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Discovery of Potent Protease-Activated Receptor 4 Antagonists with *in vivo* Antithrombotic Efficacy

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ABSTRACT. In an effort to identify novel antithrombotics, we have investigated PAR4 antagonism by developing and evaluating a tool compound, **UDM-001651**, in a monkey thrombosis model. Beginning with a high-throughput screening hit, we identified an imidazothiadiazole-based PAR4 antagonist chemotype. Detailed structure-activity relationship (SAR) studies enabled optimization to a potent, selective, and orally bioavailable PAR4 antagonist, **UDM-001651**. **UDM-001651** was evaluated in a monkey thrombosis model and shown to have robust antithrombotic efficacy and no prolongation of kidney bleeding time. This combination of excellent efficacy and safety margin strongly validates PAR4 antagonism as a promising antithrombotic mechanism.

Introduction

Atherothrombotic cardiovascular and cerebrovascular diseases are the leading cause of morbidity and mortality in developed countries and claim more lives each year than cancer in the United States.[1] Atherothrombotic diseases, such as acute coronary syndrome and stroke, originate with a gradual build-up of plaque in the arterial wall. The eventual rupture of an atherosclerotic plaque exposes a prothrombotic milieu to the blood and activates platelets at the site of the injury, leading to the formation of a potentially occlusive thrombus and subsequent ischemia. Antiplatelet agents, such as aspirin and clopidogrel, inhibit thrombus formation and significantly reduce ischemic events; thus, they are cornerstone therapies for the medical management of atherothrombotic diseases.[2] Recently, more potent and efficacious platelet $P2Y_{12}$ receptor antagonists, such as prasugrel and ticagrelor, have been studied extensively and found to be associated with increased bleeding risks, limiting their utility to improve clinical outcomes.[3-7] Significant unmet medical need remains and novel antiplatelet agents with better efficacy and reduced bleeding risk are an important research goal.

Thrombin, the central enzyme in the coagulation cascade, activates platelets with subnanomolar potency. It binds to two G-protein coupled receptors on human platelets,[8, 9] the protease-activated receptor 1 (PAR1) and protease-activated receptor 4 (PAR4),[10] and cleaves at a specific site within the N-terminal sequence of each receptor. The newly exposed, shortened N-termini serve as tethered ligands that activate their respective receptors through intramolecular interactions. Synthetic peptide analogs based on the thrombin cleaved N-terminal sequence can also activate the corresponding receptors in the absence of proteolytic activation.[11, 12] Targeting PAR1 or PAR4 with an antagonist is expected to reduce platelet activation without inhibiting the other coagulation functions of thrombin.

PAR1 has generated widespread interest as an antithrombotic target, as evidenced by pre-clinical research activity and the development of two PAR1 antagonist clinical candidates, vorapaxar (**1**) and atopaxar (**2**), Figure 1. Phase III clinical studies of vorapaxar in combination with aspirin and clopidogrel demonstrated incremental reduction of ischemic events at the price of a significant increase in intracranial

bleeding.[13, 14] In 2014, the U.S. Food and Drug Administration approved use of vorapaxar to reduce thrombotic cardiovascular events in patients with prior myocardial infarction or peripheral arterial disease. The drug is contraindicated for patients with a history of stroke, transient ischemic attack, or intracranial hemorrhage because of bleeding risk.[15] Atopaxar has completed phase II clinical trials in acute coronary syndromes and coronary artery disease, and demonstrated reductions in ischemia and numerical increases in major bleeding. Liver enzyme elevations and QT interval prolongation were also noted in atopaxar treated patients.[16, 17] Further development of atopaxar has not been reported and is presumed to have been discontinued.

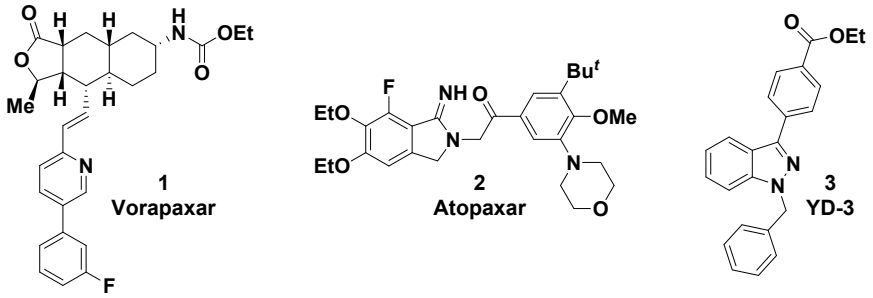


Figure 1. Chemical structures of PAR1 and PAR4 antagonists

It is hypothesized that improved separation between effects on thrombosis and hemostasis may be achieved by selectively targeting the late stage of thrombus formation and vessel occlusion.[18, 19] PAR1 is proteolytically activated at low concentrations of thrombin and induces a rapid and transient calcium signal, whereas PAR4 is cleaved at relatively high concentrations of thrombin and induces a sustained calcium signal with a slower onset.[20-22] Based on these signaling differences, it appears that PAR1 is important for triggering platelet activation, while PAR4 is required for sustaining platelet activation. Thus, inhibiting PAR4, while maintaining thrombin signaling through PAR1, could potentially inhibit occlusive thrombosis while preserving hemostasis. Selective PAR4 antagonism may comprise a novel antiplatelet mechanism with a lower risk of bleeding than existing antiplatelet therapies. We have recently disclosed preclinical studies[23, 24] that demonstrate an outstanding efficacy and bleeding profile for BMS-986120, a PAR4 antagonist clinical candidate. The early stages of the medicinal chemistry effort that led to BMS-986120 are described in this manuscript. Further optimization culminating in BMS-986120 will be reported subsequently.

In contrast to the vigorous investigation of PAR1 as an antithrombotic target, there were very few reported PAR4 antagonists at the outset of this work. Only a single chemical class of indazoles, typified by the compound **YD-3** (**3**, Figure 1) had been reported.[25-27] **YD-3** was shown to possess modest activity as judged by the inhibition observed in a GYPGKF-induced platelet aggregation assay (IC_{50} 130 nM).[26, 28] Recent work on the **YD-3** lead led to a nitroindole, **ML354**, with 70 nM IC_{50} in a GYPGKF-induced platelet aggregation assay, low *in vitro* metabolic stability, and 70-fold selectivity vs. PAR1.[29,30] Further optimization of this indole chemotype has not yet led to compounds with activity against platelet aggregation in whole blood or *in vivo* antithrombotic efficacy.[31,32]

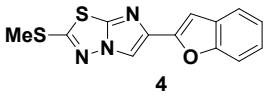
In this report, we describe the identification and optimization of PAR4 antagonists with potent activity in platelet rich plasma from an imidazothiadiazole high-throughput screening lead. We also demonstrate *in vivo* antithrombotic efficacy and low bleeding liability in a cynomolgous (cyno) monkey electrolytic carotid artery thrombosis (ECAT)

model, confirming the therapeutic potential of PAR4 antagonism as a novel antiplatelet approach.

Results and Discussion

Like the rest of the PAR family, PAR4 couples to the G protein Gq, which upon receptor activation induces intracellular signaling events resulting in an increase in cytosolic calcium.[10] To screen for molecules possessing PAR4 antagonist activity, a calcium mobilization assay (PAR4 fluorescent imaging plate reader (FLIPR)) was developed using a HEK293-PAR4 cell line, an aequorin reporter,[33] and a PAR4 agonist peptide AYPGKF.[11] YD-3 showed inhibitory activity in this assay (PAR4 FLIPR IC_{50} 410 nM). From a screening campaign of the BMS compound collection, 199 compounds were identified which inhibited PAR4 agonist peptide-induced calcium signaling and did not elicit agonist activity when tested in the absence of an added PAR4 agonist. The PAR4-dependent antiplatelet effects of these compounds were then evaluated in a γ-thrombin-induced washed platelet aggregation assay (γ-Thr WP). γ-Thrombin is a proteolytic fragment of thrombin which specifically activates PAR4.[34] The imidazo[2,1-

b][1,3,4]-thiadiazole(IDT)-based compound **4** (Figure 2) was one of the most potent hits identified by the high throughput screen (HTS) (PAR4 FLIPR IC_{50} 17 nM, γ-Thr WP IC_{50} 500 nM).



4

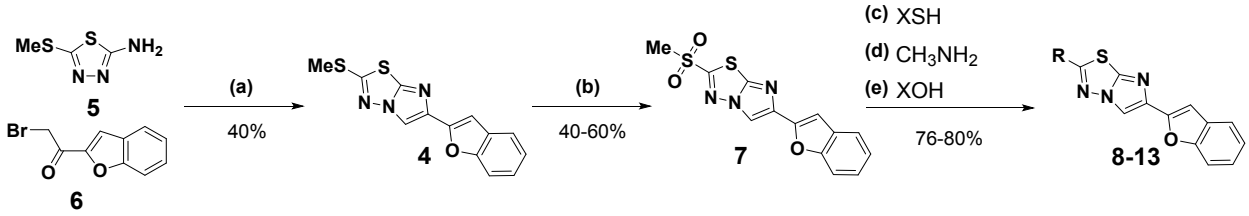
Figure 2. Chemical structure of the PAR4 antagonist HTS hit

Lead profiling of **4** demonstrated poor *in vitro* liver microsomal stability across species, and no significant activity in CYP, hERG, or PXR assays. Biotransformation studies indicated that the methylthio group was oxidized to the sulfoxide and sulfone. Both metabolites, but not the parent methyl thioether, react rapidly with glutathione and *N*-acetylcysteine. In addition, oxidative metabolites of the benzofuran ring were detected. Early SAR exploration was directed at improving potency in the PAR4 FLIPR and platelet aggregation assays and improving metabolic stability.

Initial SAR optimization of the IDT scaffold was conducted using the synthetic approach shown in Scheme 1. IDT **4** was obtained by condensation of 5-(methylthio)-

1,3,4-thiadiazol-2-amine **5** with bromoketone **6**. Activation of the thiomethyl group with mCPBA gave sulfone **7**, which was converted to a series of methylthio replacements upon treatment with various nucleophiles (thiols, amines, alcohols) in the presence of a base. The regiochemistry of the cycloaddition was verified by an x-ray crystal structure of compound **4** (supplementary material).

Scheme 1. General synthetic route for imidazothiadiazoles with 2-position modifications

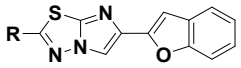


Reagents and conditions: (a) EtOH, μwave: 150 °C, 5 min; (b) *m*-CPBA, THF; (c) XSH, MeOH, 70 °C; (d) CH_3NH_2, DMF, μwave: 70 °C, 10 min (e) NaOMe, MeOH, rt or XOH, K_2CO_3, DMF, rt.

Table 1 summarizes SAR for the 2-position of the IDT scaffold generated by this synthetic approach. Incorporation of increased polarity as in sulfone **7** or steric bulk as in the ethylthio and phenylthio analogs **8** and **9**, respectively, decreased potency by 74- to 260-fold, relative to the parent methylthio compound **4.** Importantly, the methoxy

analog **10** retained the potent activity of **4** in both the PAR4 FLIPR and γ-Thr WP assays, and possessed improved human liver microsome metabolic stability based on percent of parent remaining at 10 min. Follow up in a five timepoint kinetic assay, however, showed no improvement in human liver microsome $t_{1/2}$ (compound **4**, $t_{1/2}$ = 5.4 min vs compound **10**, $t_{1/2}$ = 6.9 min). As observed for the thioethers, larger ether substituents had successively lower potency (**10** vs. **11** and **12**). The methylamine analog **13** also had modest activity, especially in the platelet aggregation assay, suggesting that amines are not favored.

Table 1. Imidazothiadiazole 2-position structure-activity relationships



Cpd	R	PAR4 FLIPR IC_{50} (nM)[a]	γ-Thr WP IC_{50} (nM)[a]	Liver microsome stability (% remaining, H, R)
4	MeS	11	270	25, 4
7	$MeSO_2$	820	>30000	ND
8	EtS	2900	ND	45, 35
9	PhS	1900	ND	ND

10	MeO	4.0	240	80, 31
11	EtO	67	2100	57, 37
12	*i*-PrO	760	>30000	ND
13	MeNH	110	>30000	65, 2
14	Me	37	>30000	20, 4
15	Et	38	2400	30, 3
16	*c*-Pr	530	>30000	62, 53

[a] Reported IC_{50} values are typically the mean of at least 2 experiments. ND = not determined

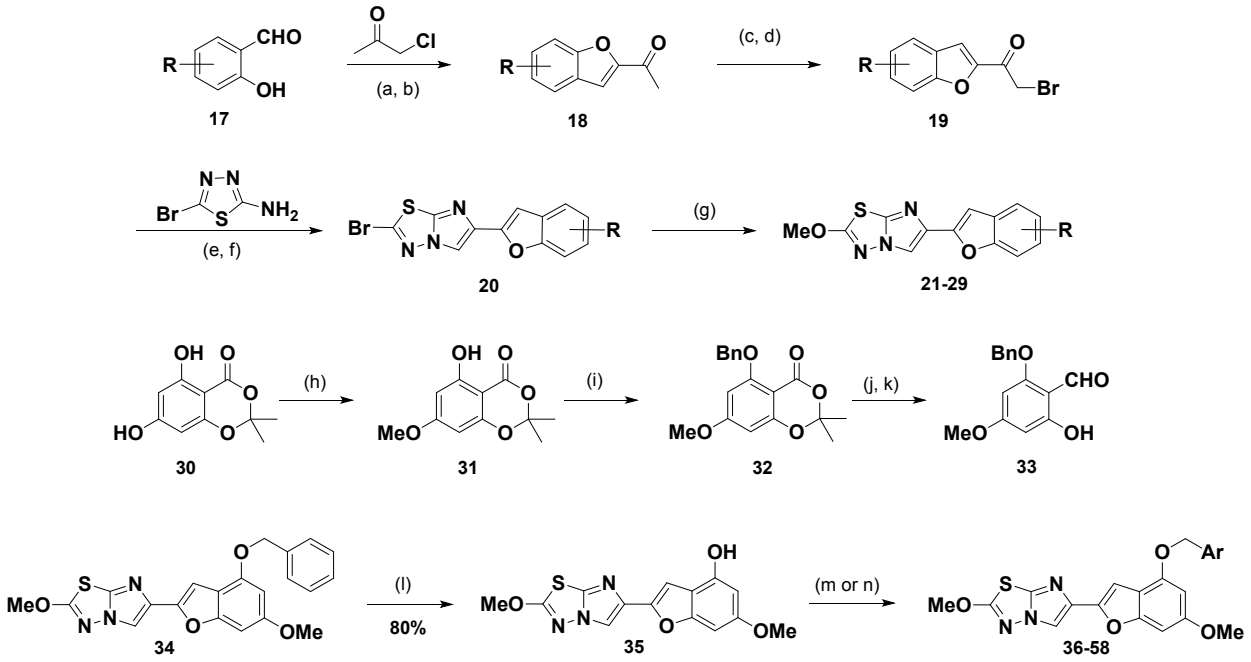
Results from the exploration of carbon-based replacements for sulfur are also shown in Table 1.[23] Methyl analog **14** shows at least 3-fold reduced potency relative to **4** and **10**. Increasing the size of the alkyl group to ethyl (compound **15**) maintained potency, with continued poor metabolic stability, while the cyclopropyl analog **16** lost 14-fold potency. A detailed biotransformation analysis on **15** identified the ethyl group as the principal site of metabolism. While metabolic stability can be improved with substitution on the ethyl moiety, both the putative hydroxy metabolites and bulkier alkyl alternatives were found to possess weak PAR4 inhibitory activity.

