Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b00143 • Publication Date (Web): 17 Mar 2016

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Potent Non-Nucleoside Inhibitors of Dengue Viral RNA-Dependent RNA Polymerase From a Fragment Hit Using Structure-Based Drug Design

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KEYWORDS: Dengue, RNA-dependent RNA polymerase, Fragment-based drug discovery, Non-nucleoside inhibitor

ABSTRACT: The discovery and optimization of non-nucleoside dengue viral RNA-dependent-RNA polymerase (RdRp) inhibitors are described. An X-ray-based fragment screen of Novartis' fragment collection resulted in the identification of a biphenyl acetic acid fragment **3**, which bound in the palm subdomain of RdRp. Subsequent optimization of the fragment hit **3**, relying

on structure-based design, resulted in a >1000-fold improvement in potency *in vitro* and acquired anti-dengue activity against all four serotypes with low micromolar EC_{50} in cell-based assays. The lead candidate **27** interacts with a novel binding pocket in the palm subdomain of the RdRp and exerts a promising activity against all clinically-relevant dengue serotypes.

INTRODUCTION

Dengue is a mosquito-borne viral disease causing flu-like symptoms, occasionally developing into the potentially life-threatening complications, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). It is now widespread in over 100 countries, threatening 40% of the world's population.[1] There are estimated to be approximately 390 million dengue infections annually, 96 million of which exhibit disease symptoms, including 500,000 cases of severe dengue and 22,000 deaths.[2] Sanofi's dengue vaccine recently gained regulatory approval in Mexico, Brazil, and the Philippines, but has its limitations since it requires a three-dose regimen and is limited to individuals aged 9 to 45 years old. An antiviral therapy is not yet available for treatment of dengue fever. Therefore, there is an urgent need to develop safe and effective drugs for the treatment of dengue infection.[3]

Dengue viruses (DENV) belong to the genus Flavivirus of the family *Flaviviridae*, and are divided into four distinct but closely related serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Infection with one serotype confers life-long immunity, however a secondary infection by a different serotype can increase the risk of developing severe dengue, because cross-immunity to the other serotypes is only partial and temporary.[4] Therefore an ideal anti-dengue drug should exert pan-serotype activity.

The dengue viral genome is a single-stranded RNA of about 11,000 nucleotides, and encodes three structural proteins and seven nonstructural proteins. The nonstructural protein 5 (NS5) acts as a methyltransferase and an RNA-dependent RNA polymerase (RdRp), both of which are essential for viral replication. DENV NS5 RdRp performs both minus-strand and plus-strand RNA synthesis during replication. Since it has no mammalian counterpart and its protein sequence is conserved across all four serotypes with more than 65% homology, it offers an attractive opportunity for discovery of new antiviral agents.[5] The 3D crystal structure of DENV NS5 RdRp adopts a classical polymerase shape, resembling a right-hand that consists of fingers, palm, and thumb subdomains (Figure 1). The catalytic active site of the DENV RdRp is defined by a conserved GDD motif comprising two aspartic acid residues (Asp^{663}, Asp^{664}), which are located in the palm subdomain. These Asp residues are involved in the coordination of two metal ions that are essential to the catalytic mechanism for incoming nucleotide incorporation. The priming loop, which regulates RNA-template binding and polymerization, points from the thumb subdomain towards the active site. [6-8]

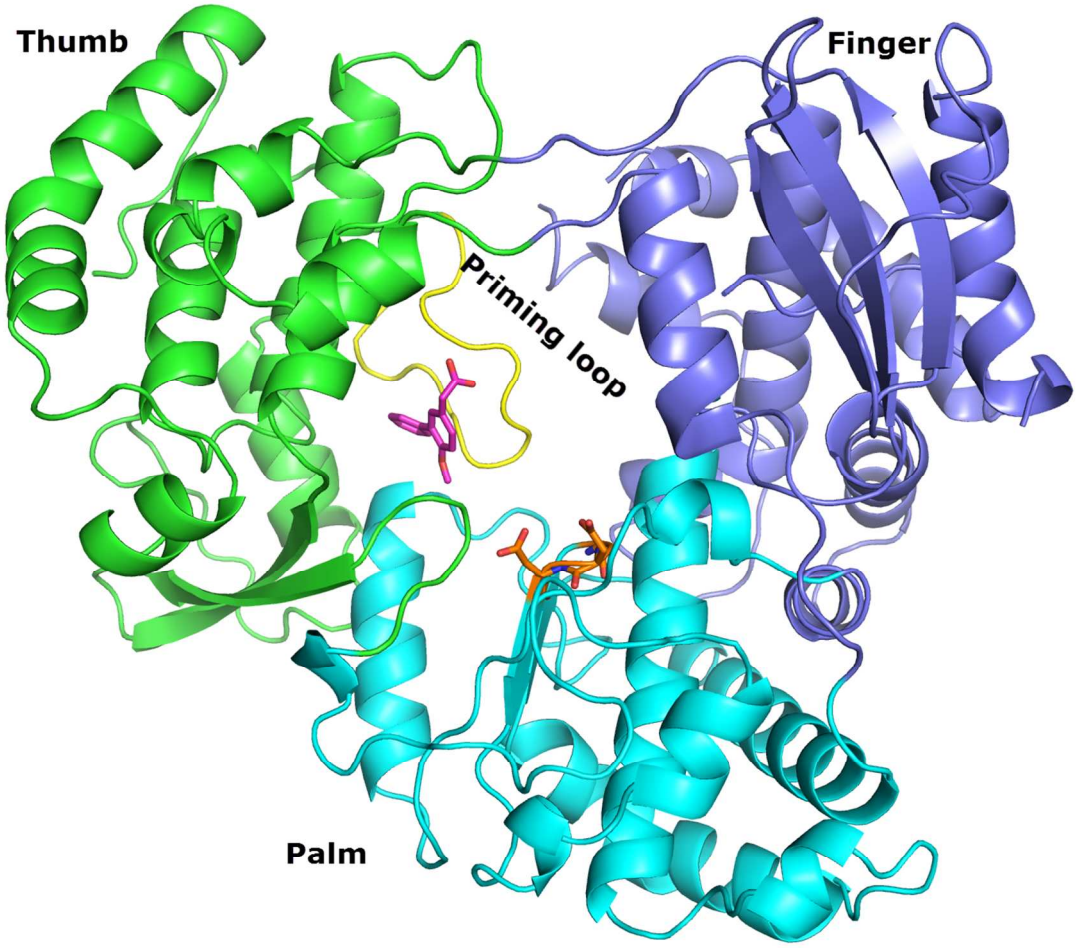


Figure 1. Crystal structure of **3** (magenta) bound to the RdRp domain of DENV-3 protein NS5 shown with the protein as a cartoon (PDB ID 5F3T). The palm subdomain (cyan) contains the active-site residues (Asp^{663} and Asp^{664} from the GDD motif, represented as orange sticks), and the fingers and thumb subdomains are colored purple and green, respectively. The priming loop is colored yellow.

In recent years, there has been a growing interest in DENV NS5, with several groups reporting RdRp inhibitors.[9-14] Our own research efforts on the discovery of DENV NS5 RdRp inhibitors have led to the identification of nucleos(t)ide inhibitors, which act as chain terminators during RNA synthesis by their 5'-triphosphate metabolites.[15,16] As a complementary activity, high-

throughput screening (HTS) of the Novartis corporate compound archive was conducted using either a radioactive scintillation proximity[17] or fluorescent-coupled assays[18] measuring the elongation activity of the enzyme. Previously we reported that our HTS campaigns led to the identification of an *N*-sulfonyl anthranilic acid hit **1** with an IC_{50} of 7.2 μM. Subsequent structure-activity relationship (SAR) exploration improved the potency to an IC_{50} of 0.26 μM. However this medicinal chemistry optimization ended up with a highly lipophilic molecule **2** (clogP 6.6), which had potentially unfavorable drug-like properties as a lead candidate. In addition, compound **2** did not exhibit any antiviral activity in cell culture (Figure 2).[17, 19]

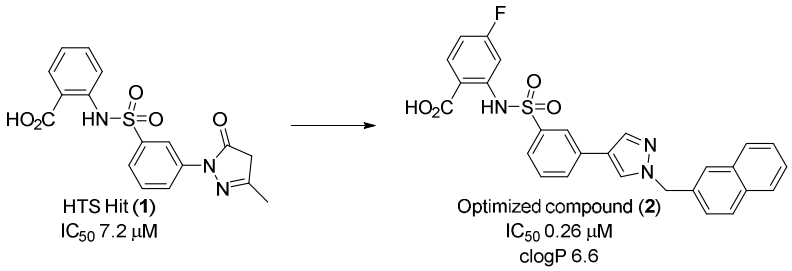


Figure 2. *N*-sulfonyl anthranilic derivatives as non-nucleoside inhibitors of the dengue RdRp from an HTS hit.

As reported in the previous publications, several hits from the HTS campaigns were rigorously followed up, however we failed to identify specific DENV NS5 RdRp inhibitors with good physicochemical properties, because of false positives due to impurities or binding to the large RNA template tunnel space.[20] The fact that multiple HTS campaigns to identify RdRp enzyme inhibitors did not provide viable hits, led us to consider fragment screening to identify novel and tractable hits that could be optimized to potent leads with good drug-like properties. As a part of fragment screening campaigns, we undertook an X-ray crystallographic screen, which provides unambiguous proof of binding to the target site and reveals the binding mode of the hits, thus

providing clear direction for their optimization. Herein, we describe our approach to fragment-based screening followed by structure-based drug design efforts to identify potent non-nucleoside inhibitors of the dengue NS5 polymerase.

RESULTS AND DISCUSSION

A fragment screen of the Novartis fragment collection using X-ray crystallography identified a single hit; a biphenyl acetic acid fragment, **3** (IC_{50} 734 μM, SPR-*K*d 613 μM, LE 0.24), which bound in the palm subdomain (Figure 1, 3).[21] A related analog from the Novartis archive, **4** (IC_{50} 769 μM, SPR-*K*d >200 μM, LE 0.26) was also found to bind in the same pocket using X-ray crystallography but it bound with the opposite orientation of the carboxylic acid moiety, suggesting that the binding was mainly from the biphenyl moiety. Each carboxylic acid formed an H-bond interaction with the priming loop (Figure 3).

In the course of our research for pan-serotype DENV RdRp inhibitors, we primarily used DENV serotype 4. DENV-4 was used for the biochemical enzyme-inhibition assay and for biophysical binding assays using isothermal titration calorimetry (ITC) or surface-plasmon resonance (SPR) for investigating SAR. DENV-3 was used for X-ray crystallography since the methodology is well established[20] and as yet there is no DENV-4 RdRp crystal structure. In addition, selected compounds were evaluated by RdRp biochemical and cell-based assays in all four DENV serotypes to confirm pan-serotype activity.

For biophysical binding analyses we used a combination of ITC and SPR. Generally the data between the two assays correlated, although SPR consistently showed a higher affinity, probably because the SPR assay was performed at 4 °C. Once the SPR assay was developed it was used as

the biophysical assay of choice to develop SAR because of its higher throughput and because of the limitations of using ITC for high-affinity binding.

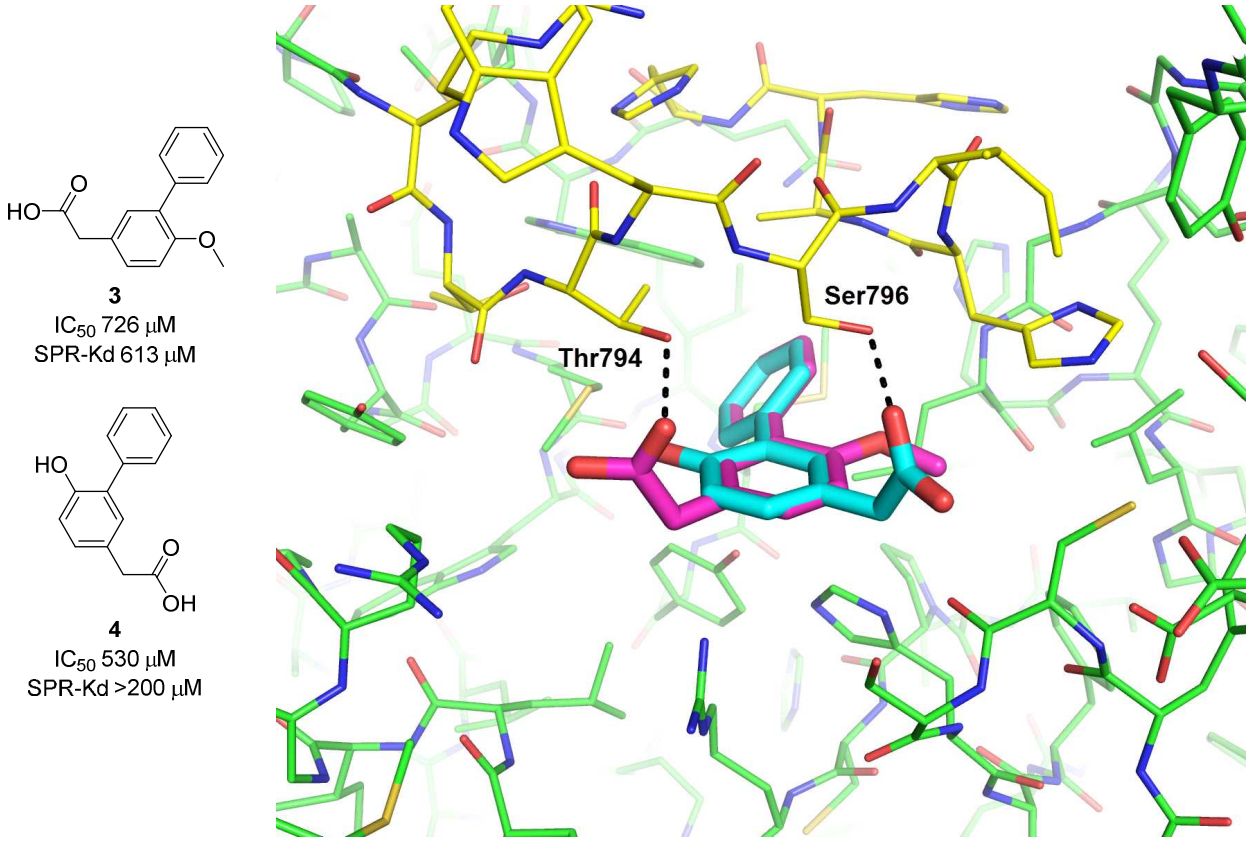


Figure 3. Overlay of co-crystal structures of **3** (magenta) and **4** (cyan) bound to the RdRp domain of DENV-3 NS5. The compounds are shown as sticks and the protein as lines. The residues in the palm and thumb subdomains are green and in the priming loop yellow. H-bond interaction of carboxylic acid groups with the priming loop is shown by dotted lines.

To initiate elaboration of the fragment hits, the biphenyl acetic acid moiety was merged to provide the bis-acid **5** (IC_{50} 177 μM, ITC-*K*d 154 μM). The initial optimization efforts were focused on filling the narrow and deep cavity from the distal aromatic ring of the bis-acid **5**. The SAR of the distal benzene ring of **5** was explored as shown in Table 1. Halogenated phenyl rings

6-8 and the five-membered furan ring **9** did not improve potency, however the thiophene ring **10** was found to provide a 10-fold increase in potency (IC_{50} 15 μM, ITC-*K*d 28 μM). The co-crystal structure of **10** with the NS5 polymerase domain suggested that this higher affinity is potentially due to the noncovalent interaction of the sulfur of the thiophene with the oxygen of the OH side-chain of Ser^{796} (Figure 4A).[22] In addition, the X-ray structure suggested that the 2-postion of the thiophene ring provided the correct vector to grow the compound toward the narrow cavity (Figure 4B).

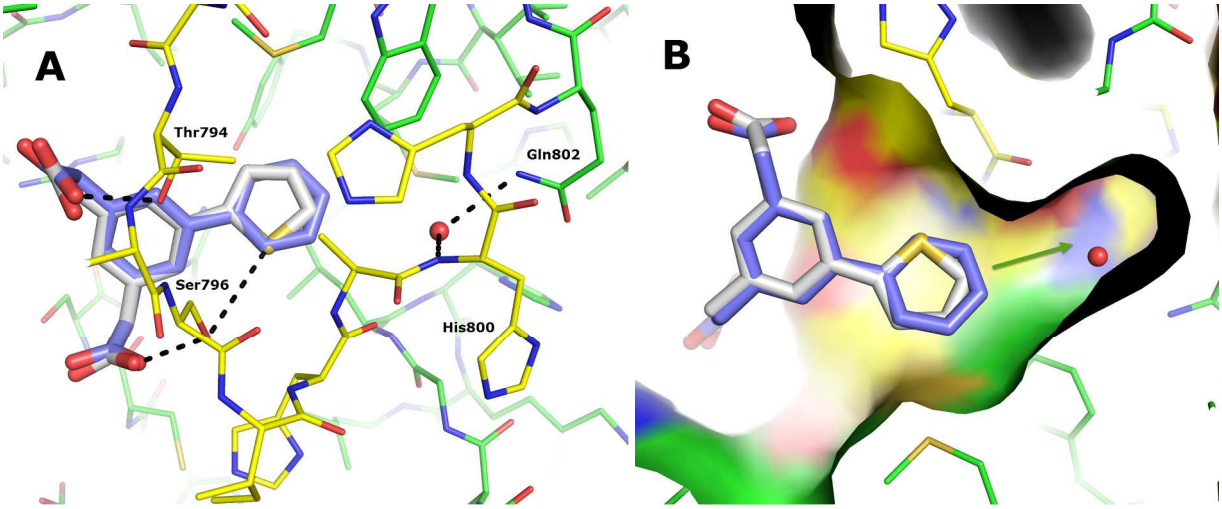
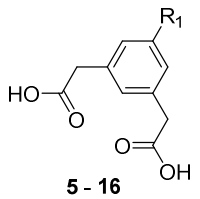


Figure 4. (A) Overlay of co-crystal structures of **5** (purple) and **10** (grey) bound to the RdRp domain of DENV-3 NS5. A water molecule is displayed as a red sphere, forming H-bonds with His^{800} and Gln^{802}. The compounds are shown as sticks and the amino acid residues as lines. The palm and thumb subdomains are green and the priming loop is yellow. (B) Alternate view of **5** and **10** from the opposite site (180 degree rotation) compared to (A) shown in surface representation. The green arrow highlights the vector off the 2-position of the thiophene to grow toward the inner cavity.

