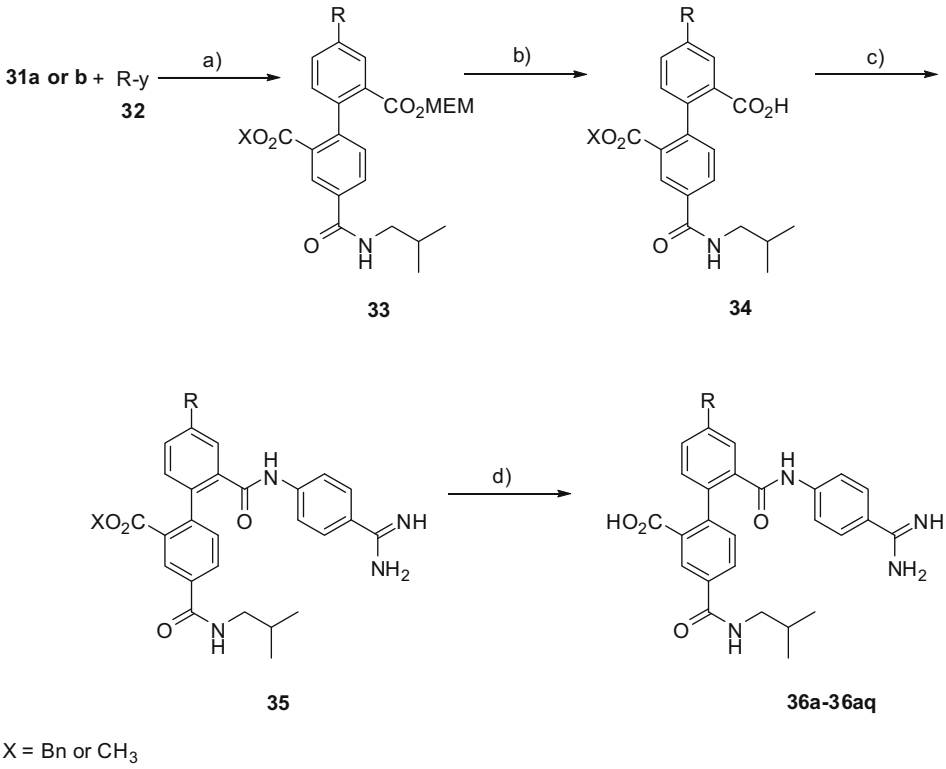


Scheme 4. Reagents: (a) $Pd[(C_6H_5)_3P]_4$, $NaHCO_3$, DME/H_2O; (b) $NaClO_2$, *t*-BuOH, CH_3CN, 2-methyl-2-butene, water; (c) 4-amino-benzamidine, DCC, pyridine; and (d) NaOH, water.



Scheme 5. Reagents: (a) $Pd[(C_6H_5)_3P]_4$, K_3PO_4, KBr, dioxane; (b) $NaClO_2$, *t*-BuOH, CH_3CN, 2-methyl-2-butene, water; (c) MEM-Cl, $[CH(CH_3)_2]_2N–C_2H_5$, CH_2Cl_2; (d) H_2, Pd/C, ethanol; (e) $C_6H_5CH_2Br$, $NaHCO_3$, DMF; and (f) $C_6H_5N(SO_2CF_3)_2$, Et_3N, CH_2Cl_2.

or boronic acid derivative) resulted in biaryl compound **33** with the appropriate R group as used in the compound **32** precursors. Other R substituents, such as alkyl, vicinal diol, azido, and amino groups were generated through functionalization of alkene or aldehyde groups present in the appropriate compound **33** as described below. Compounds **33c** (R = ethyl) and **33e** (R = n-propyl) were obtained by catalytic hydrogenation of the olefinic groups on **33b** (R = vinyl) and **33d** (R = allyl). The vicinal diol **33s** [R = CH(OH)-



Scheme 6. Reagents: (a) $Pd[(C_6H_5)_3P]_4$, K_3PO_4, KBr, dioxane or $Pd[(C_6H_5)_3P]_4$, $NaHCO_3$, DME/H_2O or $[(C_6H_5)_3P]_2PdCl_2$ with or without $(C_2H_5)_4NCl$, DMF; (b) 6 N HCl, DME; (c) 4-aminobenzamidine, DCC, pyridine; and (d) NaOH, water.

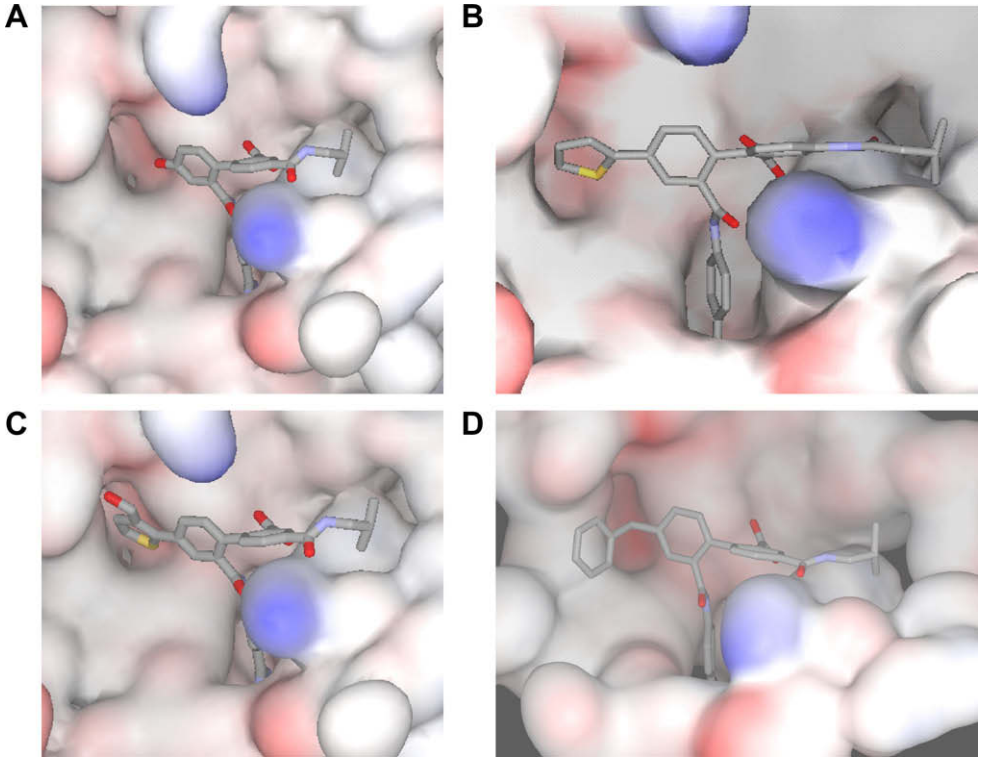


Figure 3. Crystal structures of **36a**, **36aa**, **36aj**, and **36w**, shown in A, B, C, and D, respectively. All the inhibitors are shown as sticks and FVIIa is shown as a Connolly surface colored by charge (red for negative and blue for positive). Systematic attempts were made to fill up the S2 site of the enzyme with an appropriate R group. While the hydroxyl (A) is too small, the thiophene rings (B and C) are optimal. The phenyl ring with a CH_2 linker, being large, turns away to bind in the S3 site of FVIIa. All the crystal structures were refined to R_{free} values 30–31% at 2.5–2.1 Å resolution.

CH_2OH] was obtained by the dihydroxylation of vinyl compound **33b** using osmium tetraoxide. Oxidative cleavage of diol **33s** with sodium metaperiodate followed by sodium borohydride reduction of the resulting aldehyde gave the alcohol that was treated with methanesulfonyl chloride to yield the mesylate. The mesylate was treated with sodium azide to furnish azido **33t** (R = azidomethyl). Sodium borohydride reduction of the formyl groups on compounds obtained by Suzuki coupling of formyl-substituted furan and thiophene boronic acids with **31a** and **31b** gave **33ai** (R = 2-hydroxymethylthiophen-3-yl), **33aj** (R = 3-hydroxymethylthiophen-2-yl), **33ak** (R = 3-hydroxymethylthiophen-4-yl), **33al** (R = 2-hydroxymethylthiophen-5-yl), **33ao** (R = 2-hydroxymethylfuran-3-yl), **33ap** (R = 3-hydroxymethylfuran-4-yl), and **33aq** (R = 3-hydroxymethylfuran-2-yl). Compound **33am** (R = 3-azidomethylthiophen-4-yl) was obtained from **33ak** (R = 3-hydroxymethylthiophen-4-yl) through its mesylate and subsequent reaction with sodium azide.

Compounds **33b–33aq** were converted to **34b–34aq** through acid hydrolysis with HCl in DME, and the coupling of resultant **34b–34aq** with 4-aminobenzamidine using DCC as the coupling agent yielded **35b–35aq**. Finally, basic hydrolysis of **35b–35aq** gave the target compounds **36b–36aq.**

For the preparation of **36a**, MEM ester in **31a** was hydrolyzed to acid **34a** (R = OSO_2CF_3) with magnesium bromide ethereate, which was further reacted with 4-aminobenzamidine hydrochloride to yield **35a** (R = OSO_2CF_3). Basic treatment of **35a** hydrolyzed both triflate and ester and generated desired **36a**. For the preparation of **36ah**, the formyl group in **33ah** (R = 2-formylthiophen-3-yl) was oxidized with sodium chlorite to give **33ah** (R = 2-carboxythiophen-3-yl) and the carboxy group was protected as benzyl ester with benzyl bromide and sodium bicarbonate to give **33ah** (R = 2-benzyloxycarbonylthiophen-3-yl). The subsequent coupling with 4-aminobenzamidine hydrochloride and basic hydrolysis generated the desired target **36ah**. Amino compounds **36u** and **36an** were obtained from the corresponding azido derivatives **36t** and **36am** through catalytic hydrogenation.

4. Structure–activity relationships

On the core structure **1** having three phenyl rings (A, B, and C with amidine), the first carboxyl group was introduced on Ring B to interact with positively charged Lys 192 **9**. The IC_{50} value of **9** on TF/FVIIa was 5.72 μM. Further introduction of the isobutylaminocarbonyl group on Ring B **21** filling the hydrophobic pocket increased binding to TF/FVIIa resulting in an IC_{50} value of 0.12 μM.

Replacing the hydrogen atom in **21** with a hydroxyl group (**36a**, Fig. 3A) did not seem to affect the binding affinity (Table 1). The next two compounds, **36b** and **36c**, differ only at the terminal ends by their vinyl and ethyl groups, the 10-fold better binding of **36b** results from the restricted rotational freedom and relatively smaller size of the vinyl group. The difference in the activities of **36d** (propenyl) and **36e** (propyl) compounds shows similar trend although to a lesser extent. These results also alerted us to the fact that this medium-sized S2 cavity of FVIIa is very sensitive even to small changes to the occupying chemical groups. Based upon the better activity of compounds with the unsaturated chain **36b** and **36d** and the size of pocket, we decided not to synthesize any compound without unsaturation and restricted the length to only four carbon atoms. The relatively lower binding of compounds with three to four carbon chain length **36d**, **36e**, **36i**, **36k**, and **36l** with respect to **36b** further illustrated that the size of the pocket is not big enough to accommodate optimally these groups. On the same lines, compounds with only two carbon atom length **36f** and **36g** and unsaturation have better activity. The better activity of allene **36h** (three carbon atoms but two double bonds) could also be explained by overall shorter length because of two double bonds. The variations in the binding constants are also probably the result of a delicate balance of steric hindrance with the S2 cavity and finding a suitable conformation dictated by the rotational freedom resulting from the C–C bonds on the R group. Compounds **36k** and **36l** have bulkier branched carbon chains, which are too big to fit into the narrower S2 cavity away from the catalytic triad, hence they show lower activity compared to **36b**.

Further, we explored the effects of the hydrophilic groups while keeping the carbon chain only to four atoms and unsaturated chain in most of the cases. The objective of synthesizing these compounds with longer flexible carbon chains with hydrogen bonding capabilities was in an attempt to penetrate the narrow end of the S2 site and displace the structurally conserved water molecule, mentioned in the 'Structure-Based Drug Design' Section. Compounds **36m-u** (Table 2) have hydrophilic terminal group atoms in the R position. The X-ray structure of **36m** complexed with sTF·FVIIa revealed that the hydroxyl group extended into the solvent region, rather than binding into the deeper end of the S2 cavity, which translated into reduced activity. All these compounds except **36n** showed reduced activity probably due to the same reason as explained for **36m**. Modeling studies on **36n** (Fig. 2) showed that the hydroxyl on the R group was very close (less than 1.50 Å) to the water molecule present in the active site. Potentially, the water molecule was displaced on binding, but the hydroxyl group was not able to duplicate the hydrogen bonding network in this region. As seen from Figure 2, the distance between the water and Thr98 hydroxyl group is 3 Å compared to the distance between the hydroxy group on **36n** and Thr98 which is 3.45 Å. Similarly, the distance between water and Tyr94 hydroxyl group is 3 Å, while the distance between the hydroxyl group on **36n** and Tyr94 hydroxy is 3.77 Å. Due to its ability to displace the water molecule in the S-2 region, which is a unique feature of FVIIa among all the serine proteases of the clotting cascade, the selectivity profile of **36n** (Table 2) was one of the best in this category. The poor binding of **36u** and **36s** could also be explained due to the fact that polar groups bound better when they could reach deep into the S2 cavity, but **36s** and **36u** place the hydroxy and amino groups at the mouth of the S2 site which is lined by hydrophobic residues Thr98, Thr99, Gly97, and Trp215.

Compounds **36v–y** (Table 3) have six-membered phenyl, benzyl, and pyridyl rings as R groups. Compounds **36x** and **36y** bind with less affinity probably due to the placement of the nitrogen atom on the pyridyl ring in a hydrophobic environment, while the other two compounds **36v** and **36w** with phenyl rings as R groups bind much better as they place the aromatic rings in the hydrophobic cavity. Structural studies revealed that in **36w**, the terminal phenyl ring preceded by one carbon atom spacer swings over and stacks above the Trp215 in an edgewise fashion in the crystal structure (Fig. 3D), therefore resulting into a compound having better activity than **36v**, which is without a spacer.

At this juncture, modeling studies suggested that smaller five-membered rings would be better suited as R groups. Compounds **36z**–**36aq** (Table 4) containing a five-membered heterocyclic ring were synthesized to validate our modeling studies and continue the quest for a potent and selective inhibitor. Five-membered ring compounds with one hetero atom, thiophenes **36z**, **36aa**, furans **36ab**, **36ac**, and pyrrole **36ad** and with two hetero atoms, thiazole **36af** were prepared and tested to obtain IC_{50} value on TF·FVIIa. One compound **36ag** with one carbon spacer and a thiophene ring was also prepared. The results indicated that the compounds with one hetero atom (with and without spacer) had similar IC_{50} values as obtained for alkene **36b** and alkyne **36f**, which means that these five-membered rings fit in these pockets quite optimally. However,

36af with two hetero atoms showed reduced activity. Structural studies done on **36aa** (Fig. 3B) indicated that the five-membered rings stacked parallel to the catalytic His57 residue, confining the sulfur atom to point up or down. The vinyl group in **36b** potentially binds in the same manner as these heterocycles using Pi-stacking with His57 residue.

We then decided to introduce substituents on these ring systems (Table 4) and we chose to introduce methyl at the N atom in pyrrole; carboxyl, hydroxymethyl, azidomethyl, and aminomethyl on thiophene; and hydroxymethyl on furan. Methyl at the pyrrole nitrogen **36ae**, carboxyl at the 2-position of thiophen-3-yl **36ah**, hydroxymethyl at the 3-position of thiophen-2-yl **36aj**, and hydroxymethyl at the 2-position of thiophen-5-yl **36al** were not tolerated well and resulted in poor binding compared to the unsubstituted analogs. The methyl group in pyrrole probably disrupted the stacking of pyrrole ring parallel to His57 and carboxyl and hydroxymethyl in thiophene disrupted the arrangement and also offered steric hindrances to protein residues by confining the sulfur atom in the five-membered ring in a less optimal position. However, **36aq** in the furan series, which is equivalent to **36aj** in the thiophene series in terms of positions of the oxygen atom and the hydroxymethyl group, maintained the activity probably because the oxygen atom is less bulky than the sulfur atom. Other compounds in the furan series such as **36ao** and **36ap** also maintained good activity. The position of the hydroxymethyl, azidomethyl, and aminomethyl in the thiophene series **36ai**, **36ak**, **36am**, and **36an** probably does not disturb the position of the sulfur atom, maintaining optimal activity. The crystal structures of the complexes of **36ak** and **36aq** with TF·FVIIa were also determined which further confirm the fact that these five-membered rings stack parallel to His57 in spite of the hydroxymethyl group being present.

5. Conclusion

In conclusion, we have discovered a novel series of biphenyl derivatives that are highly potent and selective TF·FVIIa inhibitors with the help of structure-based drug design. Parallel synthesis coupled with structure-based design allowed us to make a small library to explore the S2 pocket of the active site. As a result, a number of compounds were discovered to have an IC_{50} value for the inhibition of TF·FVIIa in the low nanomole range. Since it is important to have good selectivity also over other related serine proteases, X-ray crystal structure determination of a number of complexes of potent inhibitors with TF·FVIIa guided us to design selective inhibitors. We have identified a series of unique TF/FVIIa inhibitors that have selectivity over related serine proteases potentially due to the binding of pharmacophores in the S2 pocket which is exemplified by X-ray crystal structures.

6. Experimental

6.1. Crystallization, data collection, and refinement

The best crystals of the sTF·FVIIa inhibitor(15 mg/mL) were grown by a hanging drop method at room temperature from 2-μL drops made of one part protein solution and one part well solution (14% [w/v] PEG 4 K, 0.1 M $MgCl_2$, 0.1 M ADA buffer (pH 6.5) hanging over 0.5 mL of well solution). X-ray diffraction data were measured in house with an R-AXIS IV++ imaging plate detector with radiation generated from a Rigaku RU-200 rotating anode generator operating at 5 kW power. All crystals used for data collection were passed through a cryo-protectant solution containing 12% glycerol and then flash-frozen in situ using an X-Stream Cryostat (Rigaku MSC). Data were collected at a crystal-to-plate distance of 120 mm, with an oscillation angle of 1.0° and an exposure time of 2 min/frame. Typically, 100° of data were sufficient to generate essentially complete data sets. Data were collected and processed using the CRYSTAL CLEAR suite of programs. Crystals of the sTF·FVIIa inhibitor ternary complex crystals are orthorhombic with one molecule per asymmetric unit. Overall R_{symm} were usually in the range of 10–11% for these data. Crystals of this ternary complex belong to the orthorhombic crystal system with space group $P2_12_12_1$ and are isomorphous to the earlier reported DPhe-Phe-Arg (cmck) (DFFR) inhibited FVIIa-sTF (PDB entry 1DAN) crystals. For all the computations and analyses, CNX (Accelrys) suite of programs were used. Structure solution of the ternary complex began with a rigid body rotation-translation refinement using the coordinates of FVIIa·sTF (from the PDB entry 1DAN) minus the inhibitor and water molecules with the temperature factor for all the atoms set to average B factor(30 $Å^3$) for the entire complex. The refinements generally started at 32–35% R factor (R_{free} 34–36%). After the convergence of the rigid body refinement at 28–30% R factor (R_{free} 31–33%) and one round of temperature factor and positional coordinate refinement including the water molecules (restricted to 100), picked using the automatic water picking routine implemented in CNX, the R factor dropped to 24–26% (R_{free} 28–29%). The inhibitor was easily located and modeled in the $(2|F_o| - |F_c|)$ and $(|F_o| - F_c|)$ electron density maps. For most inhibitor complexes the computation was deemed sufficient and stopped at this stage if the electron density maps were clear enough and allowed us to interpret the binding and conformation of the inhibitor unambiguously. Initial coordinates and stereochemical features for the inhibitor-portion were prepared using the builder module of Quanta.