In order to improve potency by gaining additional binding interactions and to modulate the benzofuran metabolism, we next explored substitution on the benzofuran ring. Analogs with benzofuran substitution were synthesized according to Scheme 2 and are shown in Table 2. The substituted benzofurans were obtained by condensing appropriately substituted 2-hydroxy-benzaldehydes **17** with chloroacetone, followed by bromination of the resulting 2-acetylbenzofurans **18**. Reaction of 2-amino-5-bromo-1,3,4-thiadiazole with the resulting α-bromomethylketones **19** provided the bromo-IDT derivatives **20**. Displacement of the bromide with sodium methoxide gave the methoxy-IDT derivatives **21-29**.

To prepare benzofuran analogs bearing alkoxy substituents at the 4- and 6-positions, acetonide **30** was regioselectively derivatized at the *p*-phenol with the desired alcohol using a Mitsunobu reaction as described by Kamisuki, et al.[35] Subsequent alkylation at the remaining phenol with an appropriate bromide provided the bis-alkoxy intermediate **32**, which was reduced with DIBAL and hydrolyzed to afford the desired 2-hydroxy-4,6-dialkoxy-benzaldehydes **33**. These benzaldehydes were then reacted as shown for

intermediate **17** to give analogs **34-38**. Alternatively, analogs **39-58** were prepared from analog **34** after benzyl deprotection and alkylation or Mitsunobu reaction of the resulting phenol **35.** In the benzyl deprotection step, the use of BCl_3 with pentamethylbenzene as cation scavenger was crucial for high yields.[36]

Scheme 2. General synthetic route for the preparation of benzofuranyl imidazothiadiazoles with benzofuran modifications.



Reagents and conditions: (a) Cs_2CO_3, DMF, 22 °C, 18 h; (b) *p*-TsOH, 0.1 eq, THF, rt, 1 h; (c) LiHMDS, TMSCl, THF, -78 °C; (d) NBS, -25 to 0 °C, 1.5 h; (e) IPA, 88 °C, 19 h; (f) 150 °C, 30 min, μwave; (g) NaOMe, MeOH, DCM, rt, 1 h; (h) DIAD, Ph_3P, MeOH, THF, rt; (i) BnBr, K_2CO_3, DMF (j) DIBAL, -78 °C, CH_2Cl_2 (k) MeOH, HCl; (l) BCl_3,

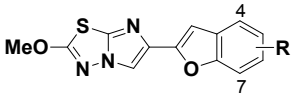
pentamethylbenzene, -78 °C, DCM; (m) $ArCH_2Br$, K_2CO_3, DMF, rt; (n) $ArCH_2OH$, Bu_3P, ADDP, THF, rt

As the potency of our PAR4 antagonists improved, we began to measure antiplatelet activity with a γ-thrombin-induced platelet-rich plasma aggregation assay (γ-Thr PRP), enabling evaluation of antiplatelet effects in the presence of plasma proteins. As shown in Table 2, addition of methoxy substituents at the 4-, 6-, and 7-positions of the benzofuran ring maintains single digit nanomolar potency in the PAR4 FLIPR assay, and importantly, improves inhibitory potency 4- to 30-fold in the γ-Thr PRP assay (compounds **21, 24,** and **26**). However, a methoxy group is not tolerated at the 5-position (compound **23**). Increasing the size of the substitution, as with benzyloxy groups, was detrimental to potency but beneficial for improving microsomal stability (compounds **22** and **25**).

Exploration of several di-substitution patterns (compounds **27-29** and **34**) showed that 4,6-disubstitution provided the best antagonist activity. The 4,6-dimethoxy and 4-benzyloxy-6-methoxy analogs **29** and **34** were noteworthy in that they possess low

micromolar γ-Thr PRP activity with significantly improved metabolic stability relative to unsubstituted **10**.

Table 2. Benzofuran substituent structure-activity relationships



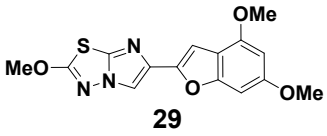
Cpd	R	PAR4 FLIPR IC_{50} (nM)[a]	γ-Thr WP IC_{50} (nM)[a]	γ-Thr PRP IC_{50} (nM)[a]	Liver microsome stability (% remaining, H, R)
10	H	4.0	240	15000	80, 31
21	4-OMe	7.6	350	1300	40, 42
22	4-OBn	24	3100	4100	85, 75
23	5-OMe	220	>30000	>30000	30, 38
24	6-OMe	1.2	120	480	57, 55
25	6-OBn	26	1800	>30000	94, 68
26	7-OMe	2.8	240	3500	25, 11
27	6-OMe,7-OMe	1800	>30000	>30000	20, 3
28	4-OMe,7-OMe	11	2100	10000	55, 28

29	4-OMe,6-OMe	0.99	74	530	86, 70
34	4-OBn, 6-OMe	4.2	1000	1600	100, 77

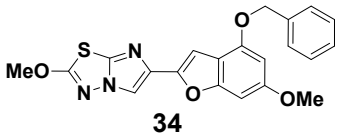
[a] Reported IC_{50} values are typically the mean of at least 2 experiments.

In light of their combination of anti-platelet potency and microsomal stability, the pharmacokinetic profiles of compounds **29** and **34** were assessed in rat as summarized in Table 3. The 4,6-dimethoxy analog **29** demonstrated high clearance and poor oral bioavailability. Compound **34**, bearing a bulky benzyloxy substituent at the benzofuran 4-position, showed a promising pharmacokinetic profile with low clearance, long half-life, and appreciable bioavailability.

Table 3. Metabolic stability and pharmacokinetic data[a] for compounds **29** and **34**



29



34

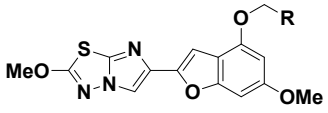
Compound	**29**	**34**
liver microsome $t_{1/2}$, min (H, R)	17, 22	>120, 40
Rat CL, mL/min/kg	33	3.7
$t_{1/2}$, h	3.2	12

F, %	1	27

[a] iv dose 0.5 mpk, iv vehicle 25/75 propylene glycol/PEG400 at 2 ml/kg, 10 min infusion; po dose 2 mpk, po vehicle 5/5/90 EtOH/TPGS/PEG300 at 5 mL/kg

With a promising combination of γ-Thr PRP potency and favorable pharmacokinetic properties established for compound **34**, a series of extended aryl analogs were prepared in an attempt to further drive potency (Table 4, compounds **36-38**). Although the γ-Thr PRP potency improved up to 3-fold, metabolic stability dropped significantly in the extended aryl analogs, focusing further investigation on compounds that retained the 4-benzyloxy group of compound **34**.

Table 4. Structure activity relationships at the benzofuran 4-position



Cpd	R	PAR4 FLIPR IC_{50} (nM)[a]	γ-Thr PRP IC_{50} (nM)[a]	Liver microsome stability (% remaining, H, R)
34	-Ph	4.2	1600	100, 77
36	$-CH_2Ph$	1.1	780	89, 43

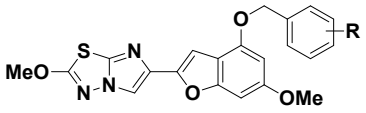
37	-CH_2OPh	0.81	480	65, 36
38	-CH_2OCH_2Ph	1.8	500	75, 25

[a] Reported IC_{50} values are typically the mean of at least 2 experiments.

With the objective of improving potency and maintaining pharmacokinetic properties, we investigated substitution on the benzyloxy moiety of compound **34**. Table 5 summarizes the biological activity of analogs bearing small substituents, which were synthesized according to the second synthetic sequence described in Scheme 2, from key intermediate **35**. Addition of substituents at the ortho position of the 4-benzyloxy substituent in compound **34** did not improve γ-Thr PRP potency (compounds **39-42**), despite a slight gain in PAR4 FLIPR activity for the methyl and chloro analogs **39** and **40**. Similar results were observed with substitution at the para position (compounds **49-51**). In contrast, introduction of substituents at the meta position generated more promising results (compounds **43-48**). Two analogs bearing lipophilic substituents displayed submicromolar potency in the γ-Thr PRP assay along with good metabolic stability (compounds **43** and **48**), while more polar analogs bearing a hydroxyl group (**45**

and **46**) were less active. The cyano analog **47** was slightly more potent than the unsubstituted analog **34** in the same assay, while the methoxy analog **44** lost potency. It is noteworthy that in most of these mono-substituted analogs, stability in human and rat liver microsomes was similar to parent compound **34**.

Table 5. Structure-activity relationships for 4-benzyloxy analogs



Cpd	R	PAR4 FLIPR IC_{50} (nM)[a]	γ-Thr PRP IC_{50} (nM)[a]	Liver microsome stability (% remaining, H, R)
34	H	4.2	1600	100, 77
39	2-Me	1.6	3600	90, 46
40	2-Cl	2.3	> 3000	95, 78
41	2-OMe	19	>1000	82, 28
42	2-CF_3	3.4	>1000	ND
43	3-Cl	3.3	680	82, 79
44	3-OMe	9.4	> 1000	69, 34
45	3-OH	3.1	2700	97, 49
46	3-CH_2OH	27	> 1000	ND
47	3-CN	0.98	820	100, ND
48	3-CF_3	3.1	440	93, 79

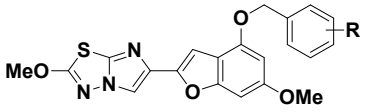
49	4-CF_3	4.3	1800	79, 90
50	4-Cl	11	> 1000	ND
51	4-OMe	1.6	> 3000	73, 64

[a] Reported IC_{50} values are typically the mean of at least 2 experiments. ND = not determined

In light of the enhanced γ-Thr PRP activity for certain analogs with meta substituents on the 4-benzyloxy moiety, a more thorough investigation of structure-activity relationships at this position was undertaken. The synthesis of these analogs followed the second synthetic sequence described in Scheme 2. The results for key analogs are summarized in Table 6. The addition of a phenyl ring attached with various linkers at the meta position of the benzyloxy side chain was found to be beneficial. The PAR4 FLIPR activity of these compounds remained in the low nanomolar range and the nature of the linker was found to affect inhibition of platelet aggregation in plasma. Single atom linkers, as in the aryl ether and thioether analogs **52** and **53**, show 4-fold improved γ-Thr PRP potency relative to **34**. Most notably, however, extension to a benzyloxy group (compound **54, UDM-001651**) enhanced the antiplatelet potency by 64-fold as

compared to the unsubstituted analog **34**, with a γ-Thr PRP IC_{50} of 25 nM. Alternative two atom linkers, such as the reverse ether **55** or ethylene **56** significantly impaired the γ-Thr PRP activity relative to **UDM-001651**, indicating that both linker conformation and polarity is important for antiplatelet activity. Lastly, the ortho and para benzyloxy regioisomers **57** and **58** showed similar potency to parent compound **34.**

Table 6. Structure-activity relationships for 4-benzyloxy analogs with aryl substituents



Cpd	R	PAR4 FLIPR IC_{50} (nM)[a]	γ-Thr PRP IC_{50} (nM)[a]	Liver microsome stability (% remaining, H, R)
34	H	4.2	1600	100, 77
52	3-OPh	1.2	390	86, 42
53	3-SPh	0.89	410	80, 45
54	3-OCH_2Ph	2.4	25	85, 76
55	3-CH_2OPh	3.2	600	86, 64
56	3-$(CH_2)_2$Ph	2.4	1200	99, 68
57	2-OCH_2Ph	3.2	2500	94, 22
58	4-OCH_2Ph	1.3	550	88, 84

[a] Reported IC_{50} values are typically the mean of at least 2 experiments.

Given its excellent inhibitory potency in platelet-rich plasma, **UDM-001651** was further characterized both *in vitro* and *in vivo*. Direct binding to PAR4 expressed on HEK293 cell membranes was studied using a previously described binding assay (Table 7).[24] The data demonstrate that UDM-001651 has single digit nanomolar affinity for PAR4 using both saturation binding and kinetic assays, with rapid and reversible binding kinetics.

Table 7. Binding parameters for UDM-001651 to PAR4 [a]

Saturation binding	K_d (nM): 1.4 ± 0.4
Association kinetics	K_d (nM): 3.0 ± 0.3
	K_{on} (nM^{-1} min^{-1}): 0.004 ± 0.001
	K_{off} (min^{-1}): 0.013 ± 0.001

[a] Reported values are means ± standard deviation, *n* = 3.

In vitro profiling data are summarized in Table 8. **UDM-001651** was found to have excellent *in vitro* stability in human, rat, mouse, dog, and monkey liver microsomes and did not have any CYP, PXR, cardiac ion channel, or cytotoxicity issues. The compound

showed very high protein binding across species. The selectivity of **UDM-001651** was tested using a variety of platelet agonists in a human platelet aggregation assay (Table 9). **UDM-001651** showed potent inhibition of platelet aggregation triggered by a PAR4 agonist peptide, but no measurable inhibition of the effects of a PAR1 agonist peptide, ADP, collagen, or the thromboxane A2 receptor agonist U46619 on platelets. Taken together, these *in vitro* data indicate **UDM-001651** has excellent properties as a tool compound for mechanistic investigations of PAR4 inhibition. Pharmacokinetic data for **UDM-001651** are summarized in Table 10. The compound shows low clearance, 6-7 h half-life, and modest bioavailability in rat, dog, and monkey. Importantly, the oral exposure was sufficient to enable *in vivo* efficacy studies in our monkey ECAT model.[37]

Table 8. *In vitro* profiling assay data for **UDM-001651**

LM $t_{1/2}$, min (H, R, M, D, Mk)	86, 20, 120, 100, 48
HLM CYP IC_{50}, μM	all > 40
PXR EC_{50}, μM	> 50
cytotoxicity TC_{50}, μM	> 50
hERG, Na patch clamp % inh. @ 10 μM	≤ 10
Protein binding, % bound (H, R, M, D Mk)	99.6, 99.8, 99.5, ≥ 99.8, >

Table 9. Inhibition of human platelet aggregation by **UDM-001651**

platelet agonist	platelet aggregation IC_{50} (nM) [a]
PAR4 agonist peptide	8
PAR1 agonist peptide	> 30,000
ADP	> 30,000
collagen	> 30,000
U46619	> 30,000

[a] Reported IC_{50} values are typically the mean of at least 2 experiments.

Table 10. Pharmacokinetic data for **UDM-001651**

species	rat [a]	dog [b]	monkey [b]
CL, mL/min/kg	10	1.6	5.2
$t_{1/2}$, h	6.9	7.0	6.2
F, %	11	26	11

[a] iv dose 0.5 mpk, iv vehicle 25/75 propylene glycol/PEG400 at 2 ml/kg, 10 min infusion; po dose 2 mpk, po vehicle 5/5/90 EtOH/TPGS/PEG300 at 5 mL/kg

[b] iv dose 0.5 mpk, iv vehicle PEG400 at 0.8 ml/kg, 10 min infusion; po dose 2 mpk, po vehicle 40/60 TPGS/PEG400 at 0.5 mL/kg

In the monkey ECAT model, **UDM-001651** was dosed orally at 0.4, 2, and 10 mg/kg (n = 7 per dose) while the vehicle (40/60 TPGS/PEG400) was dosed orally at 2.5 ml/kg (n = 8). As shown in Figure 3, **UDM-001651** demonstrated dose-dependent antithrombotic

efficacy, achieving maximal thrombus weight reduction of 38% at 2 and 10 mg/kg. Furthermore, the compound did not increase monkey kidney bleeding time even at the highest dose (Figure 4, P = 0.2, 10 mg/kg versus vehicle). Overall, UDM-001651 showed an effective antithrombotic profile with no prolongation of bleeding time, demonstrating that PAR4 inhibition is an attractive approach to antithrombotic therapy.

