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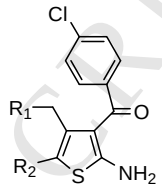

R_1=spiro-and fused-piperidines, anilines, acyclic alkyl amines, substituted phenol and thiophenol.
R_2=H, Br or C_6H_5

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Abstract: Allosteric enhancers for the A_1 adenosine receptor represent a novel and unique drug design strategy to augment the response to endogenous adenosine in a site- and event- specific manner. We have previously investigated a detailed structure-activity relationship study around a wide series of 2-amino-3-aroyl-4-[(4-arylpiperazin-1-yl)methyl]thiophene derivatives as potent allosteric enhancers of the A_1 adenosine receptor. In this manuscript we report our investigation on the influence on allosteric enhancer activity of further substitution at the 4-position of the 2-amino-3-(4-chlorobenzoyl)-thiophene system to explore bulk tolerance by replacement of the arylpiperazine moiety with a series of fused indole nuclei corresponding to 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole, 1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indole, tetrahydro-γ-carboline, tetrahydroisoquinoline, spiro-1,3-benzodioxolepiperidine, aliphatic tertiary amine, *N*-alkylaniline, aryl ether and aryl thioether templates. The 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole derivatives **3a-c** and **3e** 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. This study also shows that it is possible to obtain a good separation between allosteric enhancement and antagonistic activity at the A_1 adenosine receptor.

Keywords: A_1 adenosine receptor; allosteric enhancer; G protein-coupled receptors; 2-amino-3-benzoylthiophene.

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]. Activation of A_1 receptors induces inhibition of the enzyme adenylate cyclase mediated by an inhibitory G-protein (Gi) with a consequent reduction in cAMP levels [2]. The A_1 adenosine receptors (A_1AR) are expressed in high density in the brain (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 [3]. Effects mediated by selective activation of the A_1AR include neuro- and

cardioprotection, an antiarrhythmic effect, hypotension, reduction of neuropathic pain and inhibition of lipolysis in adipose tissue [4].

Due to the ubiquitous presence of adenosine receptors, agents which can selectively activate A_1ARs within specific tissues have attracted much attention as potential therapeutic agents [5]. Although numerous full agonists with high affinity for A_1AR have been developed, their clinical use is hampered by severe cardiovascular side effects caused by their strong hypotensive action, depression of the central nervous system (CNS) and numerous peripheral side effects on other organs. Unfortunately, agonists that selectively activate A_1AR in a specific target tissue have not been found. An alternative approach toward selective A_1AR activation is provided by the concept of allosteric modulation [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 increases the action of endogenous adenosine or selective full agonists [7]. Allosteric enhancers (AEs) of A_1AR function might provide a more selective therapeutic effect than full agonists [8]. Such agents might synergize with endogenous adenosine, with minimal effects in the absence of adenosine. Their action would therefore be limited to times and locations at which significant release of adenosine occurred [9].

PD 81,723 (**1a**, Chart 1) was one of the first allosteric enhancers acting at the A_1AR, enhancing the functional effects of adenosine or its analogues, and having no effect on agonist binding at the other adenosine receptor subtypes [10]. Among the synthesized compounds [11], derivative **1b** {T-62, (2-amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)(4-chlorophenyl)methanone}, was taken into Phase II clinical trials by King Pharmaceuticals for the treatment of neuropathic pain associated with hyperalgesia and allodynia [12].

A wide series of 2-amino-3-aroyl thiophene derivatives with general structure **2**, characterized by the presence of different phenyl-substituted piperazine moieties attached to the 4-position of the thiophene ring by a methylene unit, have been identified as potent AEs at the A_1AR [13]. The nature and the position of substituents on the phenyl ring tethered to the piperazine seemed to exert

a fundamental influence on the AE activity. Based on this information, chemical modifications of compounds with general formula **2** were started. In the present article, we have focused our attention on the systematic modification of the 4-position of the 2-amino-3-(4-chlorobenzoyl) thiophene ring, examining the effects on AE activity due to the replacement of the arylpiperazine moiety with different chemical functionalities. In this new series of compounds, the 2-amino-3-(4-chlorobenzoyl) thiophene nucleus was maintained, as this was shown to be an essential feature for AE activity.

In the first series of compounds, the arylpiperazine moiety was replaced by 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole or 1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indole moieties to furnish derivatives **3a-d** and **3e-g**, respectively. The pyrazino-indole system might be viewed as a conformationally restricted aryl piperazine, in which the *ortho* position of the phenyl ring is tethered to the carbon at the 2'-position of the piperazine moiety by a methanylylidene (-CH=) or methanediyl (-CH_2-) linker. In this way, a five-membered ring was introduced to freeze the free rotation of the C-N bond between the phenyl and the anilinic nitrogen of the piperazine moiety. Thus, derivatives **3a-g** have been synthesized in order to restrict the conformational freedom of the phenylpiperazine system, thereby constraining the corresponding "open" counterparts with general structure **2** into a near planar conformation. It is noteworthy that the 8-position of the pyrazino[1,2-*a*]indole nucleus corresponds to the 4'-position of the phenyl ring linked to the piperazine moiety in compounds with general structure **2**. A further simplification of the phenylpiperazine moiety was represented by the 1,2,3,4-tetrahydroisoquinoline system (TIQ), exemplified by compound **3h**.

Derivatives **3i-l** are characterized by the presence of a 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (or tetrahydro-γ-carboline) moiety, as regioisomeric analogues of the 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole system that characterized analogues **3a-d**.

While compounds **3a-g** and **3i-l** were characterized by the presence of an indole nucleus fused with a piperidine ring, joining the 4'-position of the piperidine nucleus to the 2-position of a benzodioxole ring, furnished the spiropiperidine derivatives **3m-q.** As a further molecular

simplification of derivatives with general structure **2**, the phenylpiperazine moiety was replaced with structurally simpler and less bulky substituents, such as *N*-alkylanilines (**3r-v**), *N*-methyl benzylamine (**3w**) or aliphatic tertiary amines (**3x-z**) (Chart 2). The influence of substituents at the 5-position of the thiophene ring (phenyl or bromine) on AE activity, combined with a tertiary amine as the 4-substituent, was evaluated by the synthesis of compounds **3aa-aj** and **3ak-al**, respectively. Finally, the nitrogen of aniline derivatives **3r-v** was replaced with an oxygen or a sulphur, to yield aryl ethers (**3am-ap**) and aryl thioether (**3aq**), respectively.

2. Chemistry

The 4-bromomethyl-5-bromothiophene analogue **4** [13a] was employed as a common intermediate for the synthesis of compounds **3a-z** by a three-step procedure (Scheme 1). Thiophene derivative **4** was coupled with the appropriate tetrahydropyrazino[1,2-*a*]indoles **5a-d** [14], hexahydropyrazino[1,2-*a*]indoles **5e-g** [15], tetrahydroisoquinoline **5h**, tetrahydro-γ-carbolines **5i-l** [16], spiropiperidines **5m-q** [17] and secondary amines **5w-z** in dichloromethane, to afford the derivatives **6a-q** and **6w-z**, respectively, in acceptable yields. Compounds **6r-v** were prepared by condensation of **4** with anilines **5r-v** in acetonitrile in the presence of potassium carbonate at 60 °C. Starting from the 5-bromothiophene derivatives **6a-z**, dehalogenation by catalytic hydrogenation using 10% Pd/C furnished the thiophene derivatives **7a-z**. The subsequent treatment with ethanolic hydrazine furnished the final compounds **3a-z**.

The 4-bromomethyl-5-phenyl analogue **8** [18] was transformed into derivatives **6aa-aj** by treatment with the appropriate amine in dichloromethane in the presence of TEA. The isolated compounds **6aa-aj** were converted to the final compounds **3aa-aj** by treatment with hydrazine in ethanol (Scheme 2)**.**

Compound **3ak** was prepared by the condensation of **4** with diallyl amine, to furnish the 5–bromo derivative **6ak**, followed by removal of the N-protected phthaloyl group by the use of hydrazine (Scheme 3). Compound **3al** was prepared by treatment of **6z** with hydrazine in ethanol at reflux.

Finally, reaction of **4** with the appropriate phenol or thiophenol in acetonitrile in the presence of potassium carbonate at 60 °C afforded the intermediates **6am-aq** (Scheme 3). The subsequent hydrogenation in the presence of 10% Pd/C furnished the 5-unsubstituted thiophene derivatives **7am-aq**, which were converted to the final compounds **3am-aq** by treatment with hydrazine in ethanol**.**

3. Results and Discussion

3.1. Functional assays

To assess the biological activity of the novel series of synthesized compounds **3a-aq**, we initially screened all molecules using a functional assay, evaluating their ability to inhibit forskolin-stimulated cAMP production *via* the hA_1-AR in intact Chinese hamster ovary (CHO) cells. When this receptor is in an active conformation in CHO , it cause a measurable inhibition of adenylyl cyclase activity. Allosteric enhancers are thought to stabilize the active conformation of the A_1-AR, leading to a reduction in the cAMP content of the cells.

The reference compound PD 81,723 and the new derivatives **3a-aq** were evaluated at a concentration of 10 μM alone (Figure 1A) or at a concentration of 100 nM in the presence of the orthosteric agonist CCPA (1 pM) to assess enhancement of the A_1-AR agonist activity (Figure 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 agonist. The degree of inhibition of cAMP production was similar under the two conditions tested.

The tetrahydropyrazino[1,2-a]indoles **3a-e**, tetrahydroisoquinoline **3h**, spiro-1,3-benzodioxole-pipridines **3m-q** and aliphatic amines **3w** and **3ad** represent the most active compounds to emerge from this investigation, inhibiting the percentage of cAMP production from 36% to 72% (Table 1). In the series of 5-phenyl derivatives **3aa-aj**, greater allosteric enhancement was seen with longer aliphatic chains among the symmetrically di-substituted aliphatic amines attached to a methylene

at the 4-position of the thiophene ring. Thus the *N*,*N*-di-*n*-propylamine (**3ac**) and *N*,*N*-diallylamine (**3ad**) are more active than the *N*,*N*-diethylamine (**3ab**) and significantly more active than the *N*,*N*-dimethylamine (**3aa**). The piperidine derivative (**3ah**) had activity similar to that of the *N*,*N*-diethyl analogue (**3ab**). Replacing the *N*,*N*-di-*n*-propylamine (**3ac**) with the branched *N*,*N*-di-isopropylamine (**3ae**) led to a significant reduction in activity, while the sterically more demanding *N*,*N*-dicyclohexylamine (**3ag**) was more active. Interestingly, disubstituted amines attached to a with two different substituents on nitrogen (**3af**, **3ai**, **3aj**), one being a methyl group, were essentially equipotent to the *N*,*N*-dimethyl analogue (**3aa**).