6.2. General methods

Commercially available solvents and reagents were used as received. All reactions were conducted under a dry nitrogen atmosphere. Melting points were obtained in open capillary tubes in a Mel-Temp II melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Mass spectra were obtained on a Fison Trio 2000 quadrupole mass spectrometer. ^1H NMR spectra were recorded on a Bruker AM300 or Bruker AM360 spectrometer using tetramethylsilane as internal standard. IR spectra were run on a Biorad FTS-7 FTIR spectrometer. Flash column chromatography was carried out using 230–400 mesh silica gel. Thin-layer chromatography was used as an indicator for the completion of the reactions and was performed on K6F Silica Gel 60A plates. The spots on TLC were visualized by UV and/or spraying the plate with 1 M ammonium sulfate in 1 N sulfuric acid and heating the plate on a hot plate. Organic solvent extracts in the isolation procedures were dried over anhydrous magnesium sulfate. Abbreviations used: Boc = tert-butoxycarbonyl; Bn = benzyl; THF = tetrahydrofuran; DMF = dimethylformamide; TFA = trifluoroacetic acid; CMA-80 = chloroform (80), methanol (18), and ammonium hydroxide (2) mixture.

6.3. Methods

6.3.1. Method A. Conversion of acid to amide

6.3.1.1. Method A-1. The carboxylic acid (1.0 mmol) was dissolved in $SOCl_2$ (12.6 mmol), DMF (few drops) was added, and the solution was refluxed for 2 h, then evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (3 mL) and the solution was cooled (ice-H_2O bath) while the appropriate amine (1.2–5.0 mmol) was added with stirring. The solution was stirred at 20–25 °C overnight

before it was washed with 1 N HCl followed by saturated $NaHCO_3$ solution, H_2O, and brine in the order given. Evaporation in vacuo followed, and the product was purified by recrystallization or flash chromatography.

6.3.1.2. Method A-2. A solution of the acid (1.0 mmol) in CH_2Cl_2 (5 mL) was treated with a solution of oxalyl chloride in CH_2Cl_2 (2 M, 1.25 mL, 2.5 mmol) followed by DMF (one drop). The reaction mixture was stirred at 20–25 °C for 2 h, then evaporated in vacuo. The residue was dissolved in CH_2Cl_2, and the evaporation was repeated. The residue was again dissolved in CH_2Cl_2 (10 mL), and the solution was treated with the appropriate amine (1.2 mmol) and Et_3N (3 mmol). The reaction mixture was stirred for 16 h, washed successively with H_2O and brine, then dried and concentrated in vacuo. The residue was purified by recrystallization or flash chromatography to furnish the amide.

6.3.2. Method B. Conversion of phenolic hydroxyl to triflate

6.3.2.1. Method B-1. A solution of the phenol (1.0 mmol) in CH_2Cl_2 (2.5 mL) under N_2 was treated with pyridine (5 mmol) and the solution was cooled to −10 °C. The cold mixture was then treated dropwise for 10 min with a solution of triflic anhydride (2.0 mmol) in CH_2Cl_2 (2.5 mL). The stirred reaction mixture was allowed to warm to 20–25 °C, then maintained for 16 h before it was treated with saturated $NaHCO_3$ solution. The organic layer was separated, washed successively with 1 N HCl, saturated $NaHCO_3$, H_2O, and brine, then dried and evaporated in vacuo. The residue was purified by recrystallization or flash column chromatography to give the desired triflate.

6.3.2.2. Method B-2. A solution of the phenolic compound (1.0 mmol) in DMF (10 mL) was treated with N-phenylbis(trifluoromethanesulfonamide (1.1 mmol) and Et_3N (2.0 mmol), and the mixture was stirred at 20–25 °C overnight. After addition of ice water, the mixture was extracted twice with Et_2O, and the combined Et_2O solution was washed with brine, dried, and evaporated to give the desired triflate, which was purified by recrystallization or flash column chromatography.

6.3.3. Method C. Conversion of acid to ester (MEM/benzyl)

6.3.3.1. Method C-1 (MEM ester). A mixture of the carboxylic acid (1.0 mmol), $NaHCO_3$ (1.05 mmol), and MEM chloride (1.05 mmol) in DMF (10 mL) was stirred at 20–25 °C for 24 h. Cold H_2O was added and the mixture was extracted twice with Et_2O. The Et_2O solution was washed with brine, dried, and concentrated in vacuo. The residue was purified by recrystallization or flash column chromatography to give the MEM ester.

6.3.3.2. Method C-2 (MEM ester). To a solution of aromatic acid (1.0 mmol) in THF (10 mL) were added diisopropylethylamine (2.0 mmol) and 2-methoxyethoxymethyl chloride (1.1 mmol). The reaction mixture was stirred at room temperature for 3 h and was diluted with ether (25 mL). The reaction mixture was washed with water (10 mL) and brine (10 mL), then dried and concentrated in vacuo to obtain the product as a colorless oil. The product was purified by flash column chromatography to furnish the desired product.

6.3.3.3. Method C-3 (benzyl ester). To a solution of aromatic acid (1.0 mmol) in DMF (10 mL) were added $NaHCO_3$ (1.05 mmol) and benzyl bromide (1.05 mmol). The mixture was stirred at 20–25 °C for 24 h, then quenched with ice water and extracted twice with EtOAc. The organic layers were combined, washed with water and brine, dried, and concentrated in vacuo to furnish the crude product. Purification by crystallization or flash column chromatography gave the desired ester.

6.3.4. Method D. Coupling reactions through aryl triflate/halide

6.3.4.1. Method D-1. Coupling of boronic acid with triflate or halide. A mixture of triflate or halide (1.0 mmol), aryl boronic acid (1.5 mmol), potassium phosphate (3.0 mmol), KBr (2.4 mmol), and tetrakis(triphenylphosphine)palladium (0.05 mmol) in dioxane (10 mL) was heated at reflux overnight under Ar. The reaction mixture was cooled, quenched with water and extracted with EtOAc. The organic layers were combined, dried, and concentrated in vacuo. Purification by flash column chromatography or crystallization gave the coupled product.

6.3.4.2. Method D-2. Coupling of boronic acid with triflate. A mixture of triflate (1.0 mmol), aryl boronic acid (2.0 mmol), $NaHCO_3$ (3.0 mmol), and tetrakis(triphenylphosphine)palladium (0.05 mmol) or bis(triphenylphosphine)palladium(II)chloride (0.05 mmol) in DME/water (9:1, 10 mL) was heated at reflux for 16 h. The reaction mixture was cooled, quenched with water, and extracted with EtOAc. The organic layer was dried and concentrated in vacuo. Purification by flash column chromatography or crystallization gave the coupled product.

6.3.4.3. Method D-3. Coupling of tributyltin derivative with triflate. A mixture of triflate (1.0 mmol), tributyltin derivative (3.0 mmol), tetraethylammonium chloride (6.0 mmol), and bis(triphenylphosphine)palladium(II)chloride (0.05 mmol) in DMF (10 mL) was heated at 70 °C overnight under Ar. The reaction mixture was cooled, quenched with water (20 mL), and extracted with EtOAc (2 × 10 mL). The organic layers were combined, dried, and concentrated in vacuo. Purification by flash column chromatography or crystallization gave the coupled product.

6.3.4.4. Method D-4. Coupling of trimethyltin derivative with triflate. A mixture of triflate (1.0 mmol), trimethyltin derivative (3.0 mmol), and bis(triphenylphosphine)palladium(II)chloride (0.05 mmol) in THF (10 mL) was heated at 70 °C overnight under Ar. The reaction mixture was cooled, quenched with water, and extracted with EtOAc (2 × 10 mL). The organic layers were combined, dried, and concentrated in vacuo. Purification by flash column chromatography or crystallization gave the coupled product.

6.3.5. Method E. Oxidation of aryl aldehyde to acid

A mixture of aldehyde (1.0 mmol), tert-butanol (5 mL), water (2 mL) and acetonitrile (1 mL, additional amount may have been added until the reaction mixture was homogenous) was stirred at room temperature. The solution was cooled in an ice bath and 2-methyl-2-butene (1 mL), $NaClO_2$ (6 mmol), and NaH_2PO_4 (1.6 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. If the solid separated out, the mixture was filtered to collect the solid, the desired product. If no solid separated out, then the reaction mixture was concentrated in vacuo to remove acetonitrile, diluted with water (10 mL), and extracted with EtOAc (2 × 10 mL). The organic layers were combined, washed with water and brine, then dried and concentrated in vacuo to furnish crude acid. Purification was achieved, if needed, by crystallization or using flash column chromatography to obtain pure acid.

6.3.6. Method F. Conversion of aromatic benzyl ether to aromatic phenol, benzyl ester to acid, alkene to alkane, azide to amine

To a solution of appropriate substrate (1.0 mmol) in ethanol (10 mL) was added 10% Pd/C. The reaction mixture was hydrogenated at 50 psi for 2–24 h (until all starting material disappeared as confirmed by MS and TLC analysis). The catalyst was removed by filtration through a pad of Celite under N_2. The filtrate was concentrated in vacuo to furnish the product, which was purified by flash column chromatography or crystallization.

6.3.7. Method G. Hydrolysis of ester to acid

6.3.7.1. Method G-1. Hydrolysis of MEM ester to acid. To a solution of MEM ester (1.0 mmol) in DME (8 mL) was added 6 N HCl (2 mL) and the solution was stirred at 20–25 °C for 16 h. The reaction mixture was neutralized with solid $NaHCO_3$ (18 mmol) and concentrated in vacuo. The reaction mixture was acidified with 0.5 N HCl (20 mL) and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with brine (20 mL), dried, and concentrated in vacuo to furnish crude product. Purification of the crude by flash column chromatography gave the product. Alternatively, the crude reaction mixture was diluted with water (10 mL) and concentrated in vacuo to remove DME. The solid obtained was collected by filtration and dried in vacuo to furnish pure acid.

6.3.7.2. Method G-2. Hydrolysis of ester to acid. To a solution of ester (1.0 mmol) in MeOH (10 mL) was added 1 N NaOH (10 mmol). The reaction mixture was stirred at room temperature for 2–3 h, filtered through a plug of cotton, and concentrated in vacuo to remove MeOH. The pH of the aqueous layer was adjusted to below 7. The solid separated out was collected by filtration, washed with water and dried in vacuo to furnish the desired acid.

6.3.8. Method H. Coupling of acid with amino compounds

To a solution of acid (1.0 mmol) in DMF (5 mL) was added corresponding amine (1.1 mmol) and the solution was stirred at room temperature until it was homogenous. Pyridine (5 mL) was added to the reaction mixture followed by 1,3-dicyclohexylcarbodiimide, DCC (1.2 mmol) and was stirred for 16 h at room temperature. The mixture was quenched with 6 N HCl (10 mL), diluted with ice cold water (10 mL), and extracted with $CHCl_3$ (2 × 10 mL). The organic layers were combined, washed with brine (10 mL), dried, and filtered. Purification of the crude by flash column chromatography gave the product as a solid. If the product was soluble in water, then the reaction mixture was concentrated in vacuo to remove pyridine and DMF and was purified by flash column chromatography.

6.3.9. Method I. Reduction of aldehyde to alcohol

To a solution of aldehyde (1.0 mmol) in THF (10 mL) was added $NaBH_4$ (0.4 mmol). The reaction mixture was stirred for 30 min and quenched with glacial acetic acid (0.3 mL). The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (2 × 10 mL). The organic layers were combined and washed with brine (10 mL), dried, filtered, and concentrated in vacuo to obtain the crude product that was purified by flash column chromatography.

6.3.10. Method J. Conversion of vinyl group to diol

To a solution of vinyl compound (1.0 mmol) in THF/t-butanol (1:1, 10 mL) and water (2.0 mL) were added 4-methylmorpholine N-oxide (2.5 mmol) and osmium tetraoxide (1 mL, 2.5 wt % in t-butanol, 0.1 mmol). The reaction mixture was stirred at room temperature for 2 h and quenched with a saturated aqueous solution of Na_2SO_3 (5 mL). The reaction mixture was stirred at room temperature for 30 min, then diluted with brine (10 mL) and EtOAc (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL). The organic layers were combined, washed with brine (10 mL), dried, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography to furnish the desired diol.

6.3.11. Method K. Conversion of diol to aldehyde

To a solution of diol (1 mmol) in DME/water (9:1, 10 mL) was added $NaIO_4$ (3.0 mmol) and stirred at room temperature for 30 min. The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and washed with brine (10 mL), dried, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography to furnish the desired aldehyde.

6.3.12. Method L. Conversion of alcohol to mesylate

To a solution of alcohol (1.0 mmol) in DME (10 mL) was added dimethylaminopyridine (0.10 mmol), methanesulfonyl chloride (3.0 mmol), and diisopropylethylamine or triethylamine (5.0 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with water (10 mL) and was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine, dried, filtered, and concentrated in vacuo. The residue obtained was purified by column chromatography to furnish the desired mesylate.

6.3.13. Method M. Conversion of mesylate to azide

To a solution of mesylate (1.0 mmol) in DMSO (10 mL) was added NaN_3 (25 mmol) and the mixture was heated at 100 °C for 16 h. The cooled reaction mixture was diluted with cold water (25 mL), then extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with water (10 mL), then with brine (10 mL). The dried and filtered solution was then concentrated in vacuo. The residue obtained was purified by column chromatography to furnish the desired azido compound.

6.3.14. Method N. Conversion of nitrile to amidine

A mixture of nitrile (1.0 mmol) and saturated methanolic HCl solution (freshly prepared by bubbling HCl gas or prepared in situ by premixing methanol and acetyl chloride at ice cold temperature) was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo to furnish methyl imidate. To the residue of methyl imidate was added MeOH (40 mL), and ammonia gas was bubbled in at reflux temperature for 16 h or until the reaction was complete. The reaction mixture was concentrated in vacuo and dried to furnish the desired amidine. Alternatively, the methyl imidate was dissolved in methanol and ammonium acetate (10.0 mmol) added. The reaction mixture was concentrated in vacuo and was purified by flash column chromatography to obtain the corresponding amidine.

6.3.14.1. Methyl 2′-(4-carbamimidoylphenylcarbamoyl)biphenyl-2-carboxylate 8. Compound **4** was prepared from 2-bromobenzoic acid (50 mmol) and 4-cyanoaniline according to Method A-1 in 70% yield. Compound **4** (20 mmol scale) and 2-formylphenyl boronic acid (Aldrich) gave **6** in 25% yield using Method D-1. Further conversion of **6** (5.3 mmol) to **7** was carried out using Method E in 46% yield. Compound **8** was prepared from **7** (2.4 mmol scale) using Method N in 45% yield. ^{1}H NMR (DMSO-d_6) δ 10.45 (s, 1H), 9.19 (br s, 2H), 8.92 (br s, 2H), 7.74 (m, 4H), 7.64 (d, J = 6.3 Hz, 1H), 7.57 (m, 3H), 7.41 (m, 2H), 7.30 (t, *J* = 8.5 Hz, 1H), 3.51 (s, 3H); IR (KBr) 3059, 1674, 1597, 1513, 1484, 1325, 1257 cm^{-1}; MS (ES$^+$) 374.26 [100% (M+1)]. Anal. ($C_{22}H_{19}N_3O_3$·HCl·0.75H_2O) C, H, N.

6.3.14.2. 2′-(4-Carbamimidoylphenylcarbamoyl)biphenyl-2-carboxylic acid 9. Compound **9** was prepared from **8** (0.8 mmol) according to Method G-2 in 63% yield. ^{1}H NMR (DMSO-d_6) δ 13.73 (br s, 1H), 9.05 (br s, 4H), 7.63 (d, J = 8.5 Hz, 2H), 7.57 (m, 3H), 7.41 (m, 3H), 7.18 (m, 1H), 7.10 (m, 1H), 7.03 (m, 1H), 6.80 (d, *J* = 6.4 Hz, 1H); IR (KBr) 3057, 1668, 1601, 1640, 1576, 1557, 1539, 1482, 1388, 1331, 1157 cm^{-1}; MS (ES$^+$) 360.32 [100% (M+1)]. Anal. ($C_{21}H_{17}N_3O_3$·0.75 HCl) C, H, N.

6.3.14.3. Methyl 2-hydroxy-5-(isobutylcarbamoyl)benzoate 11. It was prepared from 4-hydroxy-3-methoxycarbonylbenzoic acid[20] (75.0 mmol) following Method A-1 in 80% yield. ^{1}H NMR ($CDCl_3$): δ

10.79 (s, 1H), 8.49 (t, *J* = 5.8 Hz, 1H), 8.32 (d, *J* = 2.5 and 8.5 Hz, 1H), 8.00 (dd, *J* = 8.5 and 2.5 Hz, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 3.92 (s, 3H), 3.06 (dd, *J* = 6.8 and 6.0 Hz, 2H), 1.83 (m, 1H), 0.86 (d, *J* = 6.8 Hz, 6H); IR (KBr) 3370, 2961, 1679, 1637, 1548, 1490, 1440, 1283, 1243, 1182, 1085 cm^{-1}; MS (ES^+) 252.46 [50% (M+1)]. Anal. ($C_{13}H_{17}NO_4$) C, H, N.

6.3.14.4. Methyl 5-(isobutylcarbamoyl)-2-(trifluoromethylsulfonyloxy)benzoate 12. It was prepared from **11** (17.17 mol) following Method B-1 in 90.5% yield. ^1H NMR ($CDCl_3$): δ 8.41 (d, *J* = 2.4 Hz, 1H), 8.11 (d, *J* = 2.4 and 8.5 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 6.34 (br s, 1H), 4.02 (s, 3H), 3.32 (m, 2H), 1.98 (m, 1H), 0.98 (d, *J* = 6.8 Hz, 6H); IR (KBr) 3081, 2959, 1728, 1647, 1430, 1280 cm^{-1}; MS (ES^+) 384.1 [100% (M+1)]. Anal. ($C_{14}F_3H_{16}NO_6S$) C, H, N.

6.3.14.5. Benzyl 5-formyl-2-hydroxybenzoate 14. It was prepared from 5-formyl-2-hydroxybenzoic acid **13** (Aldrich, 300 mmol) following Method C-3 in 90% yield. ^1H NMR (DMSO-d_6) δ 11.23 (s, 1H), 9.85 (s, 1H), 8.30 (s, 1H), 7.98 (dd, *J* = 8.6 and 2.6 Hz, 1H), 7.47 (d, *J* = 7.7 Hz, 2H), 7.40 (t, *J* = 7.7 and 6.9 Hz, 2H), 7.34 (t, *J* = 7.7 and 6.9 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 5.38 (s, 2H); IR (KBr) 3180, 1676, 1580, 1189 cm^{-1}; MS (ES^+) 256.81 [50% (M+1)]. Anal. ($C_{15}H_{12}O_4$) C, H, N.

6.3.14.6. Benzyl 5-(isobutylcarbamoyl)-2-(trifluoromethylsulfonyloxy)benzoate 17. Compound **15** was prepared from **14** (75 mmol) following Method B-2, which was converted to **16** using Method E in quantitative yield in both steps. Compound **17** was synthesized from **16** (32 mmol) following Method A-2 in 69% yield; mp 93 °C. ^1H NMR (DMSO-d_6): δ 8.83 (t, *J* = 6.0 Hz, 1H), 8.49 (d, *J* = 2.6 Hz, 1H), 8.23 (dd, *J* = 8.6 and 1.7 Hz, 1H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.49 (m, 2H), 7.41 (m, 3H), 5.43 (s, 2H), 3.10 (t, *J* = 6.9 Hz, 2H), 2.29 (m, 1H), 0.89 (d, *J* = 6.9 Hz, 6H); IR (KBr) 3344, 2965, 1734, 1638, 1435, 1203, 1135 cm^{-1}; MS (ES^+) 459.68 (M+1)]. Anal. ($C_{20}H_{20}F_3NO_6S$) C, H, N.