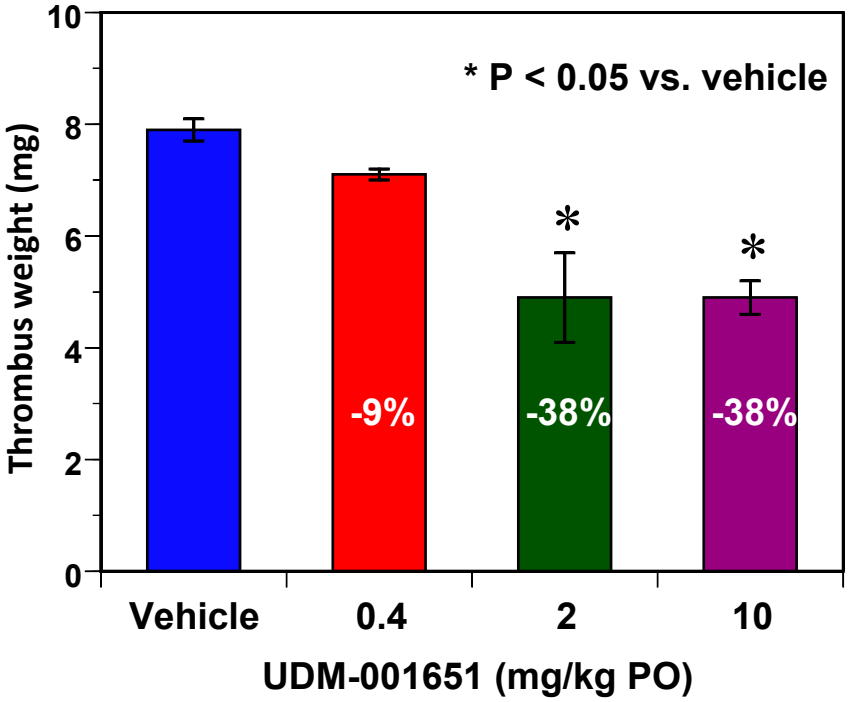


Figure 3. Antithrombotic efficacy of UDM-001651 in the monkey ECAT model

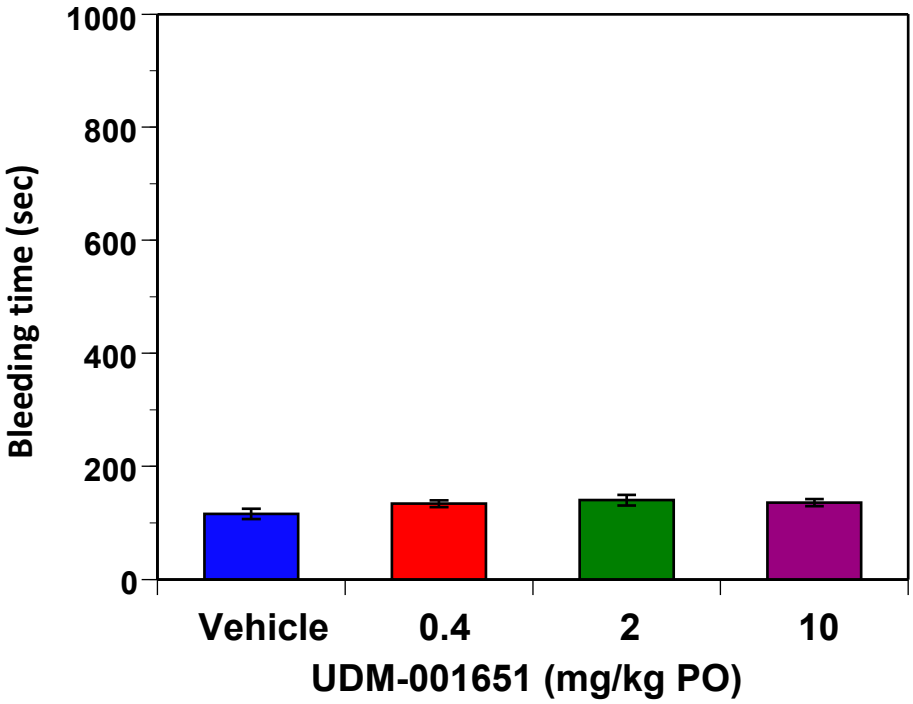


Figure 4. Profile of **UDM-001651** in the monkey kidney bleeding time model

Conclusion

In an effort to advance antithrombotic therapy, we have explored PAR4 antagonism as a novel antiplatelet mechanism, and developed a tool compound, **UDM-001651**, suitable for *in vivo* target validation. We identified an IDT-based chemotype from an HTS designed to detect PAR4 antagonism, with compound **4** as the initial hit. Detailed SAR investigations around **4** led to the discovery of **UDM-001651**, a lead compound with nanomolar antagonist potency against PAR4 in platelet rich plasma, excellent

selectivity, and oral bioavailability. Evaluation of **UDM-001651** in the monkey ECAT model revealed robust antithrombotic efficacy and no prolongation of bleeding time, demonstrating an excellent safety margin for PAR4 antagonism. These results validated PAR4 as an antithrombotic target for our drug discovery effort, and strongly supported further investigation of this mechanism towards the discovery of PAR4 antagonist clinical candidates.

Experimental

HTS protocol. Screening of the BMS compound collection for PAR4 antagonists was accomplished via aequorin-based detection of agonist induced calcium mobilization.[38] The synthetic peptide AYPGKF, corresponding to the activation sequence derived from native *N*-terminus cleavage of the PAR4 receptor, served as the agonist in the assay. Following confirmation of primary hits, potency of leads was confirmed in a concentration response manner against the PAR4 receptor. Compounds were further evaluated in calcium signaling assays to rule out PAR1 antagonist activity,

using a selective PAR1 agonist peptide, SFFLRR,[39] and HEK293 cells endogenously expressing PAR1 receptors.

Synthesis of compounds **4, 7-16, 21-29, 34-58**. Detailed synthetic procedures and analytical data are provided in the supporting information. All compounds with biological data have shown ≥ 95% purity as determined by analytical HPLC, with the exception of compounds **25** (92% purity), **41** (94% purity), **42** (89% purity) and **44** (85% purity).

FLIPR Assay in PAR4-Expressing HEK293 Cells. The activity of the PAR4 antagonists was tested in HEK293 cells expressing human PAR4 (HEK293-PAR4) by monitoring PAR4 agonist peptide (AYPGKF- or H-Ala-Phe(4-F)-Pro-Gly-Trp-Leu-Val-Lys-Asn-Gly-NH_2)-induced intracellular calcium mobilization. Briefly, HEK293 Aequorin cells that stably express the full length human PAR4 (PAR4 Clone 1.2A, Arctic ID 383940) were utilized for this assay. In addition to PAR4, these cells express endogenous PAR1 and can elicit calcium signalling upon stimulation with a selective PAR1 AP. Therefore, the same cells were also used to determine selectivity against PAR1 and agonist activity for both receptors. HEK293 Aequorin PAR4 Clone 1.2A cells

were grown in DMEM (Life Technology Catalog #10564-011) containing 10% heat-inactivated FBS, 1% Penicillin-Streptomycin, 10 μg/mL blasticidin and 100 μg/mL Zeocin at 37°C with 5% CO_2. Cells were plated overnight prior to the experiment in a black 384-well clear bottom plate at 10,000 cells /well in 30 μL growth medium and incubated in a humidified chamber at 37°C with 5% CO_2 overnight. Prior to compound addition, cell medium was replaced with 40 μL of 1X calcium and magnesium-containing Hank's Buffered Saline Solution (HBSS) (with 20 mM HEPES) and 1:1000 diluted fluorescent calcium indicator (Codex Biosolutions Catalog #CB080500-111). After a 30 min incubation period at 37°C and a further 30 min incubation and equilibration period at RT, 20 μL test compound was added at various concentrations at 0.17% DMSO final concentration. Changes in fluorescence intensity were measured using a Functional Drug Screening System (FDSS, Hamamatsu Photonics, Japan) to determine agonist activities. The cells were then incubated for 30 min at RT followed by addition of 20 μL of agonist peptide for antagonist activity measurement using an FDSS. The PAR4 AP and PAR1 AP (SFFLRR6) were routinely tested to ensure a proper response at the EC_{50} value in the assay.

Washed Platelet Aggregation Assays. (a) *Preparation of washed platelets (WP).* Human blood was collected in ACD (85 mM tri-sodium citrate, 78 mM citric acid, 110 mM D-glucose, pH 4.4) at a ratio of 1.4 ml per 10 ml blood. PRP was isolated by centrifugation at 170 g for 14 min and platelets were further pelleted by centrifugation at 1300 g for 6 min. Platelets were washed once with 10 ml ACD containing 1 mg/ml bovine serum albumin. Platelets were resuspended at ~2.5X10^8/ml in Tyrode's Buffer (137 mM NaCl, 2 mM KCl, 1.0 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, 20 mM HEPES pH 7.4). (b) *Assay method.* The ability of the compounds to inhibit platelet aggregation induced by gamma-thrombin was tested in a 96-well microplate aggregation assay format. Briefly, washed platelet suspension (100 µl) was pre-incubated for 5 min at room temperature with varying concentrations of compounds. Aggregation was initiated by gamma thrombin (Haematologic Technologies, Essex Junction, VT), which was titrated daily to achieve 80% platelet aggregation. Refludan at 1 U/mL (Berlex, Montville, NJ) was added to the gamma thrombin sample to prevent PAR1 activation induced by residual alpha-thrombin contamination. The plate was then placed into a 37 °C Molecular Devices (Sunnyvale, CA) SPECTRAMAX® Plus Plate Reader. The plate was

mixed for 10 seconds before the first read and 50 sec between each read for up to 15 min at 405 nM. Data was collected with SOFTMAX® 4.71 software. The plate also included an untreated control sample which served as ODmax, while buffer containing no platelets was the ODmin. Platelet aggregation was determined by subtracting the ODmax from the ODmin for the 100% aggregation value. In experimental samples, the observed transmission was subtracted from the minimum value and then compared to the 100% aggregation value to determine the percentage aggregation. IC_{50} values are determined using Excel Fit software. Most reported IC_{50} values are an average of at least two independent determinations.

Platelet-rich Plasma Aggregation Assay. (a) Preparation of platelet-rich plasma (PRP). Fresh blood was drawn by antecubital venipuncture from normal healthy volunteers who are free of exposure to antiplatelet drugs following institutional informed consent and anticoagulated with a final concentration of 0.38% of sodium citrate. Following centrifugation of the blood at 250 x *g* for 10 min at room temperature, PRP was collected. (b) Assay method. PRP aggregation was conducted in a 96-well plate format

as described previously.[40] The test compounds were preincubated with PRP for 5 min at 37 °C, and aggregation was initiated by addition of 50-100 nM of γ-thrombin (Haematologic Technologies), which was titrated daily to achieve 80% platelet aggregation. To prevent PAR1 activation induced by residual α-thrombin contamination, PRP was treated with recombinant hirudin (Refludan, Berlex Laboratories) at a final concentration of 1 U/mL. Aggregation was monitored in a plate reader (Spectra Max Plus 384, Molecular Devices, Sunnyvale, CA.), and read at 405 nm with mixing between reads. The selectivity of the compounds were tested following the same protocol, except that platelets were stimulated by a PAR1 agonist peptide SFFLRR (10 μM), ADP (5 μM), collagen (25 μg/ml), or the thromboxane A2 mimetic U46619 (2 μM). All these agonists, when used alone without the compounds, induced 89-96% maximal platelet aggregation. All data are an average of at least three independent determinations.

Monkey ECAT Model. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the regulations of the Institutional Animal Care and Use Committee of the Bristol-Myers Squibb Company. Healthy cyno

monkeys (Bioculture Ltd., Mauritius Island) were used in the study. These monkeys were retired from other pharmacokinetic and pharmacodynamic studies, and had at least a 4-week washout period before this study.

Twenty-nine retired monkeys were randomly assigned to one of the four groups: (1) vehicle (n=8), (2) UDM-001651 0.4 mg/kg (n=7), (3) UDM-001651 2 mg/kg (n=7) and (4) UDM-001651 10 mg/kg (n=7). On the day of the experiment, monkeys were dosed orally by gavage with UDM-001651 or vehicle (40/60 w/w TPGS/PEG400) at 2 ml/kg, where TPGS is D-alpha tocopheryl polyethylene glycol 1000 succinate and PEG400 is polyethylene glycol 400. At 1.5 h after oral dosing, monkeys were anesthetized and surgically prepared as described before.[37] The experiment involved a combination of thrombosis and bleeding time (BT) studies in the same animal.

The arterial thrombosis model used in this study was the ECAT model.[37] Briefly, an electromagnetic flow probe was placed on a segment of an isolated carotid artery to monitor blood flow. Thrombosis was induced by electrical stimulation of the carotid artery for 5 min at 10 mA, using an external stainless-steel bipolar electrode. Carotid

blood flow was measured with a Transonic flow probe and a Transonic perivascular flowmeter (TS420 Model, Transonic Systems Inc., Ithaca, NY). It was continuously recorded over a 90-min period to monitor thrombotic occlusion. The thrombus from the injured artery was removed, blotted twice on a weighing paper to remove residual fluid, and weighed.

The BT model used in this study was the kidney BT model.[37] Briefly, a left flank incision was made to expose the left kidney and the renal capsule was removed. BT was measured by renal cortex incision. BT was defined as the time from injury until bleeding stopped without re-bleeding for 30 seconds. It was monitored up to a maximum of 20 minutes and was determined in triplicate.

Synthesis, purification, and analytical methods. All reactions were carried out using commercial grade reagents and solvents without further purification. NMR chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane. Normal phase flash chromatography was carried out on Teledyne ISCO CombiFlash systems using prepacked silica cartridges and eluted with gradients of the specified solvents. Preparative reverse phase high pressure liquid chromatography (HPLC) was carried out on C18 HPLC columns using methanol/water gradients containing 0.1% trifluoroacetic acid unless otherwise stated. All new

compounds gave satisfactory ^{1}H NMR, LC/MS and/or HRMS data. Purity of all final compounds was determined by analytical HPLC using at least one of the following conditions:

Analytical HPLC Method A: Column Phenomex Luna - C18 5 micron, 4.6 x 50 mm; Mobile Phase: A = 10% MeOH / 90% H_2O / 0.1% TFA, B = 90% MeOH / 10% H_2O / 0.1% TFA; Gradient: T = 0: 100% A, T = 4 min: 100% B, stop time: 4 min; Flow = 4.0 mL/min; UV detection wavelength: 254 nm

Analytical HPLC Method B: Column Phenomex Luna - C18 5 micron, 4.6 x 50 mm; Mobile Phase: A = MeOH:H_2O (10:90), B = MeOH:H_2O (90:10); modifier 10 mM NH_4OAc; Gradient: T = 0: 100% A, T = 4 min: 100% B, stop time: 4 min; Flow = 4.0 mL/min; UV detection wavelength: 254 nm.

Analytical HPLC Method C: Column SunFire C18 - 3.5 μm C18, 4.6 mm × 150 mm; Mobile Phase: A = 95% H_2O / 5% MeCN / 0.05% TFA, B = 5% H_2O / 95% MeCN / 0.05% TFA; Gradient: T = 0: 10% B, T =12 min: 100% B, T = 14.99 min = 100% B; T = 15 min = 10% B; Flow = 2 mL/min; monitoring UV absorbance at 220 and/or 254 nm.

Analytical HPLC Method D: Column Xbridge Phenyl C18 - 3.5 μm C18, 4.6 mm × 150 mm; Mobile Phase: A = 95% H_2O / 5% MeCN / 0.05% TFA, B = 5% H_2O / 95% MeCN / 0.05% TFA; Gradient: T = 0: 10% B, T =12 min: 100% B, T = 14.99 min = 100% B; T = 15 min = 10% B; Flow = 2 mL/min; monitoring UV absorbance at 220 and/or 254 nm.

Analytical HPLC Method E: Column Zorbax XDB-C18 3.5 micron, 4.6 x 30 mm; Mobile Phase: A = MeOH:H_2O:TFA (5:95:0.05), B = MeOH:H_2O:TFA (95:5:0.05); Gradient: T = 0: 100% A, T = 2 min: 100% B, stop time: 4 min; Flow = 3.0 mL/min; UV detection wavelength: 220 and 254 nm.

