Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 2227–2238 **2227**
DOI: 10.1021/jm901771h

The Discovery of 4-{1-[({2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]cyclopropyl}benzoic Acid (MK-2894), A Potent and Selective Prostaglandin E_2 Subtype 4 Receptor Antagonist†

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Received November 30, 2009

The discovery of highly potent and selective second generation EP_4 antagonist MK-2894 (**34d**) is discussed. This compound exhibits favorable pharmacokinetic profile in a number of preclinical species and potent anti-inflammatory activity in several animal models of pain/inflammation. It also shows favorable GI tolerability profile in rats when compared to traditional NSAID indomethacin.

Introduction

Prostanoids (prostaglandins and thromboxanes) are important lipid hormones formed from arachidonic acid metabolism. PGE_2,[a] in particular, is the principal proinflammatory prostanoid and is implicated in the pathogenesis of a number of diseases such as pain, fever, arthritis and cancer. Inhibition of PGE_2 production by NSAIDs and COX-2 inhibitors (coxibs) relieves arthritis symptoms and thus is the basis of widespread uses of these drugs as analgesics.[1] Unfortunately, the therapeutic utilities of these drugs are limited by their potential to cause either GI toxicity (by NSAIDs)[2] or CV side effects (by both NSAIDs and coxibs).[3] The CV adverse events associated with these drugs are not clearly understood, although it is speculated that inhibition of prostacyclin biosynthesis may cause the prothrombotic and hypertensive effects.[4] Therefore, there is a vast unmet medical need to discover safer alternatives for treating chronic ailments such as arthritis.

PGE_2 exerts its biological effects through four subtype EP receptors, EP_{1-4}. In a mouse model of experimental arthritis (CAIA), the $EP_4^{-/-}$ mice showed a remarkable resistance to both the incidences and symptom scores (paw swelling/redness, ankylosis) of arthritis compared to the wild type controls while the $EP_{1-3}^{-/-}$ mice showed no effect, suggesting that the effect of PGE_2 in chronic inflammation was mediated predominantly by the EP_4 receptor.[5] It is worth mentioning that EP_4 may not be the only mediator of chronic inflammation, as both IP signaling and combined EP_2/EP_4 signaling were reported to mediate joint inflammation in a mouse model of CIA.[6] Lin et al. demonstrated that EP_4, not EP_{1-3}, contributed to inflammatory pain hypersensitivity in rats.[7] Using highly selective EP_1, EP_3, and EP_4 antagonists, we demonstrated that EP_4, not EP_1 or EP_3, is the primary receptor involved in joint inflammation and pain in rodent models of rheumatoid and osteoarthritis,[8] further supporting EP_4 antagonism as a valid strategy for treating inflammatory pain. It is plausible that a selective EP_4 antagonist may ameliorate symptoms of chronic inflammation without the potential CV side effects observed with NSAIDs and COX-2 inhibitors because it does not interfere with the biosynthesis of any of the prostanoids, including prostacyclin and thromboxanes.

In addition to its role in inflammation, the EP_4 receptor has also been implicated in destabilizing atherosclerotic plaques in human,[9] in developing tumor metastasis,[10] in migraine headache,[11] and in Alzheimer's disease.[12] Therefore, EP_4 antagonism represents a potential promising new therapeutic approach for treating inflammatory pain, atherosclerosis, cancer, migraine, and Alzheimer's disease and has been the subject of recent research interests.

Previously, we disclosed the discovery of our first generation EP_4 antagonists MF498 (**1**)[8] and MF310 (**2**)[13] (Figure 1). While **2** is an extremely potent compound in the rat AIA model with an ED_{50} of 0.003 mg/kg/day, it suffers from CYP 3A mediated hydrolysis of the acylsulfonamide moiety, giving rise to the corresponding sulfonamide M1. This metabolite

†Previously disclosed as MF482 at the ACS National Meeting, Washington, DC, August 16−20, 2009.

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[a] Abbreviations: AIA, adjuvent-induced arthritis; CAIA, collagen antibody-induced arthritis; cAMP, cyclic adenosine monophosphate; CIA, collagen-induced arthritis; CFA, complete Freund's adjuvant; COX, cyclooxygenase; CV, cardiovascular; CYP, cytochrome P450; DIEA, *N*,*N*-diisopropylethylamine; DP_{1-2}, prostaglandin D_2 receptor subtypes 1 and 2; EBNA, Epstein−Barr nuclear antigen; EDTA, ethylenediaminetetraacetic acid; EP_{1-4}, prostaglandin E_2 receptor subtypes 1, 2, 3, and 4; FP, prostaglandin F_{2a} receptor; GI, gastrointestinal; GSH, reduced glutathione; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HEK, human embryonic kidney; HS, human serum; HWB, human whole blood; IP, prostaglandin I_2 receptor; IP-10, interferon-inducible protein 10; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NBS, *N*-bromosuccinimide; NCS, *N*-chlorosuccinimide; NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; SD, Sprague−Dawley; TFA, trifluoroacetic acid; TNF, tumor necrosis factor; TP, thromboxane A_2 receptor.

Published on Web 02/17/2010

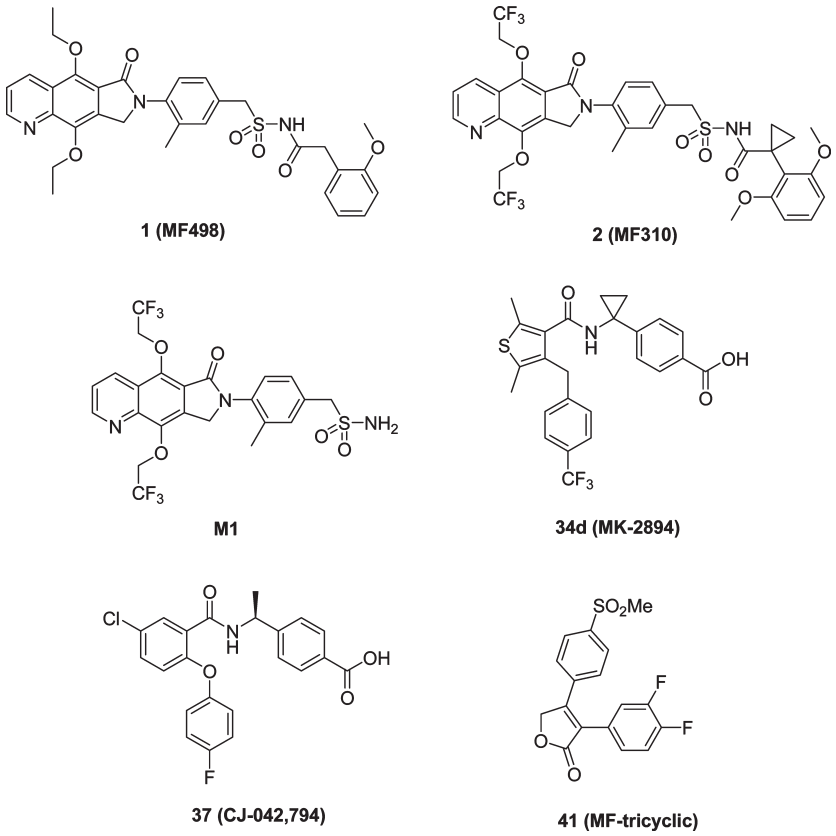


Figure 1. Chemical structure of EP_4 antagonists **1**, **2**, **34d**, and **37**, metabolite **M1**, and COX-2 inhibitor **41**.

has the potential to cause drug−drug interaction due to the fact it is a moderate time-dependent CYP 3A4 inhibitor and a CYP 3A4 inducer and has the potential to accumulate.[14] SAR effort to prevent the acylsulfonamide hydrolysis was unsuccessful, so we turned our attention to nonacylsulfonamide analogues which resulted in the discovery of a highly selective and potent second generation EP_4 antagonist, **34d** (MK-2894) (Figure 1). We herein describe the SAR leading to **34d**, its in vitro profile and pharmacokinetic properties, its in vivo efficacy in models of pain and inflammation, and its GI tolerability profile in rats.

Chemistry. The synthetic routes developed for the synthesis of the thiophene-based EP_4 antagonists are outlined in Schemes 1−4.

Compound **12** was prepared according to Scheme 1. Commercially available 3-thiophenemethanol (**3**) was brominated at the 2-position using NBS[15] to afford alcohol **4** that was subsequently treated with NCS[15] to provide the 2,5-dihalogenated thiophene derivative **5**. Oxidation under the Swern conditions gave aldehyde **6** that was reacted with 3-chlorophenylmagnesium bromide to yield alcohol **7**. Deoxygenation of **7** by treatment with TFA/triethylsilane yielded bromide **8**. Metal−bromine exchange and subsequent trapping of the resultant organometallic species with carbon dioxide gave the 2-thiophenecarboxylic acid **9**. Coupling of acid **9** with the racemic α-methylbenzylamine **10** using HATU as the coupling reagent,[16] followed by hydrolysis of the obtained methyl ester **11** under standard conditions furnished 2-thiophenecarboxamide **12**.