6.3.14.7. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 21. The synthesis of **21** began from the reaction of **17** (2.17 mmol) with **5** using Method D-2 (92% yield) to give **18** following conversions of **18**–**19** (Method E, yield 85%), **19**–**20** (Method H, yield 56%), and **20**–**21** (Method G-2, yield 36%). ^1H NMR (DMSO-d_6) δ 12.60 (br s, 1H), 9.20 (br s, 2H), 9.00 (br s, 2H), 8.50 (t, J = 5.5 Hz, 1H), 8.30 (s, 1H), 7.90 (d, *J* = 6.0 Hz, 1H), 7.80 (m, 7H), 7.60 (m, 1H), 7.40 (m, 1H), 3.15 (t, *J* = 7.5 Hz, 2H), 1.90 (m, 1H), 0.90 (d, *J* = 6.5 Hz, 6H).); IR (KBr) 3071, 2958, 1675, 1640, 1599, 1542, 1482, 1409, 1325, 1257, 1157 cm^{-1}; MS (ES^+) 459.3 [100% (M+1)]. Anal. ($C_{26}H_{26}N_4O_4{\cdot}2HCl{\cdot}H_2O$) C, H, N.

6.3.14.8. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-methoxybiphenyl-2-carboxylic acid 24. The synthesis of **24** began from the reaction of **12** (1.31 mmol) with 2-formyl-4-methoxyphenylboronic acid (Frontier Scientific) using Method D-2 (79% yield) to give **23** following conversion of **23**–**24** using Methods E, H, and G-2 in 11% yield; mp 346–350 °C. ^1H NMR (CF_3CO_2D) δ 8.43 (s, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 7.67 (q, *J* = 24.0 and 8.4 Hz, 4H), 7.56 (d, *J* = 7.7 Hz, 1H), 7.38 (s, 1H), 7.23 (s, 2H), 3.98 (s, 3H), 3.43 (d, *J* = 7.0 Hz, 2H), 2.01 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 6H); IR (KBr) 3285, 3069, 2950, 1668, 1636, 1608, 1522, 1483, 1414, 1339, 1295 cm^{-1}; MS (ES^-) 487.3 [100% (M−1)], (ES^+) 489.3 [100% (M+1)]. Anal. ($C_{27}H_{28}N_4O_5{\cdot}H_2O$) C, H, N.

6.3.14.9. 4-(Benzyloxy)-2-formylphenylboronic acid 25. The reported procedure[22] was improved as follows. To a solution of 2-bromo-5-benzyloxybenzaldehyde (60.5 g, 208 mmol) in ethanol (900 mL) were added triethylorthoformate (48.4 mL, 291 mmol) and ammonium nitrate (0.92 g, 11.5 mmol) and were stirred at room temperature for 16 h. The reaction mixture was quenched with triethylamine (1.23 mL, 1.22 mmol) and concentrated in vacuo to remove ethanol. The residue was dissolved in ether, filtered to remove any insoluble inorganic impurities, and evaporated to dryness. The residue was re-dissolved in anhydrous ether (360 mL) and cooled to −78 °C, butyl lithium (100 mL, 250 mmol) was added dropwise and the reaction mixture was stirred for 30 min after the addition was completed. Tributyl borate (73 mL, 270 mmol) in ether (200 mL) was added to the reaction mixture and was stirred at −78 °C for 2 h. The reaction mixture was allowed to warm to 0 °C and was quenched with 2 M HCl (285 mL), then heated at reflux for 1 h and cooled. The aqueous layer was separated and the organic layer was extracted twice with 1 N NaOH (150 mL). The basic extracts were combined and washed with ether (150 mL) and acidified to pH 4 using 6 N HCl. The solid separated out was collected by filtration, washed with water and hexane, and dried in vacuo to furnish desired **25** as a tan solid (50 g, 94%). ^1H NMR ($CDCl_3$) δ 4.78 (s, 2H), 5.20 (s, 2H), 7.25 (dd, *J* = 7.7 and 2.6 Hz, 1H), 7.34–7.45 (m, 5H), 7.50 (d, *J* = 2.6 Hz, 1H), 7.73 (d, *J* = 7.7 Hz, 1H), 9.85 (s, 1H); IR (KBr) 3327, 3113, 1681, 1600, 1559, 1358, 1272, 1247 cm^{-1}. Anal. ($C_{14}BH_{13}O_4$) C, H, N.

6.3.14.10. Benzyl 4′-(benzyloxy)-6′-formyl-4-(isobutylcarbamoyl) biphenyl-2-carboxylate 26a. It was prepared from **17** (4.14 mmol) and **25** following Method D-1 in 92% yield; mp 100 °C. ^1H NMR (DMSO-d_6) δ 9.63 (s, 1H), 8.72 (t, *J* = 6.0 Hz, 1H), 8.38 (d, *J* = 1.7 Hz, 1H), 8.07 (dd, *J* = 7.7 and 1.7 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.40 (m, 4H), 7.35 (m, 1H), 7.26 (m, 4H), 7.17 (d, *J* = 8.6 Hz, 1H), 6.88 (m, 2H), 5.18 (s, 2H), 5.02 (q, *J* = 13.0 and 2.5 Hz, 2H), 3.10 (t, *J* = 6.0 Hz, 2H), 1.85 (m, 1H), 0.88 (d, *J* = 6.0 Hz, 6H); IR (KBr) 3356, 3298, 2956, 1731, 1688, 1636, 1544, 1272, 1238, 1159, 1087 cm^{-1}; MS (ES^+)522.89 [100% (M+1)]. Anal. ($C_{33}H_{31}NO_5$) C, H, N.

6.3.14.11. Methyl 4′-(benzyloxy)-6'-formyl-4-(isobutylcarbamoyl) biphenyl-2-carboxylate 26b. It was prepared from **12** (80.13 mmol) and **25** following Method D-2 in 90% yield; mp 135–137 °C. ^1H NMR (DMSO-d_6) δ 9.78 (s, 1H), 8.85 (t, *J* = 5.7 Hz, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 8.20 (dd, *J* = 8.2 and 1.9 Hz, 1H), 7.55 (m, 9H), 5.35 (s, 2H), 3.69 (s, 3H), 3.23 (t, *J* = 6.5 Hz, 2H), 1.98 (m, 1H), 1.02 (d, *J* = 6.8 Hz, 6H); IR (neat) 3400, 2955, 2927, 1721, 1690, 1633, 1536, 1242 cm^{-1}; MS (ES^+) 446.3 (M+1)]. Anal. ($C_{27}H_{27}NO_5{\cdot}0.25H_2O$) C, H, N.

6.3.14.12. 4-(Benzyloxy)-2′-(benzyloxycarbonyl)-4′-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 27a. It was prepared from **26a** (3.25 mmol) following Method E in 92% yield; mp 93 °C. ^1H NMR (DMSO-d_6) δ 12.57 (s, 1H), 8.64 (t, *J* = 6.0 Hz, 1H), 8.32 (s, 1H), 7.99 (dd, *J* = 6.9 and 1.8 Hz, 1H), 7.48 (m, 2H), 7.41 (m, 3H), 7.34 (m, 1H), 7.27 (m, 4H), 7.14 (dd, *J* = 8.6 and 2.6 Hz, 1H), 7.08 (m, 3H), 5.14 (s, 2H), 5.01 (d, *J* = 5.0 Hz, 2H), 3.09 (t, *J* = 6.9 Hz, 2H), 1.85 (m, 1H), 0.86 (d, *J* = 6.9 Hz, 6H); IR (KBr) 3352, 2957, 2867, 1713, 1627, 1608, 1274, 1248, 1224, 1072 cm^{-1}; MS (ES^+) 538.86 [100% (M+1)]. Anal. ($C_{33}H_{31}NO_6$) C, H, N.

6.3.14.13. 2-Benzyl 2′-(2-methoxyethoxy)methyl 4′-(benzyloxy)-4-(isobutylcarbamoyl)biphenyl-2,2′-dicarboxylate 28a. It was prepared from **27a** (5.0 mmol) following Method C-2 in 84% yield. ^1H NMR (DMSO-d_6) δ 8.68 (t, *J* = 6.0 Hz, 1H), 8.36 (d, *J* = 1.7 Hz, 1H), 8.02 (dd, *J* = 1.7 and 8.2 Hz, 1H), 7.49 (m, 2H), 7.42 (m, 3H), 7.35 (m, 1H), 7.29 (m, 4H), 7.24 (dd, *J* = 8.3 and 2.8 Hz, 1H), 7.11 (m, 3H), 5.64 (s, 2H), 5.12 (d, *J* = 15.0 Hz, 2H), 5.02 (d, *J* = 3.8 Hz, 2H), 3.36 (dd, *J* = 3.0 and 6.0 Hz, 2H), 3.28 (dd, *J* = 3.0 and 6.0 Hz, 2H), 3.16 (s, 3H), 3.10 (t, *J* = 6.5 Hz, 2H), 1.86 (m, 1H), 0.90 (d, *J* = 6.8 Hz, 6H); IR (NaCl) 3331, 2958, 2873, 1725, 1643, 1608, 1543, 1479, 1455, 1316, 1245, 1171 cm^{-1}; MS (ES^+) 626.44 [100% (M+1)]. Anal. ($C_{37}H_{39}NO_8$) C, H, N.

6.3.14.14. 4′-Hydroxy-4-(isobutylcarbamoyl)-2′-(((2-methoxyethoxy)methoxy)carbonyl)biphenyl-2-carboxylic acid 29. It was prepared from **28a** (8.1 mmol scale) following Method F in 91% yield. ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 1H), 9.90 (s, 1H), 8.66 (t, J = 6.0 Hz, 1H), 8.34 (d, J = 1.7 Hz, 1H), 7.99 (dd, J = 7.7 and 1.7 Hz, 1H), 7.35 (d, J = 1.7 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.02 (m, 2H), 5.17 (d, J = 11.2 Hz, 2H), 3.32 (m, 2H), 3.39 (m, 2H), 3.20 (s, 3H), 3.12 (t, J = 6.0 Hz, 2H), 1.88 (m, 1H), 0.91 (d, J = 6.9 Hz, 6H); IR (KBr) 3349, 2918, 1714, 1265, 1115, 1049 cm^{-1}; MS (ES$^+$) 446.65 [100% (M+1)]. Anal. ($C_{23}H_{27}NO_8 \cdot 0.25H_2O$) C, H, N.

6.3.14.15. 2-Benzyl 2′-(2-methoxyethoxy)methyl 4′-hydroxy-4-(isobutylcarbamoyl)biphenyl-2,2′-dicarboxylate 30a. It was prepared from **29** (3.7 mmol) following Method C-3 in 64% yield. ^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H), 8.67 (t, J = 6.0 Hz, 1H), 8.32 (d, J = 2.0 Hz, 1H), 8.03 (dd, J = 8.0 and 2.0 Hz, 1H), 7.29 (m, 5H), 7.11 (m, 2H), 6.98 (m, 2H), 5.11 (d, J = 14.0 Hz, 2H), 5.04 (d, J = 3.5 Hz, 2H), 3.35 (m, 2H), 3.28 (m 2H), 3.16 (s, 3H), 3.10 (t, J = 6.0 Hz, 2H), 1.85 (m, 1H), 0.88 (d, J = 6.0 Hz, 6H); IR (KBr) 3310, 2960, 1714, 1639, 1608, 1548, 1301, 1245, 1159 cm^{-1}; MS (ES$^+$) 536.30 [100% (M+1)]. Anal. ($C_{30}H_{33}NO_8 \cdot 0.25H_2O$) C, H, N.

6.3.14.16. 2-Benzyl 2′-(2-methoxyethoxy)methyl 4-(isobutylcarbamoyl)-4′-(trifluoromethylsulfonyloxy)biphenyl-2,2′-dicarboxylate 31a. It was prepared from **30a** (2.38 mmol) following Method B-2 in 98% yield; mp 70 °C. ^{1}H NMR (DMSO-d_6) δ 8.73 (t, J = 6.0 Hz, 1H), 8.45 (d, J = 1.7 Hz, 1H), 8.07 (dd, J = 7.7 and 1.7 Hz, 1H), 7.85 (d, J = 2.6 Hz, 1H), 7.73 (dd, J = 8.6 and 2.6 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.31 (m, 3H), 7.15 (m, 2H), 5.16 (dd, J = 18.0 and 6.0 Hz, 2H), 5.04 (s, 2H), 3.40 (m, 2H), 3.29 (m, 2H), 3.16 (s, 3H), 3.12 (t, J = 6.5 Hz, 2H), 1.86 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (KBr) 3294, 2952, 1728, 1630, 1548, 1426, 1243, 1216 cm^{-1}; MS (ES$^+$) 668.15 [100%(M+1)]. Anal. ($C_{31}F_3H_{32}NO_{10}S$) C, H, N.

6.3.14.17. 2′-(2-Methoxyethoxy)methyl 2-methyl 4-(isobutylcarbamoyl)-4′-(trifluoromethylsulfonyloxy)biphenyl-2,2′-dicarboxylate 31b. The synthesis of **31b** began from **26b** (70 mmol) using Method E (quantitative yield) to give **27b** following conversions of **27b–28b** (Method C-2, 55% yield), **28b–30b** (Method F), and **30b–31b** (Method B-2, 84% yield in last two steps); mp 66–70 °C. ^{1}H NMR (DMSO-d_6) δ 8.75 (t, J = 5.6 Hz, 1H), 8.44 (d, J = 1.6 Hz, 1H), 8.11 (dd, J = 8.0 and 1.9 Hz, 1H), 8.01 (d, J = 2.9 Hz, 1H), 7.84 (dd, J = 8.4 and 2.6 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 5.23 (q, J = 18.0 and 6.0 Hz, 2H), 3.59 (s, 3H), 3.44 (m, 2H), 3.30 (m, 2H), 3.18 (s, 3H), 3.13 (t, J = 6.6 Hz, 2H), 1.88 (m, 1H), 0.91 (d, J = 6.7 Hz, 6H); IR (KBr) 3331, 2965, 1749, 1632, 1426, 1143, 933 cm^{-1}; MS (ES$^+$) 614.3 (M+23). Anal. ($C_{25}H_{28}F_3NO_{10}S$) C, H, N.

6.3.14.18. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-hydroxy-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36a. A mixture of **31a** (0.28 g, 0.41 mmol) and magnesium bromide etherate (0.43 g, 1.64 mmol) in dichloromethane (40 mL) was stirred at room temperature for 48 h. The reaction mixture was diluted with water (10 mL) and stirred for 30 min at room temperature. The reaction mixture was acidified with 1 N HCl to pH 3 and extracted with ethyl acetate (2 × 20 mL). The organic layers were combined, washed with brine (20 mL), dried, and concentrated in vacuo to furnish crude product. Purification of the crude by flash column chromatography (silica gel 14 g, eluted with 150 mL each of 50% ethyl acetate in hexane and 2.5, 5, and 7.5% (methanol in chloroform)) gave 0.14 g (57%) of **34a** (R = OSO_2CF_3) as a dark brown solid. MS (ES$^+$) 580.57 [100% (M+1)], (ES$^-$) 578.10 [100% (M−1).

Further conversion of **34a** (0.24 mmol) to **35a** was done by Method H in 53% yield; mp 175 °C. ^{1}H NMR (DMSO-d_6) δ 10.69 (s, 1H), 9.16 (br s, 2H), 8.79 (br s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.30 (d, J = 2.0 Hz, 1H), 8.06 (dd, J = 8.0 and 2.0 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.71 (m, 6H), 7.54 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.12 (m, 2H), 7.11 (m, 1H), 5.06 (s, 2H), 3.08 (t, J = 6.8 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3066, 2960, 1677, 1642, 1485, 1243, 1139 cm^{-1}; MS (ES$^+$) 697.6 [100% (M+1)]. Anal. ($C_{34}H_{31}F_3N_4O_7S \cdot HCl \cdot 0.5H_2O$) C, H, N.

Compound **36a** was prepared from **35a** (0.11 mmol) according to Method G-2 in 53% yield; mp 280 °C. ^{1}H NMR (DMSO-d_6) δ 9.68 (s, 1H), 8.99 (s, 2H), 8.77 (s, 2H), 8.40 (t, J = 6.0 Hz, 1H), 7.87 (s, 1H), 7.55 (m, 5H), 6.88 (s, 1H), 6.83 (m, 2H), 6.77 (m, 2H), 2.96 (t, J = 6.8 Hz, 2H), 1.75 (m, 1H), 0.80 (d, J = 6.8 Hz, 6H); IR (KBr) 3326, 2960, 1603, 1542, 1327 cm^{-1}; MS (ES$^+$) 475.1 [100% (M+1)]. Anal. ($C_{26}H_{26}N_4O_5S \cdot 2H_2O$) C, H, N.

6.3.14.19. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-vinylbiphenyl-2-carboxylic acid 36b. Compound **33b** was prepared from **31a** (0.75 mmol) and tributyl(vinyl)stannane **32** (Aldrich, 3.75 mmol) according to Method D-3 in 83% yield; MS (ES$^+$) 546.49 (M+Na).

Compound **34b** was prepared from **33b** (0.62 mmol) according to Method G-1 in 51% yield; MS (ES$^+$) 457.71 (M+H).

Compound **35b** was prepared from **34b** (0.32 mmol) according to Method H in 47% yield; mp >170 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.66 (s, 1H), 9.15 (br s, 2H), 8.79 (br s, 2H), 8.66 (t, J = 6.0 Hz, 1H), 8.55 (s, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.74 (m, 4H), 7.67 (t, J = 10 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.26 (m, 5H), 7.02 (d, J = 6.6 Hz, 1H), 6.87 (dd, J = 17.0 and 11.0 Hz, 2H), 6.02 (d, J = 17.0 Hz, 1H), 5.41 (d, J = 11.0 Hz, 1H), 5.05 (s, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.83 (m, 1H), 0.87 (d, J = 7.0 Hz, 6H); IR (KBr) 3263, 3063, 2958, 1676, 1641, 1484, 1324, 1257 cm^{-1}; MS (ES$^+$) 575.87 [100% (M+1)]. Anal. ($C_{35}H_{34}N_4O_4 \cdot 2HCl \cdot H_2O$) C, H, N.

Compound **36b** was prepared from **35b** (0.15 mmol) according to Method G-2 in 40% yield; mp >260 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.06 (s, 2H), 8.77 (s, 2H), 8.54 (t, J = 6.0 Hz, 1H), 8.01 (s, 1H), 7.64 (m, 7H), 7.00 (m, 2H), 6.84 (dd, J = 17.0 and 11.0 Hz, 2H), 5.94 (d, J = 17.0 Hz, 1H), 5.35 (d, J = 11.0 Hz, 1H), 3.03 (t, J = 7.0 Hz, 2H), 1.81 (m, 1H), 0.85 (d, J = 6.9 Hz, 6H); IR (KBr) 3323, 2960, 1642, 1604, 1539, 1482, 1327 cm^{-1}; MS (ES$^+$) 485.57 [100% (M+1)]. Anal. ($C_{28}H_{28}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

6.3.14.20. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-ethyl-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36c. Compound **36b** (0.2 mmol) was converted to **36c** following Method F in 55% yield. ^{1}H NMR (DMSO-d_6) δ 14.00 (br s, 1H), 9.10 (br s, 4H),8.50 (m, 1H), 8.10 (s, 1H), 7.80–7.60 (m, 6H), 7.50 (s, 1H), 7.40 (d, J = 3.0 Hz,1H), 7.00 (m, 2H), 3.00 (t, J = 7.0 Hz, 2H), 2.70 (m, 2H), 1.80 (m, 1H), 1.30 (t, J = 6.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 6H); IR (KBr) 3284, 2960, 2869, 1604, 1540, 1481 cm^{-1}; MS (ES$^+$) 487.2 [100% (M+1)]. Anal. ($C_{28}H_{30}N_4O_4 \cdot HCl$) C, H, N.