Analytical HPLC Method F: Column Supelco Ascentis C18 2.7 micron, 4.6 x 50 mm; Mobile Phase: A = acetonitrile:H_2O (5:95), B = acetonitrile:H_2O (95:5), modifier 10 mM NH_4OAc; Gradient: T = 0: 100% A, T = 5.5 min: 100% B, stop time: 7 min; Flow = 3 mL/min; UV detection wavelength: 220 nm.

Analytical HPLC Method G: Column Waters XBridge C18 1.7 micron, 2.1 x 50 mm; Mobile Phase: A = acetonitrile:H_2O:TFA (5:95:0.05), B = acetonitrile:H_2O:TFA (95:5:0.05); Gradient: T = 0: 100% A, T = 3.75 min: 100% B, stop time: 4.25 min; Flow = 1.1 mL/min; UV detection wavelength: 220 nm.

Analytical HPLC Method H: Eclipse XDB-C18 3.5 microns column (4.6 x 30 mm) eluted at 3 mL/min with a 2 min gradient from 100% A to 100% B (A: 5% acetonitrile, 94.95% water, 0.05% TFA; B: 5% water, 94.95% acetonitrile, 0.05% TFA). UV 220 and 254 nm.

Analytical HPLC Method I: Waters XBridge C18, 2.1 mm x 50 mm, 1.7 μm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0 %B to 100

%B over 3 min, then a 0.50 min hold at 100 %B; Flow: 1 mL/min; Detection: MS and UV (220 nm).

Analytical HPLC Method J: Waters XBridge C18, 2.1 mm x 50 mm, 1.7 μm particles; Mobile Phase A: 5:95 acetonitrile:water with 0.1 % trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile:water with 0.1 % trifluoroacetic acid; Temperature: 50 °C; Gradient: 0 %B to 100 %B over 3 min, then a 0.50 min hold at 100 %B; Flow: 1 mL/min; Detection: MS and UV (220 nm).

Analytical HPLC Method K: Eclipse XDB-C18 3.5 microns column (4.6 x 30 mm) eluted at 3 mL/min with a 2 min gradient from 100% A to 100% B (A: 5% methanol, 94.95% water, 0.05% TFA; B: 5% water, 94.95% methanol, 0.05% TFA). UV 220 and 254 nm.

6-(Benzofuran-2-yl)-2-(methylthio)imidazo[2,1-b][1,3,4]thiadiazole (4). 5-(Methylthio)-1,3,4-thiadiazol-2-amine (1.85 g, 12.55 mmol) and 1-(benzofuran-2-yl)-2-bromoethanone (3 g, 12.55 mmol) were dissolved in MeOH (20 mL, 0.63 M) in a microwave vial. The reaction was heated to 100 °C in the microwave for 30 min, until formation of product was observed by HPLC analysis. The reaction mixture was diluted with EtOAc, and washed with H_2O (2 x 50 mL) followed by brine (sat'd NaCl, 2 x 50 mL). The organic layer was evaporated onto silica and the crude material was purified by flash chromatography (EtOAc/hexanes 0-100%). The purity of the chromatographed material was further improved by trituration using 10% EtOAc/hexane, thus providing 1.8 g of compound **4** as a tan solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.75 -

2.79 (m, 3 H) 7.03 - 7.09 (m, 1 H) 7.18 - 7.32 (m, 2 H) 7.46 - 7.53 (m, 1 H) 7.55 - 7.63 (m, 1 H) 8.04 - 8.07 (m, 1 H). LCMS (ESI) *m/z*: 288.0 $[M + H]^+$. Purity: 98% (method A, t_R = 3.9 min).

6-(benzofuran-2-yl)-2-(methylsulfonyl)imidazo[2,1-b][1,3,4]thiadiazole (7). Compound **4** was added to a round bottom flask and dissolved in THF (109 mL). To this solution was added m-CPBA (5.66 g, 32.8 mmol). The mixture was stirred at rt under nitrogen overnight and monitored periodically by LCMS. After 1 day, approximately a 1.6 to 1.0 ratio of sulfoxide to sulfone was observed. Excess *m*-CPBA (7-8 eq.) was added and the reaction mixture was stirred overnight. The next morning only sulfone was present as evident by LCMS ([M+1]=320.0). $Na_2S_2O_3$ was added (aq, sat'd) to quench any excess oxidant. The reaction product was diluted with EtOAc, washed with $NaHCO_3$ and brine, and extracted with EtOAc (3x). The combined organics were subsequently dried over Na_2SO_4. The solvent was removed *in vacuo* and to the yellow solid residue (~11.6 g) was added MeOH. The solution was heated and then cooled to form solids. The solids were isolated by filtration to afford 1.64 g of compound **7** as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.92 (s, 1 H), 7.68 (d, *J*=7.15 Hz, 1 H), 7.61 (d, *J*=8.25 Hz, 1 H), 7.33 (t, *J*=7.15 Hz, 1 H), 7.25 - 7.30 (m, 2 H), 3.68 (s, 3 H). LCMS (ESI) *m/z*: 320.0 $[M + H]^+$. Purity: 95% (method B, t_R = 3.3 min).

6-(Benzofuran-2-yl)-2-(ethylthio)imidazo[2,1-b][1,3,4]thiadiazole (8). Compound **8** (43.7 mg, 39%), a tan solid, was obtained from 5-(ethylthio)-1,3,4-thiadiazol-2-amine (60 mg, 0.372 mmol) and 1-(benzofuran-2-yl)-2-bromoethanone (89 mg, 0.372 mmol) dissolved in EtOH and processed as described for the preparation of **4**. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H), 7.62 - 7.57 (m, 1H), 7.51 (dd, *J*=8.0, 0.8 Hz, 1H), 7.32 - 7.27 (m, 1H), 7.26 - 7.22 (m, 1H), 7.08

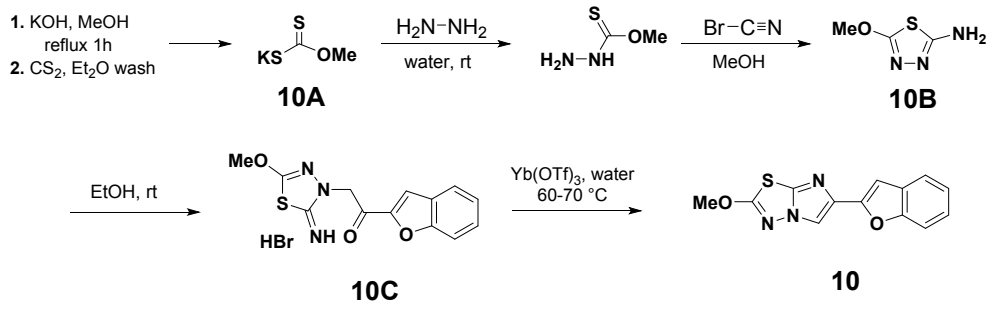
(s, 1H), 3.31 (q, *J*=7.1 Hz, 2H), 1.51 (t, *J*=7.4 Hz, 3H). LCMS (ESI) *m/z*: 302.0 $[M + H]^+$. Purity: 99% (method B, t_R = 4.0 min).

6-(benzofuran-2-yl)-2-(phenylthio)imidazo[2,1-b][1,3,4]thiadiazole (9). Compound **7** (0.01 g, 0.031 mmol) and benzenethiol (3.20 µL, 0.031 mmol) were dissolved in methanol (0.313 mL). TEA (0.013 mL, 0.094 mmol) was added at rt and the vessel was sealed (microwave tube small 0.2-1 mL). The resulting slurry was subjected to microwave conditions: 70 °C, 5 min. A white solid precipitated from of solution upon cooling. LCMS analysis of the crude mixture revealed the formation of the desired product: [M+1]=350.0. The solids were isolated by filtration and air dried to afford 8.3 mg of compound **9** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.64 (s, 1 H), 7.78 (d, *J*=6.60 Hz, 2 H), 7.63 (d, *J*=7.70 Hz, 1 H), 7.54 - 7.61 (m, 4 H), 7.23 - 7.31 (m, 2 H), 7.13 (s, 1 H). LCMS (ESI) *m/z*: 350.0 $[M + H]^+$. Purity: 99% (method B, t_R = 4.3 min).

General synthetic method A: 6-(benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (10). Compound **7** (20 mg, 0.063 mmol) was suspended in methanol (2088 µL). Sodium methoxide (5.07 mg, 0.094 mmol) was added to the vessel, the vessel was sealed, and the reaction mixture was stirred at rt. Over time, the yellow mixture became a white slurry. LCMS monitoring of the crude mixture revealed the formation of the desired product: LCMS: 3.603 min, [M+1]=270.0 {(MeOH/H_2O/NH_4OAc) Phenom. Luna C18; 50x4.6mm; 4 min Gradient}. The mixture was diluted with EtOAc, washed with brine (sat'd NaCl), and extracted with EtOAc (3x). The combined organics were subsequently dried over Na_2SO_4 and concentrated prior to purification by prep HPLC (Phenomenex Luna AXIA, 30x100mm; 17 min gradient; 0-100% MeCN/H_2O/TFA) to afford 9.3 mg (54.7%) of compound **10** as white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.93 (s, 1 H), 7.60 (d, J=7.70 Hz, 1 H), 7.48 (d, J=8.80 Hz, 1 H), 7.22-7.30 (m, 2 H), 7.10 (s, 1 H), 4.22 (s, 3 H). LCMS (ESI) *m/z*: 272.0 $[M + H]^+$. Purity: 99% (method B, t_R = 3.6 min).

Scheme 3. Synthetic route for **General synthetic method B** towards 2-methoxy-imidazothiadiazoles.



General synthetic method B (Steps 1-4): 6-(benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (10).

Step 1: **Potassium O-methyl carbonodithioate** (**10A**). Potassium hydroxide (45 g, 802 mmol) and MeOH (84 mL) were added into a round bottom flask and refluxed for 1 h. The reaction mixture was cooled to 20 °C and the potassium methoxide solution was decanted from the solids into another dry 500 mL round bottom flask. Carbon disulfide (61.1 g, 802 mmol) was added slowly in 15 min to the solution while stirring. The reaction mixture was then cooled to 0 °C and the precipitated solids were collected in a fritted funnel, washed with Et_2O (3 x 50 mL), and dried under reduced pressure for 24 h to afford 86 g of **10A** as a pink solid, which was used in the next step without further purification.

Step 2: **5-Methoxy-1,3,4-thiadiazol-2-amine** (**10B**). To **10A** (15 g, 103 mmol) in a round bottom flask was added H_2O (10 mL). The flask was cooled to 0 °C in an ice bath and hydrazine monohydrate (5.1 mL, 164 mmol) was then added dropwise to the reaction mixture. The mixture was warmed back to 20 °C and stirred upon completion of the addition. Solids precipitated within 15 min of stirring. The resulting slurry was allowed to continue to stir for 2 h at rt and then cooled to 0 °C. The pH of the heterogeneous solution was adjusted to 7 using AcOH (dropwise addition) and then the solids were isolated by filtration. The light yellow solid was dried under reduced pressure for 24 h to afford 8.5 g of the crude thiohydrazide intermediate. This material was placed in a round bottom flask and 2N NaOH solution (48 mL, 96 mmol) was added. The reaction mixture was cooled to 0 °C and a solution of CNBr (8.48 g, 80 mmol) in MeOH (8 mL) was added dropwise. The reaction was warmed to rt over a period of 1 h and stirred for 1.5 h at 20 °C. The precipitate was isolated by filtration and dried *in vacuo* to afford 5.92 g of **10B** as a brown solid. LCMS: [M+1] = 131.8; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.85 - 3.97 (s, 3 H) 6.65 - 6.80 (bs, 2 H).

Step 3: **1-(Benzofuran-2-yl)-2-(2-imino-5-methoxy-1,3,4-thiadiazol-3(2H)-yl)ethanone** (**10C**). **10B** (1 g, 7.62 mmol) was dissolved in EtOH (51 mL). 1-(Benzofuran-2-yl)-2-bromoethanone (1.82 g, 7.62 mmol) was added to the vessel, which was sealed under argon and stirred overnight at rt. Upon consumption of the starting material, the slurry was filtered and the solids were collected and air dried to afford **10C** (1.91 g) as an off white solid, which was used directly in the next step. LCMS: [M+1] = 290.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.92 (s, 2 H), 8.13 (s, 1 H), 7.92 (s, 1 H), 7.79 (s, 1 H), 7.61 (s, 1 H), 7.43 (s, 1 H), 5.83 (s, 2 H), 4.06 (s, 3 H).

Step 4: **6-(Benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole** (**10**). **10C** (300 mg, 1.037 mmol) was dissolved in H_2O (6.9 mL) and ytterbium(III) trifluoromethanesulfonate (64.3

mg, 0.104 mmol) was added. The reaction mixture was warmed to 70 °C. The slurry was stirred overnight, while monitoring via LCMS. After 21 h, the analysis indicated consumption of the starting material and clean formation of compound **10** (LCMS: [M+1] = 272.1). The mixture was cooled. The crude solids were filtered, titurated with MeOH (15 mL) followed by filtration to afford compound **10** (174.9 mg) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.48 (s, 1 H), 7.55 - 7.64 (m, 2 H), 7.21 - 7.30 (m, 2 H), 7.07 (s, 1 H), 4.19 (s, 3 H). LCMS (ESI) *m/z*: 272.0 $[M + H]^+$. Purity: 99% (method B, t_R = 3.6 min).

6-(Benzofuran-2-yl)-2-ethoxyimidazo[2,1-b][1,3,4]thiadiazole (11). Compound **7** (27 mg, 0.085 mmol) was suspended in ethanol (0.1 M, 845 μL) at rt. Potassium carbonate (35.1 mg, 0.254 mmol) was added to the vessel, and the vessel was sealed, and the reaction mixture was stirred at rt. LCMS monitoring of the crude mixture revealed the formation of the desired product: LCMS: 2.883 min, [M+1]=286.4 {(MeCN/H_2O/TFA) Phenom. Luna C18; 50x4.6mm; 4 min Gradient}. The mixture was quenched with H_2O, diluted with EtOAc, washed with brine (sat'd NaCl), and extracted with EtOAc (3x). The combined organics were subsequently dried over Na_2SO_4 and concentrated prior to purification by prep HPLC (Phenomenex Luna AXIA, 30x100mm; 17 min gradient; 0-100% MeCN/H_2O/TFA) to afford 4.31 mg of compound **11** as a solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.48 (s, 1 H), 7.65 (d, J=7.0 Hz, 1 H), 7.58 (d, J=7.9 Hz, 1 H), 7.34 - 7.22 (m, 2 H), 7.10 (s, 1 H), 4.61 (q, J=7.0 Hz, 2 H), 1.45 (t, J=7.2 Hz, 3 H). LCMS (ESI) *m/z*: 286.1 $[M + H]^+$. Purity: 100% (method I, t_R = 2.1 min; method J, t_R = 2.1 min).

6-(Benzofuran-2-yl)-2-isopropoxyimidazo[2,1-b][1,3,4]thiadiazole (12). Compound **12** (7.43 mg), a solid, was prepared using the procedure described for the synthesis of compound **11**, using isopropanol instead of ethanol. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.47 (s, 1 H), 7.64 (d, J=7.3 Hz, 1 H), 7.58 (d, J=7.9 Hz, 1 H), 7.36 - 7.24 (m, 2 H), 7.09 (s, 1 H), 5.30 - 5.17 (m, 1 H), 1.47 (d, J=6.4 Hz, 6 H). LCMS (ESI) *m/z*: 300.1 $[M + H]^+$. Purity: 98% (method I, t_R = 2.3 min; method J, t_R = 2.3 min).

