Novel Small-Molecule Atypical Chemokine Receptor 3 (ACKR3) Agonists: Design, Synthesis, and Pharmacological Evaluation for Antiplatelet Therapy

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ABSTRACT

Cardiovascular diseases are one of the leading causes of mortality worldwide. Therefore, novel therapeutic measures are urgently needed, and promising new drug targets must be explored. ACKR3, an atypical chemokine receptor, has been associated with prothrombotic events and the development of cardiovascular events. We designed, synthesized, and evaluated a series of novel small molecules ACKR3 agonists. Extensive structure-activity relationship studies resulted in several promising agonists with potencies ranging from low micromolar to nanomolar range, for example, **23** (EC_{50} = 111 nM, E_{max} = 95%) and **27** (EC_{50} = 69 nM, E_{max} = 82%) in the β-arrestin-recruitment assay. These compounds are selective for ACKR3 versus ACKR2, CXCR3, and CXCR4. Several agonists were subjected to investigations of their P-selectin expression reduction in the flow cytometry experiments. In particular, compounds **23** and **27** showed the highest potency for platelet aggregation inhibition, up to 80% and 97%, respectively. The most promising compounds, especially **27**, exhibited good solubility, metabolic stability, and no cytotoxicity, suggesting a potential tool compound for the treatment of platelet-mediated thrombosis.

Keywords: antiplatelet therapy, CXCR7/ACKR3 agonist, cardiovascular diseases, chemokine receptor, platelet aggregation

INTRODUCTION

Chemokines, also known as chemotactic cytokines, are a family of signaling proteins that act through the subfamily of class A G protein-coupled receptors (GPCRs).[1,2] Their involvement is prominent in different types of cell movement processes, such as chemotaxis, haptotaxis, chemokinesis, haptokinesis, and transcellular migration.[1] In particular, their role as chemoattractants is vital in processes such as homeostasis, the regulation of the immune system, and inflammatory signaling. Over 50 human chemokines have been identified and classified into four subfamilies (C, CC, CXC, CX3C).[3,4] The respective chemokine receptors can be divided into two main families, the classical chemokine receptors (cCKRs) and the atypical chemokine receptors (ACKRs).[1] All cCKRs transduce signals through G proteins.[1,5-7] The ACKRs share structural homology with cCKRs, but are not coupled to G proteins. Nevertheless, ACKRs play an important role by sequestering or internalizing chemokines, they shape their gradient and regulate their effect on cells expressing their respective cCKRs. Most ACKRs, notably ACKR3, have conserved the ability to recruit β-arrestin in response to chemokines.

Chemokine receptors are considered important therapeutic targets due to their key roles in various diseases, including cardiovascular diseases, multiple sclerosis, and cancer.[1,3,5,8-14] Therefore, ligands targeting chemokine receptors have been developed.[3] For example, the C-C chemokine receptor type 5 antagonist, maraviroc, has been approved for HIV treatment therapy. Also, plerixaflor, a C-X-C chemokine receptor type 4 (CXCR4) antagonist initially developed for HIV treatment, is commonly utilized in clinics to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients diagnosed with non-Hodgkin's lymphoma (NHL) or multiple myeloma (MM).[3,15,16] The ACKR family, particularly ACKR3 (previously known as C-X-C chemokine receptor type 7 or CXCR7), has gained attention in drug development efforts due to its association with

multiple diseases.[17] ACKR3 is expressed in a range of regions, including the central nervous system, the adrenal gland, endothelial cells, immune cells, and platelets.[18-22] Especially, its role in cardiovascular diseases (CVDs) has frequently been discussed in recent scientific studies.[17,23-26] CVDs are the leading cause of death worldwide, with approximately 17.9 million deaths per year, and new therapeutic strategies are in high demand.[23,27] The involvement of ACKR3 in prothrombotic events was demonstrated in 2013.[28] Rath *et al.* reported an increase in the surface expression of ACKR3 and its endogenous ligand, C-X-C motif chemokine 12 (CXCL12; also known as stromal cell-derived factor-1, SDF-1), on the platelets of patients with acute coronary syndrome.[28] This was further underlined by their 2015 publication, which linked baseline levels of ACKR3 and CXCR4 to all-cause mortality in individuals suffering from coronary artery disease (CAD).[26]

Activation of ACKR3 leads to scavenging of CXCL12, thereby reducing CXCR4 activation and subsequently promoting platelet survival, inhibition of platelet activation and aggregation, and prevention of thrombus formation (Figure 1).[24,29,30] In other cell types, ACKR3 has also been suggested to function as a decoy receptor for CXCL12 and CXCL11/ITAC-1, which are chemokines for CXCR4 and CXCR3, as well as several opioid peptides.[18,31-34]

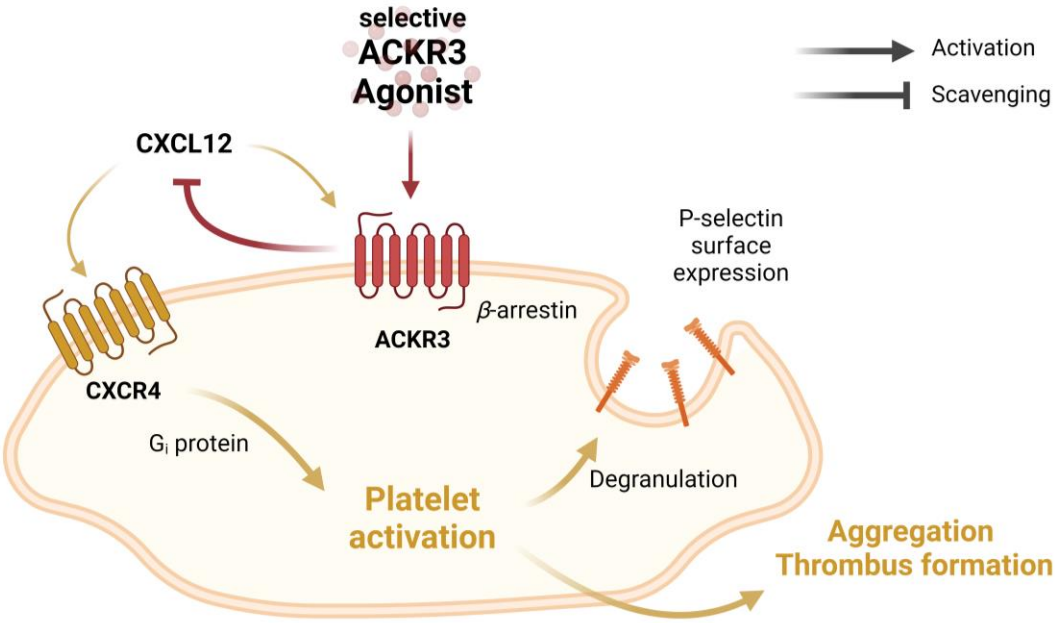


Figure 1. The mechanism of ACKR3-agonist binding and thrombus formation. Activation of ACKR3 by a selective ACKR3-agonist leads to β-arrestin recruitment and scavenging of CXCL12. Created with BioRender.com[35]

Subsequently, as knowledge regarding ACKR3's role in disease grows, several structurally distinct small-molecule ACKR3 ligands have been discovered. For example, VUF11207, a small molecule, has been utilized as a tool compound for pharmacological studies of ACKR3.[36-38] Also, CCX777 (ChemoCentryx), which has been proven to be a potent ACKR3 modulator.

We recently reported the development of first-in-class ACKR3 agonists for the modulation of platelet aggregation and degranulation.[17] In particular, the novel thiadiazolopyrimidinone-based compound showed a super-agonistic profile with an EC_{50} value of 3.5 μM (E_{max} 164%), excellent metabolic stability, and reduced platelet degranulation (P-selectin expression) by up to 97%.

In the present study, we describe the ligand-based design, development, and evaluation of novel ACKR3 agonists with high potency, efficacy, and selectivity toward ACKR3. All ACKR3 agonists were tested for their potency in β-arrestin recruitment assays and their ability to modulate platelet degranulation, specifically P-selectin/CD62P surface expression, in response to the platelet activator CRP-XL. The most promising candidates were further tested in platelet aggregation assays and subsequently evaluated for their metabolic stability, solubility, and cell toxicity. In particular, compounds **1** and **27** were demonstrated to be viable tool compounds and were employed in other promising platelet-related studies.[33,34]

RESULTS AND DISCUSSION

Design of New Compounds. Our initial goal was to develop improved ACKR3 ligands compared to our recently reported compounds and evaluate them for functional activity in platelet modulation. Yoshikawa *et al.* discovered *N*-(3-(benzyl(methyl)amino)propyl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-2-carboxamide (**I**, Figure 2) as one of the hit ACKR3 agonists by structure-based virtual screening.[39] However the agonistic activity of compound **I** was only moderate with an EC_{50} value of 2.21 μM.

In the present study, we therefore selected **I** as a lead molecule and studied its structure-activity relationships (SARs) to improve its potency. The following modifications were targeted (Figure 2) using a systematic approach. First, we removed the rigid ring moiety of the reported ligand to create a more flexible core structure (Figure 2). The resulting compound **1** already demonstrated a 5-fold improvement (EC_{50} 0.416 μM) in potency on our β-arrestin recruitment assays compared to the reported compound **I**. The new lead molecule (**1**) was divided into three main components: the head, the tail, and the connecting linker, as illustrated in Figure 2. Each part was systematically investigated to gain more insight into the SARs.

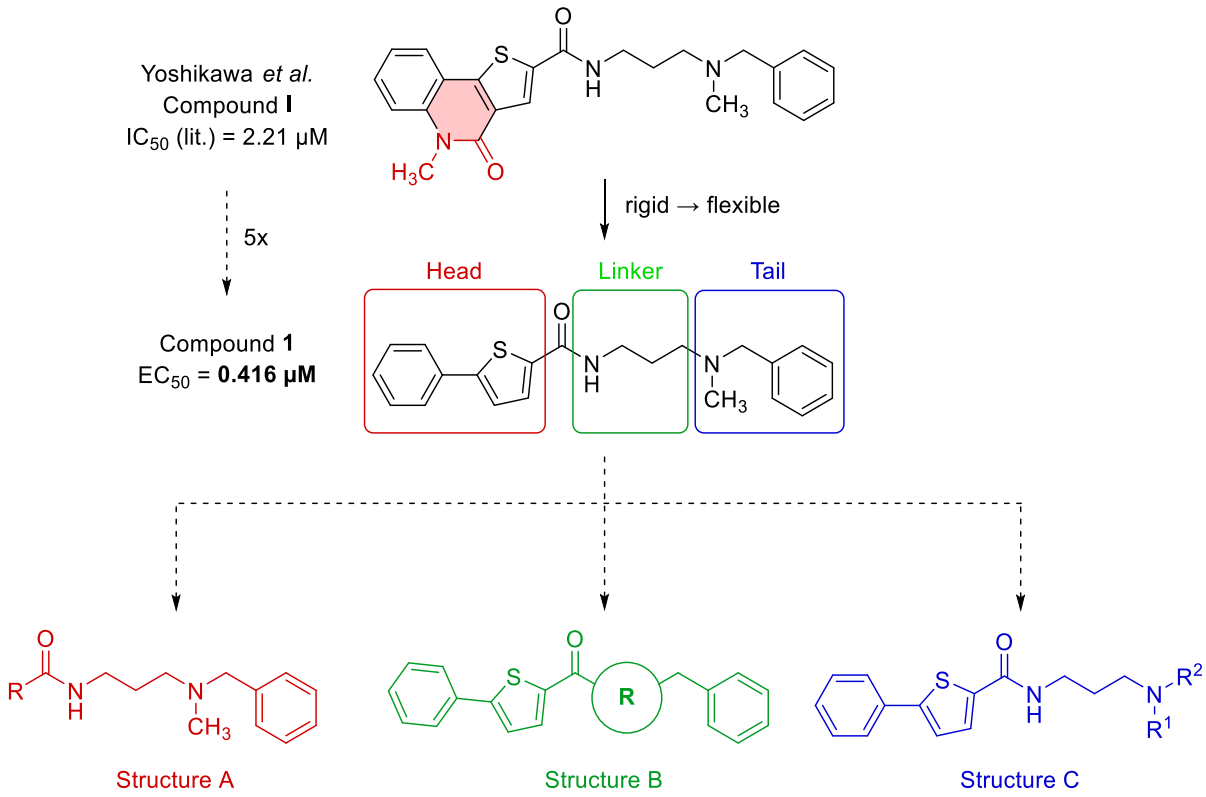
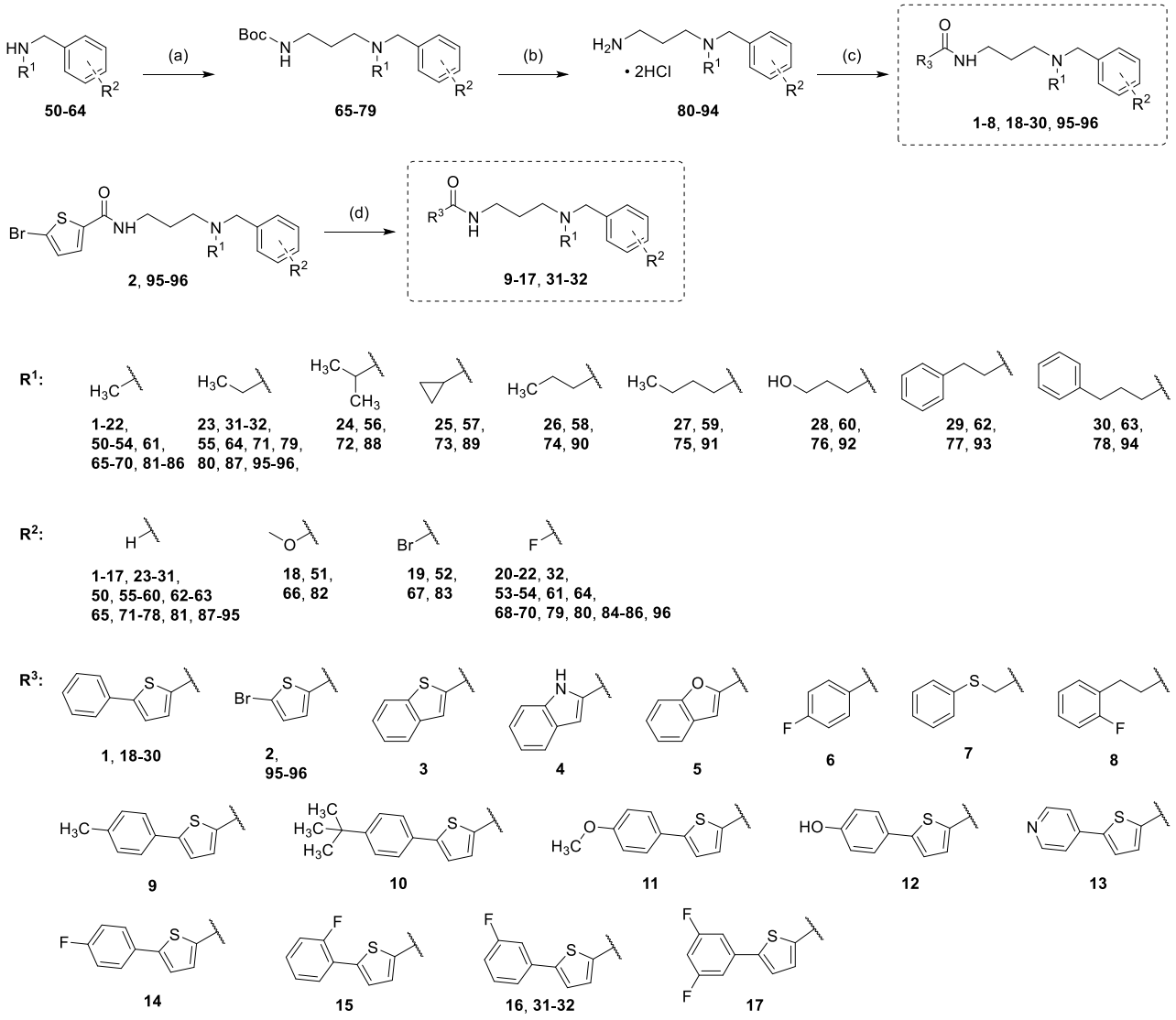


Figure 2. The ligand-based designing of new compounds **1**, A, B, and C from the compound **I** by Yoshikawa *et al.*[39]

Chemistry. The synthesis of the final compounds (**1**-**49**) was achieved in a range of different routes as outlined in Schemes 1-3. Compounds **1**-**32** were synthesized using a modular 3 to 5-step synthesis as depicted in Scheme 1. The boc-protected precursors (**65**-**79**) were synthesized by *in situ* Finkelstein reaction and nucleophile substitution reaction of the substituted benzylamines (**50**-**64**) with *tert*-butyl (3-bromopropyl)carbamate in the presence of cesium carbonate. Although the majority of benzylamines were commercially available, compounds **61**, **62**, and **63** were synthesized beforehand as shown in Scheme S1. After successful purification by flash column chromatography, the boc-group of **65**-**79** was deprotected using 4 M HCl in 1,4-dioxane to provide **80**-**94**. If possible, the solid HCl-salt of the free amine was collected using suction filtration and washed to obtain the pure product, otherwise, the solvent was evaporated, and the intermediate was used without further purification. These amines were then coupled with the appropriate carboxylic acids using hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to obtain the final compounds **1**-**8**, **18**-**30**. Compounds **9**-**17**, **31**, and **32** were synthesized from **2**, **95**, and **96** by additionally employing Suzuki-coupling reactions with the respective boronic acids or pinacol esters.

