2-Amino-3-benzoylthiophene Allosteric Enhancers of A_1 Adenosine Agonist Binding: New 3, 4-, and 5-Modifications

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2-Amino-3-aroylthiophenes are agonist allosteric enhancers (AE) at the A_1 adenosine receptor (A_1AR). Here we report the syntheses of three kinds of novel 2-aminothiophenes and assays of their AE activity at the human A_1AR (hA_1AR), namely, (1) 2-amino-4,5-diphenylthiophene-3-carboxylates, **3a**−**h**, (2) 2-amino-3-benzoyl-4,5-diphenylthiophenes, **7a**−**p**, and (3) 2-amino-5-bromo-3-benzoyl-4-phenylthiophenes, **10a**−**h**. An in vitro assay employing the A_1AR agonist [^{125}I]ABA and membranes from CHO−K1 cells stably expressing the hA_1AR measured an index of AE activity, the ability of a candidate AE to stabilize the agonist-A_1AR-G protein ternary complex, scored as the percentage of ternary complex remaining after 10 min of dissociation initiated by CPX and GTPγS. The AE activity score of 2-amino-4,5-dimethyl-3-(3-trifluoromethylbenzoyl)thiophene (PD 81,723), which was 19%, served as a standard for comparison. Two 3-carboxythiophene 3-trifluoromethylbenzyl esters, **3d** (49%) and **3f** (63%), had substantial AE activity. The 3-(1-naphthoyl) substituent of **7e** (52%) also supported AE activity. Compounds in series 3 tended to be more potent, **10a** and **10c** having scores of 91 and 80%, respectively. The activity of 2-amino-5-bromo-3-ethoxycarbonyl-4-(3-nitrophenyl)thiophene, **10h** (26%), is an exception to the rule that a 3-ethoxycarbonyl substituent cannot support AE activity.

Introdction

The A_1AR couples to various effectors through G_i and G_o proteins to initiate physiological responses such as slowing of heart rate and conduction through the atrioventricular node,[1] an antidiuretic action in the kidneys,[2] tonic suppression of CNS neuronal activity,[3] and tissue protection by the preconditioning phenomenon.[4−8] Effects such as sedation and preconditioning are potential therapeutic targets. Despite the availability of highly selective A_1AR agonists, they are unlikely to be useful drugs because the A_1AR is widely distributed through the body, and indiscriminate activation could cause side effects such as heart block, angina-like chest pain,[9] or impaired renal function.

The use of AEs to enhance the responsiveness of the A_1AR to endogenous adenosine at sites of its production is an appealing alternative to activation by exogenous agonists. That approach minimizes side effects because AEs act only on the agonist−A_1AR−G protein ternary complex,[10] limiting their action to sites and times of adenosine accumulation. The allosteric enhancement of the $GABA_A$ receptor by benzodiazepines is an example of this strategy.[11]

Bruns[12,13] discovered that 2-amino-3-benzoylthiophenes are allosteric enhancers of agonist binding to the A_1AR. As a rule, thiophenes were more potent than the corresponding benzenes. Acylation of the amino group destroyed activity. The substituent at position 3 significantly influenced activity; a 3-trifluoromethylbenzoyl group was the most potent. Large hydrophobic groups at position 4 and, to a lesser extent at position 5, promoted AE activity. The most potent compound was 2-amino-4,5-dimethyl-3-(3-trifluoromethylbenzoyl)thiophene, PD 81,723 (Figure 1), which has served as a lead compound for the development of additional AEs.[14−17] The latter studies examined additional 3-aroyl substituents and thiophenes having either no substituents at C4 and C5, 4,5-dimethylthiophenes or cycloalkyl substituents bridging C4 and C5. The present study extends this inquiry to 4,5-diaryl- and 4-aryl-5-bromothiophenes.

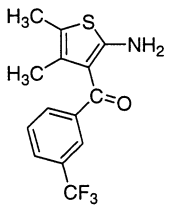

Figure 1. PD81,723.

Chemistry

The Gewald synthesis[18] (Scheme 1) is the usual route to 2-aminothiophenes. It consists of the base-catalyzed condensation of a ketone having an α-CH_2 group, **1**, with a β-ketonitrile, **2**, to form an olefin, followed by cyclization to a 2-aminothiophene, **3**, by elemental sulfur. This study developed new modifications of this synthesis to prepare 2-aminothiophenes variously substituted at the 3-, 4-, or 5-positions.

We prepared some novel 3-alkoxycarbonyl and 3-amido analogues (compounds **3a**−**h**) to further explore

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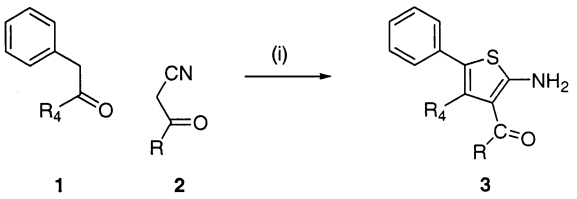
Scheme 1[a]



1 2 3

[a] (i) $TiCl_4$, pyridine then sulfur, $NHEt_2$, EtOH.

the effect of these substituents on AE activity. These syntheses started from 2-amino-4,5-diphenylthiophene-3-carboxylic acid, prepared by the titanium (IV)-catalyzed[19] coupling of deoxybenzoin, **1b**, R_4 = Ph, with ethyl cyanoacetate, **2**, R = OEt. Cyclization with sulfur formed ethyl 2-amino-4,5-diphenylthiophene-3-carboxylate, **3a**. Saponification by refluxing NaOH in aqueous ethanol released the free acid. Carbodiimide-assisted coupling of the acid with the appropriate alcohol/amine yielded the target compounds, but the instability of the acid limited yields. However, the carbodiimide-mediated condensation of cyanoacetic acid with the appropriate alcohol/amine followed by the Knoevenagel condensation and cyclization with sulfur proved a more efficient route to **3a**−**h**.

Compound **3a** also served for the synthesis of 3-aroylthiophenes **7a**−**f** (Scheme 2). As mentioned previously, refluxing in alcoholic NaOH saponified **3a**; prolonging reflux led to decarboxylation and the formation of 2-amino-4,5-diphenylthiophene, which underwent protection with either acetyl chloride or trifluoroacetic anhydride. Due to ease of removal the trifluoroacetamide proved to be the superior protecting group. Tin(IV) chloride- catalyzed Friedel−Crafts acylation with an aroyl chloride gave the protected 3-aroylthiophenes. Heating for 2 h in ethanolic 2 M NaOH at 50 °C deprotected the acetamides, and stirring overnight in K_2CO_3 at room temperature followed by heating for 2 h at 50 °C deprotected the trifluoroacetamides.