6-(benzofuran-2-yl)-2-(methyl-λ^2-azaneyl)imidazo[2,1-b][1,3,4]thiadiazole (13). Compound **7** was suspended (0.03 g, 0.094 mmol) in DMF (0.939 mL) in a microwave tube. Methanamine hydrochloride (6.34 mg, 0.094 mmol) and excess TEA (0.039 mL, 0.282 mmol) was added, the vessel was sealed, and the reaction mixture was thermally heated overnight at 70 ^{o}C. Upon cooling, analysis of the crude mixture by LCMS revealed formation of the desired product: LCMS: [M+1]=271.0. The reaction mixture was diluted with EtOAc, washed with $NaHCO_3$, extracted 3x (EtOAc), and the organics were dried and concentrated prior to purification by prep HPLC (Phenomenex Luna AXIA, 30x100mm; 17 min gradient; 0-100% MeCN/H_2O/TFA) to afford 9.7 mg (38.3%) of compound **13** as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.85 (s, 1 H), 7.63 (d, J=7.15 Hz, 1 H), 7.47 (t, J=7.15 Hz, 1 H), 7.25-7.33 (m, 3 H), 6.63 (s, 1 H), 3.09 (s, 3 H).. LCMS (ESI) *m/z*: 271.1 $[M + H]^+$. Purity: 100% (method C, t_R = 9.1 min); 100% (method D, t_R = 8.1 min).

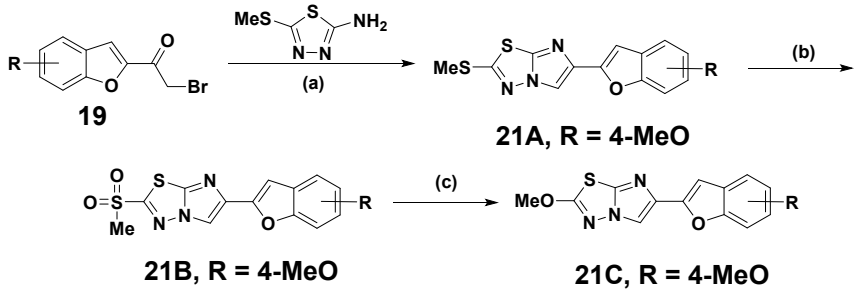
6-(Benzofuran-2-yl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole (14). Compound **14** (30.7 mg, 37%), a white solid, was prepared using the procedure described for the synthesis of compound **4**, with 5-methyl-1,3,4-thiadiazol-2-amine as a starting material. ^{1}H NMR (500 MHz, $CDCl_3$) δ

ppm 8.08 (s, 1 H), 7.61 (d, J=7.15 Hz, 1 H), 7.49 (t, J=8.80 Hz, 1 H), 7.29 (td, J=7.70, 1.10 Hz, 1 H), 7.24 (d, J=1.10 Hz, 1 H), 7.15 (s, 1H), 2.75 (s, 3 H). LCMS (ESI) *m/z*: 256.0 $[M + H]^+$. Purity: 99% (Method C, t_R = 10.4 min); 98% (method D, t_R = 9.0 min).

6-(Benzofuran-2-yl)-2-ethylimidazo[2,1-b][1,3,4]thiadiazole (15). Compound **15** (95 mg, 46%), a white solid, was prepared using the procedure described for the synthesis of compound **4**, with 5-ethyl-1,3,4-thiadiazol-2-amine as a starting material. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.61 (s, 1 H), 7.66 (d, J=7.6 Hz, 1 H), 7.60 (d, J=8.0 Hz, 1 H), 7.35 - 7.25 (m, 2 H), 7.16 (s, 1 H), 3.11 (q, J=7.6 Hz, 2 H), 1.37 (t, J=7.5 Hz, 3 H). LCMS (ESI) *m/z*: 270.0 $[M + H]^+$. Purity: 98% (method A, t_R = 3.8 min).

6-(Benzofuran-2-yl)-2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazole (16). Compound **16** (33.5 mg, 41.4%), a white solid, was prepared using the procedure described for the synthesis of compound **4**, with 5-cyclopropyl-1,3,4-thiadiazol-2-amine as a starting material. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.02 (s, 1 H), 7.61 (d, J=7.70 Hz, 1H), 7.49 (d, J=7.15 Hz, 1 H), 7.28-7.32 (m, 1 H), 7.23-7.26 (m, 1 H), 7.18 (s, 1H), 2.27 (ddd, J=13.20, 8.25, 4.95 Hz, 1 H), 1.28-1.34 (m, 2H), 1.20 (ddd, J=7.29, 4.95, 4.81 Hz, 2H). LCMS (ESI) *m/z*: 282.1 $[M + H]^+$. Purity: 100% (method C, t_R = 11.7 min); 100% (method D, t_R = 9.8 min).

Scheme 4. Initial synthetic route for benzofuranyl imidazothiadiazoles with substituent variation on the benzofuran.



Reagents and conditions: (a) EtOH, μwave: 150 °C, 5 min; (b) *m*-CPBA, THF; (c) NaOMe, MeOH, rt.

2-Methoxy-6-(4-methoxybenzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (21).

Step 1*:* **6-(4-Methoxybenzofuran-2-yl)-2-(methylthio)imidazole[2,1-b][1,3,4]thiadiazole (21A).** Compound **21A** (283 mg, 42.8%) was obtained from 5-(methylthio)-1,3,4-thiadiazol-2-amine (306 mg, 2.081 mmol) and 2-bromo-1-(4-methoxybenzofuran-2-yl)enthanone (560 mg, 2.081 mmol) using the procedure described for the synthesis of compound **4**. LCMS (ESI) *m/z*: 318.0 $[M + H]^+$.

Step 2: **6-(4-Methoxybenzofuran-2-yl)-2-(methylsulfonyl)imidazole[2,1-b][1,3,4]thiadiazole (21B)**. Compound **21B** (214 mg, 68,7%), a solid, was prepared using the procedure described the synthesis of compound **7**. LCMS (ESI) *m/z*: 350.0 $[M + H]^+$.

Step 3: **2-Methoxy-6-(4-methoxybenzofuran-2-yl)imidazole[2,1-b][1,3,4]thiadiazole (21).** Compound **21** (125 mg, 66.4%), an off-white solid, was prepared as described in general synthetic method A for synthesis of compound **10**, using compound **21D** as a starting material. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.15 (s, 1 H), 7.16 - 7.24 (m, 1 H), 7.02 - 7.12 (m, 2 H), 6.74 (d, J=7.9 Hz, 1 H), 4.24 (s, 3 H), 3.94 (s, 3 H). LCMS (ESI) *m/z*: 302.1 $[M + H]^+$. Purity: >95% (method C, t_R = 10.943 min); >95% (method D, t_R = 9.5 min).

6-(4-(Benzyloxy)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (**22**).

Step 1: **5-(Benzyloxy)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one** (**22A**). A solution of 5-hydroxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (6.00 g, 30.9 mmol) (A. Hadfield, H. Schweitzer, M.P. Trova and K. Green, Synthetic Communications, 24(7), 1025 – 1028, 1994) in *N,N*-dimethylformamide (35 mL) was treated with powdered anhydrous potassium carbonate (5.15 g, 37.26 mmol) added all at once. The resulting mixture was stirred *in vacuo* for 10 min. and then flushed with nitrogen. The reaction flask was placed in a water bath (22 ^{o}C) and treated with benzyl bromide (5.55 g, 32.16 mmol) added dropwise over 15 min. The resulting mixture was then stirred at 22 ^{o}C for 18 h. The solid formed was filtered and washed with *N,N*-dimethylformamide. The filtrate was evaporated *in vacuo* and the residual oil was diluted with ethyl acetate (300 mL), washed with cold 0.1 N hydrochloric acid, saturated sodium bicarbonate and brine. After drying over anhydrous magnesium sulfate, evaporation of the solvent gave a thick syrup. Chromatography on silica gel (4 x 13 cm, elution toluene – ethyl acetate 0 – 5 %) gave 8.78 g (100 % yield) of the title material (**22A**) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.69 (s, 6H), 5.23 (s, 2H), 6.53 (d, J = 8.2 Hz, 1H), 6.62 (d, J = 8.4 Hz, 1H), 7.24 – 7.3 (m, 1H), 7.34 – 7.4 (m, 3 H), 7.52 (broad d, J = 7.4 Hz 2H). Purity: > 99% (method K; t_R = 2.0 min).

Step 2: **2-(Benzyloxy)-6-hydroxybenzaldehyde** (**22B**). A solution of 5-(benzyloxy)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (**22A**, 4.00 g, 14.07 mmol) in dichloromethane (80 mL) was cooled to -78 ^{o}C and treated with a solution of diisobutylaluminum hydride (6.00 g, 42.2 mmol) in toluene (40 mL) added dropwise over 20 min. The resulting mixture was then stirred at -78 ^{o}C for 3 h. The reaction mixture was quenched by the careful addition of methanol (5 mL)

added dropwise over 15 min, followed by 4 N hydrochloric acid (20 mL) added dropwise over 15 min. The cooling bath was then removed and an additional 80 mL of 4 N hydrochloric acid was added over 10 min and the mixture was stirred vigorously at 22 ^{o}C for 4 h. The reaction mixture was diluted with ethyl acetate (200 mL), washed with brine, dried over anhydrous magnesium sulfate and evaporated *in vacuo*. The resulting oil was chromatographed on silica gel (4 x 10 cm, elution toluene) to give 2.25 g (70 % yield) of **22B** as a pale yellow solid. HRMS (ESI) calcd for $C_{14}H_{13}O_3$ $[M + H]^+$ *m/z* 229.0859, found 229.0859. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.12 (s, 2H), 6.43 (d, J = 8.25 Hz, 1H), 6.52 (d, J = 8.46 Hz, 1H), 7.34 – 7.4 (m, 6 H), 10.39 (s, 1H), 11.95 (s, 1H). Purity: > 99% (method G; t_R = 2.2 min).

Step 3: **1-(4-(Benzyloxy)benzofuran-2-yl)ethanone** (**22C**). A solution of 2-(benzyloxy)-6-hydroxybenzaldehyde (**22B,** 11.10 g, 48.63 mmol) (in N,N-dimethylformamide (120 mL) was treated with powdered anhydrous cesium carbonate (15.8 g, 48.63 mmol) added all at once. The resulting mixture was stirred *in vacuo* for 10 min and then flushed with nitrogen. The reaction flask was placed in a water bath (22 ^{o}C) and treated with chloroacetone (4.65 mL, 58.4 mmol) added dropwise over 10 min. The resulting mixture was then stirred at 22 ^{o}C for 18 h (no starting aldehyde left by tlc and formation of the intermediate alkylated aldehyde). The reaction mixture was then maintained under vacuum (10 mbar) for 15 min to remove any unreacted chloroacetone and flushed with nitrogen. Then anhydrous cesium carbonate (1.0 g, 3.1 mmol) was added and the mixture was heated at 55 ^{o}C and stirred for 40 h (more cesium carbonate, 1 g, was added at 24 h and 32 h) until complete conversion of the intermediate alkylated aldehyde into the benzofuran as observed by TLC. The solid was filtered and washed with N,N-dimethylformamide. The filtrate was evaporated *in vacuo* and the residual oil was diluted with ethyl acetate (400 mL), washed with cold 0.1 N hydrochloric acid, saturated sodium

bicarbonate and brine. After drying over anhydrous magnesium sulfate, evaporation of the solvent gave a thick syrup. Chromatography on silica gel (4.5 x 12 cm, elution toluene – ethyl acetate 2 – 4 %) gave 11.67 g (90 % yield) of **22C** as a light yellow solid. Recrystallization from a mixture of ethyl acetate (40 mL) and hexane (40 mL) gave colorless prisms (10.50 g). HRMS (ESI) calcd for $C_{17}H_{15}O_3$ $[M + H]^+$ *m/z* 267.1016, found 267.1022. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.56 (s, 3H), 5.20 (s, 2H), 6.73 (d, J = 8.0 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.3 – 7.5 (m, 6H), 7.63 (s, 1H). Purity: > 99% (method K; t_R = 2.1 min).

Step 4: **1-(4-(Benzyloxy)benzofuran-2-yl)-2-bromoethanone** (**22D**). A 250-mL, three-necked flask is equipped with a magnetic stirring bar and purged with a nitrogen atmosphere was charged with anhydrous tetrahydrofuran (40 mL) followed by 21.6 mL (21.6 mmol) of a 1M solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran. The mixture was cooled to -78 °C and treated with a solution of 1-(4-(benzyloxy)benzofuran-2-yl)ethanone (**22C**, 5.00 g, 18.77 mmol) in tetrahydrofuran (20 mL) added dropwise over 10 min. The resulting mixture was then stirred at -78 °C for 45 min. Then chlorotrimethylsilane (2.74 mL, 21.6 mmol) was added dropwise over 5 min and the resulting solution was stirred at -78 °C for another 20 min. The cooling bath was then removed and the mixture was allowed to warm to room temperature over 30 min. The reaction mixture was then quenched by addition to a cold solution of ethyl acetate (300 mL), saturated sodium bicarbonate (40 mL) and ice. The organic phase was rapidly dried over anhydrous magnesium sulfate (magnetic stirring) and evaporated *in vacuo* to give the silyl enol ether as an oil which is co-evaporated with toluene (20 mL). The silyl enol ether was then dissolved in dry tetrahydrofuran (80 mL), cooled to -25 °C and treated with solid sodium bicarbonate (0.10 g), followed by N-bromosuccinimide (3.34 g, 18.8 mmol) added in small portions over 10 min. The reaction mixture was allowed to warm to 0 °C over 2h and then

quenched by addition of ethyl acetate (350 mL) and saturated sodium bicarbonate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate and evaporated to give an orange oil. Chromatography on silica gel (4.5 x 12 cm, elution toluene – ethyl acetate 0 – 1 %) gave 6.13 g of 1-(4-(benzyloxy)benzofuran-2-yl)-2-bromoethanone (**22D**) as a yellow solid. Recrystallization from ethyl acetate (20 mL) and hexane (40 mL) gave pale yellow prisms (4.93 g, 76 % yield). HRMS (ESI) calcd for $C_{17}H_{14}BrO$ $[M + H]^+$ *m/z* 345.0121, found 345.0109. ^1H NMR ($CDCl_3$, 600 MHz) δ 4.39 (s, 2H), 5.20 (s, 2H), 6.75 (d, J = 7.86 Hz, 1H), 7.17 (d, J = 8.25 Hz, 1H), 7.34 – 7.46 (m, 6H), 7.78 (s, 1H). Purity: > 99% (method E; t_R = 2.2 min).

Step 5: **6-(4-(Benzyloxy)benzofuran-2-yl)-2-bromoimidazo[2,1-b][1,3,4]thiadiazole** (**22E**).

A mixture of 1-(4-(benzyloxy)benzofuran-2-yl)-2-bromoethanone (**22D**, 3.00 g, 8.69 mmol) and 5-bromo-1,3,4-thiadiazol-2-amine (1.80 g, 10.0 mmol) in isopropanol (100 mL) was heated in a pressure flask equipped with a magnetic stirring bar at 80 °C for 20 h (homogeneous after 20 min and then formation of a precipitate after 2 h). The cooled mixture was then transferred into five 20 mL microwave vials and heated in a microwave apparatus to 150 °C for 30 min. Each vial was then diluted with dichloromethane (250 mL), washed with saturated sodium bicarbonate (25 mL), brine (25 mL), and dried over anhydrous magnesium sulfate. The fractions were combined and concentrated *in vacuo*. Chromatography of the orange-brown residual solid on silica gel (4 x 10 cm, slow elution with dichloromethane) gave 2.82 g of 6-(4-(benzyloxy)benzofuran-2-yl)-2-bromoimidazo[2,1-b][1,3,4]thiadiazole (**22E**) contaminated with some 1-(4-(benzyloxy)benzofuran-2-yl)ethanone. The solid material was triturated with ethyl acetate (15 mL), filtered, washed with ethyl acetate (10 ml) and dried *in vacuo* to give 2.37 g (64 % yield) of pure title imidazothiadiazole as an off white solid which is used as such for the next step. HRMS (ESI) calcd for $C_{19}H_{13}BrN_3O_2S$ $[M + H]^+$ *m/z* 425.9906, found 425.9893. ^1H

NMR ($CDCl_3$, 600 MHz) δ 5.21 (s, 2H), 6.72 (d, J = 8.07 Hz, 1H), 7.13 (d, J = 8.26 Hz, 1H), 7.18 (broad t, 1H), 7.25 (s, 1H), 7.32 (broad t, 1H), 7.38 (broad t, 2H), 7.47 (broad d, 2H), 8.09 (s, 1H). Purity: > 99% (method E; t_R = 2.4 min).

