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Synthesis and biological evaluation of novel 2-amino-3-aroyl-4-neopentyl-5-substituted thiophene derivatives as allosteric enhancers of the A_1 adenosine receptor

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ABSTRACT

2-Amino-3-benzoyl thiophenes have been widely reported to act as allosteric enhancers at the A_1 adenosine receptor. Their activity can be increased considerably by appropriate substitutions at the 4- and 5-positions of the thiophene ring. Substituent size at the thiophene C-4 position seemed to be a factor closely related to activity, with the 4-neopentyl (2,2-dimethylpropyl) substitution showing the greatest enhanced activity. A wide series of 2-amino-3-aroyl-4-neopentylthiophene derivatives with general structure **3**, characterized by the presence of different substituents (bromine, aryl and heteroaryl) at the 5-position of the thiophene ring, have been identified as potent AEs at the A_1AR. With only one exception, all of the synthesized compounds proved to be superior to the reference compound PD 81,723 in a functional assay. Derivatives **3p**, **3u**, **3am**, **3ap** and **3ar** were the most active compounds in binding (saturation and competition) and functional cAMP studies, being able to potentiate agonist [^{3}H]CCPA binding to the A_1 receptor.

1. Introduction

Adenosine is a physiological extracellular modulator acting via four distinct G protein-coupled receptors, named A_1, A_{2A}, A_{2B} and A_3, that are widely distributed throughout the body.[1] The A_1 adenosine receptor (A_1AR) is coupled to a Gi-protein signal transduction pathway to inhibit adenylate cyclase and its activation reduces intracellular levels of cAMP.[2] A variety of adenosine mediated effects (neuro- and cardio-protection, hypotension, reduction of neuropathic pain and inhibition of lipolysis) occurs by the selective activation of the A_1AR,[3] expressed in high density in the CNS (cortex, hippocampus, cerebellum and thalamus) and fat cells, and in moderate to low levels in many other tissues, such as bladder, lung, kidney and heart.[4] Efforts to selectively target the A_1AR with modified adenosine analogues or selective orthosteric agonists have been limited by side effects due to the activation of the A_1AR in tissues other than the therapeutic target, poor receptor subtype selectivity and a tendency to cause receptor desensitization upon prolonged use.[5]

An opportunity for therapeutic intervention is provided by targeting an allosteric site on the A_1AR with an allosteric enhancer (AE).[6] The binding of an allosteric modulator to the allosteric site of the A_1AR, structurally distinct from the orthosteric binding site, induces a reversible change of the A_1AR conformation that amplifies the potency and efficacy of endogenous adenosine, an important tissue protective agent released during ischemia, hypoxia or inflammation.[7] This approach can generate selectivity in action as a consequence of both tissue-specific and receptor-specific modulation.[8] Allosteric modulation of the A_1AR may have potential therapeutic applications in the

Abbreviations: GPCRs, G protein-coupled receptors; [^{3}H]DPCPX, [^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine; [^{3}H]MRE-3008F20, [^{3}H]5-*N*-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; [^{3}H]CCPA, [^{3}H]2-chloro-N^6-cyclopentyladenosine; [^{3}H]ZM 241385, [^{3}H](4-(2-[7-amino-2-(2-furil)[1,2.4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol); CCPA, 2-chloro-N^6-cyclopentyladenosine; CHO, chinese hamster ovary; cAMP, cyclic adenosine monophosphate; AE(s), allosteric enhancer(s); hA_1AR, human A_1 adenosine receptor; NBS, *N*-bromosuccinimide; $PdCl_2$(DPPF), [1,1′-bis(diphenylphosphino) ferrocene] dichloropalladium (II) complex with dichloromethane; CsF, cesium fluoride; EWG, electron-withdrawing group; ERG, electron-releasing group; CNS, central nervous system.

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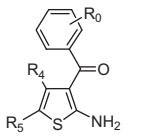
treatment of neuropathic pain, hypoxia and ischaemia-induced injury, to mitigate allodynia and as cardioprotective agents. Therefore, significant research efforts have been directed to the discovery of new small molecules acting as allosteric modulators for the A_1AR.[9]

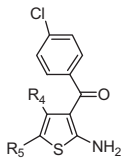
The allosteric modulation of the A_1AR by 2-amino-3-aroyl thiophenes is well documented in several review articles.[6,8–10] The 2-amino and 3-benzoyl groups were found to be crucial for the AE activity. Lipophilic substituents on the phenyl of the benzoyl moiety impart a favourable ratio of allosteric enhancement to antagonism, and include the 3-trifluoromethyl present in PD 81,723 (**1a**),[11a,11b] the 3,4-dichloro in LUF5484 (**1b**),[11c] the 4-chloro in T62 (**1c**), the 3-chloro in PD 71,605 (**1d**)[11a,11b] as well as the 4-methyl (**1e**)[11c] (Chart 1). A range of alkyl and aryl substituents in the 4- and 5-positions of the 2-amino-3-aroyl thiophene system have also been found to promote AE activity.[12] Large hydrophobic alkyl or aryl groups at the 4-position have a beneficial effect on AE activity, which increased in the order: H < Me < Phenyl, while bulky 5-alkyl or aryl substituents favoured increased competitive antagonistic properties with the resulting compounds.[13]

To further study the role of various alkyl substitutions at the 4-position of the 2-amino-3-(4-chlorobenzoyl)thiophene nucleus, the allosteric enhancement activity of compounds **2a** (methyl),[14] **2b** [isobutyl, $CH_2CH(CH_3)_2$], **2c** (*tert*-butyl, $C(CH_3)_3$) and **2d** [neopentyl, $CH_2C(CH_3)_3$] was determined by measuring the ability of the compounds at a concentration of 10 μM to reduce the cAMP content of CHO cells expressing human A_1 receptor. In this preliminary pharmacological evaluation, the neopentyl derivative **2d**, with a 35% reduction of cAMP production, was 2- to 3-fold more active than the other compounds of this series (Table 1). Compound **2d** was also more active than a small series of 4,5-disubstituted 2-amino-3-(4-chlorobenzoyl)thiophene analogues, corresponding to the 4-CH_3, 5-C_6H_5 (**2e**); 4-CH_3, 5-(4′-MeO-C_6H_4) (**2f**), 4-CH_3, 5-Br (**2g**), 4-C_6H_5, 5-CH_3 (**2h**) and 4,5-di-C_6H_5 (**2i**)[13a] derivatives, which caused a reduction of cAMP content ranging from 16% to 24%. The results obtained with compound **2d** make it possible to identify in a neopentyl moiety at the 4-position of the 2-amino-3-(4-chlorobenzoyl)thiophene scaffold an optimal substituent that is critical for interaction with the allosteric site of the A_1AR. We have therefore synthesized a first series of compounds with general formula **3**, based on the 2-amino-4-neopentyl-thiophene skeleton, containing different aroyl groups at the 3-position. Based upon previous studies, appropriate substituents on the benzoyl group were selected from among those that improved activity, such as 4-chloro (**3e**, **3j** and **3q**), 3-chloro (**3a**, **3f**, **3k** and **3r**), 3,4-dichloro (**3b**, **3g**, **3m** and **3s**), 3-trifluoromethyl (**3c**, **3h**, **3o** and **3t**) and 4-methyl (**3d**, **3i**, **3p** and **3u**). By the synthesis of compounds **3l** and **3n**, we investigated the influence on AE activity of further substitution (2-chloro and 2,4-dichloro, respectively), on the 3-benzoyl group. These molecules were also characterized by the presence of a hydrogen (**2d** and **3a–d**), bromine (**3e–i**), phenyl (**3j–p**) and 4-methoxyphenyl (**3q–u**) at the 5-position of the 2-amino-3-aroyl-4-neopentyl-thiophene system.

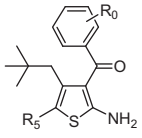
The structural refinement of the 5-phenyl and 5-(4′-methoxyphenyl)-thiophene derivatives **3j** and **3q**, respectively, led to the synthesis of analogues **3v–au**, based on the systematic modification of the 5-position of the 2-amino-3-(4-chlorobenzoyl)-4-neopentyl-thiophene ring, with the goal of evaluating the effects on AE activity due to insertion of substituents which included 3,4-dimethyl-isoxazol-4-yl (**3v**), 1*H*-pyrazol-4-yl (**3w**), isomeric thiophene (**3x** and **3y**), furan (**3z** and **3aa**) and pyridine (**3ab**, **3ac** and **3ad**), to end with the phenyl ring with electron-releasing (alkyl, alkoxy, OCF_3) and electron-withdrawing (F and Cl) groups (**3ae–3au**). By the preparation of compound **3ak**, we have also investigated the effect on AE activity due to the presence of a vinyl spacer between a 4-chlorophenyl ring and the 5-position of the thiophene ring.



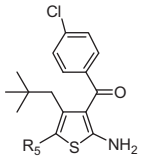
1a (PD 81,723); R_0=3-CF_3, R_4=R_5=CH_3
1b (LUF5484); R_0=3,4-Cl_2, R_4,R_5=$(CH_2)_4$
1c (T62); R_0=4-Cl, R_4,R_5=$(CH_2)_4$
1d (PD 71,605); R_0=3-Cl, R_4,R_5=$(CH_2)_4$
1e; R_0=4-CH_3, R_4,R_5=$(CH_2)_4$



2a; R_4=CH_3, R_5=H
2b; R_4=$CH_2CH(CH_3)_2$, R_5=H
2c; R_4=$C(CH_3)_3$, R_5=H
2d; R_4=$CH_2C(CH_3)_3$, R_5=H
2e; R_4=CH_3, R_5=C_6H_5
2f; R_4=CH_3, R_5=4′-$OMeC_6H_4$
2g; R_4=CH_3, R_5=Br
2h; R_4=C_6H_5, R_5=CH_3
2i; R_4=R_5=C_6H_5



3a; R_0=3-Cl, R_5=H
3b; R_0=3,4-Cl_2, R_5=H
3c; R_0=3-CF_3, R_5=H
3d; R_0=4-CH_3, R_5=H
3e; R_0=4-Cl, R_5=Br
3f; R_0=3-Cl, R_5=Br
3g; R_0=3,4-Cl_2, R_5=Br
3h; R_0=3-CF_3, R_5=Br
3i; R_0=4-CH_3, R_5=Br
3j; R_0=4-Cl, R_5=C_6H_5
3k; R_0=3-Cl, R_5=C_6H_5
3l; R_0=2-Cl, R_5=C_6H_5
3m; R_0=3,4-Cl_2, R_5=C_6H_5
3n; R_0=2,4-Cl_2, R_5=C_6H_5
3o; R_0=3-CF_3, R_5=C_6H_5
3p; R_0=4-CH_3, R_5=C_6H_5
3q; R_0=4-Cl, R_5=4′-OCH_3-C_6H_4
3r; R_0=3-Cl, R_5=4′-OCH_3-C_6H_4
3s; R_0=3,4-Cl_2, R_5=4′-OCH_3-C_6H_4
3t; R_0=3-CF_3, R_5=4′-OCH_3-C_6H_4
3u; R_0=4-CH_3, R_5=4′-OCH_3-C_6H_4



3v; R_5=3,4-($diCH_3$)-isoxazol-4-yl
3w; R_5=1H-pyrazol-4-yl
3x; R_5=thiophen-2-yl
3y; R_5=thiophen-3-yl
3z; R_5=furan-2-yl
3aa; R_5=furan-3-yl
3ab; R_5=pyridin-4-yl
3ac; R_5=pyridin-3-yl
3ad; R_5=pyridin-2-yl
3ae; R_5=4′-F-C_6H_4
3af; R_5=2′,3′-diF-C_6H_4
3ag; R_5=2′,4′-diF-C_6H_4
3ah; R_5=2′,5′-diF-C_6H_4
3ai; R_5=2′,6′-diF-C_6H_4
3aj; R_5=4′-Cl-C_6H_4
3ak; R_5=(*E*)-4′-Cl-C_6H_4CH=CH
3al; R_5=3′,4′-diCl-C_6H_4
3am; R_5=3′-OCH_3-C_6H_4
3an; R_5=2′-OCH_3-C_6H_4
3ao; R_5=4′-$OCH_3(CH_2)_2O$-C_6H_4
3ap; R_5=3′,4′-(diO-CH_2)-C_6H_3
3aq; R_5=4′-OCF_3-C_6H_4
3ar; R_5=4′-CH_3-C_6H_4
3as; R_5=3′-CH_3-C_6H_4
3at; R_5=2′-CH_3-C_6H_4
3au; R_5=4′-$(CH_3)CH$-C_6H_4

Chart 1. Chemical structures of 2-amino-3-aroyl thiophene derivatives **1a–e**, **2a–i** and **3a–au**, evaluated as allosteric modulators for the A_1 adenosine receptor.

2. Chemistry

The target compounds **2d** and **3a–au** were synthesized as shown in the reaction sequence outlined in Scheme 1. The 5-unsubstituted thiophene derivatives **2d**, **3a–d** and **5a–b** were prepared by a two-step procedure consisting of a Knoevenagel reaction of 4,4-dimethylpentan-2-one with the appropriate benzoylacetonitrile in toluene in the presence of β-alanine and acetic acid, followed by isolation and purification of the inseparable mixture of the *E*- and *Z*-olefin isomers **4a–g**. Cyclization with sulfur in ethanol in the presence of triethylamine (Gewald reaction)[15] provided the target compounds. Subsequent reaction with phthalic anhydride in acetic acid furnished, almost quantitatively, the corresponding *N*-protected phthalimido intermediates **6a–g**, which were transformed to the 5-bromothiophene derivatives **7a–g** by the chemoselective bromination with NBS in refluxing acetonitrile. These latter compounds were subjected to Suzuki cross-coupling conditions[16] in the presence of the appropriate aryl/heteroarylboronic acid under heterogeneous conditions [$PdCl_2$(dppf), CsF] in 1,4-dioxane under heating to furnish derivatives **8a–al**. For these latter compounds, as well as for the 5-bromothiophene analogues **7a–g**, the removal of the *N*-protected phthaloyl group was accomplished by the use of ethanolic hydrazine, to afford the derivatives **3e–au**.

Table 1
Effect of the novel allosteric enhancers **2a–i**, **3a–au** and of PD 81,723 in cAMP assay in hA_1 CHO cells

Compound	% Inhibition of cAMP production[a]	% Inhibition of cAMP production + CCPA[b]
2a	16 ± 1	18 ± 1
2b	17 ± 2	19 ± 2
2c	11 ± 1	18 ± 2
2d	35 ± 3	37 ± 4
2e	18 ± 2	20 ± 2
2f	24 ± 3	22 ± 2
2g	21 ± 2	25 ± 3
2h	16 ± 2	21 ± 2
2i	24 ± 2	26 ± 3
3a	45 ± 4	42 ± 4
3b	43 ± 4	41 ± 4
3c	28 ± 3	30 ± 3
3d	38 ± 4	35 ± 3
3e	48 ± 5	50 ± 5
3f	45 ± 5	48 ± 5
3g	42 ± 4	40 ± 4
3h	49 ± 5	50 ± 5
3i	61 ± 6	63 ± 6
3j	51 ± 5	53 ± 6
3k	59 ± 6	57 ± 6
3l	38 ± 3	41 ± 4
3m	56 ± 6	57 ± 6
3n	52 ± 5	55 ± 6
3o	38 ± 4	39 ± 4
3p	62 ± 6	61 ± 6
3q	44 ± 4	43 ± 4
3r	53 ± 5	56 ± 5
3s	54 ± 6	52 ± 5
3t	44 ± 4	46 ± 5
3u	64 ± 7	60 ± 5
3v	12 ± 1	19 ± 2
3w	27 ± 3	31 ± 3
3x	58 ± 5	62 ± 6
3y	62 ± 5	67 ± 7
3z	49 ± 5	47 ± 5
3aa	55 ± 6	56 ± 6
3ab	42 ± 4	46 ± 4
3ac	37 ± 4	41 ± 4
3ad	40 ± 4	43 ± 4
3ae	50 ± 5	52 ± 5
3af	60 ± 6	63 ± 6
3ag	48 ± 5	49 ± 5
3ah	46 ± 4	45 ± 4
3ai	45 ± 5	46 ± 5
3aj	57 ± 6	58 ± 6
3ak	51 ± 5	53 ± 5
3al	59 ± 6	61 ± 6
3am	55 ± 5	57 ± 6
3an	27 ± 2	29 ± 3
3ao	53 ± 5	55 ± 5
3ap	61 ± 6	59 ± 6
3aq	56 ± 6	55 ± 6
3ar	57 ± 6	58 ± 6
3as	47 ± 5	51 ± 5
3at	47 ± 5	49 ± 5
3au	56 ± 5	58 ± 5
PD 81,723	19 ± 2	22 ± 2

[a] Inhibition of the forskolin-stimulated cAMP production (in percentage) of the novel allosteric enhancers (10 μM);
[b] Inhibition of the cAMP production (in percentage) of the novel allosteric enhancers (100 nM) in the presence of CCPA (1 pM). The values are expressed as the mean ± SEM, $n = 3$ independent experiments.