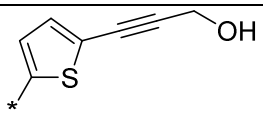
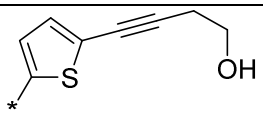
Various substituents such as Cl, Me, and Br moieties were introduced in the 2-position of the thiophene to fill the cavity, however none of them improved potency (compounds **11**-**13**). It was noted that a water molecule was bound deeper inside the narrow cavity forming two H-bond interactions with Gln^{802} and His^{800}. To interact with the water, a nitrile group was added, however cyano-thiophene **14** significantly decreased potency. As an alternative approach we attempted to displace the water molecule. A propargyl alcohol moiety was introduced to the 2-thiophene ring to provide **15** (IC_{50} 1.7 μM, ITC-*K*d 1.4 μM), which gave a 100-fold increase in potency and improved the ligand efficiency (LE) to 0.35 as compared to **5**. The homopropargyl alcohol analog **16** was found to be less potent than **15**, suggesting that the propargyl alcohol had the optimal length to displace the water molecule. Co-crystallization of **15** with the NS5 RdRp domain confirmed that the propargyl alcohol filled the narrow cavity and displaced the water molecule to form the H-bond interactions with the backbone of His^{800} and the side chain of Gln^{802} as predicted (Figure 5). Amino acid residues of the binding pocket are strictly conserved across all four serotypes except for residues Thr^{800} and Glu^{802} in DENV-2 (see supporting information Figure S3). The propargyl alcohol moiety in **15** is likely to form similar H-bond interactions with residues 800 and 802 in DENV 1-4, generating pan-serotype activity with IC_{50} values of 0.3 to 2.2 μM (Table 2).

Table 1. Enzyme inhibition and LE data for bis-acid analogs 5 – 16.



5 - 16

Compound	R_1	DENV-4 NS5	DENV-4 NS5	LE[c]

		IC_{50} (μM)[a]	ITC-*K*d (μM)[b]	
5	Ph	177	154	0.26
6	2-Cl-Ph	192		0.25
7	3-Cl-Ph	141		0.26
8	4-Cl-Ph	62		0.28
9	2-furyl	211	552	0.27
10	2-thienyl	15	28	0.36
11	5-Cl-(2-thienyl)	26	33	0.32
12	5-Me-(2-thienyl)	96		0.28
13	5-Br-(2-thienyl)	39	46	0.31
14	5-CN-(2-thienyl)	199	134	0.25
15		1.7	1.4	0.35
16		29	34	0.27

[a]Enzyme IC_{50} values were determined as described in the Experimental Section. [b]*K*d values were determined as described in the Experimental Section. [c]LE = (1.4*pIC_{50})/heavy atoms.

Table 2. Enzyme inhibition of compound 15 against other dengue serotypes.

Dengue serotype	IC_{50} (μM)
1	0.3
2	2.2
3	0.5

4	1.7

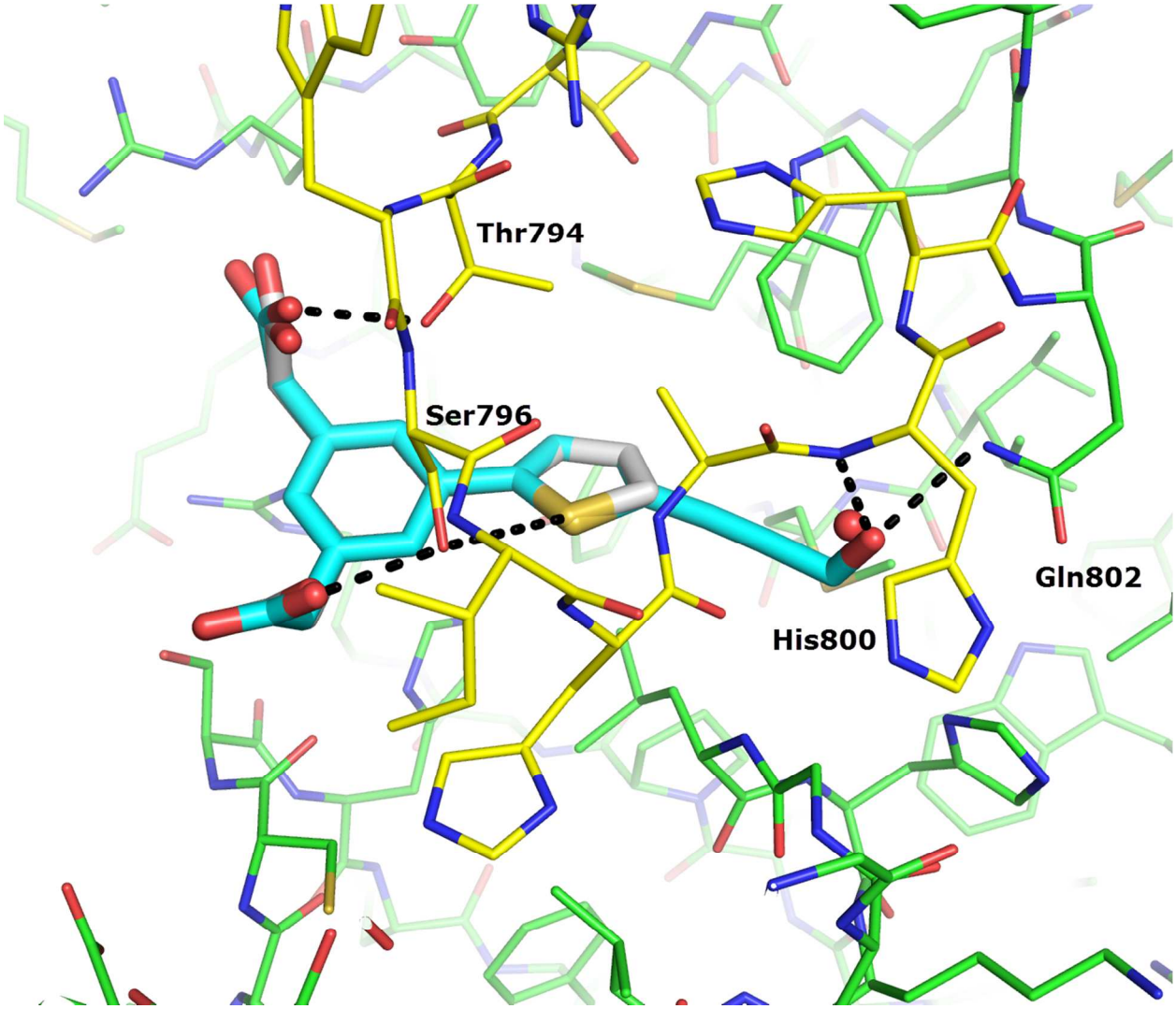


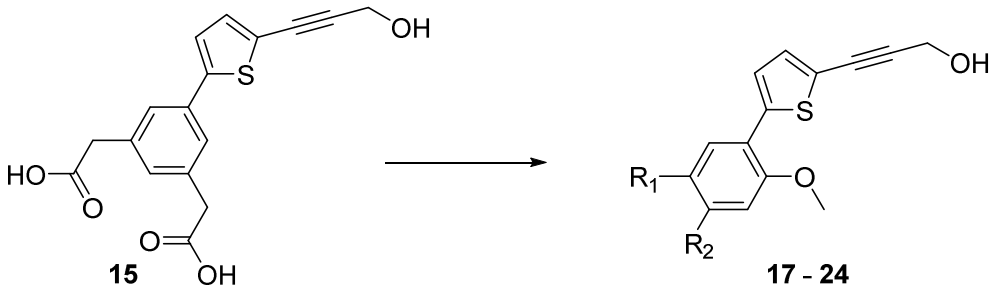
Figure 5. Overlay of co-crystal structures of **10** (grey) and **15** (cyan) with the RdRp domain of DENV-3 NS5. Residues from the palm and thumb subdomains are shown as green lines and the priming loop as yellow lines. As predicted, the hydroxyl group of the propargyl **15** is seen to substitute the water molecule present in the complex with **10**.

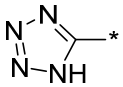
Further, we investigated the optimization of binding to the outside of the pocket. The propargyl alcohol analog **15** showed poor membrane permeability in the Caco-2 cells (Papp = 0.51 cm/s x 10^{-6}), likely due to the existence of two negatively-charged carboxylic acids. To improve cellular permeability, we revisited the mono-acid original hit **3** for the further optimization. Replacement of the distal benzene ring with the thiophene-propargyl alcohol moiety afforded **17** (IC_{50} 7.5 μM, ITC *K*d 14 μM, SPR *K*d 2.2 μM). The mono-acid **17** showed 5-fold increased Caco-2 permeability (Papp = 2.73 cm/s x 10^{-6}) as compared to the bis-acid **15**. Removing the methylene group of the phenyl acetic acid moiety of **17** provided **18**, which resulted in 3.7 fold improvement in potency (IC_{50} = 2.3 μM, ITC *K*d 3.8 μM, SPR *K*d 1.7 μM). Introduction of the methyl group on the 6-position of the methoxy benzene ring in **18** gave **19**, which inhibited the RdRp with an IC_{50} of 0.5 μM, causing a further increase in LE to 0.42 (IC_{50} = 0.53 μM, ITC *K*d 1.6 μM, SPR *K*d 0.11 μM).

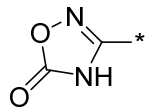
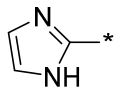
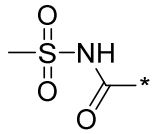
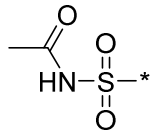
Subsequently, we sought to identify carboxylic acid bioisosteres,[23] which could have a similar geometrical topology of H-bond donor and acceptor with the CO_2H. Replacing the carboxylic acid in **18** with a well-known isostere, the tetrazole, afforded **20**, which retained inhibitory activity against RdRp (IC_{50} = 2.4 μM), however the Caco-2 permeability of **20** was not improved (Papp = 1.39 cm/s x 10^{-6}). The diazaoxazolone **21** was found to be more lipophilic and less acidic than the corresponding carboxylic acid **19**, however it showed 5-fold less potency. The non-acidic imidazole **22** lost potency significantly. Conversion of the carboxylic acid to methyl acylsulfonamide **23** offered comparable potency and binding affinity in the biochemical and the biophysical analyses, suggesting that the acylsulfonamide functionality engages similar interactions with the RdRp (IC_{50} = 0.34 μM, ITC *K*d 2.0 μM, SPR *K*d 0.12 μM). The reverse acylsulfonamide **24** was found to be less potent than **23**. Unfortunately, despite potent inhibition

against the RdRp with submicromolar IC_{50}, both **19** and **23** showed no anti-dengue activity up to 50 μM in A549 cells, which may reflect their inability to permeate cells efficiently and to reach the intracellular enzyme target. This is supported by the measured acid dissociation constant value (pKa 4.0-4.2) and distribution coefficient value (logD 0.8-1.2) at pH 7.4 for **19** and **23**. Therefore, the lack of cellular activity is likely to be the result of poor cellular permeability due to their negatively-charged carboxylic acid/acylsulfonamide functionalities and their high polarity. The Caco-2 data for **23** also indicated that it has low cellular permeability (Papp = 0.50 cm/s x 10^{-6}).

Table 3. Enzyme inhibition and LE data for mono-acid and acid bioisoster analogs 17 – 24.



cpd	R_1	R_2	D4 IC_{50} (μM)[a]	D4 ITC *K*d (μM)[b]	D4 SPR *K*d (μM)[b]	logD (pH 7.4)	pKa	LE[c]	Caco-2[d] (cm/s x10^{-6})
15			1.7	1.4				0.35	0.51
17	CH_2COOH	H	7.5	14	2.2			0.34	2.73
18	COOH	H	2.3	3.8	1.7	0.79	4.2	0.39	
19	COOH	Me	0.53	1.6	0.11	1.2	4.2	0.42	
20		H	2.4					0.36	1.39

21		Me	3.2			1.9	5.5	0.32	
22		Me	44			3.6	6.6	0.26	
23		Me	0.34	2	0.12	0.8	4.0	0.36	0.5
24		Me	2.5			0.78	4.8	0.31	

[a]Enzyme IC_{50} values were determined as described in the Experimental Section. [b]*K*d values were determined as described in the Experimental Section. [c]LE = (1.4*pIC_{50})/heavy atoms. [d]Apical to basal permeability at pH 7.4. D4 = DENV-4.

In order to gain cellular activity, our next objective was to modify the physicochemical properties of the compounds to enhance their cellular permeability as shown in Table 4. The X-ray co-crystal structure of **23** with the DENV-3 NS5 RdRp domain confirmed that it was binding in the same pocket, as shown in Figure 6. The propargyl alcohol projected into the narrow cavity and formed two H-bond interactions with His^{800} and Glu^{802}. The acylsulfonamide formed three H-bond interactions with the side chains of Thr^{794} and Arg^{729} and the backbone of Trp^{795}. The methyl group of the acylsulfonamide moiety did not make contact with the enzyme surface but was exposed to the solvent space, suggesting that modification of the methyl group could serve to influence the overall physicochemical properties of inhibitors without interfering with their affinity to the enzyme.

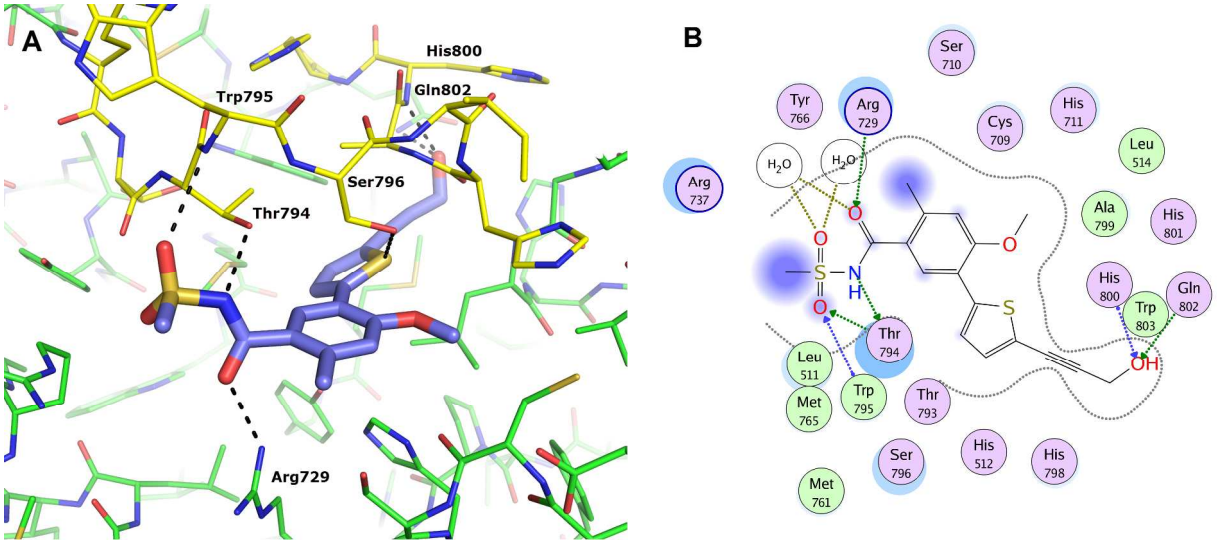
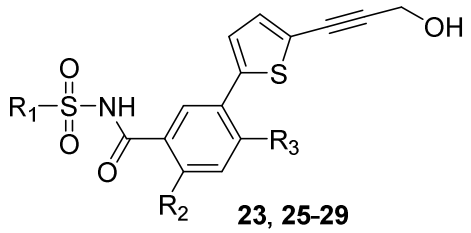


Figure 6. (A) X-ray co-crystal structure of **23** (purple) bound to the RdRp domain of DENV-3 NS5. The compound is shown as sticks and the protein residues as lines. Residues in the palm and thumb subdomains are green and in the priming loop are yellow. (B) Two-dimensional ligand-interaction map generated using Molecular Operating Environment. Hydrophobic residues are colored green, polar residues are colored light purple and charged residues have an additional blue ring. The degree of solvent exposure is shown by the blue halos. H-bond interactions to the amino acid mainchain or sidechain are shown as dashed blue or green arrows respectively, pointing towards the H-bond acceptor. Water-mediated contacts are shown as gold dashed lines.