The synthesis of the compounds bearing carboxamide and benzyl groups at the 3,4-positions of the thiophene core is illustrated in Scheme 2. 3,4-Dibromothiophene derivatives **13** and **14** were converted to the secondary benzylic alcohols **15** and **16** via monolithiation using *n*-butyllithium followed by quenching the anionic intermediates with appropriately substituted benzaldehydes. The thiophenecarboxylic acids **19** and **20** were obtained using similar procedures described for the transformation of alcohol **7** to acid **9** in Scheme 1. To prepare the 2,5-dichlorothiophene-containing analogues **27**, the desired carboxylic acid **21** was obtained by the treatment of the 2,5-unsubstituted precursor **19** with NCS in hot acetic acid.[17] Compounds **26**−**28** were obtained from acids **19**−**21** in a similar manner as described in Scheme 1 using enantiomerically pure (*S*)-α-methylbenzylamine **22**.[16]

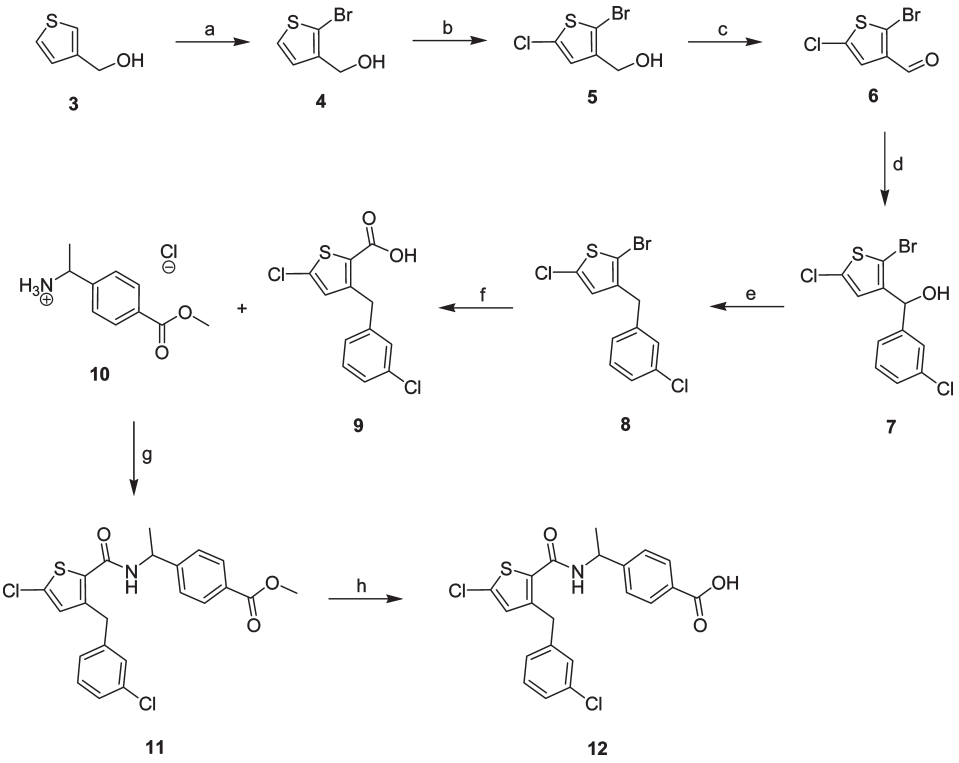
The cyclopropylbenzamide derivatives **34** were prepared by first submitting 1,4-dicyanobenzene (**29**) to the Szymoniak variation[18] of the Kulinkovich reaction in which one of the cyano groups was modified to afford the cyclopropylamine derivative **30** (Scheme 3). The other cyano group was then hydrolyzed in boiling 6 N hydrochloric acid, and the resulting carboxylic acid **31** was esterified to give the methyl ester **32**. The latter was reacted with the 2,5-dimethylthiophenecarboxylic acids **20** under the same conditions described above to provide the EP_4 antagonists **34** after hydrolysis of the methyl esters **33**.

The synthesis of the tetrazole derivative **36** was conveniently accomplished by coupling of carboxylic acid **20d** with cyclopropylamine **30**, followed by treating the resultant benzonitrile **35** with azidotributyltin in refluxing toluene (Scheme 4).[19]

Results and Discussion

SAR. EP_4 antagonists bearing a benzoic acid moiety were reported recently.[20] For example, CJ-042,794 (**37**), illustrated in Figure 1, was reported to be a potent and selective

Scheme 1. Synthesis of 2,3-Substituted[a] Thiophene **12**[b]



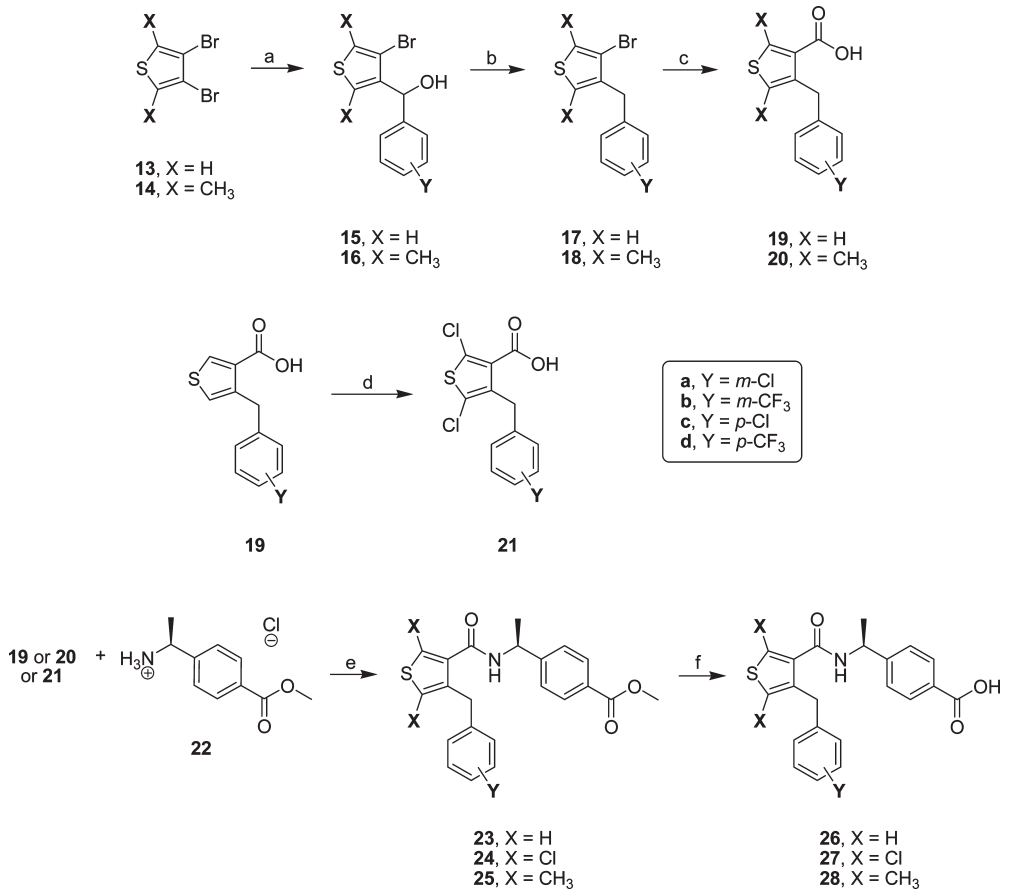
[a] The 2,3-substitution refers to the carboxamide and benzyl groups respectively. [b] Reagents and conditions: (a) NBS, THF, H_2O, 0 °C to rt; (b) NCS, THF, H_2O, rt; (c) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C to rt; (d) 3-chlorophenylmagnesium bromide, THF, Et_2O, −78 °C; (e) TFA, Et_3SiH, CH_2Cl_2, rt; (f) *n*-BuLi, CO_2, THF, −78 to 0 °C; (g) HATU, DIEA, DMF, 0 °C; (h) 1 M aq LiOH, THF, MeOH, rt.

EP_4 antagonist with good pharmacokinetic profile and was shown to be efficacious in two rat models of pain and inflammation.[20c]

Our efforts focused on compounds bearing suitable heterocyclic templates in place of the central phenyl core in **37**. By screening a number of monocyclic or bicyclic heterocycles, we discovered that thiophene is an excellent replacement. Interestingly, we observed that the regiochemistry of the carboxamide and benzyl substituents, in compounds such as **12** (Scheme 1 or Figure 2) and **26a** (Scheme 2 or Table 1, entry 1), had a profound effect on the agonist/antagonist properties of these compounds. For instance, while the 2,3-substituted analogue **12** (Figure 2) behaved as a full agonist in the EP_4 functional assay, the 3,4-substituted analogue **26a** (Table 1, entry 1) was a partial agonist (10% maximum agonist activity vs PGE_2) when the 2- and 5-positions are not substituted. This compound, however, was not selective against the EP_4 receptor. Switching over the carboxamide and benzyl substituent of **12**, such as in the fluorinated analogue **38** (Figure 2), produced a 4-fold decrease in EP_4 binding affinity with an IC_{50} of 12 nM versus 3.0 nM for **12**. Substituting the 2- and 5-positions of **26a** with chlorine atoms gave compound **27a** (Table 1, entry 2) with significantly improved potency and selectivity. It also behaved as a full antagonist in the EP_4 functional assay. This compound, unfortunately, suffered from extensive covalent protein binding when incubated with human or rat liver microsomes in vitro in the presence of cofactor NADPH, as indicated by extensive formation of a GSH adduct. Replacing the chlorine atoms with methyl groups as in compound **28a** (Table 1, entry 3) completely circumvented the covalent protein labeling issue while maintaining potency and selectivity. Interestingly, compound **28a** also behaved as a weak partial agonist. We further observed that the agonist/antagonist balance could be influenced dramatically by the substitution pattern of the benzyl group. Shifting the substituent from the meta-position to the para-position, as seen in compounds **28c** and **28d** (Table 1, entries 5 and 6), gave rise to full antagonists with further improved potency and selectivity. Interestingly, we observed that the affinity and selectivity profile of enantiomers **27a** (Table 1, entry 2) and **39** (Figure 3 and Table 1, entry 10) were essentially identical, suggesting that the chiral center was not critical for either affinity or selectivity. Removing the methyl group in **27a** gave compound **40** (Figure 3 and Table 1, entry 11), which maintained the EP_4 affinity but lost selectivity against the EP_2 receptor. Substituting the chiral α-methylbenzamide moiety in **28d** with an achiral cyclopropylbenzamide furnished the title compound **34d** with further improved affinity and selectivity. **34d** is a high affinity full antagonist against the EP_4 receptor with a K_i of 0.56 nM and an IC_{50} of 2.5 nM. It is not significantly shifted in the presence of 10% HS in either the binding assay or the functional assay (Table 2). It also exhibits exquisite selectivity against all other prostanoid receptors (>7000-fold) (Table 2) and does not display significant activity (IC_{50} < 10 μM) against a panel of >170 enzymes and receptors in a MDS Pharma screen. As shown in Table 3, **34d** exhibits favorable pharmacokinetic profile in preclinical species with moderate bioavailability (F = 18−32%), good elimination half-lives ($T_{1/2}$ = 4.5−19 h) and slow to moderate clearance rate (CL = 3.2−23 mL/min/kg).