Step 6: **6-(4-(Benzyloxy)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole** (**22**). A solution of 6-(4-(benzyloxy)benzofuran-2-yl)-2-bromoimidazo[2,1-b][1,3,4]thiadiazole (**22E**, 3.22 g, 7.55 mmol) in a mixture of dichloromethane (400 mL) and methanol (50 mL) was treated at 22 °C with 6.3 mL of a 25 wt. % solution of sodium methoxide in methanol (30.2 mmol) added in one portion. More methanol (45 mL) was added and the mixture was stirred for 40 min. The reaction mixture was quenched by the addition of 40 mL of 1 N hydrochloric acid followed by 10 ml of saturated sodium bicarbonate. The solvent was evaporated under reduced pressure and the residue was diluted with dichloromethane (400 mL), washed with brine, dried over anhydrous magnesium sulfate and evaporated *in vacuo*. Crystallization of the white solid residue from 1,2-dichloroethane (30 mL) gave 2.19 g of 6-(4-(benzyloxy)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (**22**) as a white solid. Chromatography of the mother liquors on silica gel (3 x 10 cm, elution with dichloromethane – ethyl acetate 0 - 1 %) gave another 0.46 g of product (total yield 2.65 g, 93 %). HRMS (ESI) calcd for $C_{20}H_{16}N_3O_3S$ $[M + H]^+$ *m/z* 378.0907, found 378.0911. ^1H NMR ($CDCl_3$, 600 MHz) δ 4.18 (s, 3H), 5.21 (s, 2H), 6.71 (dd, J = 7.4 Hz and J = 0.95 Hz, 1H), 7.12 – 7.17 (m, 3H), 7.32 (broad t, 1H), 7.38 (broad t, 2H), 7.47 (broad d, 2H), 7.88 (s, 1H). Purity: > 99% (method E; t_R = 2.4 min).

2-Methoxy-6-(5-methoxybenzofuran-2-yl)imidazole[2,1-b][1,3,4]thiadiazole (23).

Compound **23** (117.7 mg, 31%), a white solid, was prepared as described in general synthetic method B for preparation of compound **10,** where the 2-bromo-1-(5-methoxybenzofuran-2-

yl)ethanone intermediate was synthesized using the methods described for preparation of **21B**. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.90 (s, 1 H), 7.36 (d, J=8.80 Hz, 1 H), 7.03 (d, J=2.75 Hz, 1 H), 6.99 (s, 1 H), 6.85 (dd, J=8.80, 2.75 Hz, 1 H), 4.20 (s, 3 H), 3.84 (s, 3 H). LCMS (ESI) *m/z*: 302.1 $[M + H]^+$. Purity: 100% (method C, t_R = 10.680 min); >95% (method D, t_R = 9.3 min).

2-Methoxy-6-(6-methoxybenzofuran-2-yl)imidazole[2,1-b][1,3,4]thiadiazole (24).

Compound **24** (75 mg, 51.3%), a white solid, was prepared using the procedures described for synthesis of compound **21**, using 2-bromo-1-(6-methoxybenzofuran-2-yl)enthanone as the starting material. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.10 (s, 1 H), 7.43 (d, J=8.2 Hz, 1 H), 7.08 (d, J=1.6 Hz, 1 H), 6.94 (s, 1 H), 6.86 (dd, J=8.8, 2.2 Hz, 1 H), 4.24 (s, 3 H), 3.84 (s, 3 H). LCMS (ESI) *m/z*: 302.1 $[M + H]^+$. Purity: 95% (method C, t_R = 10.704 min); 95% (method D, t_R = 9.3 min).

6-(6-(Benzyloxy)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (25).

Step 1: **1-(6-(Benzyloxy)benzofuran-2-yl)ethan-1-one** (**25A**). 4-(Benzyloxy)-2-hydroxybenzaldehyde (15.45 g, 67.7 mmol) was weighed into a tared round bottom flask charged with cesium carbonate (33.1 g, 102 mmol). To the reactants dissolved in DMF (100 mL), 1-chloropropan-2-one (7.02 mL, 88 mmol) was added slowly over 5 min and the reaction mixture was heated at 60 °C overnight. The next day, the reaction was diluted with EtOAc (300 mL), washed with a one-to-one solution of sat'd NaCl and H_2O (50 mL, 4x), dried over $MgSO_4$, filtered and evaporated to dryness in vacuo. The residue was purified via normal phase column chromatography (ISCO) to afford 13.8 g of compound **25A**. LCMS (ESI) *m/z*: 267 $[M + H]^+$.

Step 2: **1-(6-(Benzyloxy)benzofuran-2-yl)-2-bromoethan-1-one** (**25B**). To compound **25A** (4 g, 15.02 mmol) dissolved in THF (100 mL), phenyltrimethylammonium tribromide (5.65 g, 15.02 mmol) was added. The reaction mixture was stirred at rt for 3 h until the reaction was determined to be complete. The crude mixture was poured into H_2O (300 mL), diluted with EtOAc (300 mL), washed with sat'd NaCl (10 mL, 3x), dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to afford compound **25B** (5 g, 87 % yield) as a yellow solid. LCMS (ESI) *m/z*: 346 $[M + H]^+$.

Step 3: 6-(6-(Benzyloxy)benzofuran-2-yl)-2-(methylthio)imidazo[2,1-b][1,3,4]thiadiazole (25C). Compound **25C** (2.2 g, 36.2%) was prepared from 5-(methylthio)-1,3,4-thiadiazol-2-amine (2.047 g, 13.91 mmol) and intermediate **25B** (4.8 mg, 13.91 mmol) in EtOH (90 mL) using the procedure described for synthesis of **4**. LCMS (ESI) *m/z*: 392 $[M + H]^+$.

Step 4: **6-(6-(Benzyloxy)benzofuran-2-yl)-2-(methylsulfonyl)imidazo[2,1-b][1,3,4]thiadiazole (25D)**. Compound **21D** (400 mg, 88%), a solid, was prepared using the method described for synthesis of compound **7**. LCMS (ESI) *m/z*: 426.0 $[M + H]^+$.

Step 5: **6-(6-(Benzyloxy)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (25)**. Compound **25** (3.4 mg), a yellow solid, was prepared from intermediate **25D** as described in general synthetic method A for synthesis of compound **10**. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.21 (s, 3 H) 5.13 (s, 2 H) 6.94 (s, 1 H) 7.29 - 7.37 (m, 1 H) 7.40 (s, 3 H) 7.42 - 7.46 (m, 3 H) 7.46 (s, 1 H) 7.87 (s, 1 H). LCMS (ESI) *m/z*: 378.0 $[M + H]^+$. Purity: 92% (method C, t_R = 12.819 min); 92% (method D, t_R = 11.0 min).

2-Methoxy-6-(7-methoxybenzofuran-2-yl)imidazole[2,1-b][1,3,4]thiadiazole (26). Compound **26** (112 mg, 55.3%), a white solid, was prepared using the method described for synthesis of compound **21**, using 2-bromo-1-(7-methoxybenzofuran-2-yl)enthanone as the

starting material. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.19 (s, 1 H), 7.12 - 7.18 (m. 2 H), 7.01 (s, 1 H), 6.87 (dd, J=6.2, 2.6 Hz, 1 H), 4.25 (s, 3 H), 4.00 (s, 3 H). LCMS (ESI) *m/z*: 302.0 $[M + H]^+$. Purity: 98% (method C, t_R = 10.576 min); 98% (method D, t_R = 9.3 min).

6-(6,7-Dimethoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (27).

Step 1: **1-(6,7-Dimethoxybenzofuran-2-yl)ethanone** (**27A**). 2-Hydroxy-3,4-dimethoxybenzaldehyde (1.3 g, 7.14 mmol) was dissolved in MeOH (16.4 mL) in a round bottom flask. KOH (0.400 g, 7.14 mmol), previously pulverized with a mortar and pestle, was added. The reaction mixture was heated to reflux for 30 min. The mixture was cooled and 1-chloropropan-2-one (8.56 g, 7.89 mmol) was added dropwise to the solution at 0-10 °C. The newly prepared solution was warmed to rt and stirred over 48 h. Upon reaction completion, as shown by TLC analysis, the MeOH was removed *in vacuo*, the residue was dissolved in EtOAc and H_2O was added. The crude product was washed with brine (sat'd NaCl), extracted 3x with EtOAc, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a dark oil. The crude material was purified by flash chromatography (EtOAc/hexanes 0-100%) to provide 1 g of **27A**. LCMS: [M+1] = 221.1; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 2.48 - 2.60 (s, 3 H) 3.88 - 3.95 (s, 3 H) 4.02 - 4.10 (s, 3 H) 7.05 - 7.16 (m, 1 H) 7.37 - 7.44 (m, 1 H) 7.61 - 7.68 (s, 1 H).

Step 2: **2-Bromo-1-(6,7-dimethoxybenzofuran-2-yl)ethanone** (**27B**). To **27A** (1 g, 4.54 mmol) dissolved in EtOAc (30 mL) was added copper (II) bromide (1.27 g, 5.68 mmol) and the resulting mixture was heated under Ar to reflux (80 °C) overnight. The dark solution was filtered through a plug of silica. The media was rinsed with 10% EtOAc/hexanes. The filtrate was concentrated and dried *in vacuo* and the crude material was purified by flash chromatography (EtOAc/hexanes 0-20%) to provide 710 mg of **27B**. LCMS: [M+1] = 301.0; ^{1}H

NMR (500 MHz, CD_3OD) δ ppm 3.93 - 3.95 (s, 3 H) 4.07 - 4.09 (s, 3 H) 4.53 - 4.57 (s, 2 H) 7.12 - 7.16 (m, 1 H) 7.41 - 7.45 (m, 1 H) 7.78 - 7.81 (s, 1 H).

Step 3: **6-(6,7-Dimethoxybenzofuran-2-yl)-2-(methylthio)imidazo[2,1-b]-[1,3,4]thiadiazole** (**27C**). **27C** was prepared as described in general synthetic method A for compound **10** from 5-(methylthio)-1,3,4-thiadiazol-2-amine (148 mg, 1.00 mmol) and **27B** (300 mg, 1.00 mmol). The crude product was dried onto silica gel and purified by flash chromatography (0-15% EtOAc/hexanes) to provide 135 mg of **27C** as a solid. LCMS: [M+1] = 348.1; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 2.78 - 2.85 (s, 3 H) 3.86 - 3.93 (s, 3 H) 4.06 - 4.12 (s, 3 H) 6.95 - 7.03 (m, 2 H) 7.18 - 7.24 (m, 1 H) 8.24 - 8.32 (s, 1 H).

Step 4*:* **6-(6,7-Dimethoxybenzofuran-2-yl)-2-(methylsulfonyl)imidazo-[2,1-b][1,3,4]thiadiazole** (**27D**). **27C** (135 mg, 0.398 mmol) and *m*-CPBA (335 mg, 1.94 mmol) were added to a round bottom flask containing THF (4 mL, 0.1M). The resulting mixture was stirred for 12 h at 20 °C. Upon completion, the reaction mixture was diluted with EtOAc, washed with H_2O (2 x 25 mL), followed by brine (sat'd NaCl, 2 x 25mL). The biphasic mixture was extracted with EtOAc (3x) and the combined organics were dried directly onto SiO_2 gel. The crude residue was purified by flash chromatography (0-30% EtOAc/hexanes) to provide **27D** (71 mg) as a solid. LCMS: [M+1] = 380.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.42 - 3.47 (s, 3 H) 3.93 - 3.98 (s, 3 H) 4.17 - 4.22 (s, 3 H) 6.92 - 6.97 (m, 1 H) 7.11 - 7.16 (s, 1 H) 7.20 - 7.25 (m, 1 H) 8.21 - 8.26 (s, 1 H).

Step 5**: 6-(6,7-Dimethoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole** (**27**). MeOH (3.7 mL, 0.05M) and sodium methoxide (30 mg, 0.531 mmol) were added to a round bottom flask containing **27D** (71 mg, 0.187 mmol). The resulting mixture was stirred at 20 °C for 12 h. Upon complete consumption of the starting material, the solution was diluted with

EtOAc and washed with H_2O (2 x 25 mL), followed by brine (sat'd NaCl, 2 x 25 mL). The biphasic mixture was extracted with EtOAc (3x) and the combined organics were concentrated directly onto silica. The crude residue was purified by flash chromatography (0-30% EtOAc/hexanes) to provide 40 mg of compound **27** as a solid. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 3.88 - 3.93 (s, 3 H) 4.07 - 4.13 (s, 3 H) 4.23 - 4.28 (s, 3 H) 6.93 - 7.02 (m, 2 H) 7.16 - 7.24 (m, 1 H) 8.15 - 8.20 (s, 1 H). LCMS (ESI) *m/z*: 332.1 $[M + H]^+$. Purity: 95% (method C, t_R = 10.1 min).

6-(4,7-Dimethoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (28). Compound **28** (38.6 mg, 55.7%), a pale yellow solid isolated upon final purification via flash chromatography (CH_2Cl_2/hexanes), was prepared using the methods described for synthesis of compound for **27**, using 2-hydroxy-3,6-dimethoxybenzaldehyde as the starting material. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.97 (s, 1 H), 7.12 (s, 1 H), 6.68 (d, J=8.25 Hz, 1 H), 6.53 (d, J=8.80 Hz, 1 H), 4.19 (s, 3 H), 3.98 (s, 3 H), 3.90 (s, 3 H). LCMS (ESI) *m/z*: 332.1 $[M + H]^+$. Purity: 95% (method C, t_R = 10.570 min); 98% (method D, t_R = 9.3 min).

6-(4,6-Dimethoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (29). Compound **29** (43 mg, 21.9%), an orange solid isolated upon final purification via flash chromatography (CH_2Cl_2/hexanes), was prepared using the methods described for synthesis of compound for **27**, using 2-hydroxy-4,6-dimethoxybenzaldehyde as the starting material.. ^{1}H NMR (500 MHz, CD_3OD) d ppm 8.07 (s, 1 H), 6.97 (s, 1 H), 6.71 (d, J=1.1 Hz, 1 H), 6.38 (d, J=1.6 Hz, 1 H), 4.25 (s, 3 H), 3.91 (s, 3 H), 3.84 (s, 3 H). LCMS (ESI) *m/z*: 332.1 $[M + H]^+$. Purity: 97% (method C, t_R = 10.794 min); 98% (method D, t_R = 9.4 min).