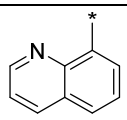
Changing the methyl to benzene sulfonamide **25** did not affect the potency against RdRp (IC_{50} 0.17 μM) and increased the lipophilicity (logD 1.7) as expected. It showed moderate cellular activity (EC_{50} 18-41 μM), whereas its acidity (pKa 3.8) was similar to those of the carboxylic acid **19** (pKa 4.2) and the methyl sulfonamide **23** (pKa 4.0). The Caco-2 data suggested that the cellular permeability of **25** was still low (Papp = 0.5 cm/s x 10^{-6}). In an attempt to improve the cellular permeability by reducing ionization of the acylsulfonamide by modifying its pKa, the 6-

methyl group of the methoxy benzene ring of **25** was replaced with the electron-donating methoxy group to afford **26**, which improved Caco-2 cell permeability (Papp = 10.8 cm/s x 10^{-6}) with similar lipophilicity (logD 1.5), reflecting reduced acidity (pKa 4.8) of the acyl sulfonamide. However, compound **26** showed only modest improvement in cellular potency (EC_{50} 7.3-37 μM) despite its increased cellular permeability. On the other hand, 3-methoxyphenyl sulfonamide **27** displayed the most potent anti-dengue activity in this series with EC_{50} of low micromolar against all four serotypes (EC_{50} 1.8-2.3 μM) without cytotoxicity up to 50 μM (CC_{50} >50 μM in HepG2 cells), while it had similar physicochemical properties (log D 1.6, pKa 4.7) and lower Caco-2 permeability (Papp = 3.91 cm/s x 10^{-6}) as compared to **26**. The exact cause of the significant improvement in the cellular potency of **27** is not known, but it may be due to difference of accessibility of the compounds to the binding site in the presence of viral and cellular proteins. Compound **27** was also confirmed to be active against all four serotypes in the biochemical assays (Table 5). When the OMe at the 4-position of the benzene ring of **27** was replaced with the electron-withdrawing chlorine, the acidity of **28** was increased to pKa 3.8, resulting in slightly reduced cellular activity (EC_{50} 5.5-13 μM). We found that the 8-quinolyl sulfonamide **29** exhibited the highest biochemical potency and binding affinity with an IC_{50} of 0.023 μM and an SPR-*K*d of 0.007 μM, although it was 2- to 6-fold less potent than **27** in the cell-based assays (EC_{50} 3.8-14 μM). The 8-quinolyl acylsulfonamide moiety of **29** may take a different binding conformation to the RdRp in the biochemical and SPR assay systems, resulting in the highest potency and binding in this series.

Table 4. Enzyme inhibition, SPR-*K*d, cellular potency, logD, pKa, and Caco-2 data for acyl sulfonamide analogs 23, 25 – 29.



23, 25-29

Cpd	R_1	R_2	R_3	D4 IC_{50} (μM)[a]	D4 SPR *K*d (μM)[b]	EC_{50} (μM, A547 cells)[c]				logD (pH 7.4)	pKa	Caco-2[d] (cm/s x10^{-6})
						D1	D2	D3	D4			
23	Me	Me	OMe	0.34	0.12	>50	>50	>50	>50	0.8	4.0	0.5
25	Ph	Me	OMe	0.17	ND	31	34	18	41	1.7	3.8	0.5
26	Ph	OMe	OMe	0.25	0.09	15	37	7.3	14	1.5	4.8	10.8
27	3-MeOPh	OMe	OMe	0.17	0.07	1.8	2.3	1.8	1.8	1.6	4.7	3.91
28	3-MeOPh	OMe	Cl	0.14	0.01	6.8	13	5.5	7.0	0.9	3.8	ND
29		Me	OMe	0.023	0.007	6.3	14	3.8	10	1.5	4.4	2.57

[a]Enzyme IC_{50} values were determined as described in the Experimental Section. [b]*K*d values were determined by SPR as described in the Experimental Section. [c]EC_{50} values were determined as described in the Experimental Section. [d]Apical to basal permeability at pH 7.4. D1 = DENV-1, D2 = DENV-2, D3 = DENV-3, D4 = DENV-4.

Table 5. Enzyme inhibition of compound 27 against other dengue serotypes.

Dengue serotype	IC_{50} (μM)
1	0.068
2	0.11

3	0.048
4	0.17

CONCLUSION

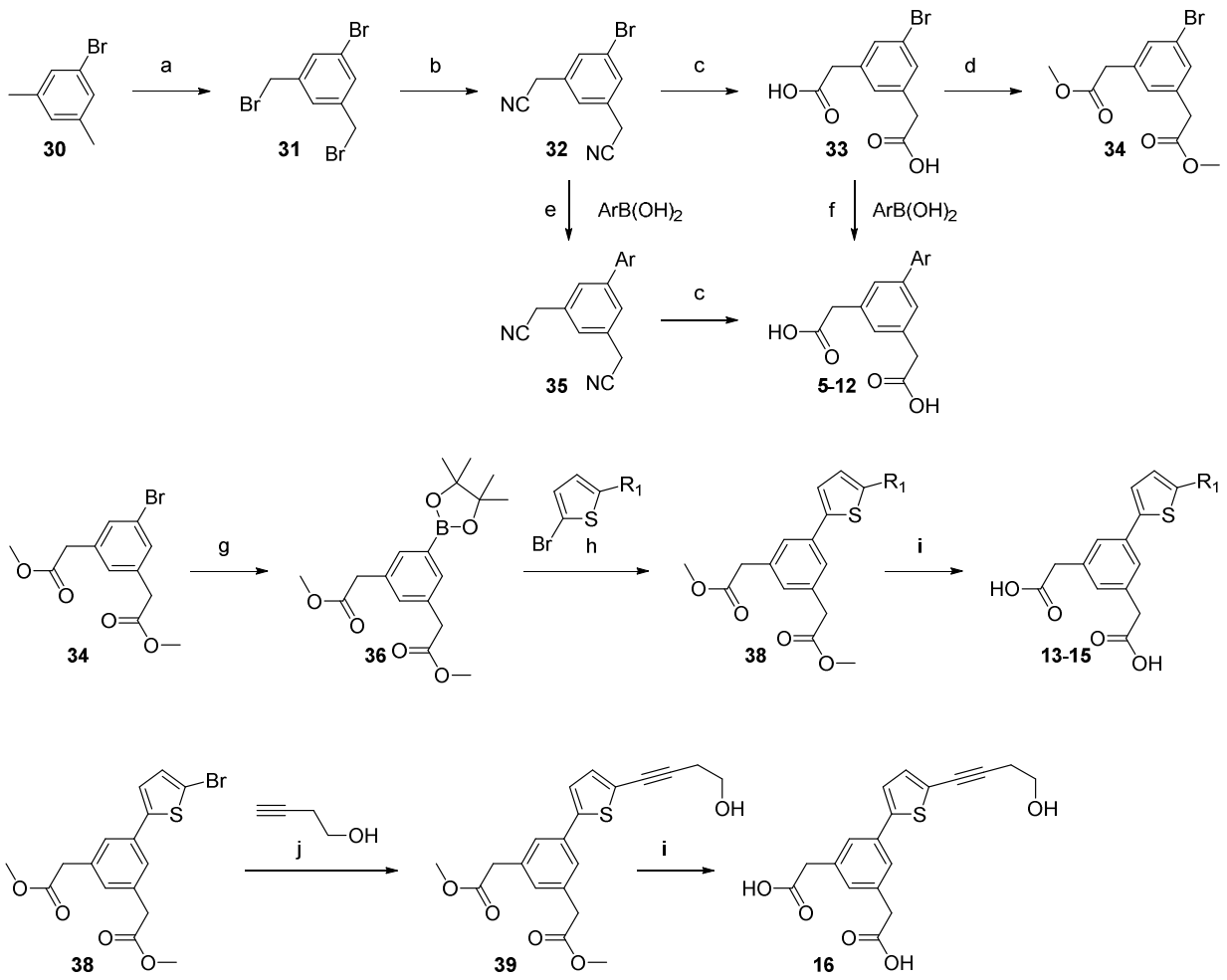
In summary, we have described a fragment-based drug discovery approach to identify novel, potent non-nucleoside inhibitors of dengue viral RNA polymerase. An X-ray-based fragment screen resulted in the identification of a biphenyl acetic acid hit **3** with an IC_{50} of ~700 μM, which is bound to a novel pocket in the palm subdomain of the RdRp. Subsequent growing and optimization of the fragment hit **3** using crystallography and computer-aided structure-based design achieved >1,000-fold improvement in the potency. Replacement of the carboxylic acid moiety with an isosteric acylsulfonamide followed by optimization of physicochemical properties led to the identification of **27**, which displayed antiviral activity in the cell-based assays for all four dengue serotypes at low micromolar concentrations. Additional biological characterization of **27** and **29** have confirmed their on-target cellular activities, which is reported elsewhere.[24] To our knowledge, this is the first class of compounds to show antiviral activity that correlates with direct evidence for interacting with a specific site of the DENV NS5 RdRp. Thus, these analogs are promising leads for further optimization and development.

CHEMISTRY

Bis-acid derivatives **5-16** described herein were prepared as shown in Scheme 1. Benzylic bromination of 3,5-dimethyl bromobenzene (**30**) followed by nucleophilic displacement of the resulting benzylic bromide **31** with potassium cyanide gave the bis-cyanide **32**,[25] which was converted to the corresponding carboxylic acid **33** and carboxylic acid ester **34**. The aromatic

ring was installed by the Suzuki-Miyaura cross-coupling methodology[26] between **33** and aromatic boronic acids to afford compounds **5-8** and **10-11**. Alternatively, the cross-coupling with the bis-cyanide **32** with aromatic boronic acids followed by hydrolysis of the cyanide gave compounds **9** and **12**. Compounds **13-15** were prepared by palladium catalyzed borylation of the aryl bromide **34** with bis(pinacolato)diboron,[27] cross-coupling with 2,5-dibormothiophene, 2-cyano-5-bromothiophene and 3-(5-bromothiophen-2-yl)prop-2-yn-1-ol (**37**), followed by alkaline hydrolysis of the ester **38**. Sonogashira reaction[28] of the bromothiophene **38** (R_1 = Br) with homopropargyl alcohol gave compound **39**, which underwent saponification to yield compound **16**.

Scheme 1. Synthesis of bis-acid derivatives 5-16[a]

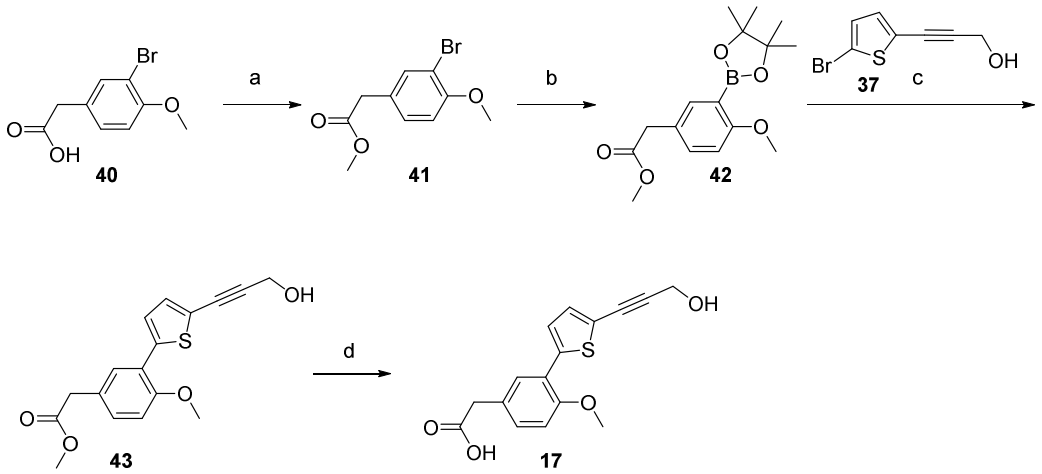


[a]Reagents and conditions: (a) NBS, AIBN, CH_3CN, reflux; (b) KCN, KI, 18-crown-6, CH_3CN; (c) *conc.* HCl, 80 ^oC; (d) H_2SO_4, MeOH, 50 ^oC; (e) $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, 1,4-dioxane, 80 ^oC; (f) $PdCl_2(dppf)$, CsF, DMF, 80 ^oC; (g) bis(pinacolato)diboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, DMSO, 90 ^oC; (h) $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, 1,4-dioxane, 80 ^oC; (i) *aq.* KOH or *aq*. LiOH, THF, MeOH; (j) $(Ph_3P)_4Pd$, CuI, *i*-Pr_2NEt, toluene, 80 ^oC.

Compound **17** was prepared from the commercially available acid **40** as shown in Scheme 2. Protection of the carboxylic acid **40** with methyl ester, palladium catalyzed borylation followed

by cross-coupling with **37** gave compound **43**. Subsequent hydrolysis of the methyl ester **43** provided compound **17**.

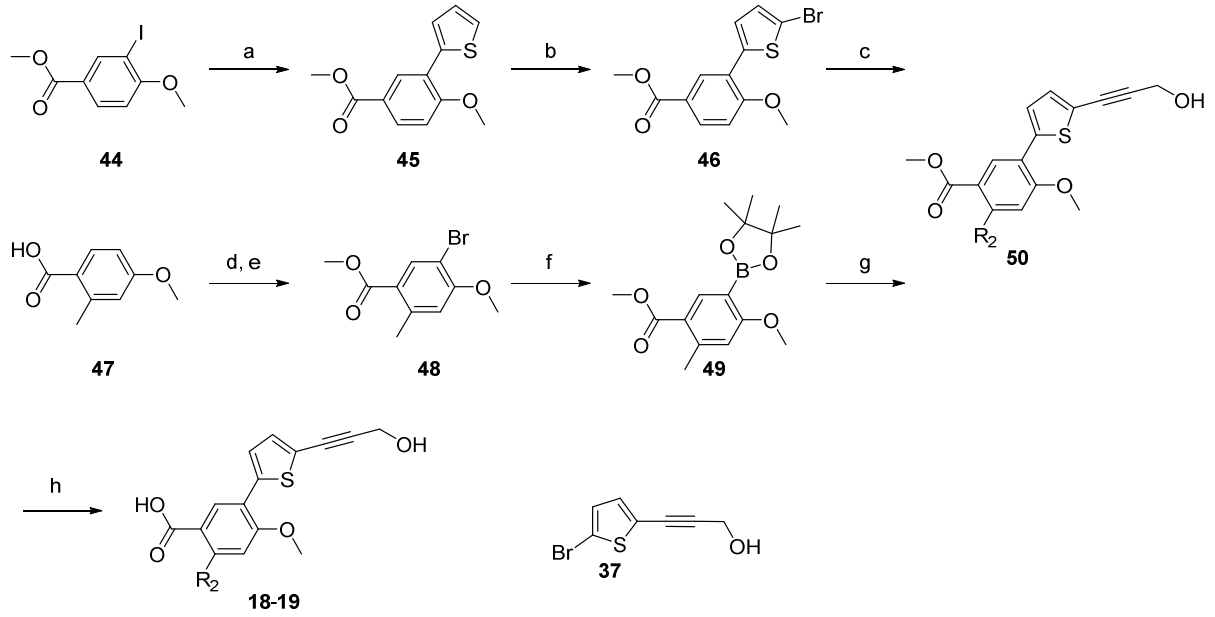
Scheme 2. Synthesis of compound 17[a]



[a]Reagents and conditions: (a) $SOCl_2$, MeOH; (b) bis(pinacolato)diboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, 1,4-dioxane, 100 ^oC; (c) $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, 1,4-dioxane, 80 ^oC; (d) *aq.* LiOH, THF.

The other mono-acid analogs **18**-**19** containing the thiophene propargyl alcohol moiety were prepared as described in Scheme 3. Suzuki-Miyaura cross-coupling of the aryl halide **44** with 2-thiophene boronic acid afforded **45**. Bromination of the thiophene **45** using *N*-bromosuccinimide (NBS) provided the 2-bromo-thiophene **46**, which was reacted with propargyl alcohol under the Sonogashira coupling condition to provide **50** (R_2 = H). Alternatively, the palladium catalyzed borylation of the aryl halide **48** followed by cross-coupling with **37** yielded **50** (R_2 = Me). The resulting esters **50** were hydrolyzed to give compounds **18**-**19**.