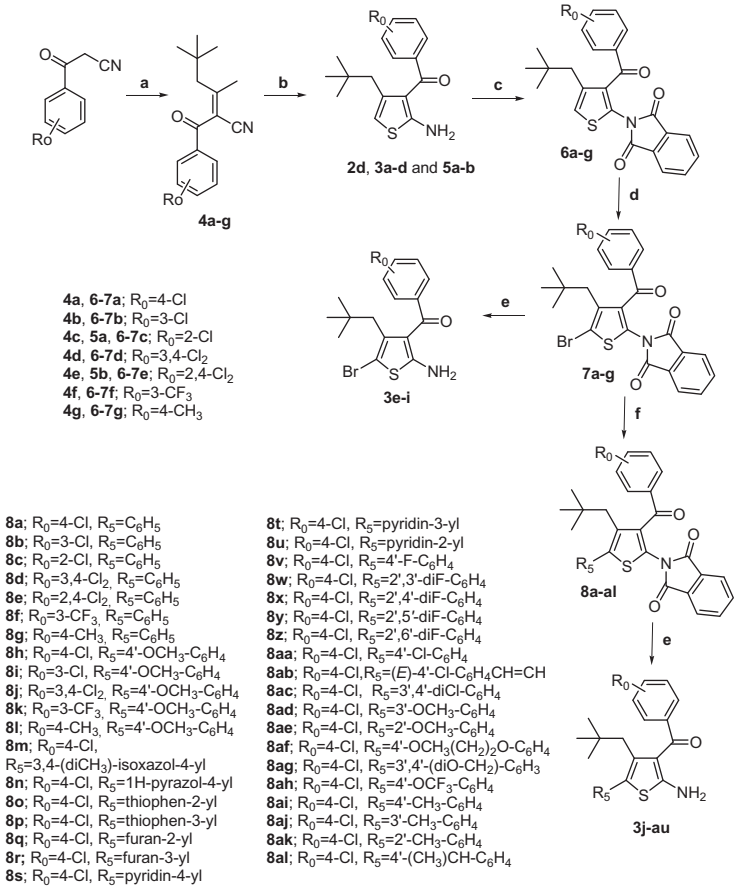
3. Biological results and discussion

3.1. Functional assays

To assess the biological activity of the synthesized compounds **2a–i** and **3a–au**, we initially screened all molecules using a functional assay, evaluating their ability to inhibit forskolin-stimulated cAMP accumulation in intact CHO cells expressing the cloned hA_1AR. When this receptor is in an active conformation in CHO cells, it causes a measurable inhibition of adenylate cyclase activity. AEs are thought to stabilize the active conformation of the A_1AR, leading to a reduction in the cAMP content of the cells.[17]

The reference compound PD 81,723 and the derivatives **2a–i** and **3a–au** were tested alone at a concentration of 10 μM (Fig. 1A) or at a concentration of 100 nM in the presence of the orthosteric agonist CCPA (1 pM) to assess enhancement of the A_1AR agonist activity (Fig. 1B).

A reduction in cAMP content is indicated in Table 1 as a percentage inhibition of cAMP production relative to control (absence of the test compound), in the absence or presence of the orthosteric



Reagents. **a**: 4,4-dimethylpentan-2-one, AcOH, β-alanine, toluene, reflux ; **b**: S_8, TEA, EtOH, reflux; **c**: phthalic anidride, AcOH, reflux; **d**: NBS, CH_3CN, reflux, **e**: NH_2NH_2, EtOH, reflux; **f**: $PdCl_2(DPPF)$, $ArB(OH)_2$, CsF, 1,4-dioxane, 65 °C.

Scheme 1.

agonist. The degree of inhibition of cAMP production was similar under the two conditions tested.

Among the 2-amino-3-aroyl-4-neopentylthiophene derivatives **2d** and **3a–au**, only one compound (**3v**) was less active than PD 81,723 at the concentration tested. This suggests that the presence of a 3,4-dimethyl-isoxazol-4-yl moiety at the 5-position of 2-amino-3-(4-chlorobenzoyl)-5-neopentyl scaffold is detrimental to the interaction of the ligand with the allosteric binding site of the A_1AR. Three of the compounds (**3c**, **3w** and **3an**) were comparable in activity to PD 81,723. All of the remaining new compounds were more active at 10 μM than PD 81,723 and decreased the percentage of cAMP production from 37% to 62% (Table 1). With only a few exceptions, the best results for inhibition of cAMP production (>50%) were observed with the presence of an aryl or heteroaryl (thiophene or furan) at the 5-position of the 2-amino-3-aroyl-4-neopentyl-thiophene.

In the series of 4-neopentyl-5-unsubstituted-thiophenes **2d** and **3a–d**, the 3-chloro (**3a**) and 3,4-dichloro (**3b**) derivatives tended to be more potent than the 4-chloro (**2d**), 3-trifluoromethyl (**3c**) and 4-methyl (**3d**) analogues, with **3c** being the least active compound in the series.

In the series of four derivatives (**3a**, **3f**, **3k** and **3r**) characterized by the presence of a common 3-chlorobenzoyl moiety at the 3-position of 2-amino-4-neopentyl-thiophene skeleton and that differ in the 5-substituent, it appeared that the most active compounds had a phenyl (**3k**) or a 4-methoxyphenyl (**3r**) at the C-5 position, while the presence of a bromine (**3f**) or the absence of a substituent (**3a**) decreased the allosteric enhancement. For compound **3k**, moving the chlorine from the 3- to the 2-position of the benzoyl moiety (compound **3l**) led to a 1.5-fold reduction in activity. The 3,4-dichlorobenzoyl derivatives **3b**, **3g**, **3m** and **3s** showed the same trend observed for the 3-chlorobenzoyl analogues **3a**, **3f**, **3k** and **3r**, with the C-5 phenyl and 4-methoxyphenyl derivatives **3m** and **3s**, respectively more active than the C-5 unsubstituted (**3b**) and bromo- (**3g**) analogues. The 3-(3,4-dichlorobenzoyl)-5-phenyl thiophene derivative **3m** showed activity comparable to that of the isomeric 2,4-dichlorobenzoyl counterpart, **3n**.

For the derivatives **3a**, **3f**, **3k** and **3r**, replacing the chlorine of the 3-chlorobenzoyl moiety with a more lipophilic and isoelectronic trifluoromethyl, to furnish compounds **3c**, **3h**, **3o** and **3t**, respectively, led to a significant reduction in activity, with the exception of the 5-bromothiophene **3h**.

For the 4-methylbenzoyl derivatives **3d**, **3i**, **3p** and **3u**, the replacement of the hydrogen (**3d**) at the 5-position of the thiophene ring with a bromine (**3i**), phenyl (**3p**) or a 4′-methoxyphenyl (**3u**) group led to a 1.5-fold reduction of cAMP production. A similar effect was observed in the series of 4-chlorobenzoyl derivatives **2d**, **3e**, **3j** and **3q**, with the C-5 substituted analogues **3e** (bromine), **3j** (phenyl) and **3q** (4′-methoxyphenyl) being more active than the C-5 unsubstituted analogue **2d**. Comparing the activities of derivatives bearing the same substituent at the 5-position of the thiophene ring (**2d** vs **3d**, **3e** vs **3i**, **3j** vs **3p**, **3q** vs **3u**), the

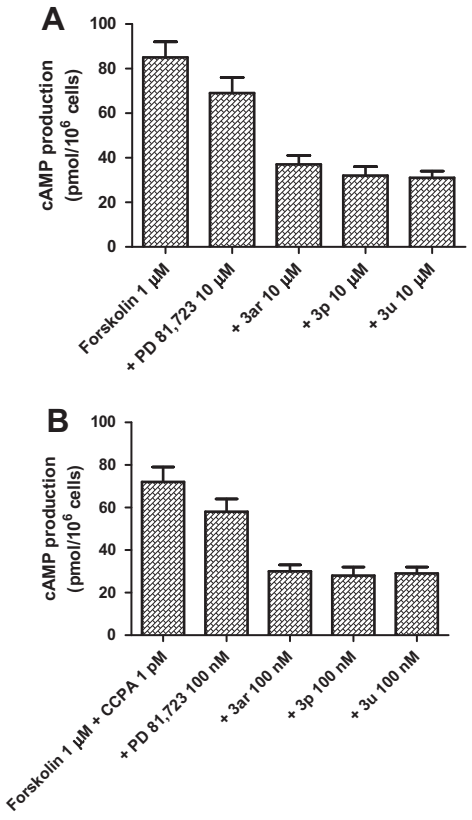


Figure 1. Histograms showing the cAMP inhibition, expressed in pmol/10^6 cells, mediated by novel allosteric enhancers at 10 μM concentration (A). The effect of the examined compounds (100 nM) was also studied in the presence of 1 pM CCPA (B). Values are expressed as mean ± SEM of three separate experiments, as described in Section 5.

substitution of the chloro at the 4-position of the benzoyl moiety with a methyl, which has similar lipophilic character but an opposite electronic effect, resulted in improved activity.

Starting from the 5-unsubstituted 4-chlorobenzoyl (**2d**), 3-trifluorobenzoyl (**3c**) and 4-methylbenzoyl (**3d**) thiophene analogues, the insertion of a bromine at the C-5 position of thiophene ring, to furnish compounds **3e**, **3h** and **3i**, respectively, caused a 1.5-fold increase in the percent inhibition of cAMP production, while there was no difference in activity between the 3-chlorobenzoyl and 3,4-dichlorobenzoyl derivatives **3a** and **3b** vs the corresponding 5-bromo analogues **3f** and **3g**, respectively.

In comparing the 5-bromothiophene derivatives **3e–i**, the 4-methylbenzoyl derivative **3i** was more active than the other compounds, which each showed a similar level of enhancement.

In contrast to the situation with the 4-chlorobenzoyl derivatives **3e** and **3j** and 4-methylbenzoyl analogues **3i** and **3p**, where replacement of bromine (**3e** and **3i**) with a phenyl (**3j** and **3p**) in the 5-position of the thiophene ring led to little change in activity, with the isomeric 3-chlorobenzoyl and 3,4-dichlorobenzoyl analogues **3f** and **3g**, respectively, replacement of bromine with phenyl group, to furnish the corresponding derivatives **3k** and **3m**, caused a substantial increase of activity. In contrast, for the 3-trifluoromethylbenzoyl compounds **3h** and **3o**, the activity of the 5-phenyl derivative **3o** was inferior to that the 5-bromo analogue **3h**.

The introduction of an electron-releasing methoxy group at the 4-position of the C-5 phenyl ring of compounds **3j**, **3k**, **3m** and **3o–p** has variable effects. Comparing **3j** and **3k** to the corresponding C-5 4′-methoxyphenyl analogues **3q** and **3r**, the substituted compounds showed slightly reduced activity, while there was no significant difference in activity between **3m** and **3p** and the related C-5 4′-methoxyphenyl analogues **3s** and **3u**. Interestingly, when a 4-methoxy group was introduced onto the 5-phenyl of compound **3o**, increased activity was observed at the tested concentration.

Molecules with the same substituent at different positions and therefore characterized by the same lipophilicity were also studied. This is the case for a group of isomeric derivatives constituted by the analogues substituted in the 3-position of the thiophene with 4-chlorobenzoyl (**2d**, **3e**, **3j** and **3q**) and 3-chlorobenzoyl (**3a**, **3f**, **3k** and **3r**) moieties. Comparing the activities of the molecules with the same substituent on the 5-position of the thiophene ring (**2d** vs **3a**, **3e** vs **3f**, **3j** vs **3k**, **3q** vs **3r**), the derivatives with the 3-chlorobenzoyl group are generally more potent than the corresponding analogues with the 4-chlorobenzoyl substituent. The exception to this trend is with the 5-bromothiophenes **3e** and **3f**, where the two compounds are effectively equal in activity.

The bioisosteric replacement of the phenyl in the 5-position of the 2-amino-3-(4-chlorobenzoyl)-4-neopentyl-thiophene scaffold (compound **3j**) by a thiophene in either regioisomeric orientation (to furnish the 2′-thienyl and 3′-thienyl derivatives **3x** and **3y**, respectively), has a benefical effect on AE activity, with no difference in the reduction of cAMP production between the two isomers. In contrast, the 3′,4′-dimethyl isoxazol-4-yl (**3v**) was not tolerated at the C-5 position of the thiophene ring, while replacement of 5-phenyl ring with an isosteric 1*H*-pyrazol-4-yl (**3w**) reduced activity by approximately one-half.

With compounds **3z–3ad**, the phenyl ring at C-5 was replaced with heterocycles that possessed heteroatoms able to form hydrogen bonds, such as the isomeric furans (**3z** and **3aa**) or pyridines (**3ab–3ad**). In addition, especially with the pyridines, we could also increase the hydrophilic properties of the molecules, since low water solubility is one of the major limitations of 2-amino-3-benzoyl thiophene derivatives. The three pyridine isomers **3ab–3ad** exhibited reduced AE activity when compared to the phenyl counterpart **3j**, while replacement of phenyl with furan-2-yl or furan-3-yl rings (**3z** and **3aa**, respectively), maintained the activity. Thus, results of functional assay of both the more hydrophilic compounds **3ab–3ad** and hydrophobic compounds **3j**, **3x** and **3y**, seem to confirm that the receptor domain of the allosteric site of the A_1AR surrounding the 5-position of thiophene ring is principally hydrophobic in nature.

Encouraged by the increased AE activity obtained with the 2-amino-4-(4-chlorobenzoyl)-4-neopentyl-5-phenylthiophene **3j**, we synthesized compounds **3q** and **3ae–3au**, to determine whether various electron-donating (alkyl, alkoxy or OCF_3) or electron-withdrawing (F and Cl) substituents on the different positions of the C-5 phenyl ring would lead to further increases in activity. Turning specifically to the 4-mono-substituted phenyl derivatives **3q**, **3ae**, **3aj**, **3ak**, **3ao**, **3aq**, **3ar**, and **3au**, they showed highly variable activity. The introduction of the weakly electron withdrawing fluorine group (compound **3e**) had little overall effect on AE activity and increasing the size of the halogen from fluorine to chlorine, to furnish the derivative **3aj**, led to a slight increase of the activity.

With the aim of determining if the presence of a second fluorine atom on the phenyl ring would lead to an increase of activity, the difluoro derivatives **3af–3ai** were prepared. The 2′,4′-difluoro-(**3ag**), 2′,5′-difluoro-(**3ah**) and 2′,6′-difluoro-(**3ai**) derivatives maintain an AE activity comparable to that of the mono-substituted 4′-F analogue **3ae**, while the activity was superior for the 2′,3′-difluoro derivative **3af**, in which the fluorines are adjacent to each other. Because the electronic properties of the di-fluorophenyl substituents in compounds **3af–3ai** are similar, the superior activity of the 2′,3′-difluoro derivative **3af** may be due to steric or hydrogen-bonding factors caused by the relative position of the two fluorine atoms on the phenyl ring.

Relative to the activity of the 4-chloro derivative **3aj**, the insertion of an additional chlorine atom to the 3-position, affording the 3′,4′-dichloro analogue **3al**, retained the activity.