As shown in Table 1, compound **3a**, characterized by the presence of a tetrahydropyrazino[1,2-*a*]indole unit linked to the 4-position of the thiophene ring by a methylene unit, had a significantly greater effect than PD 81,723. Introduction of an electron-withdrawing fluorine or chlorine at the 8'-position of the tetrahydropyrazino[1,2-*a*]indole nucleus (compounds **3b** and **3c**, respectively) maintained the activity, which was reduced by the insertion of an electron-releasing methoxy group (derivative **3d**). Replacing the tetrahydropyrazino[1,2-*a*]indole nucleus with a tetrahydroisoquinoline afforded derivative **3h**, with activity similar to that of compound **3d**.

Reduction of the C_{10}-C_{10a} double bond of the tetrahydropyrazino[1,2-*a*]indole nucleus had a contrasting effect on the activity (compounds **3e-g**). While there was no difference in activity between the unsubstituted derivatives **3a** and **3e**, the 8-chloro-substituted indoline derivative **3f** was considerably less active than the corresponding indole analogue **3c**. The data reported in Table 1 show that incorporation of a chlorine or methyl substituent at the indoline C-8 position (compounds **3f** and **3g**, respectively) led to a dramatic drop in activity relative to the unsubstituted analogue **3e**. It would be interesting to evaluate the individual optical isomers of **3e** to determine the effect of stereochemistry on activity.

In the series of tetrahydro-γ-carboline derivatives **3i-l**, the 5'-unsubstituted derivatives **3i-j** showed the least activity, while the corresponding 5'-methyl analogues **3k** and **3l** were 9- and 3-fold more

active, respectively, at a concentration of 10 μM. These latter compounds were also 1.5-fold more active than PD 81,723 when tested alone or in presence of CCPA.

Several chemically and sterically different substituents, such as fluorine (**3n**), methyl (**3o-p**) and *tert*-butyl (**3q**) in either the 4'- or 5'-position of the benzene portion of the spiro[1,3-benzodioxole-2,4'-piperidine] system were investigated. Although these modifications alter the electronic, steric and lipophilic features of this residue, there was no apparent difference in activity between compounds **3n-q**. The 4'- and 5'-methyl analogues (**3o** and **3p**, respectively) showed essentially identical activity, suggesting a lack of regiochemical preference. In addition, compound **3p** was equiactive to the more bulky and lipophilic *tert*-butyl derivative **3q** at the concentration of 10 μM. Replacement of the electron-releasing methyl in **3o** with an electron-withdrawing fluorine (**3n**), may have caused a slight increase in activity.

In the series of 5-unsubstituted thiophene derivatives **3r-z**, the aniline derivatives **3r-t** were found to be amenable to modification. The *N*-methyl-*N*-phenyl (**3r**) and *N*-ethyl-*N*-phenyl (**3s**) homologues were equiactive. Introduction of electron withdrawing substituents at the 4-position of the anilino-phenyl led to an increase in activity in the order **3v** (CF_3) = **3t** (F) > **3u** (Cl). Homologation of the *N*-methyl aniline derivative **3r** furnished the *N*-methyl-*N*-benzylamine analogue **3w**, with 2-fold improved activity at the two concentrations tested. In contrast, the presence of short-chain dialkylamines, such as *N*,*N*-diethylamine (**3x**), *N*,*N*-di-*n*-propylamine (**3y**), as well as branched-chain dialkylamines, such as *N*,*N*-di-*iso*-propylamine (**3z**) was not desirable, conferring low activity. However, when the *N*,*N*-diethylamine and *N*,*N*-di-*n*-propylamine moieties were combined with a phenyl moiety at the 5-position of the thiophene ring (compounds **3ab** and **3ac**, respectively), an increase of activity was observed that was superior to that of PD 81,723. This was not true for the *N*-methyl-*N*-benzylamine derivative **3w**, in which the corresponding 5-phenylthiophene analogue, **3aj**, showed a 10-fold reduced activity compared to **3w** when tested alone at 10 μM.

Replacing the *N*-methyl-*N*-phenyl moiety of **3r** with an arylether or arylthioether afforded derivatives **3am-aq**. The unsubstituted phenyl thioether (**3aq**) maintained activity equivalent to **3r**, while the phenyl ether (**3am**) was two fold less active than **3r**. The substituted aryl ether derivatives **3an** (*p*-CH_3) and **3ap** (*p*-F) appeared almost 2-fold less active than the corresponding *N*-methyl aniline analogues **3u** and **3t**, respectively.

As noted above, introducing a 5-phenyl moiety on 4-(*N,N*-dialkylamino)methyl thiophenes led to increased allosteric enhancer activity. Interestingly , the *N,N*-dicyclohexylamine and piperidine analogues **3ag** and **3ah**, respectively, displayed activity similar to **3ac**. Replacement of the *N,N*-di-*n*-propylamine moiety of **3ac** with the unsaturated *N,N*-diallylamine (**3ad**) retained the same level of activity. Replacing the 5-phenyl moiety of **3ad** with a bromine (**3ak**) resulted in a significant loss of activity. Compared to the *N,N*-di-*iso*-propylamine derivative **3z**, the corresponding 5-phenyl and 5-bromo analogues **3ae** and **3al**, respectively, showed two- to three-fold increased activity relative to **3z**. This suggests that activity is not only dependent on the presence of phenyl at the 5-position of thiophene ring, but also the nature of the substituent at the 4-position. This was confirmed by the reduced activity of *N,N*-dimethylamino, *N*-*tert*-butyl-*N*-methylamino and *N*-*n*-butyl-*N*-methylamino derivatives **3aa**, **3af** and **3ai**, respectively.

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 **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 (Table 2). 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 [19]. 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 12% or less. For the most active compounds in a functional assay, such as **3a-c**, **3e**, **3n** and **3q**, 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_1 AR 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 (Bmax) were evaluated in the presence and in the absence of the examined compounds (PD 81,723 and **3a-aq** at a concentration of 10 μM) and were used to calculate the increase of A_1 density (Bmax shift) (Table 3). The reference compound PD 81,723 induced a Bound and B_{MAX} shift to human A_1 adenosine receptors of 1.3- and 1.2-fold, respectively. Under the same experimental conditions, compounds **3a-e**, **3h**, **3k-w**, **3ab-ad** and **3ag-ah** 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 **3a-c, 3e, 3n,** and **3q** were the most active compounds, each causing a B_{MAX} shift of more than 5-fold. Figure 2 shows the effect of the allosteric modulators PD 81,723, **3a**, **3b** and **3n** 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 K_D shift of the tested compounds, 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 shift ranged from 1.0 ± 0.1 to 1.2± 0.1).

Table 3 also reports the derived apparent affinity (Ki) values for CCPA, based on a one-state model of analysis, in the absence and in the presence of tested enhancers. This table also shows the CCPA shift representing the ratio of apparent Ki 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 Ki value of CCPA was 15.2 ± 1.3 nM. Interestingly, a significant decrease in the

apparent Ki 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 **3a-e**, **3h**, **3k-w**, **3ab-ad** and **3ag-ah** reveal that the displacement curves are shifted left, suggesting lower Ki values for CCPA. In particular, the largest affinity shift has been observed for compounds **3a**, **3b**, **3c**, **3e** and **3n.** These molecules enhanced the apparent affinity of CCPA approximately 6.6-, 7.2-, 6.9-, 7.6- and 6.3-fold, respectively (Table 3). 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 Ki values (Table 3).

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

4. Conclusions

Encouraged by the results obtained for the arylpiperazine derivatives with general structure **2**, we continued to follow our strategy of incorporating other substituents attached to the methylene at the thiophene 4-position. Compounds **3a-d**, characterized by the presence of a tetrahydropyrazino[1,2-*a*]indole nucleus as conformationally constrained arylpiperazine analogues, appeared to be significantly more active than PD 81,723 in the functional assay. Biological data confirmed that the replacement of the arylpiperazine moiety by a tetrahydropyrazino[1,2-*a*]indole nucleus in the 4-position of thiophene ring is well tolerated. In terms of activity, reduction of the C_{10}-C_{10a} double bond of the tetrahydropyrazino[1,2-*a*]indole nucleus was tolerated only for the 8-unsubstituted hexahydropyrazino[1,2-*a*]indole derivative **3e**, which showed the same activity as the corresponding analogue **3a**.

A characteristic feature of AE at the A_1AR is the propensity to also cause antagonism at higher concentrations. None of the synthesized compounds **(3a-aq)** significantly inhibited antagonist binding at the hA_1AR, hA_2AR, or hA_3AR. Among these, derivatives **3a**, **3b**, **3c**, **3e** and **3n** 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 NMR and ^{13}C NMR spectra were determined in $CDCl_3$ or d_6-DMSO solutions and recorded with a Varian VXR-200 spectrometer or a Varian Mercury Plus 400 spectrometer. 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 and ^{13}C 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 F_{254} 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.

5.1.2. General procedure (A) for the synthesis of compounds 6a-q and 6w-ak.

To a stirred solution of compound **4** or **8** (2 mmol) in dry dichloromethane (10 mL) was added K_2CO_3 (1.1 equiv., 2.2 mmol, 304 mg). The mixture was cooled with a bath of ice/water, and then the appropriate amine (1.2 equiv., 2.4 mmol), dissolved in dichloromethane (3 mL), was added slowly over 5 min. The mixture was then stirred at room temperature for two hours, diluted with dichloromethane (10 mL), washed with water (5 mL) and then with brine (5 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a residue that was purified by column chromatography to furnish the derivatives **6a-q** and **6w-ak**.

5.1.2.1. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6a). Following general procedure (A), derivative **6a** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 69%. Yellow solid, mp 148-150 °C. ^{1}H NMR ($CDCl_3$) δ: 3.00 (s, 2H), 3.39 (t, *J*=5.8 Hz, 2H), 4.10 (t, *J*=5.8 Hz, 2H), 4.24 (s, 2H), 6.05 (s, 1H), 7.19 (m, 4H), 7.42 (m, 4H), 7.54 (m, 4H). MS (ESI): $[M]^+$=629.1, $[M+2]^+$=631.1.

5.1.2.2. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-fluoro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6b). Following general procedure (A), derivative **6b** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 78%. Yellow solid, mp 163-165 °C. ^{1}H NMR ($CDCl_3$) δ: 2.52 (t, *J*=5.4 Hz, 2H), 3.12 (s, 2H), 3.22 (s, 2H), 3.78 (t, *J*=5.6 Hz, 2H), 6.00 (s, 1H), 7.13 (m, 3H), 7.39 (m, 4H), 7.56 (m, 4H). MS (ESI): $[M]^+$=647.0, $[M+2]^+$=649.1.

5.1.2.3. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-chloro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6c). Following general procedure (A), derivative **6c** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 82%. Yellow solid, mp 220-222 °C. ^{1}H NMR ($CDCl_3$) δ: 2.46 (t, *J*=5.4 Hz, 2H), 3.09 (s, 2H), 3.34 (s, 2H), 3.78 (t, *J*=5.4 Hz, 2H), 6.00 (s, 1H), 7.11 (m, 4H), 7.33 (d, *J*=8.6 Hz, 2H),), 7.50 (s, 1H), 7.56 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.0, 48.8, 50.4, 53.6, 96.6, 109.6, 119.5, 120.9, 124.3 (2C), 125.3,

125.6, 128.5 (2C), 128.7, 129.2, 130.1 (2C), 131.0, 134.3, 135.0 (2C), 135.3, 135.8 (2C), 136.1, 138.9, 139.2, 165.5 (2C), 189.2. MS (ESI): $[M]^+$=663.1, $[M+2]^+$=665.3.