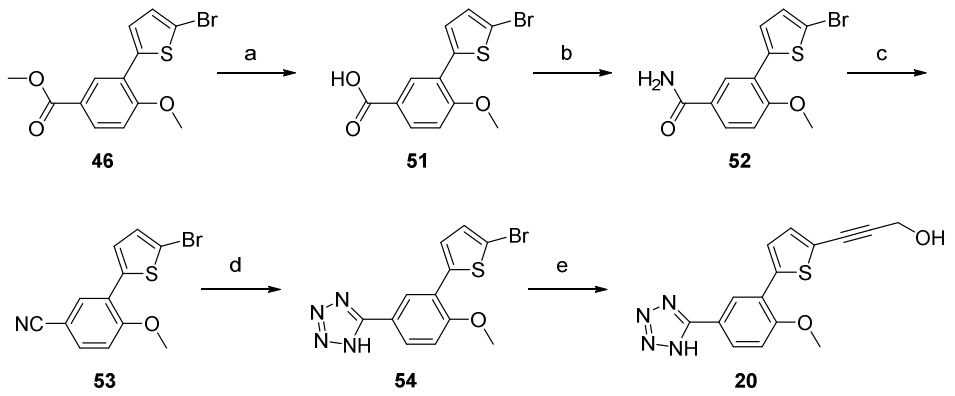
Scheme 3. Synthesis of compounds 18-19[a]



[a]Reagents and conditions: (a) 2-thiophene boronic acid, $Pd(dba)_2$, Ph_3P, K_2CO_3, 1,4-dioxane; (b) NBS, DMF; (c) propargyl alcohol, $PdCl_2(dppf)\cdot CH_2Cl_2$, CuI, Et_3N, DMF, 90 ^oC; (d) Br_2, Fe, $CHCl_3$; (e) $SOCl_2$, MeOH; (f) bis(pinacolato)diboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, 1,4-dioxane, 90 ^oC; (g) **37**, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, 1,4-dioxane, H_2O, 80 ^oC; (h) *aq.* LiOH, THF, MeOH.

Synthesis of the tetrazole analog **20** was started by hydrolysis of the ester **46** as shown in Scheme 4. Amide coupling of the acid **51** with ammonium chloride afforded the primary carboxamide **52**, which underwent dehydration to the nitrile **53**. The nitrile **53** was converted into the tetrazole **54** by treatment with sodium azide. Subsequent Sonogashira coupling of **54** with propargyl alcohol gave compound **20**.

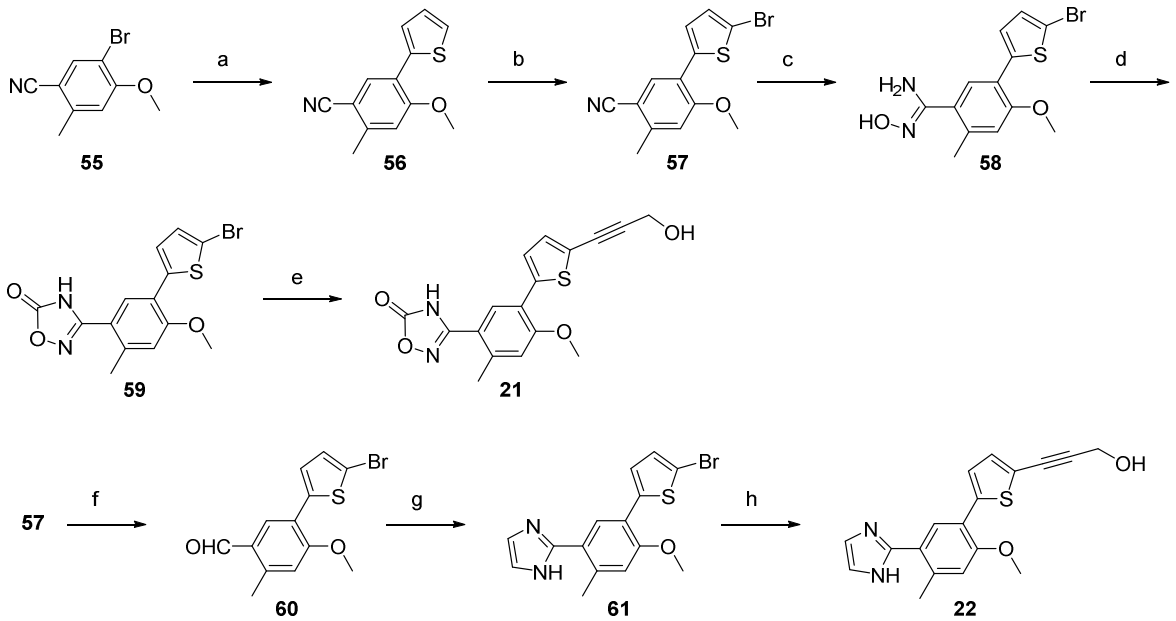
Scheme 4. Synthesis of compounds 20[a]



[a]Reagents and conditions: (a) *aq.* LiOH, THF, MeOH; (b) NH_4Cl, HATU, *i*-Pr_2NEt, DMF; (c) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (d) NaN_3, amberlyst-15, DMSO, 90 ^oC; (e) propargyl alcohol, $PdCl_2(dppf)\cdot CH_2Cl_2$, CuI, Et_3N, DMF, 80 ^oC.

The diazaoxazolone **21** was synthesized from the amidoxime **58**, which was prepared by addition of hydroxylamine to the corresponding cyano derivative **57**. Cyclization of the amidoxime **58** with carbonyldiimidazole (CDI) to the diazaoxazolone **59** followed by Sonogashira coupling with propargyl alcohol yielded compound **21**. The imidazole ring **61** was prepared by cyclization of the aldehyde **60** with glyoxal in the presence of aqueous ammonia. Subsequent introduction of the propargyl alcohol provided compound **22** (Scheme 5).

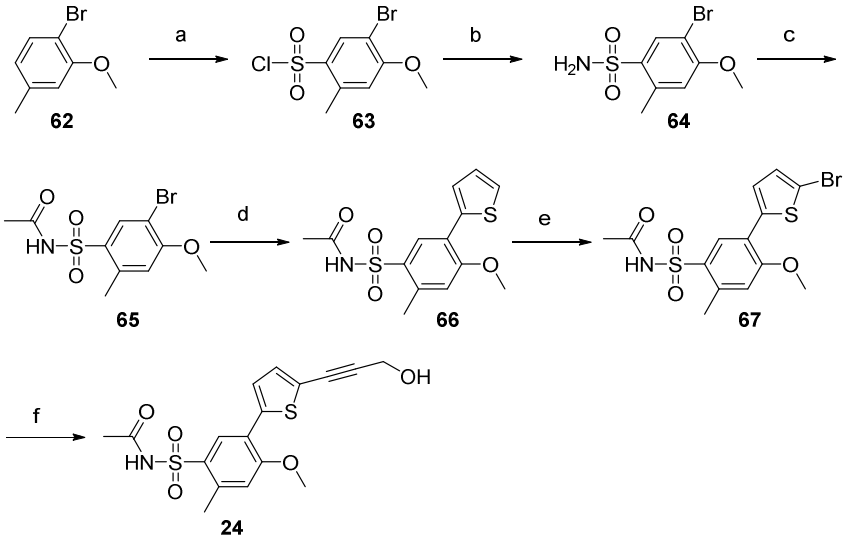
Scheme 5. Synthesis of compounds 21-22[a]



[a]Reagents and conditions: (a) $(Ph_3P)_4Pd$, K_3PO_4, *aq.* 1,4-dioxane, 80 ^oC; (b) NBS, DMF; (c) $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH, 90 ^oC; (d) CDI, DBU, 110 ^oC; (e) propargyl alcohol, $(Ph_3P)_4Pd$, CuI, *i*-Pr_2NEt, THF, 80 ^oC; (f) DIBAL-H, CH_2Cl_2; (g) glyoxal, *liq*. NH_3, MeOH; (h) propargyl alcohol, $PdCl_2(dppf){\cdot}CH_2Cl_2$, CuI, Et_3N, 1,4-dioxane, 80 ^oC.

The reverse acylsulfonamide derivative **24** was prepared as described in Scheme 6. Reaction of 2-methoxy-4-methyl bromobenzene (**62**) with chlorosulfonic acid gave the aryl sulfonyl chloride **63**, which was treated with ammonia in methanol followed by acetyl chloride to afford the reverse acylsulfonamide **65**. Cross-coupling with 2-thiophene boronic acid, bromination of the thiophene **66**, followed by introduction of propargyl alcohol yielded compound **24**.

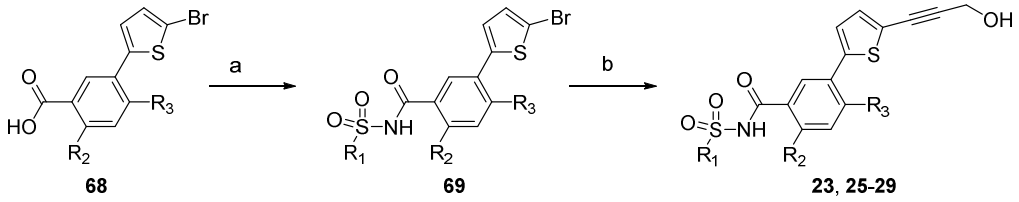
Scheme 6. Synthesis of the reverse acylsulfonamide derivative 24[a]



[a]Reagents and conditions: (a) $ClSO_3H$, CH_2Cl_2; (b) NH_3, MeOH; (c) acetyl chloride, DMAP, Et_3N, CH_2Cl_2; (d) thiophene-2-boronic acid, $(Ph_3P)_4Pd$, Na_2CO_3, *aq.* 1,2-dimethoxyethane, 80 ^oC; (e) NBS, DMF; (f) propargyl alcohol, $PdCl_2(dppf)\cdot CH_2Cl_2$, CuI, Et_3N, DMF, 80 ^oC.

Acylsulfonamide derivatives **23**, **25-29** were prepared by coupling of the benzoic acid intermediates **68** with various sulfonamides using EDCI·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) as a coupling reagent in the presence of HOBt (1-hydroxybenzotriazol) or DMAP (4-dimethylaminopyridine) as shown in Scheme 7. The resulting bromo thiophenes **69** were treated with the Sonogashira reaction to provide compounds **23**, **25-29**.

Scheme 7. Synthesis of acylsulfonamide derivatives 23, 25-29[a]



[a]Reagents and conditions: (a) EDCI·HCl, HOBt or DMAP, DMF or CH_2Cl_2; (b) propargyl alcohol, $PdCl_2(dppf)\cdot CH_2Cl_2$, CuI, Et_3N or Cs_2CO_3, 1,4-dioxane, 80 ^oC.

EXPERIMENTAL SECTION

All materials and reagents used were of the best commercially available grade and used without further purification. ^1H NMR spectra were determined on a Bruker Ultrashield 400 MHz spectrometer or a Bruker AVANCE III HD 400 MHz or a Varian Mercury 300 Plus 300 MHz NMR. Compound purity was determined by the following methods. Method 1: Waters Acquity UPLC equipped with Acquity UPLC BEH Shield RP18 column, 1.7μm, 2.1 x 50 mm using a gradient of 95:5 H_2O (0.1% formic acid)/CH_3CN over at 2.5 min, at 2.60 min then ramp to 0:100 H_2O (0.1% formic acid)/CH_3CN, hold until 4.0 min, return to 95:5 H_2O (0.1% formic acid)/CH_3CN at 4.20 min until end of run with a 0.7 mL/min flow rate. Method 2: Waters Acquity UPLC equipped with Acquity UPLC HSS T3 column, 1.8 μm, 2.1 × 50 mm using a gradient of 95:5 H_2O (0.1% formic acid)/CH_3CN to 2:98 H_2O (0.1% formic acid)/CH_3CN for 2 min run time with a 1.0 mL/min flow rate. Method 3: Agilent 1100series Ion-Trap Mass detector equipped with a Xbridge C18 column, 3.5 μm, 4.6 x 75 mm using a gradient of 95:5 5mM ammonium carbonate/CH_3CN to 20:80 5mM ammonium carbonate/CH_3CN in 4 min and hold up to 7 min with 20:80 5mM ammonium carbonate/CH_3CN with a 1.0 mL/min flow rate. Method 4: Waters Quattro Micro UPLC-LCMS equipped with a Acquity BEH C18 column, 1.7 μm, 2.1 × 100 mm using a gradient of 100:0 H_2O (0.025% trifluoro acetic acid)/CH_3CN (0.025% trifluoro acetic acid) to 20:80 H_2O (0.025% trifluoro acetic acid)/CH_3CN (0.025% trifluoro acetic acid) for 6 min run time with a 0.4 mL/min flow rate. The purity of all compounds screened in the biological assays was examined by HPLC analysis and was found to be $\geq$95 % at 254 nm.

HPLC purifications were performed using Waters Prep HPLC System with wavelength detection of 254 and 214 nm and flow rate of 20 mL/min using the following column and mobile phase conditions: A) Atlantis C-18 5 μm 19 x 150 mm column; mobile phase system of 0.1% formic acid/acetonitrile, B) XBridge C-18 5 μm 19 x 100 mm column; mobile phase system of 0.1% formic acid/acetonitrile, C) XBridge C-18 5 μm 19 x 150 mm column; mobile phase system of 10 mM ammonium bicarbonate/acetonitrile, D) Atlantis T3 5 μm 30 x 250 mm column; mobile phase system of 10 mM ammonium acetate/acetonitrile, E) Sunfire C-18 5 μm 30 x 250 mm column; mobile phase system of 0.1% formic acid/acetonitrile, F) XBridge C-18 5 μm 19 x 150 mm column; mobile phase system of 0.1% formic acid/acetonitrile. HRMS ESI-MS data were recorded using a Thermo Scientific LTQ Orbitrap XL mass spectrometer.

2,2'-([1,1'-Biphenyl]-3,5-diyl)diacetic acid (5).

A mixture of 1-bromo-3,5-dimethylbenzene (**30**) (1 g, 5.4 mmol), NBS (2.02 g, 11.35 mmol) and a catalytic amount (2-5 %) of AIBN in CH_3CN was refluxed under argon for 3 h. The solvent was evaporated under vacuum and 25 mL of CCl_4 was added. The mixture was heated in order to dissolve the crude product and, after cooling, the insoluble succinimide was eliminated by filtration and washed with more CCl_4. Evaporation of the solvent in the filtrates gave a crude product which was purified by silica gel column chromatography to yield 1-bromo-3,5-bis(bromomethyl)benzene (**31**) (890 mg, y. 48%) as a white solid. A further purification could be achieved by crystallization from ethanol. mp: 97-98.5 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.41 (s, 4H), 7.34 (brs, 1H), 7.47 (d, J = 1.5 Hz, 2H).

To a solution of 1-bromo-3,5-bis(bromomethyl)benzene (**31**) (1.35 g, 3.94 mmol) in CH_3CN (20 mL) were added KCN (0.56 g, 8.66 mmol), 18-crown-6 (0.21 g, 0.79 mmol), KI (0.065 g, 0.39

mmol), and water (1.6 mL) at room temperature. After being stirred for 24 h, the mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give 2,2'-(5-bromo-1,3-phenylene)diacetonitrile (**32**) (650 mg, y. 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.74 (s, 4H), 7.23 (s, 1H), 7.47 (s, 2H); LC-MS: m/z 235.0 (M^-).

A mixture of 2,2'-(5-bromo-1,3-phenylene)diacetonitrile (**32**) (236 mg, 1.00 mmol), phenylboronic acid (147 mg, 1.21 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (41 mg, 0.05 mmol), and potassium phosphate, tribasic (210 mg, 1.21 mmol) in 1,4-dioxane (2.5 mL) and MeOH (0.63 mL) was refluxed for 2 h. The mixture was directly purified by silica gel column chromatography to give 2,2'-([1,1'-biphenyl]-3,5-diyl)diacetonitrile (**35**) (176 mg, y. 74%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.82 (s, 4H), 7.26 (s, 1H), 7.36-7.41 (m, 1H), 7.43-7.47 (m, 2H), 7.50 (s, 2H), 7.53-7.56 (m, 2H); LC-MS: m/z 231.1 $(M-H)^-$.

A mixture of 2,2'-([1,1'-biphenyl]-3,5-diyl)diacetonitrile (**35**) (66 mg, 0.28 mmol) in *conc.* HCl (1 mL) was refluxed for 12 h. The mixture was purified by the Prep HPLC (condition B) to give 2,2'-([1,1'-biphenyl]-3,5-diyl)diacetic acid (**5**) (42 mg, y. 55%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (s, 4H), 7.14 (s, 1H), 7.37 (t, J = 7.3 Hz, 1H), 7.41 – 7.50 (m, 4H), 7.59 – 7.65 (m, 2H), 12.43 (s, 2H). LC-MS: m/z 269.3 $(M-H)^-$. HRMS (m/z) calcd for $C_{16}H_{14}O_4$ $(M-H)^-$ 269.0819, found 269.0816.

2,2'-(2'-Chloro-[1,1'-biphenyl]-3,5-diyl)diacetic acid (6). Prepared as described for **5**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (s, 4H), 7.21 (d, J = 6.7 Hz, 3H), 7.40 (qt, J = 4.2, 6.0 Hz, 3H), 7.51 – 7.61 (m, 1H), 12.39 (s, 2H). LC-MS: m/z 303.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{16}H_{13}ClO_4$ $(M-H)^-$ 303.0430, found 303.0425.