Replacing chlorine with the electron-donating methyl group (**3ar**) also maintained the activity, which was considerably reduced by the substitution of the methyl with a less lipophilic and more electron-releasing methoxy group (**3q**). For this latter compound, the reduction in activity may be attributed to electronic factors. We can exclude steric factors, due to the good activity shown by lengthening the 4′-alkoxy moiety from methoxy to methoxyethylenoxy ($CH_3OCH_2CH_2O$, derivative **3ao**), characterized by the presence of an angularity component which extends significantly above or below the plane of the C-5 phenyl ring. The replacement of the methoxyethylenoxy group with a 4′-OCF_3 (compound **3aq**) maintained the level of cAMP reduction, which was comparable to that of the 4′-Cl derivative **3aj**. Starting from this latter

Table 2
A_1AR density expressed as B_{max} values (A) obtained by [^{3}H]CCPA binding assays in hA_1CHO membranes in the presence of **2a–i**, **3a–au** and of PD 81,723 (10 μM). Modulation by the novel allosteric enhancers (10 μM) on the CCPA affinity (CCPA K_i shift) in [^{3}H]DPCPX competition binding experiments (B)[a]

Compound	(A)		(B)	
	B_{max} (fmol/mg protein)	B_{max} shift (fold of increase)	CCPA K_i (nM)	CCPA K_i shift (fold of increase)
2a	798 ± 72	1.5 ± 0.1	9.8 ± 0.7	1.6 ± 0.1
2b	833 ± 68	1.6 ± 0.1	4.5 ± 0.5	3.4 ± 0.3
2c	679 ± 62	1.3 ± 0.1	9.2 ± 0.9	1.6 ± 0.2
2d	2038 ± 186	3.9 ± 0.3	9.5 ± 0.8	1.6 ± 0.1
2e	771 ± 64	1.5 ± 0.1	10.6 ± 0.9	1.5 ± 0.1
2f	1094 ± 102	2.1 ± 0.2	8.5 ± 0.8	1.8 ± 0.2
2g	932 ± 82	1.8 ± 0.1	8.1 ± 0.7	1.9 ± 0.2
2h	731 ± 66	1.4 ± 0.1	9.7 ± 0.8	1.6 ± 0.2
2i	1126 ± 107	2.1 ± 0.2	9.3 ± 0.8	1.7 ± 0.1
3a	2241 ± 214	4.3 ± 0.4	9.8 ± 0.9	1.5 ± 0.1
3b	2148 ± 206	4.1 ± 0.4	7.5 ± 0.7	2.0 ± 0.2
3c	1359 ± 125	2.6 ± 0.2	8.8 ± 0.9	1.7 ± 0.2
3d	1726 ± 163	3.3 ± 0.3	7.7 ± 0.8	2.0 ± 0.2
3e	3591 ± 316	6.8 ± 0.5	3.5 ± 0.3	4.5 ± 0.4
3f	1877 ± 169	3.6 ± 0.3	6.2 ± 0.6	2.4 ± 0.2
3g	2346 ± 224	4.5 ± 0.4	3.8 ± 0.3	4.0 ± 0.4
3h	2286 ± 218	4.4 ± 0.4	5.1 ± 0.6	3.0 ± 0.3
3i	3351 ± 312	6.4 ± 0.6	3.5 ± 0.4	4.3 ± 0.4
3j	3649 ± 324	6.9 ± 0.7	3.3 ± 0.3	4.8 ± 0.5
3k	3286 ± 318	6.3 ± 0.6	4.8 ± 0.5	3.1 ± 0.3
3l	2863 ± 256	5.4 ± 0.5	4.0 ± 0.4	3.9 ± 0.3
3m	3072 ± 279	5.9 ± 0.5	3.2 ± 0.3	4.8 ± 0.5
3n	3603 ± 277	6.8 ± 0.7	3.5 ± 0.4	4.4 ± 0.4
3o	2087 ± 211	4.0 ± 0.4	4.2 ± 0.4	3.6 ± 0.4
3p	3493 ± 322	6.7 ± 0.6	3.0 ± 0.3	5.0 ± 0.5
3q	3198 ± 284	6.1 ± 0.6	3.6 ± 0.4	4.3 ± 0.4
3r	3139 ± 314	6.0 ± 0.6	4.1 ± 0.4	3.7 ± 0.4
3s	3138 ± 302	6.0 ± 0.5	3.5 ± 0.4	4.3 ± 0.4
3t	2084 ± 198	4.0 ± 0.4	8.4 ± 0.8	1.8 ± 0.2
3u	3861 ± 376	7.4 ± 0.7	2.8 ± 0.3	5.4 ± 0.5
3v	735 ± 65	1.4 ± 0.1	13.3 ± 1.2	1.1 ± 0.1
3w	1308 ± 138	2.5 ± 0.3	8.7 ± 0.9	1.7 ± 0.2
3x	3237 ± 318	6.2 ± 0.6	3.9 ± 0.4	3.9 ± 0.4
3y	3332 ± 341	6.4 ± 0.7	3.2 ± 0.3	4.7 ± 0.5
3z	2668 ± 262	5.1 ± 0.5	4.3 ± 0.4	3.5 ± 0.4
3aa	2922 ± 297	5.6 ± 0.6	4.8 ± 0.5	3.1 ± 0.3
3ab	2086 ± 204	4.0 ± 0.4	5.4 ± 0.5	2.8 ± 0.3
3ac	1928 ± 194	3.7 ± 0.4	6.9 ± 0.7	2.2 ± 0.2
3ad	1831 ± 176	3.5 ± 0.3	6.8 ± 0.7	2.2 ± 0.2
3ae	2876 ± 277	5.5 ± 0.5	4.1 ± 0.4	3.7 ± 0.3
3af	3176 ± 311	6.1 ± 0.6	3.1 ± 0.3	4.9 ± 0.5
3ag	2758 ± 261	5.3 ± 0.5	4.2 ± 0.4	3.6 ± 0.4
3ah	2768 ± 284	5.3 ± 0.6	4.4 ± 0.4	3.4 ± 0.3
3ai	2194 ± 208	4.2 ± 0.4	6.3 ± 0.6	2.4 ± 0.2
3aj	3022 ± 309	5.8 ± 0.6	3.8 ± 0.4	4.0 ± 0.4
3ak	2509 ± 252	4.8 ± 0.5	5.3 ± 0.5	2.8 ± 0.3
3al	3143 ± 313	6.0 ± 0.6	3.5 ± 0.3	4.3 ± 0.4
3am	3292 ± 317	6.3 ± 0.6	2.6 ± 0.3	5.8 ± 0.6
3an	1322 ± 113	2.5 ± 0.2	8.2 ± 0.8	1.9 ± 0.2
3ao	2879 ± 270	5.5 ± 0.5	4.6 ± 0.5	3.3 ± 0.3
3ap	3449 ± 338	6.6 ± 0.6	2.9 ± 0.3	5.2 ± 0.5
3aq	3337 ± 346	6.4 ± 0.7	3.3 ± 0.3	4.6 ± 0.5
3ar	3345 ± 346	6.4 ± 0.7	2.5 ± 0.3	6.0 ± 0.6
3as	2449 ± 234	4.7 ± 0.4	3.7 ± 0.3	4.1 ± 0.4
3at	2138 ± 218	4.1 ± 0.5	5.5 ± 0.5	2.7 ± 0.3
3au	2932 ± 296	5.6 ± 0.6	3.7 ± 0.4	4.1 ± 0.4
PD 81,723	685 ± 62	1.3 ± 0.1	10.4 ± 0.9	1.5 ± 0.1

(A) = B_{max} (fmol/mg protein) and B_{max} shift obtained in [^{3}H]CCPA saturation binding experiments performed in the absence (B_{max} = 522 ± 46 fmol/mg protein) or in the presence of 10 μM enhancers.
(B) = K_i values of CCPA in the presence of 10 μM test compounds and CCPA shift = K_i(CCPA)/K_i(CCPA + 10 μM enhancers) where the K_i of CCPA was 15.1 ± 1.6 nM.
[a] The values are expressed as the mean ± SEM, n = 3 independent experiments.

compound, the insertion of a vinyl (CH=CH) spacer between the 5-position of thiophene ring and the 4′-chlorophenyl moiety, to afford the (*E*)-4′-chlorocinnamic derivative **3ak**, resulted in a slight reduction in activity relative to **3aj**.

For compounds **3q**, **3am** and **3an**, the position of the methoxy substituent on the C-5 phenyl ring had influence on AE activity, which increased in the order: *ortho* < < *para* < *meta*. Placing the methoxy group on the 2-position of the phenyl ring reduced the activity by approximately one-half relative to that of **3j**. An increase of activity was observed when the methoxy substituent was moved from the 2′-position to either the 3′-(**3am**) or the 4′-(**3q**) position, which was more evident for the 3′-derivative **3am**. Also the presence of a 3′,4′-methylenedioxy moiety on the phenyl ring (**3ap**) led to an increase in the AE activity relative to **3j**.

Among the three isomeric C-5 tolyl-thiophene derivatives **3ar**-**3at**, the 4′-methyl derivative **3ar** was more active than the 3′-methyl and 2′-methyl counterparts (**3as** and **3at**, respectively), with these two latter compounds being equiactive. By replacing the 4′-methyl group (**3ar**) with the more lipophilic and sterically demanding isopropyl group (**3au**) activity was maintained.

By comparing the effects of ERG's and EWG's on the phenyl at the C-5 position of the thiophene ring, no clear influence on allosteric enhancement was observed. In fact, several compounds characterized by the presence of substituents with opposite electronic effects showed the same activity. For example, compound **3aj** containing the electron-withdrawing chloro group showed the same activity as compound **3ar** containing the electron-donating methyl group.

3.2. Antagonistic activity

Many of the currently available allosteric enhancers of agonist binding to the hA_1AR have several non-specific actions. These non-specific actions include antagonism at the A_1 adenosine receptor, especially at higher concentrations. The ability of compounds **2d** and **3a**–**aq** to displace the binding of [^{3}H]DPCPX, [^{3}H]ZM241385 and [^{3}H]MRE-3008-F20 at human A_1, A_{2A} and A_3 ARs were evaluated in CHO cells at a concentration of 10 μM. The prototype enhancer PD 81,723 did not inhibit the binding of the radiolabeled antagonists to A_1 and A_{2A} ARs, but at 10 μM, it reduced by 21% the binding of [^{3}H]MRE-3008-F20 to A_3ARs.[18] None of the examined derivatives significantly inhibited the specific binding of the radioligands to A_1, A_{2A} and A_3ARs, causing inhibition of radioligand binding of 11% or less (Table S1). For the most active compounds in functional assays, such as **3i**, **3k**, **3p**, **3u**, **3x**–**y**, **3af**, **3aj**, **3al** and **3ap**–**ar** it was possible to achieve a good separation between high efficacy in the inhibition of cAMP production and binding to the orthosteric site.

3.3. Effect of enhancers on A_1AR binding parameters

Saturation and competition experiments of the selective adenosine A_1 agonist [^{3}H]CCPA to A_1 receptors were performed to determine if the novel compounds modified the agonist binding parameters. From these experiments, A_1 receptor affinity (K_D) and density (B_{max}) were evaluated in the presence and in the absence of the examined compounds (PD 81,723, **2a**–**i** and **3a**–**au** at a concentration of 10 μM) and were used to calculate the increase of A_1 density (B_{max} shift) (Table 2).

The reference compound PD 81,723 induced a B_{max} shift to human A_1 adenosine receptors of 1.3-fold. Under the same experimental conditions, with the exception of compounds **2a**, **2c**, **2e**–**i** and **3v**, all of the new tested compounds were significantly more potent than PD 81,723. From the receptor density calculated in the presence and in the absence of the novel enhancers, the derivatives **3e**, **3i**–**k**, **3n**, **3p**, **3q**–**s**, **3x**–**y**, **3af**, **3al**–**3am** and **3ap**–**3ar** were the most active compounds, each causing a B_{max} shift of more than 6-fold. Figure 2 shows the effect of the allosteric modulators PD 81,723, **3p**, **3u** and **3ar** at 10 μM concentration in [^{3}H]CCPA saturation binding experiments on A_1AR binding parameters such as affinity and density. Interestingly, no differences were found in affinity values, suggesting that the enhancers were not able to modify the K_D values of the high affinity binding sites labeled by [^{3}H]CCPA (K_D ranged from 1.0 ± 0.1 to 1.2 ± 0.1).

Table 2 also reports the derived apparent affinity (K_i) values for CCPA, based on a one-state model of analysis, in the absence and in the presence of the tested enhancers. This table also shows the CCPA shift representing the ratio of apparent K_i values in the absence and in the presence of the tested compounds at 10 μM concentration. In the hA_1CHO membranes, by using [^{3}H]DPCPX as radioligand, the K_i value of CCPA was 15.2 ± 1.3 nM. Interestingly, a significant decrease in the apparent K_i value was due to the presence of the putative allosteric enhancers, suggesting an increase in the high-affinity binding sites. In the presence of PD 81,723, the affinity of CCPA increased by 1.5-fold. The CCPA affinity data in the presence of the derivatives **2b**, **2f**–**g**, **2i**, **3b**–**u** and **3w**–**au** reveal that the displacement curves are shifted left, suggesting even lower K_i values for CCPA. In particular, the largest affinity shift has been observed for compounds **3p**, **3u**, **3am**, **3ap** and **3ar**. These molecules enhanced the apparent affinity of CCPA approximately 5.0, 5.4-, 5.8-, 5.2- and 6.0-fold, respectively, being twice as active as the 5-unsubstituted derivatives **2b** and **3a**–**d** (Table 2) in this assay. Thus, the enhancers were able to mediate a shift of the A_1 receptors towards the high affinity state as suggested from the increase of the CCPA affinity expressed as K_i values (Table 2). In Figure 3, representative binding curves for the displacement of [^{3}H]DPCPX by different concentrations of CCPA alone and in the presence of PD 81,723, **3p**, **3u** and **3ar** at 10 μM concentration are shown, demonstrating the apparent increase of the CCPA affinity in the presence of novel enhancers.

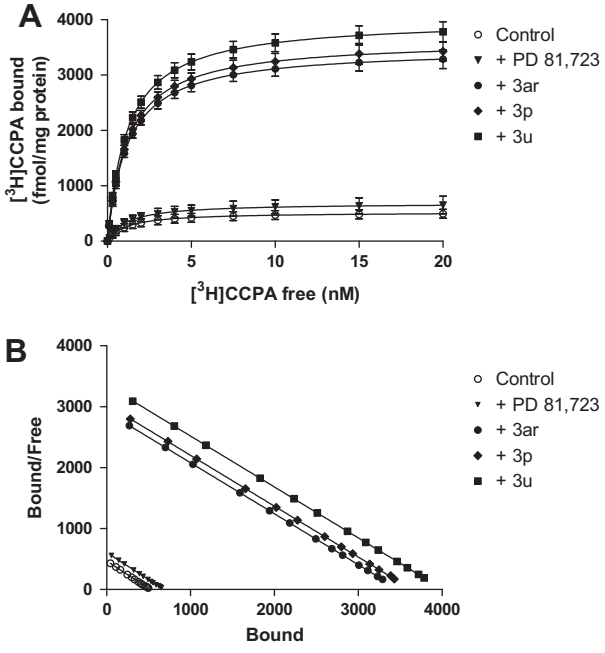


Figure 2. [^{3}H]-CCPA saturation binding curves at human A_1 adenosine receptors (A). Under control conditions, K_D value was 1.1 ± 0.1 nM and the B_{max} was 522 ± 46 fmol/mg protein. In the presence of novel enhancers (10 μM), K_D values were similar to those obtained in controls and B_{max} values were as reported in Table 2. Values are the means and vertical lines are the SEM of three separate experiments, as described in Section 5. Scatchard plots of the same experimental data (B).

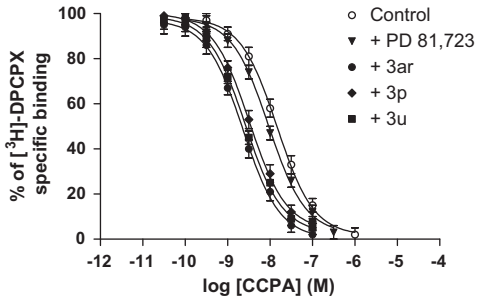


Figure 3. Inhibition curves of specific [^{3}H]-DPCPX binding to human A_1ARs of CCPA in the absence and in the presence of novel enhancers (10 μM). Affinity values were calculated by using a one-state model of analysis. Values are the means and vertical lines are the SEM of three separate experiments as described in Section 5.

The results obtained from the competition and saturation experiments confirmed the good enhancer activity of most of the synthesized compounds, in agreement with the cAMP functional assay.