6.3.14.21. 4′-Allyl-2′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36d. Compound **33d** was prepared from **31a** (0.75 mmol scale) and allyltributylstannane **32** (Aldrich, 2.25 mmol) according to Method D-3 in 89% yield. Compound **33d** (0.66 mmol) was converted to **34d** following Method G-1 and **35d** was obtained from **34d** according to Method H in 96% yield in both steps; mp 190 °C. ^{1}H NMR (DMSO-d_6) δ 10.56 (s, 1H), 9.15 (br s, 2H), 8.84 (br s, 2H), 8.64 (t, J = 6.0 Hz, 1H), 8.19 (d, J = 2.0 Hz, 1H), 7.99 (d, J = 7.0 Hz, 1H), 7.70 (m, 4H), 7.46 (s, 1H), 7.36 (m, 2H), 7.24 (m, 4H), 7.05 (s, 1H), 7.00 (s, 1H), 6.00 (m, 1H), 5.18 (d, J = 16.0 Hz, 1H), 5.10 (d, J = 11.0 Hz, 1H), 5.00 (s, 2H), 3.47 (d, J = 6.0 Hz, 2H), 3.03 (t, J = 6.0 Hz, 2H), 1.79 (m, 1H), 0.83 (d, J = 6.8 Hz, 6H); IR (KBr) 3263, 3067, 2958, 1675, 1640, 1484, 1324, 1258 cm^{-1}; MS (ES$^+$) 589.5 [100% (M+1)]. Anal. ($C_{36}H_{36}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

Compound **36d** was prepared from **35d** (0.22 mmol) according to Method G-2 in 69% yield; mp >320 °C. ^{1}H NMR (DMSO-d_6) δ 13.96 (s, 1H), 9.02 (s, 2H), 8.85 (s, 2H), 8.46 (t, J = 6.0 Hz, 1H), 7.91 (s, 1H), 7.58 (m, 4H), 7.39 (s, 1H), 7.25 (d, J = 7.8 Hz, 1H), 6.92 (d, J = 7.7 Hz, 1H), 6.87 (d, J = 7.7 Hz, 1H), 6.01 (m, 1H), 5.17 (d, J = 16.7 Hz, 1H), 5.08 (d, J = 10.0 Hz, 1H), 3.45 (d, J = 6.0 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 1.78 (m, 1H), 0.83 (d, J = 6.8 Hz, 6H); IR (KBr) 3304, 3084, 2953, 1664, 1608, 1545, 1334 cm^{-1}; MS (ES$^+$) 499.3 [100% (M+1)]. Anal. ($C_{29}H_{30}N_4O_4$·Na·0.25H_2O) C, H, N.

6.3.14.22. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-propylbiphenyl-2-carboxylic acid 36e. Compound **36d** (0.17 mmol) was converted to **36e** following Method F in 71% yield; mp 335 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.05 (s, 2H), 8.67 (s, 2H), 8.47 (t, J = 6.0 and 5.0 Hz, 1H), 7.95 (m, 1H), 7.95 (m, 1H), 7.63 (m, 5H), 7.40 (s, 1H), 7.38 (d, J = 7.7 Hz, 1H), 6.92 (m, 2H), 3.02 (t, J = 6.8 Hz, 2H), 2.64 (m, 2H), 1.80 (m, 1H), 1.66 (m, 2H), 0.96 (t, J = 8.0 and 6.5 Hz, 3H), 0.85 (d, J = 6.8 Hz, 6H); IR (KBr) 3302, 3067, 2956, 1662, 1636, 1608, 1550, 1334, 852 cm^{-1}; MS (ES$^-$) 499.31 [100% (M−1)]. Anal. ($C_{29}H_{32}N_4O_4$·1.25H_2O) C, H, N.

6.3.14.23. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-ethynyl-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36f. Compound **33f** was prepared from **31a** (0.75 mmol) and trimethylsilylacetylene (Aldrich, 2.25 mmol) following Method D-3 in 50% yield. Compound **33f** (0.38 mmol) was converted to **34f** using Method G-1 followed by treatment with tetrabutylammonium fluoride to remove trimethylsilyl in 58% yield. Compound **35f** was obtained from **34f** (0.2 mmol) using Method H in 70% yield; mp >185 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.73 (s, 1H), 9.17 (br s, 2H), 8.82 (br s, 2H), 8.71 (t, J = 6.0 Hz, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 8.0 and 2.0 Hz, 1H), 7.79 (s, 1H), 7.75 (s, 4H), 7.65 (dd, J = 8.0 and 2.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.29 (m, 3H), 7.05 (d, J = 5.5 Hz, 2H), 5.04 (d, J = 11.0 Hz, 2H), 4.41 (s, 1H), 3.09 (t, J = 6.0 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3064, 2958, 1675, 1642, 1484, 1324, 1257 cm^{-1}; MS (ES$^+$) 573.3 [100% (M+1)]. Anal. ($C_{35}H_{32}N_4O_4$·HCl·2H_2O) C, H, N.

Compound **36f** was prepared from **35f** (0.12 mmol) according to Method G-2 in 68% yield; mp >275 °C. ^{1}H NMR (DMSO-d_6) δ 9.08 (s, 2H), 8.86 (s, 2H), 8.54 (t, J = 6.0 Hz, 1H), 8.03 (m, 1H), 7.62 (m, 6H), 7.08 (d, J = 7.5 Hz, 1H), 6.99 (m, 1H), 4.32 (s, 1H), 3.03 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 7.0 Hz, 2H), 1.82 (m, 1H), 0.87 (d, J = 6.9 Hz, 6H); IR (KBr) 3288, 2958, 1665, 1642, 1604, 1542, 1482, 1411, 1325 cm^{-1}; MS (ES$^+$) 483.3 [100% (M+1)]. Anal. ($C_{28}H_{26}N_4O_4$·HCl·0.75H_2O) C, H, N.

6.3.14.24. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(prop-1-en-2-yl)biphenyl-2-carboxylic acid 36g. Compound **33g** was prepared from **31a** (0.75 mmol scale) and tributyl(prop-1-en-2-yl)stannane[23] **32** (2.25 mmol) according to Method D-3 in 70% yield. Compound **33g** (0.37 mmol scale) was converted to **34g** following Method G-1 and **35g** was obtained from **34g** according to Method H in 58% yield in both steps. ^{1}H NMR (DMSO-d_6) δ 10.80 (br s, 1H), 9.10 (br s, 2H), 8.90 (2 br s, 2H), 8.60 (m, 1H), 8.20 (s, 1H), 8.00 (m, 1H), 7.80–7.60 (m, 6H), 7.40 (d, J = 6.9 Hz, 1H), 7.30 (m, 4H), 7.00 (d, J = 6.7 Hz, 2H), 5.60 (m, 1H), 5.20 (m, 1H), 5.00 (br s, 2H), 3.10 (t, J = 6.8 Hz, 2H), 2.20 (s, 3H), 1.80 (m, 1H), 0.95 (d, J = 6.7 Hz, 6H); IR (KBr) 3262, 3063, 2965, 1675, 1483, 1325, 1257, 1154 cm^{-1}; MS (ES$^+$) 589.4 [100% (M+1)], MS (ES$^-$) 587.5 [100% (M−1)]. Anal. ($C_{36}H_{36}N_4O_4$·HCl·1.5H_2O) C, H, N.

Compound **36g** was prepared from **35g** (0.15 mmol) according to Method G-2 in 72% yield; mp >275 °C. ^{1}H NMR (DMSO-d_6) δ 9.08 (s, 2H), 8.82 (s, 2H), 8.53 (t, J = 6.0 Hz, 1H), 8.04 (m, 1H), 7.67 (m, 8H), 7.04 (m, 2H), 5.55 (s, 1H), 5.20 (s, 1H), 3.04 (t, J = 6.9 Hz, 2H), 2.19 (s, 3H), 1.81 (m, 1H), 0.87 (d, J = 6.9 Hz, 6H); IR (KBr) 3288, 3084, 2959, 1659, 1643, 1604, 1536, 1482, 1411, 1328 cm^{-1}; MS (ES$^+$) 499.4 [100% (M+1)]. Anal. ($C_{29}H_{30}N_4O_4$·HCl·2H_2O) C, H, N.

6.3.14.25. 6′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(propa-1,2-dienyl)biphenyl-2-carboxylic acid 6h. Compound **33h** was prepared from **31b** (0.75 mmol scale) and buta-1,3-dienyltributylstannane **32** (Aldrich, 2.25 mmol) according to Method D-3 in 66% yield. Compound **33h** (0.56 mmol scale) was converted to **34h** following Method G-1 in 86% yield and **35h** was obtained from **34h** (0.48 mmol) according to Method H in 41% yield.

Compound **36h** was prepared from **35h** (0.16 mmol) according to Method G-2 in 11% yield; mp >220 °C. ^{1}H NMR (DMSO-d_6) δ 14.24 (s, 1H), 9.29 (br s, 2H), 9.01 (br s, 2H), 8.73 (t, J = 6.0 Hz, 1H), 8.20 (d, J = 2.0 Hz, 1H), 7.85 (m, 6H), 7.74 (d, 2.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.22 (d, J = 7.4 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 6.73 (t, J = 6.8 Hz, 1H), 5.59 (d, J = 6.8 Hz, 2H), 3.25 (t, J = 6.8 Hz, 2H), 2.04 (m, 1H), 1.08 (d, J = 6.8 Hz, 6H); IR (KBr) 3288, 2959, 1942, 1644, 1606, 1539, 1482, 1325 cm^{-1}; MS (ES$^-$) 495.1 [100% (M−1)], (ES$^+$) 497.2 [100% (M+1)]. Anal. ($C_{29}H_{28}N_4O_4$·0.75HCl·0.25H_2O·0.25$C_4H_{10}O$) C, H, N.

6.3.14.26. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(prop-1-ynyl)biphenyl-2-carboxylic acid 36i. Compound **33i** was prepared from **31b** (0.75 mmol) and tributyl(prop-1-ynyl)stannane (Aldrich, 2.25 mmol) following Method D-3 in 53% yield. Compound **33i** (0.36 mmol) was converted to **34i** using Method G-1 and **35i** was prepared from **34i** using Method H in 58% yield in both steps; MS (ES$^+$) 609.3 (M+Na).

Compound **36i** was prepared from **35i** (0.08 mmol) according to Method G-2 in 45% yield; mp >260 °C. ^{1}H NMR (DMSO-d_6) δ 13.40 (br s, 1H), 9.30 (br s, 2H), 9.10 (br s, 2H), 8.70 (t, J = 6.0 Hz, 1H), 8.30 (s, 1H), 7.95 (m, 1H), 7.70 (m, 6H), 7.60 (d, J = 8.0 Hz, 1H), 7.20 (m, 2H), 3.10 (t, J = 6.8 Hz, 2H), 2.10 (s, 3H), 1.90 (m, 1H), 0.95 (d, J = 6.8 Hz, 6H); IR (KBr) 3087, 1676, 1640, 1480, 1324 cm^{-1}; MS (ES$^+$) 497.2 [100% (M+1)]. Anal. ($C_{29}H_{28}N_4O_4$·HCl·H_2O) C, H, N.

6.3.14.27. (Z)-4′-(But-2-enyl)-6′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36j. Compound **33j** was prepared from **31b** (0.75 mmol) and (*Z*)-but-2-enyltributylstannane[24] (2.25 mmol) following Method D-3 in 34% yield. Compound **33j** (0.30 mmol) was converted to **34j** using Method G-1 in 99% yield and **35j** was prepared from **34j** using Method H in 36% yield.

Compound **36j** was prepared from **35j** (0.12 mmol) according to Method G-2 in 96% yield; mp 230 °C. ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 1H), 9.50 (s, 2H), 8.85 (s, 2H), 8.65 (m, 1H), 8.25 (s, 1H), 7.95 (d, J = 2.17 Hz, 1H), 7.75 (m, 5H), 7.50 (d, J = 1.7 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 7.35 (d, J = 1.7 Hz, 1H), 7.18 (m, 1H), 2.90 (m, 2H), 2.70 (m, 1H), 1.85 (m, 1H), 1.60 (m, 2H), 1.25 (m, 1H), 1.10 (m, 1H), 0.88 (m, 2H), 0.82 (m, 6H); IR (KBr), 3074, 2959, 2870, 1675, 1640, 1409, 1324 cm^{-1}; MS (ES$^+$) 513.3 [100% (M+1)]. Anal. ($C_{30}H_{32}N_4O_4$·HCl·2H_2O) C, H, N.

6.3.14.28. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(3-methylbut-2-enyl)biphenyl-2-carboxylic acid 36k. Compound **33k** was prepared from **31a** (0.75 mmol scale) and tributyl(4-methylpent-3-enyl)stannane **32** (Aldrich, 2.25 mmol) according to Method D-3 in 50% yield. Compound **33k** (0.44 mmol) was converted to **34k** following Method G-1. Compound **35k** was obtained from **34k** according to Method H in 84% yield in both steps; mp >180 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.52 (s, 1H), 9.23 (br s, 2H), 8.81 (br s, 2H), 8.66 (t, J = 6.0 Hz, 1H), 8.21 (s, 1H), 8.02 (m, 1H), 7.72 (m, 5H), 7.52 (m, 2H), 7.23 (m, 5H), 7.03 (m, 2H), 5.11 (m, 1H), 5.04 (s, 2H), 3.43 (d, J = 8.0 Hz, 1H), 3.06 (t, J = 6.0 Hz, 2H), 1.85 (m, 1H), 1.74 (s, 3H), 1.47 (s, 3H), 0.86 (d,

J = 6.8 Hz, 6H); IR (KBr) 3263, 3065, 2961, 1675, 1641, 1542, 1484, 1324, 1258 cm^{-1}; MS (ES^+) 617.6 [100% (M+1)]. Anal. ($C_{38}H_{40}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

Compound **36k** was prepared from **35k** (0.24 mmol) according to Method G-2 in 87% yield; mp >280 °C. ^1H NMR (DMSO-d_6) δ 13.80 (s, 1H), 9.04 (s, 2H), 8.96 (s, 2H), 8.47 (t, J = 6.0 Hz, 1H), 7.93 (s, 1H), 7.61 (m, 6H), 7.42 (m, 1H), 6.91 (m, 2H), 6.07 (dd, J = 17.0 and 9.0 Hz, 1H), 5.35 (m, 1H), 5.09 (dd, J = 17.0 and 11.0 Hz, 1H), 3.38 (d, J = 6.5 Hz, 1H), 3.00 (t, J = 7.0 Hz, 2H), 1.78 (m, 1H), 1.72 (s, 3H), 1.41 (s, 3H), 0.84 (d, J = 6.9 Hz, 6H); IR (KBr) 3303, 3084, 2962, 1640, 1605, 1541, 1481, 1412, 1327 cm^{-1}; MS (ES^+) 527.5 [100% (M+1)]. Anal. ($C_{31}H_{34}N_4O_4 \cdot HCl \cdot 0.5H_2O$) C, H, N.

6.3.14.29. (Z)-2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(3-methylbuta-1,3-dienyl)biphenyl-2-carboxylic acid 36l. Compound **33l** was prepared from **31a** (0.75 mmol scale) and (E)-tributyl(3-methylbuta-1,3-dienyl)stannane 32 (Frontier Scientific, 2.25 mmol) according to Method D-3 in 68% yield. Compound **33l** (0.51 mmol) was converted to **34l** following Method G-1 and **35l** was obtained from **34l** according to Method H in 69% yield in both steps; mp >240 °C (dec). ^1H NMR (DMSO-d_6) δ 10.91 (s, 1H), 8.87 (br s, 4H), 8.69 (t, J = 6.0 Hz, 1H), 8.26 (d, J = 2.0 Hz, 1H), 8.03 (dd, J = 9.0 and 2.0 Hz, 1H), 7.85 (s, 1H), 7.73 (m, 5H), 7.42 (d, J = 9.0 Hz, 1H), 7.23 (m, 5H), 7.04 (d, J = 6.0 Hz, 2H), 6.73 (d, J = 16.0 Hz, 1H), 5.28 (s, 1H), 5.18 (s, 1H), 5.04 (s, 2H), 3.09 (t, J = 6.0 Hz, 2H), 1.99 (s, 3H), 1.84 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (KBr) 3061, 2957, 1673, 1640, 1482, 1323, 1255 cm^{-1}; MS (ES^+) 615.4 [100% (M+1)]. Anal. ($C_{38}H_{38}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

Compound **36l** was prepared from **35l** (0.21 mmol) according to Method G-2 in 58% yield; mp >240 °C. ^1H NMR (DMSO-d_6) δ 9.01 (s, 2H), 8.88 (s, 2H), 8.50 (t, J = 6.0 Hz, 1H), 8.07 (m, 1H), 7.73 (m, 1H), 7.63 (m, 7H), 7.11 (d, J = 17.0 Hz, 1H), 7.01 (d, J = 17.0 Hz, 1H), 6.97 (m, 1H), 6.69 (d, J = 17.0 Hz, 1H), 5.24 (s, 1H), 5.14 (s, 1H), 3.03 (t, J = 6.0 Hz, 2H), 1.92 (s, 3H), 1.81 (m, 1H), 0.84 (d, J = 6.9 Hz, 6H); IR (KBr) 3276, 2958, 1658, 1642, 1536, 1482, 1411, 1326 cm^{-1}; MS (ES^+) 525.4 [100% (M+1)]. Anal. ($C_{31}H_{32}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

6.3.14.30. (Z)-2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-hydroxyprop-1-enyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36m. Compound **33m** was prepared from **31a** (0.75 mmol scale) and (E)-3-(tributylstannyl)prop-2-en-1-ol[25] **32** (2.25 mmol) according to Method D-3 in 56% yield. Compound **33m** (0.42 mmol scale) was converted to **34m** following Method G-1 and **35m** was obtained from **34m** according to Method H in 68% yield in both steps; mp >180 °C (dec). ^1H NMR (DMSO-d_6) δ 10.66 (s, 1H), 9.18 (br s, 2H), 8.83 (br s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.23 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 7.8 and 2.0 Hz, 1H), 7.74 (s, 4H), 7.54 (s, 1H), 7.43 (m, 2H), 7.31 (d, J = 7.8 Hz, 1H), 7.25 (m, 3H), 7.04 (d, J = 6.7 Hz, 2H), 6.57 (d, J = 11.5 Hz, 1H), 5.95 (m, 1H), 5.02 (s, 2H), 5.01 (t, J = 5.5 Hz, 1H), 4.33 (t, J = 5.5 Hz, 2H), 3.09 (t, J = 6.0 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3262, 3063, 2957, 2926, 1677, 1641, 1482, 1324, 1255 cm^{-1}; MS (ES^+) 605.3 [100% (M+1)]. Anal. ($C_{36}H_{36}N_4O_5 \cdot HCl \cdot 1.5H_2O$) C, H, N.

Compound **36m** was prepared from **35** (0.15 mmol) according to Method G-2 in 53% yield; mp >220 °C. ^1H NMR (DMSO-d_6) δ 9.11 (s, 2H), 8.86 (s, 2H), 8.57 (t, J = = 6.0 Hz, 1H), 8.13 (m, 1H), 7.53 (m, 2H), 7.74 (m, 6H), 7.37 (d, J = 7.0 Hz, 1H), 7.17 (m, 2H), 6.54 (d, J = 12.0 Hz, 1H), 5.91 (m, 1H), 4.99 (m, 1H), 4.31 (m, 2H), 3.06 (t, J = 6.9 Hz, 2H), 1.83 (m, 1H), 0.87 (d, J = = 6.9 Hz, 6H); IR (KBr) 3290, 2958, 1664, 1604, 1541, 1482, 1411, 1325 cm^{-1}; MS (ES^+) 515.4 [100% (M+1)]. Anal. ($C_{29}H_{30}N_4O_5 \cdot HCl \cdot H_2O$) C, H, N.