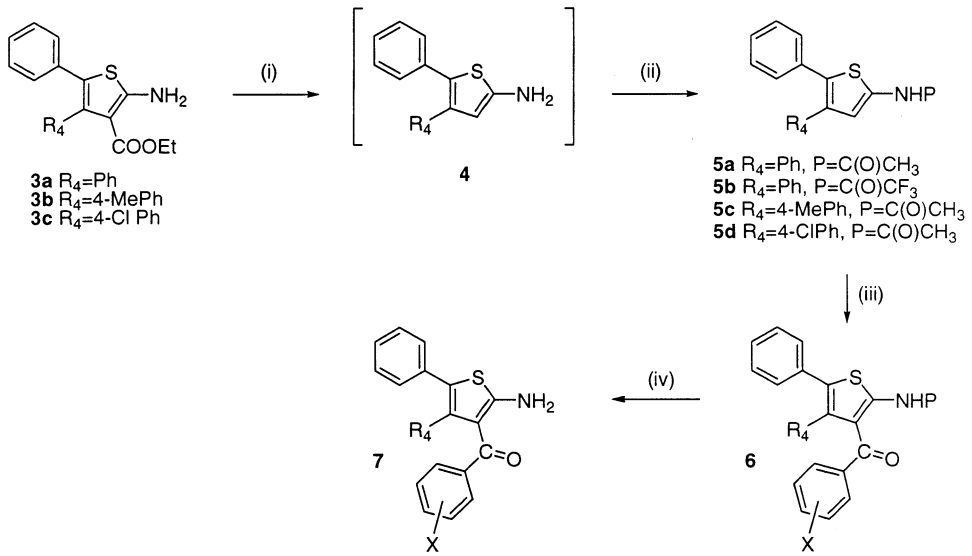
Preparations of compounds **7g**−**p** began with the synthesis of substituted deoxybenzoins by the $AlCl_3$-catalyzed Friedel−Crafts acylation of either toluene or chlorobenzene, which formed the para-substituted products exclusively. The Gewald synthesis proceeding from these deoxybenzoins gave the expected ethyl 3-carboxylates (Scheme 2). Saponification and decarboxylation of these esters was more difficult. It was necessary to reflux ethyl 2-amino-4-(4-chlorophenyl)-5-phenylthiophene-3-carboxylate (**3c**) with KOH in methanol for 3 days to effect saponification and decarboxylation. In the case of the 4-(4-methylphenyl) analogue, a significant amount of the thiophene-3-carboxylic acid was still present after refluxing with KOH. Warming an ethanolic solution of the acid with 1 equiv of oxalic acid effected complete decarboxylation. After protection of the amino group, these thiophenes underwent Freidel−Craft acylation at position 3, as described above. Deprotection afforded 4-(4-methylphenyl)- and 4-(4-chlorophenyl)thiophenes **7g**−**l** and **7m**−**p**, respectively.

We also explored a more direct route to **7a**−**p**, namely, the synthesis of 2-amino-3-ethoxycarbonyl-5-phenylthiophene by the condensation of phenylacetaldehyde with ethyl cyanoacetate followed by cyclization with sulfur. We intended to halogenate position 4 and use palladium coupling to introduce a substituted phenyl group. However, under a variety of conditions that halogenation failed.

Attempts to transform 2-amino-3-cyano-4,5-diphenylthiophenes into 3-aroyl thiophenes by copper(I)-mediated coupling[20] with phenylmagnesium bromide failed.

Gewald prepared 2-amino-3-cyano-4-phenylthiophene by condensing acetophenone with malonitrile, brominating the methyl group of the olefin and then cyclizing with NaSH, which coincidentally eliminated the bromine atom.[21] He also prepared 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene, as well as its 3-cyano analogue from 2-chlorocyclohexanone, noting that this haloketone was the only one able to form an olefin. The present study broadens the scope of that approach (Scheme 3), showing that α-bromoacetophenones **8a**−**g** can react with benzoylacetonitrile and ethyl cyanoacetate to form olefins. Cyclization with sulfur rather than NaSH gave 2-amino-3-aroyl-5-bromo-4-phenyl-

Scheme 2[a]



3a R_4=Ph
3b R_4=4-MePh
3c R_4=4-Cl Ph

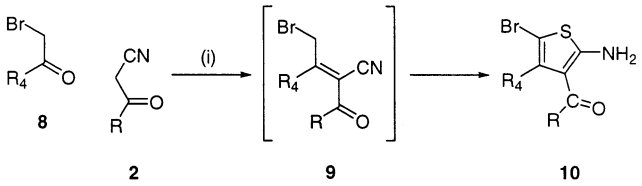
4

5a R_4=Ph, P=C(O)CH_3
5b R_4=Ph, P=C(O)CF_3
5c R_4=4-MePh, P=C(O)CH_3
5d R_4=4-ClPh, P=C(O)CH_3

6 **7**

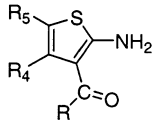
[a] (i) NaOH or KOH (then oxalic acid); (ii) Ac-Cl or $[CF_3C(O)]_2O$; (iii) R(O)Cl, $SnCl_4$; (iv) NaOH for acetyl deprotection, K_2CO_3 for trifluoroacetyl deprotection.

Scheme 3[a]



[a] (i) Sulfur, $NHEt_2$, EtOH.

Table 1



compd	R	R_4	R_5	activity (%)[a]
3a	OEt	Ph	Ph	not tested
3b	OEt	4-Me Ph	Ph	not tested
3c	OEt	4-Cl Ph	Ph	42.1 ± 1.9 (3)
3d	OBn	H	Ph	8.0 ± 2.4 (3)
3e	OBn	Ph	Ph	18.6 ± 1.5 (3)
3f	O(3-CF_3 Bn)	Ph	Ph	48.0 ± 8.0 (3)
3g	O(3-CF_3 Bn)	4-Me Ph	Ph	62.6 ± 10.8 (3)
3h	NBn_2	Ph	Ph	14.1 ± 3.6 (3)
7a	Ph	Ph	Ph	10.7 ± 2.0 (3)
7b	3-Cl Ph	Ph	Ph	9.4 ± 1.2 (3)
7c	4-Cl Ph	Ph	Ph	10.8 ± 2.0 (3)
7d	4-Ph Ph	Ph	Ph	1.5 ± 0.8 (3)
7e	1-naphth	Ph	Ph	52.3 ± 5.8 (3)
7f	2-naphth	Ph	Ph	4.2 ± 2.2 (3)
7g	Ph	4-Me Ph	Ph	13.4 ± 1.9 (3)
7h	3-Cl Ph	4-Me Ph	Ph	8.1 ± 4.2 (3)
7i	4-Cl Ph	4-Me Ph	Ph	8.4 ± 0.5 (3)
7j	4-Ph Ph	4-Me Ph	Ph	3.6 ± 2.3 (3)
7k	2-naphth	4-Me Ph	Ph	10.9 ± 3.5 (3)
7l	Bn	4-Me Ph	Ph	1.9 ± 0.5 (3)
7m	Ph	4-Cl Ph	Ph	5.6 ± 2.5 (3)
7n	4-Cl Ph	4-Cl Ph	Ph	8.7 ± 1.9 (3)
7o	4-Ph Ph	4-Cl Ph	Ph	10.5 ± 2.1 (3)
7p	1-naphth	4-Cl Ph	Ph	11.9 ± 1.6 (3)
10a	Ph	3-CF_3 Ph	Br	91.0 ± 5.6 (3)
10b	Ph	3-NO_2 Ph	Br	21.8 ± 3.3 (3)
10c	Ph	4-CF_3 Ph	Br	80.4 ± 2.0 (3)
10d	Ph	4-NO_2 Ph	Br	68.3 ± 1.5 (3)
10e	Ph	4-CN Ph	Br	20.3 ± 3.7 (3)
10f	Ph	4-Ph Ph	Br	42.7 ± 7.0 (3)
10g	Ph	2-naphth	Br	49.7 ± 5.4 (3)
10h	OEt	3-NO_2 Ph	Br	26.0 ± 2.6 (3)
PD 81,723	3-CF_3 Ph	Me	Me	19 ± 2.9 (3)[17]

[a] Concentration of allosteric enhancer was 100 μM. All data are mean ± SEM with *n* values shown in parentheses.

thiophenes **10a**−**g** and, from ethyl cyanoacetate, the 3-ethoxycarbonyl analogue **10h**.