Scheme 1. Synthesis of compounds **1-32**[a]



[a]Reagents and conditions: (a) *tert*-butyl (3-bromopropyl)carbamate, Cs_2CO_3, KI, acetone, rt, 72 h, 39%-69%; (b) 4 M HCl in 1,4-dioxane, rt, 1 h, 55%-99%; (c) R^3-CO_2H, DIPEA, HATU, dimethylformamide (DMF), rt, 16 h, 5%-89%; (d) respective boronic acid (pinacol ester for **10**), K_2CO_3, XPhos Pd G4, water/1,4-dioxane (1:4), 100°C, 16 h, 20%-79%.

The final compounds **33-43** were synthesized according to the synthetic Scheme 2. Compound **33** was synthesized from the coupling reaction of 5-phenylthiophene-2-carboxylic acid and *tert*-butyl (3-aminopropyl)carbamate in the presence of HATU and DIPEA in DMF. The boc-deprotection of **33** and subsequent reductive amination with benzaldehyde afforded compound **34**. Compounds **35-43** were obtained similarly to **33** in a short amide coupling reaction of 5-phenylthiophene-2-carboxylic acid with various amines in the presence of HATU and DIPEA in DMF. Some of these intermediate amines were commercially available, while compounds **108-113** were synthesized as outlined in Scheme S2. Compound **108** was synthesized starting with commercially available tetrahydroisoquinoline and employing a Finkelstein reaction and nucleophile substitution reaction with *tert*-butyl (3-bromopropyl)carbamate to give intermediate **103**. The deprotection of **103** gave the desired precursor amine as hydrochloride salt (**108**; Scheme S2, A, SI).

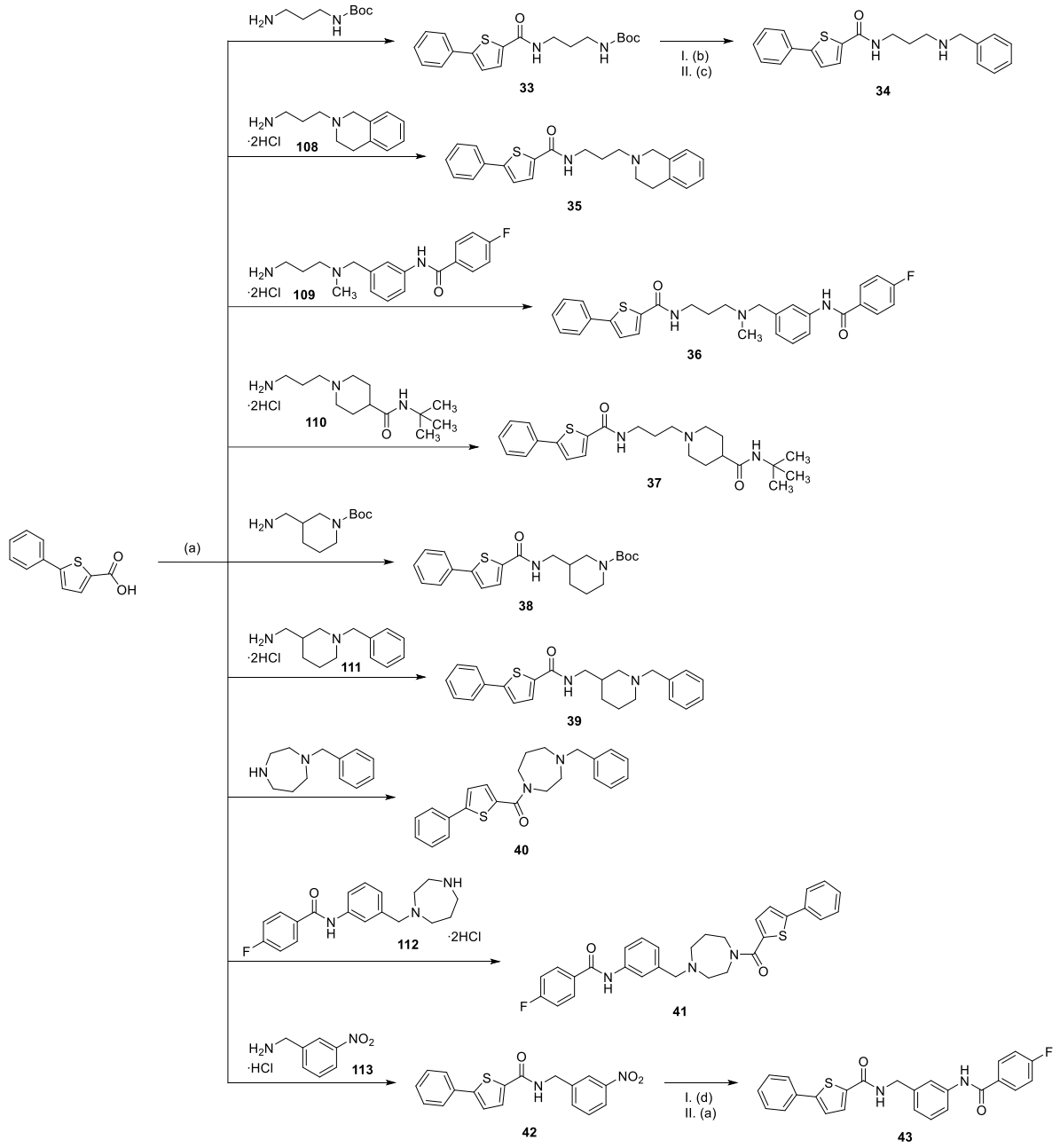
The synthesis of intermediates **109** and **112** shares the same synthetic route. Acetal-protected amino benzaldehyde was first coupled with 4-fluorobenzoic acid. The resulting amide **98** was deprotected and gave aldehyde **101**. Reductive amination of **101** with the respective amine (*tert*-butyl (3-(methylamino)propyl)carbamate for **109** and *tert*-butyl 1,4-diazepane-1-carboxylate for **112**) yielded boc-protected intermediates **104** and **107**. Deprotection gave the desired hydrochloride salts of the amine (**109** and **112**; Scheme S2, B, SI).

Amine **110** was prepared beginning with saponification of the *N*-boc-protected piperidine carboxylic acid ethyl ester (SI). Subsequently, an amide coupling reaction with the resulting carboxylic acid (**97**) and *tert*-butyl amine gave the intermediate **99**, which was deprotected with 4 M HCl in 1,4-dioxane to yield **102**. Finally, **102** was treated as described above: *in situ* Finkelstein reaction with nucleophile substitution (intermediate **105**) and boc-deprotection gave compound **110** (Scheme S2, C, SI).

Intermediate **111** was synthesized in 2 steps. At first, reductive amination of benzaldehyde with boc-protected 3-methylpiperidine gave the intermediate **106**. Subsequent boc-deprotection using 4 M HCl in 1,4-dioxane yielded the desired compound (Scheme S2, D, SI).

The precursor **113** was synthesized in multiple steps, starting with 1-(bromomethyl)-3-nitrobenzene and employing a Gabriel synthesis using the Ing-Manske procedure to create the desired primary amine. For easier handling, 4 M HCl in 1,4-dioxane was added to the product to precipitate the HCl salt of the amine (**113**; Scheme S2, E, SI). The precursor was then coupled with 5-phenylthiophene-2-carboxylic acid to give compound **42**. The nitro group in **42** was reduced and subsequently coupled with 4-fluoro benzoic acid to yield compound **43** (Scheme 2).

Scheme 2. Synthesis of compounds **33-43**[a]

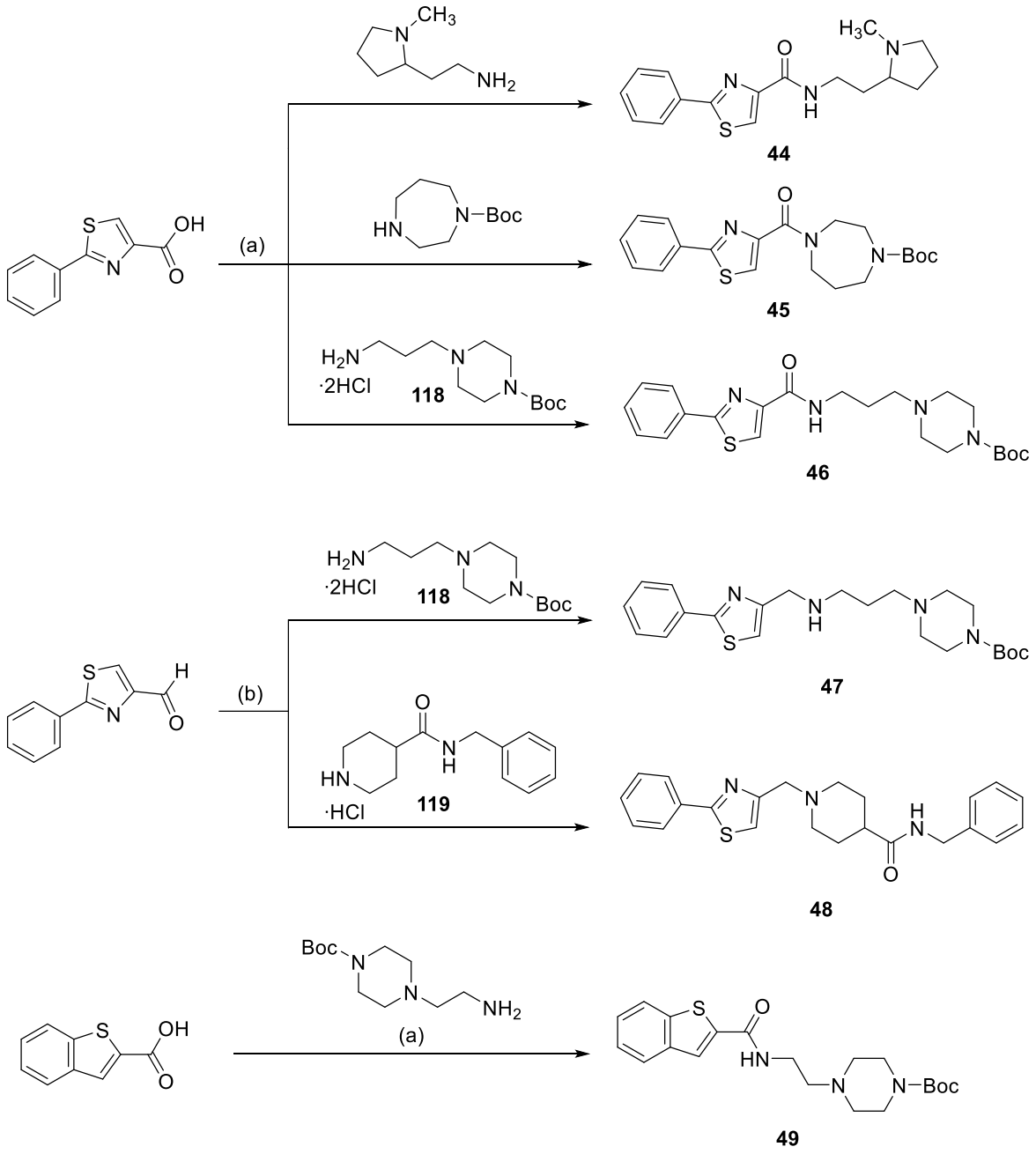


[a]Reagents and conditions: (a) DIPEA, HATU, DMF, rt, 16 h, 9%-quant.; (b) 4 M HCl in 1,4-dioxane, rt, 1 h, 99%; (c) benzaldehyde, $NaBH(OAc)_3$, Et_3N, DCE, rt, 16 h, 30%; (d) Pd/C 10%, H_2, EtOH, rt, 16 h.

Compounds **44** and **45** were synthesized in a similar manner by amide coupling of commercially available amines with 2-phenylthiazole-4-carboxylic acid as shown in Scheme

3. The precursor **118** for compounds **46** and **47** was synthesized in a 2-step route (Scheme S3, A). Nucleophile substitution of mono boc-protected piperazine and *N*-(3-bromopropyl)phthalimide gave intermediate **116**. Subsequent hydrazinolysis in ethanol yielded the desired intermediate **118**, which was either used in an amide coupling reaction to give compound **46** or in a reductive amination reaction to give compound **47** (Scheme 3). Similarly, compound **48** was generated with a reductive amination reaction of precursor **119** and phenylthiazole carbaldehyde using sodium triacetoxyborohydride and triethylamine in dichloroethane. The precursor **119** was synthesized from compound **97**, which was, in this case, coupled with benzylamine to yield compound **117**. Subsequent deprotection of **117** with HCl in 1,4-dioxane gave the desired intermediate **119** (Scheme S3, B, SI). Compound **49** was synthesized by employing a short one-step amide coupling reaction to benzo[*b*]thiophene-2-carboxylic acid with *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (Scheme 3).

Scheme 3. Synthesis of compounds **44-49**[a]



[a]Reagents and conditions: (a) DIPEA, HATU, DMF, rt, 16 h, 39%-83%; (b) $NaBH(OAc)_3$, AcOH, THF, rt, 16 h, 7% (for **47**) or $NaBH(OAc)_3$, Et_3N, DCE rt, 16 h, 71% (for **48**).

Structure-Activity Relationship Studies. Compound **I** (EC_{50} = 2.21 μM), which was reported by Yoshikawa *et al.* (Figure 2) (EC_{50} = 2.21 μM) was used as a starting compound for the structure-activity relationship studies.[39] First, we chose to remove the rigidity by deleting the

N-methylamide moiety from the thieno[3,2-*c*]quinoline part (head, structure A in Table 1). This led to a flexible phenylthiophene derivative **1** (EC_{50} = 0.416 μM), which improved the agonistic potency by ~5-fold in a β-arrestin recruitment assay. The replacement of phenyl at the thiophene core in **1** with 5-bromo led to a compound **2** (EC_{50} = 1.62 μM) with reduced activity. Attempts to fuse the phenyl moiety to thiophene in **1** reduced the activity in general, as exemplified by the benzothiophene (**3**, EC_{50} = 0.775 μM), indole (**4**, EC_{50} = 2.67 μM), and benzofuran (**5**, EC_{50} = 1.48 μM) derivatives. Also, efforts to change the 5-phenylthiophene unit in **1** to different aromatic structures, such as 4-fluorophenyl (**6**, EC_{50} = 17.0 μM), methyl phenylsulfane (**7**, EC_{50} = 8.09 μM), and 1-fluoro-2-ethylbenzene (**8**, EC_{50} = 6.42 μM) drastically reduced the agonistic activity compared to **1**. Therefore, our efforts were focused on investigating the role of the phenylthiophene moiety. The introduction of 4-methyl at the phenyl moiety in **1** resulted in compound **9** (EC_{50} = 0.576 μM) maintaining similar activity compared to **1**. However, sterically demanding substituents like 4-*tert*-butyl (**10**, EC_{50} = 10.23 μM) diminished activity. The electron-donating 4-methoxy (**11**, EC_{50} = 2.13 μM), 4-hydroxy (**11**, EC_{50} = 1.75 μM), or the replacement of the phenyl with pyridine (**12**, EC_{50} = 1.72 μM) showed only moderate agonistic potency ranging from 1.72 to 2.13 μM. Interestingly, smaller substituents like fluoro at the different positions of the phenyl ring in **1** were well tolerated and even slightly improved the agonistic potency in some cases (compounds **14**-**16**). For example, the 4-fluoro (**14**, EC_{50} = 0.387 μM) and 3-fluoro (**16**, EC_{50} = 0.267 μM) derivatives showed 1.09-1.56-fold improved potency compared to **1**. The 2-fluoro derivative (**15**, EC_{50} = 0.507 μM) exhibited similar, but the 3,5-difluoro derivative (**17**, EC_{50} = 0.758 μM) slightly reduced potency.

Summarizing our findings towards "head" optimization in **1** suggests that the 5-phenylthiophene moiety and the small substituents like fluoro on the phenyl unit are highly preferable for the binding to ACKR3.

Our next effort was to focus on the right-hand side (tail, structure B in Table 1) of molecule **1**. At first, a series of substituents was introduced at the different positions on the phenyl moiety of the tail. The rank order of potency for substituents at various positions of the phenyl ring is as follows: 3-fluoro (**22**, EC_{50} = 0.249 μM) > 2-fluoro (**21**, EC_{50} = 0.302 μM) > 4-fluoro (**20**, EC_{50} = 0.370 μM) > 4-bromo (**19**, EC_{50} = 0.668 μM) > 4-methoxy (**18**, EC_{50} = 0.930 μM). These results revealed that only the small fluoro substituent was well tolerated, especially the 3-fluoro substitution, which significantly improved the agonistic potency among other substitutions.