5.1.2.4. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-methoxy-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6d). Following general procedure (A), derivative **6d** was purified by column chromatography (EtOAc:dichloromethane 0.5:9.5). Yield: 82%. Yellow solid, mp 133-135 °C. ^{1}H NMR ($CDCl_3$) δ: 2.47 (t, *J*=5.6 Hz, 2H), 3.12 (s, 2H), 3.36 (s, 2H), 3.83 (t, *J*=5.6 Hz, 2H), 3.87 (s, 3H), 6.02 (s, 1H), 7.13 (m, 4H), 7.35 (d, *J*=8.6 Hz, 2H),), 7.53 (s, 1H), 7.54 (m, 4H). MS (ESI): $[M]^+$=659.1, $[M+2]^+$=661.1.

5.1.2.5. (R,S)-2-[5-Bromo-3-(4-chlorobenzoyl)-4-(1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6e). Following general procedure (A), derivative **6e** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 68%. Yellow solid, mp 138-140 °C. ^{1}H NMR ($CDCl_3$) δ: 2.56 (dd, *J*=15.6 and 9.6 Hz, 2H), 2.88 (m, 2H), 2.97 (m, 4H), 3.09 (d, *J*=11.6 Hz, 1H), 3.53 (m, 2H), 6.43 (d, *J*=7.6 Hz, 1H), 6.66 (d, *J*=8.0 Hz, 1H), 7.09 (m, 2H), 7.36 (d, *J*=8.8 Hz, 2H), 7.48 (d, *J*=8.8 Hz, 2H), 7.65 (m, 4H). MS (ESI): $[M]^+$=631.1, $[M+2]^+$=633.1.

5.1.2.6. (R,S)-2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-chloro-1,2.3,4,10,10a-hexahydro -1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6f). Following general procedure (A), derivative **6f** was purified by column chromatography (EtOAc:dichloromethane 0.5:9.5). Yield: 74%. Yellow solid, mp 153-155 °C. ^{1}H NMR ($CDCl_3$) δ: 2.56 (dd, *J*=15.6 and 9.6 Hz, 2H), 2.84 (m, 2H), 2.95 (m, 4H), 3.04 (d, *J*=11.6 Hz, 1H), 3.48 (m, 2H), 6.33 (d, *J*=8.0 Hz, 1H), 7.00 (d, *J*=8.0 Hz, 1H), 7.02 (s, 1H), 7.44 (d, *J*=8.8 Hz, 2H), 7.58 (d, *J*=8.8 Hz, 2H), 7.62 (m, 4H). MS (ESI): $[M]^+$=665.0, $[M+2]^+$=667.1.

5.1.2.7. (R,S)-2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-methyl-1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6g). Following general

procedure (A), derivative **6g** was purified by column chromatography (EtOAc:dichloromethane 0.5:9.5). Yield: 77%. Yellow solid, mp 141-143 °C. ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H), 2.52 (dd, *J*=15.6 and 9.6 Hz, 2H), 2.84 (m, 2H), 2.98 (m, 4H), 3.06 (d, *J*=11.6 Hz, 1H), 3.52 (m, 2H), 6.10 (s, 1H), 6.96 (d, *J*=8.0 Hz, 1H), 7.18 (d, *J*=8.0 Hz, 1H), 7.34 (m, 2H), 7.48 (d, *J*=8.4 Hz, 2H), 7.62 (m, 4H). MS (ESI): $[M]^+$=645.0, $[M+2]^+$=647.1.

5.1.2.8. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(1,2,3,4-tetrahydro-1H-isoquinolin-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6h). Following general procedure (A), derivative **6h** was purified by column chromatography (EtOAc:petroleum ether 3:7). Yield: 76%. Yellow solid, mp 101-103 °C. ^{1}H NMR ($CDCl_3$) δ: 2.23 (t, *J*=5.4 Hz, 2H), 2.61 (t, *J*=5.6 Hz, 2H), 2.89 (s, 2H), 3.28 (s, 2H), 6.87 (d, *J*=7.8 Hz, 1H), 7.19 (m, 3H), 7.33 (m, 6H), 7.50 (t, *J*=8.8 Hz, 1H), 7.73 (t, *J*=8.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.2, 49.3, 54.3, 54.9, 124.3 (2C), 125.3, 125.5, 125.8, 126.1, 126.5, 127.9, 128.4 (2C), 128.6 (2C), 128.9, 130.1 (2C), 131.1, 133.8, 134.1, 134.9, 136.1, 138.4, 138.7, 165.7 (2C), 189.0. MS (ESI): $[M]^+$=590.0, $[M+2]^+$=592.1.

5.1.2.9. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6i). Following general procedure (A), derivative **6i** was purified by column chromatography (EtOAc:petroleum ether 2:8). Yield: 72%. Yellow solid, mp 152-154 °C. ^{1}H NMR ($CDCl_3$) δ: 2.76 (t, *J*=5.8 Hz, 2H), 3.22 (t, *J*=5.8 Hz, 2H), 3.44 (s, 2H), 4.06 (s, 2H), 7.12 (m, 4H), 7.24 (d, *J*=8.6 Hz, 1H); 7.36 (d, *J*=8.8 Hz, 2H), 7.42 (d, *J*=7.6 Hz, 1H), 7.65 (m, 4H), 7.82 (bs, 1H). MS (ESI): $[M]^+$=629.0, $[M+2]^+$=631.2.

5.1.2.10. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-chloro-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6j). Following general procedure (A), derivative **6j** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 55%. Yellow solid, mp 150-152 °C. ^{1}H NMR ($CDCl_3$) δ: 2.76 (t, *J*=5.8 Hz, 2H), 3.22 (t, *J*=5.6 Hz, 2H), 3.72 (s, 2H), 4.01 (s, 2H), 7.03 (d, *J*=8.6 Hz, 1H), 7.17 (d, *J*=8.6 Hz, 2H), 7.25 (d, *J*=8.6 Hz, 1H), 7.37 (s, 1H); 7.46 (d, *J*=8.6 Hz, 2H), 7.62 (m, 4H), 7.82 (bs, 1H). MS (ESI): $[M]^+$=663.0, $[M+2]^+$=665.1.

5.1.2.11. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6k). Following general procedure (A), derivative **6k** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 73%. Yellow solid, mp 134-136 °C. ^{1}H NMR ($CDCl_3$) δ: 2.76 (t, *J*=5.6 Hz, 2H), 3.27 (t, *J*=5.6 Hz, 2H), 3.62 (s, 3H), 4.22 (m, 4H), 7.10 (m, 4H), 7.26 (d, *J*=8.6 Hz, 2H); 7.44 (d, *J*=8.6 Hz, 2H), 7.65 (m, 4H). MS (ESI): $[M]^+$=643.1, $[M+2]^+$=645.3.

5.1.2.12. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(8-chloro-5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (6l). Following general procedure (A), derivative **6l** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 81%. Yellow solid, mp 155-157 °C. ^{1}H NMR ($CDCl_3$) δ: 2.74 (t, *J*=5.6 Hz, 2H), 3.25 (t, *J*=5.6 Hz, 2H), 3.60 (s, 3H), 3.62 (s, 2H), 4.01 (s, 2H), 7.16 (m, 4H), 7.37 (s, 1H); 7.46 (d, *J*=8.6 Hz, 2H), 7.64 (m, 4H). MS (ESI): $[M]^+$=677.1, $[M+2]^+$=679.2.

5.1.2.13. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (6m). Following general procedure (A), derivative **6m** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 58%. White solid, mp 229-231 °C. ^{1}H NMR ($CDCl_3$) δ: 1.98 (t, *J*=6.0 Hz, 4H), 3.06 (t, *J*=6.0 Hz, 4H), 3.49 (s, 2H), 6.78 (m, 4H), 7.36 (d, *J*=8.6 Hz, 2H), 7.43 (d, *J*=8.6 Hz, 2H), 7.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.3 (2C), 49.5 (2C), 54.3, 108.6 (2C), 110.3, 115.9, 121.2 (2C), 124.3 (2C), 128.7 (2C), 130.1 (2C), 131.2 (2C), 135.0 (2C), 135.9 (2C), 136.3, 138.0, 138.9, 147.0 (2C), 165.6 (2C), 188.9. MS (ESI): $[M]^+$=648.1, $[M+2]^+$=650.1.

5.1.2.14. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(4-fluorospiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (6n). Following general procedure (A), derivative **6n** was purified by column chromatography (EtOAc:petroleum ether 2:8). Yield: 56%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.19 (t, *J*=6.0 Hz, 4H), 3.07 (t, *J*=6.0 Hz, 4H), 3.44 (s, 2H), 6.56 (d, *J*=8.4 Hz,

1H), 6.62 (m, 1H), 6.76 (m, 1H), 7.37 (d, *J*=8.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 2H), 7.84 (m, 4H). MS (ESI): $[M]^+$=666.0, $[M+2]^+$=668.1.

5.1.2.15. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(4-methylspiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (6o). Following general procedure (A), derivative **6o** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 74%. Yellow solid, mp 194-196 °C. ^{1}H NMR ($CDCl_3$) δ: 1.97 (t, *J*=5.6 Hz, 4H), 2.20 (s, 3H), 3.02 (m, 4H), 3.49 (s, 2H), 6.62 (m, 2H), 6.68 (t, *J*=8.0 Hz, 1H), 7.44 (d, *J*=8.6 Hz, 2H), 7.57 (d, *J*=8.6 Hz, 2H), 7.68 (m, 4H). MS (ESI): $[M]^+$=662.0, $[M+2]^+$=664.1.

5.1.2.16. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(5-methylspiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (6p). Following general procedure (A), derivative **6p** was purified by column chromatography (EtOAc:petroleum ether 2:8). Yield: 68%. White solid, mp 142-144 °C. ^{1}H NMR ($CDCl_3$) δ: 1.96 (t, *J*=6.0 Hz, 4H), 2.26 (s, 3H), 3.02 (t, *J*=6.0 Hz, 4H), 3.24 (s, 2H), 6.60 (m, 2H), 6.64 (d, *J*=8.0 Hz, 1H), 7.44 (d, *J*=8.6 Hz, 2H), 7.56 (d, *J*=8.6 Hz, 2H), 7.65 (m, 4H). MS (ESI): $[M]^+$=662.1, $[M+2]^+$=664.1.