4. Conclusions

The current study describes the synthesis and biological evaluation of a novel series of 2-amino-3-benzoyl-4-neopentyl thiophene derivatives, with variable modifications at the 5-position of the thiophene as well as in the benzoyl system. The presence of a neopentyl at the 4-position and a heteroaryl or variably substituted phenyl at the 5-position of 2-amino-3-aroyl-thiophene skeleton represented the best combination to afford a series of compounds with improved AE activity. Among the 4-neopentyl derivatives **2d** and **3a**–**au**, the 5-(3′,4′-dimethyl-isoxazol-4′yl) thiophene derivative **3v** was the least active compound in the series. Replacement of a phenyl ring in the 5-position of the thiophene ring with heterocycles that potentially could form a hydrogen bond with the allosteric site of the A_1AR allowed the activity to be maintained with the furan derivatives **3z** and **3aa**. The activity was slightly reduced with the more hydrophilic pyridine isomers **3ab**–**3ad**. On the contrary, replacement of the phenyl ring with the two isosteric/isoelectronic thienyl moieties (**3x** and **3y**) was well tolerated and increased the AE activity, with minimal difference in the reduction of cAMP production between **3x** and **3y**. This similarity also occurred with the other isomeric pair tested, the furanyl derivatives **3z** and **3aa**. In examining the effect of ERGs and EWGs on the phenyl at the C-5 position of the thiophene, no consistent pattern of effects on AE activity was observed. Staring from the mono-fluoro derivative **3ae**, the introduction of an additional fluorine atom retained [2′,4′-diF (**3ag**), 2′,5′-diF (**3ah**) and 2′,6′-diF (**3ai**) derivatives] or increased (2′,3′-diF, compound **3af**) the activity. There was little difference in activity between the two 4′-alkylphenyl derivatives **3ar** and **3au**. In an effort to more fully examine the steric effects of the alkoxy substituent at the 4′-position of the C-5 phenyl, the OCF_3 and the more bulky $MeO(CH_2)_2O$ group (compounds **3aq** and **3ao**, respectively), were prepared, both being more active relative to the 4′-OMe derivative **3q**. The contribution of methyl and methoxy moieties to activity was position-dependent. The 3′-methoxy derivative **3am** had a greater AE activity than the corresponding 4′- and 2′-methoxy analogues (**3q** and **3am**, respectively). Turning to the effects of an electron-releasing group on the C5-phenyl moiety, we found that a 4′-methyl group (**3ar**) caused a slight increase on AE activity relative to the unsubstituted derivative **3j**, which was reduced moving the methyl group from the 4′- to the 3′- and 2′-positions.

A characteristic feature of AEs at the A_1AR is the propensity to also cause antagonism at higher concentrations. None of the 2-amino-3-aroyl-4-neopentylthiophene derivatives (**2d** and **3a**–**au)** significantly inhibited antagonist binding at the hA_1AR, hA_2AR, or hA_3AR. Among these, derivatives **3p**, **3u**, **3am**, **3ap** and **3ar** were the most active compounds in binding (saturation and displacement) experiments and functional cAMP assays.

5. Experimental section

5.1. Chemistry

5.1.1. Materials and methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 and Varian 400 Mercury Plus spectrometer, respectively. Chemical shifts (δ) are given in parts per million (ppm) downfield and J values are given in hertz. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing ESI Micromass ZMD 2000 mass spectrometer. Melting points (mp) were determined on a Buchi–Tottoli apparatus and are uncorrected. Elemental analyses were conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara and were performed on a Yanagimoto MT-5 CHN recorder analyzer. All tested compounds yielded data consistent with a purity of at least 95% as compared with the theoretical values. All reactions were performed under an inert atmosphere of dry nitrogen, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates) and visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230–400 mesh silica gel and the solvent system indicated in the procedure. All commercially available compounds were used without further purification. Organic solutions were dried over anhydrous Na_2SO_4. Dichloromethane (DCM) was distilled from calcium chloride and stored over molecular sieves (3 Å). Petroleum ether refers to the fraction boiling at 40–60 °C. Compounds **2a** and **2i** were synthesized following the synthetic procedures reported in the references 14 and 13a, respectively.

5.2. General procedure (A) for the synthesis of compounds 4a–g[12b]

A mixture of 4,4-dimethylpentan-2-one (2.8 mL, 20 mmol) and the appropriate aroylacetonitrile (20 mmol), acetic acid (2.6 mL, 43.3 mmol), β-alanine (180 mg, 2 mmol) and benzene (70 mL) was heated to reflux in a Dean–Stark system. After 10 h, a second addition of acetic acid (2.6 mL) and β-alanine (180 mg, 2 mmol) was made. After 24 h, the reaction mixture was cooled to room temperature, diluted with ethyl acetate (50 mL), washed with water (30 mL), brine (30 mL), dried (Na_2SO_4), and finally concentrated in vacuo. The crude residue, constituting a mixture of *E*- and *Z*-isomers, was purified by column chromatography on silica gel to furnish the derivatives **4a**–**g**.

5.2.1. (*E*/*Z*)-2-(4-Chlorobenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4a)

Following general procedure A, using 3-(4-chlorophenyl)-3-oxo-propionitrile as β-ketonitrile, the crude was residue purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (19:1). Fractions containing the Knoevenagel adduct **4a**, as a mixture of geometric isomers, were combined and concentrated to afford the desired product as a yellow oil (yield 64%). ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 9H), 1.13 (s, 9H), 2.11 (s, 3H), 2.38 (s, 3H), 2.54 (s, 2H), 2.60 (s, 2H), 7.47 (d, J = 6.4 Hz, 2 × 2H), 7.86 (d, J = 6.4 Hz, 2 × 2H). MS (ESI): $[M+1]^+$ = 276.2.

5.2.2. (*E*/*Z*)-2-(3-Chlorobenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4b)

Following general procedure A, using 3-(3-chlorophenyl)-3-oxo-propionitrile as β-ketonitrile, the crude residue purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (9:1), afforded the desired product **4b** as a yellow oil (yield: 53%). ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 9H), 1.13 (s, 9H), 2.11 (s, 3H), 2.38 (s, 3H), 2.54 (s, 2H), 2.60 (s, 2H), 7.44 (t, J = 7.6 Hz, 2 × 1H), 7.58 (d, J = 7.4 Hz, 2 × 1H), 7.78 (d, J = 7.4 Hz, 2 × 1H), 7.89 (s, 2 × 1H). MS (ESI): $[M+1]^+$ = 276.1.

5.2.3. (*E*/*Z*)-2-(2-Chlorobenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4c)

Following general procedure A, using 3-(2-chlorophenyl)-3-oxo-propionitrile as β-ketonitrile, the crude residue purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (19:1), afforded the desired product **4c** as a colourless oil (yield: 77%). ^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 9H), 1.14 (s, 9H), 2.12 (s, 3H), 2.34 (s, 3H), 2.52 (s, 2H), 2.62 (s, 2H), 7.32 (m, 4H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$ = 276.2.

5.2.4. (*E*/*Z*)-2-(3,4-Dichlorobenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4d)

Following general procedure A, using 3-(3,4-dichlorophenyl)-3-oxo-propionitrile (20 mmol) as β-ketonitrile, the crude residue, constituting a mixture of *E*- and *Z*-isomers, was purified by column chromatography on silica gel, eluting with petroleum ether:ethyl acetate (19:1) to afford the desired product **4d** as a yellow oil (yield: 55%). ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 9H), 1.13 (s, 9H), 2.13 (s, 3H), 2.39 (s, 3H), 2.55 (s, 2H), 2.61 (s, 2H), 7.62 (d, J = 6.4 Hz, 2 × 1H), 7.80 (m, 2 × 1H), 7.99 (s, 1H) 8.00 (s, 1H). MS (ESI): $[M+1]^+$ = 310.1.

5.2.5. (*E*/*Z*)-2-(2,4-Dichlorobenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4e)

Following general procedure A, using 3-(2,4-dichlorophenyl)-3-oxo-propionitrile (20 mmol) as β-ketonitrile, the crude residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (19:1), to afford the desired product **4e** as a colourless oil (yield: 78%) δ: ^{1}H NMR ($CDCl_3$) δ: 1.03 (s, 9H), 1.09 (s, 9H), 2.29 (s, 3H), 2.31 (s, 3H), 2.62 (s, 2H), 2.83 (s, 2H), 6.95 (s, 1H), 7.01 (d, J = 6.4 Hz, 1H), 7.35 (d, J = 6.4 Hz, 1H), 7.36 (s, 1H), 7.54 (m, 2H). MS (ESI): $[M+1]^+$ = 310.1.

5.2.6. (*E*/*Z*)-2-(3-Trifluoromethylbenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4f)

Following general procedure A, using 3-(3-trifluoromethylphenyl)-3-oxo-propionitrile as β-ketonitrile, the crude residue, constituting a mixture of *E*- and *Z*-isomers, was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (19:1) to afford the desired product **4f** as a yellow oil (Yield: 44%). ^{1}H NMR ($CDCl_3$) δ: 1.14 (s, 9H), 1.16 (s, 9H), 2.15 (s, 2H), 2.42 (s, 2H), 2.58 (s, 3H), 2.63 (s, 3H), 7.63 (m, 2 × 1H), 7.83 (m, 2 × 1H), 8.03 (m, 2 × 1H), 8.18 (s, 2 × 1H). MS (ESI): $[M+1]^+$ = 310.3.

5.2.7. (*E*/*Z*)-2-(4-Methylbenzoyl)-3,5,5,-trimethylhex-2-enenitrile (4g)

Following general procedure A, using 3-(4-methylphenyl)-3-oxo-propionitrile as β-ketonitrile, the crude residue constituting a mixture of *E*- and *Z*-isomers, was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (9:1) to afford the desired product **4g** as a yellow oil (yield: 50%). ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, 9H), 1.13 (s, 9H), 2.36 (s, 3H), 2.43 (s, 3H), 2.50 (s, 2H), 2.58 (s, 3H), 7.28 (d, J = 8.2 Hz, 2 × 2H), 7.84 (d, J = 8.2 Hz, 2 × 2H). MS (ESI): $[M+1]^+$ = 256.2.

5.3. General procedure (B) for the synthesis of compounds 2d, 3a–d and 5a–b[12b]

A mixture of the Knoevenagel's adduct (6.3 mmol), triethylamine (1.1 mL, 7.65 mmol), and sulfur (243 mg, 7.6 mmol) in ethanol (20 mL) was heated to reflux for 2 h. After cooling to room temperature, the mixture was concentrated and the residue dissolved in ethyl acetate (20 mL). The organic solution was washed with 0.5 N HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), water (5 mL), brine (5 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent. Fractions containing the desired product were combined and concentrated to afford the desired product.

5.3.1. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](4-chlorophenyl)methanone (2d)

Following general procedure B, derivative **2d** was purified by column chromatography using petroleum ether/ethyl acetate 9:1 as eluent. Yellow oil. Yield: 78%. ^{1}H NMR ($CDCl_3$) δ: 0.64 (s, 9H), 2.12 (s, 2H), 5.92 (br s, 2H), 7.38 (s, 1H), 7.39 (d, J = 6.4 Hz, 2H), 7.57 (d, J = 6.4 Hz, 2H). MS (ESI): $[M+1]^+$ = 308.1. Anal. ($C_{16}H_{18}ClNOS$) C, H, N.

5.3.2. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](3-chlorophenyl)methanone (3a)

Following general procedure B, derivative **3a** was purified by column chromatography using petroleum ether/ethyl acetate 9:1 as eluent. Yellow oil. Yield: 76%. ^{1}H NMR ($CDCl_3$) δ: 0.64 (s, 9H), 2.10 (s, 2H), 5.92 (s, 1H), 6.04 (s, 2H), 7.36 (t, J = 6.8 Hz, 1H), 7.39 (d, J = 6.8 Hz, 1H), 7.56 (d, J = 6.8 Hz, 1H), 7.58 (s, 1H). MS (ESI): $[M+1]^+$ = 308.2. Anal. ($C_{16}H_{18}ClNOS$) C, H, N.

5.3.3. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](3,4-dichlorophenyl)methanone (3b)

Following general procedure B, derivative **3b** was purified by column chromatography using petroleum ether/ethyl acetate 9:1 as eluent. Yellow oil. Yield: 78%. ^{1}H NMR ($CDCl_3$) δ: 0.65 (s, 9H), 2.12 (s, 2H), 5.93 (s, 1H), 6.00 (br s, 2H), 7.41 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 7.2 Hz, 1H), 7.69 (s, 1H). MS (ESI): $[M+1]^+$ = 342.3. Anal. ($C_{16}H_{17}Cl_2NOS$) C, H, N.

5.3.4. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](3-trifluoromethylphenyl)methanone (3c)

Following general procedure B, derivative **3c** was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 19:1 as eluent. Yellow oil. Yield: 84%. ^{1}H NMR ($CDCl_3$) δ: 0.60 (s, 9H), 2.03 (s, 2H), 5.93 (s, 1H), 6.14 (br s, 2H), 7.56 (t, J = 7.8 Hz, 1H), 7.75 (m, 2H), 7.84 (s, 1H). MS (ESI): $[M+1]^+$ = 342.4. Anal. ($C_{17}H_{18}F_3NOS$) C, H, N.

5.3.5. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](4-methylphenyl)methanone (3d)

Following general procedure B, derivative **3d**, the crude residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 7:3 as eluent. Yellow solid. Yield: 68%, mp: 112–114 °C. ^{1}H NMR ($CDCl_3$) δ: 0.64 (s, 9H), 2.17 (s, 2H), 2.41 (s, 3H), 5.69 (br s, 2H), 5.92 (s, 1H), 7.21 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.97,

29.17 (3C), 31.34, 42.89, 105.45, 115.56, 128.67 (4C), 136.12, 138.05, 141.2, 162.81, 191.83. MS (ESI): $[M+1]^+$ = 288.2. Anal. ($C_{17}H_{21}NOS$) C, H, N.

5.3.6. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](2-chlorophenyl)methanone (5a)

Following general procedure B, derivative **5a** was purified by column chromatography using petroleum ether/ethyl acetate 9:1 as eluent. Yield: 72%. Yellow solid, mp: 130–131 °C. ^{1}H NMR ($CDCl_3$) δ: 0.65 (s, 9H), 1.91 (s, 2H), 5.83 (s, 1H), 6.76 (br s, 1H), 7.39 (m, 4H). MS (ESI): $[M+1]^+$ = 308.1.

5.3.7. 2-Amino-5-(2,2-dimethylpropyl)thiophen-3-yl](2,4-dichlorophenyl)methanone (5b)

Following general procedure B, derivative **5b** was purified by column chromatography using petroleum ether/ethyl acetate 9:1 as eluent. Yellow solid. Yield: 74%, mp: 138–139 °C. ^{1}H NMR ($CDCl_3$) δ: 0.67 (s, 9H), 1.93 (s, 2H), 5.84 (br s, 2H), 6.02 (s, 1H), 7.31 (s, 1H), 7.39 (d, J = 6.4 Hz, 1H), 7.44 (d, J = 6.4 Hz, 1H). MS (ESI): $[M+1]^+$ = 342.2.

5.4. General procedure (C) for the synthesis of compounds 6a–g

To a solution of 2-amino-3-aroyl-4-(2,2-dimethylpropyl)thiophene (5 mmol) in acetic acid (20 mL), was added phthalic anhydride (0.88 g, 5.9 mmol) and the mixture heated to reflux for 6 h. The solvent was removed in vacuo and the residue dissolved in ethyl acetate (20 mL). The organic solution was washed with a saturated aqueous solution of $NaHCO_3$ (5 mL), water (5 mL), brine (5 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was then stirred for 1 h with petroleum ether (20 mL), furnishing the desired product by filtration. Alternatively, the residue was purified by column chromatography (eluent EtOAc-petroleum ether) on silica gel.

5.4.1. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (6a)

Following general procedure C, the crude product was stirred for 1 h with petroleum ether (20 mL) furnishing the desired product **6a** by filtration as a yellow solid. Yield: 77%, mp 179–181 °C. ^{1}H NMR ($CDCl_3$) δ: 0.62 (s, 9H), 2.68 (s, 2H), 7.09 (s, 1H), 7.18 (d, J = 6.4 Hz, 2H), 7.65 (d, J = 6.4 Hz, 2H), 7.72 (m, 4H). MS (ESI): $[M+1]^+$ = 438.1.

5.4.2. 2-[3-(3-Chlorobenzoyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (6b)

Following general procedure C, the residue was purified by column chromatography on silica gel using petroleum ether and ethyl acetate (8.5:1.5) as eluent, to furnish the compound **6b** as a brown solid. Yield: 78%, mp 142–144 °C. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 9H), 2.72 (s, 2H), 7.08 (s, 1H), 7.30 (t, J = 8.2 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.73 (m, 4H), 7.82 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H). MS (ESI): $[M+1]^+$ = 438.2.