2,2'-(3'-Chloro-[1,1'-biphenyl]-3,5-diyl)diacetic acid (7). Prepared as described for **5**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.63 (s, 4H), 7.18 (s, 1H), 7.41 – 7.53 (m, 4H), 7.58 – 7.63 (m, 1H), 7.68 (t, J = 1.8 Hz, 1H), 12.44 (s, 2H). LC-MS: m/z 303.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{16}H_{13}ClO_4$ $(M-H)^-$ 303.0430, found 303.0426.

2,2'-(4'-Chloro-[1,1'-biphenyl]-3,5-diyl)diacetic acid (8). Prepared as described for **5**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.63 (s, 4H), 7.17 (s, 1H), 7.44 (s, 2H), 7.50 – 7.56 (m, 2H), 7.62 – 7.70 (m, 2H), 12.40 (s, 2H). LC-MS: m/z 303.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{16}H_{13}ClO_4$ $(M-H)^-$ 303.0430, found 303.0425.

2,2'-(5-(Furan-2-yl)-1,3-phenylene)diacetic acid (9).

A mixture of 2,2'-(5-bromo-1,3-phenylene)diacetonitrile (**32**) (112 mg, 0.28 mmol) in *conc.* HC1 (1.2 mL) was refluxed for 18 h. The mixture was purified by the Prep HPLC (condition B) to give 2,2'-(5-bromo-1,3-phenylene)diacetic acid (**33**) (112 mg, y. 86%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.58 (s, 4H), 7.15 (s, 1H), 7.37 (s, 2H), 12.45 (brs, 2H).

A mixture of 2,2'-(5-bromo-1,3-phenylene)diacetic acid (**33**) (50 mg, 0.18 mmol), furan-2-boronic acid (62 mg, 0.55 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (33 mg, 0.04 mmol), and CsF (152 mg, 1.00 mmol) in DMF (2.6 mL) was stirred at 80 ^oC for 2 h. The mixture was filtered and washed with MeOH. The filtrate was concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition B) to give 2,2'-(5-(furan-2-yl)-1,3-phenylene)diacetic acid (**9**) (25 mg, y. 52%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.59 (s, 4H), 6.59 (dd, J = 1.8, 3.4 Hz, 1H), 6.91 (d, J = 2.8 Hz, 1H), 7.06 (s, 1H), 7.49 (d, J = 1.3 Hz, 2H), 7.74 (d, J = 1.2 Hz, 1H), 12.41 (s, 2H). LC-MS: m/z 259.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{14}H_{12}O_5$ $(M-H)^-$ 259.0612, found 259.0611.

2,2'-(5-(Thiophen-2-yl)-1,3-phenylene)diacetic acid (10). Prepared as described for **5**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.52 (d, J = 4.5 Hz, 4H), 6.98 (dd, J = 2.7, 5.7 Hz, 1H), 7.06 (s, 1H), 7.15 – 7.25 (m, 2H), 7.34 (s, 2H), 9.66 (s, 2H). LC-MS: m/z 275.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{14}H_{12}O_4S$ $(M-H)^-$ 275.0384, found 275.0381.

2,2'-(5-(5-Chlorothiophen-2-yl)-1,3-phenylene)diacetic acid (11). Prepared as described for **5**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.59 (s, 4H), 7.11 (s, 1H), 7.14 – 7.17 (m, 1H), 7.33 – 7.42 (m, 3H), 12.41 (s, 2H). LC-MS: m/z 309.0 $(M-H)^-$. HRMS (m/z) calcd for $C_{14}H_{11}ClO_4S$ $(M-H)^-$ 308.9994, found 308.9990.

2,2'-(5-(5-Methylthiophen-2-yl)-1,3-phenylene)diacetic acid (12). Prepared as described for **9**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.47 (s, 3H), 3.58 (s, 4H), 6.82 (dd, J = 1.1, 3.5 Hz, 1H), 7.04 (s, 1H), 7.26 (d, J = 3.5 Hz, 1H), 7.36 (d, J = 1.1 Hz, 2H), 12.38 (s, 2H). LC-MS: m/z 289.0 $(M-H)^+$. HRMS (m/z) calcd for $C_{15}H_{14}O_4S$ $(M-H)^-$ 289.0540, found 289.0536.

2,2'-(5-(5-Bromothiophen-2-yl)-1,3-phenylene)diacetic acid (13).

A mixture of dimethyl 2,2'-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-phenylene)diacetate (**36**) (700 mg, 2.0 mmol), 2,5-dibromothiophene (681 mg, 2.8 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (82 mg, 0.10 mmol), and potassium phosphate tribasic (420 mg, 2.4 mmol) in 1,4-dioxane (9 mL) and MeOH (2 mL) was stirred at 80 °C under for 2 h. After the reaction mixture was concentrated *in vacuo*, the residue was purified by the Prep HPLC (condition B) to give dimethyl 2,2'-(5-(5-bromothiophen-2-yl)-1,3-phenylene)diacetate (**38,** R_1 = Br) (160 mg, y. 21%). LC-MS: m/z 383.04 $(M\ (Br^{79})+H)^+$, 385.06 $(M\ (Br^{81})+H)^+$.

To a suspension of dimethyl 2,2'-(5-(5-bromothiophen-2-yl)-1,3-phenylene)diacetate (**38,** R_1 = Br) (20 mg, 0.052 mmol) in THF (1 mL) and MeOH (1 mL) was added *aq.* KOH (4 M, 0.14 mL, 0.56 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was

concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition B) to give 2,2'-(5-(5-bromothiophen-2-yl)-1,3-phenylene)diacetic acid (**13**) (18 mg, y. 97%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.60 (s, 4H), 7.12 (s, 1H), 7.26 (d, J = 3.9 Hz, 1H), 7.32 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 1.2 Hz, 2H), 12.42 (s, 2H). HRMS (m/z) calcd for $C_{14}H_{11}BrO_4S$ $(M-H)^-$ 352.9489, found 352.9486.

2,2'-(5-(5-Cyanothiophen-2-yl)-1,3-phenylene)diacetic acid (14).

2,2'-(5-Bromo-1,3-phenylene)diacetic acid (**33**) (50 g, 0.18 mol) was dissolved in 1 L of MeOH. 5 mL of concentrated sulfuric acid was added. The resulting solution was stirred at 50 ^{o}C for 72 h. The solvent was removed. To the residue was added EtOAc and ice water. The organic layer was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give dimethyl 2,2'-(5-bromo-1,3-phenylene)diacetate (**34**) (50 g, y. 94%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.58 (s, 4H), 3.70 (s, 6H), 7.13 (s, 1H), 7.36 (s, 2H).

Dimethyl 2,2'-(5-bromo-1,3-phenylene)diacetate (**34**) (0.128 g, 0.425 mmol), bis(pinacolato)diboron (0.140 g, 0.553 mmol), KOAc (0.125 g, 1.275 mmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.031 g, 0.043 mmol) were placed in a reaction vial. After 3 mL of DMSO was added, the reaction vessel was flushed with argon and heated to 90 °C for 2 h. After the reaction mixture was allowed to cool to room temperature, the dark brown mixture was filtered through a pad of celite and the pad of celite was washed with EtOAc. The organic filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition A) to give dimethyl 2,2'-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-phenylene)diacetate (**36**) (0.148 g, y. 39%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.36 (s, 12H), 3.65 (s, 4H), 3.71 (s, 6H), 7.34 (s, 1H), 7.64 (s, 2H); LC-MS: m/z 349.2 $(M+H)^+$.

Dimethyl 2,2'-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-phenylene)diacetate (**36**) (0.234 g, 0.671 mmol), 5-bromothiophene-2-carbonitrile (0.126 g, 0.671 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.027 g, 0.034 mmol), and potassium phosphate tribasic (0.171 g, 0.806 mmol) were placed in a reaction vial. To this mixture were added 1,4-dioxane (3.6 mL) and MeOH (0.9 mL). The reaction was allowed to heat to 80 °C under argon for 2.5 h to give a brown reaction mixture. After cooling to room temperature, the reaction mixture was purified by silica gel column chromatography to give dimethyl 2,2'-(5-(5-cyanothiophen-2-yl)-1,3-phenylene)diacetate (**38**, R_1 = CN) (0.221 g, y. 50%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.64 (s, 6H), 3.77 (s, 4H), 7.25 (brs, 1H), 7.58 (d, 2H, J = 4.0 Hz), 7.66 (d, 1H, J = 4.0 Hz), 8.00 (d, 1H, J = 4.0 Hz); LC-MS: m/z 330.15 $(M+H)^+$.

To a solution of dimethyl 2,2'-(5-(5-cyanothiophen-2-yl)-1,3-phenylene)diacetate (**38**, R_1 = CN) (0.1 g, 0.304 mmol) in MeOH (1.5 mL) was added *aq*. KOH (4 M, 0.304 mL, 1.214 mmol) at 0 °C. The reaction was allowed to stir at room temperature for 20 h. The reaction mixture was extracted with *tert*-butyl methyl ether and the aqueous layer was collected. The collected aqueous layer was acidified to pH < 4.0 using 1 *N* HCl. The acidified aqueous layer was further extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition A) to give 2,2'-(5-(5-cyanothiophen-2-yl)-1,3-phenylene)diacetic acid (**14**) (31 mg, y.32 %). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.32 (br, 2H), 3.64 (s, 4H), 7.23 (s, 1H), 7.55 (d, 2H, J = 1.3 Hz), 7.66 (d, 1H, J = 3.8 Hz), 8.00 (d, 1H, J = 4.0 Hz); LC-MS: m/z 300.3 $(M-H)^-$. HRMS (m/z) calcd for $C_{15}H_{11}NO_4S$ $(M-H)^-$ 300.0336, found 300.0331.

2,2'-(5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-1,3-phenylene)diacetic acid (15). Prepared as described for **14**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.60 (s, 4H), 4.34 (s, 2H), 5.33

(s, 1H), 7.12 (s, 1H), 7.29 (d, J = 3.8 Hz, 1H), 7.42 (d, J = 4.1 Hz, 3H). LC-MS: m/z 331.0 $(M+H)^+$. HRMS (m/z) calcd for $C_{17}H_{14}O_5S$ $(M-H)^-$ 329.0489, found 329.0484.

2,2'-(5-(5-(4-Hydroxybut-1-yn-1-yl)thiophen-2-yl)-1,3-phenylene)diacetic acid (16).

A mixture of dimethyl 2,2'-(5-(5-bromothiophen-2-yl)-1,3-phenylene)diacetate (**38**, R_1 = Br) (30 mg, 0.078 mmol), but-3yn-1-ol (0.012 mL, 0.157 mmol), $Pd(Ph_3P)_4$ (4.9 mg, 0.004 mmol), CuI (1 mg, 0.005 mmol), and *i*-Pr_2NH (0.011 mL, 0.078 mmol) in toluene (2.5 mL) was stirred at 100 ^oC for 24 h. After the solvent was concentrated, the residue was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition B) to give dimethyl 2,2'-(5-(5-(4-hydroxybut-1-yn-1-yl)thiophen-2-yl)-1,3-phenylene)diacetate (**39**) (15 mg, y. 52%).

To a suspension of dimethyl 2,2'-(5-(5-(4-hydroxybut-1-yn-1-yl)thiophen-2-yl)-1,3-phenylene)diacetate (**39**) (15 mg, 0.04 mmol) in THF (1 mL) and MeOH (1 mL) was added *aq.* KOH (4 M, 0.1 mL, 0.4 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition B) to give 2,2'-(5-(5-(4-Hydroxybut-1-yn-1-yl)thiophen-2-yl)-1,3-phenylene)diacetic acid (**16**) (12 mg, y. 88%). ^1H NMR (DMSO-d_6, 400 MHz) δ 2.61 (t, J = 6.7 Hz, 2H), 3.56 – 3.61 (m, 6H), 7.11 (s, 1H), 7.21 (d, J = 3.8 Hz, 1H), 7.36 – 7.44 (m, 3H). LC-MS: m/z 345.0 $(M+H)^+$. HRMS (m/z) calcd for $C_{18}H_{16}O_5S$ $(M-H)^-$ 343.0646, found 343.0639.

2-(3-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxyphenyl)acetic acid (17).

To a solution of 3-bromo-4-methyloxyphenylacetic acid (**40**) (20 g, 82 mmol) in MeOH (170 mL) under ice-bath was added thionyl chloride (11.9 mL, 163 mmol) slowly. The resulting clear colourless reaction mixture was allowed to stir at room temperature for 2.5 h. The mixture was concentrated, re-dissolved in CH_2Cl_2, and washed with *aq.* NaOH (1 M, 500 mL). The aqueous

layer was further extracted with CH_2Cl_2 (2 x 250 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to give a clear colorless liquor which in turn precipitated to give methyl 2-(3-bromo-4-methoxyphenyl)acetate as a white solid (**41**) (21.0 g, y. 100%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (s, 3H) 3.64 (s, 2H) 3.83 (s, 3H) 7.06 (d, J = 8.5 Hz, 1H) 7.25 (dd, J = 8.4, 2.1 Hz, 1H) 7.49 (d, J = 2.0 Hz, 1H).

A mixture of methyl 2-(3-bromo-4-methoxyphenyl)acetate (**41**) (0.78 g, 3.01 mmol), bis(pinacolato)diboron (0.92 g, 3.61 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.044 g, 0.060 mmol), and KOAc (0.59 g, 6.02 mmol) in 1,4-dioxane (12 mL) was stirred at 100 ^{o}C for 24 h. After concentration, the residue was purified by silica gel column chromatography to give methyl 2-(4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (**42**) (1.80 g, y. 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 12H), 3.54 (s, 2H), 3.65 (s, 3H), 3.80 (s, 3H), 6.80 (d, J = 8.4 Hz, 1H), 7.30 (dd, J = 8.8, 2.4 Hz, 1H), 7.52 (d, J = 2.4 Hz, 1H).

A mixture of methyl 2-(4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (**42**) (150 mg, 0.49 mmol), 3-(5-bromothiophen-2-yl)prop-2-yn-1-ol (**37**) (106 mg, 0.49 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (20 mg, 0.024 mmol), and potassium phosphate tribasic (102 mg, 0.59 mmol) in 1,4-dioxane (2.2 mL) and MeOH (0.49 mL) was stirred at 80 ^{o}C for 2 h. After concentration, the residue was purified by silica gel column chromatography to give methyl 2-(3-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxyphenyl)acetate (**43**) (40 mg, y. 26%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (s, 3H), 3.67 (s, 2H), 3.91 (s, 3H), 4.33 (s, 2H), 5.39 (s, 1H), 7.11 (d, J = 8.5 Hz, 1H), 7.22 (dd, J = 2.1, 8.5 Hz, 1H), 7.26 (d, J = 3.9 Hz, 1H), 7.52 (d, J = 4.0 Hz, 1H), 7.67 (d, J = 2.1 Hz, 1H).

To a suspension of methyl 2-(3-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxyphenyl)acetate (**43**) (20 mg, 0.063 mmol) in THF (0.33 mL) was added a solution of

LiOH (4.5 mg, 0.19 mmol) in water (0.16 mL). The reaction mixture was stirred at room temperature for 2 h, then neutralized with acetic acid. After the mixture was concentrated, the residue was purified by the Prep HPLC (condition B) to give 2-(3-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxyphenyl)acetic acid (**17**) (18 mg, y. 94%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.54 (s, 2H), 3.90 (s, 3H), 4.33 (s, 2H), 7.00 – 7.34 (m, 3H), 7.51 (s, 1H), 7.65 (s, 1H). LC-MS: m/z 303.0 $(M+H)^+$. HRMS (m/z) calcd for $C_{16}H_{14}O_4S$ $(M-H)^-$ 301.0540, found 301.0536.

3-(5-bromothiophen-2-yl)prop-2-yn-1-ol (37).

A mixture of 2,5-dibromothiophene (1.65 g, 6.82 mmol), propargyl alcohol (0.27 mL, 4.55 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.16 g, 0.23 mmol), CuI (59 mg, 0.31 mmol), and *i*-Pr_2NH (0.65 mL, 4.55 mmol) in THF (9 mL) was stirred at 60 °C for 1 h. After the mixture was concentrated, the residue was purified by silica gel column chromatography to give 3-(5-bromothiophen-2-yl)prop-2-yn-1-ol (0.62 g, y. 63%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.74 (s, 1H), 4.47 (s, 2H), 6.90 (d, J = 3.9 Hz, 1H), 6.93 (d, J = 3.9 Hz, 1H).

3-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxybenzoic acid (18).

A mixture of methyl 3-iodo-4-methoxybenzoate (**44**) (4.0 g, 13.7 mmol), *aq.* K_2CO_3 (2 M, 80 mL, 160 mmol), $Pd(Ph_3P)_4$ (0.43 g, 1.64 mmol) and thiophene-2-boronic acid (3.56 g, 27.7 mmol**)** in 1,4-dioxane (80 mL) was purged with nitrogen for 15 min. Then, $Pd(dba)_2$ (0.24 g, 0.41 mmol) was added and the reaction mixture was stirred at 110 °C for 20 h. The reaction mixture was cooled to room temperature, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, and concentrated *in vacuo* to obtain methyl 4-methoxy-3-(thiophen-2-yl)benzoate (**45**) (6.5 g), which was used as such in the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ

3.91 (s, 3H), 3.99 (s, 3H), 7.0 (d, J = 8.8 Hz, 1H), 7.08-7.13 (m, 1H), 7.36 (d, J = 4.8 Hz, 1H), 7.55 (d, J = 2.8 Hz, 1H), 7.6 (dd, J = 2.4, 8.4 Hz, 1H), 8.33 (d, J = 2.4 Hz, 1H).