5.1.2.17. 2-[5-Bromo-4-(5-t-butylspiro-benzo[1,3-d]-dioxol-2-yl)-3-(4-chlorobenzoyl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (6q). Following general procedure (A), derivative **6q** was purified by column chromatography (EtOAc:petroleum ether 3:7). Yield: 77%. White solid, mp 105-107 °C. ^{1}H NMR ($CDCl_3$) δ: 1.28 (s, 9H), 2.08 (t, *J*=6.0 Hz, 4H), 3.24 (t, *J*=6.0 Hz, 4H), 3.48 (s, 2H), 6.66 (d, *J*=8.4 Hz, 1H), 6.79 (d, *J*=8.4 Hz, 1H), 6.84 (s, 1H), 7.38 (d, *J*=8.6 Hz, 2H), 7.45 (d, *J*=8.6 Hz, 2H), 7.66 (m, 4H). MS (ESI): $[M]^+$=704.1, $[M+2]^+$=706.2.

5.1.2.18. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-benzyl-N-methylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6w). Following general procedure (A), the product **6w** was purified by column chromatography (EtOAc-petroleum ether 3-7 as eluent). Yield 78%. Yellow solid, mp 184-186 °C. ^{1}H NMR ($CDCl_3$) δ: 2.48 (s, 3H), 3.44 (s, 2H), 4.13 (s, 2H), 6.28 (d, *J*=8.4 Hz, 2H), 6.54 (t, *J*=7.2

Hz, 1H), 7.02 (m, 4H), 7.40 (d, *J*=8.4 Hz, 2H), 7.72 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.8, 54.7, 61.0, 110.8, 124.3 (2C), 126.9, 128.1 (2C), 128.7 (2C), 128.8 (2C), 130.4 (2C), 131.2 (2C), 134.9 (2C), 135.2, 135.8, 136.0, 138.1, 138.6, 139.0, 165.6 (2C), 189.0. MS (ESI): $[M]^+$=578.1, $[M+2]^+$=580.2.

5.1.2.19. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N,N-diethylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6x). Following general procedure (A), the product **6x** was purified by column chromatography (EtOAc-petroleum ether 1.5:8.5 as eluent). Yield 54%. Brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, *J*=7.4 Hz, 6H), 2.17 (q, *J*=7.4 Hz, 4H), 3.43 (s, 2H), 7.32 (d, *J*=8.6 Hz, 2H), 7.66 (d, *J*=8.6 Hz, 2H), 7.75 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.88 (2C), 44. 6(2C), 50.8, 124.1 (2C), 128.4 (2C), 130.1 (2C), 130.5, 131.1 (2C), 134.8 (2C), 135.1, 135.9, 137.1, 138.6, 139.7, 165.6 (2C), 188.4. MS (ESI): $[M]^+$=530.2, $[M+2]^+$=532.2.

5.1.2.20. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N,N-dipropylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6y). Following general procedure (A), the product **6y** was purified by column chromatography (EtOAc-petroleum ether 3:7 as eluent). Yield 88%. Yellow solid, mp 143-145 °C. ^{1}H NMR ($CDCl_3$) δ: 0.63 (t, *J*=7.6 Hz, 6H), 1.19 (m, 4H), 1.83 (t, *J*=7.4 Hz, 4H), 3.27 (s, 2H), 7.23 (d, *J*=8.6 Hz, 2H), 7.38 (d, *J*=8.6 Hz, 2H), 7.52 (m, 4H). MS (ESI): $[M]^+$=558.1, $[M+2]^+$=560.2.

5.1.2.21. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N,N-diisopropylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6z). Following general procedure (A), the product **6z** was purified by column chromatography (EtOAc-petroleum ether 1.5:8.5 as eluent). Yield 53%. Yellow solid, mp 144-146 °C. ^{1}H NMR ($CDCl_3$) δ: 0.74 (d, *J*=6.8 Hz, 12H), 2.66 (m, 2H), 3.64 (s, 2H), 7.26 (d, *J*=8.6 Hz, 2H), 7.61 (d, *J*=8.6 Hz, 2H), 7.68 (m, 4H). MS (ESI): $[M]^+$=558.2, $[M+2]^+$=560.3.

5.1.2.22. 2-[3-(4-Chlorobenzoyl)-4-(N,N-dimethylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6aa). Following general procedure (A), the product **6aa** was purified by column chromatography (EtOAc as eluent). Yield 61%. Yellow solid, mp 106-108 °C. ^{1}H NMR ($CDCl_3$) δ:

1.61 (s, 6H), 3.09 (s, 2H), 7.40 (m, 7H), 7.45 (m, 4H), 7.66 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M]^+$=500.0, $[M+2]^+$=502.0.

5.1.2.23. 2-[3-(4-Chlorobenzoyl)-4-(N,N-diethylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ab). Following general procedure (A), the product **6ab** was purified by column chromatography (EtOAc:petroleum ether 3:7 as eluent). Yield 62%. Yellow solid, mp 176-178 °C. ^{1}H NMR ($CDCl_3$) δ: 0.50 (t, *J*=7.2 Hz, 6H), 2.11 (q, *J*=7.2 Hz, 4H), 3.46 (s, 2H), 7.31 (d, *J*=8.8 Hz, 2H), 7.43 (m, 5H), 7.74 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.68 (2C), 44.4 (2C), 49.9, 124.1 (2C), 128.4 (2C), 128.7 (2C), 129.6, 130.0 (2C), 130.6, 131.4 (2C), 132.8, 134.7 (2C), 134.9, 136.4, 136.7, 138.3 (2C), 139.4, 139.6, 166.1 (2C), 189.1. MS (ESI): $[M]^+$=528.1, $[M+2]^+$=530.2.

5.1.2.24. 2-[3-(4-Chlorobenzoyl)-4-(N,N-dipropylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ac). Following general procedure (A), the product 6ac was purified by column chromatography (EtOAc:petroleum ether 3:7 as eluent). Yield 61%. Yellow solid, mp 175-177 °C. ^{1}H NMR ($CDCl_3$) δ: 0.55 (t, *J*=7.2 Hz, 6H), 1.42 (m, 4H), 2.02 (d, *J*=7.2 Hz, 4H), 3.24 (s, 2H), 7.36 (d, *J*=8.8 Hz, 2H), 7.41 (m, 7H), 7.55 (m, 4H). MS (ESI): $[M]^+$=556.1, $[M+2]^+$=558.2.

5.1.2.25. 2-[3-(4-Chlorobenzoyl)-4-(N,N-diallylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ad). Following general procedure (A), the product **6ad** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 66%. Yellow solid, mp 183-185 °C. ^{1}H NMR ($CDCl_3$) δ: 2.67 (d, *J*=6.4 Hz, 4H), 3.49 (s, 2H), 4.84 (m, 4H), 5.33 (m, 2H), 7.31 (d, *J*=8.8 Hz, 2H), 7.49 (m, 3H), 7.74 (m, 8H). MS (ESI): $[M]^+$=552.1, $[M+2]^+$=554.2.

5.1.2.26. 2-[3-(4-Chlorobenzoyl)-4-(N,N-diisopropylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ae). Following general procedure (A), the product **6ae** was purified by column chromatography (EtOAc:petroleum ether 3:7 as eluent). Yield 53%. White solid, mp 168-170 °C. ^{1}H NMR ($CDCl_3$) δ: 0.57 (d, *J*=6.6 Hz, 12H), 2.62 (m, 2H), 3.71 (s, 2H), 7.22 (d, *J*=8.6 Hz, 2H), 7.47 (m, 2H), 7.74 (m, 9H). MS (ESI): $[M]^+$=556.1, $[M+2]^+$=558.2.

5.1.2.27. 2-[3-(4-Chlorobenzoyl)-4-(N-t-butyl-N-methylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6af). Following general procedure (A), the product **6af** was purified by column chromatography (EtOAc:petroleum ether 3:7 as eluent). Yield 64%. Brown solid, mp 221-223 °C. ^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 9H), 1.72 (s, 3H), 3.89 (s, 2H), 7.29 (d, *J*=8.6 Hz, 2H), 7.46 (m, 5H), 7.72 (m, 2H), 7.82 (m, 4H). MS (ESI): $[M]^+$=542.1, $[M+2]^+$=544.2.

5.1.2.28. 2-[3-(4-Chlorobenzoyl)-4-(N,N-dicyclohexylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ag). Following general procedure (A), the product **6ag** was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 52%. Yellow solid, mp 98-100 °C. ^{1}H NMR ($CDCl_3$) δ: 0.92 (m, 12H), 1.51 (m, 8H), 2.17 (m, 2H), 3.81 (s, 2H), 7.22 (d, *J*=8.4 Hz, 2H), 7.47 (m, 5H), 7.74 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.8 (4C), 25.1 (2C), 28.9 (4C), 43.2 (2C), 61.2, 123.9 (2C), 128.4 (2C), 129.1, 129.5 (2C), 129.9 (2C), 130.1, 130.5 (2C), 130.9, 131.2, 131.5 (2C), 132.3 (2C), 135.1, 135.4, 135.7, 139.9, 165.3 (2C), 185.6. MS (ESI): $[M]^+$=636.2, $[M+2]^+$=638.2.

5.1.2.29. 2-[3-(4-Chlorobenzoyl)-5-phenyl-4-((piperidin-1-yl)methyl)-thiophen-2-yl]-isoindole-1,3-dione (6ah). Following general procedure (A), the product **6ah** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 47%. Yellow solid, mp 187-189 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (m, 6H), 1.65 (m, 4H), 2.98 (s, 2H), 7.22 (d, *J*=8.8 Hz, 2H), 7.53 (m, 3H), 7.73 (m, 8H). MS (ESI): $[M]^+$=540.1, $[M+2]^+$=540.2.

5.1.2.30. 2-[3-(4-Chlorobenzoyl)-4-(N-n-butyl-N-methylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6ai). Following general procedure (A), the product **6ai** was purified by column chromatography (EtOAc:petroleum ether 1:1 as eluent). Yield 63%. Yellow solid, mp 75-77 °C. ^{1}H NMR ($CDCl_3$) δ: 0.76 (t, *J*=7.2 Hz, 3H), 0.86 (m, 4H), 1.58 (s, 3H), 1.69 (t, *J*=7.2 Hz, 2H), 3.16 (s, 2H), 7.40 (m, 4H), 7.48 (m, 4H), 7.67 (m, 5H). MS (ESI): $[M]^+$=542.1, $[M+2]^+$=544.2.

5.1.2.31. 2-[3-(4-Chlorobenzoyl)-4-(N-benzyl-N-methylaminomethyl)-5-phenyl-thiophen-2-yl]-isoindole-1,3-dione (6aj). Following general procedure (A), derivative **6aj** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 61%. Yellow solid, mp 106-107 °C. ^{1}H NMR ($CDCl_3$) δ: 1.56 (s, 3H), 3.27 (s, 2H), 4.75 (s, 2H), 7.18 (d, *J*=8.4 Hz, 2H), 7.36 (m, 8H), 7.45 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.6, 53.6, 60.7, 124.1 (2C), 126.8 (2C), 127.9 (2C), 128.6 (2C), 128.8, 128.9 (2C), 129.7, 130.1 (2C), 130.4, 130.9, 131.3 (2C), 132.6, 134.7 (4C), 135.0, 135.5, 136.2, 137.9, 138.6, 166.1 (2C), 189.8. MS (ESI): $[M]^+$=576.1, $[M+2]^+$=578.2.