To obtain deeper insight into the SARs, we subsequently replaced *N*-methyl with hydrogen, several alkyls, arylalkyls, and hydroxy alkyls (see **23**-**30**, **34**). The rank order of potency for these substitutions is as follows: *N*-butyl (**27**, EC_{50} = 0.069 μM) > *N*-ethyl (**23**, EC_{50} = 0.111 μM) ~ *N*-propyl (**26**, EC_{50} = 0.111 μM) > 2-phenylethyl (**29**, EC_{50} = 0.479 μM) > 3-hydroxypropyl (**28**, EC_{50} = 0.571 μM) > cyclopropyl (**25**, EC_{50} = 0.630 μM) > 2-propyl (**24**, EC_{50} = 0.709 μM) > H (**34**, EC_{50} = 1.29 μM) > 3-phenylpropyl (**30**, EC_{50} = 1.64 μM). In the first place, these results revealed that *N*-substitution is important for enhancing the agonistic potency because the compound with free NH (**34**), being one of the least potent agonists, implies that there might be no need for a hydrogen donating bond interaction at this position. The compounds with ethyl (**23**) or *N*-propyl (**26**) improved agonistic activity 3.7-fold compared to **1**. The compound with *N*-butyl (**27**) was found to be optimal. In fact, compound **27** was found to be the best agonist in the present study, with an EC_{50} of 0.069 μM. The polar 3-hydroxypropyl (**28**) and 2-phenylethyl (**29**) showed similar levels of agonistic activity to **1**, but 3-phenylpropyl substitution reduced the activity. The steric cyclopropyl (**25**) and 2-propyl (**24**) substitutions did not show any beneficial activity.

The other *N*-ethylated compounds (**31**, EC_{50} = 0.334 μM and **32**, EC_{50} = 0.846 μM) also showed good to moderate agonistic activity compared to **1**. The replacement of *N*-benzyl with

tert-butyl abolished the activity (**33**), suggesting the bulky substituent is not favorable for activating ACKR3. Interestingly, the rigidification of *N*-ethyl to dihydroisoquinoline resulted in compound **35** (EC_{50} of 0.150 μM) having improved activity over **1** and similar activity as the corresponding *N*-methylated compound (**23**). Further expansion of the tail section as it in compounds **36** (EC_{50} = 1.1 μM) and **37** (EC_{50} = 2.5 μM) led to reduced activity.

In the next experiments, we focused on demonstrating the linker in the molecules (structure C, Table 1). First, the flexible propyl (C = 3) in **1** was rigidified to piperidine (**38**, EC_{50} = 4.677 μM and **39**, EC_{50} = 1.04 μM) or diazepane leading to a cell toxic compound (**40**, EC_{50} = n.d.) or abolished activity (**41**, EC_{50} > 50 μM). Shortening the length of the linker (C = 3 to C = 1) also resulted in reduced activity. See, for example, compounds **42** (EC_{50} = 0.790 μM) and **43** (EC_{50} = 3.35 μM). Other experiments, like changing the thiophene ring to the more polar thiazole derivatives (**44**-**48**; structure D, Table 1), showed only moderate agonistic activity with compound **48** reaching the maximum level of activity at an EC_{50} of 1.5 μM. Also, compound **49** (structure E, Table 1) with a short linker and the piperazine tail group dropped in agonistic potency.

Efficacy. The endogenous ligand of ACKR3, CXCL12, was set at 100% efficacy in β-arrestin recruitment assays to serve as the standard for the determination of each compound's efficacy. As shown in Table 1, most of the highly active compounds were found to be full agonists of ACKR3, with efficacies in the same range as CXCL12. For example, the highly active compounds **16**, **23**, **26**, **27**, and **35** showed efficacies ranging from 82% to 103% of the maximal effect observed for CXCL12. Agonists **1**, **3**, **9**, **17**, **18**, **19**, **20**, **21**, **22**, **24**, **28**, **29**, **31**, and **32**, also showed efficacies ranging from 73% to 96% compared to CXCL12, although their potency was slightly reduced compared to the above-mentioned compounds. For some compounds, the efficacy was not correlated to their potency, e.g., **6**-**8**, **37**, **44**, **47**, and **48**. Compounds **11**, **12**, **15**, **19**, **25**, **41**-**43**, and **46** showed moderate efficacies, ranging from 50% to 76%, suggesting

partial agonism with respect to CXCL12. Agonists **10**, **14**, **38**, and **49** had significantly lower efficacies among all, ranging from 18% to 46%. Concentration-response curves for the best ACKR3 agonists (**1**, **9**, **16**, **20**, **21**, **23**, **26**, **27**, **35**) in *β*-arrestin recruitment assays are shown in Figure 3.

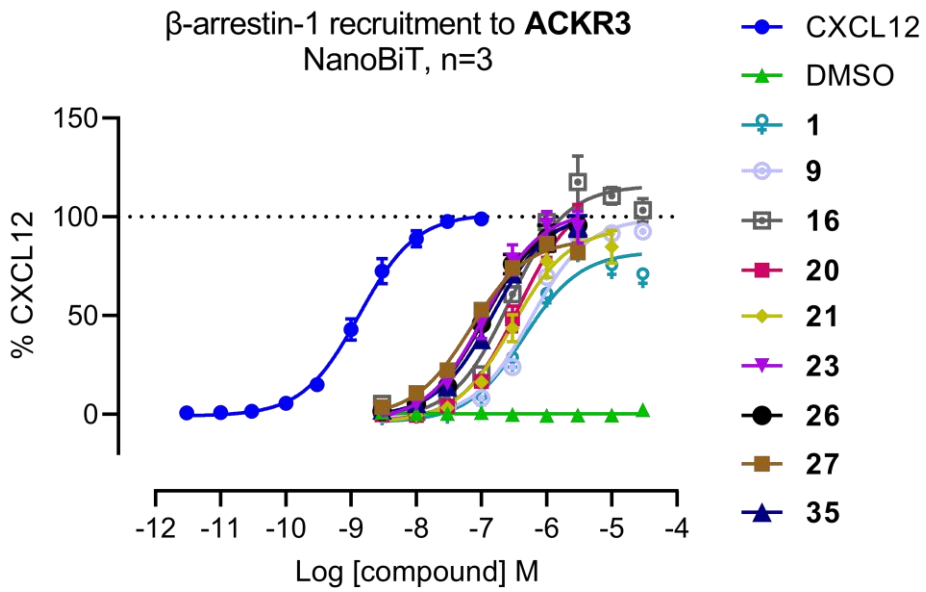


Figure 3. *β*-arrestin recruitment activity of selected potent compounds **1** (EC_{50} = 0.416 μM, E_{max} = 73%), **9** (EC_{50} = 0.576 μM, E_{max} = 93%), **16** (EC_{50} = 0.267 μM, E_{max} = 103%), **20** (EC_{50} = 0.370 μM, E_{max} = 96%), **21** (EC_{50} = 0.302 μM, E_{max} = 85%), **23** (EC_{50} = 0.111 μM, E_{max} = 95%), **26** (EC_{50} = 0.111 μM, E_{max} = 96%), **27** (EC_{50} = 0.069 μM, E_{max} = 82%), **35** (EC_{50} = 0.150 μM, E_{max} = 95%), and the positive control CXCL12 to ACKR3 using a NanoLuc complementation-based assay in HEK293T cells. The efficacy of CXCL12 at 300 nM was set as 100%. Data are the mean ± SEM (for details, see the Experimental Section).

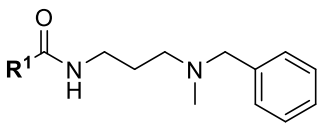
Platelet Degranulation Studies of Derivatives. To determine the functional activity of all active agonists on platelet modulation, reduction of P-selectin surface expression was tested. P-selectin is a surface protein involved in cell adhesion mechanisms and can be found on

activated platelets. After induction of CRP (0.5µg/ml)-induced platelet activation in platelet-rich plasma (PRP), treatment with the respective compound should therefore lead to a significant reduction in P-selectin expression. Fluorochrome-conjugated antibodies against P-selectin were used to determine surface expression compared to activated platelets (PRP). Compound **1** showed superior inhibition of platelet degranulation (P-selectin expression reduction = 76%) compared to VUF11207 (65%). Although structural changes of the "head" moiety (Structure A, Table 1) of **1** generally decreased its agonistic activity, functional activity was often significantly enhanced. For example, in some cases, the reduction in P-selectin expression reached 91%. In particular, compounds **3** (90%) and **9** (91%) demonstrated high platelet degranulation reduction. Compound **5** (35%) showed a weaker reduction in P-selectin expression compared to **4** (80%). However, both compounds showed moderate agonistic potency. The changes in the "tail" compartment showed a different trend in terms of platelet modulation activity. For example, the agnostic potency of some compounds did not transfer to their P-selectin expression reduction. Compounds **21**, **25**, **29**, and **35** showed weak P-selectin expression reduction despite their agonistic potency in the low micromolar range. This could probably be due to the fractions of the ligand trapped by plasma proteins of the PRP depending on the polarity of the compound. Indeed, several highly potent agonists exhibited excellent P-selectin expression reduction compared to the commercially available VUF11207 (P-selectin expression reduction = 65%), for example, compounds **23**, **26**, **27**, **28**, and **31**. The platelet degranulation potency was also highly sensitive toward "linker"-changes (Structure C, Table 1). Only compound **40** showed a good P-selectin expression reduction (76%), while the active compound **42** showed only 17%.

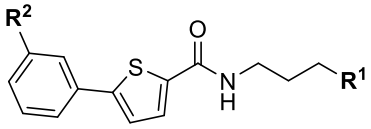
It is interesting to note that, after the replacement of 5-phenylthiophene with 5-phenylthiazole, the resulting derivatives showed excellent P-selectin expression reduction, despite their agonistic potency in the micromolar range. For example, the compound **47** reached 100%,

although its EC_{50} is only 4.2 μM. This suggested once again that the polarity of the compounds might be important for effective P-selectin expression reduction.

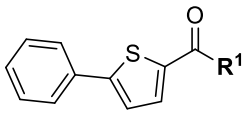
Table 1. Chemical structures, potencies, efficacies, and platelet degranulation modulation activity of **1**-**49** as ACKR3 agonists.



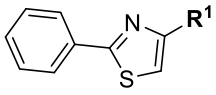
Structure A
1-17



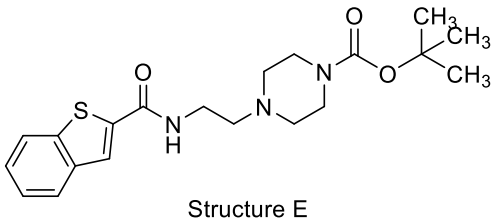
Structure B
18-37
31, **32**: R^2 = -F



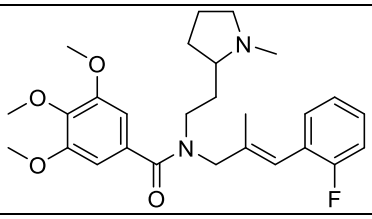
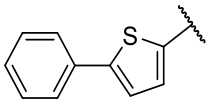
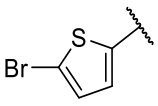
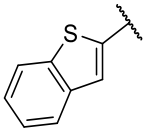
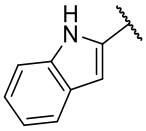
Structure C
38-43

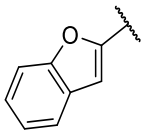
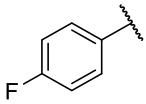
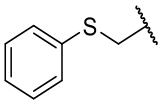
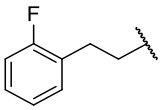
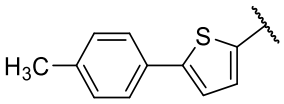
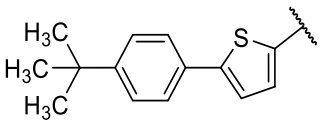
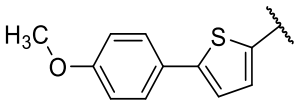
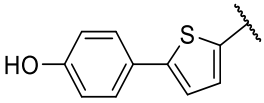
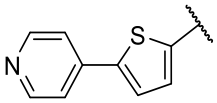
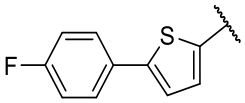
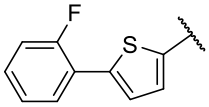
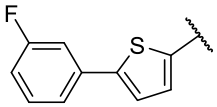
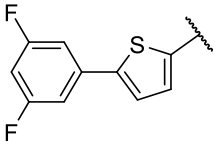


Structure D
44-48

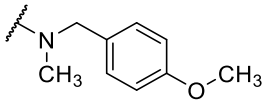
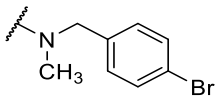
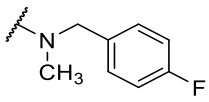
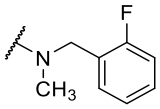
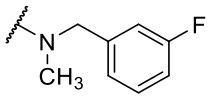
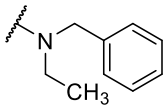
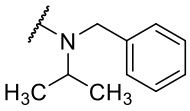
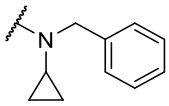
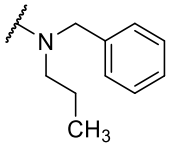
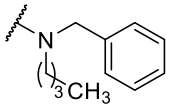
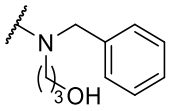
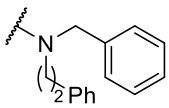
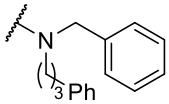
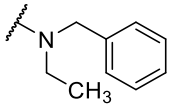


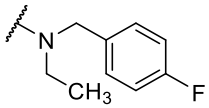
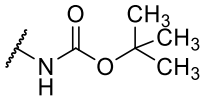
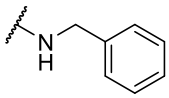
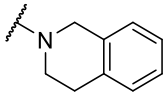
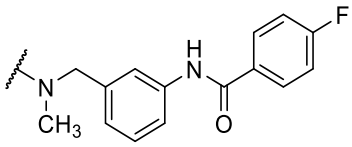
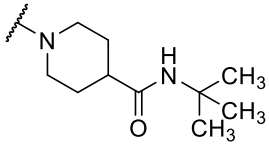
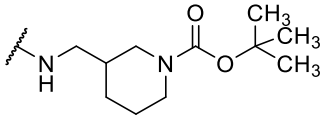
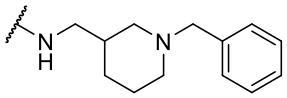
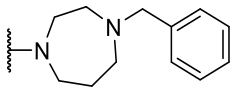
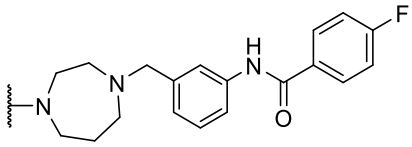
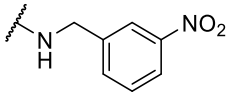
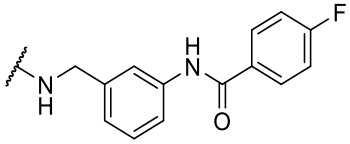
Structure E
49

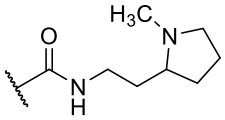
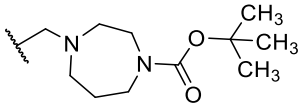
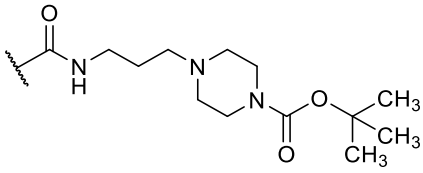
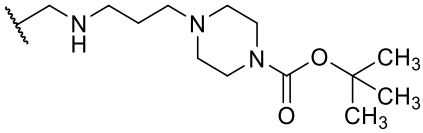
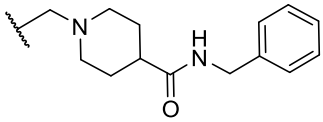
Compound	R^1	ACKR3		Flow cytometry
		β-arrestin recruitment EC_{50} (μM)[a]	Efficacy (E_{max}) *vs* CXCL12 (%)[b]	P-selectin expression reduction (%)[c]
Structure A: Head optimization				
VUF11207		0.044 [0.033–0.056]	95	65±6
1		0.416 [0.322–0.626]	73	76±10
2		1.62 [1.29–2.06]	80	–
3		0.775 [0.508–0.824]	89	91±10
4		2.67 [1.88–2.73]	81	85±14

5		1.48 [0.877–1.56]	83	35±4
6		17.0 [15.2–19.1]	80	–
7		8.09 [5.31–12.4]	99	–
8		6.42 [5.54–7.45]	90	–
9		0.576 [0.436–0.760]	93	99±5
10		10.23 [5.72–18.6]	46	–
11		2.13 [1.60–2.83]	70	–
12		1.75 [0.901–3.31]	64	–
13		1.72 [1.36–2.17]	80	–
14		0.382 [0.143–0.999]	33	95±9
15		0.507 [0.245–1.05]	62	78±12
16		0.267 [0.181–0.395]	103	92±4
17		0.758 [0.482–1.23]	86	89±5

Structure B: Tail optimization

18		0.930 [0.590–1.47]	93	89±5
19		0.668 [0.373–1.26]	76	98±4
20		0.370 [0.237–0.589]	96	94±6
21		0.302 [0.185–0.493]	85	43±10
22		0.249 [0.148–0.416]	81	72±14
23		0.111 [0.068–0.180]	95	95±6
24		0.709 [0.552–0.921]	84	94±4
25		0.630 [0.402–0.989]	64	17±7
26		0.111 [0.083–0.149]	96	80±8
27		0.069 [0.052–0.091]	82	80±7
28		0.571 [0.414–0.790]	87	89±4
29		0.479 [0.311–0.737]	84	1±8
30		1.64 [1.05–2.56]	81	1±5
31	 R^2 = -F	0.334 [0.267–0.416]	85	100±1