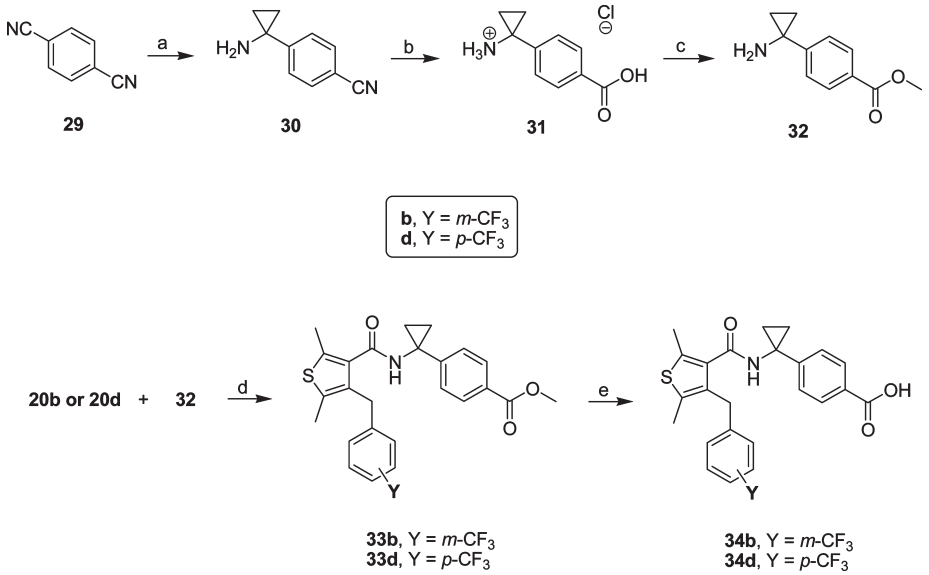
We also prepared the corresponding tetrazole analogue **36** (Scheme 4 and Table 1, entry 9) for comparison because tetrazole is a classical carboxylic acid bioisostere. While **36**

Scheme 2. Synthesis of 3,4-Substituted[a] Thiophene-containing EP_4 Antagonists **26**−**28**[b]



[a] The 3,4-substitution refers to the carboxamide and benzyl groups respectively. [b] Reagents and conditions: (a) *n*-BuLi, YC_6H_4CHO, Et_2O, −78 to 0 °C; (b) TFA, Et_3SiH, CH_2Cl_2, 0 °C; (c) *n*-BuLi, CO_2, Et_2O/THF, −78 to 0 °C; (d) NCS, AcOH, 110 °C; (e) HATU, DIEA, DMF, 0 °C; (f) 1 M aq LiOH, THF, MeOH, rt or 50 °C.

Scheme 3. Synthesis of Cyclopropylbenzamide EP_4 Antagonists **34**[a]

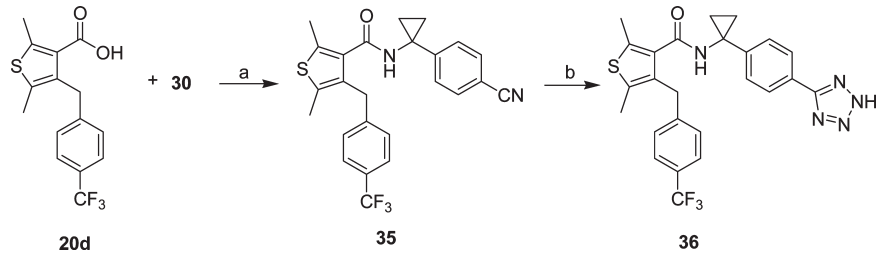


[a] Reagents and conditions: (a) $Ti(O^iPr)_4$, EtMgBr, $BF_3 \cdot Et_2O$, CH_2Cl_2, rt; (b) 6 N HCl, reflux; (c) cat. HCl, MeOH, reflux; (d) HATU, DIEA, DMF, 0 °C; (e) 1 M aq LiOH, THF, MeOH, 50 °C.

maintained comparable affinity, selectivity (except for EP_1, Ki = 500 nM), and functional potency in vitro, it exhibited poor oral bioavailability (F = 1%) in rat, and as a result, was not pursued further.

Biology. The radio ligand binding assays to determine affinity and selectivity of compounds were performed according to reported literature procedures in membranes prepared from HEK 293(EBNA) clonal cell lines overexpressing

Scheme 4. Synthesis of Tetrazole Derivative **36**[a]



[a] Reagents and conditions: (a) HATU, DIEA, DMF, 0 °C; (b) Bu_3SnN_3, toluene, reflux.

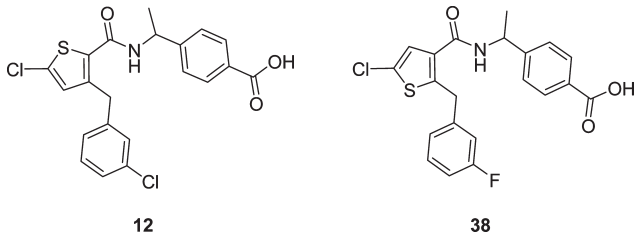


Figure 2. Structure of 2,3-Substituted thiophene-containing EP_4 ligands **12** and **38**.

the human prostanoid receptors EP_{1-4}, DP_{1-2}, TP, FP, and IP and their respective 3*H*-prostaglandins or thromboxane,[21] and was further described in detail in our recent publication.[8] The EP_4 functional assay (PGE_2-induced cAMP accumulation in the same cell line) to determine EP_4 agonist/antagonist activity was performed according to the procedure reported by Clark et al.[8] A partial agonist generally is unable to completely inhibit the PGE_2 effect, and can partially potentiate cAMP accumulation in the absence of PGE_2. The EP_4 binding and functional assays were performed in the absence and/or presence of 10% HS in order to assess the extent of serum protein binding of compounds. The HWB assay (blockade of inhibition of TNF_α-induced IP-10 release by EP_4 agonist L-902688 in HWB) was performed similarly to the procedure reported by Murase et al.,[20d] except that a specific EP_4 agonist, L-902688, was used instead of PGE_2.[22]

In Vivo Efficacy and Tolerability. The AIA model and GI tolerability model in male SD rats were described in ref 8. The acute carrageenan-induced mechanical hyperalgesia model in SD rats was performed according to a published literature procedure.[23] In this model, **34d** displayed a dose-dependent inhibition of pain response when measured at 3 h post subplantar injection of carrageenan and 1 h post oral dose of compound (Figure 4a). The ED_{50} was established as 0.36 mg/kg with a corresponding EC_{50} of 250 nM. The maximum inhibition reached was 60−70% [vs 70% for COX-2 inhibitor MF-tricyclic (**41**)[24] (Figure 1) in the same experiment] when compared to the vehicle control.

In the rat AIA model, **34d** exhibited potent activity in inhibiting chronic paw swelling, in both the primary paw and the secondary paw, in a dose-dependent manner, with an ED_{50} of 0.02 mg/kg/day and an EC_{50} at 24 h after the last dose of ∼1 nM, reflecting its potent in vitro activity (Figure 4b, only data from the secondary paw is shown). Complete inhibition of the secondary paw swelling was achieved at an ED_{100} of 0.1 mg/kg/day with a plasma concentration of 4 nM at 24 h after the final dose. In comparison, potent COX-2 inhibitors such as rofecoxib[25] and **41** (Figure 1),[24] in the same model but different experiments, exhibited comparable maximum efficacy but were much less potent with ED_{50}s of 0.7 and 0.5 mg/kg/day, respectively. In addition, the potency and efficacy of EP_4 antagonist **34d** is clearly superior to that reported for **37** (Figure 1) in the same model (ED_{80} = 11.5 mg/kg).[20c]

As illustrated in Figure 5, **34d** exhibited excellent GI tolerability in the rat model of GI mucosal permeability. Dosing **34d** at 3, 10, and 30 mg/kg/day for 5 days did not cause significant ^{51}Cr leakage, while dosing indomethacin at 5 mg/kg/day for three days resulted in significant increase in urinary ^{51}Cr excretion due to increased GI mucosal permeability. This result corroborates well with results obtained with **1**[8] and **37**,[20b] further demonstrating that a highly selective EP_4 antagonist may have superior GI tolerability profile compared to traditional NSAIDs.

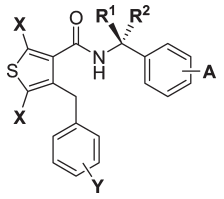
Conclusion

We have described the discovery and preclinical characterization of the highly potent and selective EP_4 antagonist **34d**. In rodent models of pain and inflammation, **34d** exhibited comparable maximum efficacy to coxibs and NSAIDs but was significantly more potent. In the rat GI tolerability model measuring mucosal permeability, **34d** exhibited no obvious effect to the GI mucosal integrity at doses up to 30 mg/kg for 5 days while indomethacin caused significant GI mucosal damage in 3 days. Overall, **34d** displays the desired potency, selectivity, pharmacokinetic, and GI tolerability profile for further development and represents a potential safer alternative for treating pain and inflammation than traditional NSAIDs and coxibs.