To a stirred solution of methyl 4-methoxy-3-(thiophen-2-yl)benzoate (**45**) (1.0 g, 4.03 mmol) in DMF was added NBS (1.07 g, 6.05 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h, then was poured into water (50 mL), and extracted with diethyl ether. The combined organic extracts were dried over Na_2SO_4 and concentrated to afford methyl 3-(5-bromothiophen-2-yl)-4-methoxybenzoate (**46**) (0.9 g, y. 68% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 3H), 3.99 (s, 3H), 7.00 (d, J = 8.8 Hz, 1H), 7.05 (d, J = 4.0 Hz, 1H), 7.32 (d, J = 4.0 Hz, 1H), 7.96 (dd, J = 2.0, 8.8 Hz, 1H), 8.29 (d, J = 2.4 Hz, 1H).

To a stirred solution of methyl 3-(5-bromothiophen-2-yl)-4-methoxybenzoate (**46**) (200 mg, 0.611 mmol) in DMF (4 mL) were added CuI (23 mg, 0.12 mmol), propargyl alcohol (41 mg, 0.73 mmol) followed by Et_3N (617 mg, 6.11 mmol). The reaction mixture was purged with argon for 15 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (50 mg, 0.061 mmol) and the mixture was stirred at 80 °C for 8 h. The reaction mixture was cooled to room temperature and diluted with EtOAc, filtered through a pad of celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford methyl 3-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-4-methoxybenzoate (**50**, R_2 = H) (130 mg, y. 70%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.85 (s, 3H), 4.01 (s, 3H), 4.34 (d, J = 7.6 Hz, 2H), 5.36-5.45 (m, 1H), 7.28 (d, J = 4.8 Hz, 1H), 7.30 (s, 1H), 7.62 (d, J = 5.2 Hz, 1H), 7.93 (dd, J = 3.2, 12.0 Hz, 1H), 8.26 (d, J = 2.4 Hz, 1H); LC-MS: m/z: 303.1 $(M+H)^+$.

To a stirred solution of methyl 3-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-4-methoxybenzoate (**50**, R_2 = H) (370 mg, 1.22 mmol) in MeOH (6.0 mL), THF (6.0 mL), and water (6.0 mL) was

added lithium hydroxide monohydrate (170 mg, 7.34 mmol) and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated and residue was dissolved in water (40 mL), acidified with *aq.* citric acid. The precipitated solid was collected by filtration, washed with water and dried under vacuum to afford 3-(5-(3-hydroxyprop-1-ynyl) thiophen-2-yl)-4-methoxybenzoic acid (**18**) (270 mg, y. 76%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.97 (s, 3H), 4.34 (s, 2H), 5.3-5.5 (m, 1H), 7.19 (d, J = 7.2 Hz, 1H), 7.27 (d, J = 4.8 Hz, 1H), 7.55 (d, J = 4.4 Hz, 1H), 7.91 (d, J = 10.8 Hz, 1H), 8.26 (s, 1H); LC-MS: m/z: 288.9 $(M+H)^+$. HRMS (m/z) calcd for $C_{15}H_{12}O_4S$ $(M-H)^-$ 287.0384, found 287.0380.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methylbenzoic acid (19).

To a solution of 4-methoxy-2-methylbenzoic acid (**47**) (5.00 g, 30.1 mmol) in $CHCl_3$ (30 mL) was added iron powder (1.17 g, 45.2 mmol) followed by bromine (4.87 g, 30.1 mmol). The reaction mixture was stirred at room temperature for 16 h. Then, the stirring bar was removed (which took away iron powder with that) and the mixture was diluted with $CHCl_3$. To this mixture was added saturated *aq.* $NaHSO_4$, which led to formation of a white precipitate. The precipitated solid was filtered, washed with water and dried under vacuum to afford 5-bromo-4-methoxy-2-methylbenzoic acid (2.4 g, y. 32%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.56 (s, 3H), 3.91 (s, 3H), 7.08 (s, 1H), 8.00 (s, 1H), 12.70 (brs, 1H); LC-MS: m/z 242.97 (M (Br^{79})-H)$^-$, 244.97 (M (Br^{81})-H)$^-$.

To a solution of 5-bromo-4-methoxy-2-methylbenzoic acid (1.40 g, 5.43 mmol) in MeOH (10 mL) was slowly added thionyl chloride (4 mL). The mixture was refluxed for 3 h, then cooled to room temperature. After the bulk of solvent was evaporated, the residue was suspended in water and extracted with EtOAc. The combined organic extracts were dried over Na_2CO_3, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford

methyl 5-bromo-4-methoxy-2-methylbenzoate (**48**) (1.00 g, y. 68%). LC-MS: m/z 257.99 (M (Br^{79})+H)$^+$, 259.99 (M (Br^{81})+H)$^+$.

To a solution of methyl 5-bromo-4-methoxy-2-methylbenzoate (**48**) (0.50 g, 1.94 mmol) and bis(pinacolato)diboron (0.59 g, 2.33 mmol) in 1,4-dioxane (15 mL) were added KOAc (0.79 g, 5.81 mmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.14 g, 0.19 mmol). After being stirred at 90 ^oC for 4 h, the dark brown mixture was cooled to room temperature and filtered through a pad of celite. The filtrate was concentrated to obtain methyl 4-methoxy-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**49**) (0.5 g), which was used as such in the next step. LC-MS: m/z 306.98 (M+H)$^+$.

A mixture of methyl 4-methoxy-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**49**) (0.500 g), 3-(5-bromothiophen-2-yl)prop-2-yn-1-ol (**37**) (0.423 g) and K_2CO_3 (0.627 g, 4.90 mmol) in 1,4-dioxane (9 mL) and water (1 mL) was purged with argon for 10 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.12 g, 0.16 mmol) and the mixture was stirred at 80 ^oC for 3 h. The dark brown mixture was cooled to room temperature and filtered through a pad of celite. The celite pad was washed with EtOAc. The combined filtrates were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford methyl 5-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-4-methoxy-2-methylbenzoate (**50**, R_2 = Me) (0.15 g, 70% purity by LCMS). LC-MS: m/z 316.85 (M+H)$^+$.

To a solution of methyl 5-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-4-methoxy-2-methylbenzoate (**50**, R_2 = Me) (50 mg) in THF (2 mL), MeOH (2 mL), and water (1 mL) was added lithium hydroxide monohydrate (19 mg, 0.47 mmol). The reaction mixture was stirred at room temperature for 12 h. After the reaction mixture was concentrated, the residue was

dissolved in water and washed with EtOAc. The aqueous layer was acidified with 2 *N* HCl until pH = 2 and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2CO_3, filtered, and concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition C) to give 5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methylbenzoic acid (**19**) (15 mg, y. 9% over 3 steps). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.58 (s, 3H), 3.98 (s, 3H), 4.33 (d, *J* = 5.9 Hz, 2H), 5.87 (t, *J* = 5.8 Hz, 1H), 7.09 (s, 1H), 7.26 (d, *J* = 3.9 Hz, 1H), 7.51 (d, *J* = 4.0 Hz, 1H), 8.19 (s, 1H), 12.7 (s, 1H). LC-MS: m/z 301.03 $(M-H)^-$. HRMS (m/z) calcd for $C_{16}H_{14}O_4S$ $(M-H)^-$ 301.0540, found 301.0535.

3-(5-(2-Methoxy-5-(1H-tetrazol-5-yl)phenyl)thiophen-2-yl)prop-2-yn-1-ol (20).

To a stirred solution of methyl 3-(5-bromothiophen-2-yl)-4-methoxybenzoate (**46**) (1.5 g, 4.60 mmol) in MeOH (5 mL), THF (5 mL), and water (5 mL) was added lithium hydroxide monohydrate (0.26 g, 6.90 mmol) and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated, the residue was dissolved in water and the resulting solution was washed with diethyl ether. The aqueous layer was acidified with *aq.* citric acid and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to yield 3-(5-bromothiophen-2-yl)-4-methoxybenzoic acid (**51**) (1.0 g, y. 71%), which was used as such in the next step. LC-MS: m/z: 311.2 $(M-H)^-$.

To a stirred solution of 3-(5-bromothiophen-2-yl)-4-methoxybenzoic acid (**51**) (1.0 g, 3.20 mmol) in DMF (5 mL) was added HATU (1.8 g, 4.80 mmol) followed by ammonium chloride (0.25 g, 4.80 mmol) and *i*-Pr_2NEt (0.62 g, 4.80 mmol). The reaction mixture was stirred at room temperature for 5 h and then poured into water, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to obtain 3-(5-bromothiophen-2-yl)-

4-methoxybenzamide (**52**) (850 mg) as a pale brown solid, which was used as such in the next step. LC-MS: m/z: 311.9 $(M+H)^+$.

To a stirred solution of 3-(5-bromothiophen-2-yl)-4-methoxybenzamide (**52**) (850 mg, 2.73 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.7 mL, 5.46 mmol) and $(CF_3CO)_2O$ (0.86 g, 4.09 mmol) at 0 °C and resulting mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with a mixture of water and CH_2Cl_2. The organic layer was separated and aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 3-(5-bromothiophen-2-yl)-4-methoxybenzonitrile (**53**) (350 mg, 43% over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.00 (s, 3H), 7.02 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 4.0 Hz, 1H), 7.24 (d, J = 4.0 Hz, 1H), 7.56 (dd, J = 1.6, 8.4 Hz, 1H), 7.86 (d, J = 2.4 Hz, 1H).

To a stirred solution of 3-(5-bromothiophen-2-yl)-4-methoxybenzonitrile (**53**) (350 mg, 1.194 mmol) in DMSO (5 mL) were added NaN_3 (116 mg, 1.791 mmol) and amberlyst-15 (30 mg), and the mixture was stirred at 80 °C for 8 h. The reaction mixture was filtered and the filtrate was diluted with water. The resultant solid was collected by filtration, washed with water, diethyl ether, and dried to afford 5-(3-(5-bromothiophen-2-yl)-4-methoxyphenyl)-1H-tetrazole (**54**) (200 mg, y. 50%). LC-MS: m/z: 337.04 $(M+H)^+$.

To a stirred solution of 5-(3-(5-bromothiophen-2-yl)-4-methoxyphenyl)-1H-tetrazole (**54**) (150 mg, 0.446 mmol) in DMF (5 mL) were added CuI (16 mg, 0.089 mmol), propargyl alcohol (29 mg, 0.517 mmol) followed by Et_2NH (0.4 mL, 4.46 mmol). The reaction mixture was purged with argon for 15 min, $PdCl_2(dppf)\cdot CH_2Cl_2$ (36 mg, 0.044 mmol) was added. The reaction mixture was stirred at 80 °C for 8 h, then cooled to room temperature, and poured into water. The resulting solid was collected by filtration. This solid material was dissolved in EtOAc and

the solution was filtered through a pad of celite. The filtrate was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to yield a crude material, which was purified by the Prep HPLC (condition F) to afford 3-(5-(2-methoxy-5-(1H-tetrazol-5-yl)phenyl)thiophen-2-yl)prop-2-yn-1-ol (**20**) (3 mg, y. 2%) as pale yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.97 (s, 3H), 4.34 (d, J = 5.6 Hz, 2H), 5.30-5.40 (m, 1H), 7.26 (brs, 1H), 7.30 (brs, 1H), 7.62 (brs, 1H), 7.90-8.20 (m, 2H), 11.90 (brs, 1H). LC-MS: m/z: 313.09 $(M+H)^+$. HRMS (m/z) calcd for $C_{15}H_{12}N_4O_2S$ $(M+H)^+$ 313.0754, found 313.0751.

3-(5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methylphenyl)-1,2,4-oxadiazol-5(4H)-one (21).

A solution of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzonitrile (**57**) (100 mg, 0.32 mmol), hydroxylamine hydrochloride (23 mg, 0.32 mmol), and $NaHCO_3$ (30 mg, 0.36 mmol) in MeOH (0.44 mL) were taken in a glass pressure reactor with teflon screw cap. The resulting reaction mixture was heated at 90 °C for 18 h. After the solvent was concentrated, the residue was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give 5-(5-bromothiophen-2-yl)-*N*′-hydroxy-4-methoxy-2-methylbenzimidamide (**58**) (105 mg, y. 95%).

A mixture of 5-(5-bromothiophen-2-yl)-*N*′-hydroxy-4-methoxy-2-methylbenzimidamide (**58**) (105 mg, 0.31 mmol), CDI (75 mg, 0.46 mmol), and DBU (52 mg, 0.34 mmol) in 1,4-dioxane (1.5 mL) was stirred at 110 °C for 1.5 h. To this reaction mixture was added CDI (25 mg, 0.15 mmol) and the reaction mixture was stirred at 110 °C for an additional 1.5 h. After the solvent was concentrated, the residue was diluted with EtOAc, washed with 1*N* HCl (x 3) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by the Prep

HPLC (condition B) to give 3-(5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenyl)-1,2,4-oxadiazol-5(4H)-one (**59**) (42 mg, y. 35%).

A mixture of 3-(5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenyl)-1,2,4-oxadiazol-5(4H)-one (**59**) (20 mg, 0.054 mmol), propargyl alcohol (6 mg, 0.11 mmol), $Pd(Ph_3P)_4$ (3.4 mg, 0.003 mmol), CuI (0.7 mg, 0.004 mmol), and *i*-Pr_2NH (5.5 mg, 0.054 mmol) in THF (1 mL) was stirred at 60 ^oC for 2 h. The mixture was purified by the Prep HPLC (condition B) to give 3-(5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methylphenyl)-1,2,4-oxadiazol-5(4H)-one (**21**) (12 mg, y. 64%). ^1H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 3H), 4.00 (s, 3H), 4.34 (s, 2H), 5.32 (s, 1H), 7.22 (s, 1H), 7.31 (d, J = 4.0 Hz, 1H), 7.62 (s, 1H), 8.02 (s, 1H), 12.67 (s, 1H). LC-MS: m/z 343.0 $(M+H)^+$. HRMS (m/z) calcd for $C_{17}H_{14}N_2O_4S$ $(M+H)^+$ 343.0743, found 343.0744.

3-(5-(5-(1H-Imidazol-2-yl)-2-methoxy-4-methylphenyl)thiophen-2-yl)prop-2-yn-1-ol (22).

A mixture of 5-bromo-4-methoxy-2-methylbenzonitrile (**55**) (1.00 g, 4.40 mmol), thiophene-2-boronic acid (0.739 g, 7.70 mmol), and potassium phosphate tribasic (1.88 g, 8.80 mmol) in 1,4-dioxane (15 mL) and water (5 mL) was purged with argon for 5 min. To this mixture was added $Pd(Ph_3P)_4$ (0.513 g, 0.44 mmol), and the reaction mixture was stirred at 80 ^oC for 2 h. After cooling to room temperature, the mixture was diluted with EtOAc and filtered through a pad of celite. The filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 4-methoxy-2-methyl-5-(thiophen-2-yl)benzonitrile (**56**) (500 mg, 84% purity by LCMS). LC-MS: m/z 230.12 $(M+H)^+$.

To a solution of 4-methoxy-2-methyl-5-(thiophen-2-yl)benzonitrile (**56**) (0.200 g) in DMF (5 mL) was added NBS (0.124 g, 0.70 mmol). After being stirred at room temperature for 1 h, the

reaction mixture was poured into ice-cold water. The precipitated white solid was filtered and dried under vacuum to afford 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzonitrile (**57**) (0.2 g, 84% purity by LCMS). LC-MS: m/z 308.1 (M (Br^{79})+H)$^+$, 310.2 (M (Br^{81})+H)$^+$.

To a solution of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzonitrile (**57**) (0.2 g) in CH_2Cl_2 (10 mL) was added DIBAL-H (1.0 M in toluene, 0.12 mL, 0.12 mmol) at 0 ^oC. The reaction mixture was stirred at room temperature for 1 h, then the reaction was quenched with 1 *N* HCl (5 mL). The mixture was extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzaldehyde (**60**) (0.2 g, 78% purity by LCMS), which was used as such in the next step. LC-MS: m/z 310.94 (M (Br^{79})+H)$^+$, 311.97(M (Br^{81})+H)$^+$.

To a solution of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzaldehyde (**60**) (200 mg) in MeOH (5 mL) at 0 ^oC was added glyoxal (40% aq solution, 0.032 mL, 0.7 mmol) followed by aqueous ammonia (0.04 mL), and the reaction mixture was stirred at room temperature for 20 h. After the bulk of solvent was concentrated, the residue was purified by silica gel column chromatography to afford 2-(5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenyl)-1H-imidazole (**61**) (90 mg, 76% purity by LCMS), which was used as such in the next step. LC-MS: m/z 349.14 (M (Br^{79})+H)$^+$, 351.14 (M (Br^{81})+H)$^+$.