5.1.2.32. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N,N-diallylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6ak). Following general procedure (A), derivative **6ak** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5). Yield: 92%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.69 (d, *J*=6.6 Hz, 4H), 3.92 (s, 2H), 4.89 (m, 4H), 5.29 (m, 2H), 7.26 (d, *J*=8.6 Hz, 2H), 7.64 (d, *J*=8.6 Hz, 2H), 7.77 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 50.9, 55.6 (2C), 110.5, 117.9 (2C), 124.2 (2C), 128.6 (2C), 130.3 (2C), 131.2, 134.2 (2C), 134.9 (4C), 135.8, 136.8, 138.8, 138.9, 165.6 (2C), 188.6. MS (ESI): $[M]^+$=554.2, $[M+2]^+$=556.2.

5.1.3. General procedure (B) for the synthesis of compounds (6r-v).

To a stirred solution of **4** (540 mg, 1 mmol) in dry acetonitrile (10 mL) was added the appropriate aniline **5r-v** (2 equiv., 2 mmol,) and K_2CO_3 (207 mg, 3 equiv., 1.5 mmol). The mixture was then heated at 60°C for 1 h. The solvent was removed under reduced pressure and the residue partitioned between a mixture of dichloromethane (15 mL) and water (5 mL). The organic phase was washed with brine (5 mL), dried (Na_2SO_4), then concentrated *in vacuo* to give a residue that was purified by column chromatography.

5.1.3.1. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-methyl-N-phenyl-amino-methyl)-thiophen-2-yl]-isoindole-1,3-dione (6r). Following general procedure (B), the product **6r** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 95%. Yellow solid, mp 186-188

°C. ^{1}H NMR ($CDCl_3$) δ: 2.56 (s, 3H), 4.29 (s, 2H), 6.33 (d, *J*=8.6 Hz, 2H), 6.69 (t, *J*=7.2 Hz, 1H), 7.02 (m, 4H), 7.42 (d, *J*=8.6 Hz, 2H), 7.78 (m, 4H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 50.1, 54.8, 110.7, 113.6 (2C), 117.9, 123.7 (2C), 128.2 (2C), 128.4 (2C), 129.7, 130.6, 131.3, 134.4 (2C), 135.1 (2C), 136.7, 137.8 (2C), 142.3, 148.9, 185.4 (2C), 187.8. MS (ESI): $[M]^+$=564.0, $[M+2]^+$=566.1.

5.1.3.2. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N'-ethyl-N-phenyl-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6s). Following general procedure (B), the product **6s** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 86%. Yellow solid, mp 158-160 °C. ^{1}H NMR ($CDCl_3$) δ: 0.89 (t, *J*=7.2 Hz, 3H), 2.96 (q, *J*=7.2 Hz, 2H), 4.24 (s, 2H), 6.29 (d, *J*=8.8 Hz, 2H), 6.92 (t, *J*=7.2 Hz, 1H), 7.02 (m, 4H), 7.35 (d, *J*=8.6 Hz, 2H), 7.73 (m, 4H). MS (ESI): $[M]^+$=578.2, $[M+2]^+$=580.3.

5.1.3.3. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-(4-fluorophenyl)-N-methylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6t). Following general procedure (B), the product **6t** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 52%. Yellow solid, mp 188-190 °C. ^{1}H NMR ($CDCl_3$) δ: 2.50 (s, 3H), 4.22 (s, 2H), 6.35 (m, 2H), 6.76 (t, *J*=8.4 Hz, 2H), 7.11 (d, *J*=8.6 Hz, 2H), 7.45 (d, *J*=8.6 Hz, 2H), 7.78 (m, 4H). MS (ESI): $[M]^+$=582.1, $[M+2]^+$=584.3.

5.1.3.4. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-(4-chlorophenyl)-N-methylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6u). Following the general procedure (B), the product **6u** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 90%. Yellow solid, mp 122-124 °C. ^{1}H NMR ($CDCl_3$) δ: 2.54 (s, 3H), 4.29 (s, 2H), 6.28 (d, *J*=9.0 Hz, 2H), 6.96 (d, *J*=9.0 Hz, 2H), 7.11 (d, *J*=8.6 Hz, 2H), 7.41 (d, *J*=8.6 Hz, 2H), 7.43 (m, 4H). MS (ESI): $[M]^+$=598.1, $[M+2]^+$=600.1.

5.1.3.5. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-methyl-N-(4-trifluoromethylphenyl)-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6v). Following the general procedure (B), the product **6v** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 74%.Yellow solid, mp 189-191 °C. ^{1}H NMR ($CDCl_3$) δ: 2.66 (s, 3H), 4.43 (s, 2H), 6.33 (d, *J*=8.8 Hz, 2H), 7.04 (d, *J*=8.8 Hz, 2H), 7.27 (d, *J*=6.8 Hz, 2H), 7.35 (d, *J*=6.8 Hz, 2H), 7.72 (m, 4H). MS (ESI): $[M]^+$=632.1, $[M+2]^+$=634.1.

5.1.4. General procedure (C) for the synthesis of compounds (6am-aq).

To a stirred solution of **4** (270 mg, 0.5 mmol) in dry acetonitrile (5 mL) was added the appropriate phenol or thiophenol (1 equiv., 1 mmol) and K_2CO_3 (1 equiv., 69 mg, 0.5 mmol). The mixture was then heated at 60°C for 1 h. After this time, the appropriate phenol or thiophenol (1 equiv., 1 mmol,) and K_2CO_3 (1 equiv., 69 mg, 0.5 mmol) were added and the mixture was heated at 60°C for 1 h further. The solvent was removed under reduced pressure and the residue partitioned between a mixture of dichloromethane (15 mL) and water (5 mL). The organic phase was washed with brine (5 mL), dried (Na_2SO_4), then concentrated under reduced pressure to give a residue that was purified by column chromatography.

5.1.4.1. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (6am). Following general procedure (C), the product **6am** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 72%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 4.87 (s, 2H), 6.36 (d, *J*=8.4 Hz, 2H), 6.72 (t, *J*=7.2 Hz, 1H), 7.12 (m, 4H), 7.44 (d, *J*=8.6 Hz, 2H), 7.82 (m, 4H). MS (ESI): $[M]^+$=551.0, $[M+2]^+$=553.1.

5.1.4.2. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(4-(methyl)phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (6an). Following general procedure (C), the product **6an** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 56%. White solid, mp 97-100 °C. ^{1}H NMR ($CDCl_3$) δ: 2.28 (s, 3H), 4.97 (s, 2H), 6.54 (d, *J*=8.8 Hz, 2H), 6.94 (d, *J*=8.8 Hz, 2H), 7.18

(d, *J*=8.6 Hz, 2H), 7.62 (d, *J*=8.6 Hz, 2H), 7.78 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 62.3, 113.2, 125.2 (2C), 127.8, 128.9 (2C), 129.5 (2C), 130.8 (2C), 131.2 (2C), 132.4, 133.4, 134.0, 134.6, 135.3 (2C), 136.1 (2C), 137.3, 159.4, 164.5 (2C), 186.4. MS (ESI): $[M]^+$=565.1, $[M+2]^+$=567.2.

5.1.4.3. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(4-(isopropyl)phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (6ao). Following general procedure (C), the product **6ao** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 47%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.16 (d, *J*=6.8 Hz, 6H), 2.81 (m, 1H), 4.98 (s, 2H), 6.58 (d, *J*=8.8 Hz, 2H), 7.00 (d, *J*=8.8 Hz, 2H), 7.21 (d, *J*=8.6 Hz, 2H), 7.64 (d, *J*=8.6 Hz, 2H), 7.78 (m, 4H). MS (ESI): $[M]^+$=593.1, $[M+2]^+$=595.2.

5.1.4.4. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(4-(fluoro)phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (6ap). Following general procedure (C), the **6ap** product was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 65%. White solid, mp 112-114 °C. ^{1}H NMR ($CDCl_3$) δ: 4.98 (s, 2H), 6.63 (d, *J*=8.8 Hz, 2H), 6.89 (d, *J*=8.8 Hz, 2H), 7.21 (m, 2H), 7.63 (d, *J*=8.6 Hz, 2H), 7.76 (m, 4H). MS (ESI): $[M]^+$=569.1, $[M+2]^+$=571.2.

5.1.4.5. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(phenylthiomethyl)-thiophen-2-yl]-isoindole-1,3-dione (6aq). Following general procedure (C), the product **6aq** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 69%. Yellow solid, mp 127-129 °C. ^{1}H NMR ($CDCl_3$) δ: 4.14 (s, 2H), 6.32 (d, *J*=8.4 Hz, 2H), 6.68 (m, 1H), 7.06 (m, 4H), 7.34 (d, *J*=8.6 Hz, 2H), 7.74 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.4, 112.5, 124.2 (2C), 127.6, 128.7 (2C), 129.0 (2C), 130.6 (2C), 130.8 (2C), 132.1, 133.7, 134.2, 134.8, 135.1 (2C), 135.7 (2C), 137.3, 139.5, 165.5 (2C), 189.1. MS (ESI): $[M]^+$=567.2, $[M+2]^+$=569.3.

5.1.5. General procedure (D) for the synthesis of compounds 7a-z and 7am-aq.

A solution of derivative **6a-z** or **6am-aq** (2 mmol) in DMF (20 mL), containing Et_3N (0.3 mL, 2 mmol, 1 equiv.) was hydrogenated over 120 mg of 10% Pd/C at 60 p.s.i for 3 h. The catalyst was removed by filtration, the filtrate concentrated, and the residue dissolved in dichloromethane (20 mL), washed with water (5 mL), brine (5 mL), dried (Na_2SO_4). After filtering, the solvent was removed *in vacuo* to obtain a residue that was purified by column chromatography.

5.1.5.1. 2-[3-(4-Chlorobenzoyl)-4-(1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7a). Following general procedure (D), derivative **7a** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 68%. White solid, mp 148-150 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (s, 2H), 3.42 (t, *J*=5.6 Hz, 2H), 4.12 (t, *J*=5.6 Hz, 2H), 4.36 (s, 2H), 6.04 (s, 1H), 6.26 (s, 1H), 7.17 (m, 4H), 7.42 (m, 4H), 7.584 (m, 4H). MS (ESI): $[M+1]^+$= 552.2.

5.1.5.2. 2-[3-(4-Chlorobenzoyl)-4-(8-fluoro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7b). Following general procedure (D), derivative **7b** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 68%. Yellow solid, mp 145-147 °C. ^{1}H NMR ($CDCl_3$) δ: 2.48 (t, *J*=5.4 Hz, 2H), 3.16 (s, 2H), 3.26 (s, 2H), 3.82 (t, *J*=5.6 Hz, 2H), 6.04 (s, 1H), 6.26 (s, 1H), 7.17 (m, 4H), 7.42 (m, 4H), 7.58 (m, 4H). MS (ESI): $[M+1]^+$= 570.3.