Results and Discussion

Table 1 summarizes the results of the screening assays. Compounds **3a**−**h** (series 1) included three types of 2-amino-4,5-diphenylthiophenes which possess ester or amide substituents in the 3-position. Compounds **3a**−**c** were ethyl esters, **3d**−**g** were benzyl or substituted benzyl esters and **3h**, was a dibenzylamide. Few compounds with ester groups in the 3-position have been evaluated for AE activity. Bruns et al. tested two 2-amino-3-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines using an assay that measured the dissociation of [^{3}H]-N^6-cyclohexyladenosine ([^{3}H]CHA) from rat brain membranes after treatment with the allosteric enhancer and (*R*)-PIA.[12] These compounds enhanced equilibrium binding of N^6-cyclohexyladenosine by 7% and 66%, respectively, at 100 μM (compared with 321% for PD81,723 at the same concentration, which are roughly equivalent to <1 and 5% in our assay), which led him to conclude that substitution of ethoxycarbonyl for benzoyl reduced, but did not destroy activity. In contrast, compound **3c** had substantial activity. Compounds **3d**−**g** are benzyl esters of thiophene-3-carboxylic acids and have a potency ranking **3d** < **3e** < **3f** < **3g**. Although the series is small, it suggests that the 4-phenyl group of **3e** contributes additively to activity, as does the 3-OCF_3 group of **3f** and **3g** as well as the 4-(*p*-methylphenyl) group of **3g**.

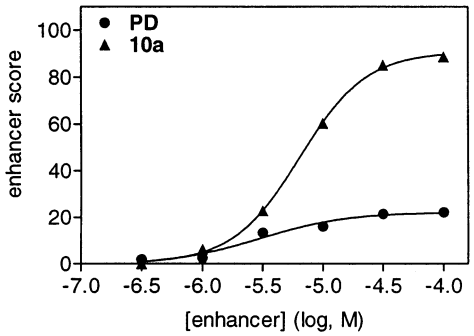


Figure 2. Dose response curves for PD81,723 and **10a**. EC_{50} values for PD81,723 and compound **10a** were 8.4 μM and 6.36 μM, respectively.

Compounds **7a**−**p** (series 2) are 2-amino-3-aroyl-4,5-diphenylphenylthiophenes having modifications of either the 3- and/or 5-substituent. Only one, **7e**, showed significant activity, recording an AE score of 52%. The uniformly modest activity of the other members of this series offers no clues to account for the activity of **7e**.

In the 5-bromothiophenes series **10a**−**h** (series 3) showed allosteric enhancer activity at least as high as that of PD 81,273 with several compounds having substantially higher activity (**10a**, **c**, **d**, **f**, **g**). The first three have in common a strong electron-withdrawing group in either the meta or para position of the 4-phenyl group. That activity owes to electronic effects is uncertain because compounds **10b** and **10e** likewise have strong electron-withdrawing groups in that substituent but only modest activity. Compounds **10f** and **10g** have very large 4-aryl groups and substantial enhancer activity. That activity is reminiscent of the enhanced activity conferred by large 3-aroyl groups shown previously[17] and raises the possibility that the two types of bulky substituents bind to a common site.

Figure 2 compares the concentration-dependence of the allosteric enhancer effects of PD 81,723 and **10a**. Both have the same EC_{50}, but the maximum score of **10a** is twice that of PD 81,723. Such a result is consistent with the mechanism of these enhancers, retarded dissociation of the ligand−receptor-G protein ternary complex.

Figure 3 compares the activities of PD 81,723 and **10a** as antagonists of ligand binding to the A_1AR. At a concentration of 100 μM **10a** inhibited the binding of the antagonist [^{3}H]CPX by 29%, less than the 85% inhibition exerted by PD 81,723.

The allosteric enhancers prepared in this study proved to be quite stable upon standing. The purity of selected analogues was checked (by TLC and microanalysis) after they had been standing for >6 months at room temperature, and no degradation was observed.

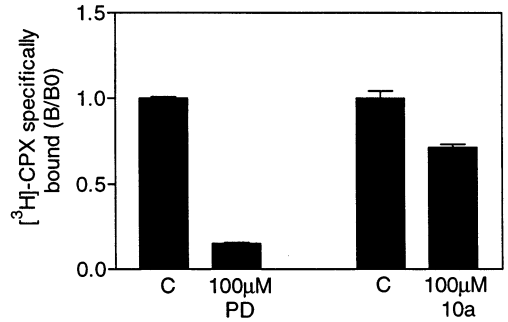


Figure 3. Effects of PD and **10a** on antagonist binding to A_1AR Membranes. % Inhibition values for PD81,723 and compound **10a** at 100 μM were 85.3% (±0.33%, $n = 3$) and 28.6% (±1.2%, $n = 3$), respectively.

To be useful for potential therapeutic indications, such as preconditioning, sedation, or for the management of pain,[22] there are several properties of allosteric enhancers that should be optimized. It is desirable to improve potency and efficacy, and to minimize the competitive antagonist activity of some compounds that is not required for enhancer activity. It will also be necessary to produce compounds that are stable and lack toxicity. The compounds described here are clearly improved compared to existing aminothiophenes in terms of efficacy, potency, lack of antagonist activity, and stability. Additional studies will be required to determine if these and other new compounds that are currently under development in several laboratories are safe and effective in animals.

Experimental Section

Chemical Procedures. Merck Kieselgel 60 and 60 F_{254} were used for column and thin-layer chromatography. Melting points were determined on Electrothermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz Unity Plus spectrometer using TMS as an internal standard. Electrospray mass spectral data was obtained on a Fisons VG Micromass Platform II spectrometer. Elemental analyses were performed by the Microanalytical Service, Department of Chemistry, University of Queensland.

Representative Procedure for Deoxybenzoin Formation. Phenylacetyl chloride (4.73 g, 30 mmol) was stirred with the appropriate substituted benzene (excess) at 0 °C. Aluminum(III) chloride (4.4 g, 33 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were evaporated and triturated with hexane to afforded the target compound.

1-(4-Methylphenyl)-2-phenylethanone (1b). Yield 89%. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 4.28 (s, 2H, CH_2), 7.35−7.26 (m, 7H, ArH), 7.94 (d, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.6, 45.3, 126.7, 128.6, 128.7, 129.2, 129.4, 134.0, 134.7, 143.9, 197.3.

1-(4-Chlorophenyl)-2-phenylethanone (1c). Yield 74%. ^{1}H NMR ($CDCl_3$) δ 4.26 (s, 2H, CH_2), 7.35−7.29 (m, 5H, ArH), 7.43 (d, 2H, $J = 8.3$, ArH), 7.95 (d, 2H, $J = 8.3$, ArH). ^{13}C NMR ($CDCl_3$) δ 45.5, 127.0, 128.7, 128.9, 129.3, 130.0, 134.1, 134.8, 139.6, 196.4.