To a solution of 2-(5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenyl)-1H-imidazole (**61**) (90 mg) in 1,4-dioxane (14 mL) were added propargyl alcohol (21 mg, 0.38 mmol), Et_3N (269 mg, 2.66 mmol), and CuI (9 mg, 0.047 mmol). After the mixture was purged with argon for 5 min, $PdCl_2(dppf)\cdot CH_2Cl_2$ (21 mg, 0.025 mmol) was added and the mixture was stirred at 80 ^oC for 2 h. After cooling to room temperature, the mixture was diluted with EtOAc and filtered

through a pad of celite. The filtrate was washed with water and brine, dried over Na_2CO_3, filtered, and concentrated to obtain a dark brown gum, which was purified by the Prep HPLC (condition C) to give 3-(5-(5-(1H-imidazol-2-yl)-2-methoxy-4-methylphenyl)thiophen-2-yl)prop-2-yn-1-ol (**22**) (25 mg, y. 19% over 5 steps). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.58 (s, 3H), 3.96 (s, 3H), 4.33 (d, J = 5.9 Hz, 2H), 5.36 (t, J = 5.9 Hz, 1H), 7.04 (s, 1H), 7.09 (s, 1H), 7.23 (s, 1H), 7.28 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.95 (s, 1H), 12.2 (s, 1H); LC-MS: m/z 325.29 $(M+H)^+$. HRMS (m/z) calcd for $C_{18}H_{16}N_2O_sS$ $(M+H)^+$ 325.1005, found 325.1003.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methyl-*N*-(phenylsulfonyl)benzamide (23).

A mixture of methyl 5-bromo-4-methoxy-2-methylbenzoate (**48**) (1.50 g, 5.80 mmol), 4,4,5,5-tetramethyl-2-(thiophen-2-yl)-1,3,2-dioxaborolane (1.46 g, 6.95 mmol) and K_2CO_3 (2.40 g, 17.4 mmol) in 1,4-dioxane (60 mL) and water (15 mL) was purged with argon for 5 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.42 g, 0.58 mmol), and the mixture was stirred at 80 ^oC for 4 h. The dark brown mixture was cooled to room temperature and filtered through a pad of celite. The celite pad was washed with EtOAc, the filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford methyl 4-methoxy-2-methyl-5-(thiophen-2-yl)benzoate (0.7 g, 77% purity by LCMS), which was used as such in the next step. LC-MS: m/z 261.04 $(M+H)^+$.

To a solution of methyl 4-methoxy-2-methyl-5-(thiophen-2-yl)benzoate (700 mg) in DMF (5 mL) was added NBS (428 mg, 2.40 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-cold water, the precipitated white solid was filtered and dried under vacuum to give methyl methyl 5-(5-bromothiophen-2-yl)-4-methoxy-2-

methylbenzoate (700 mg, y. 35% over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.65 (s. 3H), 3.89 (s, 3H), 3.97 (s, 3H), 6.79 (s, 1H), 7.02 (d, J = 4.0 Hz, 1H), 7.24 (d, J = 4.0 Hz, 1H), 8.22 (s, 1H). LC-MS: m/z 340.98 (M (Br^{79})+H)$^+$, 343.00 (M (Br^{81})+H)$^+$.

To a solution of methyl 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzoate (600 mg, 1.76 mmol) in THF (3 mL) - MeOH (3 mL) - water (3 mL) was added lithium hydroxide monohydrate (295 mg, 7.10 mmol), and the mixture was stirred at 40 ^{o}C for 4 h. After the reaction mixture was concentrated, the residue was dissolved in water and washed with diethyl ether. The aqueous extract was acidified with *aq.* citric acid until pH = 2, the precipitated solid was filtered and dried under vacuum to give 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzoic acid (**68**, R_2 = Me, R_3 = OMe) (400 mg, y. 70%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.56 (s, 3H), 3.91 (s, 3H), 6.90 (s, 1H), 7.18 (d, J = 4.4 Hz, 1H), 7.33 (d, J = 4.4 Hz, 1H), 8.04 (s, 1H). LC-MS: m/z 327.09 (M (Br^{79})+H)$^+$, 329.07 (M (Br^{81})+H)$^+$.

To a mixture of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzoic acid (**68**, R_2 = Me, R_3 = OMe) (300 mg, 0.920 mmol) and methanesulfonamide (131 mg, 1.38 mmol) in DMF (5 mL) were added EDCI·HCl (265 mg, 1.38 mmol), HOBt (186 mg, 1.38 mmol), and Et_3N (218 mg, 1.84 mmol). After the mixture was stirred at 50 ^{o}C for 16 h, the brown solution was diluted with ice-water and extracted with EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give 5-(5-bromothiophen-2-yl)-4-methoxy-2-methyl-*N*-(methylsulfonyl)benzamide (**69**, R_1 = R_2 = Me, R_3 = OMe) (100 mg), which was used as such in the next step.

A solution of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methyl-*N*-(methylsulfonyl)benzamide (**69**, R_1 = R_2 = Me, R_3 = OMe) (100 mg), propargyl alcohol (21 mg, 0.37 mmol), Et_3N (255 mg, 2.48 mmol) and CuI (9 mg, 0.049 mmol) in DMF (3 mL) was purged with argon for 5 min. To this

mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (19 mg, 0.024 mmol), and the mixture was stirred at 80 ^oC for 3 h. The dark brown mixture was cooled to room temperature, diluted with water and extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give 5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methyl-*N*-(methylsulfonyl)benzamide (**23**) (28 mg, y. 8% over 2 steps). ^1H NMR (DMSO-d_6, 400 MHz) δ 2.47 (s, 3H), 3.38 (s, 3H), 3.98 (s, 3H), 4.34 (brs, 2H), 5.37 (brs, 1H), 7.11 (s, 1H), 7.30 (d, J = 3.9 Hz, 1H), 7.63 (d, J = 3.9 Hz, 1H), 7.95 (s, 1H), 12.0 (s, 1H). LC-MS: m/z 380.00 $(M+H)^+$. HRMS (m/z) calcd for $C_{17}H_{17}NO_5S_2$ $(M+H)^+$ 380.0621, found 380.0616.

_N_-((5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2 methylphenyl)sulfonyl)acetamide (24).

To a solution of 1-bromo-2-methoxy-4-methylbenzene (**62**) (5.0 g, 24.8 mmol) in CH_2Cl_2 (20 mL) was added chlorosulfonic acid (5.0 mL, 149.2 mmol) at room temperature and the mixture was stirred at 80 °C for 2 h. Then the reaction mixture was poured into ice-cold water, extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was dissolved in ammonia solution in MeOH (20 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. After concentration, the residue was washed with n-hexane to afford 5-bromo-4-methoxy-2-methylbenzenesulfonamide (**64**) (5.0 g, y. 72%) as white solid. LC-MS: m/z 279.93 $(M(Br^{79})+H)^+$, 281.91 $(M(Br^{81})+H)^+$.

To a solution of 5-bromo-4-methoxy-2-methylbenzenesulfonamide (**64**) (1.0 g, 3.50 mmol), Et_3N (0.75mL, 5.3 mmol), and DMAP (0.043 g, 0.35 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added acetyl chloride (0.27 mL, 3.9 mmol) slowly. After being stirred at room temperature for 5 h, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and

concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford *N*-(5-bromo-4-methoxy-2-methylphenylsulfonyl)acetamide (**65**) (0.74 g, y. 66%). LC-MS: m/z 319.92 (M (Br^{79})-H)$^-$, 321.90 (M (Br^{81})-H)$^-$.

A mixture of *N*-(5-bromo-4-methoxy-2-methylphenylsulfonyl)acetamide (**65**) (0.74 g, 2.2 mmol), thiophene-2-boronic acid (0.44 g, 3.4 mmol), and Na_2CO_3 (0.60 g, 5.7 mmol) in 1,2-dimethoxyethane and water (3:1, 10 mL) was purged with argon for 10 min. To this mixture was added $PdCl_2(PPh_3)_2$ (0.16 g, 0.22 mmol) and the reaction mixture was stirred at 80 °C for 2 h. After dilution with EtOAc, the mixture was filtered through celite pad. The filtrate was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford *N*-(4-methoxy-2-methyl-5-(thiophen-2-yl)phenylsulfonyl)acetamide (**66**) (0.20 g, y. 28%). LC-MS: m/z 326.3 $(M+H)^+$.

To a solution of *N*-(4-methoxy-2-methyl-5-(thiophen-2-yl)phenylsulfonyl)acetamide (**66**) (0.20 g, 0.61 mmol) in DMF (5 mL) was added NBS (0.11 g, 0.61 mmol) and the mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with water, and the precipitates were filtered and washed with n-hexane to afford *N*-((5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenyl)sulfonyl)acetamide (**67**) (0.10 g, y. 41%). LC-MS: m/z 403.90 (M (Br^{79})+H)$^+$, 405.92 (M (Br^{81})+H)$^+$.

A mixture of *N*-(5-(5-bromothiophen-2-yl)-4-methoxy-2-methylphenylsulfonyl)acetamide (**67**) (100 mg, 0.24 mmol), propargyl alcohol (20 mg, 0.37 mmol), CuI (9 mg, 0.04 mmol), and Et_3N (0.34 mL, 2.4 mmol) in 1,4-dioxane (5 mL) was purged with argon for 10 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (20 mg, 0.02 mmol) and the mixture was stirred at 80 °C for 16 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, filtered through celite pad, and the filtrate was concentrated. The residue was purified by the Prep HPLC

(condition D) to afford *N*-(5-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-4-methoxy-2-methylphenylsulfonyl)acetamide (**24**) (18 mg, y. 20%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.92 (s, 3H), 2.60 (s, 3H), δ 4.0 (s, 3H), 4.33 (d, J = 6.0 Hz, 2H), 5.39 (t, J = 6.0 Hz, 1H), 7.20 (s, 1H), 7.28 (d, J = 4.0 Hz, 1H), 7.46 (d, J = 4.0 Hz, 1H), 8.17 (s, 1H), 12.15 (brs, 1H). LC-MS: m/z 380.04 $(M+H)^+$. HRMS (m/z) calcd for $C_{17}H_{17}NO_5S_2$ $(M+H)^+$ 380.0621, found 380.0617.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methyl-*N*-(phenylsulfonyl)benzamide (25). Prepared as described for **23**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.25 (s, 3H), 3.95 (s, 3H), 4.34 (brs, 2H), 5.36 (brs, 1H), 7.05 (s, 1H), 7.29 (d, J = 3.9 Hz, 1H), 7.64 (d, J = 3.9 Hz, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.72-7.76 (m, 1H), 7.90 (s, 1H), 8.00-8.02 (m, 2H), 12.4 (s, 1H). LC-MS: m/z 442.3 $(M+H)^+$. HRMS (m/z) calcd for $C_{22}H_{19}NO_5S_2$ $(M+H)^+$ 442.0777, found 442.0771.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-2,4-dimethoxy-*N*-(phenylsulfonyl)benzamide (26).

To a solution of 5-bromo-2,4-dihydroxybenzoic acid (5.00 g, 21.7 mmol) in acetone (70 mL) was added K_2CO_3 (18.0 g, 130 mmol) followed by iodomethane (18.5 g, 130 mmol) at room temperature, and the reaction mixture was refluxed for 16 h. After the bulk of solvent was concentrated *in vacuo*, the residue was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, concentrated to afford methyl 5-bromo-2,4-dimethoxybenzoate (4.00 g, y. 67%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.82 (s, 3H), 3.92 (s, 3H), 3,96 (s, 3H), 6.72 (s, 1H), 7.96 (s, 1H). LC-MS: m/z 275.08 $(M\ (Br^{79})+H)^+$, 277.10 $(M\ (Br^{81})+H)^+$.

A mixture of methyl 5-bromo-2,4-dimethoxybenzoate (1.00 g, 3.64 mmol), thiophene-2-boronic acid (0.698 g, 5.45 mmol) and potassium phosphate tribasic (1.31 g, 10.9 mmol) in 1,4-dioxane (24 mL) and water (6 mL) was purged with argon for 5 min. To this mixture was added

$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.296 g, 0.636 mmol), and the mixture was stirred at 80 ^oC for 2 h. The dark brown mixture was cooled to room temperature and filtered through a pad of celite. The celite pad was washed with EtOAc, and the filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford methyl 2,4-dimethoxy-5-(thiophen-2-yl)benzoate (0.8 g, y. 79%). ^1H NMR ($CDCl_3$, 400 MHz) δ 3.85 (s, 3H), 3.97 (s, 3H), 3.99 (s, 3H), 6.55 (s, 1H), 7.07 (dd, J = 4.8, 4.0 Hz, 1H), 7.28 (d, J = 4.4 Hz, 1H), 7.41 (d, J = 2.8 Hz, 1H), 8.17 (s, 1H). LC-MS: m/z 279.11 $(M+H)^+$.

To a solution of methyl 2,4-dimethoxy-5-(thiophen-2-yl)benzoate (800 mg, 2.88 mmol) in DMF (8 mL) was added NBS (512 mg, 2.88 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-cold water, the precipitated white solid was filtered and dried under vacuum to afford methyl 5-(5-bromothiophen-2-yl)-2,4-dimethoxybenzoate (800 mg, y. 78%). ^1H NMR ($CDCl_3$, 400 MHz) δ 3.86 (s, 3H), 3.97 (s, 3H), 3.00 (s, 3H), 6.56 (s, 1H), 7.02 (d, J = 4.0 Hz, 1H), 7.16 (d, J = 3.6 Hz, 1H), 8.12 (s, 1H). LC-MS: m/z 357.2 $(M\ (Br^{79})+H)^+$, 359.2 $(M\ (Br^{81})+H)^+$.

To a solution of methyl 5-(5-bromothiophen-2-yl)-2,4-dimethoxybenzoate (800 mg, 2.25 mmol) in THF (8 mL) - MeOH (8 mL) - water (8 mL) was added lithium hydroxide monohydrate (283 mg, 6.74 mmol), and the mixture was stirred at room temperature for 4 h. After the reaction mixture was concentrated, the residue was dissolved in water (100 mL) and acidified with *aq.* citric acid until pH = 2. The precipitated solid was filtered and dried under vacuum to 5-(5-bromothiophen-2-yl)-2,4-dimethoxybenzoic acid (**68**, $R_2 = R_3 = OMe$) (600 mg, y. 78%). LC-MS: m/z 343.2 $(M\ (Br^{79})+H)^+$, 345.2 $(M\ (Br^{81})+H)^+$.

To a mixture of 5-(5-bromothiophen-2-yl)-2,4-dimethoxybenzoic acid (**68**, $R_2 = R_3 = OMe$) (150 mg, 0.438 mmol) and benzenesulfonamide (103 mg, 0.657 mmol) in CH_2Cl_2 (3 mL) were added EDCI·HCl (168 mg, 0.877 mmol) and DMAP (160 mg, 1.31 mmol), and the mixture was stirred at room temperature for 8 h. The brown solution was poured into ice-cold water, and extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 5-(5-bromothiophen-2-yl)-2,4-dimethoxy-*N*-(phenylsulfonyl)benzamide (**69**, R_1 = Ph, $R_2 = R_3 =$ OMe) (120 mg, y. 57%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.95 (s, 3H), 4.05 (s, 3H), 6.81 (s, 1H), 7.17 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 4.4 Hz, 1H), 7.61-7.69 (m, 2H), 7.69-7.77 (m, 1H), 7.78 (s, 1H), 8.01 (d, J = 7.6 Hz, 2H), 11.64 (brs, 1H); LC-MS: m/z 479.8 (M (Br^{79})-H)$^-$, 481.9 (M (Br^{81})-H)$^-$.

To a solution of 5-(5-bromothiophen-2-yl)-2,4-dimethoxy-*N*-(phenylsulfonyl)benzamide (**69**, R_1 = Ph, $R_2 = R_3 =$ OMe) (100 mg, 0.207 mmol) in 1,4-dioxane (5 mL) were added propargyl alcohol (15 mg, 0.27 mmol), Et_3N (210 mg, 2.15 mmol), and CuI (8 mg, 0.043 mmol), and the mixture was purged with argon for 5 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (17 mg, 0.021 mmol), and the mixture was stirred at 80 ^{o}C for 3 h. After cooling to room temperature, the mixture was diluted with EtOAc and filtered through a pad of celite. The filtrate was washed with water and brine, dried over Na_2CO_3, filtered, and concentrated to obtain a dark brown gum, which was purified by the Prep HPLC (condition E) to give 5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-2,4-dimethoxy-*N*-(phenylsulfonyl)benzamide (**26**) (23 mg, y. 24%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.95 (s, 3H), 4.02 (s, 3H), 4.32 (brs, 2H), 5.35 (brs, 1H), 6.82 (s, 1H), 7.21 (d, J = 3.4 Hz, 1H), 7.44 (d, J = 3.4 Hz, 1H), 7.66 (t, J = 7.4 Hz, 2H), 7.73 (t, J = 7.3

Hz, 1H), 7.80 (s, 1H), 8.01 (d, J = 7.3 Hz, 2H), 11.7 (s, 1H). LC-MS: m/z 455.94 $(M-H)^-$. HRMS (m/z) calcd for $C_{22}H_{19}NO_6S_2$ $(M+H)^+$ 458.0727, found 458.0718.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-2,4-dimethoxy-*N*-((3-methoxyphenyl)sulfonyl)benzamide (27). Prepared as described for **26**.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.84 (s, 3H), 3.96 (s, 3H), 4.03 (s, 3H), 4.32 (brs, 2H), 5.85 (brs, 1H), 6.83 (s, 1H), 7.21 (d, J = 4.0 Hz, 1H), 7.29-7.32 (m, 1H), 7.44 (d, J = 4.4 Hz, 1H), 7.49 (br, 1H), 7.57 (d, J = 4.8 Hz, 2H), 7.81 (s, 1H), 11.6 (s, 1H). LC-MS: m/z 488.09 $(M+H)^+$. HRMS (m/z) calcd for $C_{23}H_{21}NO_7S_2$ $(M+H)^+$ 488.0832, found 488.0822.