5.1.5.3. 2-[3-(4-Chlorobenzoyl)-4-(8-chloro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7c). Following general procedure (D), derivative **7c** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 68%. Yellow solid, mp 145-147 °C. ^{1}H NMR ($CDCl_3$) δ: 2.43(t, *J*=5.4 Hz, 2H), 3.14 (s, 2H), 3.25 (s, 2H), 3.79 (t, *J*=5.4 Hz, 2H), 6.01 (s, 1H), 6.63 (s, 1H), 7.12 (m, 4H), 7.39 (m, 2H),), 7.52 (s, 1H), 7.68 (m, 4H). MS (ESI): $[M+1]^+$= 586.2.

5.1.5.4. 2-[3-(4-Chlorobenzoyl)-4-(8-methoxy-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7d). Following general procedure (D), derivative **7d** was purified by column chromatography (EtOAc:dichloromethane 2:8). Yield: 74%. Yellow solid, mp 135-137 °C. ^{1}H NMR ($CDCl_3$) δ: 2.42 (t, *J*=5.4 Hz, 2H), 3.13 (s, 2H), 3.26 (s, 2H), 3.79 (t, *J*=5.4 Hz, 2H), 3.84 (s, 3H), 6.02 (s, 1H), 6.64 (s, 1H), 7.15 (m, 4H), 7.35 (m, 2H), 7.56 (s, 1H), 7.71 (m, 4H). MS (ESI): $[M+1]^+$= 582.2.

5.1.5.5. 2-[3-(4-Chlorobenzoyl)-4-(1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7e). Following general procedure (D), derivative **7e** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 71%. Yellow solid, mp 130-131 °C. ^{1}H NMR ($CDCl_3$) δ: 2.54 (m, 2H), 2.90 (m, 2H), 2.96 (m, 4H), 3.11 (d, *J*=11.6 Hz, 1H), 3.44 (m, 2H), 6.40 (d, *J*=7.6 Hz, 1H), 6.66 (m, 2H), 7.12 (m, 2H), 7.24 (d, *J*=8.8 Hz, 2H), 7.48 (d, *J*=8.8 Hz, 2H), 7.65 (m, 4H). MS (ESI): $[M+1]^+$= 554.3.

5.1.5.6. 2-[3-(4-Chlorobenzoyl)-4-(8-chloro-1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7f). Following general procedure (D), derivative **7f** was purified by column chromatography (EtOAc:dichloromethane 2:8). Yield: 70%. Yellow solid, mp 138-140 °C. ^{1}H NMR ($CDCl_3$) δ: 2.44 (m, 2H), 2.82 (m, 2H), 2.92 (m, 4H), 3.10 (d, *J*=11.4 Hz, 1H), 3.28 (m, 2H), 6.30 (d, *J*=8.0 Hz, 1H), 6.62 (s, 1H), 7.00 (d, *J*=8.0 Hz, 1H), 7.04 (s, 1H), 7.46 (d, *J*=8.8 Hz, 2H), 7.60 (d, *J*=8.8 Hz, 2H), 7.68 (m, 4H). MS (ESI): $[M+1]^+$= 588.3.

5.1.5.7. 2-[3-(4-Chlorobenzoyl)-4-(1,2.3,4,10,10a-hexahydro-8-methyl-1H-pyrazino[1,2-a]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7g). Following general procedure (D), derivative **7g** was purified by column chromatography (EtOAc:dichloromethane 0.5:9.5). Yield: 64%. Yellow solid, mp 134-136 °C. ^{1}H NMR ($CDCl_3$) δ: 2.21 (s, 3H), 2.42 (m, 2H), 2.74 (m, 2H), 2.90 (m, 4H), 3.08 (d, *J*=11.4 Hz, 1H), 3.22 (m, 2H), 6.12 (s, 1H), 7.00 (d, *J*=8.0 Hz, 1H), 7.11 (m, 2H), 7.36 (m, 2H), 7.44 (d, *J*=8.4 Hz, 2H), 7.64 (m, 4H). MS (ESI): $[M+1]^+$= 568.2.

5.1.5.8. 2-[3-(4-Chlorobenzoyl)-4-(1,2,3,4-tetrahydro-1H-isoquinolin-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7h). Following general procedure (D), derivative **7h** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 89%. White solid, mp 98-100 °C. ^{1}H NMR ($CDCl_3$) δ: 2.25 (t, *J*=5.4 Hz, 2H), 2.64 (t, *J*=5.6 Hz, 2H), 2.94 (s, 2H), 3.22 (s, 2H), 6.71 (s, 1H), 6.89 (d, *J*=7.8 Hz, 1H), 7.13 (m, 3H), 7.35 (m, 6H), 7.54 (t, *J*=8.8 Hz, 1H), 7.78 (t, *J*=8.8 Hz, 1H). MS (ESI): $[M+1]^+$= 513.3.

5.1.5.9. 2-[3-(4-Chlorobenzoyl)-4-(2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7i). Following general procedure (D), derivative **7i** was purified by column chromatography (EtOAc:petroleum ether 1:1). Yield: 52%. Yellow solid, mp 112-114 °C. ^{1}H NMR ($CDCl_3$) δ: 2.72 (t, *J*=5.8 Hz, 2H), 3.20 (t, *J*=5.8 Hz, 2H), 3.46 (s, 2H), 3.84 (s, 2H), 6.84 (s, 1H), 7.06 (m, 4H), 7.22 (d, *J*=8.6 Hz, 1H); 7.34 (d, *J*=8.6 Hz, 2H), 7.44 (d, *J*=7.6 Hz, 1H), 7.62 (m, 4H), 7.88 (bs, 1H). MS (ESI): $[M+1]^+$= 552.2.

5.1.5.10. 2-[3-(4-Chlorobenzoyl)-4-(8-chloro-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7j). Following general procedure (D), derivative **7j** was purified by column chromatography (EtOAc:petroleum ether 9:1). Yield: 55%. Yellow solid, mp 165-167 °C. ^{1}H NMR ($CDCl_3$) δ: 2.74 (t, *J*=5.8 Hz, 2H), 3.24 (t, *J*=5.6 Hz, 2H), 3.70 (s, 2H), 4.00 (s, 2H), 7.00 (d, *J*=8.6 Hz, 1H), 7.04 (s, 1H), 7.15 (d, *J*=8.6 Hz, 2H), 7.24 (d, *J*=8.6 Hz, 1H), 7.38 (s, 1H); 7.46 (d, *J*=8.6 Hz, 2H), 7.60 (m, 4H), 7.82 (bs, 1H). MS (ESI): $[M+1]^+$= 586.1.

5.1.5.11. 2-[3-(4-Chlorobenzoyl)-4-(5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7k). Following general procedure (D), derivative **7k** was purified by column chromatography (EtOAc:petroleum ether 7:3). Yield: 55%. Yellow solid, mp 124-126 °C. ^{1}H NMR ($CDCl_3$) δ: 2.76 (t, *J*=5.6 Hz, 2H), 3.27 (t, *J*=5.6 Hz, 2H), 3.62 (s, 3H), 4.22 (m, 4H), 7.10 (m, 5H), 7.32 (d, *J*=8.6 Hz, 2H); 7.46 (d, *J*=8.6 Hz, 2H), 7.66 (m, 4H). MS (ESI): $[M+1]^+$= 566.2.

5.1.5.12. 2-[3-(4-Chlorobenzoyl)-4-(8-chloro-5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-ylmethyl)-thiophen-2-yl]-isoindole-1,3-dione (7l). Following general procedure (D), derivative **7l** was purified by column chromatography (EtOAc:petroleum ether 9:1). Yield: 65%. Yellow solid, mp 145-147 °C. ^{1}H NMR ($CDCl_3$) δ: 2.72 (t, *J*=5.6 Hz, 2H), 3.24 (t, *J*=5.6 Hz, 2H), 3.54 (s, 3H), 3.63 (s, 2H), 4.12 (s, 2H), 6.84 (s, 1H), 7.11 (d, *J*=8.0 Hz, 2H), 7.16 (m, 2H), 7.32 (m, 3H), 7.64 (m, 4H). MS (ESI): $[M+1]^+$= 600.5.

5.1.5.13. 2-[3-(4-Chlorobenzoyl)-4-(spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (7m). Following general procedure (D), derivative **7m** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 95%. Yellow solid, mp 142-144 °C. ^{1}H NMR ($CDCl_3$) δ: 2.07 (t, *J*=6.0 Hz, 4H), 3.19 (t, *J*=6.0 Hz, 4H), 3.49 (s, 2H), 6.76 (m, 5H), 7.38 (d, *J*=8.6 Hz, 2H), 7.44 (d, *J*=8.6 Hz, 2H), 7.64 (m, 4H). MS (ESI): $[M+1]^+$= 571.2.

5.1.5.14. 2-[3-(4-Chlorobenzoyl)-4-(4-fluoro-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (7n). Following general procedure (D), derivative **7n** was purified by column chromatography (EtOAc:dichloromethane 0.25:9.75). Yield: 55%. Yellow solid, mp 210-212 °C. ^{1}H NMR ($CDCl_3$) δ: 2.08 (t, *J*=6.0 Hz, 4H), 3.04 (t, *J*=6.0 Hz, 4H), 3.34 (s, 2H), 6.56 (d, *J*=8.4 Hz, 1H), 6.64 (m, 2H), 6.74 (m, 1H), 7.42 (d, *J*=8.6 Hz, 2H), 7.56 (d, *J*=8.6 Hz, 2H),7.64 (m, 4H). MS (ESI): $[M+1]^+$= 589.2.

5.1.5.15. 2-[3-(4-Chlorobenzoyl)-4-(4-methyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (7o). Following general procedure (D), derivative **7o** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 79%. White solid, mp 166-168 °C. ^{1}H NMR ($CDCl_3$) δ: 1.89 (t, *J*=5.6 Hz, 4H), 2.20 (s, 3H), 3.13 (m, 4H), 3.43 (s, 2H), 6.63 (m, 2H), 6.70 (m, 2H), 7.44 (d, *J*=8.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 2H), 7.63 (m, 4H). MS (ESI): $[M+1]^+$= 585.3.

5.1.5.16. 2-[3-(4-Chlorobenzoyl)-4-(5-methyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (7p). Following general procedure (D), derivative **7p** was purified by column chromatography (EtOAc:petroleum ether 2:8). Yield: 77%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.97 (t, *J*=6.0 Hz, 4H), 2.26 (s, 3H), 3,37 (t, *J*=6.0 Hz, 4H), 3.57 (s, 2H), 6.44 (m, 3H), 6.50 (d, *J*=8.0 Hz, 1H), 7.42 (d, *J*=8.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 2H), 7.67 (m, 4H). MS (ESI): $[M+1]^+$= 585.1.