6.3.14.31. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-hydroxyprop-1-en-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36n. Compound **33n** was prepared from **31a** (0.75 mmol scale) and 2-(tributylstannyl)prop-2-en-1-ol[26] **32** (2.25 mmol) according to Method D-3 in 46% yield. Compound **33n** (0.36 mmol) was converted to **34n** following Method G-1 in 95% yield and **35n** was obtained from **34n** (0.34 mmol) according to Method H in 25% yield; mp 200–225 °C. ^1H NMR (DMSO-d_6) δ 10.60 (s, 1H), 9.17 (s, 2H), 8.85 (s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 2.0 and 7.9 Hz, 1H), 7.75 (m, 6H), 7.65 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.25 (m, 3H), 7.04 (d, J = 6.8 Hz, 2 H), 5.68 (s 1H), 5.46(s, 1H), 5.20 (t, J = 5.8 Hz, 1H), 5.04 (br s, 1H), 4.44 (d, J = 5.6 Hz, 2H), 3.09 (t, J = 6.10 Hz, 2H), 1.89 (m, 1H), 0.88 (d, J = 6.0 Hz, 6H); IR (KBr) 3257, 2957, 2869, 1676, 1641, 1605, 1543, 1483 cm^{-1}; MS (ES^+) 605.5 [100% (M+1)]. Anal. ($C_{36}H_{36}N_4O_5 \cdot HCl \cdot 1.5H_2O$) C, H, N.

Compound **36n** was prepared from **35n** (0.08 mmol) according to Method G-2 in 41% yield. ^1H NMR (DMSO-d_6) δ 9.50 (br s, 2H), 8.77 (br s, 2H), 8.49 (t, J = 6.0 Hz, 1H), 7.98 (m, 1H), 7.63 (m, 7H), 7.55 (d, J = 6.9 Hz, 1H), 7.01 (d, J = 7.9 Hz, 1H), 6.99 (m, 1H), 5.55 (s, 1H), 5.38 (s, 1H), 5.13 (t, J = 5.0 Hz, 1H), 4.39 (d, J = 5.0 Hz, 2H), 3.02 (t, J = 6.0 Hz, 2H), 1.81 (m, 1H), 0.86 (d, J = 6.9 Hz, 6H); IR (KBr) 3221, 2958, 1659, 1605, 1541, 1482, 1412, 1328 cm^{-1}; MS (ES^+) 515.4 [100% (M+1)]. Anal. ($C_{29}H_{30}N_4O_5 \cdot HCl \cdot 0.25H_2O$) C, H, N.

6.3.14.32. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(4-hydroxybut-1-en-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36o. Compound **33o** was prepared from **31a** (0.75 mmol scale) and tert-butyldimethyl(3-(tributylstannyl)but-3-enyloxy)silane[27] **32** (2.25 mmol) according to Method D-3. Compound **33o** was converted to **34o** following Method G-1 and **35o** was obtained from **34o** according to Method H in 25% yield in three steps; mp >180 °C (dec). ^1H NMR (DMSO-d_6) δ 10.68 (s, 1H), 9.18 (br s, 2H), 8.82 (br s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.25 (s, 1H), 8.03 (dd, J = 7.8 and 2.0 Hz, 1H), 7.75 (m, 6H), 7.66 (dd, J = 7.8 and 2.0 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.29 (m, 5H), 7.05 (d, J = 7.0 Hz, 2H), 5.05 (s, 2H), 4.65 (t, J = 5.0 Hz, 1H), 3.60 (m, 2H), 3.09 (t, J = 6.0 Hz, 2H), 2.75 (t, J = 7.0 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3262, 3064, 2956, 1677, 1641, 1483, 1324, 1255 cm^{-1}; MS (ES^+) 619.4 [100% (M+1)]. Anal. ($C_{37}H_{38}N_4O_5 \cdot HCl \cdot 2H_2O$) C, H, N.

Compound **36o** was prepared from **35o** (0.15 mmol) according to Method G-2 in 70% yield; mp >255 °C. ^1H NMR (DMSO-d_6) δ 9.08 (s, 2H), 8.82 (s, 2H), 8.54 (t, J = 6.0 Hz, 1H), 8.05 (m, 1H), 7.63 (m, 8H), 7.06 (m, 2H), 5.52 (s, 1H), 5.20 (s, 1H), 4.63 (t, J = 5.0 Hz, 1H), 3.56 (m, 2H), 3.05 (t, J = 6.0 Hz, 2H), 2.71 (t, J = 7.0 Hz, 2H), 1.82 (m, 1H), 0.87 (d, J = 6.9 Hz, 6H); IR (KBr) 3089, 2957, 1643, 1604, 1542, 1482, 1411, 1325 cm^{-1}; MS (ES^+) 529.4 [100% (M+1)]. Anal. ($C_{30}H_{32}N_4O_5 \cdot HCl \cdot 0.25H_2O$) C, H, N.

6.3.14.33. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-hydroxyprop-1-ynyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36p. Compound **33p** was prepared from **31a** (0.75 mmol) and prop-2-yn-1-ol (Aldrich, 2.25 mmol) following Method D-3 in 42% yield. Compound **33p** (0.31 mmol) was converted to **34p** using Method G-1 and **35p** was prepared from **34p** using Method H in 43% yield in both steps. ^1H NMR (DMSO-d_6) δ 10.71 (s, 1H), 9.15 (br s, 2H), 8.76 (br s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.23 (s, 1H), 8.02 (dd, J = 10.0 and 2.0 Hz, 1H), 7.73 (m, 5H), 7.58 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.25 (m, 3H), 7.03 (d, J = 7.0 Hz, 2H), 5.42 (t, J = 6.0 Hz, 1H), 5.00 (m, 2H), 4.36 (d, J = 6.0 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.72 (m, 1H), 0.87 (d, J = 6.8 Hz, 6H); IR (KBr) 3275, 2957, 1675, 1637, 1480, 1324, 1257 cm^{-1}; MS (ES^+) 603.4 [100% (M+1)]. Anal. ($C_{36}H_{34}N_4O_5 \cdot HCl \cdot 1.5H_2O$) C, H, N.

Compound **36p** was prepared from **35p** (0.12 mmol) according to Method G-2 in 80% yield; mp >260 °C. ^1H NMR (DMSO-d_6) δ 8.99 (s, 2H), 8.86 (s, 2H), 8.52 (t, J = 6.0 Hz, 1H), 8.03 (m, 1H), 7.63 (m, 7H), 7.50 (d, J = 7.0 Hz, 1H), 7.07 (d, J = 7.0 Hz, 1H), 7.12 (m, 1H), 5.40 (t, J = 6.0 Hz, 1H), 4.33 (d, J = 6.0 Hz, 2H), 3.01 (t,

J = 7.0 Hz, 2H), 1.80 (m, 1H), 0.84 (d, J = 6.9 Hz, 6H); IR (KBr) 3298, 2959, 1667, 1642, 1604, 1542, 1481, 1411, 1327 cm^{-1}; MS (ES^+) 513.4 [100% (M+1)]. Anal. ($C_{29}H_{28}N_4O_5 \cdot HCl$) C, H, N.

6.3.14.34. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(4-hydroxybut-1-ynyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36q. Compound **33q** was prepared from **31a** (0.75 mmol) and but-3-yn-1-ol (Aldrich, 2.63 mmol) following Method D-3 in 57% yield. Compound **33q** (0.43 mmol) was converted to **34q** using Method G-1 and **35q** was prepared from **34q** using Method H in 59% yield in both steps; mp >225 °C. ^1H NMR (DMSO-d_6) δ 10.71 (s, 1H), 9.16 (s, 2H), 8.81 (s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.25 (s, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.73 (m, 5H), 7.69 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 8.9 Hz, 1H), 7.26 (m, 3H), 7.03 (m, 2H), 5.02 (br s, 2H), 4.95 (t, J = 5.0 Hz, 1H), 3.62 (q, J = 6.0 and 12.8 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 2.62 (t, J = 6.0 Hz, 2H), 1.83 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3263, 3064, 2956, 1677, 1640, 1482, 1324, 1257 cm^{-1}; MS (ES^+) 617.4 [100% (M+1)]. Anal. ($C_{37}H_{36}N_4O_5 \cdot HCl \cdot 2H_2O$) C, H, N.

Compound **36q** was prepared from **35q** (0.23 mmol) according to Method G-2 in 91% yield; mp >200 °C. ^1H NMR (DMSO-d_6) δ 9.06 (s, 2H), 8.78 (s, 2H), 8.52 (t, J = 6.0 Hz, 1H), 8.01 (d, J = 6.8 Hz, 1H), 7.62 (m, 7H), 7.46 (d, J = 6.8 Hz, 1H), 7.00 (m, 2H), 4.94 (t, J = 6.0 Hz, 1H), 3.60 (q, J = 6.0 and 12.8 Hz, 2H), 3.01 (t, J = 6.0 Hz, 2H), 2.58 (t, J = 6.0 Hz, 2H), 1.82 (m, 1H), 0.85 (d, J = 6.8 Hz, 6H); IR (KBr) 3381, 2958, 1665, 1604, 1544, 1481, 1326 cm^{-1}; MS (ES^+) 525.4 [100% (M+1)]. Anal. ($C_{30}H_{30}N_4O_5 \cdot HCl \cdot H_2O$) C, H, N.

6.3.14.35. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-hydroxy-3-methylbut-1-ynyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36r. Compound **33r** was prepared from **31a** (0.75 mmol) and 2-methylbut-3-yn-2-ol (Aldrich, 2.25 mmol) following Method D-3 in 66% yield. Compound **33m** (0.5 mmol) was converted to **34m** using Method G-1 and **35m** was prepared from **34m** using Method H in 20% yield in both steps; MS (ES^+) 631.5 (M+Na).

Compound **36r** was prepared from **35r** (0.06 mmol) according to Method G-2 in 50% yield; mp >260 °C. ^1H NMR (DMSO-d_6) δ 9.06 (s, 2H), 8.77 (s, 2H), 8.53 (t, J = 6.0 Hz, 1H), 8.03 (m, 1H), 7.64 (m, 7H), 7.46 (d, J = 6.9 Hz, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 5.52 (s, 1H), 3.02 (t, J = 6.8 Hz, 2H), 1.81 (m, 1H), 1.48 (s, 6H),0.85 (d, J = 6.8 Hz, 6H); IR (KBr) 3323, 2962, 1605, 1538, 1482, 1326 cm^{-1}; MS (ES^-) 539.4 [100% (M−1)]. Anal. ($C_{31}H_{32}N_4O_5 \cdot HCl \cdot H_2O$) C, H, N.

6.3.14.36. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(1,2-dihydroxyethyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36s. Compound **33b** (0.81 mmol, R = CH=CH_2) was converted to **33s** (R = $CH(OH)CH_2OH$) following Method J in 36% yield. Compound **33s** (0.22 mmol) generated **34s** using Method G-1 in quantitative yield and **34s** (0.22 mmol) was converted to **35s** using Method H in 60% yield. Compound **36s** was prepared from **35s** (0.08 mmol) using Method G-2 in 70% yield; mp >265 °C. ^1H NMR (DMSO-d_6) δ 13.83 (s, 1H), 8.90 (br s, 4H), 8.47 (t, J = 6.0 Hz, 1H), 7.95 (s, 1H), 5.30 (s, 1H), 7.61 (m, 6H), 7.40 (m, 1H), 6.95 (d, J = 7.7 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 6.64 (d, J = 9.0 Hz, 1H), 6.22 (s, 1H), 4.60 (t, J = 5.1 Hz, 1H), 3.51 (d, J = 5.6 Hz, 2H), 3.01 (t, J = 7.0 Hz, 2H), 1.80 (m, 1H), 0.85 (d, J = 6.9 Hz, 6H); IR (KBr) 3327, 2958, 1635, 1606, 1540, 1482, 1326 cm^{-1}; MS (ES^+) 519.52 [100% (M+1)]. Anal. ($C_{28}H_{30}N_4O_6 \cdot HCl \cdot 0.5H_2O$) C, H, N.

6.3.14.37. 4′-(Azidomethyl)-6′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36t. Compound **33s** (2.3 mmol, R = $CH(OH)CH_2OH$) was converted to **33t** (R = CHO) using Method K in 72% yield; **33t** (1.0 mmol, R = CHO) was converted to **33t** (R = CH_2OH) using Method I; **33t** (R = $CH_2SO_2CH_3$) was prepared using Method L, which was further converted to **33t** (R = CH_2N_3) following Method M. Further conversion of **33t** (R = CH_2N_3) to **34t** was done following Method G-1 and **35t** was prepared from **34t** following Method H. The overall yield in all these steps was 11%. ^1H NMR (DMSO-d_6) δ 10.80 (s, 1H), 9.20 (br s, 2H), 8.90 (br s, 2H), 8.70 (t, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.00 (d, J = 6.0 Hz, 1H), 7.70 (m, 4H), 7.65 (s, 1H), 7.60 (d, J = 5.0 Hz, 1H), 7.40 (d, J = 5.8 Hz, 1H), 7.35 (d, J = 6.9 Hz, 1H), 7.29 (m, 3H), 7.00 (m, 2H), 5.00 (br s, 2H), 4.60 (s, 2H), 3.01 (t, J = 6.8 Hz, 2H), 1.81 (m, 1H), 0.95 (d, J = 6.8 Hz, 6H); IR (KBr) 3262, 3063, 2957, 2099, 1675, 1639, 1482, 1324, 1255 cm^{-1}; MS (ES^+) 604.3 [100% (M+1)].

Compound **36t** was prepared from **35t** (0.008 mmol) using Method G-2; MS (ES^+) 514.25 [100% (M+1)].

The compound was submitted for testing in DMSO solution and the concentration was calculated based upon the amount of ester (**35t**) used.

6.3.14.38. 4′-(Aminomethyl)-6′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36u. Compound **36u** was prepared from **36t** (0.08 mmol) using Method F; MS (ES^+) 488.30 [100% (M+1)].

The compound was submitted for testing in DMSO solution and the concentration was calculated based upon the amount of ester (**35t**) used.

6.3.14.39. 4-{2-(2-Carboxy-4(N-(2-methyl) propyl))carboxamido) phenyl-5-phenyl}phenyl carboxamidobenzamidine 36v. Compound **33v** was prepared from **31a** (0.75 mmol) and phenylboronic acid (Aldrich, 2.25 mmol) following Method D-1 in 75% yield. ^1H NMR (DMSO-d_6) δ 8.72 (t, J = 6.0 Hz, 1H), 8.42 (d, J = 1.7 Hz, 1H), 8.11 (d, J = 1.7 Hz, 1H), 8.07 (dd, J = 7.7 and 1.7 Hz, 1H), 7.88 (dd, J = 1.7 and 7.7 Hz, 1H), 7.73 (d, J = 6.8 Hz, 2H), 7.54 (t, J = 7.7 Hz, 2H), 7.44 (m, 1H), 7.37 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 7.7 Hz, 1H), 7.21 (m, 3H), 7.09 (m, 2H), 5.17 (d, J = 17.0 Hz, 2H), 5.05 (d, J = 5.0 Hz, 2H), 3.40 (m, 2H), 3.30 (m, 2H), 3.16 (s, 3H), 3.12 (t, J = 6.0 Hz, 2H), 1.87 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (NaCl) 3328, 2959, 1723, 1642, 1542, 1475, 1308, 1239 cm^{-1}; MS (ES^+) 596.45 [100% (M+1)]. Anal. ($C_{36}H_{37}NO_7$) C, H, N.

Compound **33v** (0.5 mmol) was converted to **34v** following Method G-1 in quantitative yield. ^1H NMR (DMSO-d_6) δ 12.73 (br s, 1H), 8.73 (t, J = 6.0 Hz, 1H), 8.41 (d, J = 1.7 Hz, 1H), 8.12 (d, J = 1.7 Hz, 1H), 8.07 (dd, J = 7.7 and 1.7 Hz, 1H), 7.83 (dd, J = 7.7 and 1.7 Hz, 1H), 7.72 (d, J = 6.9 Hz, 2H), 7.54 (t, J = 7.7 Hz, 2H), 7.44 (t, J = 7.7 Hz, 1H), 7.37 (d, J = 7.7 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 7.21 (m, 3H), 7.09 (m, 2H), 5.08 (d, J = 14.0 Hz, 2H), 3.13 (t, J = 6.5 Hz, 2H), 1.88 (m, 1H), 0.91 (d, J = 6.8 Hz, 6H); IR (KBr) 3370, 2958, 1712, 1618, 1552, 1240 cm^{-1}; MS (ES^+) 507.93 [100% (M+1)]. Anal. ($C_{32}H_{29}NO_5 \cdot 0.5H_2O$) C, H, N.

Compound **35v** was prepared from **34v** (0.57 mmol) using Method H in 89% yield; mp 215 °C. ^1H NMR (DMSO-d_6) δ 10.75 (s, 1H), 9.19 (s, 2H), 8.89 (s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.29 (d, J = 1.7 Hz, 1H), 8.07 (dd, J = 7.7 and 1.7 Hz, 1H), 7.99 (d, J = 1.7 Hz, 1H), 7.87 (dd, J = 7.7 and 1.7 Hz, 1H), 7.83 (d, J = 7.7 Hz, 2H), 7.77 (m 4H), 7.54 (t, J = 7.7 Hz, 2H), 7.43 (m, 3H), 7.19 (m, 3H), 7.03 (d, J = 6.9 Hz, 2H), 5.04 (br s, 2H), 3.09 (t, J = 6.5 Hz, 2H), 1.84 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (KBr) 3060, 2958, 1717, 1675, 1484, 1259 cm^{-1}; MS (ES^+) 625.81 [100% (M+1)]. Anal. ($C_{39}H_{36}N_4O_4 \cdot HCl \cdot 1.25H_2O$) C, H, N.

Compound **36v** was prepared from **35v** (0.30 mmol) using Method G-2 in 86% yield; mp 268 °C. ^1H NMR (DMSO-d_6) δ 13.28 (s, 1H), 9.04 (s, 4H), 8.50 (t, J = 6.0 Hz, 1H), 7.97 (s, 1H), 7.82 (s, 1H), 7.74 (m, 3H), 7.62 (m, 5H), 7.50 (t, J = 7.7 Hz, 2H), 7.40 (t, J = 7.7 Hz, 1H), 7.10 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 7.7 Hz, 1H), 3.01 (t, J = 6.5 Hz, 2H), 1.80 (m, 1H), 0.85 (d, J = 6.8 Hz, 6H); IR (KBr) 2958, 1605, 1540, 1481, 1327 cm^{-1}; MS (ES^+) 535.48 [100% (M+1)]. Anal. ($C_{32}H_{30}N_4O_4 \cdot HCl \cdot 0.5H_2O$) C, H, N.

6.3.14.40. 2′-[({4-[Amino(imino)methyl]phenyl}amino)carbonyl]-4′-benzyl-4-[(isobutylamino)carbonyl]-1,1′-biphenyl-2-carboxylic acid 36w. Compound **33w** was prepared from **31b** (0.75 mmol) and benzyltributylstannane[28] (2.25 mmol) following Method D-3 in 32% yield. Compound **33w** (0.27 mmol) was converted to **34w** using Method G-1 in quantitative yield and **35w** was prepared from **34w** (0.27 mmol) using Method H in 20% yield; MS (ES^+) 563.4 (M+H).

Compound **36w** was prepared from **35w** (0.06 mmol) according to Method G-2 in 66% yield; mp >217 °C. ^1H NMR (DMSO-d_6) δ 13.64 (br s, 1H), 9.06 (s, 2H), 8.89 (s, 2H), 8.50 (t, J = 6.0 Hz, 1H), 7.98 (s, 1H), 7.62 (m, 7H), 7.43 (s, 1H), 7.33 (m, 4H), 7.20 (m, 1H), 6.95 (m, 2H), 4.04 (s, 2H), 3.02 (t, J = 6.8 Hz, 2H), 1.80 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 2958, 2927, 1606, 1481, 1325 cm^{-1}; MS (ES^-) 547.4 [100% (M−1). Anal. ($C_{33}H_{32}N_4O_4{\cdot}HCl{\cdot}H_2O$) C, H, N.