Experimental Section

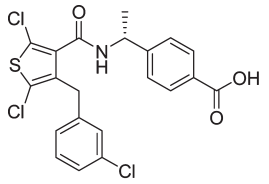
Chemistry. All commercial chemicals were used without further treatment. Anhydrous solvents were purchased from Sigma-Aldrich (Milwaukee, WI), Acros Organics (Fisher Scientific, Nepeau, ON, Canada), or American Chemicals Ltd. (Montreal, QC, Canada). All air-sensitive reactions were carried out under an atmosphere of dry nitrogen. TLC analyses were performed on Merck 60 F_{254} silica gel glass-backed plates, with spots detected visually under ultraviolet irradiation (254 nm). Purification of the crude reaction mixtures was performed as indicated using chromatography on silica gel, either by standard flash method (glass column, Merck Kieselgel 60, 230−400 mesh) or by using a CombiFlash apparatus (Teledyne Isco, Lincoln, NE) equipped with columns of appropriate size (Teledyne Isco, Lincoln, NE, or Silicycle, Quebec City, QC, Canada). All tested compounds possess a purity ≥95% (except **38**, 91%) as determined by reversed-phase HPLC and/or combustion analysis. Proton NMR spectra were obtained using a Bruker Avance 400 spectrometer, at 400 MHz, and were recorded in the indicated solvent. Chemical shifts (δ) were recorded in parts per million (ppm) and reported relative to the solvent peak. High-resolution

Table 1. In Vitro Profile of 3,4-Substituted[a] Thiophene-Containing EP_4 Antagonists[b]



							binding affinity (K_i, nM)[c]			
entry	compd	X	Y	R^1	R^2	A	EP_4	EP_2	others[d]	EP_4 functional potency (IC_{50}, nM)[e]
1	**26a**	H	*m*-Cl	CH_3	H	*p*-COOH	6.4 ± 2.6	16 ± 2	> 7282	12 ± 5
2	**27a**	Cl	*m*-Cl	CH_3	H	*p*-COOH	1.3 ± 0.6	746 ± 231	> 456	3.6 ± 2.0
3	**28a**	CH_3	*m*-Cl	CH_3	H	*p*-COOH	3.1 ± 0.4	467 ± 228	> 2226	8.6 ± 1.0
4	**28b**	CH_3	*m*-CF_3	CH_3	H	*p*-COOH	2.5 ± 0.2	800 ± 187	> 3461	4.7 ± 3.5
5	**28c**	CH_3	*p*-Cl	CH_3	H	*p*-COOH	2.9 ± 0.7	202 ± 40	> 2117	5.4
6	**28d**	CH_3	*p*-CF_3	CH_3	H	*p*-COOH	1.4 ± 0.4	1194 ± 429	> 5474	4.5 ± 1.2
7	**34b**	CH_3	*m*-CF_3	$-CH_2-$	$-CH_2-$	*p*-COOH	0.75 ± 0.20	> 29142	> 4992	nd[f]
8	**34d**	CH_3	*p*-CF_3	$-CH_2-$	$-CH_2-$	*p*-COOH	0.56 ± 0.10	5900 ± 3300	> 3900	2.5 ± 0.7
9	**36**	CH_3	*p*-CF_3	$-CH_2-$	$-CH_2-$	5-tetrazolyl	0.63 ± 0.11	> 29034	> 501	2.3
10	**39**	Cl	*m*-Cl	H	CH_3	*p*-COOH	0.87 ± 0.20	384 ± 108	> 224	1.8
11	**40**	Cl	*m*-Cl	H	H	*p*-COOH	0.72 ± 0.29	43 ± 12	> 628	1.6

[a] The 3,4-substitution refers to the carboxamide and benzyl groups, respectively. [b] Values are means from at least three experiments, except for EP_4 functional potency for compounds **28c**, **36**, **39**, and **40** ($n = 1$). [c] For details on the binding assay for all PG receptors, see ref 8. [d] Other PG receptors refers to EP_1, EP_3, DP_1, DP_2, IP, TP, FP. [e] The EP_4 functional assay measured the inhibition of PGE_2-induced cAMP accumulation in HEK 293 cells; for details, see ref 8. [f] nd: not done.



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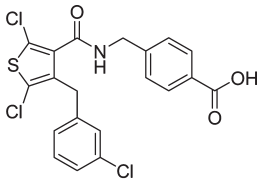

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Figure 3. (*R*)-Enantiomer (**39**) and unsubstituted analogue (**40**) of (*S*)-α-methylbenzamide derivative **27a**.

mass spectra (HRMS) were performed in the ESI mode using an Agilent Technologies MSD TOF mass spectrometer coupled to an Agilent Technologies 1200 series LC. Reversed-phase HPLC purity determinations were performed at three different wavelengths (λ_{max}, 220 and 254 nm) on an Agilent Technologies 1100 series system equipped with a Zorbax RxC18 column (150 mm × 4.6 mm, 5 μ) using an acetonitrile/water gradient containing 0.1% formic acid. Elemental analyses were performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). LC/MS/MS experiments for the pharmacokinetic studies were conducted using a Perkin-Elmer Sciex API 2000 instrument equipped with a Phenomenex Luna C18 column (50 mm × 2 mm, 5 μ) using an acetonitrile/water gradient containing 0.1% formic acid.

Pharmacokinetic Studies. All aspects of housing, care and use of the animals were in accordance with the guidelines of the Canadian Council on Animal Care and the "Guide to the Care and Use of Experimental Animals." SD rats, C57BL/6 mice, beagle dogs, and cynomolgus monkeys were fasted overnight and prior to dosing given either by oral gavage or intravenously. At various time points, blood samples were collected, and this blood was centrifuged to provide plasma samples. The plasma was stored at −10 °C until time of analysis. At this time, aliquots of plasma samples were treated with an equal amount of acetonitrile, containing 0.1% formic acid, to precipitate proteins. The samples thus obtained were vortexed and centrifuged.

Table 2. In Vitro Profile of **34d**[a]

binding affinity[b]	
receptor	K_i, nM
EP_4	0.56 ± 0.10
EP_4 + 10% HS	0.97 ± 0.23
EP_1	> 5600
EP_2	5900 ± 3300
EP_3	> 7900
DP_1	> 3900
DP_2	> 21000
IP	> 22000
TP	> 6800
FP	> 24000
rEP_4	0.70 ± 0.16
rEP_1	> 29000
rEP_2	7200
rEP_3	3300
EP_4 functional potency[c]	
cell line	IC_{50}, nM
HEK 293	2.5 ± 0.7
HEK 293 + 10% HS	2.9 ± 1.1
HWB	11 ± 9

[a] Values are means from at least three experiments, except for rEP_2 and rEP_3 ($n = 1$); HS, human serum; r, rat. [b] For details on the binding assay for all PG receptors, see ref 8. [c] The EP_4 functional potency was evaluated in HEK 293 cells, where the inhibition of PGE_2-induced cAMP accumulation is measured (for details, see ref 8) or in human whole blood (HWB) where the blockade of inhibition of TNF_α-induced IP-10 release by an EP_4 agonist was measured (for details, see ref 24).

The resulting supernatants were transferred into sample vials and subsequently analyzed by LC/MS/MS in the presence of an internal standard. Reference standard samples for each test compounds were prepared as described above, using plasma derived from the blood of untreated animals and subsequently

spiked with known concentrations of test compounds in an appropriate range.

Receptor Binding, Functional and HWB Assays. The receptor binding assays and the EP_4 funtional assay were conducted according to the procedure described in ref 8. The EP_4 HWB assay was perfomed as described in ref 22.

In Vivo Efficacy and Tolerability Models. The rat AIA model was performed under the conditions published in ref 8. The rat carrageenan-induced mechanical hyperalgesia model was conducted according to the procedure described in ref 23. The GI tolerability model in rat was described previously in ref 8 and performed accordingly.

2-Bromo-3-hydroxymethylthiophene (4). To a solution of commercially available 3-thiophenemethanol (**3**) (8.20 g, 71.8 mmol) in THF (150 mL) at 0 °C was added water (10 mL) followed by solid NBS (12.8 g, 71.8 mmol), and the solution was stirred at room temperature for 1 h. Most of the solvent was evaporated in vacuo, and the residue was redissolved in EtOAc and washed with water (3×) and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give **4** as a yellowish oil. The crude product was used in the next step directly, without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.50 (d, 1H), 7.12 (d, 1H), 4.55 (s, 2H).

(2-Bromo-5-chloro-3-thienyl)methanol (5). To a solution of **4** (13.0 g, 67.3 mmol) in a mixture of THF (100 mL) and water (10 mL) was added NCS (9.88 g, 74.0 mmol), and the resulting mixture was stirred at room temperature for 5 h and concentrated in vacuo. The residue was redissolved in EtOAc and washed with water (3×) and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give **5**. The crude product was used in the next step directly, without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.03 (s, 1H), 4.52 (d, 2H), 4.45 (t, 1H).

2-Bromo-5-chlorothiophene-3-carbaldehyde (6). To a solution of DMSO (2.10 mL, 29.7 mmol) in CH_2Cl_2 (50 mL) at −78 °C was added oxalyl chloride (1.90 mL, 26.8 mmol) dropwise, and the mixture was stirred for 30 min at room temperature. **5** (4.50 g, 19.8 mmol) in CH_2Cl_2 (25 mL) was added, and the resulting solution was stirred for 30 min. Triethylamine (6.40 mL, 45.5 mmol) was then added in one portion, and the mixture was stirred at −78 °C for 30 min and allowed to warm slowly in air. After concentration in vacuo, the residue was suspended in Et_2O and then filtered. The filtrate was concentrated in vacuo to give **6**. The crude product was used in the next step directly, without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.20 (s, 1H).

(2-Bromo-5-chloro-3-thienyl)(3-chlorophenyl)methanol (7). To a solution of **6** (2.50 g, 11.1 mmol) in a mixture of THF (20 mL) and Et_2O (20 mL) at −78 °C was added 3-chlorophenylmagnesium bromide (0.5 M in THF, 26.6 mL, 13.3 mmol) within 2 min. The mixture was stirred at the same temperature for 5 min, quenched with saturated NH_4Cl, and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and filtered. The filtrate was concentrated, and the residue was purified by flash chromatography (EtOAc/hexane 10:90 to 20:80) to give **7** (2.51 g, 67%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.52 (s, 1H), 7.39 (d, J = 5.1 Hz, 2H), 7.32 (m, 1H), 7.00 (s, 1H), 5.89 (d, J = 4.1 Hz, 1H), 5.36 (d, J = 4.1 Hz, 1H).

2-Bromo-5-chloro-3-(3-chlorobenzyl)thiophene (8). To a solution of **7** (2.50 g, 7.40 mmol) in CH_2Cl_2 (30 mL) at room temperature was added TFA (5.68 mL, 74.0 mmol) (red solution formed) followed by triethylsilane (5.91 mL, 37.0 mmol) (red solution turned to yellow). The mixture was stirred at room

Table 3. Pharmacokinetic Parameters of EP_4 antagonist **34d**[a]

species	dose po, iv (mg/kg)	F (%)	C_{max} (μM)	$T_{1/2}$ (h)	CL (mL/min/kg)	V_{dss} (L/kg)
mouse[b]	20, 5	21	1.4	15	23	7.6
rat[c]	20, 5	29	4.5	4.5	9.2	2.6
dog[c]	5, 1	32	3.3	8.8	3.2	0.91
cynomolgus monkey[c]	5, 1	18	0.05	19	0.91	7.9

[a] Oral (po) dose administered in 0.5% methocel. Intravenous (iv) dose administered in 60% PEG 200. F denotes bioavailability, C_{max} denotes the maximum concentration reached and is determined from the po data, $T_{1/2}$ is the half-life in plasma and is determined from the iv data, CL is plasma clearance, V_{dss} is the volume of distribution. [b] The corresponding sodium salt was used in this experiment. [c] The free acid was used in this experiment.