4-Chloro-5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-2-methoxy-N-((3-methoxyphenyl)sulfonyl)benzamide (28).

A mixture of methyl 5-bromo-4-chloro-2-methoxybenzoate (1.5 g, 5.3 mmol), thiophene-2-boronic acid (0.83 g, 6.5 mmol) and potassium phosphate tribasic (3.3 g, 15.9 mmol) in 1,4-dioxane (10 mL) and water (5 mL) was purged with argon for 5 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.43 g, 0.53 mmol), and the mixture was stirred at 90 ^oC for 8 h. The dark brown mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 4-chloro-2-methoxy-5-(thiophen-2-yl)benzoate (0.6 g, y. 40%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.80 (s, 3H), 3.90 (s, 3H), 7.16 (dd, J = 4.8, 3.6 Hz, 1H), 7.37 (dd, J = 3.6, 1.2 Hz, 1H), 7.39 (s, 1H), 7.67 (d, J = 4.4 Hz, 1H), 7.85 (s, 1H).

To a solution of methyl 4-chloro-2-methoxy-5-(thiophen-2-yl)benzoate (600 mg, 2.12 mmol) in DMF (20 mL) was added NBS (378 mg, 2.12 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-cold water, the precipitated white

solid was filtered and dried under vacuum to afford methyl 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxybenzoate (500 mg, y. 70%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.80 (s, 3H), 3.90 (s, 3H), 7.22 (d, J = 4.0 Hz, 1H), 7.28 (d, J = 4.0 Hz, 1H), 7.40 (s, 1H), 7.85 (s, 1H). LC-MS: m/z 361.0 (M (Br^{79})+H)$^+$, 363.0 (M (Br^{81})+H)$^+$.

To a solution of methyl 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxybenzoate (500 mg, 1.39 mmol) in THF (5 mL) - MeOH (5 mL) - water (3 mL) was added lithium hydroxide monohydrate (291 mg, 6.94 mmol), and the mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated, the residue was dissolved in water and acidified with *aq.* citric acid until pH = 2, and extracted with EtOAc. The organic extract was dried over Na_2SO_4, filtered, and concentrated to obtain 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxybenzoic acid (**68**, R_2 = OMe, R_3 = Cl) (400 mg, y. 83%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.91 (s, 3H), 7.22 (d, J = 3.6 Hz, 1H), 7.27 (d, J = 4.0 Hz, 1H), 7.36 (s, 1H), 7.82 (s, 1H), 13.00 (brs, 1H). LC-MS: m/z 345.0 (M (Br^{79})-H)$^-$, 347.0 (M (Br^{81})-H)$^-$.

To a mixture of 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxybenzoic acid (**68**, R_2 = OMe, R_3 = Cl) (200 mg, 0.57 mmol) and 3-methoxybenzenesulfonamide (118 mg, 0.64 mmol) in CH_2Cl_2 (20 mL) were added EDCI·HCl (326 mg, 1.71 mmol) and DMAP (208 mg, 1.71 mmol), and the mixture was stirred at room temperature for 8 h. The brown solution was diluted with 1 *N* HCl and extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was suspended in methanol (10 mL), stirred for 5 min, filtered, and dried under vacuum to afford 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxy-*N*-(3-methoxyphenylsulfonyl)benzamide (**69**, R_1 = 3-OMePh, R_2 = OMe, R_3 = Cl) (200 mg, y. 66%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.84 (s, 3H), 3.91 (s, 3H), 7.22 (d, J = 4.0 Hz, 1H), 7.26

(d, J = 4.0 Hz, 1H), 7.29-7.32 (m, 1H), 7.36 (s, 1H), 7.43-7.46 (m, 1H), 7.53-7.60 (m, 3H), 12.20 (brs, 1H); LC-MS: m/z 513.84 (M (Br^{79})-H)$^-$, 515.82 (M (Br^{81})-H)$^-$.

A mixture of 5-(5-bromothiophen-2-yl)-4-chloro-2-methoxy-*N*-(3-methoxyphenylsulfonyl)benzamide (**69**, R_1 = 3-OMePh, R_2 = OMe, R_3 = Cl) (180 mg, 0.35 mmol), propargyl alcohol (23 mg, 0.42 mmol), Cs_2CO_3 (340 mg, 1.04 mmol), and CuI (7 mg, 0.035 mmol) in DMF (10 mL) was purged with argon for 5 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (28 mg, 0.035 mmol), and the mixture was stirred at 90 ^oC for 2 h. The dark brown mixture was cooled to room, diluted with water, and extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by the Prep HPLC (condition E) to give 4-chloro-5-(5-(3-hydroxyprop-1-ynyl)thiophen-2-yl)-2-methoxy-*N*-(3-methoxyphenylsulfonyl)benzamide (**28**) (30 mg, y. 10%). ^1H NMR (DMSO-d_6, 400 MHz) δ 3.84 (s, 3H), 3.88 (s, 3H), 4.33 (brs, 2H), 5.39 (brs, 1H), 7.29-7.34 (m, 3H), 7.37 (s, 1H), 7.46 (s, 1H), 7.54-7.61 (m, 3H), 12.2 (brs, 1H). LC-MS: m/z 491.94 $(M+H)^+$. HRMS (m/z) calcd for $C_{22}H_{18}ClNO_6S_2$ $(M+H)^+$ 492.0337, found 492.0324.

5-(5-(3-Hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methyl-*N*-(quinolin-8-ylsulfonyl)benzamide (29).

To a mixture of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methylbenzoic acid (**68**, R_2 = Me, R_3 = OMe) (340 mg, 1.05 mmol) and quinoline-8-sulfonamide (200 mg, 0.96 mmol) in CH_2Cl_2 (15 mL) were added EDCI·HCl (548 mg, 2.88 mmol) and DMAP (351 mg, 2.88 mmol), and the mixture was stirred at room temperature for 8 h. The brown solution was diluted with 1 *N* HCl and extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was suspended in MeOH (10 mL), stirred for 5 min, filtered, and dried under vacuum to afford 5-(5-bromothiophen-2-yl)-4-methoxy-2-methyl-

N-(quinolin-8-ylsulfonyl)benzamide (**69**, R_1 = 8-quinoline, R_2 = Me, R_3 = OMe) (150 mg, 30%). LC-MS: m/z 517.30 (M (Br^{79})+H)$^+$, 519.30 (M (Br^{81})+H)$^+$.

A mixture of 5-(5-bromothiophen-2-yl)-4-methoxy-2-methyl-*N*-(quinolin-8-ylsulfonyl)benzamide (**69**, R_1 = 8-quinoline, R_2 = Me, R_3 = OMe) (150 mg, 0.29 mmol), propargyl alcohol (32 mg, 0.58 mmol), Cs_2CO_3 (282 mg, 0.87 mmol), and CuI (7 mg, 0.035 mmol) in DMF (10 mL) was purged with argon for 5 min. To this mixture was added $PdCl_2(dppf)\cdot CH_2Cl_2$ (23 mg, 0.029 mmol), and the mixture was stirred at 80 ^oC for 3 h. The reaction mixture was cooled to room temperature and filtered through a pad of celite. The celite pad was washed with EtOAc and the filtrate was concentrated *in vacuo*. The residue was suspended in MeOH (10 mL), stirred for 5 min, filtered, and dried under vacuum to give 5-(5-(3-hydroxyprop-1-yn-1-yl)thiophen-2-yl)-4-methoxy-2-methyl-*N*-(quinolin-8-ylsulfonyl)benzamide (**29**) (20 mg, y. 14%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.08 (s, 3H), 3.92 (s, 3H), 4.35 (d, J = 4.8 Hz, 2H), 5.39 (t, J = 5.2 Hz, 1H), 6.98 (s, 1H), 7.36 (d, J = 4.0 Hz, 1H), 7.60 (d, J = 4.4 Hz, 1H), 7.72 (dd, J = 8.0, 4.4 Hz, 1H), 7.85 (t, J = 8.4 Hz, 1H), 7.94 (s, 1H), 8.38-8.40 (m, 1H), 8.53-8.59 (m, 2H), 9.07 (dd, J = 4.4, 2.0 Hz, 1H), 12.8 (brs, 1H). LC-MS: m/z 493.16 (M+H)$^+$. HRMS (m/z) calcd for $C_{25}H_{20}N_2O_5S_2$ (M+H)$^+$ 493.0886, found 493.0880.

DENV biochemical enzyme Assay.

The assay comprised 100 nM *in vitro* transcribed DENV4 mini-genome viral RNA template (575nt, bearing DENV4 linked 5'-3' UTR sequences), 20 μM ATP, 20 μM GTP, 20 μM UTP, 5 μM ATTO-CTP, and 100 nM of DENV4 full length NS5, in 50 mM Tris/HCl, pH 7.5, 10 mM KCl, 1 mM $MgCl_2$, 0.3 mM $MnCl_2$, 0.001% Triton-X-100 and 10 μM cysteine.[29] Compounds from 0-20 or -100 μM concentrations were two-fold serially diluted into 384-well white opaque

plates (Corning Costar) in a final volume of 5 µL, after which 5 µL of enzyme in 1X assay buffer was added into respective wells. The plate was incubated at room temperature for 15 min, after which 5 µL of RNA and NTP mix were added to the wells to initiate the reactions. The reaction was terminated after 120 min by addition of 10 µL of 2.5X STOP buffer (200 mM NaCl, 25 mM $MgCl_2$, 1.5M DEA, pH 10; Promega) with 25 nM calf intestinal alkaline phosphatase (NEB) and incubated at room temperature for 60 min followed by reading on a Tecan Saffire II microplate reader at $excitation_{max}$ and $emission_{max}$ wavelengths 422 nm and 566 nm respectively. All datapoints were performed in duplicate wells. The IC_{50} values were determined with the nonlinear regression curve fit for sigmoidal dose-response (variable slope) from GraphPad Prism version 3.02 (GraphPad Prism, Inc.).

DENV1-4 infected cell-based assay.

Approximately 7×10^3 A549 cells per well were seeded into 384-well plates in Ham's F-12K medium containing 2 % FBS, 1mM L-glutamine and 1 % penicillin-streptomycin and incubated over-night at 37°C in 5 % CO_2. At 24 h post-seeding, cells were infected with DENV1-4 at multiplicity of infection [MOI] of 0.3-1 (DENV1, strain MY97-10245, MOI = 0.5; DENV2, strain MY97-10340, MOI = 0.3; DENV3 strain MY05-34640, MOI = 1; DENV4, strain MY01-22713; MOI = 0.5) and treated immediately with 10-point, 3-fold serially diluted compounds (at final 0.5% DMSO concentration). At 48 h post-infection, cells were fixed with 4 % paraformaldehye and stained with 4G2 antibody conjugated with Dylight 488 (for DENV envelope protein) and DRAQ5 (for nuclear DNA). Quantifications of DENV envelope protein were determined using the Opera high content imaging system and analysis software (Perkin Elmer, USA). Calculation of EC_{50} values were performed using Helios software package (Novartis, Basel).

Surface plasmon resonance (SPR) analysis.

Biotinylated DENV-3 and -4 RdRp were captured on separate flow cells in 50 mM Tris pH 7.5, 200 mM NaCl, 2 mM DTT, 0.05% Tween 20, and 3% DMSO at 4°C. Flow cell 1 was left blank. Compounds were tested in a 7-point 2-fold serial dilution, typically from 200 μM and a zero-concentration sample was subtracted from each run. Compounds were injected at a flow rate of 30 μL/min, with 45 s contact time and 60-600 s dissociation, starting from the DMSO control and finishing with the highest concentration. The experiments were performed using a Biacore T200 instrument and the data were analyzed using Biacore T200 Evaluation software, version 2.0.

Isothermal titration calorimetry (ITC) analysis.

DENV-4 RdRp (residues 274-900) in 20 mM HEPES pH 7.0, 300 mM NaCl, and 2 mM TCEP was placed in the sample cell of an iTC200 (GE healthcare), at 30-50 μM. The ligand was diluted from a 50 mM stock in DMSO into the same buffer (typically at 2.5 mM) and the same amount of DMSO was added to the protein sample. Experiments were performed at 25°C with a reference power of 10. An initial injection of 0.4 μl was followed by 15 2.6-μl injections. The heat of dilution for the compound was measured by injecting the compound at the same concentration into buffer and this was subtracted from the titration into protein sample. The injection peaks were integrated using Origin and fitted to a 1:1 binding isotherm.

ASSOCIATED CONTENT

Supporting Information

Crystallographic protocols and information for cocrystals of RdRp with **4**, **5**, **10**, **15** and **23**. Copies of ^{1}H and ^{13}C NMRs of **27** and **29**. IC_{50} values and their standard deviation for

compounds **3**-**29**. EC_{50} and CC_{50} values and their standard deviation for compounds **23** and **25**-**29**. Multiple alignment of amino acid residues of compound binding pocket. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The coordinates and structure factor files have been deposited in the Protein Data Bank under the accession codes 5HN0 (**4**), 5HMW (**5**), 5HMX (**10**), 5HMY (**15**), and 5HMZ (**23**).

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ACKNOWLEDGMENTS

The authors would like to thank the following colleagues from Novartis who participated in generating the data presented in this paper: Cheah Chen Seh, David Beer, Ghislain Bonamy, Katherine Chan, Chin Chin Lim, Hao Ying Xu, Lee Boon Heng, Wan Kah Fei, Ivan Low, Peck Gee Seah, and Prakash Vachaspati.

The authors would like to thank Drs. Ashok Kumal Jha and Joseph Shambabu Maddirala and their colleagues in GVK Biosciences for their technical assistance.

ABBREVIATIONS USED

AIBN azobisisobutyronitrile; CDI 1,1'-carbonyldiimidazole; 18-crown-6 1,4,7,10,13,16-hexaoxacyclooctadecane; DBU 1,8-diazabicyclo[5.4.0]undec-7-ene; DENV dengue virus; DHF dengue hemorrhagic fever; DSS dengue shock syndrome; DIBAL-H diisobutylaluminium hydride; DMAP 4-dimethylaminopyridine; DMF *N*,*N*-dimethylformamide; DMSO dimethyl sulfoxide; EDCI·HCl 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; $PdCl_2(dppf)\cdot CH_2Cl_2$ [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with

dichloromethane; HATU 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HOBt 1-hydroxybenzotriazole; HTS throughput screening; ITC Isothermal titration calorimetry; NBS *N*-bromosuccinimide; NS5 nonstructural protein 5; RdRp RNA-dependent RNA polymerase; SAR structure-activity relationship; SPR Surface plasmon resonance.

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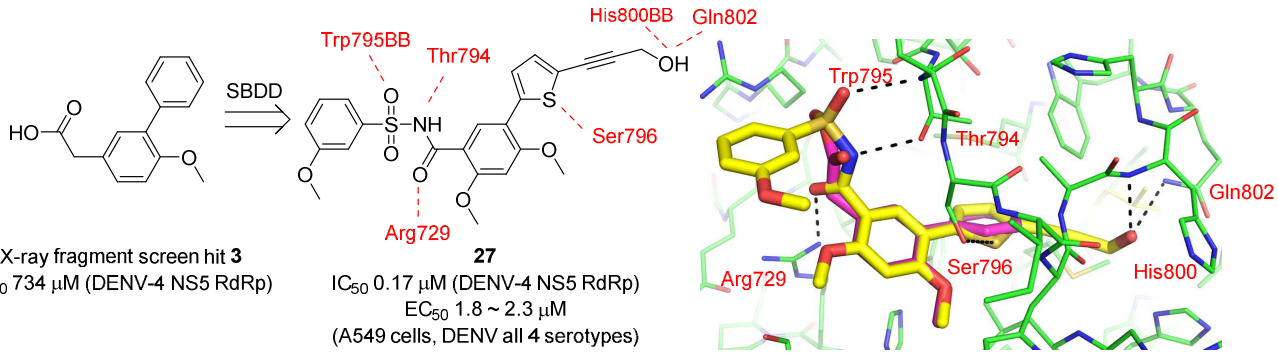

X-ray fragment screen hit **3**
IC_{50} 734 μM (DENV-4 NS5 RdRp)

27
IC_{50} 0.17 μM (DENV-4 NS5 RdRp)
EC_{50} 1.8 ~ 2.3 μM
(A549 cells, DENV all 4 serotypes)