6.3.14.41. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(pyridin-4-yl)biphenyl-2-carboxylic acid 36x. Compound **33x** was prepared from **31a** (0.75 mmol) and pyridine-4-ylboronic acid (Frontier Scientific, 2.25 mmol) following Method D-1 in 83% yield. Compound **33x** (0.0.42 mmol) was converted to **34x** following Method G-1 in 61% yield. ^1H NMR (DMSO-d_6) δ 8.71 (m, 3H), 8.40 (d, J = 2.0 Hz, 1H), 8.18 (s, 1H), 8.05 (dd, J = 7.6 and 1.7 Hz, 1H), 7.85 (m, 1H), 7.75 (d, J = 6.2 Hz, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.18 (m, 3H), 7.06 (m, 2H), 5.03 (s, 2H), 3.11 (t, J = 6.9 Hz, 2H), 1.88 (m, 1H), 0.90 (d, J = 6.9 Hz, 6H); IR (KBr) 3308, 2957, 2870, 1712, 1634, 1606, 1546, 1265 cm^{-1}; MS (ES^+) 509.49 [100% (M+1)]. Anal. ($C_{31}H_{28}N_2O_5{\cdot}1.75H_2O$) C, H, N.

Compound **35x** was prepared from **34x** (0.22 mmol) using Method H in 72% yield. ^1H NMR (DMSO-d_6) δ 10.76 (s, 1H), 9.11 (s, 2H), 8.84 (s, 2H), 8.70 (m, 4H), 8.29 (d, J = 2.0 Hz, 1H), 8.08 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 8.0 and 2.0 Hz, 1H), 7.94 (dd, J = 8.0 and 2.0 Hz, 1H), 7.87 (d, J = 6.0 Hz, 2H), 7.76 (s, 4H), 7.45 (t, J = 7.0 Hz, 2H), 7.18 (m, 3H), 7.03 (m, 1H), 5.03 (s, 2H), 3.08 (t, J = 6.9 Hz, 2H), 1.75 (m, 1H), 0.87 (d, J = 6.9 Hz, 6H); IR (KBr) 3063, 2957, 1677, 1641, 1600, 1482, 1326, 1259 cm^{-1}; MS (ES^+) 626.76 [100% (M+1)]. Anal. ($C_{38}H_{35}N_5O_4{\cdot}HCl{\cdot}1.75H_2O$) C, H, N.

Compound **36x** was prepared from **35x** (0.15 mmol) using Method G-2 in 58% yield; mp >285 °C. ^1H NMR (DMSO-d_6) δ 14.19 (s, 1H), 9.06 (br s, 2H), 8.67 (br s, 2H), 8.67 (d, J = 6.0 Hz, 2H), 8.50 (t, J = 6.0 Hz, 1H), 7.97 (m, 2H), 7.91 (dd, J = 7.7 and 2.0 Hz, 1H), 7.80 (d, J = 6.0 Hz, 2H), 7.64 (m, 6H), 7.18 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 7.7 Hz, 1H), 3.02 (t, J = 5.0 Hz, 2H), 1.82 (m, 1H), 0.80 (d, J = 6.9 Hz, 6H); IR (KBr) 3287, 2959, 1641, 1603, 1541, 1481, 1330 cm^{-1}; MS (ES^+) 536.43 (20% (M+1)]. Anal. ($C_{31}H_{29}N_5O_4{\cdot}HCl{\cdot}H_2O$) C, H, N.

6.3.14.42. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(pyridin-3-yl)biphenyl-2-carboxylic acid 36y. Compound **33y** was prepared from **31a** (0.75 mmol) and pyridine-3-ylboronic acid (Frontier Scientific, 2.25 mmol) following Method D-1 in 83% yield. Compound **33y** (0.42 mmol) was converted to **34y** following Method G-1 in 61% yield. ^1H NMR (DMSO-d_6) δ 12.70 (br s, 1H), 8.91 (d, J = 2.6 Hz, 1H), 8.68 (t, J = 6.0 Hz, 1H), 8.62 (d, J = 2.0 Hz, 1H), 8.40 (d, J = 1.7 Hz, 1H), 8.12 (m, 2H), 8.05 (dd, J = 8.6 and 1.7 Hz, 1H), 7.88 (d, J = 8.5 and 1.7 Hz, 1H), 7.53 (dd, J = 8.6 and 5.2 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.18 (m, 3H), 7.08 (m, 2H), 5.04 (d, J = 12.0 Hz, 2H), 3.11 (t, J = 6.5 Hz, 2H), 1.87 (m, 1H), 0.90 (d, J = 6.8 Hz, 6H); IR (KBr) 3403, 2958, 2418, 1709, 1655, 1540, 1261 cm^{-1}; MS (ES^+) 509.11 [100% (M+1)]. Anal. ($C_{31}H_{28}N_2O_5$) C, H, N.

Compound **35y** was prepared from **34y** (0.31 mmol) using Method H in 46% yield; mp 265 °C. ^1H NMR (DMSO-d_6) δ 10.73 (br s, 1H), 9.16 (br s, 2H), 9.05 (d, J = 1.9 Hz, 1H), 8.79 (s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.64 (dd, J = 1.2 and 5.0 Hz, 1H), 8.29 (d, J = 1.7 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.05 (m, 2H), 7.93 (dd, J = 8.0 and 1.8 Hz, 1H), 7.76 (m, 5H), 7.56 (dd, J = 8.0 and 4.3 Hz, 1H), 7.44 (d, J = 7.4 Hz, 2H), 7.18 (m, 3H), 7.00 (m, 2H), 5.00 (s, 2H), 3.08 (t, J = 6.5 Hz, 2H), 1.82 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3062, 2957, 1677, 1641, 1544, 1483, 1326, 1125 cm^{-1}; MS (ES^+) 626.44 [100% (M+1)]. Anal. ($C_{38}H_{35}N_5O_4{\cdot}2\ HCl{\cdot}0.5H_2O$) C, H, N.

Compound **36y** was prepared from **35y** (0.13 mmol) using Method G-2 in 66% yield; mp 280 °C. ^1H NMR (DMSO-d_6) δ 9.05 (br s, 2H), 8.95 (d, J = 2.1 Hz, 1H), 8.75 (s, 2H), 8.65 (dd, J = 5.0 and 1.4 Hz, 1H), 8.50 (t, J = 5.6 Hz, 1H), 8.20 (dt, J = 1.8 and 7.7 Hz, 1H), 7.99 (d, J = 2.1 Hz, 1H), 7.90 (d, J = 2.1 Hz, 1H), 7.85 (dd, J = 7.7 and 2.2 Hz, 2H), 7.65 (m, 5H), 7.55 (dd, J = 7.7 and 4.5 Hz, 1H), 7.15 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 7.7 Hz, 1H), 3.08 (t, J = 5.0 Hz, 2H), 1.82 (m, 1H), 0.90 (d, J = 6.8 Hz, 6H); IR (KBr) 3285, 2958, 1638, 1605, 1543, 1481, 1329 cm^{-1}; MS (ES^+) 536.30 (M+1). Anal. ($C_{31}H_{29}N_5O_4{\cdot}3H_2O$) C, H, N.

6.3.14.43. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(thiophen-2-yl)biphenyl-2-carboxylic acid 36z. Compound **33z** was prepared from **31a** (0.75 mmol) and thiophen-2-ylboronic acid (Aldrich, 2.25 mmol) following Method D-1 in 85% yield. ^1H NMR (DMSO-d_6) δ 8.73 (t, J = 6.0 Hz, 1H), 8.42 (s, 1H), 8.06 (m, 2H), 7.86 (d, J = 8.6 Hz, 1H), 7.64 (d, J = 5.0 Hz, 1H), 7.62 (d, J = 3.5 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.24 (d, J = 7.7 Hz, 1H), 7.21 (m, 4H), 7.08 (m, 2H), 5.16 (d, J = 17.0 Hz, 2H), 5.05 (d, J = 2.6 Hz, 2H), 3.39 (m, 2H), 3.29 (m, 2H), 3.16 (s, 3H), 3.12 (t, J = 6.0 Hz, 2H), 1.87 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (NaCl) 3342, 2959, 1716, 1643, 1542, 1478, 1295, 1240 cm^{-1}; MS (ES^+) 602.52 [100% (M+1)]. Anal. ($C_{34}H_{35}NO_7S{\cdot}0.5H_2O$) C, H, N.

Compound **33z** (0.57 mmol) was converted to **35z** using Methods G-1 and H in 89% yield in both steps. ^1H NMR (DMSO-d_6) δ 10.76 (s, 1H), 9.17 (s, 2H), 8.80 (s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.26 (dd, J = 7.7 and 1.7 Hz, 1H), 8.03 (dd, J = 7.7 and 1.7 Hz, 1H), 7.94 (d, J = 1.7 Hz, 1H), 7.82 (dd, J = 7.7 and 1.7 Hz, 1H), 7.75 (m, 4H), 7.70 (d, J = 4.3 Hz, 1H), 7.66 (d, J = 5.2 Hz, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.20 (m, 4H), 7.04 (d, J = 6.9 Hz, 2H), 5.05 (s, 2H), 3.07 (t, J = 6.9 and 6.0 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.9 Hz, 6H); IR (KBr) 3065, 2957, 2924, 1675, 1483, 1408, 1324, 1259 cm^{-1}; MS (ES^+) 631.05 [100% (M+1)]. Anal. ($C_{37}H_{34}N_4O_4S{\cdot}HCl{\cdot}1.5H_2O$) C, H, N.

Compound **36z** was prepared from **35z** (0.29 mmol) following Method G-2 in 79% yield; mp 268 °C. ^1H NMR (DMSO-d_6) δ 14.95 (s, 1H), 8.97 (s, 4H), 8.50 (t, J = 6.0 Hz, 1H), 7.97 (d, J = 2.0 Hz, 1H), 7.80 (d, J = 2.0 Hz, 1H), 7.73 (dd, J = 7.9 and 2.0 Hz, 1H), 7.61 (m, 7H), 7.18 (t, J = 3.9 Hz, 1H), 7.05 (d, J = 7.9 Hz, 1H), 6.93 (d, J = 7.9 Hz, 1H), 3.01 (t, J = 6.0 Hz, 2H), 1.81 (m, 1H), 0.84 (d, J = 6.9 Hz, 6H); IR (KBr) 2957, 2868, 1642, 1604, 1540, 1481, 1411, 1326 cm^{-1}; MS (ES^+) 541.17 [100% (M+1)]. Anal. ($C_{30}H_{28}N_4O_4S{\cdot}HCl{\cdot}0.25H_2O$) C, H, N.

6.3.14.44. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(thiophen-3-yl)biphenyl-2-carboxylic acid 36aa. Compound **33aa** was prepared from **31a** (0.75 mmol) and thiophen-3-ylboronic acid (Aldrich, 2.25 mmol) following Method D-1 in 88% yield. ^1H NMR (DMSO-d_6) δ 8.68 (t, J = 6.0 Hz, 1H), 8.41 (d, J = 1.7 Hz, 1H), 8.14 (d, J = 1.7 Hz, 1H), 8.06 (dd, J = 2.0 and 8.0 Hz, 1H), 7.91 (dd, J = 1.7 and 7.7 Hz, 1H), 8.00 (m, 1H), 7.71 (dd, J = 4.8 and 3.0 Hz, 1H), 7.61 (d, J = 5.0 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.08 (m, 2H), 7.20 (m, 3H), 5.16 (d, J = 16.0 Hz, 2H), 5.04 (d, J = 4.3 Hz, 2H), 3.39 (dd, J = 5.2 and 3.4 Hz, 2H), 3.30 (m, 2H), 3.16 (s, 3H), 3.12 (t, J = 6.0 and 6.8 Hz, 2H), 1.87 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (NaCl) 3331, 2959, 1721, 1643, 1543, 1296, 1240 cm^{-1}; MS (ES^+) 602.27 [100% (M+1)]. Anal. ($C_{34}H_{35}NO_7S$) C, H, N.

Compound **33aa** (0.61 mmol) was converted to **34aa** following Method G-1 in 98% yield; ^1H NMR (DMSO-d_6) δ 12.70 (brs, 1H), 8.72 (t, J = 6.0 Hz, 1H), 8.39 (d, J = 1.7 Hz, 1H), 8.15 (m, 1H), 8.07

(dd, J = 1.7 and 7.7 Hz, 1H), 8.02 (d, J = 1.7 Hz, 1H), 7.88 (dd, J = 7.2 and 1.7 Hz, 1H), 7.70 (dd, J = 2.6 and 4.3 Hz, 1H), 7.63 (d, J = 5.2 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.22 (m, 4H), 7.09 (m, 2H), 5.07 (d, J = 13.0 Hz, 2H), 3.12 (t, J = 6.0 Hz, 2H), 1.88 (m, 1H) 0.92 (d, J = 6.9 Hz, 6H); IR (KBr) 3296, 2961, 2870, 1721, 1691, 1630, 1545, 1242 cm^{-1}; MS (ES^+) 514.06 [100% (M+1)]. Anal. ($C_{30}H_{27}NO_5S$) C, H, N.

Compound **35aa** was prepared from **34aa** (0.6 mmol) using Method H in 74% yield; mp 210 °C. ^1H NMR (DMSO-d_6) δ 10.73 (s, 1H), 9.17 (s, 2H), 8.82 (s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.23 (d, J = 1.7 Hz, 1H), 8.05 (m, 3H), 7.90 (dd, J = 1.7 and 7.7 Hz, 1H), 7.72 (m, 6H), 7.43 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.20 (m, 3H), 7.02 (d, J = 6.8 Hz, 2H), 5.04 (s, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.9 Hz, 6H); IR (KBr) 3265, 3065, 2958, 1675, 1483, 1408, 1324, 1259 cm^{-1}; MS (ES^+) 631.82 [100% (M+1)]. Anal. ($C_{37}H_{34}N_4O_4S{\cdot}HCl{\cdot}1.25H_2O$) C, H, N.

Compound **36aa** was prepared from **35aa** (0.3 mmol) using Method G-2 in 93% yield; mp 265 °C. ^1H NMR (DMSO-d_6) δ 13.24 (s, 1H), 9.05 (s, 2H), 8.90 (s, 2H), 8.49 (t, J = 6.0 Hz, 1H), 7.97 (s, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.65 (m, 1H), 7.62 (m, 6H), 7.05 (d, J = 7.7 Hz, 1H), 6.93 (d, J = 7.7 Hz, 1H), 3.01 (t, J = 6.0 Hz, 2H), 1.81 (m, 1H), 0.85 (d, J = 6.9 Hz, 6H); IR (KBr) 2957, 1642, 1604, 1540, 1481, 1411, 1325 cm^{-1}; MS (ES^+) 541.42 [100% (M+1)]. Anal. ($C_{30}H_{28}N_4O_4S{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

6.3.14.45. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(furan-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ab. Compound **33ab** was prepared from **31a** (0.75 mmol) and furan-2-ylboronic acid (Lancaster, 2.25 mmol) following Method D-1 in 71% yield. Compound **33ab** (0.41 mmol) was converted to **34ab** following Method G-1 in 69% yield.

Compound **35ab** was prepared from **34ab** (0.29 mmol) using Method H in 56% yield; mp 260 °C. ^1H NMR (DMSO-d_6) δ 10.77 (br s, 1H), 9.12 (br s, 2H), 8.88 (br s, 2H), 8.67 (t, J = 6.2 Hz, 1H), 8.25 (d, J = 1.8 Hz, 1H), 8.03 (dd, J = 1.7 and 8.0 Hz, 1H), 7.98 (d, J = 1.2 Hz, 1H), 7.85 (m, 2H), 7.74 (m, 5H), 7.42 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.16 (m, 3H), 7.03 (m, 2H), 6.67 (m, 1H), 5.04 (s, 2H), 3.06 (t, J = 6.2 Hz, 2H), 1.83 (m, 1H), 0.87 (d, J = 6.7 Hz, 6H); IR (KBr) 3605, 2958, 1676, 1641, 1487, 1324, 1258 cm^{-1}; MS (ES^+) 615.75 [100% (M+1)]. Anal. ($C_{37}H_{34}N_4O_5{\cdot}HCl{\cdot}1.5H_2O$) C, H, N.

Compound **36ab** was prepared from **35ab** (0.15 mmol) using Method G-2 in 76% yield; mp 290 °C. ^1H NMR (DMSO-d_6) δ 9.07 (s, 2H), 8.86 (s, 2H), 8.53 (t, J = 5.0 Hz, 1H), 8.03 (s, 1H), 7.89 (d, J = 1.4 Hz, 1H), 7.78 (m, 2H), 7.65 (m, 6H), 7.10 (m, 2H), 7.08 (d, J = 7.0 Hz, 1H), 6.64 (dd, J = 3.5 and 2.0 Hz, 1H), 3.03 (t, J = 6.0 Hz, 2H), 1.81 (m, 1H), 0.86 (d, J = 6.9 Hz, 6H); IR (KBr) 3306, 2958, 1640, 1603, 1541, 1482, 1411, 1369, 1328 cm^{-1}; MS (ES^+) 525.43 [100% (M+1)]. Anal. ($C_{30}H_{28}N_4O_4S{\cdot}HCl{\cdot}0.75H_2O$) C, H, N.

6.3.14.46. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(furan-3-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ac. Compound **33ac** was prepared from **31a** (0.75 mmol) and furan-3-ylboronic acid (Lancaster, 2.25 mmol) following Method D-1 in 55% yield. Compound **33ac** (0.41 mmol) was converted to **34ac** following Method G-1 in 69% yield. ^1H NMR (DMSO-d_6) δ 12.58 (br s, 1H), 8.66 (t, J = 6.0 Hz, 1H), 8.36 (d, J = 1.7 Hz, 1H), 8.29 (s, 1H), 8.04 (m, 2H), 7.78 (t, J = 1.7 Hz, 1H), 7.75 (dd, J = 8.5 and 1.7 Hz, 1H), 7.31 (d, J = 7.7 Hz, 1H), 7.21 (m, 3H), 7.16 (d, J = 8.6 Hz, 1H), 7.06 (m, 2H), 7.02 (s, 1H), 5.03 (d, J = 10.0 Hz, 2H), 3.12 (t, J = 6.0 Hz, 2H), 1.86 (m, 1H), 0.89 (d, J = 6.0 Hz, 6H); IR (KBr) 3281, 2960, 1726, 1692, 1632, 1547, 1243, 1162 cm^{-1}; MS (ES^+) 498.49 [100% (M+1)]. Anal. ($C_{30}H_{27}NO_6$) C, H, N.

Compound **35ac** was prepared from **34ac** (0.24 mmol) using Method H in 68% yield; mp 260 °C. ^1H NMR (DMSO-d_6) δ 10.67 (s, 1H), 9.14 (br s, 2H), 8.80 (br s, 2H), 8.63 (t, J = 6.0 Hz, 1H), 8.33 (s, 1H), 8.25 (d, J = 1.9 Hz, 1H), 8.02 (dd, J = 8.0 and 1.7 Hz, 1H), 7.92 (d, J = 1.7 Hz, 1H), 7.78 (m, 5H), 7.42 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.21 (m, 3H), 7.11 (d, J = 1.7 Hz, 1H), 7.03 (dd, J = 1.7 and 8.0 Hz, 2H), 5.05 (s, 2H), 3.07 (t, J = 6.2 Hz, 2H), 1.73 (m, 1H), 0.87 (d, J = 6.2 Hz, 6H); IR (KBr) 3263, 3065, 2958, 1676, 1484, 1326, 1257 cm^{-1}; MS (ES^+) 615.75 [100% (M+1)]. Anal. ($C_{37}H_{34}N_4O_4S{\cdot}HCl{\cdot}2H_2O$) C, H, N.