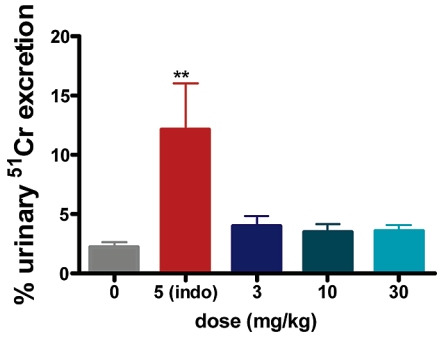

Figure 5. GI tolerability of compound **34d** in rats vs NSAID indomethacin based on urinary ^{51}Cr-excretion after a single oral dose of ^{51}Cr-EDTA on day 4 for **34d** treated animals and on day 2 for indomethacin treated animals (n = 5/group for compound treatment and 4/group for vehicle; $^{**}p < 0.05$ vs vehicle, 1 way ANOVA/Dunnett's test). Indomethacin: 5 mg/kg once daily in 0.5% methocel for 3 days; **34d**: 3, 10, and 30 mg/kg in 0.5% methocel once daily for 5 days.

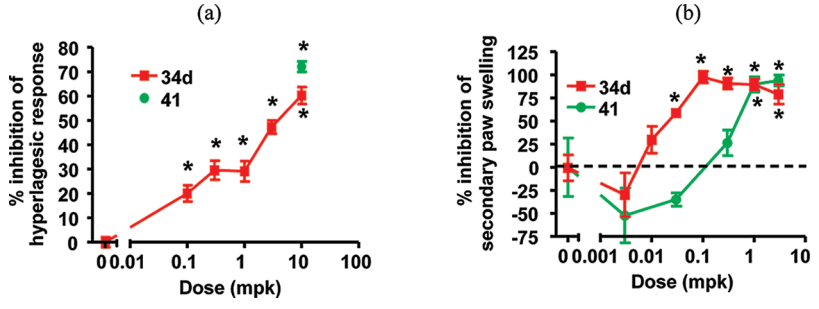

Figure 4. In vivo activity of compound **34d**. (a) Dose-dependent inhibition of hyperalgesic response in the rat carrageenan-induced hyperalgesia model (red) in comparison to COX-2 inhibitor **41** at 10 mg/kg (green) from the same experiment (n = 10−20/group, $^{*}p <$ 0.01 vs vehicle by 1 way ANOVA/Dunnett's test, at single oral doses of ≥0.1 mg/kg at 3 h after carrageenan challenge and 1 h after oral dosing of compounds). (b) Inhibition of secondary paw swelling in female Lewis rats with AIA (red) in comparison with COX-2 inhibitor **41** (green) from a separate experiment (n = 7/group for compound and vehicle, $^{*}p < 0.02$ vs vehicle by 1 way ANOVA/Dunnett's test at doses ≥0.01 mg/kg; compounds were dosed in 0.5% methocel once daily for 9 days starting from day 9 after injection of CFA in the primary paw on day 0).

temperature for 30 min and concentrated. Residual TFA was coevaporated with toluene and the residue dried under high vacuum. The crude was purified by flash chromatography (hexane) to give **8** as a colorless oil (1.57 g, 66%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.39−7.21 (m, 4H), 6.93 (s, 1H), 3.95 (s, 2H).

5-Chloro-3-(3-chlorobenzyl)thiophene-2-carboxylic Acid (9). To a solution of **8** (1.56 g, 4.84 mmol) in THF (15 mL) at −78 °C was added *n*-BuLi (2.5 M in hexane, 2.13 mL, 5.32 mmol) dropwise, and the mixture was stirred for 5 min at the same temperature. An excess of CO_2 gas was bubbled into the reaction mixture, and the latter was allowed to warm to 0 °C, quenched with 1 N HCl, and extracted with EtOAc. The organic layer was washed successively with saturated NH_4Cl and brine, dried ($MgSO_4$), and filtered. The filtrate was concentrated and the residue was recrystallized from Et_2O/hexane to give **9** as a white solid (1.26 g, 91%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.39−7.23 (m, 4H), 7.04 (s, 1H), 4.40 (s, 2H).

Methyl 4-[1-({[5-Chloro-3-(3-chlorobenzyl)-2-thienyl]carbonyl}amino)ethyl]benzoate (11). To a solution of **9** (200 mg, 0.696 mmol) and (±)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**10**) [prepared from (±)-1-(4-bromophenyl)ethylamine according to ref 16] (180 mg, 0.835 mmol) in DMF (3 mL) at 0 °C were added successively HATU (317 mg, 0.835 mmol) and DIEA (304 μL, 1.74 mmol) dropwise. The mixture was stirred at 0 °C for 15 min and diluted with a mixture of water, EtOAc, and Et_2O. The organic layer was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude was purified by chromatography using the CombiFlash system (EtOAc/hexane 10:90 to 40:60, 15 min) to give **11** as a white solid (300 mg, 96%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.99 (d, J = 8.3 Hz, 2H), 7.82 (br d, J = 7.6 Hz, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.34−7.20 (m, 4H), 6.98 (s, 1H), 5.30 (m, 1H), 4.30 (d, J_{AB} = 14.6 Hz, 1H), 4.26 (d, J_{AB} = 14.7 Hz, 1H), 3.89 (s, 3H), 1.57 (d, J = 7.1 Hz, 3H).

4-[1-({[5-Chloro-3-(3-chlorobenzyl)-2-thienyl]carbonyl}amino)-ethyl]benzoic Acid (12). To a solution of **11** (81.0 mg, 0.181 mmol) in a mixture of THF (1.5 mL) and MeOH (1.5 mL) was added a solution of LiOH (aq 1 M, 900 μL, 0.900 mmol). The resulting mixture was stirred at room temperature for 18 h and concentrated. The residue was diluted with 1 N HCl and extracted with EtOAc. The organic layer was dried (Na_2SO_4), filtered, and concentrated to give **12** as a white solid (78 mg, 99%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.20 (br s, 1H), 8.02 (d, J = 8.2 Hz, 2H), 7.79 (br d, J = 7.7 Hz, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.34 (s, 1H), 7.31−7.20 (m, 3H), 6.98 (s, 1H), 5.34−5.26 (m, 1H), 4.31 (d, J_{AB} = 14.7 Hz, 1H), 4.26 (d, J_{AB} = 14.7 Hz, 1H), 1.57 (d, J = 7.1 Hz, 3H). HRMS calcd for $C_{21}H_{16}Cl_2NO_3S$ [$(M - H)^-$], 432.0233; found, 432.0229.

(4-Bromo-3-thienyl)(3-chlorophenyl)methanol (15a). Commercially available 3,4-dibromothiophene (**13**) (15.5 g, 64.1 mmol) was added dropwise to a solution of *n*-BuLi (2.5 M in hexane, 25.6 mL, 64.1 mmol) in Et_2O (50 mL) at −78 °C. After 1.5 h, 3-chlorobenzaldehyde (7.29 mL, 64.1 mmol) was added dropwise to the beige suspension. The resulting solution was stirred at −78 °C for 1 h and allowed to warm to 0 °C. After 1 h, the reaction was quenched by the addition of 25% aqueous NH_4OAc. The aqueous layer was extracted with EtOAc (2×), and the combined organics were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to afford the **15a** as a pale-yellow oil (19.5 g, 100%). The crude product was used directly without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.54 (m, 2H), 7.47 (s, 1H), 7.39−7.28 (m, 3H), 5.89 (d, J = 4.6 Hz, 1H), 5.12 (d, J = 4.5 Hz, 1H).

3-Bromo-4-(3-chlorobenzyl)thiophene (17a). To **15a** (144 mg, 0.474 mmol) in CH_2Cl_2 (1 mL) at 0 °C were successively added triethylsilane (303 μL, 1.90 mmol) quickly and TFA (364 μL, 4.74 mmol) dropwise. After 30 min, the reaction mixture was concentrated to dryness and the residue was dissolved in $CHCl_3$ and washed with 5% $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$, and the combined organics were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (toluene/hexane 5:95) to afford **17a** as a colorless oil (110 mg, 81%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.57 (d, J = 3.4 Hz, 1H), 7.37−7.20 (m, 5H), 4.01 (s, 2H).

4-(3-Chlorobenzyl)thiophene-3-carboxylic Acid (19a). To a solution of *n*-BuLi (2.5 M in hexane, 8.65 mL, 21.6 mmol) in Et_2O (40 mL) at −78 °C was added a solution of **17a** (6.22 g, 21.6 mmol) in THF (10 mL) dropwise, and the resulting yellow solution was stirred for 30 min at the same temperature. An excess of CO_2 gas was bubbled into the reaction mixture, and the latter was stirred at −78 °C for 30 min, allowed to warm to 0 °C, and quenched with 1 N NaOH. The aqueous layer was washed with Et_2O (2×) and acidified (pH 2) with 6 N HCl. The off-white precipitate was extracted with $CHCl_3$ (5×), and the combined organic layers were washed successively with water and brine, dried (Na_2SO_4), filtered, and concentrated give **19a** as an off-white solid (4.75 g, 87%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.05 (br s, 1H), 8.32 (d, J = 3.4 Hz, 1H), 7.33−7.27 (m, 2H), 7.23−7.18 (m, 3H), 4.31 (s, 2H).