32	 R^2 = -F	0.846 [0.634–1.15]	78	100±1
33		no activity	–	0±4
34		1.29 [0.958–1.73]	112	99±1
35		0.150 [0.113–0.200]	95	29±10
36		1.1 [0.567–7.41]	86	–
37		2.5 [1.34–9.41]	102	–
Structure C: Linker optimization				
38		4.68 [2.44–9.28]	18	–
39		1.04 [0.748–1.46]	90	44±10
40		–*	–	73±6
41		> 50	50	–
42		0.790 [0.550–1.14]	67	19±4
43		3.35 [2.67–4.23]	53	–
Structure D: Thiazole derivates				

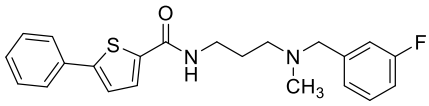
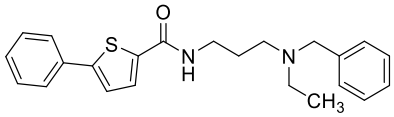
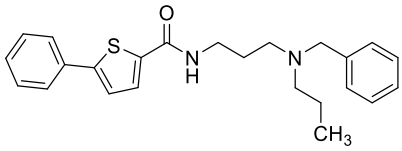
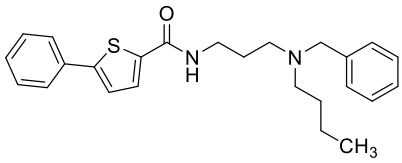
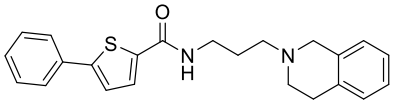
44		3.00 [2.48–3.64]	121	–
45		–*	–	89±5
46		4.96 [3.60–6.90]	65	86±2
47		4.20 [3.20–5.51]	89	101±2
48		1.1 [0.598–2.06]	110	–
Structure E: Shortened Linker				
49	for structure see above	7.37 [6.02–8.97]	25	72±12

[a]Average value of three independent experiments with 95% CI (for details, see the Experimental Section). [b]The efficacy of CXCL12 at 300 nM was set as 100%; average value of three independent experiments. [c]PRP was pre-incubated with compound (100 μM) and then treated with CRP-XL (1 μg/mL). Data are expressed as a percentage of P-selectin-MFI (% ± SEM) normalized to CRP-XL-treated vehicle PRP (DMSO 0.1%) and untreated control from at least three individual experiments in duplicate. Dash (–) = Not tested.*EC_{50} could not be calculated due to cell toxicity.

Selectivity studies. To assess selectivity, the most promising compounds (**22**, **23**, **26**, **27**, **35**) were chosen and examined in a β-arrestin recruitment assay for their ability to modulate closely related receptors including ACKR2, CXCR3, and CXCR4 (Table 2) We were particularly interested in CXCR4 selectivity, as it shares the endogenous ligand CXCL12 with ACKR3, which is known to be a scavenger receptor for CXCR4 signaling.[40] Also studies have shown

that CXCR4's activation often leads to physiological effects opposite to those of ACKR3.[24,41] As shown in Table 2 and Figure 4, none of the tested compounds showed agonistic activity against any specific receptors at the tested concentration of 10 μM and exhibited high selectivity towards ACKR3.

Table 2. Selectivity of ACKR3 agonists against ACKR2, CXCR3, and CXCR4.

		β-arrestin recruitment EC_{50} (μM)[a]			
Cmpd	Structure	ACKR3	ACKR2	CXCR3	CXCR4
22		0.249 [0.148–0.416]	> 10	> 10	> 10
23		0.111 [0.068–0.180]	> 10	> 10	> 10
26		0.111 [0.083–0.149]	> 10	> 10	> 10
27		0.069 [0.052–0.091]	> 10	> 10	> 10
35		0.150 [0.113–0.200]	> 10	> 10	> 10

[a]Average value of three independent experiments with 95% CI (for details, see the Experimental Section).

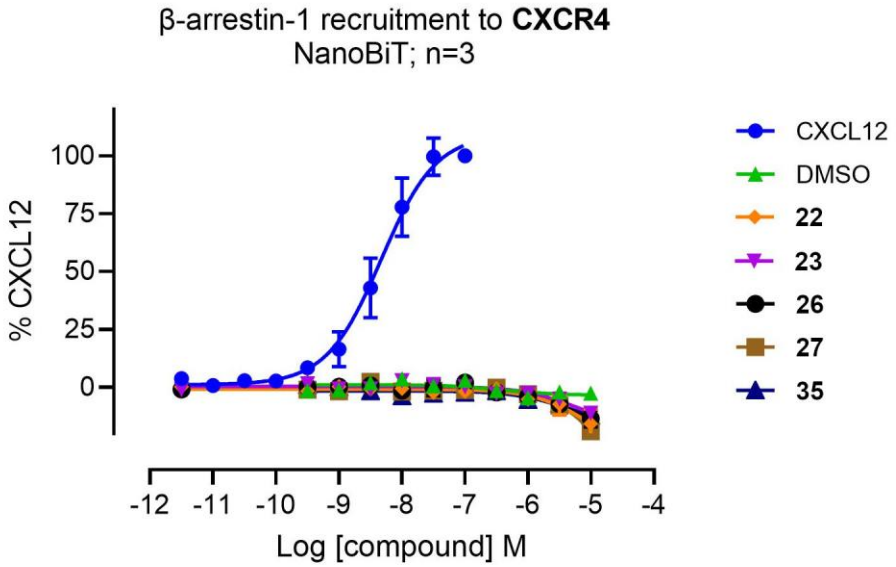


Figure 4. *β*-arrestin recruitment activity of selected most potent compounds **22**, **23**, **26**, **27**, **35** and the positive control CXCL12 to CXCR4 using a NanoLuc complementation-based assay in HEK293T cells. Data are the mean ± SEM (for details, see the Experimental Section).

Metabolic stability, cell viability, and solubility studies.

The most promising compound **27** was subjected to metabolic stability testing, as this is a crucial factor for subsequent *in vivo* studies. As shown in Figure 5, compound **27** was stable in human liver microsomes (HLMs) over the course of 60 min. Around 69% of compound **27** remained, while the rest was determined to be a mixture of different metabolites (Figure S1, SI). The most prominent metabolites were identified to be the de-benzylated compound, the hydroxylated metabolite and the de-alkylation of the *N*-butyl chain. Subsequently, we studied the metabolism of **27** in mouse liver microsomes (MLMs) (Figure S2, SI). The substance showed lower metabolic stability, with only 24% remaining after 60 min. To test whether metabolites still exhibit platelet activity, we synthesized the most promising de-alkylated metabolite, which is compound **34** (see Table 1). This compound still showed high functional

activity (P-selectin expression reduction = 99%) in flow cytometry experiments, although its agonistic activity was reduced.

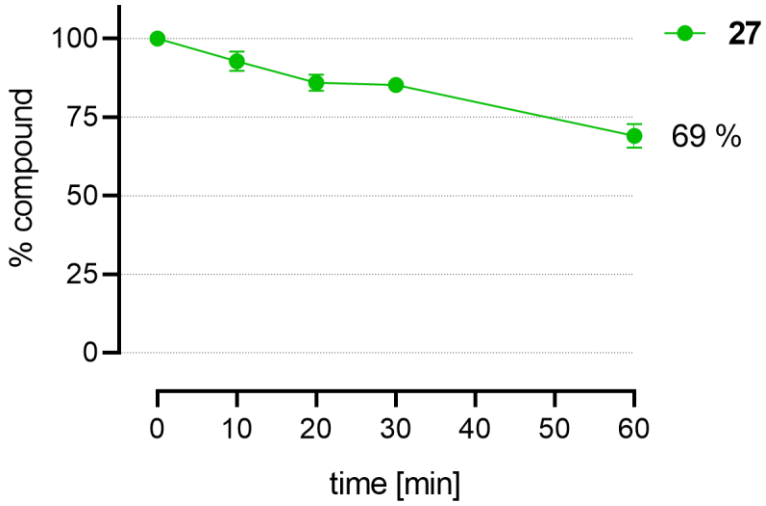


Figure 5. *In vitro* metabolic stability studies of compound **27** in human liver microsomes (20 mg/mL, male, pooled). The compound was tested at a concentration of 100 μM. Data are the mean ± ½ SD (for details, see the Experimental Section).

The potential cytotoxicity of compounds **23** and **27** was tested in cell viability assays on HEK293 cells. Both compounds were studied in different concentrations up to 1 mM. After several hours of incubation ranging from 1 to 72 h, the compounds were well tolerated by the cells at concentrations of 1 μM and below compared to VUF11207 (Figure S3, SI). This indicates that the compounds are not cytotoxic at effective concentrations.

For additional evaluations, we conducted kinetic solubility experiments on both lead compounds. Both compounds **23** and **27** were found to be moderately to well soluble in phosphate-buffered saline (PBS, pH 7.4), showing kinetic solubilities of 28 μM and 75 μM, respectively (Table S1, SI).

Platelet aggregation studies. Subsequently, agonists **23** and **27** were further evaluated for their functional activity by conducting ADP-induced platelet aggregation experiments. All tested compounds (**23**, Aggr. reduction = 65 ± 9%; **27**, Aggr. reduction = 40 ± 12%), including the reference compound VUF11207, showed a reduction in platelet aggregation compared to the control. Notably, compound **23** (Aggr. reduction = 65 ± 9%) showed the highest potency for platelet aggregation inhibition (Figure 6). Combined with its excellent potency in reducing P-selectin expression tested in the flow cytometry experiments, compound **23** shows great potential as a tool compound for further experiments.

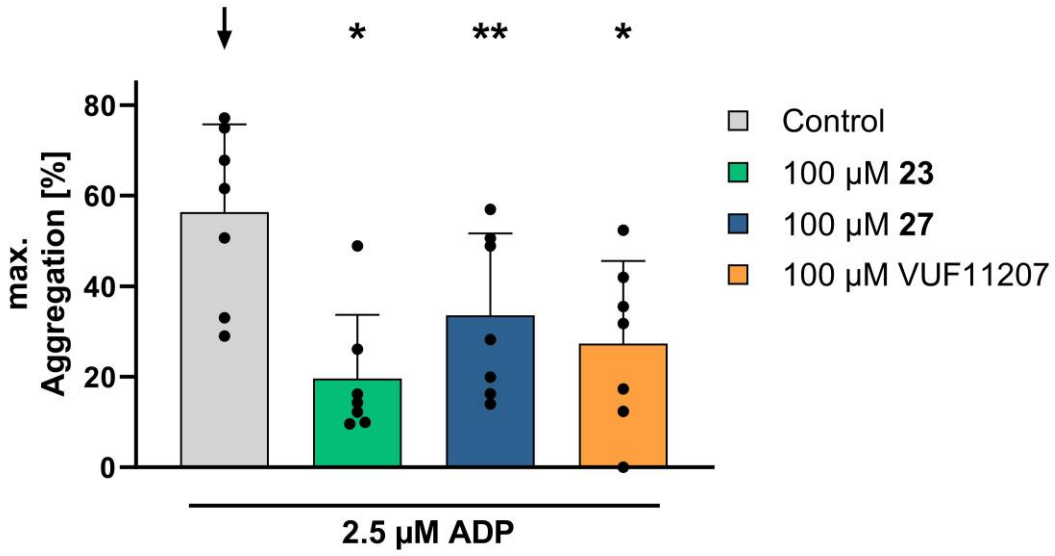


Figure 6. Aggregation of platelets as % of max. aggregation. 1 x 108 platelets were preincubated with respective agonist (100 µM, 15 min, 37 °C). Subsequently, platelets were activated with 2.5 µM ADP and aggregation was analyzed using a light transmission aggregometer (5 min at 1,000 rpm and 37 °C).

CONCLUSIONS

In conclusion, we designed and synthesized a series of 49 novel compounds and evaluated them as ACKR3 agonists in a β-arrestin recruitment assay and platelet modulators in a flow cytometry assay. The molecular design involved optimization efforts of three segments, starting with compound **I**, which showed weak ACKR3 agonistic activity (EC_{50} value of 2.21 μM). Structure-activity relationship studies led to the identification of highly active ACKR3 agonists **23** (EC_{50} = 0.111 μM), **26** (EC_{50} = 0.111 μM), and **27** (EC_{50} = 0.069 μM), which demonstrated excellent P-selectin expression reduction up to 97%. All three agonists are highly selective at ACKR3 versus other closely related receptors, including ACKR2, CXCR3, and CXCR4. ADP-induced platelet aggregation experiments demonstrated that compounds **23** and **27** exhibited potent inhibitory effects on platelet aggregation (65% and 40% aggregation reduction, respectively). Further experiments have shown that compound **27** exhibited metabolic stability, low cytotoxicity, and moderate kinetic solubility, rendering it a suitable tool compound for future *in vitro* and *in vivo* studies.

EXPERIMENTAL SECTION

Chemistry and Methods.

Starting materials, reagents, and (anhydrous) solvents were commercially available and purchased from a range of manufacturers and used without further purification. Thin-layer chromatography (TLC) with Macherey-Nagel precoated 60 F254 silica plates was used for reaction controls. TLC-spots were visualized either by ultraviolet (UV) light (254 nm/365 nm) or staining solutions. For purification purposes, flash column chromatography was carried out using Grace Davison Davisil LC60A (20–45 μm) or Merck Geduran Si60 (mesh 63–200 μm) using a LaFlash (VWR International GmbH, Darmstadt, Germany) automated flash chromatography system. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at ambient temperature. Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal control tetramethylsilane (TMS), and the spectra were calibrated against the residual solvent peak of the used deuterated solvent (either DMSO-*d6* or $CDCl_3$). Coupling constants (*J*) are expressed in hertz (Hz). The purity of all compounds was determined by RP-HPLC using an Agilent 1100 Series LC with a Phenomenex Luna C8 analytical column (150 x 4.6 mm, 5 μm) and detected by a UV DAD detector at 254 nm and 230 nm wavelength. Elution was carried out with the following gradient: [A = 0.01 M KH_2PO_4, pH 2.30, B = MeOH] 40% B to 85% B in 8 min, 85% B for 5 min, 85% to 40% B in 1 min, 40% B for 2 min, stop time 16 min, flow 1.5 mL/min. Mass spectra analysis were obtained from an Advion expression compact mass spectrometer (electrospray ionization, ESI) with a TLC plate reader system (using the following settings: ESI voltage 3.50 kV, capillary voltage 187 V, source voltage 44 V, capillary temperature 250 °C, desolvation gas temperature 250 °C, gas flow 5 L/min). High-resolution mass spectra (HRMS) were determined for final compounds by the mass spectrometry department, Institute of Organic Chemistry, Eberhard Karls University Tübingen on a Bruker maXis 4G ESI-TOF (Bruker Daltonik GmbH, Bremen, Germany). The

instrument was operated in ESI positive mode, and settings were as follows: nebulizer gas 1.2 bar, gas flow 6.0 L/min, source temperature 200 °C, capillary voltage +4500 V, end plate offset −500 V. The m/z range was from 80 to 1050 m/z. All final compounds are >95% pure determined by above mentioned HPLC procedure (Pages S84-S132, SI). Compound NMR spectra are accessible in the SI (Pages S35-S83). Synthesis of precursors is available in SI (Pages S5-S34).

Synthesis. *General Procedure I: Amide Coupling.* The solution of carboxylic acid and HATU (1.2 eq.) dissolved in dry DMF (2.5 mL) was stirred for 15 minutes. Amine (1 eq.) and DIPEA (3 eq.) were then added to the mixture, which was allowed to stir at room temperature overnight. After the reaction was completed, the mixture was diluted with DCM (10 mL) and washed with water (3 x 10 mL) and brine (3 x 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified via flash chromatography or suction filtration.

General Procedure II: Suzuki Coupling. To a vial containing the organohalide precursor in 1,4-dioxane (2 mL) and water (0.5 mL), the boronic acid or boronic acid pinacol ester (1.1 eq.), XPhos Pd G4 (0.05 eq.), and K_2CO_3 (3 eq.) were added. The mixture was degassed with argon and heated to 100 °C for 16 h. After completion of the reaction monitored by TLC, the mixture was diluted with EtOAc (10 mL) and washed with water (3x 10 mL) and brine (3x 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified via flash chromatography (SiO_2; DCM/MeOH).

General Procedure III: Reductive Amination. The carbaldehyde (1 - 1.1 eq.) was dissolved in THF or DCE (5 mL), and one drop of acetic acid or triethylamine was added to the solution.

After 15 min of continuous stirring, the amine was added and the mixture was allowed to stir for 1 h. The mixture was cooled to 0 °C and sodium triacetoxyborohydride (1.5 eq.) was added portion wise. After completion of the reaction, the mixture was diluted with EtOAc (10 mL) and washed with water (3x 10 mL) and brine (3x 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified via flash chromatography (SiO_2; DCM/MeOH).