5.4.3. 2-[3-(2-Chlorobenzoyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (6c)

Following general procedure C, the residue was triturated with petroleum ether (20 mL), furnishing the desired product **6c** as a yellow solid. Yield: 73%; mp: 157–158 °C. ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, 9H), 2.72 (s, 2H), 7.07 (m, 4H), 7.44 (d, J = 6.4 Hz, 1H), 7.70 (m, 4H). MS (ESI): $[M+1]^+$ = 438.1.

5.4.4. 2-[3-(3,4-Dichlorobenzoyl)-4-(2,2-dimethylpropyl) thiophen-2-yl]isoindoline-1,3-dione (6d)

Following general procedure C, the residue was triturated with petroleum ether (20 mL), furnishing the desired product **6d** as a yellow solid. Yield: 83%, mp 131–133 °C. ^{1}H NMR ($CDCl_3$) δ: 0.82 (s, 9H), 2.69 (s, 2H), 7.10 (s, 1H), 7.27 (t, J = 8.2 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.72 (m, 5H). MS (ESI): $[M+1]^+$ = 472.1.

5.4.5. 2-[3-(2,4-Dichlorobenzoyl)-4-(2,2-dimethylpropyl) thiophen-2-yl]isoindoline-1,3-dione (6e)

Following general procedure C, the residue was triturated with petroleum ether (20 mL), furnishing the desired product **6e** as a yellow solid. Yield: 68%; mp: 134–136 °C. ^{1}H NMR ($CDCl_3$) δ: 0.68 (s, 9H), 2.69 (s, 2H), 7.07 (d, J = 6.4 Hz, 1H), 7.15 (s, 1H), 7.16 (s, 1H), 7.36 (d, J = 6.4 Hz, 1H), 7.75 (m, 4H). MS (ESI): $[M+1]^+$ = 472.2.

5.4.6. 2-[4-(2,2-Dimethylpropyl)-3-(3-trifluoromethylbenzoyl)-thiophen-2-yl]isoindoline-1,3-dione (6f)

Following general procedure C, the residue was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate 4:1, to furnish the compound **6f** as a brown oil. Yield: 83%. ^{1}H NMR ($CDCl_3$) δ: 0.68 (s, 9H), 2.74 (s, 2H), 7.11 (s, 1H), 7.34 (t, J = 8.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.70 (m, 4H), 7.80 (d, J = 8.4 Hz, 1H), 7.93 (s, 1H). MS (ESI): $[M+1]^+$ = 472.1.

5.4.7. 2-[4-(2,2-Dimethylpropyl)-3-(4-methylbenzoyl)-thiophen-2-yl]isoindoline-1,3-dione (6g)

Following general procedure C, the residue was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate 4:1, to furnish the compound **6g** as a pink solid. Yield: 73%; mp 169–170 °C. ^{1}H NMR ($CDCl_3$) δ: 0.82 (s, 9H), 2.14 (s, 3H), 2.71 (s, 2H), 6.98 (s, 1H), 7.04 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.72 (m, 4H). MS (ESI): $[M+1]^+$ = 418.2.

5.5. General procedure (D) for the synthesis of compounds 7a–g

To a solution of thiophene derivative **6a**–**g** (0.5 mmol) in CH_3CN (5 mL) was added NBS (180 mg, 2 mmol). The mixture was heated to reflux for 2 h, then cooled to room temperature. The solvent was removed under reduced pressure, and the residue, dissolved in EtOAc (10 mL), was sequentially washed with a saturated aqueous solution of $NaHCO_3$ (2 mL), water (1 mL), brine (1 mL), dried (Na_2SO_4), filtered, and concentrated to give a residue purified by column chromatography on silica gel or triturated with petroleum ether.

5.5.1. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(2,2-dimethylpropyl) thiophen-2-yl]isoindoline-1,3-dione (7a)

Following general procedure D, after workup as described previously, the residue was triturated with petroleum ether for 10 min, to furnish **7a** as a white solid. Yield: 96%, mp 180–183 °C. ^{1}H NMR ($CDCl_3$) δ: 0.85 (s, 9H), 2.79 (s, 2H), 7.18 (d, J = 6.4 Hz, 2H), 7.62 (d, J = 6.4 Hz, 2H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$ = 516.1, $[M+3]^+$ = 518.2.

5.5.2. 2-[5-Bromo-3-(3-chlorobenzoyl)-4-(2,2-dimethylpropyl) thiophen-2-yl]isoindoline-1,3-dione (7b)

Following general procedure D, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 17:3 as eluent, to give the desired compound **7b** as a yellow solid. Yield: 87%. mp: 143–144 °C. ^{1}H NMR ($CDCl_3$) δ: 0.86 (s, 9H), 2.63 (s, 2H), 7.16 (m, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.67 (s, 1H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$ = 516.0, $[M+3]^+$ = 518.1.

5.5.3. 2-[5-Bromo-3-(2-chlorobenzoyl)-4-(2,2-dimethylpropyl) thiophen-2-yl]isoindoline-1,3-dione (7c)

Following general procedure D, after workup as described previously, the residue was triturated with petroleum ether for 10 min, to furnish **7c** as a yellow solid. Yield: 96%, mp

135–136 °C. ^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 9H), 2.76 (s, 2H), 7.12 (m, 3H), 7.42 (d, J = 6.4 Hz, 1H), 7.71 (m, 4H). MS (ESI): $[M+1]^+$ = 516.1, $[M+3]^+$ = 518.1.

5.5.4. 2-[5-Bromo-3-(3,4-dichlorobenzoyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindol-ine-1,3-dione (7d)

Following general procedure D, after workup as described previously, the residue was purified by column chromatography on silica gel eluting with a mixture of petroleum ether/ethyl acetate 9:1 as eluent, to give the desired compound **7d** as a yellow solid. Yield: 78%. mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ: 0.85 (s, 9H), 2.80 (s, 2H), 7.27 (d, J = 8.2 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.76 (m, 5H). MS (ESI): $[M+1]^+$ = 550.8, $[M+3]^+$ = 552.8.

5.5.5. 2-[5-Bromo-3-(2,4-dichlorobenzoyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindol-ine-1,3-dione (7e)

Following general procedure D, after workup as described previously, the residue was triturated with a mixture of diethyl ether/petroleum ether for 30 min to give the desired product **7e** as a yellow solid. Yield: 84%, mp 165–166 °C. ^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 9H), 2.77 (s, 2H), 7.09 (d, J = 8.4 Hz, 1H), 7.16 (s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.78 (m, 4H). MS (ESI): $[M+1]^+$ = 550.6, $[M+3]^+$ = 552.7.

5.5.6. 2-[5-Bromo-4-(2,2-dimethylpropyl)-3-(3-trifluoromethylbenzoyl)thiophen-2-yl]isoindoline-1,3-dione (7f)

Following general procedure D, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of petroleum ether/ethyl acetate 9:1 to give the desired product **7f** as a yellow oil. Yield: 87%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, 9H), 2.86 (s, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.70 (m, 4H), 7.83 (d, J = 7.6 Hz, 1H), 7.94 (s, 1H). MS (ESI): $[M+1]^+$ = 550.1, $[M+3]^+$ = 552.1.

5.5.7. 2-[5-Bromo-4-(2,2-dimethylpropyl)-3-(4-methylbenzoyl) thiophen-2-yl]isoindoline-1,3-dione (7g)

Following general procedure D, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of petroleum ether/ethyl acetate 4:1 to give the desired product **7g** as a white solid. Yield: 80%, mp 169–170 °C. ^{1}H NMR ($CDCl_3$) δ: 0.86 (s, 9H), 2.13 (s, 2H), 2.82 (s, 3H), 6.97 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 7.8 Hz, 2H), 7.68 (m, 4H). MS (ESI): $[M+1]^+$ = 495.9, $[M+3]^+$ = 497.9.

5.6. General procedure (*E*) for the synthesis of compounds 8a–al

A stirred suspension of bromothiophene **7a**–**g** (0.5 mmol) and the appropriate aryl/heteroarylboronic acid (0.75 mmol) in dioxane (6 mL containing 2 drops of water) was degassed under a stream of nitrogen over 10 min, then treated with $PdCl_2$(DPPF) (41 mg, 0.05 mmol) and CsF (190 mg, 1.25 mmol). The reaction mixture was heated under nitrogen at 45 °C for 30 min, then at 65 °C for 6 h (or 95 °C for 18 h for compounds **8s**–**u**). The reaction mixture was cooled to ambient temperature, diluted with CH_2Cl_2 (10 mL), filtered on a pad of celite and evaporated in vacuo. The residue was dissolved with CH_2Cl_2 (15 mL), and the resultant solution was washed sequentially with water (5 mL) and brine (5 mL). The organic layer was dried and evaporated, and the residue was purified by column chromatography on silica gel.

5.6.1. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-phenylthiophen-2-yl]isoindoline-1,3-dione (8a)

Following general procedure E, after workup as described previously, the residue was purified by flash chromatography on silica gel using light petroleum ether/ethyl acetate 9:1 as eluent, affording compound **8a** as a white solid. Yield: 90%, mp 115–117 °C.^{1}H NMR ($CDCl_3$) δ: 0.56 (s, 9H), 2.94 (s, 2H) 7.26 (d, 2H, J = 6.4 Hz), 7.42 (d, 2H, J = 6.4 Hz), 7.52 (m, 2H), 7.74 (m, 7H). MS (ESI): $[M+1]^+$ = 514.1.

5.6.2. 2-[3-(3-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(phenyl)thiophen-2-yl]isoindoline-1,3-dione (8b)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 9:1 as eluent. affording the desired intermediate **8b** as a white solid. Yield: 62%, mp 91–93 °C. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.97 (s, 2H), 7.17 (d, 1H, J = 7.6 Hz), 7.49 (m, 6H), 7.78 (m, 6H). MS (ESI): $[M+1]^+$ = 514.1.

5.6.3. 2-[3-(2-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(phenyl)thiophen-2-yl]isoindoline-1,3-dione (8c)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 4:1 as eluent affording the desired intermediate **8c** as a cream colored solid. Yield: 77%, mp 87–90 °C. ^{1}H NMR ($CDCl_3$) δ: 0.90 (s, 9H), 2.82 (s, 2H), 7.22 (m, 3H), 7.54 (m, 5H), 7.62 (d, J = 6.4 Hz, 1H), 7.81 (m, 4H). MS (ESI): $[M+1]^+$ = 514.2.

5.6.4. 2-[3-(3,4-Dichlorobenzoyl)-4-(2,2-dimethylpropyl)-5-phenylthiophen-2-yl]isoindoline-1,3-dione (8d)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 19:1 as eluent, affording the desired intermediate **8d** as a yellow solid. Yield: 55%, mp 171–173 °C. ^{1}H NMR ($CDCl_3$) δ: 0.56 (s, 9H), 2.79 (s, 2H), 7.32 (s, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.52 (m, 5H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$ = 548.1.

5.6.5. 2-[3-(2,4-Dichlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(phenyl)thiophen-2-yl]iso-indoline-1,3-dione (8e)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 9:1 as eluent, affording the desired intermediate **8e** as a yellow oil. Yield: 89%. ^{1}H NMR ($CDCl_3$): δ 0.88 (s, 9H), 2.74 (s, 2H), 7.11 (d, J = 6.4 Hz, 1H), 7.18 (s, 1H), 7.45 (m, 6H), 7.78 (m, 4H). MS (ESI): $[M+1]^+$ = 548.2.

5.6.6. 2-[4-(2,2-Dimethylpropyl)-5-(phenyl)-3-(3-trifluoromethylbenzoyl)thiophen-2-yl]isoindoline-1,3-dione (8f)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (4:1) as eluent, affording the desired intermediate **8f** as a yellow solid. Yield: 63%, mp 170–172 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.99 (s, 2H), 7.39 (d, J = 7.6 Hz, 1H), 7.43 (m, 6H), 7.71 (m, 4H), 7.86 (d, J = 7.6 Hz, 1H), 8.08 (s, 1H). MS (ESI): $[M+1]^+$ = 548.2.

5.6.7. 2-[4-(2,2-Dimethylpropyl)-3-(4-methylbenzoyl)-5-(phenyl)thiophen-2-yl]isoindoline-1,3-dione (8g)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 17:3 as eluent, affording the desired intermediate **8g** as a yellow solid. Yield: 61%, mp 95–96 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.13 (s, 3H), 2.96 (s, 2H), 6.98 (d, J = 7.8 Hz, 2H), 7.52 (m, 5H), 7.64 (d, J = 7.8 Hz, 2H), 7.72 (m, 4H). MS (ESI): $[M+1]^+$ = 494.0.

5.6.8. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8h)

Following general procedure E, after workup as described previously, the crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether 3:17, to furnish the desired intermediate **8h** as a yellow solid. Yield: 55%, mp 170–172 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.90 (s, 2H), 3.86 (s, 3H), 6.95 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 9.0 Hz, 2H), 7.43 (d, J = 9.0 Hz, 2H), 7.72 (m, 6H). MS (ESI): $[M+1]^+$ = 544.0.

5.6.9. 2-[3-(3-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl)thiophen-2-yl]isoindoline-1,3-dione (8i)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 9:1 as eluent affording the desired intermediate **8i** as a yellow solid. Yield: 58%, mp 110–112 °C. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.93 (s, 2H), 3.87 (s, 3H), 6.96 (d, J = 8.8 Hz, 2H), 7.13 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.74 (m, 6H). MS (ESI): $[M+1]^+$ = 544.2.

5.6.10. 2-[3-(3,4-Dichlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl)thiophen-2-yl]isoindoline-1,3-dione (8j)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 19:1 as eluent, affording the desired intermediate **8j** as a yellow solid. Yield: 63%, mp 81–83 °C. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.91 (s, 2H), 3.87 (s, 3H), 6.96 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.77 (m, 4H), 7.84 (s, 1H). MS (ESI): $[M+1]^+$ = 578.1.

5.6.11. 2-[4-(2,2-Dimethylpropyl)-5-(4-methoxyphenyl)-3-(3-trifluoromethylbenzoyl)-thiophen-2-yl]isoindoline-1,3-dione (8k)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 19:1 as eluent, affording the desired intermediate **8k** as a yellow solid. Yield: 54%, mp 156–158 °C. ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 9H), 2.95 (s, 2H), 3.87 (s, 3H), 7.00 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.71 (m, 5H), 7.88 (d, J = 7.6 Hz, 1H), 8.03 (s, 1H). MS (ESI): $[M+1]^+$ = 578.2.

5.6.12. 2-[4-(2,2-Dimethylpropyl)-5-(4-methoxyphenyl)-3-(4-methylbenzoyl)-thiophen-2-yl]isoindoline-1,3-dione (8l)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 19:1 as eluent, affording the desired intermediate **8l** as a yellow oil. Yield: 63%. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.12 (s, 3H), 2.86 (s, 2H), 3.86 (s, 3H), 6.75 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 6.2 Hz, 2H), 7.02 (d, J = 6.2 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.69 (m, 4H). MS (ESI): $[M+1]^+$ = 524.3.

5.6.13. 2-[3-(4-Chlorobenzoyl)-5-(3,5-dimethylisoxazol-4-yl)-4-(2,2-dimethylpropyl)-thiophen-2-yl]isoindoline-1,3-dione (8m)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride followed by 2% ethyl acetate in methylene chloride to afford the desired product **8m** as a pale tan solid. Yield: 65%, mp 212–214 °C. ^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 9H), 2.31 (s, 3H), 2.44 (s, 3H), 2.64 (s, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.79 (m, 2H). MS (ESI): $[M+1]^+$ = 533.0.

5.6.14. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(pyrazol-4-yl)thiophen-2-yl]isoindoline-1,3-dione (8n)

A solution of **7a** and 1-(*tert*-butyloxycarbonyl)pyrazole-4-boronic acid pinacol ester (1.7 mmol, 500 mg) in anhydrous dioxane (12 mL) was degassed under a stream of nitrogen for approximately 10 min. To the solution was added [1,1′bis(diphenylphosphino)ferrocene]-dichloropalladium (II) methylene chloride complex (0.10 mmol, 82 mg) and CsF (2.75 mmol, 420 mg). The mixture was heated under nitrogen to 40 °C for 1 h, then to 60 °C for three days. After cooling to room temperature, the reaction mixture was diluted with methylene chloride (35 mL), filtered through a pad of Celite, and the combined filtrates concentrated. The residue was dissolved in 1:1 v/v mixture of trifluoroacetic acid and toluene (20 mL), stirred at ambient temperature for one hour, concentrated, dissolved in 1:1 v/v mixture of acetic acid and toluene, heated to reflux for two hours, and cooled to room temperature. The mixture was diluted with 2-propanol (6 mL), washed with a saturated aqueous solution of sodium bicarbonate (10 mL), and the aqueous layer back-extracted with methylene chloride containing a small amount of 2-propanol (2 × 15 mL). The combined organic phases were dried (MgSO4), filtered, and concentrated, then purified by column chromatography on silica gel, eluting with a gradient of 2% → 20% ethyl acetate in methylene chloride to furnish the desired intermediate **8n** as a pale tan solid. Yield: 37%, mp 297–299 °C. ^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 9H), 2.89 (s, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.70 (m, 2H), 7.78 (m, 2H), 7.79 (s, 2H). MS (ESI): $[M+1]^+$ = 504.0.