Representative Procedure for Cyanoacetic Acid Coupling. Cyanoacetic acid (10 mmol) and the appropriate alcohol/amine (10 mmol) were dissolved in dichloromethane (40 mL) and cooled to 0 °C. A solution of *N*,*N*-dicyclohexylcarbodiimide (10 mmol) and *N*,*N*-(dimethylamino)pyridine (catalytic) in dichloromethane (20 mL) was added, and the reaction mixture was stirred for 1 h at 0 °C. During this period a solid precipitated which was subsequently collected by suction filtration. No further purification was required.

Cyanoacetic Acid Benzyl Ester (2b). Yield 100%. ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 7.38 (s, 5H, ArH). ^{13}C NMR ($CDCl_3$) δ 24.6, 68.4, 112.9, 128.5, 128.6, 128.8, 134.3, 162.8.

***N,N*-Dibenzyl-2-cyanoacetamide (2c).** Yield 98%. ^{1}H NMR ($CDCl_3$) δ 3.54 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 7.15−7.40 (m, 10H, ArH). ^{13}C NMR ($CDCl_3$) δ 25.2, 49.4, 50.5, 114.0, 126.0, 127.8, 128.1, 128.3, 128.7, 129.2, 134.8, 135.9, 162.5.

Representative Procedure for 2-Amino-4,5-diphenylthiophene-3-carboxylate/carboxamide Formation. Neat $TiCl_4$ (4.9 mL, 45 mmol) was added dropwise into 60 mL of dry THF at 0 °C, followed by a solution of deoxybenzoin (4.33 g, 22 mmol) and the substituted cyanoacetate/cyanoacetamide (30 mmol) in THF. The ice bath was removed, and dry pyridine (1.5 mL) was added. The dark blue solution was stirred for 1 h. More pyridine (4.5 mL) was added, and the reaction mixture stirred overnight at room temperature. Workup with 10% HCl, followed by extraction with ethyl acetate, washing the combined organic layers with 2 M NaOH, drying over $MgSO_4$, and evaporation of the solvents afforded the crude Knoevenagel product. This product was taken up in THF (20 mL) and stirred at room temperature with sulfur (710 mg, 22.2 mmol) and diethylamine (4 mL) for 1 h. The volatiles were removed in vacuo to afford the crude product, which was recrystallized from hot ethanol.

Ethyl 2-Amino-4,5-diphenylthiophene-3-carboxylate (3a). Yield 55%. ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H, $J = 7.1$, CH_2CH_3), 3.95 (q, 2H, $J = 7.1$, CH_2CH_3), 5.28 (br s, 2H, CH_2), 7.28−7.03 (m, 10H, ArH). ^{13}C NMR ($CDCl_3$) δ 13.4, 59.3, 108.3, 121.2, 126.4, 126.6, 127.5, 128.1, 128.7, 130.1, 133.8, 136.3, 137.9, 162.0, 165.9.

Ethyl 2-Amino-4-(4-methylphenyl)-5-phenylthiophene-3-carboxylate (3b). Yield 83%. ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 2.05 (s, 3H, CH_3) 3.96 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 5.68 (br s, 2H, NH_2), 7.01−7.32 (m, 9H, ArH).

Ethyl 2-Amino-4-(4-chlorophenyl)-5-phenylthiophene-3-carboxylate (3c). Yield 72%. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 4.00 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 5.83 (br s, 2H, NH_2), 7.03−7.27 (m, 9H, ArH). ^{13}C NMR ($CDCl_3$) δ 13.5, 59.4, 107.7, 121.4, 126.6, 127.6, 128.2, 128.8, 131.5, 132.5, 133.4, 134.7, 136.3, 162.4, 165.7.

Benzyl 2-Amino-5-phenylthiophene-3-carboxylate (3d). Yield 61%. mp 96−98 °C. ^{1}H NMR ($CDCl_3$) δ 5.32 (s, 2H, CH_2), 5.79 (br s, 2H, NH_2), 7.45−7.22 (m, 11H, ArH). ^{13}C NMR ($CDCl_3$) δ 65.5, 107.5, 121.0, 124.6, 125.0, 126.6, 128.0, 128.1, 128.5, 128.7, 133.8, 136.4, 162.4, 165.0. ESMS 332 ($M + Na^+$). Anal. ($C_{18}H_{15}NO_2S$) C, H, N.

Benzyl 2-Amino-4,5-diphenylthiophene-3-carboxylate (3e). Yield 34%. mp 112−113 °C. ^{1}H NMR ($CDCl_3$) δ 5.02 (s, 2H, CH_2), 6.05 (br s, 2H, NH_2), 6.85−7.26 (m, 15H, ArH). ^{13}C NMR (DMSO) δ 64.4, 105.1, 118.9, 126.4, 126.7, 127.3, 127.4, 127.7, 128.0, 128.3 (2C), 129.8, 134.8, 135.7, 136.2, 137.6, 164.0, 164.7. Anal. ($C_{24}H_{19}NO_2S$) C, H, N.

3-Trifluoromethylbenzyl 2-Amino-4,5-diphenylthiophene-3-carboxylate (3f). Yield 45%. mp 109−111 °C. ^{1}H NMR ($CDCl_3$) δ 5.07 (s, 2H, CH_2), 6.37 (br s, 2H, NH_2), 7.02−7.55 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 64.4, 107.1, 121.1, 123.9 (q, $J = 272.3$ Hz), 124.4 (q, $J = 3.5$ Hz), 124.5 (q, $J = 3.8$), 126.4, 126.7, 127.5, 128.0, 128.5, 128.6, 129.9, 130.3 (q, $J = 32.5$ Hz), 131.1, 133.6, 135.8, 136.6, 137.5, 163.3, 165.5. Anal. ($C_{25}H_{18}F_3NO_2S$) C, H, N.

3-Trifluoromethylbenzyl 2-Amino-4-(4-methylphenyl)-5-phenylthiophene-3-carboxylate (3 g). Yield 45%. mp 108−110 °C. ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 5.98 (br s, 2H, NH_2), 6.92−7.58 (m, 13H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.4, 64.4, 107.4, 121.1, 123.9 (q, $J = 272.6$ Hz), 124.4 (q, $J = 3.8$ Hz), 124.5 (q, $J = 3.8$), 126.4, 128.1, 128.3, 128.5, 128.7, 129.8, 130.3 (q, $J = 32.2$ Hz), 131.2, 133.8, 134.4, 135.9, 136.2, 136.7, 163.1, 165.6. ESMS 468 ($M + H^+$). Anal. ($C_{26}H_{20}F_3NO_2S$) C, H, N.

***N,N*-Dibenzyl 2-Amino-4,5-diphenylthiophene-3-carboxamide (3h).** Yield 52%. mp 122−123 °C. ^{1}H NMR (DMSO-d_6) δ 4.25 (br s, 4H, 2 × CH_2), 6.58 (br s, 2H, NH_2), 7.08−7.39 (m, 20H, ArH). ^{13}C NMR (DMSO-d_6) δ 49.5, 116.5, 122.5, 126.8, 127.5, 127.7, 127.9, 128.4, 128.7, 128.8, 129.4, 130.3, 134.7, 135.5, 136.4, 137.2, 151.7, 167.8. ESMS 475 ($M + H^+$). Anal. ($C_{31}H_{26}N_2OS$) C, H, N.