N-(3-(Benzyl(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(1)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.84 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.84 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (2–7%). Yield: 150 mg (49%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.47 – 7.37 (m, 5H), 7.36 – 7.28 (m, 4H), 7.21 (d, *J* = 3.9 Hz, 1H), 3.74 (s, 2H), 3.56 (q, *J* = 5.9 Hz, 2H), 2.80 (t, *J* = 5.4 Hz, 2H), 2.42 (s, 3H), 1.92 (quint, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8, 149.1, 137.5, 135.5, 133.7, 130.0, 129.5, 129.2, 128.9, 128.6, 128.4, 126.2, 123.7, 62.7, 56.2, 40.7, 39.3, 25.2. HRMS (ESI+): calcd. *m/z* 364.16093 for $C_{22}H_{24}N_2OS$. Found 365.16845 $[M+H]^+$. TLC-MS (ESI+): 365.2 $[M+H]^+$. HPLC t_R = 5.78 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide ***(2)***. The title compound was prepared from 5-bromothiophene-2-carboxylic acid (1.04 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 1.04 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (3–7%). Yield: 235 mg (62%), pale brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.36 – 7.26 (m, 5H), 6.93 (dd, *J* = 12.9, 3.9 Hz, 2H), 3.54 (s, 2H), 3.50 (q, *J* = 5.3 Hz, 2H), 2.61 (t, *J* = 5.6 Hz,

2H), 2.27 (s, 3H), 1.77 (quint, J = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.9, 141.2, 138.0, 130.6, 129.6, 128.6, 127.8, 127.7, 117.3, 77.5, 77.2, 76.8, 63.4, 57.4, 41.6, 40.7, 25.1. HRMS (ESI+): calcd. *m/z* 366.04015/368.03810 for $C_{16}H_{19}BrN_2OS$. Found 367.04773/369.04569 $[M+H]^+$. TLC-MS (ESI+): 367.1/369.2 $[M+H]^+$. HPLC t_R = 4.71 min.

*N-(3-(Benzyl(methyl)amino)propyl)benzo[b]thiophene-2-carboxamide (**3**).* The title compound was prepared from benzo[*b*]thiophene-2-carboxylic acid (0.6 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.6 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (2–5%). Yield: 73 mg (36%), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.71 (d, J = 7.3 Hz, 1H), 7.60 (s, 1H), 7.44 – 7.33 (m, 4H), 7.34 – 7.27 (m, 3H), 3.70 (s, 2H), 3.58 (q, J = 5.3 Hz, 2H), 2.77 (t, J = 5.7 Hz, 2H), 2.39 (s, 3H), 1.91 (quint, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7, 141.0, 139.4, 139.1, 136.1, 130.0, 128.8, 128.2, 126.2, 125.2, 125.1, 124.8, 122.7, 62.8, 56.5, 41.0, 39.9, 25.0. HRMS (ESI+): calcd. *m/z* 338.14528 for $C_{20}H_{22}N_2OS$. Found 339.15304 $[M+H]^+$. TLC-MS (ESI+): 339.2 $[M+H]^+$. HPLC t_R = 4.07 min.

*N-(3-(Benzyl(methyl)amino)propyl)-1H-indole-2-carboxamide (**4**).* The title compound was prepared from 1*H*-indole-2-carboxylic acid (0.84 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.84 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (2–7%). Yield: 151 mg (56%), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 8.20 (s, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.32 – 7.22 (m, 5H), 7.22 – 7.14 (m, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.55 (s, 1H), 3.56 (s, 2H), 3.52 (quint, J = 5.9 Hz, 2H), 2.63 (t, J = 5.7 Hz, 2H), 2.26 (s, 3H), 1.78 (quint, J = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 136.9, 136.5, 131.1,

129.9, 128.8, 128.0, 127.8, 124.3, 122.0, 120.5, 112.2, 102.6, 63.0, 56.9, 41.1, 39.6, 25.2. HRMS (ESI+): calcd. *m/z* 321.18411 for $C_{20}H_{23}N_3O$. Found 322.19178 $[M+H]^+$. TLC-MS: (ESI+) 322.2 $[M+H]^+$. HPLC t_R = 1.09 min.

*N-(3-(Benzyl(methyl)amino)propyl)benzofuran-2-carboxamide (**5**).* The title compound was prepared from benzofuran-2-carboxylic acid (0.16 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.16 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–4%). Yield: 15 mg (30%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.39 – 7.27 (m, 4H), 7.26 – 7.09 (m, 5H), 3.57 (s, 2H), 3.52 (q, *J* = 5.7 Hz, 2H), 2.60 (t, *J* = 5.9 Hz, 2H), 2.26 (s, 3H), 1.81 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 154.9, 149.3, 137.6, 129.6, 128.7, 127.8, 127.7, 126.7, 123.7, 122.7, 111.9, 110.0, 63.0, 56.4, 41.6, 39.2, 25.7. HRMS (ESI+): calcd. *m/z* 322.16813 for $C_{20}H_{22}N_2O_2$. Found 323.17569 $[M+H]^+$. TLC-MS (ESI+): 323.2 $[M+H]^+$. HPLC t_R = 4.12 min.

*N-(3-(Benzyl(methyl)amino)propyl)-4-fluorobenzamide (**6**).* The title compound was prepared from 4-fluorobenzoic acid (0.33 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.33 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (3–7%). Yield: 41 mg (41%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.72 – 7.60 (m, 2H), 7.31 – 7.18 (m, 5H), 6.99 (t, *J* = 8.6 Hz, 2H), 3.63 – 3.46 (m, 4H), 2.64 (t, *J* = 5.7 Hz, 2H), 2.26 (s, 3H), 1.82 (quint, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 164.6 (d, *J* = 251.0 Hz), 138.0, 130.9 (d, *J* = 3.1 Hz), 129.5, 129.4, 129.3, 128.6, 127.6, 115.5, 115.3, 63.2, 57.2, 41.5, 40.5, 25.3. HRMS (ESI+): calcd. *m/z* 300.16379 for $C_{18}H_{21}FN_2O$. Found 301.17125 $[M+H]^+$. TLC-MS (ESI+): 301.2 $[M+H]^+$. HPLC t_R = 3.62 min.

*N-(3-(Benzyl(methyl)amino)propyl)-2-(phenylthio)acetamide (**7**).* The title compound was prepared from 2-(phenylthio)acetic acid (0.36 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 0.36 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 28 mg (24%), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.32 – 7.13 (m, 10H), 3.59 (s, 2H), 3.41 (s, 2H), 3.30 (q, *J* = 6.3 Hz, 2H), 2.32 (t, *J* = 6.3 Hz, 2H), 2.09 (s, 3H), 1.60 (quint, *J* = 6.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 138.3, 135.3, 129.3, 129.3, 128.4, 127.6, 127.3, 126.4, 62.8, 55.3, 41.9, 39.1, 37.1, 26.0. HRMS (ESI+): calcd. *m/z* 328.16093 for $C_{19}H_{24}N_2OS$. Found 329.16856 $[M+H]^+$. TLC-MS (ESI+): 329.2 $[M+H]^+$. HPLC t_R = 4.08 min

*N-(3-(Benzyl(methyl)amino)propyl)-3-(2-fluorophenyl)propanamide (**8**).* The title compound was prepared from 3-(2-fluorophenyl)propanoic acid (1.67 mmol) and N^1-benzyl-N^1-methylpropane-1,3-diamine dihydrochloride (**81**, 1.67 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (2–6%). Yield: 470 mg (86%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.19 (m, 5H), 7.19 – 7.09 (m, 2H), 7.04 – 6.89 (m, 2H), 6.72 (s, 1H), 3.47 (s, 2H), 3.25 (q, *J* = 5.7 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.42 (t, *J* = 6.1 Hz, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 1.61 (quint, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3, 161.2 (d, *J* = 245.0 Hz), 137.7, 130.8 (d, *J* = 4.9 Hz), 129.3, 128.6, 128.1 (d, *J* = 8.1 Hz), 127.9, 127.7 (d, *J* = 4.6 Hz), 124.2, 124.2, 115.3 (d, *J* = 22.0 Hz), 62.6, 55.4, 41.5, 38.6, 36.8, 36.8, 25.7, 25.3, 25.3. HRMS (ESI+): calcd. *m/z* 328.19509 for $C_{20}H_{25}FN_2O$. Found 329.20254 $[M+H]^+$. TLC-MS (ESI+): 329.3 $[M+H]^+$. HPLC t_R = 4.51 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(p-tolyl)thiophene-2-carboxamide ***(9)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.22 mmol) and *p*-tolylboronic acid (0.24 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (4–8%). Yield: 30 mg (37%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.29 – 7.20 (m, 9H), 6.92 (d, *J* = 3.8 Hz, 1H), 3.56 – 3.48 (m, 4H), 2.58 (t, *J* = 5.7 Hz, 2H), 2.39 (s, 3H), 2.27 (s, 3H), 1.78 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 147.4, 138.9, 138.3, 136.2, 133.6, 131.0, 130.4, 129.6, 128.5, 128.0, 127.5, 126.9, 126.1, 63.3, 57.0, 41.8, 40.4, 25.4, 21.2. HRMS (ESI+): calcd. *m/z* 378.17658 for $C_{23}H_{26}N_2OS$. Found 379.18445 $[M+H]^+$. TLC-MS (ESI+): 379.2 $[M+H]^+$. HPLC t_R = 6.24 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(4-(tert-butyl)phenyl)thiophene-2-carboxamide ***(10)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.22 mmol) and (4-(*tert*-butyl)phenyl)boronic acid (0.25 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (6–10%). Yield: 27 mg (29%), brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.43 (dd, *J* = 45.9, 8.4 Hz, 4H), 7.28 – 7.20 (m, 6H), 7.11 (d, *J* = 3.8 Hz, 1H), 3.54 – 3.46 (m, 4H), 2.57 (t, *J* = 5.8 Hz, 2H), 2.25 (s, 3H), 1.76 (quint, *J* = 6.0 Hz, 2H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 151.7, 148.5, 138.2, 137.9, 131.0, 129.6, 128.9, 128.6, 127.5, 126.0, 125.9, 123.0, 63.3, 57.1, 41.7, 40.5, 34.8, 31.3, 25.4. HRMS (ESI+): calcd. *m/z* 420.22353 for $C_{26}H_{32}N_2OS$. Found 421.23131 $[M+H]^+$. TLC-MS (ESI+): 421.2 $[M+H]^+$. HPLC t_R = 7.97 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(4-methoxyphenyl)thiophene-2-carboxamide ***(11)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.19 mmol) and (4-methoxyphenyl)boronic acid (0.21 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (4–8%). Yield: 57 mg (78%), yellow-brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.53 – 7.39 (m, 2H), 7.30 – 7.21 (m, 5H), 7.20 (d, *J* = 3.8 Hz, 1H), 7.01 (d, *J* = 3.8 Hz, 1H), 6.93 – 6.82 (m, 2H), 3.78 (s, 3H), 3.55 – 3.45 (m, 4H), 2.55 (t, *J* = 5.7 Hz, 2H), 2.23 (s, 3H), 1.74 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 160.0, 148.4, 138.3, 137.3, 129.6, 128.9, 128.5, 127.5, 126.7, 122.3, 114.5, 63.3, 57.1, 55.5, 41.8, 40.4, 25.5. HRMS (ESI+): calcd. *m/z* 394.17150 for $C_{23}H_{26}N_2O_2S$. Found 395.17891 $[M+H]^+$. TLC-MS (ESI+): 395.2 $[M+H]^+$. HPLC t_R = 5.55 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(4-hydroxyphenyl)thiophene-2-carboxamide ***(12)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.21 mmol) and (4-hydroxyphenyl)boronic acid (0.23 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (5–8%). Yield: 21 mg (26%), brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.37 (d, *J* = 10.8 Hz, 2H), 7.28 – 7.17 (m, 6H), 6.99 (d, *J* = 3.8 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.36 (s, 1H), 3.56 (s, 2H), 3.51 (q, *J* = 5.1 Hz, 2H), 2.61 (t, *J* = 5.8 Hz, 2H), 2.27 (s, 3H), 1.80 (quint, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.6, 157.6, 149.4, 137.3, 136.1, 129.8, 129.5, 128.6, 127.8, 127.6, 125.6, 122.1, 116.3, 63.0, 56.8, 41.5, 40.4, 25.2. HRMS (ESI+): calcd. *m/z* 380.15585 for $C_{22}H_{24}N_2O_2S$. Found 381.16359 $[M+H]^+$. TLC-MS (ESI+): 381.2 $[M+H]^+$. HPLC t_R = 4.04 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(pyridin-4-yl)thiophene-2-carboxamide ***(13)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.27 mmol) and pyridin-4-ylboronic acid hydrate (0.3 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (5–8%). Yield: 36 mg (36%), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 5.6 Hz, 2H), 8.35 (s, 1H), 7.44 – 7.36 (m, 2H), 7.30 (d, *J* = 4.5 Hz, 1H), 7.28 – 7.23 (m, 5H), 7.21 (d, *J* = 3.9 Hz, 1H), 3.57 – 3.49 (m, 4H), 2.61 (t, *J* = 5.6 Hz, 2H), 2.25 (s, 3H), 1.78 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.2, 150.6, 144.6, 140.9, 140.8, 138.2, 129.7, 128.7, 128.6, 127.6, 125.5, 120.1, 63.4, 57.6, 41.7, 40.8, 25.1. TLC-MS (ESI+): calcd. *m/z* 365.16 for $C_{21}H_{23}N_3OS$. Found 366.2 $[M+H]^+$. HPLC t_R = 1.64 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(4-fluorophenyl)thiophene-2-carboxamide ***(14)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.26 mmol) and 2-(4-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.29 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (2–6%). Yield: 20 mg (20%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.59 – 7.49 (m, 2H), 7.33 – 7.27 (m, 5H), 7.24 (d, *J* = 3.7 Hz, 1H), 7.14 – 7.02 (m, 3H), 3.59 – 3.51 (m, 4H), 2.63 (t, *J* = 5.7 Hz, 2H), 2.29 (s, 3H), 1.81 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (d, *J* = 248.4 Hz), 161.8, 147.2, 138.5, 138.1, 130.1 (d, *J* = 3.4 Hz), 129.7, 128.8, 128.6, 128.0, 127.9, 127.6, 123.4, 116.2, 116.0, 63.3, 57.3, 41.6, 40.5, 25.3. HRMS (ESI+): calcd. *m/z* 382.15151 for $C_{22}H_{23}FN_2OS$. Found 383.15948 $[M+H]^+$. TLC-MS (ESI+): 383.2 $[M+H]^+$. HPLC t_R = 5.96 min.

*N-(3-(Benzyl(methyl)amino)propyl)-5-(2-fluorophenyl)thiophene-2-carboxamide (**15**).* The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.22 mmol) and (2-fluorophenyl)boronic acid (0.24 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (2–6%). Yield: 20 mg (24%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.63 – 7.49 (m, 1H), 7.33 – 7.29 (m, 1H), 7.29 – 7.20 (m, 7H), 7.17 – 7.06 (m, 2H), 3.59 – 3.44 (m, 4H), 2.58 (t, *J* = 5.6 Hz, 2H), 2.26 (s, 3H), 1.77 (quint, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 159.3 (d, *J* = 251.2 Hz), 141.1 (d, *J* = 3.5 Hz), 139.2 (d, *J* = 4.0 Hz), 138.0, 129.7, 129.7, 129.6, 129.0 (d, *J* = 3.1 Hz), 128.6, 128.5, 127.6, 126.7 (d, *J* = 7.1 Hz), 124.7 (d, *J* = 3.6 Hz), 121.8 (d, *J* = 12.5 Hz), 116.6 (d, *J* = 22.4 Hz), 63.2, 57.0, 41.7, 40.4, 25.3. TLC-MS (ESI+): calcd. *m/z* 382.15 for $C_{22}H_{23}FN_2OS$. Found 383.2 $[M+H]^+$. HPLC t_R = 6.04 min.

*N-(3-(Benzyl(methyl)amino)propyl)-5-(3-fluorophenyl)thiophene-2-carboxamide (**16**).* The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.22 mmol) and (3-fluorophenyl)boronic acid (0.24 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (4–8%). Yield: 63 mg (79%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.34 – 7.29 (m, 2H), 7.29 – 7.22 (m, 6H), 7.21 (d, *J* = 3.9 Hz, 1H), 7.13 (d, *J* = 3.9 Hz, 1H), 7.03 – 6.93 (m, 1H), 3.54 – 3.49 (m, 4H), 2.58 (t, *J* = 5.6 Hz, 2H), 2.24 (s, 3H), 1.76 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2 (d, *J* = 246.5 Hz), 161.6, 146.7 (d, *J* = 2.7 Hz), 139.2, 138.3, 135.9 (d, *J* = 8.2 Hz), 130.7 (d, *J* = 8.6 Hz), 129.6, 128.8, 128.6, 127.5, 124.1, 121.9 (d, *J* = 2.9 Hz), 115.2 (d, *J* = 21.3 Hz), 113.0 (d, *J* = 23.0 Hz), 63.4, 57.4, 41.7, 40.6, 25.4. HRMS (ESI+): calcd. *m/z* 382.15151 for $C_{22}H_{23}FN_2OS$. Found 383.15942 $[M+H]^+$. TLC-MS (ESI+): 383.2 $[M+H]^+$. HPLC t_R = 5.94 min.