5.1.5.17. 2-[3-(4-Chlorobenzoyl)-4-(5-t-butyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-ylmethyl-thiophen-2-yl]-isoindole-1,3-dione (7q). Following general procedure (D), derivative **7q** was purified by column chromatography (EtOAc:dichloromethane 1:9). Yield: 88%. Yellow solid, mp 188-190 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (s, 9H), 2.08 (t, *J*=6.0 Hz, 4H), 3.22 (t, *J*=6.0 Hz, 4H), 3.44 (s, 2H), 6.69 (d, *J*=8.4 Hz, 1H), 6.79 (m, 2H), 6.85 (s, 1H), 7.36 (d, *J*=8.6 Hz, 2H), 7.48 (d, *J*=8.6 Hz, 2H), 7.66 (m, 4H). MS (ESI): $[M+1]^+$= 627.3.

5.1.5.18. 2-[3-(4-Chlorobenzoyl)-4-(N-methyl-N-phenyl-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7r). Following general procedure (D), the product **7r** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 51%. Yellow solid, mp 149-150 °C. ^{1}H NMR ($CDCl_3$) δ: 2.93 (s, 3H), 4.49 (s, 2H), 6.62 (d, *J*=8.6 Hz, 2H), 6.66 (t, *J*=7.2 Hz, 1H), 7.14 (m, 5H), 7.58 (d, *J*=8.6 Hz, 2H), 7.76 (m, 4H). MS (ESI): $[M+1]^+$= 487.1.

5.1.5.19. 2-[5-Bromo-3-(4-chlorobenzoyl)-4-(N-ethyl-N-phenyl-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7s). Following general procedure (D), the product **7s** was purified by column chromatography (EtOAc:-petroleum ether 1.5:8.5 as eluent). Yield 56%. Yellow solid, mp 153-155 °C. ^{1}H NMR ($CDCl_3$) δ: 1.14 (t, *J*=7.0 Hz, 3H), 3.37 (q, *J*=7.0 Hz, 2H), 4.48 (s, 2H), 6.60 (d, *J*=8.8 Hz, 2H), 6.64 (t, *J*=7.2 Hz, 1H), 7.16 (m, 5H), 7.58 (d, *J*=8.8 Hz, 2H), 7.76 (m, 4H). MS (ESI): $[M+1]^+$= 501.2.

5.1.5.20. 2-[3-(4-Chlorobenzoyl)-4-(N-(4-fluorophenyl)-N-methyl-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7t). Following general procedure (D), the product **7t** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 45%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 2.84 (s, 3H), 4.42 (s, 2H), 6.56 (m, 2H), 6.87 (t, *J*=8.6 Hz, 2H), 7.16 (d, *J*=8.6 Hz, 2H), 7.22 (s, 1H), 7.55 (d, *J*=8.6 Hz, 2H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$= 505.1.

5.1.5.21. 2-[3-(4-Chlorobenzoyl)-4-(N-(4-chlorophenyl)-N-methyl-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7u). Following general procedure (D), the product **7u** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 55%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.90 (s, 3H), 4.49 (s, 2H), 6.53 (d, *J*=9.2 Hz, 2H), 7.09 (d, *J*=9.2 Hz, 2H), 7.13 (s, 1H), 7.19 (d, *J*=8.8 Hz, 2H), 7.56 (d, *J*=8.8 Hz, 2H), 7.74 (m, 4H). MS (ESI): $[M+1]^+$= 521.4.

5.1.5.22. 2-[3-(4-Chlorobenzoyl)-4-(N-methyl-N-(4-trifluoromethylphenyl)-aminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7v). Following general procedure (D), the product **7v** was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 87%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.02 (s, 3H), 4.60 (s, 2H), 6.64 (d, *J*=8.8 Hz, 2H), 7.09 (s, 1H), 7.16 (d, *J*=8.8 Hz, 2H), 7.42 (d, *J*=8.8 Hz, 2H), 7.57 (d, *J*=8.8 Hz, 2H), 7.77 (m, 4H). MS (ESI): $[M+1]^+$= 555.0.

5.1.5.23. 2-[3-(4-Chlorobenzoyl)-4-(N-benzyl-N-methylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7w). Following general procedure (D), the product **7w** was purified by column chromatography (EtOAc:petroleum ether 3:7 as eluent). Yield 56%. Yellow solid, mp 134-136 °C. ^{1}H NMR ($CDCl_3$) δ: 2.90 (s, 3H), 3.44 (s, 2H), 4.49 (s, 2H), 6.60 (d, *J*=8.4 Hz, 2H), 6.62 (t, *J*=7.2 Hz, 1H), 7.08 (m, 5H), 7.50 (d, *J*=8.4 Hz, 2H), 7.72 (m, 4H). MS (ESI): $[M+1]^+$= 501.2.

5.1.5.24. 2-[3-(4-Chlorobenzoyl)-4-(N,N-diethylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7x). Following general procedure (D), the product **7x** was purified by column chromatography (EtOAc:petroleum ether 1:1 as eluent). Yield 68%. Brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.18 (t, *J*=6.8

Hz, 6H), 2.04 (q, *J*=6.8 Hz, 4H), 3.16 (s, 2H), 7.17 (s, 1H), 7.49 (d, *J*=7.8 Hz, 2H), 7.56 (d, *J*=7.8 Hz, 2H), 7.65 (m, 4H). MS (ESI): $[M+1]^+$= 453.1.

5.1.5.25. 2-[3-(4-Chlorobenzoyl)-4-(N,N-dipropylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7y). Following general procedure (D), the product **7y** was purified by column chromatography (EtOAc:petroleum ether 6:4 as eluent). Yield 48%. Brown oil. ^{1}H NMR ($CDCl_3$) δ: 0.75 (t, *J*=7.2 Hz, 6H), 1.28 (m, 4H), 2.17 (t, *J*=7.2 Hz, 4H), 3.48 (s, 2H), 5.30 (s, 1H), 7.22 (d, *J*=8.0 Hz, 2H), 7.66 (d, *J*=7.8 Hz, 2H), 7.73 (m, 4H). MS (ESI): $[M+1]^+$= 481.1.

5.1.5.26. 2-[3-(4-Chlorobenzoyl)-4-(N,N-diisopropylaminomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7z). Following general procedure (D), the product **7z** was purified by column chromatography (EtOAc:petroleum ether 4:6 as eluent). Yield 56%. Brown oil. ^{1}H NMR ($CDCl_3$) δ: 0.87 (d, *J*=6.6 Hz, 12H), 2.90 (m, 2H), 3.62 (s, 2H), 5.29 (s, 1H), 7.20 (d, *J*=8.6 Hz, 2H), 7.68 (d, *J*=8.6 Hz, 2H), 7.78 (m, 4H). MS (ESI): $[M+1]^+$= 481.2.

5.1.5.27. 2-[3-(4-Chlorobenzoyl)-4-(phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (7am). Following general procedure (D), the product **7am** was purified by column chromatography (EtOAc:petroleum ether 4:6 as eluent). Yield 64%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 5.10 (s, 2H), 6.74 (d, *J*=8.6 Hz, 2H), 6.82 (s, 1H), 7.19 (m, 5H), 7.57 (d, *J*=8.6 Hz, 2H), 7.76 (m, 4H). MS (ESI): $[M+1]^+$= 474.0.

5.1.5.28. 2-[3-(4-Chlorobenzoyl)-4-(4-(methyl)-phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (7an). Following general procedure (D), the product **7an** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 45%. White solid, mp 84-86 °C. ^{1}H NMR ($CDCl_3$) δ: 2.26 (s, 3H), 5.09 (s, 2H), 6.72 (d, *J*=8.6 Hz, 2H), 7.01 (d, *J*=8.8 Hz, 2H), 7.19 (d, *J*=8.8 Hz, 2H), 7.51 (s, 1H), 7.56 (d, *J*=8.6 Hz, 2H), 7.75 (m, 4H). MS (ESI): $[M+1]^+$= 488.1.

5.1.5.29. 2-[3-(4-Chlorobenzoyl)-4-(4-(isopropyl)-phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (7ao). Following general procedure (D), the product **7ao** was purified by column

chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 45%. White solid, mp 104-106 °C. ^{1}H NMR ($CDCl_3$) δ: 1.19 (d, *J*=7.0 Hz, 6H), 2.82 (m, 1H), 5.09 (s, 2H), 6.75 (d, *J*=8.6 Hz, 2H), 7.07 (d, *J*=8.8 Hz, 2H), 7.19 (d, *J*=8.8 Hz, 2H), 7.51 (s, 1H), 7.59 (d, *J*=8.6 Hz, 2H), 7.79 (m, 4H). MS (ESI): $[M+1]^+$= 516.1.

5.1.5.30. 2-[3-(4-Chlorobenzoyl)-4-(4-(fluoro)-phenyloxymethyl)-thiophen-2-yl]-isoindole-1,3-dione (7ap). Following general procedure (D), the product **7ap** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 54%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 5.09 (s, 2H), 6.79 (m, 2H), 6.92 (d, *J*=8.4 Hz, 2H), 7.16 (d, *J*=8.4 Hz, 2H), 7.50 (s, 1H), 7.59 (d, *J*=8.4 Hz, 2H), 7.77 (m, 4H). MS (ESI): $[M+1]^+$= 492.1.

5.1.5.31. 2-[3-(4-Chlorobenzoyl)-4-(phenylthiomethyl)-thiophen-2-yl]-isoindole-1,3-dione (7aq). Following general procedure (D), the product **7aq** was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 83%. White solid, mp 178-180 °C. ^{1}H NMR ($CDCl_3$) δ: 4.19 (s, 2H), 7.15 (d, *J*=8.6 Hz, 2H), 7.22 (m, 6H), 7.64 (d, *J*=8.6 Hz, 2H), 7.76 (m, 4H). MS (ESI): $[M+1]^+$= 490.1.

5.1.6. General procedure (E) for the synthesis of compounds (3a-aq).

A stirred suspension of the appropriate thiophene derivatives **6aa-6ak, 7a-7z, or 7am-7aq** (0.5 mmol) and 100% hydrazine monohydrate (1.2 eq, 0.6 mmol, 29 μL) in abs. ethanol (10 mL) was refluxed for 1 h. The solvent was evaporated and the residue was partitioned between dichloromethane (10 mL) and water (5 mL). The separated organic phase was washed with brine (2 mL), dried (Na_2SO_4), then concentrated *in vacuo* to obtain a residue that was purified by column chromatography to give the desired products **3a-aq**.

5.1.6.1. [2-Amino-4-((1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl]-(4-chlorophenyl)methanone (3a). Following general procedure (E), derivative **3a** was purified by column chromatography (dichloromethane as eluent). Yield: 51%. Yellow solid, mp 75-77 °C. ^{1}H

NMR (d_6-DMSO) δ: 2.34 (t, *J*=5.2 Hz, 2H), 3.09 (s, 2H), 3.21 (s, 2H), 3.81 (t, *J*=5.2 Hz, 2H), 6.03 (s, 1H), 6.35 (s, 1H), 7.00 (t, *J*=6.8 Hz, 1H), 7.05 (t, *J*=6.8 Hz, 1H), 7.28 (d, *J*=7.6 Hz, 1H), 7.39 (m, 3H), 7.48 (d, *J*=8.4 Hz, 2H), 7.66 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 41.1, 49.3, 49.9, 56.9, 95.7, 106.4, 109.0, 112.4, 119.3, 119.4, 119.9, 127.6, 127.8 (2C), 129.3 (2C), 134.2, 134.7, 135.2, 135.4, 140.4, 16.1, 190.5. MS (ESI): $[M+1]^+$= 422.2. Anal. calcd for $C_{23}H_{20}ClN_3OS$: C, 65.47; H, 4.78; N, 9.96; found: C, 65.21; H, 4.48; N, 9.78.