***N*-(4,5-Diphenylthiophen-2-yl)acetamide (5a).** Ethyl 2-amino-4,5-diphenylthiophene-3-carboxylate (4.0 g, mmol) was refluxed in an ethanol (20 mL)/2 M NaOH (20 mL) solution for 48 h. The reaction mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL) and water (20 mL). Evaporation in vacuo gave crude 2-amino-4,5-diphenylthiophene. This material was dissolved in dry pyridine (5 mL) and cooled to 0 °C. Acetyl chloride (1.5 mL) was added, and the reaction was stirred at room temperature for 1 h. The reaction mixture was poured onto ice, and the resultant precipitate collected and washed with water. Recrystallization from ethanol gave pure **5a** (yield 64%). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CH_3), 6.72 (s, 1H, H-3), 7.20−7.28 (m, 10H, ArH), 8.54 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 23.2, 114.9, 126.8, 126.9, 128.3, 128.4, 129.0, 129.2, 130.5, 134.2, 134.6, 136.6, 137.6, 166.9.

***N*-(4,5-Diphenylthiophen-2-yl)-2,2,2-trifluoroacetamide (5b).** Crude 2-amino-4,5-diphenylthiophene (3.07 g, 12.2 mmol), prepared using the procedure described above, was stirred with triethylamine (3 mL) and trifluoroacetic anhydride (3.15 g, 15 mmol) in dry chloroform (20 mL) at room temperature overnight. The reaction mixture was diluted with chloroform, washed with brine (20 mL) and water (20 mL), and then evaporated in vacuo. Column chromatography with ethyl acetate/hexane (4:1) as an eluent afforded pure **5b** (yield 72%). ^{1}H NMR ($CDCl_3$) δ 7.01 (s, 1H, H-3), 7.35−7.26 (m, 10H, ArH), 8.99 (br s, 1H, NH_2). ^{13}C NMR ($CDCl_3$) δ 115.6 (q, J = 285.2 Hz), 118.8, 127.2, 127.6, 128.4, 128.5, 129.0, 129.2, 129.5, 133.4, 134.1, 135.4, 135.8, 153.6 (q, J = 38.6 Hz).

***N*-(5-Phenyl-4-(4-methylphenyl)thiophen-2-yl)acetamide (5c).** Ethyl 2-Amino-4-(4-methylphenyl)-5-phenylthiophene-3-carboxylate (10.3 g, 30.5 mmol) was refluxed overnight with KOH (5.6 g, 99.7 mmol) in aqueous methanol (100 mL). The reaction mixture was evaporated, taken up in ethyl acetate (100 mL), and washed with water. The ethyl acetate portion was evaporated and the residue dissolved in ethanol (50 mL) and stirred with oxalic acid (2.70 g, 30 mmol) at 55 °C for 1 h. Evaporation of the ethanol afforded crude 2-amino-4-(4-methylphenyl)-5-diphenylthiophene which was dissolved in dry dichloromethane (20 mL) and treated with acetyl chloride (4.49 g, 61 mmol) and dry pyridine (5 mL). The solution was stirred for 1 h at room temperature. After evaporation of the volatiles, the residue was taken up in ethyl acetate (100 mL) and washed with 1 M HCl. Drying, filtration, and evaporation of the ethyl acetate solution afforded the crude product which was purified by column chromatography (silica gel was used as the stationary phase and ethyl acetate/hexane 1:2 → 1:1 as the eluent, yield 25%). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.72 (s, 1H, H-3), 7.03−7.28 (m, 9H, ArH), 8.75 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 21.1, 23.2, 115.1, 126.9, 128.3, 128.8, 129.0, 129.1, 133.6, 134.3, 134.6, 136.5, 137.5, 167.0.

***N*-[4-(4-Chlorophenyl)-5-phenylthiophen-2-yl]acetamide (5d).** Ethyl 2-amino-4-(4-chlorophenyl)-5-phenylthiophene-3-carboxylate (0.99 g, 2.77 mmol) and KOH (5.6 g, 100 mmol) were refluxed in aqueous methanol (100 mL) for 3 days. The reaction mixture was evaporated, taken up in ethyl acetate (100 mL), and washed with water. The ethyl acetate portion was evaporated, and the residue was dissolved in dry dichloromethane (20 mL) and treated with acetyl chloride (0.43 g, 5.48 mmol) and dry pyridine (5 mL). The solution was stirred for 1 h at room temperature. After evaporation of the volatiles, the residue was taken up in ethyl acetate (100 mL) and washed with 1 M HCl. Drying, filtration, and evaporation of the ethyl acetate solution afforded the crude product which was purified by column chromatography (silica gel was used as the stationary phase and ethyl acetate/hexane 1:2 → 1:1 as the eluent, yield 26%). ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, CH_3), 6.69 (s, 1H, H-3), 7.13−7.262 (m, 9H, ArH), 8.41 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 23.3, 114.5, 127.2, 128.5 (2C), 129.2, 130.2, 130.9, 132.7, 133.3, 133.8, 134.9, 137.7, 166.9.

Representative Procedure for Friedel−Crafts Acylation. *N*-(4,5-Diphenylthiophen-2-yl)acetamide (0.235 mg, 0.8 mmol) and benzoyl chloride (140 mg, 1 mmol) were dissolved in 1,2-dichloroethane (5 mL). Tin(IV) chloride (443 mg, 1.7 mmol) was added, and the reaction was refluxed until the starting material was no longer apparent by TLC (3 h). The reaction was quenched with ice, and the organic phase was washed sequentially with 2 M HCl, water, and 2 M NaOH. Drying and evaporation gave a solid that was purified by chromatography on silica gel eluted with ethyl acetate−hexane (4:1).

***N*-(3-Benzoyl-4,5-Diphenylthiophen-2-yl)acetamide (6a).** Yield 59%. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 6.88−8.13 (m, 15H, ArH), 11.15 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 23.6, 126.6, 127.2, 127.3, 127.6, 128.2, 128.4, 128.9, 129.3, 130.0, 130.8, 131.4, 133.3, 133.6, 135.0, 138.6, 148.6, 167.9, 171.5, 195.4.

***N*-[3-(3-Chlorobenzoyl)-4,5-diphenylthiophen-2-yl]-2,2,2-trifluoroacetamide (6b).** Yield 53%. ^{1}H NMR ($CDCl_3$) δ 6.83−7.29 (m, 14H, ArH), 12.12 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 115.4 (q, J = 286.9 Hz), 124.5, 126.7, 127.0, 127.3, 128.0, 128.5, 129.0, 129.2, 129.5, 130.6, 131.7, 132.8, 133.0, 133.5, 134.3, 135.1, 139.4, 145.9, 155.4 (q, J = 39.8 Hz), 194.0.

***N*-[3-(4-Chlorobenzoyl)-4,5-diphenylthiophen-2-yl]-2,2,2-trifluoroacetamide (6c).** Yield 70%. ^{1}H NMR ($CDCl_3$) δ 6.84−7.39 (m, 14H, ArH), 12.08 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 115.4 (q, J = 286.9 Hz), 124.7, 127.5, 127.9, 128.2, 128.7, 129.2, 129.7, 130.6, 130.7, 130.9, 132.4, 134.1, 135.0, 136.2, 138.1, 145.5, 154.3 (q, J = 39.8 Hz), 194.1.

***N*-[3-Biphenyl-4-carbonyl)-4,5-diphenylthiophen-2-yl]-2,2,2-trifluoroacetamide (6d).** Yield 89%. ^{1}H NMR ($CDCl_3$) δ 6.88−7.43 (m, 19H, ArH), 12.16 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 115.5 (q, J = 287.2 Hz), 125.3, 126.2, 126.9, 127.1, 127.8, 127.9, 128.0, 128.5, 128.8, 129.6 (2C), 130.9, 132.6, 132.8, 134.4, 135.3, 136.6, 139.9, 144.5, 145.2, 154.3 (q, J = 39.5 Hz), 195.0.