N-(3-(Benzyl(methyl)amino)propyl)-5-(3,5-difluorophenyl)thiophene-2-carboxamide ***(17)***. The title compound was prepared from *N*-(3-(benzyl(methyl)amino)propyl)-5-bromothiophene-2-carboxamide (**2**, 0.19 mmol) and (3,5-difluorophenyl)boronic acid (0.21 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (2–7%). Yield: 26 mg (34%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.33 – 7.26 (m, 5H), 7.18 (dd, *J* = 18.3, 3.9 Hz, 2H), 7.10 – 7.01 (m, 2H), 6.82 – 6.72 (m, 1H), 3.59 – 3.50 (m, 4H), 2.63 (t, *J* = 5.6 Hz, 2H), 2.28 (s, 3H), 1.80 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.4 (dd, *J* = 249.0, 13.2 Hz), 161.4, 145.4 (t, *J* = 3.0 Hz), 139.8, 138.3, 136.8 (t, *J* = 10.3 Hz), 129.7, 128.8, 128.6, 127.7, 124.7, 109.0 (dd, *J* = 26.7, 7.6 Hz), 103.6 (t, *J* = 25.5 Hz), 63.5, 57.7, 41.7, 40.9, 25.1. TLC-MS (ESI+): calcd. *m/z* 400.14 for $C_{22}H_{22}F_2N_2OS$. Found 401.2 $[M+H]^+$. HPLC t_R = 6.63 min.

N-(3-((4-Methoxybenzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(18)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.29 mmol) and N^1-(4-methoxybenzyl)-N^1-methylpropane-1,3-diamine dihydrochloride (**82**, 0.29 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 33 mg (28%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.61 – 7.55 (m, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.28 (m, 2H), 7.25 – 7.21 (m, 2H), 7.19 (d, *J* = 3.9 Hz, 1H), 6.87 – 6.79 (m, 2H), 3.74 (s, 3H), 3.59 – 3.49 (m, 4H), 2.64 (t, *J* = 5.7 Hz, 2H), 2.31 (s, 3H), 1.83 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 159.2, 148.4, 133.8, 131.2, 131.0, 129.2, 129.2, 129.0, 128.5, 126.2, 123.4, 114.0, 62.5, 55.3, 29.8, 25.1, 22.8, 14.3. HRMS (ESI+): calcd. *m/z* 394.17150 for $C_{23}H_{26}N_2O_2S$. Found 395.17939 $[M+H]^+$. TLC-MS (ESI+): 395.2 $[M+H]^+$. HPLC t_R = 6.07 min.

*N-(3-((4-Bromobenzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**19**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.29 mmol) and N^1-(4-bromobenzyl)-N^1-methylpropane-1,3-diamine dihydrochloride (**83**, 0.29 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 63 mg (48%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (t, *J* = 4.6 Hz, 1H), 7.59 – 7.51 (m, 2H), 7.43 – 7.33 (m, 4H), 7.32 – 7.26 (m, 2H), 7.20 – 7.13 (m, 3H), 3.57 – 3.42 (m, 4H), 2.58 (t, *J* = 5.9 Hz, 2H), 2.25 (s, 3H), 1.78 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.0, 148.5, 137.9, 136.9, 133.6, 131.7, 131.2, 129.1, 129.1, 128.5, 126.1, 123.5, 121.5, 62.4, 56.8, 41.5, 40.1, 25.4. HRMS (ESI+): calcd. *m/z* 442.07/444.07 for $C_{22}H_{23}BrN_2OS$. Found 443.07891/445.07696 $[M+H]^+$. TLC-MS (ESI+): 465.1 $[M+Na]^+$. HPLC t_R = 6.86 min.

*N-(3-((4-Fluorobenzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**20**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.44 mmol) and N^1-(4-fluorobenzyl)-N^1-methylpropane-1,3-diamine dihydrochloride (**84**, 0.44 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–6%). Yield: 90 mg (53%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.54 (d, *J* = 9.6 Hz, 2H), 7.40 – 7.33 (m, 2H), 7.33 – 7.29 (m, 2H), 7.28 – 7.23 (m, 2H), 7.17 (d, *J* = 3.8 Hz, 1H), 6.96 (t, *J* = 8.6 Hz, 2H), 3.54 – 3.48 (m, 4H), 2.62 (t, *J* = 12.1 Hz, 2H), 2.26 (s, 3H), 1.79 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.3 (d, *J* = 245.9 Hz), 162.1, 148.5, 137.9, 133.6, 133.5 (d, *J* = 3.6 Hz), 131.2 (d, *J* = 8.1 Hz), 129.1, 129.1, 128.5, 126.1, 123.4, 115.4 (d, *J* = 21.3 Hz), 62.2, 56.8, 41.3, 40.1, 25.3. HRMS (ESI+): calcd. *m/z* 382.15151 for $C_{22}H_{23}FN_2OS$. Found 383.15955 $[M+H]^+$. TLC-MS (ESI+): 383.2 $[M+H]^+$. HPLC t_R = 6.09 min.

*N-(3-((2-Fluorobenzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**21**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.29 mmol) and N^1-(2-fluorobenzyl)-N^1-methylpropane-1,3-diamine dihydrochloride (**85**, 0.29 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 100 mg (89%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (t, *J* = 4.7 Hz, 1H), 7.56 – 7.52 (m, 2H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 3H), 7.28 – 7.21 (m, 1H), 7.15 (d, *J* = 3.9 Hz, 1H), 7.10 – 7.04 (m, 1H), 7.04 – 6.97 (m, 1H), 3.66 (s, 2H), 3.51 (q, *J* = 5.4 Hz, 2H), 2.69 (t, *J* = 5.7 Hz, 2H), 2.31 (s, 3H), 1.82 (quint, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 161.6 (d, *J* = 247.0 Hz), 148.4, 138.1, 133.7, 132.2 (d, *J* = 4.3 Hz), 129.8 (d, *J* = 8.3 Hz), 129.1, 128.9, 128.4, 126.1, 124.2 (d, *J* = 3.6 Hz), 123.4, 115.7 (d, *J* = 22.0 Hz), 57.0, 55.7 (d, *J* = 1.5 Hz), 41.2, 40.2, 25.2. HRMS (ESI+): calcd. *m/z* 382.15151 for $C_{22}H_{23}FN_2OS$. Found 383.15920 $[M+H]^+$. TLC-MS (ESI+): 383.2 $[M+H]^+$. HPLC t_R = 5.90 min.

*N-(3-((3-Fluorobenzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**22**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.29 mmol) and N^1-(3-fluorobenzyl)-N^1-methylpropane-1,3-diamine dihydrochloride (**86**, 0.29 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 33 mg (29%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (t, *J* = 4.4 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.41 – 7.34 (m, 2H), 7.33 – 7.28 (m, 2H), 7.25 – 7.16 (m, 2H), 7.11 – 7.00 (m, 2H), 6.98 – 6.90 (m, 1H), 3.60 – 3.49 (m, 4H), 2.54 (t, *J* = 5.9 Hz, 2H), 2.27 (s, 3H), 1.74 (quint, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0 (d, *J* = 246.2 Hz), 162.0, 148.5, 140.7 (d, *J* = 5.6 Hz), 138.0, 133.7, 130.1 (d, *J* = 8.1 Hz), 129.1, 129.0, 128.5, 126.2, 125.1 (d, *J* = 2.9 Hz), 123.5, 116.3 (d, *J* = 21.1 Hz), 114.5 (d, *J* = 21.0 Hz), 62.7 (d, *J* =

1.8 Hz), 57.0, 41.6, 40.2, 25.5. HRMS (ESI+): calcd. *m/z* 382.15151 for $C_{22}H_{23}FN_2OS$. Found 383.15913 $[M+H]^+$. TLC-MS (ESI+): 383.2 $[M+H]^+$. HPLC t_R = 6.16 min.

N-(3-(Benzyl(ethyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(23)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.44 mmol) and N^1-benzyl-N^1-ethylpropane-1,3-diamine dihydrochloride (**87**, 0.44 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–6%). Yield: 70 mg (42%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.61 – 7.52 (m, 2H), 7.39 – 7.33 (m, 2H), 7.32 – 7.26 (m, 4H), 7.26 – 7.21 (m, 3H), 7.16 (d, *J* = 3.9 Hz, 1H), 3.58 (s, 2H), 3.47 (q, *J* = 3.9 Hz, 2H), 2.66 – 2.52 (m, 4H), 1.75 (quint, *J* = 5.9 Hz, 2H), 1.08 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 148.3, 138.5, 138.3, 133.8, 129.5, 129.1, 128.8, 128.5, 128.4, 127.4, 126.1, 123.3, 58.4, 52.4, 47.1, 40.3, 25.2, 11.1. HRMS (ESI+): calcd. *m/z* 378.17658 for $C_{23}H_{26}N_2OS$. Found 379.18423 $[M+H]^+$. TLC-MS (ESI+): 379.2 $[M+H]^+$. HPLC t_R = 6.04 min.

N-(3-(Benzyl(isopropyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(24)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.42 mmol) and N^1-benzyl-N^1-isopropylpropane-1,3-diamine dihydrochloride (**88**, 0.42 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–3%). Yield: 30 mg (18%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.56 (m, 2H), 7.45 – 7.32 (m, 6H), 7.31 – 7.22 (m, 3H), 7.20 – 7.11 (m, 2H), 3.56 (s, 2H), 3.51 – 3.44 (m, 2H), 3.00 (hept, *J* = 6.5 Hz, 1H), 2.52 (t, *J* = 5.5 Hz, 2H), 1.64 (quint, *J* = 6.0 Hz, 2H), 1.07 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 148.4, 140.2, 138.3, 133.8, 129.4, 129.1, 128.7, 128.5, 128.4, 127.2, 126.2, 123.3, 54.3, 49.0, 48.0, 39.9, 26.1, 17.5. TLC-MS (ESI+): calcd. *m/z* 392.19 for $C_{24}H_{28}N_2OS$. Found 393.2 $[M+H]^+$. HPLC t_R = 5.51 min.

*N-(3-(Benzyl(cyclopropyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**25**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.54 mmol) and N^1-benzyl-N^1-cyclopropylpropane-1,3-diamine (**89**, 0.54 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with PE/EtOAc (10–40%). Yield: 55 mg (26%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.54 (m, 2H), 7.42 – 7.35 (m, 2H), 7.34 – 7.29 (m, 1H), 7.28 – 7.25 (m, 4H), 7.25 – 7.21 (m, 3H), 7.20 (d, *J* = 3.7 Hz, 1H), 3.76 (s, 2H), 3.43 (q, *J* = 5.7 Hz, 2H), 2.70 (t, *J* = 6.0 Hz, 2H), 1.86 – 1.71 (m, 3H), 0.57 – 0.51 (m, 2H), 0.50 – 0.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 148.4, 138.1, 137.8, 133.8, 129.9, 129.2, 128.9, 128.5, 128.3, 127.3, 126.2, 123.4, 60.0, 54.4, 40.3, 37.7, 25.4, 7.4. TLC-MS (ESI+): calcd. *m/z* 390.18 for $C_{24}H_{26}N_2OS$. Found 391.2 $[M+H]^+$. HPLC t_R = 6.19 min.

*N-(3-(Benzyl(propyl)amino)propyl)-5-phenylthiophene-2-carboxamide (**26**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.36 mmol) and N^1-benzyl-N^1-propylpropane-1,3-diamine dihydrochloride (**90**, 0.36 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 36 mg (26%), colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.57 (m, 3H), 7.43 – 7.38 (m, 4H), 7.38 – 7.32 (m, 4H), 7.22 (d, *J* = 3.9 Hz, 1H), 3.84 (s, 2H), 3.51 (q, *J* = 5.7 Hz, 2H), 2.87 (s, 2H), 2.80 (s, 1H), 2.69 (t, *J* = 7.3 Hz, 2H), 1.95 (quint, *J* = 5.9, 5.5 Hz, 2H), 1.69 – 1.57 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 149.5, 136.8, 133.6, 130.0, 129.7, 129.2, 129.1, 128.7, 126.2, 123.9, 58.4, 54.9, 51.9, 38.7, 25.2, 18.6, 11.6. HRMS (ESI+): calcd. *m/z* 392.19223 for $C_{24}H_{28}N_2OS$. Found 393.19974 $[M+H]^+$. TLC-MS (ESI+): 393.2 $[M+H]^+$. HPLC t_R = 6.35 min.

N-(3-(Benzyl(butyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(27)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.46 mmol) and N^1-benzyl-N^1-butylpropane-1,3-diamine dihydrochloride (**91**, 0.46 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–3%). Yield: 13 mg (7%), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.50 (m, 3H), 7.44 – 7.37 (m, 2H), 7.36 – 7.30 (m, 3H), 7.30 – 7.28 (m, 1H), 7.28 – 7.22 (m, 3H), 7.20 (d, *J* = 3.8 Hz, 1H), 3.60 (s, 2H), 3.50 (q, *J* = 5.5 Hz, 2H), 2.60 (t, *J* = 5.6 Hz, 2H), 2.51 (t, *J* = 7.6 Hz, 2H), 1.77 (quint, *J* = 5.8 Hz, 2H), 1.59 – 1.50 (m, 2H), 1.33 – 1.26 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 148.4, 138.3, 133.9, 129.6, 129.2, 128.8, 128.6, 128.5, 127.4, 126.2, 123.4, 59.1, 53.6, 52.9, 40.1, 28.7, 25.6, 20.9, 14.2. HRMS (ESI+): calcd. *m/z* 406.20788 for $C_{25}H_{30}N_2OS$. Found 407.21556 $[M+H]^+$. TLC-MS (ESI+): 407.2 $[M+H]^+$. HPLC t_R = 6.82 min.

N-(3-(Benzyl(3-hydroxypropyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(28)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (1.2 mmol) and 3-((3-aminopropyl)(benzyl)amino)propan-1-ol dihydrochloride (**92**, 1.2 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–4%). Yield: 25 mg (5%), colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.50 (m, 2H), 7.43 (d, *J* = 3.9 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.35 – 7.32 (m, 4H), 7.32 – 7.25 (m, 2H), 7.22 (d, *J* = 3.9 Hz, 1H), 7.10 (t, *J* = 6.0 Hz, 1H), 4.24 (s, 1H), 3.82 – 3.61 (m, 4H), 3.44 (q, *J* = 6.3 Hz, 2H), 2.82 (t, *J* = 6.1 Hz, 2H), 2.71 (t, *J* = 6.6 Hz, 2H), 1.91 (quint, *J* = 6.5 Hz, 2H), 1.79 (quint, *J* = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7, 149.1, 137.5, 135.8, 133.7, 129.7, 129.3, 129.2, 129.0, 128.6, 128.3, 126.2, 123.7, 63.2, 58.7, 53.6, 51.0, 37.6, 27.3, 25.9. TLC-MS (ESI+): calcd. *m/z* 408.19 for $C_{24}H_{28}N_2O_2S$. Found 431.3 $[M+Na]^+$. HPLC t_R = 5.53 min.

N-(3-(Benzyl(phenethyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(29)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.45 mmol) and N^1-benzyl-N^1-phenethylpropane-1,3-diamine dihydrochloride (**93**, 0.45 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with PE/EtOAc (0–60%). Yield: 24 mg (12%), white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.50 (m, 2H), 7.38 – 7.31 (m, 2H), 7.30 – 7.25 (m, 4H), 7.25 – 7.16 (m, 5H), 7.16 – 7.04 (m, 5H), 3.63 (s, 2H), 3.39 (q, *J* = 5.7 Hz, 2H), 2.86 – 2.70 (m, 4H), 2.61 (t, *J* = 5.9 Hz, 2H), 1.71 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 148.4, 140.3, 138.9, 138.2, 133.8, 129.4, 129.1, 128.8, 128.8, 128.6, 128.5, 127.4, 126.2, 126.2, 123.3, 59.0, 55.5, 52.6, 39.8, 33.0, 25.9. TLC-MS (ESI+): calcd. *m/z* 454.21 for $C_{29}H_{30}N_2OS$. Found 477.1 $[M+Na]^+$. HPLC t_R = 7.14 min.

N-(3-(Benzyl(3-phenylpropyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(30)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.42 mmol) and N^1-benzyl-N^1 -(3-phenylpropyl)propane-1,3-diamine dihydrochloride (**94**, 0.42 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–6%). Yield: 19 mg (10%), colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 2H), 7.50 – 7.40 (m, 3H), 7.39 – 7.35 (m, 1H), 7.35 – 7.30 (m, 4H), 7.30 – 7.23 (m, 4H), 7.23 – 7.18 (m, 2H), 7.18 – 7.10 (m, 2H), 3.64 (s, 2H), 3.53 – 3.48 (m, 2H), 2.74 – 2.49 (m, 6H), 1.92 (quint, *J* = 7.8 Hz, 2H), 1.78 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.0, 148.5, 141.9, 138.6, 138.0, 133.8, 129.5, 129.1, 129.0, 128.6, 128.5, 128.4, 127.5, 126.2, 126.0, 123.4, 59.0, 53.3, 52.8, 39.8, 33.8, 28.3, 25.7. TLC-MS (ESI+): calcd. *m/z* 468.22 for $C_{30}H_{32}N_2OS$. Found 491.2 $[M+Na]^+$. HPLC t_R = 7.46 min.