Methyl 4-[(1*S*)-1-({[4-(3-Chlorobenzyl)-3-thienyl]carbonyl}amino)ethyl]benzoate (23a). To a solution of **19a** (380 mg, 1.50 mmol) and (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (294 mg, 1.36 mmol) in DMF (1.5 mL) at 0 °C were added successively HATU (518 mg, 1.36 mmol) and DIEA (548 μL, 3.14 mmol) dropwise. The mixture was stirred at 0 °C for 1 h, poured into 1:1 saturated $NaHCO_3$/water (15 mL), and extracted with EtOAc (3×). The combined organic layers were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 40:60 to 95:5) to give **23a** as a white solid (520 mg, 92%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.99−7.90 (m, 4H), 7.51 (d, J = 8.0 Hz, 2H), 7.25−7.12 (m, 5H), 5.26 (m, 1H), 4.25 (d, J_{AB} = 15.0 Hz, 1H), 4.21 (d, J_{AB} = 15.0 Hz, 1H), 3.89 (s, 3H), 1.52 (d, J = 7.1 Hz, 3H).

4-[(1*S*)-1-({[4-(3-Chlorobenzyl)-3-thienyl]carbonyl}amino)ethyl]-benzoic Acid (26a). To a solution of **23a** (100 mg, 0.242 mmol) in a mixture of THF (2.5 mL) and MeOH (1.25 mL) was added a solution of LiOH (aq 0.5 M, 1.45 mL, 0.725 mmol). The resulting mixture was stirred at room temperature for 60 h, acidified with 6 N HCl (1 mL), diluted with Et_2O (30 mL), and washed with water and brine. CH_2Cl_2 (50 mL) was added to the organic layer to dissolve the suspended portion of product. The aqueous layer was further extracted with CH_2Cl_2, and the combined organic fractions were dried (Na_2SO_4), filtered, and concentrated to provide pure **26a** as a white solid (87 mg, 90%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.64 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.82 (d, J = 3.2 Hz, 1H), 7.34 (d, J = 8.2 Hz, 2H), 7.16−7.10 (m, 3H), 7.07 (s, 1H), 7.02−6.98 (m, 1H), 5.10 (m, 1H), 4.16 (d, J_{AB} = 15.3 Hz, 1H), 4.10 (d, J_{AB} = 15.3 Hz, 1H), 1.45 (d, J = 7.1 Hz, 3H). HRMS calcd for $C_{21}H_{17}ClNO_3S$ [$(M - H)^-$], 398.0623; found, 398.0616. Anal. ($C_{21}H_{18}ClNO_3S$) H, N. C: calcd, 63.07; found, 62.36.

2,5-Dichloro-4-(3-chlorobenzyl)thiophene-3-carboxylic Acid (21a). NCS (4.46 g, 33.4 mmol) was added to **19a** (4.02 g, 15.9 mmol) in AcOH (40 mL), and the resulting mixture was heated at 110 °C for 2 h and allowed to cool to room temperature. The solvent was coevaporated with toluene (3×), and the residue was partitioned between $CHCl_3$ (250 mL) and water (100 mL). The organic layer was washed with water (3×) and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 20:80 to 30:70 containing 0.5% AcOH) to afford **21a** as a beige solid (3.60 g, 70%) after residual AcOH was coevaporated with toluene. ^{1}H NMR (400 MHz, acetone-d_6) δ 11.91 (br s, 1H), 7.34−7.27 (m, 1H), 7.26−7.18 (m, 2H), 7.19−7.11 (m, 1H), 4.27 (s, 2H).

Methyl 4-[(1*S*)-1-({[2,5-Dichloro-4-(3-chlorobenzyl)-3-thienyl]carbonyl}amino)ethyl]benzoate (24a). The acid **21a** (1.90 g, 5.91 mmol), (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (1.27 g, 5.91 mmol), HATU (2.25 g, 5.91 mmol),

and DIEA (2.35 mL, 13.6 mmol) were reacted in DMF (28 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/toluene 2:98 to 5:95, 20 min) to afford **24a** as a white solid (2.14 g, 75%). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.11 (br d, J = 7.8 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.28−7.18 (m, 3H), 7.12−7.07 (m, 1H), 5.23 (m, 1H), 4.07 (d, J_{AB} = 15.3 Hz, 1H), 4.00 (d, J_{AB} = 15.3 Hz, 1H), 3.89 (s, 3H), 1.48 (d, J = 7.1 Hz, 3H).

4-[(1*S*)-1-({[2,5-Dichloro-4-(3-chlorobenzyl)-3-thienyl]carbonyl}amino)ethyl]benzoic Acid (27a). To a solution of **24a** (2.11 g, 4.37 mmol) in a mixture of THF (90 mL) and MeOH (45 mL) was added a solution of LiOH (aq 1 M, 13.1 mL, 13.1 mmol). The resulting mixture was heated at 50 °C for 4 h, cooled to room temperature, and acidified with 1 N HCl (14.0 mL). Water was added until precipitation, and the solid was extracted with EtOAc (3×). The combined organics were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was stirred in Et_2O, collected by filtration, rinsed with the same solvent, and dried to provide pure **27a** as a white solid (1.84 g, 90%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.17 (br s, 1 H), 8.11 (br d, J = 7.8 Hz, 1H), 7.99 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.29−7.18 (m, 3H), 7.13−7.07 (m, 1H), 5.24 (m, 1H), 4.07 (d, J_{AB} = 15.3 Hz, 1H), 4.01 (d, J_{AB} = 15.3 Hz, 1H), 1.48 (d, J = 7.0 Hz, 3H). HRMS calcd for $C_{21}H_{15}Cl_3NO_3S$ [$(M - H)^-$], 465.9844; found, 465.9836. Anal. ($C_{21}H_{16}Cl_3NO_3S$) C, H, N.

(4-Bromo-2,5-dimethyl-3-thienyl)(3-chlorophenyl)methanol (16a). A solution of 3,4-dibromo-2,5-dimethylthiophene[26] (**14**) (3.00 g, 11.1 mmol) in THF (50 mL) was treated with *n*-BuLi (2.5 M in hexane, 4.44 mL, 11.1 mmol) and 3-chlorobenzaldehyde (1.26 mL, 11.1 mmol) under the conditions described above for the preparation of **15a.** The crude product was purified by chromatography using the CombiFlash system (EtOAc/hexane 2.98 to 12:88, 20 min) to afford **16a** as a yellow oil (2.92 g, 79%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.47 (s, 1H), 7.39−7.25 (m, 3H), 6.08 (d, J = 4.4 Hz, 1H), 5.07 (d, J = 4.3 Hz, 1H), 2.35 (s, 6H).

3-Bromo-4-(3-chlorobenzyl)-2,5-dimethylthiophene (18a). Triethylsilane (5.55 mL, 34.7 mmol) and TFA (6.68 mL, 86.8 mmol) were successively added to a solution of **16a** (2.88 g, 8.68 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was stirred at the same temperature for 30 min and concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed with 5% aqueous $NaHCO_3$, water, and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by chromatography using the CombiFlash system (EtOAc/hexane 2:98 to 5:95, 20 min) to afford **18a** as a colorless oil (2.42 g, 88%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.31 (t, J = 7.8 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.17 (s, 1H), 7.13 (d, J = 7.77 Hz, 1H), 3.98 (s, 2H), 2.40 (s, 3H), 2.35 (s, 3H).

4-(3-Chlorobenzyl)-2,5-dimethylthiophene-3-carboxylic Acid (20a). A solution of **18a** (2.39 g, 7.57 mmol) in THF (30 mL) was treated with *n*-BuLi (2.5 M in hexane, 3.03 mL, 7.57 mmol) and CO_2 gas under the conditions described above for the preparation of **19a**. The reaction was quenched with 25% aqueous NH_4OAc and extracted with EtOAc. The combined organics were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 25:75 containing 0.25% AcOH) to afford **20a** as a beige solid (1.23 g, 58%) after residual AcOH was coevaporated with toluene. ^{1}H NMR (400 MHz, acetone-d_6) δ 11.01 (br s, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.21−7.11 (m, 2H), 7.07 (d, J = 7.7 Hz, 1H), 4.23 (s, 2H), 2.63 (s, 3H), 2.34 (s, 3H).

Methyl 4-[(1*S*)-1-({[4-(3-Chlorobenzyl)-2,5-dimethyl-3-thienyl]carbonyl}amino)ethyl]benzoate (25a). The acid **20a** (97 mg, 0.345 mmol), (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (78 mg, 0.363 mmol), HATU (138 mg, 0.363 mmol), and DIEA (139 μL, 0.795 mmol) were reacted in DMF (1 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$ 0:100 to 2:98) to provide **25a** as a white solid (130 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.11−7.17 (m, 2H), 7.03 (s, 1H), 6.93 (d, J = 6.6 Hz, 1H), 5.69 (br d, J = 7.9 Hz, 1H), 5.18 (m, 1H), 3.94 (s, 2H), 3.92 (s, 3H), 2.44 (s, 3H), 2.31 (s, 3H), 1.35 (d, J = 7.0 Hz, 3H).

4-[(1*S*)-1-({[4-(3-Chlorobenzyl)-2,5-dimethyl-3-thienyl]carbonyl}amino)ethyl]benzoic Acid (28a). The ester **25a** (130 mg, 0.294 mmol) in THF (3 mL) and MeOH (1.5 mL) was treated with LiOH (aq 0.5 M, 1.76 mL, 0.882 mmol) for 17 h, under the conditions described above for the preparation of **27a**, but at room temperature. Pure **28a** was obtained as a white solid without purification (115 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.68 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 7.23−7.17 (m, 2H), 7.09 (s, 1H), 7.04−6.99 (m, 1H), 5.09 (m, 1H), 3.90 (d, J_{AB} = 15.3 Hz, 1H), 3.83 (d, J_{AB} = 15.3 Hz, 1H), 2.35 (s, 3H), 2.29 (s, 3H), 1.34 (d, J = 7.0 Hz, 3H). HRMS calcd for $C_{23}H_{21}ClNO_3S$ [$(M - H)^-$], 426.0936; found, 426.0930.

(4-Bromo-2,5-dimethyl-3-thienyl)[3-(trifluoromethyl)phenyl]methanol (16b). A solution of 3,4-dibromo-2,5-dimethylthiophene[26] (**14**) (3.00 g, 11.1 mmol) in a mixture of Et_2O (40 mL) and THF (20 mL) was treated with *n*-BuLi (2.5 M in hexane, 4.44 mL, 11.1 mmol) and 3-(trifluoromethyl)benzaldehyde (1.48 mL, 11.1 mmol) under the conditions described above for the preparation of **15a**. The pale-brown oil obtained (**16b**) was used without further purification (3.93 g, 97%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.83 (s, 1H), 7.63−7.53 (m, 3H), 6.17 (d, J = 4.4 Hz, 1H), 5.18 (d, J = 4.2 Hz, 1H), 2.34 (s, 6H).