***N*-[3-(Naphthalene-1-carbonyl)-4,5-diphenylthiophen-2-yl]-2,2,2-trifluoroacetamide (6e).** Used without purification for the preparation of compound **7f**.

***N*-[3-(Naphthalene-2-carbonyl)-4,5-diphenylthiophen-2-yl]-2,2,2-trifluoroacetamide (6f).** Yield 91%. ^{1}H NMR ($CDCl_3$) δ 6.62−7.79 (m, 17H, ArH), 12.09 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 115.5 (q, J = 273.7 Hz), 124.3, 125.5, 126.3, 126.7, 127.5, 127.6, 127.7, 127.8, 128.0, 128.5, 129.0, 129.5, 130.5, 131.3, 131.4, 132.6, 132.8, 134.5, 134.7, 135.0, 135.5, 145.0, 154.3 (q, J = 39.5 Hz), 195.3.

N-**[3-Benzoyl-5-phenyl-4-(4-methylphenyl)thiophen-2-yl]acetamide (6g).** Yield 98%. ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.66−8.16 (m, 14H, ArH), 11.42 (br s, 1H, NH).

***N*-[3-(3-Chlorobenzoyl)-5-phenyl-4-(4-methylphenyl)-thiophen-2-yl]acetamide (6h).** Yield 58%. ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 6.73−8.09 (m, 13H, ArH), 11.27 (br s, 1H, NH).

***N*-[3-(4-Chlorobenzoyl)-5-phenyl-4-(4-methylphenyl)-thiophen-2-yl]acetamide (6i).** Yield 60%. ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.71−7.23 (m, 13H, ArH), 11.21 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 20.9, 23.7, 122.1, 127.2, 127.5, 128.2, 128.5, 129.4, 130.0, 130.2, 130.7, 131.8, 133.3, 134.2, 136.6, 137.1, 137.3, 149.2, 167.7, 194.1.

***N*-[3-(4-Phenylbenzoyl)-5-phenyl-4-(4-methylphenyl)-thiophen-2-yl]acetamide (6j).** Yield 60%. ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 6H, 2 × CH_3), 6.83−8.03 (m, 18H, ArH), 11.21 (br s, 1H, NH).

***N*-[3-(Naphthalene-1-carbonyl)-5-phenyl-4-(4-methylphenyl)thiophen-2-yl]acetamide (6k).** Yield 93%. ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.22−7.65 (m, 16H, ArH), 12.04 (br s, 1H, NH).

***N*-(5-Phenyl-3-phenylacetyl-4-(4-methylphenyl)thiophen-2-yl)acetamide (6l).** Yield 44%. ^{1}H NMR ($CDCl_3$) δ 2.28

(s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.45 (s, 2H, CH_2), 6.98−7.30 (m, 14H, ArH), 12.12 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 21.2, 23.6, 48.4, 122.2, 126.6, 127.0, 128.1, 128.2, 129.2, 129.4, 129.6, 130.3, 130.5, 133.3, 133.6, 133.9, 134.8, 137.7, 149.5, 168.0, 198.1.

***N*-[3-Benzoyl-4-(4-chlorophenyl)-5-phenylthiophen-2-yl]acetamide (6m).** Yield 92%. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 6.77 (d, 2H, J = 8.7 Hz, 5-C_6H_4Cl), 6.85 (d, 2H, J = 8.7 Hz, 5-C_6H_4Cl), 7.02−8.15 (m, 10H, ArH), 11.20 (br s, 1H, NH).

***N*-[3-(4-Chlorobenzoyl-4-(4-chlorophenyl)-5-phenylthiophen-2-yl]acetamide (6n).** Yield 37%. Compound **6n** was deprotected directly.

***N*-[3-(Biphenyl-4-carbonyl)-4-(4-chlorophenyl)-5-phenylthiophen-2-yl]acetamide (6o).** Yield 17%. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, CH_3), 6.79−7.78 (m, 18H, ArH), 11.28 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 23.7, 122.2, 126.4, 127.1, 127.3, 127.5, 127.9, 128.4, 128.7, 128.9, 129.4, 129.5, 130.7, 132.2, 132.7, 133.0, 133.8, 137.3, 140.1, 144.6, 149.3, 167.9, 194.7.

***N*-[3-(Naphthylene-1-carbonyl)-4-(4-chlorophenyl)-5-phenylthiophen-2-yl]acetamide (6p).** Yield 66%. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 6.40−7.93 (m, 16H, ArH), 12.16 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 23.8, 124.2, 124.5, 124.7, 125.9, 126.0, 126.2, 126.6, 126.9, 127.3, 127.9, 128.2, 128.2, 128.6, 129.3, 130.4, 131.0, 131.5, 132.8, 132.9, 133.0, 134.3, 137.6, 168.3, 196.2.

Representative Procedure for Deprotection. Method A. The acetamide (0.63 mmol) was dissolved in warm EtOH (2 mL) and stirred with 2 M NaOH (2 mL) for 5 h at 50 °C. The initial red color faded, and a precipitate formed and was collected by filtration. Additional product was obtained by EtOAc extraction.

Method B. The trifluoroacetamide (0.54 mmol) was stirred with aqueous K_2CO_3 in ethanol at room-temperature overnight and then warmed to 50 °C for 2 h to complete the reaction.

(2-Amino-4,5-diphenylthiophen-3-yl)phenylmethanone (7a). Yield 46%. mp 155 °C. ^{1}H NMR ($CDCl_3$) δ 5.17 (br s, 2H, NH_2), 6.86−7.34 (m, 15H, ArH). ^{13}C NMR (DMSO) δ 115.7, 118.7, 126.4, 126.6, 127.1, 127.5, 128.1, 128.4, 128.7, 129.9, 130.3, 133.5, 135.6, 135.9, 140.2, 165.1, 192.0. ESMS 356 (M + H^+). Anal. ($C_{23}H_{17}$NOS) C, H, N.

(2-Amino-4,5-diphenylthiophen-3-yl)(3-chlorophenyl)-methanone (7b). Yield 54%. mp 143−144 °C. ^{1}H NMR ($CDCl_3$) δ 6.57 (br s, 2H, NH_2), 6.88−7.26 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 117.1, 120.8, 126.2, 126.6, 126.8, 127.7, 128.1, 128.6, 128.7, 129.2, 129.8, 130.4, 132.9, 133.6, 135.6, 137.7, 141.6, 165.5, 191.9. ESMS 390 (M + H^+). Anal. ($C_{23}H_{16}$ClNOS) C, H, N.

(2-Amino-4,5-diphenylthiophen-3-yl)(4-chlorophenyl)-methanone (7c). Yield 58%. mp 151−152 °C. ^{1}H NMR ($CDCl_3$) δ 5.53 (br s, 2H, NH_2), 6.82−7.26 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 117.4, 120.9, 126.5, 126.8, 127.3, 127.7, 128.2, 129.2, 129.5, 129.8, 130.7, 133.7, 135.7, 136.0, 138.4, 164.8, 192.4. Anal. ($C_{23}H_{16}$ClNOS) C, H, N.