5-(Benzyloxy)-7-methoxy-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (32). A solution of **31** (30.00 g, 0.134 mol, see Kamisuki, S. et al. *Tetrahedron* **2004**, *60*, 5695-5700 for preparation) in *N,N*-dimethylformamide (400 mL) was treated with powdered anhydrous potassium carbonate (19.41 g, 0.14 mol), added all at once. The resulting mixture was stirred *in vacuo* for 10 min. and then flushed with nitrogen. The reaction flask was placed in a water bath (22 °C) and treated with benzyl bromide (24.03 g, 0.14 mol) added dropwise over 15 min. The resulting mixture was then stirred at 22 °C for 18 h (no starting material remained by tlc). The solid was filtered and washed with *N,N*-dimethylformamide. The filtrate was concentrated *in vacuo* and the residual oil was diluted with ethyl acetate (500 mL), washed with cold 0.1 N hydrochloric acid, saturated sodium bicarbonate, and brine. After drying over anhydrous magnesium sulfate, evaporation of the solvent gave a thick syrup. Crystallization from ethyl acetate (50 mL) and hexane (150 mL) gave 35.17 g of **32** as large colorless prisms. Chromatography of the mother liquors on silica gel (4 x 13 cm column, elution with toluene - ethyl acetate 0-5%) gave 6.64 g of additional material to afford a total yield of 41.81 g (99%). HRMS (ESI) calcd for $C_{18}H_{19}O_5$ $[M+H]^+$ *m/z* 315.1227, found 315.1386. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.68 (s, 6H), 3.77 (s, 3H), 5.19 (s, 2H), 5.19 (s, 2H), 6.04 (d, J = 2.03 Hz, 1H), 6.15 (d, *J* = 2.03 Hz, 1H), 7.27 (broad t, 1H), 7.36 (broad t, 2H), 7.52 (broad d, 2H). Purity: > 99% (method E; t_R = 2.0 min).

2-(Benzyloxy)-6-hydroxy-4-methoxybenzaldehyde (33). A solution of **32** (6.76 g, 21.5 mmol) in dichloromethane (120 mL) was cooled to -78 °C and treated with 43 mL (64.5 mmol) of a 1.5 M solution of diisobutylaluminum hydride in toluene, added dropwise over 20 min. The resulting mixture was stirred at -78 °C for 3 h. The reaction mixture was quenched by the careful addition

of methanol (5 mL) added dropwise over 15 min, followed by 1N hydrochloric acid (50 mL) added dropwise over 15 min. The cooling bath was then removed and an additional 150 mL of 1N hydrochloric acid was added over 20 min. The mixture was stirred at 22 °C for 2 h and diluted with dichloromethane (400 mL). The organic phase was collected and the aqueous phase (pH ~ 1) was extracted with dichloromethane (3 x 50 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residual oil was diluted with tetrahydrofuran (70 mL), treated with 10 mL of 0.1N hydrochloric acid and stirred at 20 °C for 2 h. The reaction mixture was diluted with ethyl acetate (300 mL), washed with brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to give a clear oil. Chromatography on silica gel (4 x 13 cm, elution with toluene) gave 4.08 g (73% yield) of **33** as a clear oil which solidified to a white solid on standing. HRMS (ESI) calcd for $C_{15}H_{15}O_4$ $[M+H]^+$ *m/z* 259.0965, found 259.1153. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.80 (s, 3H), 5.07 (s, 2H), 5.97 (d, J = 2.1 Hz, 1H), 6.01 (d, J = 2.1 Hz, 1H), 7.3 - 7.4 (m, 5 H), 10.15 (s, 1H), 12.49 (s, 1H). Purity: > 95% (method E; t_R = 2.1 min).

6-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-*b*][1,3,4]thiadiazole (34).

Step 1: **1-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)ethanone (34A).** A solution of **33** (3.46 g, 13.4 mmol) in N,N-dimethylformamide (50 mL) was treated with powdered anhydrous cesium carbonate (4.58 g, 14.05 mmol) added all at once. The resulting mixture was stirred *in vacuo* for 10 min. and then flushed with nitrogen. The reaction flask was placed in a water bath (22 °C) and treated with chloroacetone (1.74 g, 18.7 mmol) added dropwise over 5 min. The resulting mixture was then stirred at 22 °C for 18 h (no starting aldehyde left by tlc and formation of the

intermediate alkylated aldehyde). The solid was filtered and washed with *N,N*-dimethylformamide. The filtrate was evaporated *in vacuo* and the residual oil was diluted with ethyl acetate (300 mL), washed with cold 0.1 N hydrochloric acid, saturated sodium bicarbonate and brine. After drying over anhydrous magnesium sulfate, evaporation of the solvent gave a thick syrup. This syrup was diluted with tetrahydrofuran (50 mL) and ethyl acetate (50 mL), treated with *p*-toluenesulfonic acid monohydrate (0.2 g) and stirred at 20 °C for 1 h (tlc indicated complete cyclization of the intermediate alkylated aldehyde). The reaction mixture was diluted with ethyl acetate (300 mL), washed with saturated sodium bicarbonate and brine. After drying over anhydrous magnesium sulfate, evaporation of the solvent gave a thick syrup. Chromatography on silica gel (4 x 12 cm, elution toluene - ethyl acetate 2-4%) gave 3.51 g (88% yield) of **34A** as a yellow solid. Recrystallization from ethyl acetate (10 mL) and hexane (20 mL) gave the title material as large yellow prisms (3.15 g). HRMS (ESI) calcd for $C_{18}H_{17}O_4$ $[M+H]^+$ *m/z* 297.1121, found 297.1092. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.51 (s, 3H), 3.82 (s, 3H), 5.13 (s, 2H), 6.37 (d, *J* = 1.77 Hz, 1H), 6.63 (broad s, 1H), 7.34 (broad t, 1H), 7.39 (broad t, 2H), 7.44 (broad d, 2H), 7.55 (d, J = 0.7 Hz,1H). Purity: > 99% (method E; t_R = 2.1 min).

Step 2: **1-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoethanone (34B).** A 250-mL, three-necked flask equipped with a magnetic stirring bar and purged with a nitrogen atmosphere was charged with anhydrous tetrahydrofuran (25 mL) followed by 9.3 mL (9.3 mmol) of a 1 M solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran. The mixture was cooled to -78 °C and treated with a solution of **34A** (2.40 g, 8.1 mmol) in tetrahydrofuran (20 mL) added dropwise over 10 min. The resulting mixture was then stirred at -78 °C for 45 min. Chlorotrimethylsilane (1.18 mL, 9.31 mmol) was added dropwise over 5 min and the resulting solution was stirred at -78 °C for another 20 min. The cooling bath was removed and the

mixture was allowed to warm to room temperature over 30 min. The reaction mixture was then quenched by addition to a cold solution of ethyl acetate (200 mL), saturated sodium bicarbonate (30 mL) and ice. The organic phase was rapidly dried over anhydrous magnesium sulfate (magnetic stirring) and evaporated *in vacuo* to give the silyl enol ether as an oil which was co-evaporated with toluene (20 mL). The silyl enol ether was then dissolved in dry tetrahydrofuran (40 mL), cooled to -20 °C and treated with solid sodium bicarbonate (0.10 g) followed by *N*-bromosuccinimide (1.44 g, 8.1 mmol), added in small portions over 15 min. The reaction mixture was allowed to warm to 0 °C over 2 h and then quenched by addition of ethyl acetate (300 mL) and saturated sodium bicarbonate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate and evaporated to give an orange oil. Chromatography on silica gel (4 x 12 cm, elution with toluene - ethyl acetate 0-5%) gave 2.62 g (86% yield) of **34B** as a yellow solid. Recrystallization from ethyl acetate (10 mL) and hexane (20 mL) gave yellow prisms (2.30 g). HRMS (ESI) calcd for $C_{18}H_{16}BrO_4$ $[M+H]^+$ *m/z* 375.0226, found 375.0277. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.84 (s, 3H), 4.33 (s, 2H), 5.14 (s, 2H), 6.38 (d, J = 1.76 Hz, 1H), 6.64 (broad s, 1H), 7.35 (broad t, 1H), 7.40 (broad t, 2H), 7.44 (broad d, 2H), 7.70 (s, 1H). Purity: > 95% (method E; t_R = 2.2 min).

Step 3: **6-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoimidazo[2,1-*b*][1,3,4]thiadiazole (34C).** A mixture of **34B** (3.00 g, 8.0 mmol) and 5-bromo-1,3,4-thiadiazol-2-amine (1.65 g, 9.16 mmol) in isopropanol (100 mL) was heated in a pressure flask equipped with a magnetic stirring bar at 78-80 °C for 18 h (homogeneous after 20 min and then formation of a precipitate after 2 h). The cooled mixture was transferred into five 20 mL microwave vials and then heated in a microwave apparatus to 150 °C for 30 min. Each vial was then diluted with dichloromethane (250 mL), washed with saturated sodium bicarbonate (25 mL) and brine (25

mL), and dried over anhydrous magnesium sulfate. The fractions were combined and concentrated *in vacuo*. Chromatography of the orange-brown residual solid on silica gel (4 x 10 cm, slow elution with dichloromethane due to poor solubility) gave 2.96 g of the title imidazothiadiazole contaminated with some 1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)ethanone. The solid material was triturated with ethyl acetate (20 mL), filtered, washed with ethyl acetate (10 ml) and dried *in vacuo* to give 2.34 g (64% yield) of pure **34C** as an off white solid which was used as such for the next step. HRMS (ESI) calcd for $C_{20}H_{15}BrN_3O_3S$ $[M+H]^+$ *m/z* 456.00175, found 456.00397. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.82 (s, 3H), 5.16 (s, 2H), 6.38 (d, J = 1.67 Hz, 1H), 6.66 (broad s, 1H), 7.15 (s, 1H), 7.31 (broad t, 1H), 7.38 (broad t, 2H), 7.45 (broad d, 2H), 8.02 (s, 1H). Purity: > 95% (method E; t_R = 2.4 min).

Step 4: **6-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-*b*][1,3,4]thiadiazole (34).** A solution of **34C** (2.30 g, 5.04 mmol) in a mixture of dichloromethane (180 mL) and methanol (45 mL) was treated at 22 °C with 4.2 mL of a 25 wt. % solution of sodium methoxide in methanol (0.2 mmol) added in one portion. More methanol (45 mL) was added and the mixture was stirred for 1 h. The reaction mixture was quenched by the addition of 25 mL of 1N hydrochloric acid, followed by 20 ml of saturated sodium bicarbonate. The solvent was evaporated under reduced pressure and the residue was diluted with dichloromethane (400 mL), washed with brine, dried over anhydrous magnesium sulfate and evaporated *in vacuo*. Chromatography of the residue on silica gel (3 x 10 cm, elution with dichloromethane - ethyl acetate 0-4%) gave 1.70 g (83% yield) of **34** as a white solid. This material was recrystallized from ethyl acetate (30 mL per gram, 80% recovery) to give white needles. HRMS (ESI) calcd for $C_{21}H_{18}N_3O_4S$ $[M+H]^+$ *m/z* 408.1013, found 408.1024. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.81 (s, 3H), 4.18 (s, 3H), 5.16 (s, 2H), 6.37 (d, J = 1.75 Hz, 1H), 6.67

(broad s, 1H), 7.07 (s, 1H), 7.31 (broad t, 1H), 7.37 (broad t, 2H), 7.45 (broad d, 2H), 7.81 (s, 1H). Purity: > 99% (method H; t_R = 2.1 min).

6-Methoxy-2-(2-methoxyimidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)benzofuran-4-ol (35). A mixture of **34** (1.250 g, 3.06 mmol) and pentamethylbenzene (3.17 g, 21.4 mmol) in dichloromethane (200 mL) was cooled to -78 °C under a nitrogen atmosphere and then treated immediately (to avoid crystallization) with 8 mL (8 mmol) of a 1 M solution of boron trichloride in dichloromethane, added dropwise over 3 min. The resulting mixture was stirred at -78 °C for 1 h. The reaction mixture was then quenched by the addition of a solution of sodium bicarbonate (6 g) in water (100 mL) added in one portion. The cooling bath was removed and the resulting mixture was stirred at room temperature for 1 h. The solid formed was filtered, washed successively with water (50 mL) and dichloromethane (50 mL). The filter cake was allowed to soak with anhydrous ethanol (15 ml) and then sucked dry. The white solid obtained was then dried under vacuum for 24 h to give 0.788 g (80% yield) of pure title material (> 95% by HPLC). The combined filtrate and washings were diluted with dichloromethane (600 mL) and stirred in a warm water bath until the organic phase was clear with no apparent solid in suspension. The organic phase was collected, dried over anhydrous magnesium sulfate and rapidly filtered while still warm. The filtrate was evaporated and the residue (product and pentamethylbenzene) was triturated with toluene (20 mL), the solid collected and washed with toluene (20 mL) to give 0.186 g (19% yield, 99% combined yield) of **35** as a tan solid. HRMS (ESI) calcd for $C_{14}H_{12}N_3O_4S$ $[M+H]^+$ *m/z* 318.0543, found 318.0578. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 3.71 (s, 3H), 4.16 (s, 3H), 6.21 (d, *J* = 1.87 Hz, 1H), 6.61 (broad s, 1H), 6.95 (s, 1H), 8.29 (s, 1H), 9.96 (s, 1H). Purity: >95% (method E; t_R = 2.0 min).

General Synthetic Method C: Mitsunobu Reaction with Compound 35

A mixture of 6-methoxy-2-(2-methoxyimidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)benzofuran-4-ol (**35**) (0.100 g, 0.315 mmol), triphenylphosphine (1.2 equiv) and the selected alcohol (1.2 equiv) in anhydrous tetrahydrofuran (10 mL) and under nitrogen was treated at 23 °C with a solution of diisopropyl azodicarboxylate (1.2 equiv) in tetrahydrofuran (2 mL) added dropwise over 10 min. The heterogeneous mixture usually became homogeneous after 20 min (with sonication) and the reaction was complete by LCMS analysis after 3 h. The reaction mixture was then diluted with dichloromethane, washed with brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent and chromatography of the residue on silica gel gave the coupled product in 40 – 80 % yield.

2-Methoxy-6-(6-methoxy-4-phenethoxybenzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (36). Prepared according to general synthetic method C with 2-phenylethanol to give **36** (56 % yield). MS (ESI): calcd for $C_{22}H_{20}N_3O_4S$ $[M+H]^+$ *m/z* 422.1169, found 422.1222. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.37 (d, *J* = 7.65 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.23 (t, *J* = 7.2 Hz, 1H), 6.86 (s, 1H), 6.79 (s, 1H), 6.44 (s, 1H), 4.30 (t, *J* = 6.4 Hz, 2H), 4.20 (s, 3H), 3.79 (s, 3H), 3.10 (t, *J* = 6.4 Hz, 2H). Purity: > 95% (method E; t_R = 2.4 min).

2-Methoxy-6-(6-methoxy-4-(2-phenoxyethoxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (37). Prepared according to general synthetic method C with 2-phenoxylethanol to give **37**. MS (ESI): calcd for $C_{22}H_{20}N_3O_5S$ $[M+H]^+$ *m/z* 418.1118, found 422.1135. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* =

7.3 Hz, 1H), 7.02 (d, *J* = 7.9 Hz, 2H), 6.96 (t, *J* = 7.3 Hz, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.51 (d, *J* = 1.8 Hz, 1H), 4.44 – 4.47 (m, 2H), 4.37 – 4.40 (m, 2H), 4.20 (s, 3H), 3.81 (s, 3H). Purity: > 95% (method E; t_R = 2.3 min).

6-(4-(2-(Benzyloxy)ethoxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (38). Prepared according to general synthetic method C with 2-(benzyloxy)ethanol to give **38**. MS (ESI): calcd for $C_{23}H_{22}N_3O_5S$ $[M+H]^+$ *m/z* 452.1275, found 452.1317. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.33 – 7.41 (m, 4H), 7.25 – 7.33 (m, 1H), 6.93 (s, 1H), 6.81 (s, 1H), 6.46 (s, 1H), 4.61 (s, 2H), 4.28 (t, *J* = 4.4 Hz, 2H), 4.20 (s, 3H), 3.83 (t, *J* = 4.4 Hz, 2H), 3.80 (s, 3H). Purity: > 95% (method E; t_R = 2.6 min).

2-Methoxy-6-(6-methoxy-4-((2-methylbenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (39). Prepared according to general synthetic method C with 2-methylbenzyl alcohol to give **39 (**75 % yield**)**. MS (ESI): calcd for $C_{22}H_{20}N_3O_4S$ $[M+H]^+$ *m/z* 422.1175, found 422.1166. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.44 - 7.51 (m, 1H), 7.18 - 7.33 (m, 3H), 6.95 (s, 1H), 6.84 (s, 1H), 6.59 (d, *J* = 2.05 Hz, 1H), 5.23 (s, 2H), 4.20 (s, 3H), 3.81 (s, 3H), 2.36 (s, 3H). Purity: > 99% (method E; t_R = 2.4 min).