3-Bromo-2,5-dimethyl-4-[3-(trifluoromethyl)benzyl]thiophene (18b). Triethylsilane (5.63 mL, 35.3 mmol) and TFA (6.77 mL, 88.2 mmol) were successively added to a solution of **16b** (3.22 g, 8.82 mmol) in CH_2Cl_2 (20 mL) under the conditions described above for the preparation of **18a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/hexane 0:100 to 2:98, 26 min) to afford **18b** as a colorless liquid (2.53 g, 82%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.57−7.48 (m, 3H), 7.44 (m, 1H), 4.08 (s, 2H), 2.42 (s, 3H), 2.36 (s, 3H).

2,5-Dimethyl-4-[3-(trifluoromethyl)benzyl]thiophene-3-carboxylic Acid (20b). A solution of **18b** (2.50 g, 7.16 mmol) in THF (30 mL) was treated with *n*-BuLi (2.5 M in hexane, 2.86 mL, 7.16 mmol) and CO_2 gas under the conditions described above for the preparation of **20a**. The crude product was purified by flash chromatography (EtOAc/hexane 20:80 to 25:75 containing 0.25% AcOH) to afford **20b** as a beige solid (1.20 g, 53%) after residual AcOH was coevaporated with toluene. ^{1}H NMR (400 MHz, acetone-d_6) δ 11.03 (br s, 1H), 7.51−7.43 (m, 3H), 7.39 (m, 1H), 4.32 (s, 2H), 2.64 (s, 3H), 2.36 (s, 3H).

Methyl 4-{(1*S*)-1-[({2,5-Dimethyl-4-[3-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]ethyl}benzoate (25b). The acid **20b** (200 mg, 0.636 mmol), (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (137 mg, 0.636 mmol), HATU (242 mg, 0.636 mmol), and DIEA (253 μL, 1.46 mmol) were reacted in DMF (3.5 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/toluene 2:98 to 10:90, 20 min) to provide **25b** as an off-white solid (209 mg, 69%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.92 (d, J = 8.0 Hz, 2H), 7.70 (br d, J = 8.0 Hz, 1H), 7.49−7.34 (m, 6H), 5.22 (m, 1H), 4.07 (d, J_{AB} = 15.6 Hz, 1H), 4.02 (d, J_{AB} = 15.6 Hz, 1H), 3.88 (s, 3H), 2.41 (s, 3H), 2.32 (s, 3H), 1.45 (d, J = 7.1 Hz, 3H).

4-{(1*S*)-1-[({2,5-Dimethyl-4-[3-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]ethyl}benzoic Acid (28b). The ester **25b** (202 mg, 0.425 mmol) in THF (8 mL) and MeOH (4 mL) was treated with LiOH (aq 1 M, 1.27 mL, 1.27 mmol) for 4 h under the conditions described above for the preparation of **27a**. The crude solid was triturated in 1:4 $CHCl_3$/hexane, collected by filtration, rinsed with hexane, and dried to afford pure **28b** as a white solid (162 mg, 82%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.15 (br s, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.70 (br d, J = 8.0 Hz,

1H), 7.51−7.37 (m, 6H), 5.24 (m, 1H), 4.08 (d, J_{AB} = 15.7 Hz, 1H), 4.02 (d, J_{AB} = 15.7 Hz, 1H), 2.42 (s, 3H), 2.33 (s, 3H), 1.45 (d, J = 7.1 Hz, 3H). HRMS calcd for $C_{24}H_{21}F_3NO_3S$ [(M − H)$^-$], 460.1200; found, 460.1194. Anal. ($C_{24}H_{22}F_3NO_3S$) C, H, N.

(4-Bromo-2,5-dimethyl-3-thienyl)(4-chlorophenyl)methanol (16c). A solution of 3,4-dibromo-2,5-dimethylthiophene[26] (**14**) (1.00 g, 3.70 mmol) in THF (15 mL) was treated with *n*-BuLi (1.6 M in hexane, 2.32 mL, 3.70 mmol) and 4-chlorobenzaldehyde (521 mg, 3.70 mmol) under the conditions described above for the preparation of **15a**. The crude product was purified by flash chromatography (EtOAc/hexane 10:90 to 25:75) to afford **16c** (975 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 4H), 6.05 (d, J = 6.0 Hz, 1H), 2.48 (d, J = 6.0 Hz, 1H), 2.36 (s, 3H), 2.34 (s, 3H).

3-Bromo-4-(4-chlorobenzyl)-2,5-dimethylthiophene (18c). Triethylsilane (1.88 mL, 11.8 mmol) and TFA (2.26 mL, 29.4 mmol) were successively added to a solution of **16c** (975 mg, 2.94 mmol) in CH_2Cl_2 (5 mL) under the conditions described above for the preparation of **18a**. The crude product was purified by flash chromatography (hexane) to afford **18c** as a white solid (835 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 8.0 Hz, 2H), 3.91 (s, 2H), 2.39 (s, 3H), 2.35 (s, 3H).

4-(4-Chlorobenzyl)-2,5-dimethylthiophene-3-carboxylic Acid (20c). A solution of **18c** (824 mg, 2.61 mmol) in a mixture of THF (5 mL) and Et_2O (10 mL) was treated with *n*-BuLi (1.6 M in hexane, 1.63 mL, 2.61 mmol) and CO_2 gas under the conditions described above for the preparation of **20a**. Pure **20c** was obtained as a light-yellow solid without further purification (472 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 8.0 Hz, 2H), 4.18 (s, 2H), 2.70 (s, 3H), 2.32 (s, 3H).

Methyl 4-[(1*S*)-1-({[4-(4-Chlorobenzyl)-2,5-dimethyl-3-thienyl]carbonyl}amino)ethyl]benzoate (25c). The acid **20c** (472 mg, 1.68 mmol), (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (363 mg, 1.68 mmol), HATU (639 mg, 1.68 mmol), and DIEA (675 μL, 3.87 mmol) were reacted in DMF (10 mL) under the conditions described above for the preparation of **23a** (an additional 2 mL of DMF needed to be added and the mixture was allowed to reach room temperature for homogeneity). The crude product was purified by flash chromatography (MeOH/CH_2Cl_2 0:100 to 2:98) to provide **25c** as a white solid (540 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.20−7.17 (m, 4H), 6.98 (d, J = 8.2 Hz, 2H), 5.58 (br d, J = 8.0 Hz, 1H), 5.18 (m, 1H), 3.97−3.88 (m, 5H), 2.43 (s, 3H), 2.32 (s, 3H), 1.38 (d, J = 8.0 Hz, 3H).

4-[(1*S*)-1-({[4-(4-Chlorobenzyl)-2,5-dimethyl-3-thienyl]carbonyl}amino)ethyl]benzoic Acid (28c). The ester **25c** (540 mg, 1.22 mmol) in THF (12 mL) and MeOH (4 mL) was treated with LiOH (aq 0.5 M, 7.33 mL, 3.67 mmol) for 2 h under the conditions described above for the preparation of **27a**. Pure **28c** was obtained as a white solid without further purification (520 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 8.61 (d, J = 8.2 Hz, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.3 Hz, 2H), 7.02 (d, J = 8.2 Hz, 2H), 5.09 (m, 1H), 3.87 (d, J_{AB} = 15.3 Hz, 1H), 3.79 (d, J_{AB} = 15.2 Hz, 1H), 2.34 (s, 3H), 2.29 (s, 3H), 1.34 (d, J = 7.0 Hz, 3H). HRMS calcd for $C_{23}H_{21}ClNO_3S$ [(M − H)$^-$], 426.0936; found, 426.0930.

(4-Bromo-2,5-dimethyl-3-thienyl)[4-(trifluoromethyl)phenyl]methanol (16d). A solution of 3,4-dibromo-2,5-dimethylthiophene[26] (**14**) (3.00 g, 11.1 mmol) in THF (50 mL) was treated with *n*-BuLi (2.5 M in hexane, 4.44 mL, 11.1 mmol) and 4-(trifluoromethyl)benzaldehyde (1.48 mL, 11.1 mmol) under the conditions described above for the preparation of **15a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/hexane 2.98 to 15:85, 26 min) to afford **16d** as a yellow oil (2.93 g, 72%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.69 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 6.16 (d, J = 4.4 Hz, 1H), 5.15 (d, J = 4.2 Hz, 1H), 2.34 (s, 6H).

3-Bromo-2,5-dimethyl-4-[4-(trifluoromethyl)benzyl]thiophene (18d). Triethylsilane (5.11 mL, 32.0 mmol) and TFA (6.14 mL, 80.0 mmol) were successively added to a solution of **16d** (2.92 g, 8.00 mmol) in CH_2Cl_2 (18 mL) under the conditions described above for the preparation of **18a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/hexane 0:100 to 10:90, 28 min) to afford **18d** as a colorless liquid (2.36 g, 84%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.63 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 4.07 (s, 2H), 2.41 (s, 3H), 2.35 (s, 3H).

2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]thiophene-3-carboxylic Acid (20d). A solution of **18d** (2.34 g, 6.70 mmol) in THF (30 mL) was treated with *n*-BuLi (2.5 M in hexane, 2.68 mL, 6.70 mmol) and CO_2 gas under the conditions described above for the preparation of **20a**. The crude material was triturated in EtOAc/hexane 10:90, collected by filtration, rinsed with the same solvent followed by hexane, and dried to afford pure **20d** as an off-white solid (1.11 g, 53%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.01 (br s, 1H), 7.58 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.32 (s, 2H), 2.64 (s, 3H), 2.34 (s, 3H).