5.6.15. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(thiophen-2-yl)thiophen-2-yl]isoindoline-1,3-dione (8o)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride to furnish the desired intermediate **8o** as a pale yellow solid. Yield: 77%, mp 160–163 °C. ^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 9H), 3.03 (s, 2H), 7.09 (dd, J = 5.0 and 3.5 Hz, 1H), 7.28 (m, 3H), 7.41 (dd, J = 5.0 and 1.0 Hz, 1H), 7.74 (m, 4H), 7.80 (m, 2H). MS (ESI): $[M+1]^+$ = 520.0.

5.6.16. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(thiophen-3-yl)thiophen-2-yl]isoindoline-1,3-dione (8p)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of ethyl acetate/heptane 3:7 to furnish the desired intermediate **8p** as a very pale yellow solid. Yield: 72%, mp 175–178 °C. ^{1}H NMR ($CDCl_3$) δ: 0.61 (s, 9H), 2.94 (s, 2H), 7.20 (d, J = 8.5 Hz, 2H), 7.28 (m, 1H), 7.43 (m, 2H), 7.73 (m, 4H), 7.79 (m, 2H). MS (ESI): $[M+1]^+$ = 520.0.

5.6.17. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(furan-2-yl)thiophen-2-yl]isoindoline-1,3-dione (8q)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of methylene chloride/heptane 2:1 to furnish the desired intermediate **8q** as a pale yellow solid. Yield: 77%, mp 184–186 °C. ^{1}H NMR ($CDCl_3$) δ: 0.71 (s, 9H), 3.01 (s, 2H), 6.49 (m, 1H), 6.60 (m, 1H), 7.22 (d, J = 8.5 Hz, 2H), 7.50 (m, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.80 (m, 2H). MS (ESI): $[M+23]^+$ = 526.0.

5.6.18. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(furan-3-yl)-thiophen-2-yl]isoindoline-1,3-dione (8r)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a gradient of 50–60% methylene chloride in heptane to furnish the desired intermediate **8r** as a pale yellow solid. Yield: 55%, mp 214-217 °C. ^{1}H NMR ($CDCl_3$) δ: 0.68 (s, 9H), 2.89

(s, 2H), 6.63 (m, 1H), 7.20 (d, J = 8.5 Hz, 2H), 7.52 (m, 1H), 7.64 (m, 1H), 7.68 (d, J = 8.5 Hz, 2H), 7.73 (m, 2H), 7.78 (m, 2H). MS (ESI): $[M+1]^+$ = 504.0.

5.6.19. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(pyridin-4-yl)thiophen-2-yl]isoindoline-1,3-dione (8s)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of ethyl acetate/heptane 1:3 to furnish the desired intermediate **8s** as a very pale tan solid. Yield: 54%, mp 227–230 °C. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.97 (s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.47 (dd, J = 4.5 and 1.5 Hz, 2H), 7.70 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.80 (m, 2H), 8.71 (dd, J = 4.5 and 1.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 515.0.

5.6.20. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(pyridin-3-yl)thiophen-2-yl]isoindoline-1,3-dione (8t)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of ethyl acetate/heptane 2:3 to furnish the desired intermediate **8t** as a very pale tan solid. Yield: 24%, mp 217–220 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.93 (s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.41 (m, 1H), 7.68 (m, 4H), 7.80 (m, 2H), 7.85 (m, 1H), 8.65 (m, 1H), 8.80 (m, 1H). MS (ESI): $[M+1]^+$ = 515.0.

5.6.21. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(pyridin-2-yl)thiophen-2-yl]isoindoline-1,3-dione (8u)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with 5% ethyl acetate in heptane to furnish the desired intermediate **8u** as a very pale tan solid. Yield: 24%, mp 187–190 °C. ^{1}H NMR ($CDCl_3$) δ: 0.60 (s, 9H), 3.16 (s, 2H), 7.23 (d, J = 8.5 Hz, 2H), 7.30 (m, 1H), 7.68 (m, 1H), 7.74 (m, 4H), 7.81 (m, 3H), 8.69 (m, 1H). MS (ESI): $[M+1]^+$ = 515.0.

5.6.22. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-fluorophenyl)-thiophen-2-yl]isoindoline-1,3-dione (8v)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of methylene chloride/heptane 2:1, to furnish the desired intermediate **8v** as a very pale yellow solid. Yield: 71%, mp 171–173 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.89 (s, 2H), 7.15 (m, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.50 (m, 2H), 7.71 (m, 4H), 7.78 (m, 2H). MS (ESI): $[M+1]^+$ = 532.0.

5.6.23. 2-[3-(4-Chlorobenzoyl)-5-(2,3-difluorophenyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (8w)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride to furnish the desired intermediate **8w** as a white solid. Yield: 80%, mp 162–164 °C. ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 9H), 2.77 (s, 2H), 7.15 (m,1H), 7.27 (m, 4H), 7.75 (m, 4H), 7.81 (m, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.72 (m, 2H), 7.78 (m, 2H). MS (ESI): $[M+1]^+$ = 550.0.

5.6.24. 2-[3-(4-Chlorobenzoyl)-5-(2,4-difluorophenyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (8x)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of methylene chloride/heptane 3:2, to furnish the desired intermediate **8x** as a pale yellow solid. Yield: 68%, mp 150–152 °C. ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 9H), 2.74 (s, 2H), 7.03 (m, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.50 (m, 1H), 7.68 (m, 4H), 7.81 (m, 2H). MS (ESI): $[M+1]^+$ = 550.0.

5.6.25. 2-[3-(4-Chlorobenzoyl)-5-(2,5-difluorophenyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (8y)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of methylene chloride/heptane 3:2, to furnish the desired intermediate **8y** as a pale yellow solid. Yield: 95%, mp 165–167 °C. ^{1}H NMR ($CDCl_3$) δ: 0.60 (s, 9H), 2.78 (s, 2H), 7.07 (m, 3H), 7.23 (d, J = 8.5 Hz, 2H), 7.68 (m, 4H), 7.75 (m, 2H). MS (ESI): $[M+1]^+$ = 550.0.

5.6.26. 2-[3-(4-Chlorobenzoyl)-5-(2,6-difluorophenyl)-4-(2,2-dimethylpropyl)-thiophen-2-yl]isoindoline-1,3-dione (8z)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of methylene chloride/heptane 2:1, to afford the desired product **8z** as a pale beige solid. Yield: 48%, mp 114–116 °C. ^{1}H NMR ($CDCl_3$) δ: 0.61 (s, 9H), 2.66 (s, 2H), 7.03 (t, J = 8.0 Hz, 2H), 7.24 (d, J = 8.5 Hz, 2H), 7.37 (m, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.75 (m, 2H), 7.81 (m, 2H). MS (ESI): $[M+1]^+$ = 550.0.

5.6.27. 2-[3-(4-Chlorobenzoyl)-5-(4-chlorophenyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (8aa)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of heptane/ethyl acetate 4:1, to furnish the desired intermediate **8aa** as a pale yellow solid. Yield: 97%, mp 110–112 °C. ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 9H), 2.90 (s, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.37 (dd, J = 2.0 and 8.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.72 (m, 2H), 7.78 (m, 2H). MS (ESI): $[M+1]^+$ = 548.5.

5.6.28. (*E*)-2-[3-(4-Chlorobenzoyl)-5-(2-(4-chlorophenyl)ethen-1-yl)-4-(2,2-dimethyl-propyl)thiophen-2-yl]isoindoline-1,3-dione (8ab)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride/heptane 3:1, to afford the desired product **8ab** as a pale yellow foam. Yield: 29%. ^{1}H NMR ($CDCl_3$) δ: 0.84 (s, 9H), 2.87 (s, 2H), 6.90 (d, J = 16.0 Hz, 1H), 7.20 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 16.0 Hz, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.80 (m, 2H). MS (ESI): $[M+1]^+$ = 574.1.

5.6.29. 2-[3-(4-Chlorobenzoyl)-5-(3,4-dichlorophenyl)-4-(2,2-dimethylpropyl)thiophen-2-yl]isoindoline-1,3-dione (8ac)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of heptane/ethyl acetate 4:1, to furnish the desired intermediate **8ac** as a pale tan solid. Yield: 95%. mp 210–213 °C. ^{1}H NMR ($CDCl_3$) δ: 0.59 (s, 9H), 2.90 (s, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.37 (dd, J = 2.0 and 8.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.72 (m, 2H), 7.78 (m, 2H). MS (ESI): $[M+23]^+$ = 604.0.

5.6.30. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(3-methoxyphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8ad)

Following general procedure E, after workup as described previously, the crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether 3:17, to furnish the desired intermediate **8ad** as a yellow solid. Yield: 55%, mp 153–156 °C. ^{1}H NMR ($CDCl_3$) δ: 0.62 (s, 9H), 2.94 (s, 2H), 3.86 (s, 3H), 6.96 (m, 1H), 7.03 (m, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.38 (t, J = 7.6 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.75 (m, 4H). MS (ESI): $[M+1]^+$ = 544.0.

5.6.31. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(2-methoxyphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8ae)

Following general procedure E, after workup as described previously, the crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether 3:17, to furnish the desired intermediate **8ae** as a yellow solid. Yield: 55%, mp 115–117 °C. ^{1}H NMR ($CDCl_3$) δ: 0.56 (s, 9H), 2.73 (s, 2H), 3.93 (s, 3H), 6.94 (d, J = 8.8 Hz, 2H), 7.04 (m, 2H), 7.230 (d, J = 8.8 Hz, 2H), 7.41 (m, 2H), 7.75 (m, 4H). MS (ESI): $[M+1]^+$ = 544.1.

5.6.32. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-(2-(methoxy)ethoxy)phenyl)-thiophen-2-yl]isoindoline-1,3-dione (8af)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of ethyl acetate/heptane 1:3, to furnish the desired intermediate **8af** as a very pale tan solid. Yield: 54%, mp 227–230 °C. ^{1}H NMR ($CDCl_3$) δ: 0.58 (s, 9H), 2.97 (s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.47 (dd, J = 4.5 and 1.5 Hz, 2H), 7.70 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.80 (m, 2H), 8.71 (dd, J = 4.5 and 1.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 588.2.

5.6.33. 2-[5-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-chlorobenzoyl)-4-(2,2-dimethylpropyl)thio-phen-2-yl]isoindoline-1,3-dione (8ag)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride to furnish the desired intermediate **8ag** as a pale yellow solid. Yield: 95%, mp 213–215 °C. ^{1}H NMR ($CDCl_3$) δ: 0.61 (s, 9H), 2.89 (s, 2H), 6.03 (s, 2H), 6.88 (d, J = 8.5 Hz, 1H), 6.98 (m, 2H), 7.20 (d, J = 8.5 Hz, 2H), 7.68 (m, 4H), 7.75 (m, 2H). MS (ESI): $[M+1]^+$ = 558.0.

5.6.34. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-(trifluoromethoxy)phenyl)-thiophen-2-yl]isoindoline-1,3-dione (8ah)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with methylene chloride/heptane 2:1, to afford the desired product **8ah** as a white solid. Yield: 84%, mp 99–102 °C. ^{1}H NMR ($CDCl_3$): δ 0.57 (s, 9H), 2.91 (s, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.70 (d, J = 8.5 Hz, 2H), 7.74 (m, 2H), 7.79 (m, 2H). MS (ESI): $[M+1]^+$ = 598.2.

5.6.35. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(4-methylphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8ai)

Following general procedure E, after workup as described previously, the crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether 2:8, to furnish the desired intermediate **8ai** as a yellow solid. Yield: 63%, mp 162–165 °C. ^{1}H NMR ($CDCl_3$) δ: 0.84 (s, 9H), 1.57 (s, 3H), 2.79 (s, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.74 (m, 8H). MS (ESI): $[M+1]^+$ = 528.2.

5.6.36. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(3-methylphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8aj)

Following general procedure E, after workup as described previously, the crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether1:4, to furnish the desired intermediate **8aj** as a yellow solid. Yield: 58%. mp 149–151 °C. ^{1}H NMR ($CDCl_3$) δ: 0.85 (s, 9H), 1.56 (s, 3H), 2.79 (s, 2H), 7.18 (d, J = 8.6 Hz, 2H), 7.24 (s, 1H), 7.34 (d, J = 4.8 Hz, 1H), 7.63 (d, J = 8.6 Hz, 2H), 7.74 (m, 6H). MS (ESI): $[M+1]^+$ = 528.2.

5.6.37. 2-[3-(4-Chlorobenzoyl)-4-(2,2-dimethylpropyl)-5-(2-methylphenyl)-thiophen-2-yl]isoindoline-1,3-dione (8ak)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a gradient of methylene chloride/heptane (from 2:1 to 4:1), to furnish the desired intermediate **8ak** as a yellow solid. Yield: 72%, mp 154–156 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.41 (s, 3H), 2.92 (s, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.71 (m, 4H), 7.77 (m, 2H). MS (ESI): m/z 528.0 (M+H). MS (ESI): $[M+1]^+$ = 528.1.

5.6.38. 2-[3-(4-Chlorobenzoyl)-5-(4-(1-(methyl)ethyl)phenyl)-4-(2,2-dimethylpropyl)-thiophen-2-yl]isoindoline-1,3-dione (8al)

Following general procedure E, after workup as described previously, the residue was purified by column chromatography on silica gel, eluting with a mixture of ethyl acetate/heptane 3:7, to furnish the desired intermediate **8al** as a very pale yellow solid. Yield: 74%, mp 120–122 °C. ^{1}H NMR ($CDCl_3$) δ: 0.56 (s, 9H), 1.30 (d, J = 7.0 Hz, 6H), 2.93 (s, 2H), 2.94 (m, 1H), 7.20 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.73 (m, 4H), 7.79 (m, 2H). MS (ESI): $[M+1]^+$ = 556.2.

5.7. General procedure (F) for the synthesis of compounds 3e-au[14]

A stirred suspension of thiophene derivative **7a**–**g** or **8a**–**al** (0.5 mmol) and hydrazine monohydrate (29 μL, 0.6 mmol, 1.2 equiv) in absolute EtOH (10 mL) was refluxed for 1 h. The solvent was evaporated, and the residue partitioned between CH_2Cl_2 (10 mL) and water (5 mL). The organic phase was washed with brine (2 mL), dried (Na_2SO_4) and concentrated in vacuo to obtain a residue that was purified by column chromatography on silica gel.

5.7.1. (2-Amino-4-(2,2-dimethylpropyl)-5-bromothiophen-3-yl)(4-chlorophenyl)-methanone (3e)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to afford the desired product **3e** as a yellow oil. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 9H), 2.25 (s, 2H), 5.88 (br s, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.46 (4C), 33.44, 93.45, 114.19, 128.51 (2C), 130.82 (2C), 135.24, 136.45, 138.42, 161.88, 189.76. MS (ESI): $[M]^+$ = 386.0, $[M+2]^+$ = 388.2. Anal. ($C_{16}H_{17}ClBrClNOS$) C, H, N.

5.7.2. (2-Amino-4-(2,2-dimethylpropyl)-5-bromothiophen-3-yl)(3-chlorophenyl)methanone (3f)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to afford the desired product **3f** as a yellow oil. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.70 (s, 9H), 2.24 (s, 2H), 5.93 (br s, 2H), 7.42 (d, J = 8.2 Hz, 1H), 7.49 (m, 2H), 7.57 (s, 1H). MS (ESI): $[M]^+$ = 386.0, $[M+2]^+$ = 388.1. Anal. ($C_{16}H_{17}ClBrClNOS$) C, H, N.