(2-Amino-4,5-diphenylthiophen-3-yl)biphenyl-4-ylmethanone (7d). Yield 56%. mp 188−189 °C. ^{1}H NMR ($CDCl_3$) δ 5.71 (br s, 2H, NH_2), 6.86−7.42 (m, 19H, ArH). ^{13}C NMR ($CDCl_3$) δ 117.9, 125.9, 126.3, 126.7, 127.0, 127.1, 127.6, 128.1, 128.5, 128.7, 129.0, 129.3, 130.8, 133.9, 135.9, 138.8, 140.6, 140.8, 142.7, 164.2, 195.0. ESMS 432 (M + H^+). Anal. ($C_{29}H_{21}$-NOS) C, H, N.

(2-Amino-4,5-diphenylthiophen-3-yl)naphthalen-1-ylmethanone (7e). Yield 30% (over two steps). mp 221−222 °C. ^{1}H NMR ($CDCl_3$) δ 6.44−7.46 (m, 17H, ArH). ^{13}C NMR ($CDCl_3$) δ 118.6, 120.5, 124.1, 125.4, 125.5, 125.9, 126.0, 126.2, 126.6, 127.8, 128.0, 128.7, 128.9, 129.2, 129.7, 130.2, 133.0, 133.7, 135.4, 136.2, 138.6, 165.7, 194.3. ESMS 406 (M + H^+). Anal. ($C_{27}H_{19}$NOS) C, H, N.

(2-Amino-4,5-diphenylthiophen-3-yl)naphthalen-2-ylmethanone (7f). Yield 30%. mp 212−214 °C. ^{1}H NMR ($CDCl_3$) δ 6.09 (br s, 2H, NH_2), 6.51−7.65 (m, 17H, ArH). ^{13}C NMR ($CDCl_3$) δ 118.2, 120.9, 124.8, 125.7, 126.0, 126.7, 127.0, 127.1, 127.3, 127.4, 128.1, 128.7, 129.2, 129.9, 130.3, 131.5, 133.8, 133.9, 135.9, 136.2, 137.1, 164.2, 193.7. ESMS 406 (M + H^+). Anal. ($C_{27}H_{19}$NOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-phenylmethanone (7 g). Yield 57%. mp 161−162 °C. ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 5.08 (br s, 2H, NH_2), 6.62−7.27 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.0, 117.9, 120.3, 126.6, 127.1, 128.2, 128.5, 128.6, 129.2, 129.8, 130.4, 132.8, 134.1, 135.9, 136.1, 140.0, 164.2, 194.0. Anal. ($C_{24}H_{19}$NOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-(3-chlorophenyl)methanone (7h). Yield 57%. mp 170−171 °C. ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H, CH_3), 6.18 (br s, 2H, NH_2), 6.68−7.26 (m, 13H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.0, 117.5, 120.5, 126.2, 126.6, 128.2, 128.4, 128.6, 128.7, 129.2, 129.5, 130.3, 132.7, 132.9, 133.8, 135.7, 136.2, 141.7, 165.1, 192.1. ESMS 404 (M + H^+). Anal. ($C_{24}H_{18}$ClNOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-(4-chlorophenyl)methanone (7i). Yield 97%. mp 170−172 °C. ^{1}H NMR ($CDCl_3$) δ 2.17 (s, 3H, CH_3), 5.36 (br s, 2H, NH_2), 6.69−7.25 (m, 13H, ArH) (s, 4H). ^{13}C NMR ($CDCl_3$) δ 20.9, 117.6, 120.4, 126.7, 127.2, 128.2, 128.4, 129.2, 129.7, 130.6, 132.6, 133.9, 135.7, 135.9, 136.5, 138.4, 164.9, 192.5. ESMS 404 (M + H^+). Anal. ($C_{24}H_{18}$ClNOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-(4-phenylphenyl)methanone (7j). Yield 59%. mp 188−189 °C. ^{1}H NMR (DMSO) δ 1.93 (s, 3H, CH_3), 6.63−7.24 (m, 18H, ArH), 8.09 (br s, 2H, NH_2). ^{13}C NMR (DMSO) δ 20.6, 115.9, 118.3, 125.4, 126.5, 126.7, 127.7, 128.1, 128.3, 128.6, 128.7, 128.9, 130.3, 133.0, 134.1, 135.4, 135.6, 139.0, 139.7, 141.4, 165.3, 191.7. ESMS 446 (M + H^+). Anal. ($C_{30}H_{23}$NOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-naphthalen-1-ylmethanone (7k). Yield 34%. mp 225−226 °C. ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H, CH_3), 6.19−7.86 (m, 16H, ArH). ^{13}C NMR ($CDCl_3$) δ 20.7, 118.8, 120.0, 124.2, 125.4, 125.5, 125.7, 126.1, 126.4, 127.1, 127.6, 128.0, 128.5, 128.8, 129.5, 130.1, 132.2, 132.9, 133.8, 135.3, 136.3, 138.8, 165.5, 194.4. ESMS 420 (M + H^+). Anal. ($C_{28}H_{21}$NOS) C, H, N.

(2-Amino-5-phenyl-4-(4-methylphenyl)thiophen-3-yl)-2-phenylethanone (7l). Yield 77%. mp 147−149 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.31 (s, 2H, CH_2), 6.19 (br s, 2H, NH_2), 6.96−7.27 (m, 14H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.0, 46.6, 116.3, 118.7, 126.2, 126.7, 128.1, 128.5, 128.6, 129.4, 129.7, 130.4, 134.0, 134.6, 135.2, 136.3, 137.2, 165.4, 193.8. ESMS 384 (M + H^+). Anal. ($C_{25}H_{21}$NOS) C, H, N.

[2-Amino-4-(4-chlorophenyl)-5-phenylthiophen-3-yl]-phenylmethanone (7m). Yield 72%. mp 207−208 °C. ^{1}H NMR ($CDCl_3$) δ 5.68 (br s, 2H, NH_2), 6.74−7.31 (m, 14H, ArH). ^{13}C NMR (DMSO) δ 115.4, 119.1, 126.8, 127.2, 127.5, 128.1, 128.5, 128.7, 128.8, 129.8, 132.1, 133.6, 134.2, 134.9, 140.3, 165.5, 191.8. ESMS 390 (M + H^+). Anal. ($C_{23}H_{16}$ClNOS) C, H, N.

[2-Amino-4-(4-chlorophenyl-5-phenylthiophen-3-yl](4-chlorophenyl)methanone (7n). Yield 57%. mp 211−215 °C. ^{1}H NMR ($CDCl_3$) δ 7.14−7.32 (m, 13H, ArH). ^{13}C NMR ($CDCl_3$) δ 118.0, 121.0, 126.9, 127.5, 127.9, 128.8, 128.9, 129.3, 129.8, 131.7, 131.9, 133.4, 133.7, 134.4, 136.2, 164.0, 195.9. ESMS 424 (M + H^+). Anal. ($C_{23}H_{15}Cl_2$NOS) C, H, N.

(2-Amino-4-(4-chlorophenyl-5-phenylthiophen-3-yl)(4-phenylphenyl)methanone (7o). Yield 88%. mp 205−207 °C. ^{1}H NMR ($CDCl_3$) δ 6.72 (br s, 2H, NH_2), 6.77−7.46 (m, 18H, ArH). ^{13}C NMR ($CDCl_3$) δ 117.5, 121.2, 126.2, 127.0, 127.3, 127.6, 127.7, 128.3, 128.6, 128.9, 129.4, 132.0, 132.4, 133.5, 134.5, 134.6, 138.7, 140.6, 143.4, 164.7, 193.2. ESMS 466 (M + H^+). Anal. ($C_{29}H_{20}$ClNOS) C, H, N.