Methyl 4-{(1*S*)-1-[({2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]ethyl}benzoate (25d). The acid **20d** (250 mg, 0.795 mmol), (1*S*)-1-[4-(methoxycarbonyl)phenyl]ethanaminium chloride (**22**)[16] (172 mg, 0.795 mmol), HATU (302 mg, 0.795 mmol), and DIEA (316 μL, 1.83 mmol) were reacted in DMF (5 mL) under the conditions described above for the preparation of **23a**. The precipitate formed in the $NaHCO_3$ solution was collected by filtration, successively rinsed with water, EtOH, and hexane, and dried to provide **25d** as a white solid (332 mg, 88%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.93 (d, J = 8.2 Hz, 2H), 7.65 (br d, J = 8.2 Hz, 1H), 7.48 (m, 4H), 7.29 (d, J = 8.0 Hz, 2H), 5.24 (m, 1H), 4.08 (d, J_{AB} = 15.5 Hz, 1H), 3.98 (d, J_{AB} = 15.5 Hz, 1H), 3.88 (s, 3H), 2.42 (s, 3H), 2.33 (s, 3H), 1.46 (d, J = 7.1 Hz, 3H).

4-{(1*S*)-1-[({2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]ethyl}benzoic acid (28d). The ester **25d** (327 mg, 0.688 mmol) in THF (13 mL) and MeOH (6.5 mL) was treated with LiOH (aq 1 M, 2.06 mL, 2.06 mmol) for 17 h under the conditions described above for the preparation of **27a**. The crude product was purified by chromatography using the CombiFlash system (EtOH/$CHCl_3$ 10:90) to afford **28d** as an off-white solid (185 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (br s, 1H), 8.61 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 5.09 (m, 1H), 3.99 (d, J_{AB} = 15.3 Hz, 1H), 3.89 (d, J_{AB} = 15.3 Hz, 1H), 2.35 (s, 3H), 2.32 (s, 3H), 1.32 (d, J = 7.0 Hz, 3H). HRMS calcd for $C_{24}H_{21}F_3NO_3S$ [(M − H)$^-$], 460.1200; found, 460.1192. Anal. ($C_{24}H_{22}F_3NO_3S$) C, H, N.

4-(1-Aminocyclopropyl)benzonitrile (30). To a solution of 1,4-dicyanobenzene (**29**) (3.30 g, 25.8 mmol) in CH_2Cl_2 (130 mL) was added Ti(O^{i}Pr)$_4$ (7.56 mL, 25.8 mmol) followed by EtMgBr (3 M in Et_2O, 15.5 mL, 46.4 mmol) dropwise (exothermic, gas evolution occurred after one equivalent of reagent added), and the mixture was stirred at room temperature for 45 min. $BF_3 \cdot Et_2O$ (5.71 mL, 46.4 mmol) was added and the mixture was stirred at the same temperature for 2 h, quenched with NH_4Cl and 1 N HCl, and separated. The aqueous layer was washed once with Et_2O, and the pH was adjusted to 9−10 with 10 N NaOH (precipitate formation). The mixture was filtered through celite and the cake washed with water and EtOAc. The layers were separated and the aqueous phase extracted with EtOAc. The organic layers were combined, washed with brine, dried (Na_2SO_4), filtered, and concentrated to afford **30** as a viscous oil which solidified at −20 °C. The crude was used directly without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 1.26−1.18 (m, 2H), 1.10−1.05 (m, 2H).

1-(4-Carboxyphenyl)cyclopropanaminium Chloride (31). A mixture of **30** (576 mg, 3.64 mmol) and 6 N HCl (12 mL) was heated to the reflux temperature for 40 h, cooled to room

temperature, and concentrated to dryness to afford **31** as a beige solid. The crude was used without further purification.

Methyl 4-(1-Aminocyclopropyl)benzoate (32). Aqueous HCl (12 N, 2 drops) was added to a suspension of **31** (3.64 mmol) in MeOH (5 mL). The resulting mixture was heated to the reflux temperature for 16 h, cooled to room temperature, and concentrated to dryness. The residue was partitioned between EtOAc and 5% aqueous $NaHCO_3$. The organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to afford **32** as an orange oil (472 mg, 68%). The crude was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 3.92 (s, 3H), 1.20−1.15 (m, 2H), 1.10−1.05 (m, 2H).

Methyl 4-{1-[({2,5-Dimethyl-4-[3-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]cyclopropyl}benzoate (33b). The acid **20b** (299 mg, 0.952 mmol), methyl 4-(1-aminocyclopropyl)benzoate (**32**) (182 mg, 0.952 mmol), HATU (362 mg, 0.952 mmol), and DIEA (379 μL, 2.19 mmol) were reacted in DMF (6 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/$CHCl_3$ 2:98 to 5:95 to 10:90, 30 min) to provide **33b** as an off-white solid (226 mg, 49%). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.03 (br s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 7.7 Hz, 1H), 7.49−7.43 (m, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.3 Hz, 2H), 4.12 (s, 2H), 3.86 (s, 3H), 2.49 (s, 3H), 2.31 (s, 3H), 1.32−1.25 (m, 2H), 1.24−1.18 (m, 2H).

4-{1-[({2,5-Dimethyl-4-[3-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]cyclopropyl}benzoic Acid (34b). The ester **33b** (223 mg, 0.457 mmol) in THF (8.5 mL) and MeOH (4.2 mL) was treated with LiOH (aq 1 M, 1.37 mL, 1.37 mmol) for 18 h under the conditions described above for the preparation of **27a**. The crude material was triturated in EtOAc, collected by filtration, rinsed with EtOAc/hexane 25:75 followed by hexane, and dried to afford pure **34b** as a white solid (186 mg, 86%). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.15 (br s, 1H), 8.29 (s, 1H), 7.85 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 7.7 Hz, 1H), 7.50−7.43 (m, 2H), 7.41 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.3 Hz, 2H), 4.12 (s, 2H), 2.49 (s, 3H), 2.31 (s, 3H), 1.31−1.24 (m, 2H), 1.24−1.18 (m, 2H). HRMS calcd for $C_{25}H_{21}F_3NO_3S$ [(M − H)$^-$], 472.1200; found, 472.1193.

Methyl 4-{1-[({2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]cyclopropyl}benzoate (33d). The acid **20d** (275 mg, 0.875 mmol), methyl 4-(1-aminocyclopropyl)-benzoate (**32**) (167 mg, 0.873 mmol), HATU (333 mg, 0.875 mmol), and DIEA (348 μL, 2.01 mmol) were reacted in DMF (6 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/$CHCl_3$ 2:98 to 5:95 to 10:90, 30 min) to provide **33d** as a white solid (176 mg, 41%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.98 (br s, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.3 Hz, 2H), 4.11 (s, 2H), 3.86 (s, 3H), 2.48 (s, 3H), 2.32 (s, 3H), 1.32−1.26 (m, 2H), 1.26−1.20 (m, 2H).

4-{1-[({2,5-Dimethyl-4-[4-(trifluoromethyl)benzyl]-3-thienyl}carbonyl)amino]cyclopropyl}benzoic Acid (34d). The ester **33d** (172 mg, 0.353 mmol) in THF (7 mL) and MeOH (3.5 mL) was treated with LiOH (aq 1 M, 1.06 mL, 1.06 mmol) for 17 h under the conditions described above for the preparation of **27a**. The crude material was triturated in EtOH/hexane 10:90, collected by filtration, rinsed with the same solvent followed by hexane, and dried to afford **34d** as a white solid (134 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.84 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.58 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H), 7.08 (d, J = 8.1 Hz, 2H), 4.02 (s, 2H), 2.41 (s, 3H), 2.29 (s, 3H), 1.28−1.18 (m, 2H), 1.14−1.00 (m, 2H). HRMS calcd for $C_{25}H_{21}F_3NO_3S$ [(M − H)$^-$], 472.1200; found, 472.1192. Anal. ($C_{25}H_{22}F_3NO_3S$) H, N. C: calcd, 63.41; found, 62.90.

***N*-[1-(4-Cyanophenyl)cyclopropyl]-2,5-dimethyl-4-[4-(trifluoromethyl)benzyl]thiophene-3-carboxamide (35).** The acid **20d** (286 mg, 0.910 mmol), crude 4-(1-aminocyclopropyl)benzonitrile (**30**) (144 mg, 0.910 mmol), HATU (346 mg, 0.910 mmol), and DIEA (362 μL, 2.09 mmol) were reacted in DMF (5 mL) under the conditions described above for the preparation of **23a**. The crude product was purified by chromatography using the CombiFlash system (EtOAc/$CHCl_3$ 2:98 to 5:95 to 10:90, 27 min) to provide **35** as a pale-yellow solid (179 mg, 43%). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (s, 1H), 7.58 (m, 4H), 7.34−7.28 (m, 4H), 4.11 (s, 2H), 2.48 (s, 3H), 2.33 (s, 3H), 1.35−1.29 (m, 2H), 1.29−1.22 (m, 2H).

2,5-Dimethyl-*N*-{1-[4-(2*H*-tetrazol-5-yl)phenyl]cyclopropyl}-4-[4-(trifluoromethyl)benzyl]thiophene-3-carboxamide (36). To a suspension of **35** (174 mg, 0.383 mmol) in toluene (2.5 mL) was added azidotributyltin (317 μL, 1.15 mmol), and the mixture was heated at the reflux temperature. After 20 h, the solution then obtained was allowed to cool to room temperature and AcOH (365 μL) was added. The heterogeneous mixture was stirred for 4 h, and the precipitated solid was collected by filtration, successsively rinsed with toluene and hexane, and then dried to afford **36** as an off-white solid (170 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 7.86 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.0 Hz, 2H), 7.29−7.23 (m, 4H), 4.01 (s, 2H), 2.42 (s, 3H), 2.29 (s, 3H), 1.28−1.21 (m, 2H), 1.08−1.03 (m, 2H).). HRMS calcd for $C_{25}H_{22}F_3N_5OS$ [(M − H)$^-$], 496.1424; found, 496.1420.

Acknowledgment. We thank the Comparative Medicine group at Merck Frosst for their help with the pharmacokinetic studies.

Supporting Information Available: Synthetic procedures for the preparation of test compounds **38**, **39**, and **40**, combustion analysis for compounds **12**, **27a**, **28b**, **28d**, **34d**, and **40**, and HPLC purity data for compounds **12**, **26a**, **27a**, **28a**−**d**, **34b**, **34d**, **36**, **38**, **39**, and **40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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