Compound **36ac** was prepared from **35ac** (0.15 mmol) using Method G-2 in 64% yield; mp 300 °C. ^1H NMR (DMSO-d_6) δ 14.10 (s, 1H), 9.05 (br s, 2H), 8.79 (br s, 2H), 8.47 (t, J = 5.6 Hz, 1H), 8.30 (s, 1H), 7.96 (d, J = 2.0 Hz, 1H), 7.78 (m, 2H), 7.63 (m, 7H), 7.05 (m, 1H), 7.01 (d, J = 7.7 Hz, 1H), 6.92 (d, J = 7.7 Hz, 1H), 3.02 (t, J = 4.9 Hz, 2H), 1.81 (m, 1H), 0.85 (d, J = 6.3 Hz, 6H); IR (KBr) 2958, 1641, 1603, 1541, 1481, 1411, 1328 cm^{-1}; MS (ES^+) 525.36 [100% (M+1)]. Anal. ($C_{30}H_{28}N_4O_5{\cdot}HCl{\cdot}1.25H_2O$) C, H, N.

6.3.14.47. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(1H-pyrrol-2-yl)biphenyl-2-carboxylic acid 36ad. Compound **33ad** was prepared from **31a** (0.65 mmol) and N-*tert*-butoxycarbonylpyrrol-2-ylboronic acid (Frontier Scientific, 2.24 mmol) following Method D-1 in 60% yield. Compound **33ad** (0.4 mmol) was converted to **34ad** following Method G-1 in 92% yield. Compound **35ad** was prepared from **34ad** (0.34 mmol) using Method H in 74% yield; mp >225 °C (dec). ^1H NMR (DMSO-d_6) δ 10.58 (s, 1H), 9.15 (br s, 2H), 8.77 (br s, 2H), 8.66 (t, J = 6.0 Hz, 1H), 8.26 (d, J = 1.7 Hz, 1H), 8.05 (dd, J = 8.6 and 1.7 Hz, 1H), 7.74 (m, 5H), 7.61 (dd, J = 1.7 and 7.7, 1H), 7.45 (d, J = 1.7 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.33 (d, 7.7 Hz, 1H), 7.26 (m, 3H), 7.11 (m, 2H), 6.51 (s, 1H), 6.37 (t, J = 3.4 Hz, 1H), 5.07 (s, 2H), 3.11 (t, J = 6.0 Hz, 2H), 1.87 (m, 1H), 1.41 (s, 9H), 0.90 (d, J = 6.9 Hz, 6H); IR (KBr) 3271, 2960, 1737, 1678, 1643, 1608, 1485, 1315, 1145 cm^{-1}; MS (ES^+) 714.78 [100% (M+1)]. Anal. ($C_{42}H_{43}N_5O_6{\cdot}HCl{\cdot}H_2O$) C, H, N.

Compound **36ad** was prepared from **35ad** (0.45 mmol) using Method G-2 in 78% yield; mp >270 °C (dec). ^1H NMR (DMSO-d_6) δ 11.45 (s, 1H), 9.08 (br s, 2H), 8.88 (br s, 2H), 8.75 (t, J = 6.0 Hz, 1H), 8.04 (br s, 1H), 7.88 (m, 1H), 7.70 (m, 8H), 7.03 (m, 2H), 6.90 (m, 1H), 6.62 (m, 1H), 6.17 (m, 1H), 3.07 (t, J = 6.0 Hz, 2H), 1.84 (m, 1H), 0.86 (d, J = 6.9 Hz, 6H); IR (KBr) 3292, 2959, 1643, 1604, 1540, 1481, 1328 cm^{-1}; MS (ES^+) 524.65 [100% (M+1)]. Anal. ($C_{30}H_{29}N_5O_4{\cdot}HCl{\cdot}H_2O$) C, H, N.

6.3.14.48. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(1-methyl-1H-pyrrol-2-yl)biphenyl-2-carboxylic acid 36ae. Compound **33ae** was prepared from **31a** (0.75 mmol) and 1-methyl-2-(tributylstannyl)-1H-pyrrole (Frontier Scientific, 2.7 mmol) following Method D-3 in quantitative yield. Compound **33ae** (0.75 mmol) was converted to **34ae** following Method G-1 and **35ae** was prepared from **34ae** using Method H in 79% yield for both steps. ^1H NMR (DMSO-d_6) δ 10.66 (s, 1H), 9.20 (s, 2H), 8.86 (s, 2H), 8.66 (t, J = 6.0 Hz, 1H), 8.24 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 7.8 and 2.0 Hz, 1H), 7.69 (m, 4H), 7.68 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.24 (m, 3H), 7.09 (m, 2H), 6.92 (s, 1H), 6.40 (s, 1H), 6.17 (t, J = 4.0 Hz, 1H), 5.10 (br s, 2H), 3.74 (s, 3H), 3.09 (t, J = 6.0 Hz, 2H), 1.83 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3264, 3093, 1675, 1641, 1483, 1325, 1259 cm^{-1}; MS (ES^+) 628.65 [100% (M+1)]. Anal. ($C_{38}H_{37}N_5O_4{\cdot}HCl{\cdot}1.5H_2O$) C, H, N.

Compound **36ae** was prepared from **35ae** (0.27 mmol) using Method G-2 in 63% yield; mp >260 °C. ^1H NMR (DMSO-d_6) δ 9.10 (s, 2H), 8.84 (s, 2H), 8.56 (t, J = 6.0 Hz, 1H), 8.08 (br s, 1H), 7.67 (m, 7H), 7.58 (d, J = 7.9 Hz, 1H), 7.11 (m, 2H), 6.91 (br s, 1H), 6.31 (br s, 1H), 6.11 (t, J = 3.0 Hz, 1H), 3.74 (s, 3H), 3.05 (t, J = 6.0 Hz, 2H), 1.83 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3099, 2957, 1642, 1603, 1541, 1481 cm^{-1}; MS (ES^+) 538.64 [100% (M+1)]. Anal. ($C_{31}H_{31}N_5O_4{\cdot}HCl{\cdot}0.75H_2O$) C, H, N.

6.3.14.49. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(thiazol-2-yl)biphenyl-2-carboxylic acid 36af. Compound **33af** was prepared from **31a** (0.75 mmol) and 2-(trimethylstannyl)thiazole[29] (2.25 mmol) following Method D-4 in 38% yield. Compound **33af** (0.28 mmol) was converted to **34af** following Method G-1 in 50% yield. Compound **35af** was prepared from **34af** (0.37 mmol) using Method H in 30% yield; mp 205–210 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.84 (s, 1H), 9.16 (s, 2H), 8.78 (s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 8.19 (s, 1H), 8.09 (dd, J = 2.0 and 7.7 Hz, 1H), 8.04 (dd, J = 2.0 and 7.7 Hz, 1H), 8.01 (d, J = 4.0 Hz, 1H), 7.89 (d, J = 3.0 Hz, 1H), 7.73 (m, 4H), 7.44 (dd, J = 3.0 and 7.8 Hz, 2H), 7.16 (m, 3H), 7.30 (s, 1H), 7.05 (s, 1H), 5.03 (br s, 2H), 3.06 (t, J = 6.5 Hz, 2H), 1.82 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 3061, 2956, 1675, 1641, 1483, 1323, 1255 cm^{-1}; MS (ES$^+$) 632.4 [100% (M+1)]. Anal. ($C_{36}H_{33}N_5O_4S$·2 HCl) C, H, N.

Compound **36af** was prepared from **35af** (0.1 mmol) using Method G-2 in 51% yield; mp 288–292 °C. ^{1}H NMR (DMSO-d_6) δ 14.08 (br s, 1H), 9.06 (s, 2H), 8.79 (s, 2H), 8.51 (t, J = 6.0 Hz, 1H), 8.11 (d, J = 2.0 Hz, 1H), 8.01 (m, 3H), 7.85 (d, J = 3.0 Hz, 1H), 7.63 (m, 6H), 7.17 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 3.02 (t, J = 6.5 Hz, 2H), 1.81 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 3311, 2960, 1605, 1542, 1482, 1328 cm^{-1}; MS (ES$^+$) 542.2 [100% (M+1)]. Anal. ($C_{27}H_{27}N_5O_4S$·HCl·0.75H_2O) C, H, N.

6.3.14.50. 2′-(4-Carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)-4′-(thiophen-2-ylmethyl)biphenyl-2-carboxylic acid 36ag. To a solution of 2-hydroxymethylthiophene (Aldrich, 20 g, 174 mmol) in dichloromethane (200 mL) cooled to 0 °C PBr_3 (5.6 mL, 58 mmol) was added dropwise over a period of 10 min and was stirred at room temperature for 3 h. The reaction mixture was quenched with water (100 mL) and the organic layer was separated. The aqueous layer was extracted with dichloromethane (50 mL). The organic layers were combined, washed with brine (50 mL), dried, filtered, and concentrated in vacuo to furnish crude product. The crude product was distilled in vacuo (50 °C) to furnish 2-bromomethylthiophene (22 g, 75%) as a colorless oil which was stored in the freezer until further use.

^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 1.3 and 5.0 Hz, 1H), 7.09 (m, 1H), 6.92 (dd, J = 3.5 and 5 Hz, 1H), 4.73 (s, 2H).

To lithium clippings (2.76 g, 400 mmol) in THF (200 mL) cooled to −40 °C was added dropwise tributyltin chloride (Aldrich, 10.8 mL, 40 mmol) in THF (50 mL) over a period of 15 min. The reaction mixture was allowed to warm to room temperature and was stirred for 16 h. The reaction mixture was filtered through glass wool to remove insoluble impurities and cooled to −40 °C. A freshly prepared solution of 2-bromomethylthiophene (7.08 g, 40 mmol) was added dropwise over a period of 10 min and was stirred at room temperature overnight. The reaction mixture was quenched with saturated aqueous ammonium chloride solution (50 mL) and was extracted with ether (2 × 50 mL). The organic layers were combined, washed with brine (50 mL), dried, filtered, and concentrated in vacuo to furnish 2-tributyltinmethylthiophene (14 g) NMR analysis showed product was 60% pure and was used as such without further purification. ^{1}H NMR ($CDCl_3$): δ 7.32 (d, J = 5 Hz, 1H), 7.08 (m, 1H), 6.94 (dd, J = 3.5 and 5 Hz, 1H), 1.84 (m, 2H), 1.64 (m, 6H), 1.36 (m, 12H), 0.94 (m, 9H).

Compound **33ag** was prepared from **31b** (0.75 mmol) and tributyl(thiophen-2-ylmethyl)stannane following Method D-3 in 21% yield. Compound **33ag** (0.49 mmol) was converted to **34ag** following Method G-1 in 80% yield. Compound **35ag** was prepared from **34ag** (0.33 mmol) using Method H in 10% yield. ^{1}H NMR (DMSO-d_6) δ 10.59 (br s, 1H), 9.16 (s, 2H), 8.85 (s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.21 (s, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.73 (m, 4H), 7.58 (s, 1H), 7.50–7.38 (m, 3H), 7.32 (d, J = 8.1 Hz, 1H), 7.03 (d, J = 7.5 Hz, 2H), 4.31 (s, 2H), 3.55 (s, 3H), 3.07 (t, J = 6.8 Hz, 2H), 1.85 (m, 1H), 0.87 (d, J = 6.8 Hz, 6H); MS (ES$^-$) 567.3 [100% (M−1), (ES$^+$) 569.3 [100% (M+1)]. Anal. ($C_{32}H_{32}N_4O_4S$·HCl·NH_4Cl·2.5H_2O) C, H, N.

Compound **36ag** was prepared from **35ag** (0.00185 mmol) using Method G-2. in 51% yield; MS (ES$^-$) 553.3 [100% (M−1)].

Pure sample could not be isolated. This was submitted in DMSO solution for biological activity.

6.3.14.51. 3-(2-(4-Carbamimidoylphenylcarbamoyl)-2′-carboxy-4′-(isobutylcarbamoyl)biphenyl-4-yl)thiophene-2-carboxylic acid 36ah. Compound **33ah** (R = 2-formylthiophen-3-yl) was prepared from **31a** (1.5 mmol) and 2-formylthiophen-3-ylboronic acid (Lancaster, 6.0 mmol) following Method D-1 in 88% yield. Compound **33ah** (R = 2-formylthiophen-3-yl, 0.13 mmol) was converted to **33ah** (R = 2-carboxylthiophen-3-yl) using Method E in 20% yield, which was converted to **33ah** (R = 2-benzyloxycarbonylthiophen-3-yl) following Method C-3 in 80% yield. Compound **33ah** (R = 2-benzyloxycarbonylthiophen-3-yl, 0.125 mmol) was converted to **34ah** following Method G-1 in 95% yield. Compound **35ah** was prepared from **34ah** (0.11 mmol) using Method H in 76% yield; mp 130 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.73 (s, 1H), 9.17 (br s, 2H), 8.80 (br s, 2H), 8.70 (t, J = 6.0 Hz, 1H), 8.23 (d, J = 2.0 Hz, 1H), 8.05 (m, 2H), 7.80 (d, J = 2.0 Hz, 1H), 7.70 (m, 4H), 7.20-7.50 (m, 10H), 7.10 (m, 3H), 5.35 (s, 2H), 5.03 (s, 2H), 3.15 (t, J = 6.0 Hz, 2H), 1.87 (m, 1H), 0.90 (d, J = 6.9 Hz, 6H); IR (KBr) 2955, 2923, 2853, 1677, 1606, 1408, 1323, 1423, cm^{-1}; MS (ES$^+$) 765.4 [100% (M+1)]. Anal. ($C_{45}H_{40}N_4O_6$·5 HCl·2.25H_2O) C, H, N.

Compound **36ah** was prepared from **35ah** (0.017 mmol) using Method G-2; MS (ES$^+$) 585.4 [100% (M+1)].

Pure sample could not be isolated. This was submitted in DMSO solution for biological activity.

6.3.14.52. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(2-(hydroxymethyl)thiophen-3-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ai. Compound **33ah** (R = 2-formylthiophen-3-yl, 0.36 mmol) was converted to **33ai** (R = 2-hydroxymethylthiophen-3-yl) using Method I in 93% yield. Compound **33ai** (R = 2-hydroxymethylthiophen-3-yl, 0.33 mmol) was converted to **34ai** following Method G-1 in 61% yield. Compound **35ai** was prepared from **34ai** (0.2 mmol) using Method H in 48% yield. ^{1}H NMR (DMSO-d_6) δ 10.64 (s, 1H), 9.14 (br s, 2H), 8.79 (br s, 2H), 8.67 (t, J = 6.0 Hz, 1H), 8,24 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.74 (m, 6H), 7.58 (d, J = 5.0 Hz, 1H), 7.44 (m, d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 5.0 Hz, 1H), 7.21 (m, 3H), 7.03 (d, J = 5.8 Hz, 2H), 5.69 (t, J = 5.0 Hz, 1H), 5.04 (br s, 2H), 4.71 (d, J = 5.0 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.83 (m, 1H), 0.87 (d, J = 6.8 Hz, 6H); IR (KBr) 3259, 3064, 2956, 1677, 1641, 1482, 1323, 1258 cm^{-1}; MS (ES$^+$) 661.74 [100% (M+1)]. Anal. ($C_{38}H_{36}N_4O_5S$·HCl·1.75H_2O) C, H, N.

Compound **36ai** was prepared from **35ai** (0.09 mmol) using Method G-2 in 54% yield; mp >290 °C. ^{1}H NMR (DMSO-d_6) δ 9.05 (br s, 2H), 8.78 (s, 2H), 8.52 (t, J = 6.0 Hz, 1H), 8.02 (br s, 1H), 7.65 (m, 6H), 7.53 (d, J = 5.0 Hz, 1H), 7.54 (d, J = 5.0 Hz, 1H), 7.26 (d, J = 5.0 Hz, 1H), 7.10 (m, 1H), 6.99 (m, 2H), 5.64 (t, J = 5.0 Hz, 1H), 4.71 (d, J = 5.0 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.73 (m, 1H), 0.84 (d, J = 6.9 Hz, 6H); IR (KBr) 3294, 2958, 1644, 1604, 1541, 1481, 1411, 1326 cm^{-1}; MS (ES$^+$) 571.56 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_4S$·HCl·0.75H_2O) C, H, N.

6.3.14.53. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-(hydroxymethyl)thiophen-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36aj. Compound **33aj** (R = 3-formylthiophen-2-yl) was prepared from **31a** (0.75 mmol) and 3-formylthiophen-2-ylboronic acid (Frontier Scientific, 2.25 mmol) following Method D-1 in 54% yield. Compound **33aj** (R = 3-formylthiophen-2-yl, 0.49 mmol) was converted to **33aj** (R = 3-hydroxymethylthiophen-2-yl) using Method I in 67% yield. Compound **33aj** (R = 3-

hydroxymethylthiophen-2-yl, 0.33 mmol) was converted to **34aj** following Method G-1 in 61% yield. Compound **35aj** was prepared from **34aj** (0.2 mmol) using Method H in 61% yield; mp 200 °C. ^{1}H NMR (DMSO-d_6) δ 10.70 (s, 1H), 9.15 (br s, 2H), 8.77 (br s, 2H), 8.67 (t, J = 6.0 Hz, 1H), 8.25 (s, 1H), 8.04 (d, J = 7.0 Hz, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.71 (m, 4H), 7.70 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 6.0 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.22 (m, 4H), 7.05 (s, 1H), 7.03 (d, J = 2.0 Hz, 1H), 5.31 (t, J = 6.0 Hz, 1H), 5.04 (br s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 1.82 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 3263, 3064, 2957, 1674, 1643, 1480, 1324, 1257 cm^{-1}; MS (ES$^+$) 661.74 [100% (M+1)]. Anal. ($C_{38}H_{36}N_4O_5S$·HCl·2H_2O) C, H, N.

Compound **36aj** was prepared from **35aj** (0.09 mmol) using Method G-2 in 53% yield; mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 9.05 (br s, 2H), 8.81 (br s, 2H), 8.49 (t, J = 6.0 Hz, 1H), 8.02 (s, 1H), 7.68 (s, 1H), 7.62 (m, 7H), 7.53 (d, J = 6.0 Hz, 1H), 7.21 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 7.0 Hz, 1H), 7.01 (s, 1H), 5.25 (t, J = 5.0 Hz, 1H), 4.51 (d, J = 5.0 Hz, 2H), 3.01 (t, J = 6.0 Hz, 2H), 1.81 (m, 1H), 0.85 (d, J = 6.8 Hz, 6H),; IR (KBr) 3281, 3099, 2957, 1643, 1604, 1541, 1326, 1266 cm^{-1}; MS (ES$^+$) 571.64 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_5S$·HCl·H_2O) C, H, N.

6.3.14.54. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(4-(hydroxymethyl)thiophen-3-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ak. Compound **33ak** (R = 4-formylthiophen-3-yl) was prepared from **31a** (0.75 mmol) and 4-formylthiophen-3-ylboronic acid (Digital, 2.25 mmol) following Method D-1 in 70% yield. Compound **33ak** (R = 4-formylthiophen-3-yl, 0.53 mmol) was converted to **33ak** (R = 4-hydroxymethylthiophen-3-yl) using Method I in 72% yield. Compound **33ak** (R = 4-hydroxymethylthiophen-3-yl, 0.41 mmol) was converted to **34ak** following Method G-1 in 88% yield. Compound **35ak** was prepared from **34ak** (0.35 mmol) using Method H in 26% yield; mp 175 °C. ^{1}H NMR (DMSO-d_6) δ 10.60 (s, 1H), 9.17 (br s, 1H), 8.80 (br s, 2H), 8.65 (t, J = 6.0 Hz, 1H), 8.25 (s, 1H), 8.04 (d, J = 7.0 Hz, 1H), 7.80 (m, 2H), 7.70 (m, 5H), 7.60 (d, J = 2.0 Hz, 1H), 7.40-7.10 (m, 6H), 7.15 (m 2H), 5.4 (br s, 1H), 5.00 (s, 2H), 4.50 (s, 2H), 3.09 (d, J = 6.0 Hz, 2H), 1.89 (m, 1H), 0.88 (d, J = 6.9 Hz, 6H); IR (KBr) 3257, 2857, 2857, 1675, 1640, 1604, 1542, 1425 cm^{-1}; MS (ES$^+$) 659.5 [100% (M+1)]. Anal. ($C_{36}H_{36}N_4O_5S$·HCl·2.5H_2O) C, H, N.