6-(4-((2-Chlorobenzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (40). Prepared according to general synthetic method C with 2-chlorobenzyl alcohol to give **40**. MS (ESI): calcd for $C_{21}H_{17}ClN_3O_4S$ $[M+H]^+$ *m/z* 442.0623, found 442.0628. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.64 - 7.71 (m, 1H), 7.54 (dd, *J* = 3.66, 5.71 Hz,

1H), 7.38 - 7.45 (m, 2H), 6.98 (s, 1H), 6.86 (br s, 1H), 6.55 (br s, 1H), 5.30 (s, 2H), 4.20 (s, 3H), 3.80 (s, 3H). Purity: > 99% (method E; t_R = 2.5 min).

2-Methoxy-6-(6-methoxy-4-((2-methoxybenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (41). Prepared according to general synthetic method C with 2-methoxybenzyl alcohol to give **41** (4.2 mg, 22%). MS (ESI): calcd for $C_{22}H_{20}N_3O_5S$ $[M+H]^+$ *m/z* 438.1, found 437.9. Purity: 94% (method G; t_R = 2.3 min).

2-Methoxy-6-(6-methoxy-4-((2-(trifluoromethyl)benzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (42). Prepared according to general synthetic method C with 2-(trifluoromethyl)benzyl alcohol to give **42** (2.0 mg, 8.4%). MS (ESI): calcd for $C_{22}H_{17}F_3N_3O_4S$ $[M+H]^+$ *m/z* 476.1, found 475.8. Purity: 89% (method G; t_R = 2.4 min).

6-(4-((3-Chlorobenzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (43). Prepared according to general synthetic method C with 3-chlorolbenzyl alcohol to give **43** (70 % yield). LCMS (ESI): calcd for $C_{21}H_{17}ClN_3O_4S$ $[M+H]^+$ *m/z* 442.0623, found 442.0583. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.82 (s, 1H), 7.46 (s, 1H), 7.28 - 7.32 (m, 3H), 7.07 (s, 1H), 6.68 (d, *J* = 0.93 Hz, 1H), 6.34 (d, *J* = 1.81 Hz, 1H), 5.13 (s, 2H), 4.18 (s, 3H), 3.81 (s, 3H). Purity: > 99% (method E; t_R = 2.4 min).

2-Methoxy-6-(6-methoxy-4-((3-methoxybenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (44). Prepared according to general synthetic method C with 3-

methoxybenzyl alcohol to give **44** (2.8 mg, 10%). MS (ESI): calcd for $C_{22}H_{20}N_3O_5S$ $[M+H]^+$ *m/z* 438.1, found 437.9. Purity: 85% (method G; t_R = 2.3 min).

3-(((6-Methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)phenol (45). *Step 1*. 6-Methoxy-2-(2-methoxyimidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)benzofuran-4-ol (**35**) was reacted with (3-(tert-butyldimethylsilyloxy)benzyl alcohol (P.A. Grieco and C.J. Markworth. Tetrahedron Lett., 40, 1999, 665-666) according to general synthetic method C to give 6-(4-((3-((tert-butyldimethylsilyl)oxy)benzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (61 % yield). MS (ESI): calcd for $C_{27}H_{32}N_3O_5SSi$ $[M+H]^+$ *m/z* 538.1826, found 538.183. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.04 (t, *J* = 7.8 Hz, 1H), 6.86 – 6.88 (m, 2H), 6.74 (s, 1H), 6.60 (dd, *J* = 8.03, 1.84 Hz, 1H), 6.49 (s, 1H), 6.18 (d, *J* = 1.17 Hz, 1H), 4.94 (s, 2H), 4.01 (s, 3H), 3.63 (s, 3H), 0.78 (s, 9H), 0.00 (s, 6H). Purity: > 99% (method E; t_R = 2.7 min).

Step 2. A solution of 6-(4-((3-((tert-butyldimethylsilyl)oxy)benzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (0.115 g, 0.21 mmol) in tetrahydrofuran (6 mL) was treated at 23 °C with acetic acid (0.1 mL, 0.017 mmol) followed by tetrabutylammonium fluoride hydrate (0.20 g, 0.76 mmol) added in one portion. After 1.5 hour, the reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate, brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent and chromatography of the residue on silica gel (gradient of ethyl acetate in dichloromethane) gave 0.069 g (77 % yield) of compound **45** as a white solid. MS (ESI): calcd for $C_{21}H_{18}N_3O_5S$ $[M+H]^+$ *m/z* 424.0962, found 424.0972. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.34 (s, 1H), 7.15 (t, *J* = 8.0 Hz, 1H),

6.93 (s, 1H), 6.84 – 6.87 (m, 2H), 6.77 (d, J = 0.83 Hz, 1H), 6.67 (d, J = 8.43 Hz, 1H), 6.45 (d, J = 1.8 Hz, 1H), 5.13 (s, 2H), 4.16 (s, 3H), 3.74 (s, 3H). Purity: > 99% (method E; t_R = 2.2 min).

(3-{[(6-methoxy-2-{2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl}-1-benzofuran-4-yl)oxy]methyl}phenyl)methanol (46). Prepared according to general synthetic method C with 3-hydroxymethylbenzyl alcohol to give **46** (0.8 mg, 3.7%). MS (ESI): calcd for $C_{22}H_{20}N_3O_5S$ $[M+H]^+$ *m/z* 438.1, found 437.8. Purity: > 99% (method G; t_R = 1.9 min).

3-(((6-Methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)benzonitrile (47). Prepared according to general synthetic method C with 3-(hydroxymethyl)benzonitrile to give **47** (74 % yield). MS (ESI): calcd for $C_{22}H_{17}N_4O_4S$ $[M+H]^+$ *m/z* 433.0965, found 433.0946. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.83 (s, 1H), 7.75 (s, 1H), 7.69 (d, J = 7.84 Hz, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.05 (s, 1H), 6.70 (s, 1H), 6.32 (d, J = 1.74 Hz, 1H), 5.18 (s, 2H), 4.18 (s, 3H), 3.82 (s, 3H). Purity: > 95% (method E; t_R = 2.3 min).

2-Methoxy-6-(6-methoxy-4-((3-(trifluoromethyl)benzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (48). Prepared according to general synthetic method C with 3-trifluoromethylbenzyl alcohol to give **48** (0.8 mg, 3 % yield). MS (ESI): calcd for $C_{22}H_{17}F_3N_3O_4S$ $[M+H]^+$ *m/z* 476.1, found 476.0. Purity: 97% (method F; t_R = 3.5 min).

2-Methoxy-6-(6-methoxy-4-((4-(trifluoromethyl)benzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (49). Prepared according to general synthetic method C with 4-

trifluoromethylbenzyl alcohol to give **49** (2.7 mg, 10 % yield). MS (ESI): calcd for $C_{22}H_{17}F_3N_3O_4S$ $[M+H]^+$ *m/z* 476.1, found 476.0. Purity: 98.3% (method F; t_R = 3.5 min).

6-(4-((4-Chlorobenzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (50). Prepared according to general synthetic method C with 4-chlorobenzyl alcohol to give **50**. MS (ESI): calcd for $C_{21}H_{17}ClN_3O_4S$ $[M+H]^+$ *m/z* 442.0623, found 442.0628. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.54 (d, *J* = 8.35 Hz, 2H), 7.48 (d, *J* = 8.35 Hz, 2H), 6.98 (s, 1H), 6.83 (s, 1H), 6.51 (d, *J* = 2.05 Hz, 1H), 5.25 (s, 2H), 4.20 (s, 3H), 3.79 (s, 3H). Purity: 97% (method E; t_R = 2.4 min).

2-Methoxy-6-(6-methoxy-4-((4-methoxybenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (51). Prepared according to general synthetic method C with 4-methoxybenzyl alcohol to give **51**. MS (ESI): calcd for $C_{22}H_{20}N_3O_5S$ $[M+H]^+$ *m/z* 438.1119, found 438.1124. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.43 (d, *J* = 8.79 Hz, 2H), 6.96 (d, *J* = 8.79 Hz, 2H), 6.93 (s, 1H), 6.81 (s, 1H), 6.52 (d, *J* = 2.05 Hz, 1H), 5.16 (s, 2H), 4.20 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H). Purity: > 99% (method E; t_R = 2.4 min).

2-Methoxy-6-(6-methoxy-4-((3-phenoxybenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (52). Prepared according to general synthetic method C with 3-phenoxybenzyl alcohol to give **52** (66 % yield). MS (ESI): calcd for $C_{27}H_{22}N_3O_5S$ $[M+H]^+$ *m/z* 500.1275, found 500.127. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.3 - 7.34 (m, 3H), 7.19 (d, *J* =7.62 Hz, 1H), 7.11 (s, 1H), 7.08 (t, *J* =7.4 Hz, 1H), 7.03 (s, 1H), 7.01 (d, *J* =7.72 Hz,

2H), 6.94 (dd, *J* =8.08, 2.06 Hz, 1H), 6.67 (d, *J* =0.92 Hz, 1H), 6.34 (d, *J* =1.8 Hz, 1H), 5.13 (s, 2H), 4.18 (s, 3H), 3.81 (s, 3H). Purity: > 99% (method E; t_R = 2.5 min).

2-Methoxy-6-(6-methoxy-4-((3-(phenylthio)benzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (53). Prepared according to general synthetic method C with 3-(phenylthio)-benzenemethanol to give **53** (31 % yield). MS (ESI): calcd for $C_{27}H_{22}N_3O_4S_2$ $[M+H]^+$ *m/z* 516.1946, found 516.109. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.82 (s, 1H), 7.40 (s, 1H), 7.5 - 7.36 (m, 8H), 7.01 (s, 1H), 6.67 (d, *J* =0.82 Hz, 1H), 6.32 (d, *J* =1.76 Hz, 1H), 5.11 (s, 2H), 4.19 (s, 3H), 3.81 (s, 3H). Purity: > 99% (method E; t_R = 2.6 min).

6-(4-((3-(Benzyloxy)benzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (54). Prepared according to general synthetic method C with 3-benzyloxybenzyl alcohol to give **54** (70 % yield). HRMS (ESI) calcd for $C_{28}H_{24}N_3O_5S$ $[M+H]^+$ *m/z* 514.1431, found 514.1406. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm, 3.79 (s, 3H), 4.19 (s, 3H), 5.12 (s, 2H), 5.22 (s, 2H), 6.51 (d, *J* = 1.57 Hz, 1H), 6.82 (s, 1H), 6.95 - 7.01 (m, 2H), 7.07 (d, *J* = 7.43 Hz, 1H), 7.15 (s, 1H), 7.32 (t, *J* = 7.83 Hz, 2H), 7.36 - 7.42 (m, 2H), 7.42 - 7.48 (m, 2H), 8.36 (s, 1H). ^{13}C NMR (DMSO-d_6, 100.5 MHz) δ 55.68, 60.37, 69.16, 69.37, 88.84, 95.93, 97.94, 111.18, 112.28, 113.77, 114.13, 119.73, 127.67, 127.80, 128.40, 129.61, 134.42, 136.99, 138.54, 149.17, 151.71, 155.47, 158.45, 158.61, 169.41. Purity: > 99% (method E; t_R = 2.5 min).

2-Methoxy-6-(6-methoxy-4-((3-(phenoxymethyl)benzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazole (55). Prepared according to general synthetic method C with (3-(phenoxymethyl)phenyl)methanol to give **55**. HRMS (ESI) calcd for $C_{28}H_{24}N_3O_5S$ $[M+H]^+$ *m/z*

514.1431, found 514.1439. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.84 (s, 3H), 4.20 (s, 3H), 5.10 (s, 2H), 5.20 (s, 2H), 6.39 (d, J = 1.7 Hz, 1H), 6.70 (s, 1H), 6.95 - 7.0 (m, 3H), 7.09 (s, 1H), 7.28 - 7.31 (m, 2 H), 7.41 - 7.45 (m, 3H), 7.55 (s, 1H), 7.84 (s, 1H). Purity: 95% (method E; t_R = 2.5 min).

2-Methoxy-6-(6-methoxy-4-((3-phenethylbenzyl)oxy)benzofuran-2-yl)imidazo[2,1-b][1,3,4]thiadiazol: (56). Prepared according to general synthetic method C with (3-phenethylphenyl)methanol to give **56**. HRMS (ESI) calcd for $C_{29}H_{26}N_3O_4S$ $[M+H]^+$ *m/z* 512.1639, found 512.165. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.94 (s, 4H), 3.84 (s, 3H), 4.20 (s, 3H), 5.15 (s, 2H), 5.20 (s, 2H), 6.40 (s, 1H), 6.69 (s, 1H), 7.09 (s, 1H), 7.19 – 7.21 (m, 4H), 7.27 - 7.31 (m, 2 H), 7.39 (d, J = 7.9 Hz, 2H), 7.84 (s, 1H). Purity: 95% (method E; t_R = 2.6 min).

6-(4-((2-(Benzyloxy)benzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (57). Prepared according to general synthetic method C with (2-(benzyloxy)phenyl)methanol to give **57** (57 % yield). MS (ESI): calcd for $C_{28}H_{24}N_3O_5S$ $[M+H]^+$ *m/z* 514.1431, found 514.1448. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.55 (d, *J* =7.4 Hz, 1H), 7.40 (d, *J* =7.43 Hz, 2H), 7.34 (t, *J* =7.5 Hz, 2H), 7.25 - 7.30 (m, 2H), 7.09 (s, 1H), 6.98 (t, 1H), 6.94 (d, *J* =8.22 Hz, 1H), 6.66 (s, 1H), 6.41 (d, *J* =1.51 Hz, 1H), 5.27 (s, 2H), 5.12 (s, 2H), 4.18 (s, 3H), 3.80 (s, 3H). Purity: > 99% (method E; t_R = 2.5 min).

6-(4-((4-(Benzyloxy)benzyl)oxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (58). Prepared according to general synthetic method C with (4-(benzyloxy)phenyl)methanol to give **58** (44 % yield). MS (ESI): calcd for $C_{28}H_{24}N_3O_5S$ $[M+H]^+$

m/z 514.1431, found 514.1453. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.42 (d, *J* =7.5 Hz, 2H), 7.36 - 7.38 (m, 4H), 7.31 (t, *J* =7.2 Hz, 1H), 7.04 (s, 1H), 6.97 (d, *J* =8.35 Hz, 2H), 6.66 (s, 1H), 6.38 (s, 1H), 5.09 (s, 2H), 5.07 (s, 2H), 4.18 (s, 3H), 3.82 (s, 3H). Purity: > 99% (method E; t_R = 2.5 min).

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge:

Proton and carbon NMR spectra for compound **54**

Single crystal x-ray data for compound **4**

Biotransformation studies of compounds **4** and **15**

Molecular Formula strings are available as a csv file.

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ABBREVIATIONS

ECAT electrolytic carotid artery thrombosis; FLIPR fluorescent imaging plate reader; HTS high thoughput screening; IDT imidazo[2,1-*b*][1,3,4]-thiadiazole; PAR protease-activated receptor; SAR structure-activity relationship

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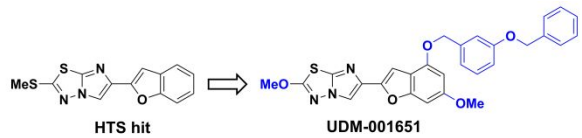

HTS hit

PAR4 FLIPR IC_{50}, nM 11
LM stability, % rem (H, R) 25, 4

UDM-001651

PAR4 FLIPR IC_{50}, nM 2.4
γ-Thr PRP IC_{50}, nM 25
LM $t_{1/2}$, min (H,Mk) 86, 48

Robust antithrombotic efficacy
No bleeding time prolongation