5.7.3. (2-Amino-4-(2,2-dimethylpropyl)-5-bromothiophen-3-yl)(3,4-dichloro-phenyl)methanone (3g)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to afford the desired product **3g** as a yellow solid. Yield: 78%, mp 134–136 °C. ^{1}H NMR ($CDCl_3$) δ: 0.71 (s, 9H), 2.26 (s, 2H), 5.92 (br s, 2H), 7.42 (d, J = 7.6 Hz, 1H), 7.49 (s, 1H), 7.72 (d. J = 7.8 Hz, 1H). MS (ESI): $[M]^+$ = 419.1, $[M+2]^+$ = 421.1. Anal. ($C_{16}H_{16}ClBrCl_2NOS$) C, H, N.

5.7.4. (2-Amino-4-(2,2-dimethylpropyl)-5-bromothiophen-3-yl) (3-trifluoromethylphenyl)methanone (3h)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to afford the desired product **3h** as a yellow oil. Yield 93%. ^{1}H NMR ($CDCl_3$) δ: 0.68 (s, 9H), 2.18 (s, 2H), 6.03 (br s, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.73 (m, 2H), 7.85 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.43 (4C), 33.43, 93.64, 113.88, 122.21, 125.15, 128.04, 129.42, 129.69, 133.01, 135.04, 140.52, 162.76, 189.19. MS (ESI): $[M]^+$ = 419.8, $[M+2]^+$ = 421.9. Anal. ($C_{17}H_{17}BrF_3NOS$) C, H, N.

5.7.5. (2-Amino-4-(2,2-dimethylpropyl)-5-bromothiophen-3-yl) (4-methylphenyl)methanone (3i)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (9:1) to afford the desired product **3i** as a brown solid. Yield 75%, mp 123–124 °C. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 9H), 2.24 (s, 2H), 2.43 (s, 3H), 5.88 (br s, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.03, 29.44 (4C), 33.36, 93.03, 114.79, 128.89 (2C), 129.00 (2C), 135.56, 137.10, 141.88, 160.96, 191.07. MS (ESI): $[M+1]^+$ = 365.7, $[M+3]^+$ = 367.9. Anal. ($C_{17}H_{20}BrNOS$) C, H, N.

5.7.6. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (4-chlorophenyl)-methanone (3j)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 3:17, to afford the desired product **3j** as a yellow solid. Yield: 89%, mp 153–155 °C. ^{1}H NMR ($CDCl_3$) δ: 0.44 (s, 9H), 2.35 (s, 2H), 6.33 (br s, 2H), 7.37 (m, 5H), 7.46 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.34 (4C), 33.42, 116.29, 121.49, 126.94, 128.42 (2C), 128.57 (2C), 129.93 (2C), 130.82 (2C), 131.13, 134.82, 136.07, 139.16, 161.81, 190.34. MS (ESI): $[M+1]^+$ = 384.1. Anal. ($C_{22}H_{22}ClNOS$) C, H, N.

5.7.7. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (3-chlorophenyl)-methanone (3k)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with petroleum ether/ethyl acetate 9:1, to give the desired product **3k** as a yellow solid. Yield: 95%, mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.34 (s, 2H), 6.02 (br s, 2H), 7.39 (m, 5H), 7.48 (d, J = 7.2 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.68 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.35 (4C), 33.43, 116.07, 121.54, 126.99, 127.64, 128.26, 128.60 (2C), 129.93, 130.29 (2C), 130.99, 131.07, 133.06, 134.77, 142.46, 162.39, 189.81. MS (ESI): $[M+1]^+$ = 384.0. Anal. ($C_{22}H_{22}ClNOS$) C, H, N.

5.7.8. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (2-chlorophenyl)-methanone (3l)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 1:4, to give the desired product **3l** as a yellow oil. Yield: 90%. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 9H), 2.25 (s, 2H), 5.88 (br s, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.68 (m, 5H). MS (ESI): $[M+1]^+$ = 384.2. Anal. ($C_{22}H_{22}ClNOS$) C, H, N.

5.7.9. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (3,4-dichlorophenyl)-methanone (3m)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to give the desired product **3m** as a yellow solid. Yield: 95%, mp 194–196 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.36 (s, 2H), 5.96 (br s, 2H), 7.37 (m, 2H), 7.54 (m, 5H), 7.79 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.37 (4C), 33.48, 115.82, 121.79, 127.01, 128.60 (2C), 129.23, 129.88 (2C), 130.50, 130.70, 130.99, 131.27, 134.03, 134.72, 140.54, 162.28, 188.62. MS (ESI): $[M+1]^+$ = 418.1. Anal. ($C_{22}H_{21}Cl_2NOS$) C, H, N.

5.7.10. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (2,4-dichlorophenyl)-methanone (3n)

Following the general procedure (F), the residue was crystallized from petroleum ether to give the desired product **3n** as a yellow solid. Yield: 81%, mp 166–167 °C. ^{1}H NMR ($CDCl_3$) δ: 0.54 (s, 9H), 2.28 (s, 2H), 6.68 (br s, 2H), 7.32 (d, J = 6.4 Hz, 1H), 7.38 (m, 6H), 7.46 (d, J = 6.4 Hz, 1H). MS (ESI): $[M+1]^+$ = 418.1. Anal. ($C_{22}H_{21}Cl_2NOS$) C, H, N.

5.7.11. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (3-trifluoromethylphenyl)-methanone (3o)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with petroleum ether/ethyl acetate 9:1, to give the desired product **3o** as a yellow solid. Yield: 83%, mp 116–118 °C. ^{1}H NMR ($CDCl_3$) δ: 0.42 (s, 9H), 2.26 (s, 2H), 6.12 (br s, 2H), 7.32 (m, 5H), 7.59 (t, J = 7.6 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.95 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.28 (4C), 33.41, 115.94, 121.75, 125.18, 122.12, 125.18, 127.04, 127.70, 128.62 (2C), 129.64 (2C), 129.90, 130.86, 132.95, 134.71, 141.25, 162.68, 189.73. MS (ESI): $[M+1]^+$ = 418.2. Anal. ($C_{23}H_{22}F_3NOS$) C, H, N.

5.7.12. (2-Amino-4-(2,2-dimethylpropyl)-5-phenylthiophen-3-yl) (4-methylphenyl)-methanone (3p)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with petroleum ether/ethyl acetate 17:3, to give the desired product **3p** as a yellow solid. Yield: 81%, mp 78–80 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.42 (s, 3H), 2.78 (s, 2H), 5.77 (br s, 2H), 7.23 (m, 5H), 7.36 (d, J = 7.2 Hz, 2H), 7.64 (d, J = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.00, 29.33 (4C), 33.35, 126.00, 128.35, 128.54 (2C), 128.82 (2C), 129.00, 129.18 (2C), 129.94 (2C), 131.53, 134.95, 137.79, 144.78, 160.85, 191.67. MS (ESI): $[M+1]^+$ = 364.2. Anal. ($C_{23}H_{25}NOS$) C, H, N.

5.7.13. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3q)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 17:3, to afford the desired product **3q** as a yellow solid. Yield: 90%, mp 150–152 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.35 (s, 2H), 3.83 (s, 3H), 5.91 (br s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.36 (4C), 33.32, 54.99, 113.97 (2C), 116.16, 121.42, 126.87, 128.38 (2C), 130.39, 130.80 (2C), 131.21 (2C), 135.99, 139.25, 158.20, 161.51, 190.25. MS (ESI): $[M+1]^+$ = 414.2. Anal. ($C_{23}H_{24}ClNO_2S$) C, H, N.

5.7.14. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl) thiophen-3-yl)(3-chloro-phenyl)methanone (3r)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 17:3, to afford the desired product **3r** as a yellow solid. Yield: 88%, mp 74–76 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.30 (s, 2H), 3.83 (s, 3H), 6.02 (br s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 8.8 Hz, 2H), 7.33 (m, 1H), 7.42 (d, J = 7.2 Hz, 1H), 7.52 (d, J = 7.4 Hz, 1H), 7.67 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.36 (4C), 33.32, 54.98, 113.99 (2C), 115.94, 121.48, 126.80, 127.62, 128.26, 129.32, 130.26, 130.99, 131.21 (2C), 133.03, 142.55, 158.21, 162.12, 189.71. MS (ESI): $[M+1]^+$ = 414.1. Anal. ($C_{23}H_{24}ClNO_2S$) C, H, N.

5.7.15. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl) thiophen-3-yl)(3,4-dichloro-phenyl)methanone (3s)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether 3:17, to furnish the desired product **3s** as a yellow solid. Yield 95%, mp 182–184 °C, ^{1}H NMR ($CDCl_3$) δ: 0.46 (s, 9H), 2.31 (s, 2H), 3.84 (s, 3H), 6.00 (br s, 2H), 6.89 (d, J = 6.6 Hz, 1H), 7.29 (d, J = 6.6 Hz, 1H), 7.33 (d, J = 7.4 Hz, 2H), 7.54 (d, J = 7.4 Hz, 2H), 7.79 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.40 (4C), 33.38, 54.99, 114.00 (2C), 115.70, 121.73, 126.76, 129.22, 130.23, 130.49, 130.55, 130.67, 131.17 (2C), 133.96, 140.64, 158.24, 162.04, 188.51. MS (ESI): $[M+1]^+$ = 448.2. Anal. ($C_{23}H_{23}Cl_2NO_2S$) C, H, N.

5.7.16. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl) thiophen-3-yl)(3-trifluoro-methylphenyl)methanone (3t)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 9:1, to give the desired product **3t** as a yellow solid. Yield: 75%, mp 154–156 °C. ^{1}H NMR ($CDCl_3$) δ: 0.43 (s, 9H), 2.22 (s, 2H), 3.83 (s, 3H), 6.11 (br s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H), 7.61 (t, J = 7.6 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.30 (4C), 33.31, 54.98, 114.03 (2C), 115.81, 121.69, 125.18, 126.74, 127.66, 128.46, 128.88, 129.61, 130.13, 131.18 (2C), 132.93, 141.34, 158.26, 162.42, 189.63. MS (ESI): $[M+1]^+$ = 448.0. Anal. ($C_{24}H_{24}F_3NO_2S$) C, H, N.

5.7.17. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methoxyphenyl) thiophen-3-yl)(4-methyl-phenyl)methanone (3u)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 17:3, to give the desired product **3u** as a yellow solid. Yield: 81%, mp 78–80 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.42 (s, 3H), 2.78 (s, 2H), 5.60 (s, 1H), 5.77 (br s, 2H), 7.23 (m, 6H), 7.36 (d, J = 7.2 Hz, 2H), 7.64 (d, J = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.00, 29.36, 33.26 (3C), 33.32, 54.98, 113.94 (2C), 121.15, 128.54 (2C), 129.12, 129.56 (2C), 130.81, 131.21 (2C), 135.47, 137.87, 141.36, 158.13, 160.52, 191.63. MS (ESI): $[M+1]^+$ = 393.8. Anal. ($C_{24}H_{27}NO_2S$) C, H, N.

5.7.18. (2-Amino-5-(3,5-dimethylisoxazol-4-yl)-4-(2,2-dimethylpropyl)thiophen-3-yl)(4-chlorophenyl)methanone (3v)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 99:1, to provide the desired product **3v** as a yellow solid. Yield: 83%, mp 247–250 °C. ^{1}H NMR ($CDCl_3$) δ: 0.53 (s, 9H), 2.03 (br s, 2H), 2.23 (s, 3H), 2.36 (s, 3H), 5.90 (br s, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 403.0. Anal. ($C_{21}H_{23}ClN_2O_2S$) C, H, N.

5.7.19. (2-Amino-4-(2,2-dimethylpropyl)-5-(pyrazol-4-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3w)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with methylene chloride/ethyl acetate 3:1, to provide the desired product **3w** as a yellow solid. Yield: 90%, mp 80–82 °C. ^{1}H NMR ($CDCl_3$) δ: 0.54 (s, 9H), 2.33 (s, 2H), 5.89 (br s, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.65 (m, 3H). MS (ESI): $[M+1]^+$ = 374.0. Anal. ($C_{19}H_{20}ClN_3OS$) C, H, N.

5.7.20. (2-Amino-4-(2,2-dimethylpropyl)-5-(thiophen-2-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3x)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with heptane/ethyl acetate 17:3, to provide the desired product **3x** as a yellow solid. Yield: 94%, mp 164–166 °C. ^{1}H NMR ($CDCl_3$) δ: 0.55 (s, 9H), 2.48 (s, 2H), 5.94 (br s, 2H), 7.01 (dd, J = 5.0 and 3.5 Hz, 1H), 7.04 (dd, J = 3.5 and 1.0 Hz, 1H), 7.27 (m, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 390.0. Anal. ($C_{20}H_{20}ClNOS_2$) C, H, N.

5.7.21. (2-Amino-4-(2,2-dimethylpropyl)-5-(thiophen-3-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3y)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with heptane/ethyl acetate 22:3, to provide the desired product **3y** as a yellow solid. Yield: 93%, mp 171–173 °C. ^{1}H NMR ($CDCl_3$) δ: 0.49 (s, 9H), 2.36 (s, 2H), 5.91 (br s, 2H), 7.14 (dd, J = 5.0 and 1.5 Hz, 1H), 7.23 (dd, J = 3.0 and 1.5 Hz, 1H), 7.34 (dd, J = 5.0 and 3.0 Hz, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 390.0. Anal. ($C_{20}H_{20}ClNOS_2$) C, H, N.

5.7.22. (2-Amino-4-(2,2-dimethylpropyl)-5-(furan-2-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3z)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with heptane/ethyl acetate 22:3, to provide the desired product **3z** as a yellow-orange solid. Yield: 93%, mp 119–121 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (s, 9H), 2.49 (s, 2H), 5.99 (br s, 2H), 6.34 (m, 1H), 6.42 (m, 1H), 7.39 (m, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 374.0. Anal. ($C_{20}H_{20}ClNO_2S$) C, H, N.

5.7.23. (2-Amino-4-(2,2-dimethylpropyl)-5-(furan-3-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3aa)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, the residue was purified by column chromatography on silica, eluting with methylene chloride, providing the desired product **3aa** as a yellow solid. Yield: 85%, mp 98–100 °C. ^{1}H NMR ($CDCl_3$) δ: 0.55 (s, 9H), 2.33 (s, 2H), 6.50 (br s, 2H), 7.42 (d, J = 8.5 Hz, 1H), 7.44 (m, 1H), 7.50 (m, 1H), 7.30 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 374.0. Anal. ($C_{20}H_{20}ClNO_2S$) C, H, N.

5.7.24. (2-Amino-4-(2,2-dimethylpropyl)-5-(pyridine-4-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3ab)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 22:3, to provide the desired product **3ab** as a yellow solid. Yield: 87%, mp 180–182 °C. ^{1}H NMR ($CDCl_3$) δ: 0.47 (s, 9H), 2.44 (s, 2H), 6.04 (br s, 2H), 7.32 (dd, J = 4.5 and 1.5 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 8.59 (dd, J = 4.5 and 1.5 H, 2H). MS (ESI): $[M+1]^+$ = 385.0. Anal. ($C_{21}H_{21}ClNO_2S$) C, H, N.

5.7.25. (2-Amino-4-(2,2-dimethylpropyl)-5-(pyridin-3-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3ac)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 2:1, to provide the desired product **3ac** as a very pale tan solid. Yield: 85%, mp 212–214 °C. ^{1}H NMR ($CDCl_3$) δ: 0.46 (s, 9H), 2.35 (s, 2H), 5.97 (br s, 2H), 7.32 (m, 1H), 7.45 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.70 (m, 1H), 8.54 (m, 1H), 8.66 (m, 1H). MS (ESI): $[M+1]^+$ = 385.0. Anal. ($C_{21}H_{21}ClNO_2S$) C, H, N.

5.7.26. (2-Amino-4-(2,2-dimethylpropyl)-5-(pyridin-2-yl) thiophen-3-yl)(4-chlorophenyl)-methanone (3ad)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl

acetate 3:1, to provide the desired product **3ad** as a yellow solid. Yield: 87%, mp 170–172 °C. ^{1}H NMR ($CDCl_3$) δ: 0.51 (s, 9H), 2.75 (s, 2H), 6.09 (br s, 2H), 7.11 (m, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.51 (m, 1H), 7.62 (m, 3H), 8.58 (m, 1H). MS (ESI): $[M+1]^+$ = 385.0. Anal. ($C_{21}H_{21}ClNO_2S$) C, H, N.