Compound **36ak** was prepared from **35ak** (0.07 mmol) using Method G-2 in 37% yield; mp >260 °C (dec). ^{1}H NMR (DMSO-d_6) δ 14.11 (br s, 1H), 9.05 (br s, 2H), 8.75 (br s, 2H), 8.49 (t, J = 6.0 Hz, 1H), 7.97 (s, 1H), 7.73 (s, 1H), 7.67 (d, J = 3.0 Hz, 1H), 7.61 (m, 7H), 7.54 (d, J = 3.0 Hz, 1H), 7.06 (d, J = 6.9 Hz, 1H), 6.89 (d, J = 6.9 Hz, 1H), 5.23 (t, J = 5.0 Hz, 1H), 5.42 (d, J = 5.0 Hz, 2H), 3.09 (t, J = 6.0 Hz, 2H), 1.74 (m, 1H) 0.86 (d, J = 6.9 Hz, 6H); IR (KBr) 3095, 2958, 1661, 1603, 1541, 1481, 1411, 1325 cm^{-1}; MS (ES$^+$) 571.3 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_5S$·HCl) C, H, N.

6.3.14.55. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(5-(hydroxymethyl)thiophen-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36al. Compound **33al** (R = 5-formylthiophen-2-yl) was prepared from **31a** (0.75 mmol) and 5-formylthiophen-2-ylboronic acid (Frontier Scientific, 2.25 mmol) following Method D-2; **33al** (R = 4-formylthiophen-3-yl) was converted to **33al** (R = 5-hydroxymethylthiophen-2-yl) using Method I; **33al** (R = 4-hydroxymethylthiophen-3-yl) was converted to **34al** following Method G-1; **35al** was prepared from **34al** using Method H and 36al was prepared from **35al** following Method G-2 in overall yield of 10%; mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 9.10 (br s, 2H), 8.80 (br s, 2H), 8.50 (t, J = 6.0 Hz, 1H), 8.02 (s, 1H), 7.68 (s, 1H), 7.62 (m, 6H), 7.53 (d, J = 5.8 Hz, 1H), 7.15 (d, J = 6.0 Hz, 1H), 7.13 (m, 1H), 7.01 (s, 1H), 5.50 (t, J = 5.0 Hz, 1H), 4.70 (d, J = 5.0 Hz, 2H), 3.01 (m, 2H), 1.80 (m, 1H), 0.85 (d, J = 6.8 Hz, 6H); IR (KBr) 3335, 2957, 1644, 1604, 1540, 1481, 1326 cm^{-1}; MS (ES$^+$) 571.2 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_5S$·HCl·2.5H_2O) C, H, N.

6.3.14.56. 4′-(4-(Azidomethyl)thiophen-3-yl)-6′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36am. Compound **33ak** (R = 4-hydroxymethylthiophen-3-yl, 0.65 mmol) was converted to **33am** (R = 4-methanesulfonyloxymethylthiophen-3-yl) using Method L in 77% yield. Compound **33am** (R = 4-methanesulfonyloxymethylthiophen-3-yl, 1.1 mmol) was converted to **33am** (R = 4-azidomethylthiophen-3-yl) following Method M in 59% yield. Compound **33am** (0.66 mmol) was converted to **34am** using Method G-1 and then to **35am** using Method H in 21% yield; mp >200 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.73 (s, 1H), 9.19 (br s, 2H), 8.88 (br s, 2H), 8.71 (t, J = 6.0 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 8.07 (dd, J = 7.7 and 2.0 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.80 (d, J = 2.0 Hz, 1H), 7.83 (m, 5H), 7.72 (dd, J = 2.0 and 7.7 Hz, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.41 (d, J = 7.7 Hz, 1H), 4.56 (s, 2H), 3.56 (s, 3H), 3.11 (t, J = 6.8 Hz, 2H), 1.87 (m, 1H), 0.92 (d, J = 6.8 Hz, 6H); IR (KBr) 3061, 2956, 2096, 1719, 1675, 1642, 1483, 1324, 1255 cm^{-1}; MS (ES$^-$) 608.2 [100% (M^{-1}), (ES$^+$) 610.3 [100% (M+1)]. Anal. ($C_{32}H_{31}N_7O_4S$·0.1 $C_{32}H_{32}N_4O_4S$·HCl·1.5H_2O):* C, H, N.

*NMR and CHN analysis indicates 20% contamination of 4-[2-{2-methoxycarbonyl-4-(N-(2-methyl)propyl)aminocarbonyl}phenyl-5-(4-methylthiophene-3-yl)]phenylcarbonylaminobenzamidine dihydrochloride hydrate.

Compound **36am** was prepared from **35am** (0.006 mmol) using Method G-2. MS (ES$^+$) 596.2 [100% (M+1)].

Pure sample could not be isolated. This was submitted in DMSO solution for biological activity testing.

6.3.14.57. 4′-(4-(Aminomethyl)thiophen-3-yl)-2′-(4-carbamimidoylphenylcarbamoyl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36an. Compound **35am** (0.1 mmol) was converted to **35an** following Method F in 58% yield; mp >230 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.74 (s, 1H), 8.71 (t, J = 6.0 Hz, 1H), 8.27 (s, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.74 (m, 8H), 7.44 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 3.96 (s, 2H), 3.57 (s, 3H), 3.11 (t, J = 6.8 Hz, 2H), 1.86 (m, 1H), 0.91 (d, J = 6.8 Hz, 6H); IR (KBr) 3061, 2957, 1677, 1641, 1483, 1324, 1256 cm^{-1}; MS (ES$^-$) 582.2 [100% (M−1)]. Anal. ($C_{32}H_{33}N_5O_4S$.0.2$C_{32}H_{32}N_4O_4S$·2 HCl·H_2O):* C, H, N.

*NMR and CHN analysis indicates 20% contamination of 4-[2-{2-methoxycarbonyl-4-(N-(2-methyl)propyl)aminocarbonyl}phenyl-5-(4-methylthiophene-3-yl)]phenylcarbonylaminobenzamidine dihydrochloride hydrate.

Compound **35an** (0.05 mmol) was converted to **36an** following Method G-2 in 72% yield; mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 14.00 (br s, 1H), 8.52 (t, J = 6.0 Hz, 1H), 7.98 (s, 1H), 7.63 (m, 8H), 7.07 (d, J = 7.7 Hz, 1H), 6.96 (d, J = 7.7 Hz, 1H), 3.83 (s, 2H), 3.02 (t, J = 6.8 Hz, 2H), 1.81 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 3292, 3094, 2959, 1641, 1606, 1325, cm^{-1}; MS (ES$^-$) 568.1 [100% (M−1)]. Anal. ($C_{31}H_{31}N_5O_4S$.0.15$C_{31}H_{30}$-N_4O_4S·2.5H_2O):* C, H, N.

*MS, NMR, and CHN analysis indicates 15% contamination of 4-[2-{2-Carboxy-4-(N-(2-methyl)propyl)aminocarbonyl}phenyl-5-(4-methylthiophene-3-yl)]phenylcarbonyl aminobenzamidine.

6.3.14.58. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(2-(hydroxymethyl)furan-3-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ao. Compound **33ao** (R = 2-formylfuran-3-yl) was prepared from **31b** (0.85 mmol) and 2-formylfuran-3-ylboronic acid (Lancaster, 2.54 mmol) following Method D-2 in 94% yield. Compound **33ao** (R = 2-formylfuran-3-yl, 0.83 mmol) was converted to **33ao** (R = 2-hydroxymethylfuran-3-yl) using Method I in 65% yield. Compound **33ao** (R = 2-hydroxymethylfuran-3-yl, 0.64 mmol) was converted to **34ao** following Method G-1 in 78% yield. Compound **35ao** was prepared from **34ao** (0.32 mmol) using Method H in 36% yield; mp >190 °C. ^{1}H NMR (DMSO-d_6) δ 10.71

(s, 0.4H), 10.69 (s, 0.6H), 9.18 (br s, 2H), 8.82 (br s, 2H), 8.69 (t, J = 6.0 Hz, 1H), 8.25 (s, 0.6H), 8.16 (s, 0.4H), 8.05 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 7.7 Hz, 0.4H), 7.87 (m, 0.4H), 7.79 (dd, J = 7.7 and 2.0 Hz, 0.6H), 7.76 (m, 0.6H), 7.81 (m, 5H), 7.45 (d, J = 8.8 Hz, 1H), 7.39 (d, J = 7.7 Hz, 0.6H), 7.37 (d, J = 7.7 Hz, 0.4H), 6.94 (d, J = 2.0 Hz, 1H), 5.48 (t, J = 5.0 Hz, 0.6H), 5.22 (t, J = 5.0 Hz, 0.4H), 4.59 (d, J = 5.0 Hz, 1.2H), 4.54 (d, J = 5.0 Hz, 0.8H), 3.57 (s, 3H), 3.10 (t, J = 6.8 Hz, 2H), 1.84 (m, 1H), 0.89 (d, J = 6.8 Hz, 6H); IR (KBr) 3094, 2957, 1718, 1654, 1601, 1482, 1327 cm^{-1}; MS (ES^-) 567.4 [100% (M−1), (ES^+) 569.4 [100% (M+1)]. Anal. ($C_{32}H_{32}N_4O_6 \cdot 2\,HCl \cdot H_2O$) C, H, N.

Compound **35ao** (0.13 mmol) was converted to **36ao** following Method G-2 in 40% yield; mp >290 °C. ^1H NMR (DMSO-d_6) δ 13.95 (br s, 1H), 9.07 (br s, 2H), 8.91 (br s, 2H), 8.51 (t, J = 6.0 and 5.0 Hz, 1H), 7.99 (m, 1H), 7.66 (m, 9H), 7.09 (d, J = 7.7 Hz, 0.6H), 7.06 (d, J = 7.7 Hz, 0.4H), 6.97 (m, 1H), 6.87 (m, 1H), 5.44 (t, J = 5.0 Hz, 0.6H), 5.19 (t, J = 5.0 Hz, 0.4H), 4.54 (d, J = 5.0 Hz, 1.2H), 4.51 (d, J = 5.0 Hz, 0.8H), 3.03 (t, J = 6.8 Hz, 2H), 1.82 (m, 1H), 0.86 (d, J = 6.8 Hz, 6H); IR (KBr) 3320, 2960, 1641, 1604, 1567, 1546, 1483, 1331 cm^{-1}; MS (ES^-) 553.4 [100% (M−1), (ES^+) 555.4 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_6 \cdot HCl$) C, H, N.

6.3.14.59. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(4-(hydroxymethyl)furan-3-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36ap. Compound **33ap** (R = 4-formylfuran-3-yl) was prepared from **31b** (0.85 mmol) and 4-formylfuran-3-ylboronic acid[30] (2.54 mmol) following Method D-2 in quantitative yield. Compound **33ap** (R = 4-formylfuran-3-yl, 0.87 mmol) was converted to **33ap** (R = 4-hydroxymethylfuran-3-yl) using Method I in 75% yield. Compound **33ap** (R = 4-hydroxymethylfuran-3-yl, 0.65 mmol) was converted to **34ap** following Method G-1 in 42% yield. Compound **35ap** was prepared from **34ap** (0.27 mmol) using Method H in 36% yield. ^1H NMR (DMSO-d_6) δ 10.68 (s, 1H), 9.17 (br s, 2H), 8.82 (br s, 2H), 8.68 (t, J = 6.0 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 8.0 and 2.0 Hz, 1H), 7.87 (m, 1H), 7.89 (dd, J = 8.0 and 2.0 Hz, 1H), 7.75 (m, 5H), 7.44 (d, J = 9.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 5.22 (t, J = 5.0 Hz, 1H), 4.54 (d, J = 5.0 Hz, 2H), 3.57 (s, 3H), 3.10 (t, J = 6.8 Hz, 2H), 1.84 (m, 1H), 0.88 (d, J = 6.8 Hz, 6H); IR (KBr) 3247, 2958, 1718, 1684, 1653, 1485, 1328, 1258 cm^{-1}; MS (ES^-) 567.4 [100% (M−1), (ES^+) 569.4 [100% (M+1)]. Anal. ($C_{32}H_{32}N_4O_6 \cdot 3\,HCl \cdot 2H_2O$) C, H, N.

Compound **35ap** (0.09 mmol) was converted to **36ap** following Method G-2 in 54% yield; mp >250 °C (dec). ^1H NMR (DMSO-d_6) δ 13.84 (br s, 1H), 9.01 (br s, 2H), 8.80 (br s, 2H), 8.46 (t, J = 6.0 Hz, 1H), 8.03 (s, 1H), 7.95 (s, 1H), 7.77 (s, 1H), 7.67 (m, 3H), 7.61 (m, 5H), 7.02 (d, J = 7.7 Hz, 1H), 6.94 (m, 1H), 5.13 (t, J = 5.0 Hz, 1H), 4.47 (m, 2H), 2.97 (t, J = 6.8 Hz, 2H), 1.78 (m, 1H), 0.80 (d, J = 6.8 Hz, 6H); IR (KBr) 3300, 2960, 1643, 1604, 1547, 14 83, 1329 cm^{-1}; MS (ES^-) 553.3 [100% (M−1), (ES^+) 555.3 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_6 \cdot HCl \cdot 1.5H_2O$) C, H, N.

6.3.14.60. 2′-(4-Carbamimidoylphenylcarbamoyl)-4′-(3-(hydroxymethyl)furan-2-yl)-4-(isobutylcarbamoyl)biphenyl-2-carboxylic acid 36aq. Compound **33aq** (R = 3-formylfuran-2-yl) was prepared from **31b** (0.85 mmol) and 3-formylfuran-2-ylboronic acid (Lancaster, 2.54 mmol) following Method D-2; **33aq** (R = 3-formylfuran-2-yl) was converted to **33aq** (R = 4-hydroxymethylfuran-3-yl) using Method I; **33aq** (R = 4-hydroxymethylfuran-3-yll) was converted to 34aq following Method G-1; **35aq** was prepared from **34aq** using Method H and **36aq** was prepared from **35aq** using Method G-2 in overall yield of 12%; mp >190 °C. ^1H NMR (DMSO-d_6) δ 14.10 (br s, 1H), 9.10 (m, 4H), 8.60 (m, 1H), 8.10 (m, 1H), 7.90 (s, 1H), 7.80-7.50 (m, 8H), 7.30 (m, 1H), 7.10 (m, 1H), 6.70 (s, 1H), 5.40 (br s, 1H), 4.60 (m, 2H), 3.10 (m, 2H), 1.90 (m, 1H), 0.90 (d, J = 6.8 Hz, 6H); IR (KBr) 3313, 2958, 1642, 1603, 1542, 1481, 1326, 1265, 1003 cm^{-1}; (ES^+) 555.1 [100% (M+1)]. Anal. ($C_{31}H_{30}N_4O_6 \cdot HCl \cdot 0.5H_2O$) C, H, N.

6.4. Biological assays

The prepared compounds were tested for inhibition of target enzyme TF·FVIIa, and for related serine proteases, such as factor Xa, plasmin, thrombin, and trypsin to determine the selectivity. An amidolytic assay based upon the absorbance of p-Nitroanilide (pNA) at OD_{405} was utilized for TF/VIIa, FXa, thrombin, and trypsin. In case of plasmin, amidolytic assay was based upon the absorbance of derivatized thiols from the hydrolysis of Z-Lys-SBzl (Bachem) in the presence of 5,5′ Dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma) at OD_{405}. The IC_{50} of the test compounds was determined by using KC4A data reduction software (Bio-Tek Instruments) to interpolate percent inhibition from observed V_{max} values. The test compound (10 mM stock solution in DMSO) was diluted 10-fold in specific assay buffers and subsequent serial 10-fold dilutions were made prior to the addition of substrate. All the IC_{50} values represent the average of at least two measurements and the difference between the two measurements was generally less than twofold.

6.5. TF·FVIIa

TF·FVIIa assay reactions were performed in a 200 μL mixture containing 4 nM FVIIa (Hematalogic Technologies), 10 nM lipidated tissue factor (Sunol Molecular), in an assay buffer containing 100 mM Tris, pH 7.2, 150 mM NaCl, 5 mM calcium chloride, 0.1% bovine serum albumin (BSA), and 10% dimethylsulfoxide (DMSO). TF and FVIIa were allowed to equilibrate at room temperature for 15 min. Test compounds dissolved in DMSO were incubated at varied concentrations with TF·FVIIa for 10 min, followed by addition of 500 μM substrate Spectrozyme-FVIIa (American Diagnostica). Reactions were incubated for 5 min at room temperature prior to measuring the change in OD_{405} nm for 10 min at 21-s intervals with a PowerwaveX (Bio-Tek Instruments) microplate reader.

6.6. Factor Xa

FXa assay reactions were performed in a 200-μL mixture containing (0.01 U/mL) FXa (Enzyme Research Laboratories), in an assay buffer containing 100 mM Tris, pH 7.2, 150 mM NaCl, 5 mM calcium chloride, 0.1% bovine serum albumin (BSA), and 1% dimethylsulfoxide (DMSO). Test compounds were added at varied concentrations and reactions were initiated by the addition of Chromozym X (Boehringer-Mannheim) at a final concentration of (0.4 mM). Reactions were incubated for 5 min at room temperature prior to measuring the change in OD_{405} nm for 10 min at 21-s intervals with a PowerwaveX (Bio-Tek Instruments) microplate reader.

6.7. Plasmin

The inhibition of TF·FVIIa test compound was assessed against a similar serine protease, plasmin, in order to determine the selectivity of the test compounds for TF·FVIIa. The assay is an amidolytic assay based upon the absorbance of derivatized thiols from the hydrolysis of Z-Lys-SBzl (Bachem) in the presence of 5,5′ Dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma) at OD_{405}. The IC_{50} of the test compounds was determined by using KC4A data reduction software (Bio-Tek Instruments) to interpolate percent inhibition from observed V_{max} values. Plasmin assay reactions were performed in a 200-μL mixture containing (0.002 U/mL) plasmin (Sigma Chemical), in an assay buffer containing 200 mM triethylamine (TEA), 10 mM $CaCl_2$, pH 7.8, and 1% dimethylsulfoxide (DMSO).

Test compounds were added at varied concentrations and reactions were initiated by the addition of 100 μL of a substrate solution consisting of 0.055 mg/mL (*Z*-Lys-SBzl) (Bachem) and 24 mg/mL DTNB. Reactions were incubated for 5 min at room temperature prior to measuring the change in OD_{405} nm for 10 min at 21-s intervals with a PowerwaveX (Bio-Tek Instruments) microplate reader.

6.8. Thrombin

Thrombin assay reactions were performed in a 200-μL mixture containing (1 U/mL) thrombin (Sigma Chemicals), in an assay buffer containing 100 mM HEPES, pH 7.5, 10 mM $CaCl_2$, and 1% dimethylsulfoxide (DMSO). Test compounds were added at varied concentrations and reactions were initiated by the addition of Nα-Benzoyl-Phe-Val-Arg-p-Nitroanalide (Sigma Chemicals) at a final concentration of 1 mM. Reactions were incubated for 5 min at room temperature prior to measuring the change in OD_{405} nm for 10 min at 21-s intervals with a PowerwaveX (Bio-Tek Instruments) microplate reader.

6.9. Trypsin

Trypsin assay reactions were performed in a 200-μL mixture containing (1 μg/mL) trypsin (Sigma Chemicals), in an assay buffer containing 200 mM triethylanolamine (TEA), pH 7.8, 10 mM $CaCl_2$, and 1% dimethylsulfoxide (DMSO). Test compounds were added at varied concentrations and reactions were initiated by the addition of Nα-Benzoyl-L-Arginine-p-Nitroanalide (L-BAPNA, HCl) (Sigma Chemicals) at a final concentration of 0.05 mg/mL. Reactions were incubated for 5 min at room temperature prior to measuring the change in OD_{405} nm for 10 min at 21-s intervals with a PowerwaveX (Bio-Tek Instruments) microplate reader.

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