N-(3-(Benzyl(ethyl)amino)propyl)-5-(3-fluorophenyl)thiophene-2-carboxamide ***(31)***. The title compound was prepared from N-(3-(benzyl(ethyl)amino)propyl)-5-bromothiophene-2-carboxamide (**95**, 0.29 mmol) and (3-fluorophenyl)boronic acid (0.32 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 29 mg (25%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.40 – 7.33 (m, 2H), 7.33 – 7.30 (m, 2H), 7.30 – 7.24 (m, 4H), 7.23 (d, *J* = 3.9 Hz, 1H), 7.18 (d, *J* = 3.9 Hz, 1H), 7.06 – 6.98 (m, 1H), 3.60 (s, 2H), 3.52 (q, *J* = 5.1 Hz, 2H), 2.69 – 2.56 (m, 4H), 1.78 (quint, *J* = 5.8 Hz, 2H), 1.11 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2 (d, *J* = 246.5 Hz), 161.7, 146.7 (d, *J* = 2.6 Hz), 139.2, 138.6, 135.9 (d, *J* = 8.1 Hz), 130.7 (d, *J* = 8.5 Hz), 129.6, 128.7, 128.6, 127.5, 124.0, 121.9 (d, *J* = 2.9 Hz), 115.2 (d, *J* = 21.4 Hz), 113.0 (d, *J* = 23.0 Hz), 58.6, 52.7, 47.1, 40.6, 25.2, 11.2. TLC-MS (ESI+): calcd. *m/z* 396.17 for $C_{23}H_{25}FN_2OS$. Found 397.2 $[M+H]^+$. HPLC t_R = 6.29 min.

N-(3-(Ethyl(4-fluorobenzyl)amino)propyl)-5-(3-fluorophenyl)thiophene-2-carboxamide ***(32)***. The title compound was prepared from 5-bromo-*N*-(3-(ethyl(4-fluorobenzyl)amino)propyl)thiophene-2-carboxamide (**96**, 0.33 mmol) and (3-fluorophenyl)boronic acid (0.36 mmol, 1.1 eq.) according to general procedure II. Purification by flash chromatography with DCM/MeOH (1–4%). Yield: 53 mg (40%), brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.40 – 7.33 (m, 2H), 7.30 – 7.23 (m, 4H), 7.20 (d, *J* = 3.9 Hz, 1H), 7.06 – 6.99 (m, 1H), 6.99 – 6.92 (m, 2H), 3.56 (s, 2H), 3.51 (q, *J* = 5.4 Hz, 2H), 2.67 – 2.50 (m, 4H), 1.78 (quint, *J* = 5.9 Hz, 2H), 1.10 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7 (dd, *J* = 349.3, 103.6 Hz), 161.6, 146.8 (d, *J* = 2.9 Hz), 139.0, 135.8 (d, *J* = 8.2 Hz), 131.1 (d, *J* = 7.8 Hz), 130.7 (d, *J* = 8.5 Hz), 129.9, 128.7, 124.1, 121.9 (d, *J* = 2.9 Hz), 115.4 (d, *J* = 21.3 Hz), 115.3 (d, *J* = 21.3 Hz), 113.0 (d, *J* = 22.9 Hz), 57.7, 52.5, 47.0,

40.4, 25.3, 11.2. TLC-MS (ESI+): calcd. *m/z* 414.16 for $C_{23}H_{24}F_2N_2OS$. Found 415.2 $[M+H]^+$. HPLC t_R = 6.44 min.

*tert-Butyl (3-(5-phenylthiophene-2-carboxamido)propyl)carbamate (**33**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (2.01 mmol) and *tert*-butyl (3-aminopropyl)carbamate (2.01 mmol, 1 eq.) according to general procedure I. Purification by suction filtration and wash with water. Yield: 637 mg (88%), brown solid. ^{1}H NMR (400 MHz, DMSO) δ 8.47 (t, *J* = 5.6 Hz, 1H), 7.76 – 7.65 (m, 3H), 7.53 (d, *J* = 3.9 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.39 – 7.33 (m, 1H), 6.80 (t, *J* = 5.0 Hz, 1H), 3.24 (q, *J* = 6.7 Hz, 2H), 2.98 (q, *J* = 6.6 Hz, 2H), 1.63 (quint, *J* = 6.9 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 160.9, 155.6, 147.2, 139.0, 133.1, 129.2, 128.8, 128.4, 125.6, 124.2, 77.5, 37.7, 36.9, 29.6, 28.2. TLC-MS (ESI+): calcd. *m/z* 360.15 for $C_{19}H_{24}N_2O_3S$. Found 382.8 $[M+Na]^+$. HPLC t_R = 8.70 min.

*N-(3-(Benzylamino)propyl)-5-phenylthiophene-2-carboxamide (**34**).* The title compound was prepared from benzaldehyde (0.51 mmol, 1 eq.), *N*-(3-aminopropyl)-5-phenylthiophene-2-carboxamide hydrochloride (**114**, 0.51 mmol), and triethylamine in DCE according to general procedure III. Purification by flash chromatography with DCM/MeOH (1–10%). Yield: 53 mg (30%), pale yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 8.78 (t, *J* = 5.8 Hz, 1H), 8.21 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.75 – 7.65 (m, 2H), 7.54 (d, *J* = 3.9 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.41 (m, 2H), 7.40 – 7.33 (m, 3H), 4.02 (s, 2H), 3.32 (q, *J* = 6.5 Hz, 2H), 2.85 (t, *J* = 7.4 Hz, 2H), 1.87 (quint, *J* = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 161.1, 147.3, 138.8, 134.5, 133.1, 129.4, 129.2, 129.1, 128.5, 128.4, 128.2, 125.6, 124.2, 50.8, 45.0, 36.6, 26.8. TLC-MS (ESI+): calcd. *m/z* 350.15 for $C_{21}H_{22}N_2OS$. Found 372.9 $[M+Na]^+$. HPLC t_R = 5.90 min.

N-(3-(3,4-Dihydroisoquinolin-2(1H)-yl)propyl)-5-phenylthiophene-2-carboxamide ***(35)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.53 mmol) and 3-(3,4-dihydroisoquinolin-2(1*H*)-yl)propan-1-amine dihydrochloride (**108**, 0.53 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH/1% NH_3 (1.5–2%). Yield: 17 mg (9%), yellow semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.40 – 7.27 (m, 5H), 7.25 – 7.12 (m, 3H), 7.09 – 7.04 (m, 1H), 7.02 (d, *J* = 3.9 Hz, 1H), 6.91 (d, *J* = 3.9 Hz, 1H), 3.75 (s, 2H), 3.62 (q, *J* = 5.1 Hz, 2H), 3.00 (t, *J* = 5.9 Hz, 2H), 2.85 (t, *J* = 6.0 Hz, 2H), 2.72 (t, *J* = 5.5 Hz, 2H), 1.81 (quint, *J* = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 148.0, 138.1, 134.3, 134.2, 133.7, 129.2, 129.0, 128.8, 128.3, 126.9, 126.7, 126.1, 126.1, 123.2, 58.9, 56.2, 51.5, 41.4, 29.4, 24.2. HRMS (ESI+): calcd. *m/z* 376.16093 for $C_{23}H_{24}N_2OS$. Found 377.16839 $[M+H]^+$. TLC-MS (ESI+): 399.3 $[M+Na]^+$. HPLC t_R = 5.19 min.

N-(3-((3-(4-Fluorobenzamido)benzyl)(methyl)amino)propyl)-5-phenylthiophene-2-carboxamide ***(36)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.34 mmol) and *N*-(3-(((3-aminopropyl)(methyl)amino)methyl)phenyl)-4-fluorobenzamide (**109**, 0.216 mg) according to general procedure I. Purification by flash chromatography with DCM/MeOH (5–8%). Yield: 96 mg (56%), pale yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 10.35 (s, 1H), 8.63 (s, 1H), 8.09 – 8.00 (m, 2H), 7.92 (s, 1H), 7.75 – 7.62 (m, 4H), 7.52 (d, *J* = 3.9 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.40 – 7.30 (m, 4H), 7.18 (s, 1H), 4.02 (s, 1H), 3.40 (q, *J* = 6.5 Hz, 3H), 2.49 – 2.20 (m, 2H), 1.98 – 1.72 (m, 2H), 1.29 – 0.63 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 164.5, 164.1 (d, *J* = 249.1 Hz), 161.1, 147.3, 139.3, 138.8, 133.1, 131.2 (d, *J* = 3.1 Hz), 130.4, 130.4, 129.2, 129.1, 128.8, 128.5, 125.6, 124.2, 115.4 (d,

J = 21.9 Hz), 54.9, 45.7, 37.0, 21.1, 8.6. TLC-MS (ESI+): calcd. *m/z* 501.19 for $C_{29}H_{28}FN_3O_2S$. Found 502.2 $[M+H]^+$. HPLC t_R = 6.71 min.

*N-(tert-Butyl)-1-(3-(5-phenylthiophene-2-carboxamido)propyl)piperidine-4-carboxamide (**37**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.48 mmol) and 1-(3-aminopropyl)-*N*-(*tert*-butyl)piperidine-4-carboxamide dihydrochloride (**110**, 0.48 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (10–20%). Yield: 94 mg (46%), white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.70 – 7.66 (m, 2H), 7.56 (d, *J* = 3.9 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.34 – 7.30 (m, 1H), 7.30 (d, *J* = 3.9 Hz, 1H), 5.25 (s, 1H), 3.55 (q, *J* = 5.4 Hz, 2H), 3.12 – 3.05 (m, 2H), 2.53 (t, *J* = 5.6 Hz, 2H), 2.06 – 1.93 (m, 3H), 1.93 – 1.88 (m, 1H), 1.87 – 1.81 (m, 3H), 1.77 (quint, *J* = 5.9 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 162.0, 148.4, 137.9, 133.8, 129.5, 129.2, 128.4, 126.3, 123.6, 58.9, 53.7, 51.1, 41.0, 29.2, 29.0, 24.5. TLC-MS (ESI+): calcd. *m/z* 427.23 for $C_{24}H_{33}N_3O_2S$. Found 428.4 $[M+H]^+$. HPLC t_R = 5.98 min.

*tert-Butyl 3-((5-phenylthiophene-2-carboxamido)methyl)piperidine-1-carboxylate (**38**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (1.47 mmol) and *tert*-butyl 3-(aminomethyl)piperidine-1-carboxylate (1.47 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–1%). Yield: 588 mg (quant.), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.55 (m, 2H), 7.52 (d, *J* = 3.9 Hz, 1H), 7.41 – 7.33 (m, 2H), 7.33 – 7.27 (m, 1H), 7.22 (d, *J* = 3.9 Hz, 1H), 3.93 – 3.40 (m, 3H), 3.35 – 2.98 (m, 2H), 2.97 – 2.74 (m, 2H), 1.95 – 1.76 (m, 2H), 1.70 – 1.56 (m, 1H), 1.44 (s, 9H), 1.41 – 1.18 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 155.3, 148.8, 137.9,

133.7, 129.1, 128.9, 128.4, 126.1, 123.5, 79.7, 47.1, 45.1, 41.6, 35.6, 28.5, 28.2, 23.7. TLC-MS (ESI+): calcd. *m/z* 400.18 for $C_{22}H_{28}N_2O_3S$. Found 423.2 $[M+Na]^+$. HPLC t_R = 9.44 min.

*N-((1-Benzylpiperidin-3-yl)methyl)-5-phenylthiophene-2-carboxamide (**39**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.5 mmol) and (1-benzylpiperidin-3-yl)methanamine hydrochloride (**111**, 0.5 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–6%). Yield: 26 mg (13%), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.48 (m, 2H), 7.38 – 7.27 (m, 3H), 7.27 – 7.16 (m, 5H), 7.14 (d, *J* = 3.9 Hz, 1H), 6.63 (s, 1H), 3.46 (dd, *J* = 57.6, 13.1 Hz, 2H), 3.37 – 3.19 (m, 2H), 2.82 – 2.42 (m, 2H), 2.27 – 2.00 (m, 2H), 1.96 – 1.80 (m, 1H), 1.73 – 1.59 (m, 2H), 1.58 – 1.45 (m, 1H), 1.23 – 1.03 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 148.7, 137.9, 137.4, 133.7, 129.5, 129.1, 129.0, 128.5, 128.4, 127.4, 126.2, 123.5, 63.4, 57.4, 53.9, 43.8, 35.6, 28.3, 24.1. TLC-MS (ESI+): calcd. *m/z* 390.18 for $C_{24}H_{26}N_2OS$. Found 390.9 $[M+H]^+$. HPLC t_R = 5.84 min.

*(4-Benzyl-1,4-diazepan-1-yl)(5-phenylthiophen-2-yl)methanone (**40**).* The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.5 mmol) and 1-benzyl-1,4-diazepane (0.5 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–3%). Yield: 152 mg (83%), white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.80 – 7.65 (m, 2H), 7.49 (d, *J* = 3.6 Hz, 1H), 7.47 – 7.41 (m, 3H), 7.40 – 7.32 (m, 2H), 7.32 – 7.27 (m, 3H), 7.26 – 7.19 (m, 1H), 3.88 – 3.63 (m, 4H), 3.61 (s, 2H), 2.76 – 2.57 (m, 4H), 1.97 – 1.74 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 162.7, 146.3, 139.0, 137.5, 132.9, 130.3, 129.2, 128.6, 128.5, 128.2, 126.9, 125.7, 123.7, 61.1, 54.4, 48.4, 45.8, 38.2, 28.3. TLC-MS (ESI+): calcd. *m/z* 376.16 for $C_{23}H_{24}N_2OS$. Found 377.2 $[M+H]^+$. HPLC t_R = 5.18 min.

4-Fluoro-N-(3-((4-(5-phenylthiophene-2-carbonyl)-1,4-diazepan-1-yl)methyl)phenyl)benzamide ***(41)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.25 mmol) and *N*-(3-((1,4-diazepan-1-yl)methyl)phenyl)-4-fluorobenzamide dihydrochloride (**112**, 0.25 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (1–3%). Yield: 80 mg (63%), pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 – 7.98 (m, 1H), 7.97 – 7.78 (m, 2H), 7.70 – 7.53 (m, 4H), 7.42 – 7.36 (m, 2H), 7.35 – 7.26 (m, 3H), 7.22 (s, 1H), 7.15 – 7.05 (m, 3H), 3.79 (s, 4H), 3.65 (s, 2H), 2.84 – 2.74 (m, 2H), 2.70 (s, 2H), 1.96 (quint, *J* = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 164.9, 164.1 (d, *J* = 59.0 Hz), 140.2, 138.2, 137.2, 133.6, 131.2 (d, *J* = 3.1 Hz), 130.1, 129.7, 129.6, 129.2, 129.2, 128.5, 126.2, 125.1, 122.8, 120.7, 119.3, 115.9 (d, *J* = 22.0 Hz), 62.3, 60.9, 54.9, 49.2, 46.8. TLC-MS (ESI+): calcd. *m/z* 513.19 for $C_{30}H_{28}FN_3O_2S$. Found 536.2 $[M+Na]^+$. HPLC t_R = 6.45 min.

N-(3-Nitrobenzyl)-5-phenylthiophene-2-carboxamide ***(42)***. The title compound was prepared from 5-phenylthiophene-2-carboxylic acid (0.98 mmol) and (3-nitrophenyl)methanamine hydrochloride (**113**, 0.98 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–1%). Yield: 230 mg (69%), white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 8.14 – 8.05 (m, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.54 (d, *J* = 3.9 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.44 – 7.30 (m, 3H), 7.26 (d, *J* = 3.8 Hz, 1H), 6.73 (t, *J* = 5.6 Hz, 1H), 4.71 (d, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 149.7, 148.6, 140.6, 136.7, 134.1, 133.5, 129.8, 129.7, 129.2, 128.8, 126.3, 123.7, 122.7, 122.6, 43.3. TLC-MS (ESI+): calcd. *m/z* 338.07 for $C_{18}H_{14}N_2O_3S$. Found 361.1 $[M+Na]^+$. HPLC t_R = 8.35 min.

N-(3-(4-Fluorobenzamido)benzyl)-5-phenylthiophene-2-carboxamide ***(43)***. The title compound was prepared from 4-fluorobenzoic acid (0.5 mmol) and *N*-(3-aminobenzyl)-5-phenylthiophene-2-carboxamide (**115**, 0.5 mmol, 1 eq.) according to general procedure I. Purification by suction filtration and washing with water. Yield: 187 mg (87%), white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.29 (s, 1H), 9.12 (s, 1H), 8.03 (s, 2H), 7.92 – 7.63 (m, 5H), 7.55 (s, 1H), 7.48 – 7.19 (m, 6H), 7.09 (s, 1H), 4.49 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.4, 164.0 (d, *J* = 249.0 Hz), 161.0, 147.5, 140.0, 139.2, 138.8, 133.1, 131.3, 130.4, 130.3, 129.2, 128.6, 128.5, 125.7, 124.3, 122.7, 119.2, 119.0, 115.3 (d, *J* = 21.7 Hz), 42.6. TLC-MS (ESI+): calcd. *m/z* 430.12 for $C_{25}H_{19}FN_2O_2S$. Found 453.2 $[M+Na]^+$. HPLC t_R = 8.79 min.