5.7.27. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-fluorophenyl) thiophen-3-yl)(4-chlorophenyl)-methanone (3ae)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 4:1, providing the desired product **3ae** as a yellow solid. Yield: 95%, mp 169–171 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.31 (s, 2H), 5.92 (br s, 2H), 7.07 (m, 2H), 7.35 (m, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 402.0. Anal. ($C_{22}H_{21}ClFNOS$) C, H, N.

5.7.28. (2-Amino-5-(2,3-difluorophenyl)-4-(2,2-dimethylpropyl) thiophen-3-yl)(4-chlorophenyl)methanone (3af)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 17:3, providing the desired product **3af** as a yellow-orange solid. Yield: 96%, mp 173–175 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.21 (s, 2H), 5.95 (br s, 2H), 7.08 (m, 3H), 7.44 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 420.0. Anal. ($C_{22}H_{20}ClF_2NOS$) C, H, N.

5.7.29. (2-Amino-5-(2,4-difluorophenyl)-4-(2,2-dimethylpropyl) thiophen-3-yl)(4-chlorophenyl)methanone (3ag)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 22:3, providing the desired product **3ag** as a yellow solid. Yield: 94%, mp 164–166 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.17 (s, 2H), 5.91 (br s, 2H), 6.85 (m, 2H), 7.29 (m, 1H), 7.44 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 420.0. Anal. ($C_{22}H_{20}ClF_2NOS$) C, H, N.

5.7.30. (2-Amino-5-(2,5-difluorophenyl)-4-(2,2-dimethylpropyl) thiophen-3-yl)(4-chlorophenyl)methanone (3ah)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 22:3, providing the desired product **3ah** as a yellow solid. Yield: 95%, mp 165–167 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.21 (s, 2H), 5.95 (s, 2H), 6.97 (m, 3H), 7.44 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 420.0. Anal. ($C_{22}H_{20}ClF_2NOS$) C, H, N.

5.7.31. (2-Amino-4-(2,2-dimethylpropyl)-5-(2,6-difluorophenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3ai)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 22:3, to provide the desired product **3ai** as a yellow solid. Yield: 87%, mp 175–177 °C. ^{1}H NMR ($CDCl_3$) δ: 0.49 (s, 9H), 2.12 (s, 2H), 5.93 (br s, 2H), 6.96 (t, J = 8.0 Hz, 2H), 7.28 (m, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 420.0. Anal. ($C_{22}H_{20}ClF_2NOS$) C, H, N.

5.7.32. (2-Amino-5-(4-chlorophenyl)-4-(2,2-dimethylpropyl) thiophen-3-yl)(4-chlorophenyl)-methanone (3aj)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/methylene chloride 1:3, providing the desired product **3aj** as a yellow solid. Yield: 87%, mp 162–164 °C.^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.33 (s, 2H), 5.93 (br s, 2H), 7.37 (m, 4H), 7.44 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 418.0. Anal. ($C_{22}H_{21}Cl_2NOS$) C, H, N.

5.7.33. (*E*)-(2-Amino-5-(2-(4-chlorophenyl)ethen-1-yl)-4-(2,2-dimethylpropyl)thiophen-3-yl)(4-chlorophenyl)methanone (3ak)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with methylene chloride/heptane 3:1, affording the desired product **3ak** as a yellow-orange solid. Yield: 80%, mp 171–174 °C. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 9H), 2.32 (s, 2H), 6.11 (br s, 2H), 6.49 (d, J = 16 Hz, 1H), 7.12 (d, J = 16 Hz, 1H),7.28 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 444.2. Anal. ($C_{24}H_{23}Cl_2NOS$) C, H, N.

5.7.34. (2-Amino-5-(3,4-dichlorophenyl)-4-(2,2-dimethylpropyl)thiophen-3-yl)(4-chlorophenyl)-methanone (3al)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with a gradient of heptane/methylene chloride (from 1:2 to 1:3), providing the desired product **3al** as a yellow solid. Yield: 87%, mp 189–191 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.33 (s, 2H), 5.95 (br s, 2H), 7.23 (dd, J = 8.5 Hz, 2H), 7.43 (m, 2H), 7.48 (d, J = 2.0 Hz, 1H), 7.63 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 452.0. Anal. ($C_{22}H_{20}Cl_3NOS$) C, H, N.

5.7.35. (2-Amino-4-(2,2-dimethylpropyl)-5-(3-methoxyphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3am)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 4:1, to afford the desired product **3am** as a yellow solid. Yield: 64%, mp 168–170 °C. ^{1}H NMR ($CDCl_3$) δ: 0.46 (s, 9H), 2.37 (s, 2H), 3.83 (s, 3H), 5.93 (br s, 2H), 6.86 (d, J = 8.2 Hz, 1H), 6.95 (s, 1H), 7.00 (m, 1H), 7.23 (m, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H). MS (ESI): $[M+1]^+$ = 414.2. Anal. ($C_{23}H_{24}ClNO_2S$) C, H, N.

5.7.36. (2-Amino-4-(2,2-dimethylpropyl)-5-(2-methoxyphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3an)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 17:3, to afford the desired product **3an** as a yellow solid. Yield: 60%, mp 180–182 °C. ^{1}H NMR ($CDCl_3$) δ: 0.54 (s, 9H), 2.16 (s, 2H), 3.83 (s, 3H), 5.85 (br s, 2H), 6.96 (m, 2H), 7.27 (m, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.21 (4C), 32.87, 55.42, 111.61, 115.73, 117.21, 120.26, 122.92, 128.35 (2C), 129.18, 130.78 (2C), 132.58, 132.62, 136.01, 139.22, 156.97, 162.01, 190.30. MS (ESI): $[M+1]^+$ = 414.1. Anal. ($C_{23}H_{24}ClNO_2S$) C, H, N.

5.7.37. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-(2-(methoxy) ethoxy)phenyl)thiophen-3-yl)(4-chlorophenyl)-methanone (3ao)

Following the general procedure (F), the residue was purified by column chromatography on silica gel eluting with methylene chloride/ethyl acetate 49:1, affording the desired product **3ao** as a yellow-orange solid. Yield: 85%, mp 168–170 °C. ^{1}H NMR ($CDCl_3$) δ: 0.44 (s, 9H), 2.31 (br s, 2H), 3.47 (s, 3H), 3.77 (t, J = 5.0 Hz, 2H), 4.14 (t, J = 5.0 Hz, 2H), 5.91 (br s, 2H), 6.93 (d, J = 8.5 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 458.0. Anal. ($C_{25}H_{28}ClNO_3S$) C, H, N.

5.7.38. (2-Amino-4-(2,2-dimethylpropyl)-5-(3,4-methylenedioxyphenyl)thiophen-3-yl)(4-chlorophenyl) methanone (3ap)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 17:3, providing the desired product **3ap** as a yellow-orange solid. Yield: 91%, mp 190–192 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.32 (s, 2H), 5.90 (br s, 2H), 6.00 (s, 2H), 6.81 (d, J = 8.5 Hz, 1H), 6.88 (m, 3H), 7.43 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 428.0. Anal. ($C_{23}H_{23}ClNO_3S$) C, H, N.

5.7.39. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-(trifluoromethoxy)phenyl)thiophen-3-yl)(4-chlorophenyl) methanone (3aq)

Following the general procedure (F), the residue was purified by column chromatography on silica, eluting with methylene chloride/ethyl acetate 49:1, affording the desired product **3aq** as a yellow solid. Yield: 83%, mp 137–139 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.34 (s, 2H), 5.92 (br s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 468.0. Anal. ($C_{23}H_{21}ClF_3NO_2S$) C, H, N.

5.7.40. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-methylphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3ar)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 4:1 to afford the desired product **3ar** as a yellow solid. Yield 78%, mp 134–136 °C. ^{1}H NMR ($CDCl_3$) δ: 0.44 (s, 9H), 2.22 (s, 3H), 2.38 (s, 2H), 5.93 (br s, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H). MS (ESI): $[M+1]^+$ = 398.0. Anal. ($C_{23}H_{24}ClNOS$) C, H, N.

5.7.41. (2-Amino-4-(2,2-dimethylpropyl)-5-(3-methylphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3as)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate 19:1, to afford the desired product **3as** as a yellow solid. Yield: 67%, mp 180–182 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 2.24 (s, 3H), 2.37 (s, 2H), 5.83 (br s, 2H), 7.19 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.42 (s, 1H); 7.56 (d, J = 8.2 Hz, 2H), 7.63 (m, 2H). MS (ESI): $[M+1]^+$ = 398.1. Anal. ($C_{23}H_{24}ClNOS$) C, H, N.

5.7.42. (2-Amino-4-(2,2-dimethylpropyl)-5-(2-methylphenyl) thiophen-3-yl)(4-chloro-phenyl)methanone (3at)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with 2% ethyl acetate in methylene chloride to afford the desired product **3at** as a yellow solid. Yield 94%, mp 160–162 °C. ^{1}H NMR ($CDCl_3$) δ: 0.45 (s, 9H), 2.34 (s, 2H), 2.37 (s, 3H), 5.91 (br s, 2H), 7.17 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 398.0. Anal. ($C_{23}H_{24}ClNOS$) C, H, N.

5.7.43. (2-Amino-4-(2,2-dimethylpropyl)-5-(4-(1-(methyl)ethyl) phenyl)thiophen-3-yl)(4-chlorophenyl)methanone (3au)

Following the general procedure (F), the residue was purified by column chromatography on silica gel, eluting with heptane/ethyl acetate 22:3, providing the desired product as a yellow solid. Yield: 87%, mp 155–157 °C. ^{1}H NMR ($CDCl_3$) δ: 0.48 (s, 9H), 1.27 (d, J = 7.0 Hz, 6H), 2.35 (s, 2H), 2.96 (m, 1H), 5.90 (br s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H). MS (ESI): $[M+1]^+$ = 426.0. Anal. ($C_{25}H_{28}ClNOS$) C, H, N.

5.8. Biology experiments

5.8.1. Materials

[^{3}H]DPCPX ([^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine; specific activity, 120 Ci/mmol) and [^{3}H]CCPA ([^{3}H]2-chloro-N^6-cyclopentyl-adenosine; specific activity, 55 Ci/mmol) were obtained from Perkin Elmer Research Products (Boston, MA); [^{3}H]ZM 241385 ([^{3}H](4-(2-[7-amino-2-(2-furil)[1,2.4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol); specific activity, 17 Ci/mmol) was obtained from Biotrend (Cologne, Germany); [^{3}H]MRE-3008-F20 ([^{3}H]5-*N*-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; specific activity, 67 Ci/mmol) was obtained from Amersham International (Buckinghamshire, UK). DPCPX (1,3-dipropyl-8-cyclopentyl-xanthine), R-PIA ((*R*)-N^6-(L-2-Phenylisopropyl)adenosine) and CCPA (2-chloro-N^6-cyclopentyladenosine) were obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and obtained from commercial sources.

5.8.2. Cell membrane preparation

The hA_1CHO, hA_{2A}CHO and hA_3CHO cells were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12, containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), geneticine (G418) 0.2 mg/mL at 37 °C in 5% CO_2/95% air. For membrane preparation the culture medium was removed and the cells were washed with phosphate-buffered saline and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris–HCl, 1 mM EDTA, pH 7.4). The cell suspension was homogenized with a Polytron, the homogenate was spun for 10 min at 1000*g* and the supernatant was then centrifuged for 30 min at 100,000*g*. The membrane pellet was suspended in 50 mM Tris–HCl buffer (pH 7.4) for A_1ARs, in 50 mM Tris–HCl, 10 mM $MgCl_2$ (pH 7.4) for A_{2A}ARs, in 50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA (pH 7.4) for A_3ARs. The membranes were incubated with 2–3 IU/mL of adenosine deaminase to reduce the endogenous adenosine. The protein concentration was determined according to a Bio-Rad method with bovine albumin as a standard reference.[19]

5.8.3. Binding experiments in hA_1CHO membranes

5.8.3.1. [^{3}H]CCPA binding experiments. Saturation binding experiments of [^{3}H]CCPA (0.05–20 nM) to hA_1CHO membranes were performed in triplicate at 25 °C for 90 min in 50 mM Tris–HCl, pH 7.4, in the absence and presence of the tested compounds at the final concentration of 10 μM.[20] Non-specific binding was defined as binding in the presence of 1 μM R-PIA.

5.8.3.2. [^{3}H]DPCPX competition binding experiments. Competition binding experiments of 1 nM [^{3}H]DPCPX were performed in triplicate in 50 mM Tris–HCl, pH 7.4, for 90 min at 25 °C. The effect of the different tested compounds at a concentration of 10 μM on the CCPA curve (0.01 nM–1 μM) was investigated.[21] Non-specific binding was defined as binding in the presence of 1 μM DPCPX.

5.8.3.3. Assay of the antagonistaActivity versus A_1, A_{2A} and A_3 ARs. A_1, A_{2A} and A_3 AR competition binding experiments were performed using 1 nM [^{3}H]DPCPX, 1 nM [^{3}H]ZM 241385 and 2 nM [^{3}H]MRE-3008-F20 as radioligands, respectively.[21–23] Membrane suspensions were incubated in 50 mM Tris–HCl, pH 7.4, at 25 °C for 120 min, in 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.4, at 4 °C for 60 min, and in 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4 at 4 °C for 120 min to study A_1, A_{2A} and A_3 ARs, respectively. Non-specific binding was defined as the binding in the presence of 1 μM DPCPX or ZM 241385 or MRE-3008-F20 for A_1, A_{2A} and A_3ARs, respectively. Inhibition was expressed as percentage of control specific binding (100%). Test agents were dissolved in DMSO

and added to the assay from a 100-fold concentrated solution in DMSO. Control incubations also contained 1% DMSO.

Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass fiber filters using a Brandel cell harvester (Brandel Instruments, Unterföhring, Germany). The filter bound radioactivity was counted by Packard Tri Carb 2810 TR scintillation counter (Perkin Elmer).

5.8.4. Effect of the novel compounds in cyclic AMP assays

Human A_1 CHO cells (10^6 cells/mL) were prepared as described above and were suspended in 0.5 mL incubation mixture phosphate buffer, containing 1.0 IU adenosine deaminase/mL and 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as a phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. The effect of allosteric enhancers were studied at 10 μM concentration that was added to the mixture for a further 10 min. The effect of allosteric enhancers (100 nM) was also studied in the presence of a low concentration of CCPA (1 pM). Forskolin 1 μM was added for 5 min and was used to stimulate the activity of adenylate cyclase. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cAMP levels by a competition protein binding assay. Samples of cAMP standards (0–10 pmol) were added to each test tube containing trizma base 0.1 M, aminophylline 8.0 mM, mercaptoethanol 6.0 mM, pH 7.4 and [^{3}H]-cAMP (at the final concentration of 1 nM). The binding protein, previously prepared from beef adrenals, was added to the samples and incubated at 4 °C for 150 min. At the end of the incubation time and after the addition of charcoal, the samples were centrifuged at 2000*g* for 10 min. The clear supernatant was mixed with 4 mL of Ultima Gold (Perkin Elmer) and counted in a Packard Tri Carb 2810 TR scintillation counter (Perkin Elmer).

5.8.5. Data analysis

Saturation and competition binding experiments were analysed with the program LIGAND, which performed weighted, non-linear, least squares curve fitting program.[24] Inhibitory binding constants, K_i, were also calculated from the IC_{50} values according to the Cheng and Prusoff equation $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C∗] is the concentration of the radioligand and K_D* its dissociation constant.[25] All experimental data are expressed as mean ± standard error of the mean (S.E.M.) of three or four independent experiments performed in duplicate.

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Supplementary data

Supplementary data associated (Supplementary data (Table S1) on antagonist activity of compounds PD 81,723, **2a–i** and **3a–au**, synthetic procedure for the preparation of compounds **2b–c** and **2e–h**. Elemental analyses of compounds **3a–au**) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc. 2013.11.043.

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18. In our experiments, the reference compound PD 81,723 (at a concentration of 10 μM) did not inhibit [^{3}H]DPCPX binding to human A1 receptors transfected in CHO cells. For the same reference compound, Bruns (Ref. 11a) showed a *Ki* value of 11 μM obtained in competition binding experiments by using [^{3}H]DPCPX as radioligand on rat membranes. Furthermore, data performed on CHO-K1cells stably expressing the human A_1 receptors (Ref. 12c) reported an inhibition of [^{3}H]DPCPX binding to human A_1 receptors by PD 81,723 only of 42 ± 7%, when tested at 100 μM. We speculate that species differences in affinity binding of PD 81,723 may explain the discrepancy between the data.
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