[2-Amino-4-(4-chlorophenyl)-5-phenylthiophen-3-yl)-(naphthalen-1-yl)methanone (7p). Yield 47%. mp 175 °C. ^{1}H NMR ($CDCl_3$) δ 5.28 (br s, 2H, NH_2), 6.33−7.81 (m, 16H, ArH). ^{13}C NMR (DMSO) δ 116.7, 119.2, 124.5, 125.1, 125.4, 125.8, 126.2, 126.3, 126.8, 127.9, 128.4, 128.6, 129.5, 130.7, 131.1, 132.5, 133.5, 134.1, 134.4, 139.2, 143.1, 166.8, 192.2. Anal. ($C_{27}H_{18}$ClNOS) C, H, N.

Representative Procedure for the Gewald Synthesis of the Substituted 2-Amino-5-bromo-4-phenylthiophenes

(10a−h). A mixture of benzoylacetonitrile (1.0 g, 6.9 mmol), 4'-trifluoromethyl-2-bromoacetophenone (1.8 g, 6.9 mmol), powdered sulfur (0.220 g, 6.9 mmol) and 1.0 mL of diethylamine in 25 mL of anhydrous ethanol was stirred at 45 °C for 5 h. The reaction mixture was stirred for 3 days at room temperature during which time a yellow color developed and a yellow precipitate formed. The crude product was collected by suction filtration and recrystallized from hexane/ethanol.

[2-Amino-5-bromo-4-(3-trifluoromethylphenyl)thiophen-3-yl]phenylmethanone (10a). Yield 48%. mp 128 °C. ^{1}H NMR ($CDCl_3$) δ 5.73 (br s, 2H, NH_2), 7.38−7.47 (m, 4H, ArH), 7.56−7.67 (m, 3H, ArH), 8.32−8.36 (m, 2H, ArH). Anal. ($C_{18}H_{11}F_3BrNOS$) C, H, N.

[2-Amino-5-bromo-4-(3-nitrophenyl)thiophen-3-yl]phenylmethanone (10b). Yield 30%. mp 166 °C. ^{1}H NMR ($CDCl_3$) δ 5.58 (br s, 2H, NH_2), 7.40−7.45 (m, 4H, ArH), 7.57−7.69 (m, 3H, ArH), 8.36−8.40 (m, 2H, ArH). Anal. ($C_{17}H_{11}BrN_2O_3S$) C, H, N.

[2-Amino-5-bromo-4-(4-trifluoromethylphenyl)thiophen-3-yl]phenylmethanone (10c). Yield 52%. mp 140 °C. ^{1}H NMR ($CDCl_3$) δ 5.62 (br s, 2H, NH_2), 7.39−7.48 (m, 5H, ArH), 7.58−7.65 (m, 2H, ArH), 7.73−7.92 (m, 2H, ArH). Anal. ($C_{18}H_{11}F_3BrNOS$) C, H, N.

[2-Amino-5-bromo-4-(4-nitrophenyl)thiophen-3-yl)phenylmethanone (10d). Yield 33%. mp 178 °C. ^{1}H NMR ($CDCl_3$) δ 5.39 (br s, 2H, NH_2), 7.39−7.47 (m, 5H, ArH), 7.57−7.71 (m, 2H, ArH), 7.90−8.33 (m, 2H, ArH). Anal. ($C_{17}H_{11}BrN_2O_3S$) C, H, N.

[2-Amino-5-bromo-4-(4-cyanophenyl)thiophen-3-yl]phenylmethanone (10e). Yield 58%. mp 122 °C. ^{1}H NMR ($CDCl_3$) δ 5.42 (br s, 2H, NH_2), 7.40−7.48 (m, 5H, ArH), 7.57−7.64 (m, 2H, ArH), 7.77−7.92 (m, 2H, ArH). Anal. ($C_{18}H_{11}BrN_2OS$) C, H, N.

[2-Amino-5-bromo-4-(4-phenylphenyl)thiophen-3-yl]-phenylmethanone (10f). Yield 48%. mp 68 °C. ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 2H, NH_2), 7.37−7.47 (m, 6H, ArH), 7.58−7.67 (m, 6H, ArH, 8.01−8.03 (m, 2H, ArH). Anal. ($C_{23}H_{16}BrN_2OS$) C, H, N.

[2-Amino-5-bromo-4-(2-naphthyl)thiophen-3-yl]phenylmethanone (10 g). Yield 39%. mp 92 °C. ^{1}H NMR ($CDCl_3$) δ 3.04 (br s, 2H, NH_2), 7.18−8.29 (m, 12H, ArH). Anal. ($C_{21}H_{14}BrN_2O_3S$) C, H, N.

Ethyl 2-Amino-5-bromo-4-(3-nitrophenyl)thiophene-3-carboxylate (10h). Ethyl cyanoacetic acid was used in place of benzoylacetonitrile for the synthesis of **10h** (yield 53%). ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_3), 4.29 (q, 2H, CH_2), 5.74 (br s, 2H, NH_2), 7.69−7.76 (m, 1H, ArH), 8.06−8.49 (m, 3H, ArH). Anal. ($C_{13}H_{11}BrN_2O_4S$) C, H, N.

Assay of AE Activity.[17] The assay of AE activity consisted of three phases: (1) binding to equilibrium of the agonist, ^{125}I-ABA to the A_1AR-G protein ternary complex; (2) stabilization of that complex by the AE, and (3) dissociation of the complex by adding a combination of an A_1AR antagonist and GTPγS, to accelerate agonist radioligand dissociation. The assay employed membranes from CHO−K1 cells stably expressing the hA_1AR. For agonist binding to equilibrium, the buffer consisted of 10 mM HEPES, pH 7.2, containing 0.5 mM $MgCl_2$, 1 U/mL adenosine deaminase, 0.5 nM ^{125}I-ABA, and 10 μg of membrane protein in a final volume of 100 μL applied to 96 well Millipore GF/C glass fiber filter plates. After 90 min at room temperature, the addition of 50 μL of a 0.3 mM solution of a candidate AE initiated stabilization of the ternary complex. Five minutes later 50 μL of a solution of 40 μM 8-CPT and 200 μM GTPγS was added to initiate the dissociation of the ternary complex. Ten minutes later membranes were filtered, washed, dried, and counted for residual ^{125}I-ABA. The percentage of specifically bound agonist remaining after 10 min of dissociation served as an index of AE activity:

$$\% \text{ AE activity} = 100 \times (B - B_o)/(B_{eq} - B_o)$$

Where B = residual binding (cpm) bound at the end of 10 min of dissociation in the presence of an AE, B_o = residual binding (cpm) at the end of 10 min of dissociation in the absence of an AE, and B_{eq} = cpm bound at the end of 90 min of equilibrium binding.

The percentage of specific binding remaining after 10 min of dissociation constitutes an index of AE activity for ranking candidate compounds. A score of 100% means no dissociation and a score of zero means complete dissociation.

Assay of A_1AR Antagonist Activity. Assays of antagonism of equilibrium binding by PD81,723 and enhancer **10a** used membranes isolated from CHOK1 cells expressing the hA_1AR. These membranes were incubated with [^{3}H]CPX (2 nM) and either PD81,723 (100 μM) or compound **10a** (100 μM) at room temperature for 90 min. Binding was done in triplicate and expressed as fraction of control.

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