N-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-phenylthiazole-4-carboxamide ***(44)***. The title compound was prepared from 2-phenylthiazole-4-carboxylic acid (0.39 mmol) and 2-(1-methylpyrrolidin-2-yl)ethan-1-amine (0.39 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (10%). Yield: 48 mg (39%), pale brown semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.98 – 7.87 (m, 3H), 7.46 – 7.40 (m, 3H), 3.73 – 3.39 (m, 3H), 3.08 – 2.93 (m, 1H), 2.80 – 2.71 (m, 1H), 2.69 (s, 3H), 2.39 – 2.02 (m, 4H), 1.99 – 1.84 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.4, 161.7, 150.5, 132.8, 130.8, 129.2, 126.7, 123.0, 66.5, 56.2, 39.3, 36.6, 31.1, 29.8, 21.7. TLC-MS (ESI+): calcd. *m/z* 315.14 for $C_{17}H_{21}N_3OS$. Found 316.2 $[M+H]^+$. HPLC t_R = 3.92 min.

tert-Butyl 4-(2-phenylthiazole-4-carbonyl)-1,4-diazepane-1-carboxylate ***(45)***. The title compound was prepared from 2-phenylthiazole-4-carboxylic acid (2.44 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (2.44 mmol, 1 eq.) according to general procedure I. Purification

by flash chromatography with PE/EtOAc (10–30%). Yield: 784 mg (83%), yellow oil. ^{1}H NMR (400 MHz, DMSO) δ 8.29 – 8.09 (m, 1H), 8.05 – 7.90 (m, 2H), 7.67 – 7.44 (m, 3H), 3.92 – 3.59 (m, 4H), 3.58 – 3.46 (m, 2H), 3.45 – 3.35 (m, 2H), 1.95 – 1.71 (m, 2H), 1.43 – 1.19 (m, 9H). ^{13}C NMR (101 MHz, DMSO) δ 166.4, 163.1, 154.4, 151.0, 132.6, 130.7, 129.3, 126.3, 126.2, 78.7, 45.3, 34.9, 28.3, 28.0, 27.8, 26.0. TLC-MS (ESI+): calcd. *m/z* 387.16 for $C_{20}H_{25}N_3O_3S$. Found 410.2 $[M+Na]^+$. HPLC t_R = 8.98 min.

tert-Butyl 4-(3-(2-phenylthiazole-4-carboxamido)propyl)piperazine-1-carboxylate ***(46)***. The title compound was prepared from 2-phenylthiazole-4-carboxylic acid (0.43 mmol) and *tert*-butyl 4-(3-aminopropyl)piperazine-1-carboxylate (**118**, 0.43 mmol, 1 eq.) according to general procedure I. Purification by suction filtration and washing with water. Yield: 128 mg (69%), white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.65 (t, 1H), 8.28 (s, 1H), 8.02 (s, 2H), 7.52 (s, 3H), 3.43 – 3.32 (m, 6H), 2.47 – 2.20 (m, 6H), 1.71 (quint, *J* = 6.7 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 167.2, 160.2, 153.8, 150.9, 132.5, 130.7, 129.2, 126.4, 123.9, 78.7, 56.2, 52.6, 43.7, 37.9, 28.0, 25.9. TLC-MS (ESI+): calcd. *m/z* 430.20 for $C_{22}H_{30}N_4O_3S$. Found 431.2 $[M+H]^+$. HPLC t_R = 5.65 min.

tert-Butyl 4-(3-(((2-phenylthiazol-4-yl)methyl)amino)propyl)piperazine-1-carboxylate ***(47)***. The title compound was prepared from 2-phenylthiazole-4-carbaldehyde (0.47 mmol, 1.1 eq.) and *tert*-butyl 4-(3-aminopropyl)piperazine-1-carboxylate (**118**, 0.43 mmol) according to general procedure III. Purification by flash chromatography with DCM/MeOH (5–8%). Yield: 13 mg (7%), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (q, *J* = 2.6 Hz, 2H), 7.54 (s, 1H), 7.48 – 7.40 (m, 3H), 4.26 (s, 2H), 3.41 (s, 4H), 3.20 (s, 2H), 2.67 – 2.36 (m, 6H), 2.00 (quint, *J* = 5.9 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 154.6, 148.8, 133.0, 130.8,

129.2, 126.7, 119.7, 80.0, 58.1, 48.8, 46.8, 29.8, 28.5, 22.1, 14.2. TLC-MS (ESI+): calcd. *m/z* 416.22 for $C_{22}H_{32}N_4O_2S$. Found 417.2 $[M+Na]^+$. HPLC t_R = 3.45 min.

N-Benzyl-1-((2-phenylthiazol-4-yl)methyl)piperidine-4-carboxamide ***(48)***. The title compound was prepared from 2-phenylthiazole-4-carbaldehyde (0.39 mmol, 1 eq.) and *N*-benzylpiperidine-4-carboxamide hydrochloride (**119**, 0.39 mmol) in presence of triethylamine in DCE according to general procedure III. Purification by flash chromatography with DCM/MeOH (2–7%). Yield: 99 mg (71%), white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 7.89 (m, 2H), 7.45 – 7.37 (m, 3H), 7.35 – 7.22 (m, 5H), 7.13 (s, 1H), 5.76 (t, *J* = 4.5 Hz, 1H), 4.44 (d, *J* = 5.6 Hz, 2H), 3.73 (d, *J* = 0.6 Hz, 2H), 3.13 – 2.98 (m, 2H), 2.21 – 2.04 (m, 3H), 1.92 – 1.75 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.9, 168.0, 155.2, 138.5, 133.9, 130.0, 129.0, 128.9, 127.9, 127.7, 126.7, 115.9, 58.7, 53.4, 43.6, 43.5, 29.2. TLC-MS (ESI+): calcd. *m/z* 391.17 for $C_{23}H_{25}N_3OS$. Found 392.2 $[M+H]^+$. HPLC t_R = 5.45 min.

tert-Butyl 4-(2-(benzo[b]thiophene-2-carboxamido)ethyl)piperazine-1-carboxylate ***(49)***. The title compound was prepared from benzo[*b*]thiophene-2-carboxylic acid (0.39 mmol) and *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (0.39 mmol, 1 eq.) according to general procedure I. Purification by flash chromatography with DCM/MeOH (0–3%). Yield: 70 mg (46%), yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.80 (m, 2H), 7.77 (s, 1H), 7.49 – 7.33 (m, 2H), 6.95 (t, *J* = 4.6 Hz, 1H), 3.57 (q, *J* = 5.6 Hz, 2H), 3.47 (t, *J* = 4.6 Hz, 4H), 2.63 (t, *J* = 6.0 Hz, 2H), 2.48 (t, *J* = 4.8 Hz, 4H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4, 154.8, 140.9, 139.2, 138.6, 126.4, 125.3, 125.1, 125.0, 122.8, 80.0, 56.5, 52.7, 43.6, 36.4, 28.5. TLC-MS (ESI+): calcd. *m/z* 389.18 for $C_{20}H_{27}N_3O_3S$. Found 412.2 $[M+Na]^+$. HPLC t_R = 5.23 min.

ACKR3 *β*-Arrestin Recruitment Assay. The potency and efficacy of final compounds **1** to **49** were evaluated by ligand-induced recruitment of *β*-arrestin to ACKR3 using a NanoLuc complementation assay (NanoBiT, Promega Corporation, Madison, WI, USA) in HEK293T cells.[42-45] In short: 5 × 106 HEK293T cells were seeded in 10-cm culture dishes and 24 h later co-transfected with pNBe vectors encoding human ACKR3 or control receptors (ACKR2, CXCR4 or CXCR3). C-terminally fused to SmBiT and *β*-arrestin-1 *N*-terminally fused to LgBiT. After 24 h, transfected cells were harvested, incubated for 15 minutes at 37 °C with 200-fold diluted Nano-Glo Live Cell substrate, and distributed into white 96-well plates (5×10^4 cells per well). The prepared cells were then treated with compounds at concentrations ranging from 0.3 nM to 100 μM. Induction of *β*-arrestin recruitment to receptors was evaluated by measuring bioluminescence with a Mithras LB940 luminometer (Berthold Technologies). For determination of a compound's efficacy and concentration-response curves, the signal recorded was compared to values for CXCL12 at 100 nM considered as full agonist reference ligand (E_{max} = 100 %). All curves were fitted to data points generated from the mean of at least three independent experiments. Results with values of $p < 0.05$ were considered statistically significant.

Flow Cytometry Assay. The inhibition of platelet degranulation was determined via flow cytometry. Platelets were treated with collagen-related peptide (CRP-XL, CambCol Laboratories, Cambridge, UK) and the platelets' activation state was determined by measuring P-selectin surface expression.[46,47] Briefly, PRP was isolated from peripheral human blood collected in citrate-phosphate-dextrose solution with adenine (CPDA) and diluted with phosphate-buffered saline (PBS) (Sigma Aldrich Co., Ltd., St. Louis, Missouri, USA)

supplemented with $CaCl_2$ and $MgCl_2$. PRP (10^6 platelets /sample) was preincubated with the respective compound (100 μM) for 15 min. Samples were then treated with 1 μg/ml CRP-XL (CambCol Laboratories, Cambridge, UK) for 30 min at room temperature in the presence of the respective fluorochrome-conjugated antibody against P-selectin (CD62P-FITC; Beckman Coulter, Brea, CA, USA). Subsequently, samples were fixed in 0.5% paraformaldehyde and analyzed using a FACS-Calibur flow cytometer (FACS-Calibur flow cytometer, Becton-Dickinson, East Rutherford, NJ, USA). Mean fluorescence intensity (MFI) was used as a quantitative measurement of platelet surface expression.

Cell Viability Assay. RealTime-Glo™ MT Cell Viability Assay (Promega Corporation, Madison, WI, USA) was performed using HEK293 cells as indicated in the manufacturer's manual to measure a compound's cytotoxicity on cells. In brief, in a white solid-bottom 96-well plate cells ($5x10^3$/well) were seeded and incubated with the respective compounds diluted in DMEM (Gibco, Carlsbad, CA, USA). After cell seeding viability reagent was added. Subsequently, cells were incubated with the compound and viability reagent containing medium at 37 °C, 5% CO_2 for 72 h. The luminescence signal was determined after 1, 24, 48, and 72 hours on a GloMax®-Multi Detection System plate reader (Promega Corporation, Madison, WI, USA).

Metabolic Stability Assay. Pooled liver microsomes from mice (male) and humans (male) were purchased from *Sekisui XenoTech, LLC*, Kansas City, KS, USA. Metabolic stability assays were performed in the presence of an NADPH-regenerating system consisting of 5 mM glucose-6-phosphate, 5 U/mL glucose-6-phosphate dehydrogenase, and 1 mM $NADP^+$. Liver microsomes (20 mg/mL), NADPH-regenerating system, and 4 mM $MgCl_2 \cdot 6\ H_2O$ in 0.1 M TRIS-HCl-buffer (pH 7.4) were preincubated for 5 min at 37 °C and 750 rpm on a shaker. The

reaction was started by adding the preheated compound at 10 mM resulting in a final concentration of 0.1 mM. The reaction was quenched at selected time points (0, 10, 20, 30, 60, and 120 min) by pipetting 100 μL of internal standard (ketoprofen) in acetonitrile at concentrations of 400 μM (compounds **1** and **16**), 600 μM (compound **26**), 800 μM (compound **27**), and 450 μM (compound **35**), respectively. The samples were vortexed for 30 s and centrifuged (21910 relative centrifugal force, 4 °C, 20 min). The supernatant was used directly for LC-MS analysis.

All compound incubations were conducted at least in triplicates. Additionally, a negative control containing BSA (20 mg/mL) instead of liver microsomes and a positive control using Verapamil instead of the compound was performed. A limit of 1% organic solvent during incubation was not exceeded. Sample separation and detection were performed on an *Alliance 2695 Separations Module* HPLC system (*Waters Corporation*, Milford, MA, USA) equipped with a *Phenomenex Kinetex* 2.6 μm XB-C18 100 Å 50 x 3 mm column (*Phenomenex Inc.*, Torrance, CA, USA) coupled to an *Alliance 2996 Photodiode Array Detector* and a *MICROMASS QUATTRO micro API* mass spectrometer (both *Waters Corporation*, Milford, MA, USA) using electrospray ionization in positive mode. Mobile phase A: 90% water, 10% acetonitrile, and additionally 0.1% formic acid (v/v), mobile phase B: 100% acetonitrile with additional 0.1% formic acid (v/v). The gradient was set to 0-2.5 min 0% B, 2.5-10 min from 0 to 40% B, 10-12 min 40% B, 12-12.01 min from 40 to 0% B, 12.01-17 min 0% B at a flow rate of 0.7 mL/min. Samples were maintained at 10 °C, the column temperature was set to 20 °C with an injection volume of 5 μL. Spray, cone, extractor, and RF lens voltages were at 4 kV, 30 V, 8 V, and 2 V, respectively. The source and desolvation temperatures were set to 120 °C and 350 °C, respectively, and the desolvation gas flow was set to 750 L/h. Data analysis was conducted using *MassLynx 4.1* software (*Waters Corporation*, Milford, MA, USA).

Solubility Assay. A 100 mL flask with phosphate-buffered saline was prepared from NaCl, KCl, Na_2HPO_4, KH_2PO_4, and distilled water. The buffer was adjusted to pH 7.4 with HCl. Afterward, 10mM stock solutions of compounds in DMSO were prepared. 10 μL of compound stock solution were added to 990 μL of PBS in Eppendorf tubes. As a reference, 10 μL of compound stock solution were added to 990 μL of HPLC-grade MeOH in Eppendorf tubes. All tubes were centrifuged (14,000 rpm, 4 °C, 20 min). Subsequently, 600 μL of supernatant were extracted carefully and filled into HPLC vials. The compound's area under the curve (AUC) was determined by RP-HPLC using an Agilent 1100 Series LC with a Phenomenex Luna C8 analytical column (150 x 4.6 mm, 5 μm) and detected by a UV DAD detector at 254 nm and 230 nm wavelength. The compound's solubility was calculated by the AUC of the compound in PBS divided by the AUC of the reference solution multiplied by the concentration of the compound in the solution.

Aggregation experiments. For the light transmission aggregation experiments, citrate anticoagulated blood from healthy donors was used. To obtain platelet-rich plasma, whole blood was centrifuged at 210 x g for 20 min at room temperature. 1 x 10^8 platelets were preincubated with 100 μM respective ACKR3 agonist for 15 min at 37 °C. Subsequently, platelets were activated with 2.5 μM ADP and aggregation was analyzed for 5 min at 1,000 rpm and 37 °C using a light transmission aggregometer (Aggregometer 490-X; Chrono-Log Corp., Havertown, PA, USA). Maximum platelet aggregation and the area under the curve were quantified using Aggrolink8 software (Chrono-Log Corp., Havertown, PA, USA).

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge at https://

Estimated metabolites of **27** after 60 min of incubation in human liver microsomes (Figure S1); *In vitro* metabolic stability studies of compounds **1**, **16**, **26**, **27**, and **35** (Figure S2); Cell viability assays of compounds **23** and **27** (Figure S3); Kinetic solubility studies of selected compounds (Table S1); Synthesis of intermediates; Synthesis of intermediates **61**-**63** (Scheme S1), Synthesis of intermediates **97**-**113** (Scheme S2); Synthesis of intermediates **116**-**119** (Scheme S3); ^{1}H and ^{13}C NMR Spectra; HPLC traces; Supplementary References.

Author Contributions:

A.B., S.A.L., T.P. designed the compounds. A.B. synthesized and analyzed the compounds. M.S., M.C. and A.C. performed potency, selectivity, and efficacy studies. V.D.-B. and A.-K.R. performed flow cytometry, platelet aggregation, and cell viability experiments. A.R. performed metabolic stability studies. S.A.L., M.G., and T.P. supervised the studies. A.B. and T.P. drafted the manuscript. All authors edited and revised the manuscript. All authors read and approved the content of the manuscript.

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ABBREVIATIONS USED

ACKR, atypical chemokine receptor; ADP, adenosine diphosphate; AUC, area under the curve; BSA, bovine serum albumin; CAD, coronary artery disease; cAMP, cyclic adenosine monophosphate; cCKRs, conventional chemokine receptors; CD62P, P-selectin; CD62P-FITC, fluorochrome-conjugated antibody against P-selectin; CPDA, citrate-phosphate-dextrose solution with adenine; CRP-XL, synthetic cross-linked collagen-related peptide; CVD, cardiovascular disease; CXCL12, C-X-C motif chemokine 12; DAD, diode array detector; DCE, 1,2-dichloroethane; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, dulbecco's modified eagle's medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EC_{50}, half maximal effective concentration; ESI, electrospray ionization; ESI-TOF, electrospray ionization-time-of-flight; FACS, fluorescence activated cell sorting; GPCR, G protein-coupled receptors; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; HIV, human immunodeficiency viruses; HLMs, human liver microsomes; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectra; LC-MS, liquid chromatography-mass spectrometry; MFI, mean fluorescence intensity; MLMs, mouse liver microsomes; NADP, nicotinamide adenine dinucleotide phosphate; NMR, nuclear magnetic resonance spectroscopy; PBS, phosphate-buffered saline; PE, petroleum ether 60-90; RP-HPLC, reversed-phase liquid chromatography; SDF-1, stromal cell-derived factor 1; SEM, standard error of the mean; SI, supporting information; THF, tetrahydrofuran; TLC, thin-layer chromatography; TLC-MS, thin-layer chromatography-mass spectrometry; TMS, tetramethylsilane; TRIS, tris(hydroxymethyl)aminomethane; UV, ultraviolet

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