5.1.6.2. [2-Amino-4-((8-fluoro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl]-(4-chlorophenyl) methanone (3b). Following general procedure (E), derivative **3b** was purified by column chromatography (dichloromethane:EtOAc 9.5:0.5). Yield: 41%. Yellow solid, mp 163-165 °C. ^{1}H NMR (d_6-DMSO) δ: 2.06 (t, *J*=4.8 Hz, 2H), 3.06 (s, 2H), 3.18 (s, 2H), 3.79 (t, *J*=4.8 Hz, 2H), 6.02 (s, 1H), 6.32 (s, 1H), 6.84 (dt, *J*=9.6 and 2.4 Hz, 1H), 7.07 (d, *J*=9.6 and 2.4 Hz, 1H), 7.27 (m, 1H), 7.39 (d, *J*=8.6 Hz, 2H), 7.44 (d, *J*=8.6 Hz, 2H), 7.65 (bs, 2H),. ^{13}C NMR (100 MHz, d_6-DMSO) δ: 41.4, 49.2, 49.9, 56.9, 96.1, 104.1, 104.4, 106.6, 107.8, 108.1, 109.9, 112.5, 127.9 (2C), 128.0 (2C), 129.4, 132.2, 136.3, 140.5, 156.2 (d, *J*=242 Hz), 166.2, 190.6. MS (ESI): $[M+1]^+$= 440.1. Anal. calcd for $C_{23}H_{19}ClFN_3OS$: C, 62.79; H, 4.35; N, 9.55; found: C, 62.56; H, 4.16; N, 9.38.

5.1.6.3. [2-Amino-4-((8-chloro-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl]-(4-chlorophenyl)methanone (3c). Following general procedure (E), derivative **3c** was purified by column chromatography (dichloromethane:EtOAc 9.5:0.5). Yield: 45%. Yellow solid, mp 178-180 °C. ^{1}H NMR (d_6-DMSO) δ: 2.34 (t, *J*=5.2 Hz, 2H), 3.08 (s, 2H), 3.20 (s, 2H), 3.81 (t, *J*=5.2 Hz, 2H), 6.04 (s, 1H), 6.34 (s, 1H), 7.04 (dd, *J*=8.4 and 2.0 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 1H), 7.41 (d, *J*=8.8 Hz, 2H), 7.47 (m, 3H), 7.68 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 41.3, 48.9, 49.7, 56.7, 95.6, 106.5, 110.5, 112.3, 118.6, 119.8, 123.9, 127.8 (2C), 128.7, 129.3 (2C), 133.9, 134.7, 135.2, 136.0, 140.4, 166.1, 190.5. MS (ESI): $[M+1]^+$= 456.3. Anal. calcd for $C_{23}H_{19}Cl_2N_3OS$: C, 60.53; H, 4.20; N, 9.21; found: C, 60.41; H, 4.03; N, 9.20.

5.1.6.4. [2-Amino-4-((8-methoxy-1,2,3,4-tetrahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3d). Following general procedure (E), derivative **3d** was purified by column chromatography (dichloromethane:EtOAc 9.5:0.5). Yield: 54%. Yellow solid, mp 168-170 °C. ^{1}H NMR (d_6-DMSO) δ: 2.31 (t, *J*=4.8 Hz, 2H), 3.07 (s, 2H), 3.17 (s, 2H), 3.72 (s, 3H), 3.76 (t, *J*=4.8 Hz, 2H), 5.95 (s, 1H), 6.34 (s, 1H), 6.69 (dd, *J*=8.8 and 2.8 Hz, 1H), 6.94 (d, *J*=2.8 Hz, 1H), 7.19 (d, *J*=8.8 Hz, 1H), 7.41 (d, *J*=8.4 Hz, 2H), 7.48 (d, *J*=8.4 Hz, 2H), 7.67 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 41.3, 49.4, 50.0, 55.3, 57.0, 95.7, 101.6, 106.5, 109,7, 109.8, 112.5, 127.9 (2C), 128.2, 129.4 (2C), 130.8, 134.8 (2C), 135.4, 140.5, 153.8, 166.2, 190.6. MS (ESI): $[M+1]^+$= 452.1. Anal. calcd for $C_{24}H_{22}ClN_3O_2S$: C, 63.78; H, 4.91; N, 9.30; found: C, 63.59; H, 4.77; N, 9.19.

5.1.6.5. [2-Amino-4-((1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3e). Following general procedure (E), derivative **3e** was purified by column chromatography (dichloromethane:EtOAc 9:1). Yield: 52%. Yellow solid, mp 70-71 °C. ^{1}H NMR ($CDCl_3$) δ: 1.86 (m, 2H), 2.42 (m, 2H), 2.78 (m, 5H), 3.42 (m, 2H), 6.07 (bs, 2H), 6.36 (d, *J*=8.0 Hz, 1H), 6.62 (d, *J*=8.0 Hz, 1H), 7.02 (m, 3H), 7.39 (d, *J*=8.4 Hz, 2H), 7.54 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 424.1. Anal. calcd for $C_{23}H_{22}ClN_3OS$: C, 65.16; H, 5.23; N, 9.91; found: C, 65.02; H, 5.04; N, 9.78.

5.1.6.6. [2-Amino-4-((8-chloro-1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3f). Following general procedure (E), derivative **3f** was purified by column chromatography (dichloromethane:EtOAc 9:1). Yield: 43%. Yellow solid, mp 133-135 °C. ^{1}H NMR ($CDCl_3$) δ: 1.86 (m, 2H), 2.42 (m, 2H), 2.78 (m, 4H), 3.12 (m, 1H), 3.42 (m, 2H), 4.86 (bs, 2H), 6.24 (d, *J*=8.0 Hz, 1H), 6.66 (s, 1H), 6.96 (d, *J*=8.0 Hz, 1H), 7.08 (s, 1H), 7.42 (d, *J*=8.8 Hz, 2H), 7.60 (d, *J*=8.8 Hz, 2H). MS (ESI): $[M+1]^+$= 458.4. Anal. calcd for $C_{23}H_{21}Cl_2N_3OS$: C, 60.26; H, 4.62; N, 9.17; found: C, 60.02; H, 4.48; N, 9.01.

5.1.6.7. [2-Amino-4-((8-methyl-1,2.3,4,10,10a-hexahydro-1H-pyrazino[1,2-a]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3g). Following general procedure (E), derivative **3g** was purified by column chromatography (dichloromethane:EtOAc 9.5:0.5). Yield: 51%. Yellow solid, mp 145-147 °C. ^{1}H NMR ($CDCl_3$) δ: 1.88 (m, 2H), 2.44 (s, 3H), 2.52 (m, 2H),3.24 (t, *J*=5.2 Hz, 2H), 3.78 (t, *J*=5.2 Hz, 2H), 3.92 (m, 1H), 4.22 (s, 2H), 6.12 (s, 1H), 6.84 (bs, 2H), 7.00 (d, *J*=8.0 Hz, 1H), 7.12 (m, 2H), 7.36 (m, 2H), 7.56 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 438.2. Anal. calcd for $C_{24}H_{24}Cl_2N_3OS$: C, 65.81; H, 5.52; N, 9.59; found: C, 65.62; H, 5.38; N, 9.41.

5.1.6.8. [2-Amino-4-((1,2,3,4-tetrahydro-1H-isoquinolin-2-yl)methyl)-thiophen-3-yl]-(4-chlorophenyl)methanone (3h). Following general procedure (E), derivative **3h** was purified by column chromatography (dichloromethane:EtOAc 8:2). Yield: 46%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.24 (t, *J*=5.6 Hz, 2H), 2.65 (t, *J*=5.6 Hz, 2H), 3.01 (s, 2H), 3.08 (s, 2H), 6.13 (bs, 2H), 6.18 (s, 1H), 7.07 (m, 4H), 7.33 (d, *J*=8.4 Hz, 2H), 7.41 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 383.2. Anal. calcd for $C_{21}H_{19}ClN_2OS$: C, 65.71; H, 5.00; N, 7.32; found: C, 65.48; H, 4.93; N, 7.11.

5.1.6.9. [2-Amino-4-((2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3i). Following general procedure (E), derivative **3i** was purified by column chromatography (petroleum ether:EtOAc 1:1). Yield: 46%. Yellow solid, mp 82-84 °C. ^{1}H NMR ($CDCl_3$) δ: 2.42 (t, *J*=5.8 Hz, 2H), 2.64 (t, *J*=5.8 Hz, 2H), 2.95 (s, 2H), 3.25 (s, 2H), 6.07 (bs, 2H), 7.02 (m, 5H), 7.36 (m, 4H); 7.72 (bs, 1H). MS (ESI): $[M+1]^+$= 422.2. Anal. calcd for $C_{23}H_{20}ClN_3OS$: C, 65.47; H, 4.78; N, 9.96; found: C, 65.23; H, 4.56; N, 9.72.

5.1.6.10. [2-Amino-4-((8-chloro-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3j). Following general procedure (E), derivative **3j** was purified by column chromatography (petroleum ether:EtOAc 0.5:9.5). Yield: 55%. Yellow solid, mp 119-120 °C. ^{1}H NMR ($CDCl_3$) δ: 2.42 (t, *J*=7.2 Hz, 2H), 2.54 (t, *J*=7.2 Hz, 2H), 3.20 (s, 2H), 3.67 (s, 2H), 6.10 (bs, 2H), 7.02 (m, 2H), 7.16 (d, *J*=8.6 Hz, 2H), 7.25 (d, *J*=8.6 Hz, 1H), 7.38 (m, 3H), 7.82 (bs,

1H). MS (ESI): $[M+1]^+$= 456.1. Anal. calcd for $C_{23}H_{19}Cl_2N_3OS$: C, 60.53; H, 4.20; N, 9.21; found: C, 60.37; H, 4.02; N, 9.01.

5.1.6.11. [2-Amino-4-((5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-yl)methyl)-thiophen-3-yl](4-chlorophenyl)methanone (3k). Following general procedure (E), derivative **3k** was purified by column chromatography (petroleum ether:EtOAc 7:3). Yield: 34%. Yellow solid, mp 78-80 °C. ^{1}H NMR (d_6-DMSO) δ: 2.24 (t, *J*=5.6 Hz, 2H), 2.56 (t, *J*=5.6 Hz, 2H), 2.98 (s, 2H), 3.11 (s, 2H), 3.56 (s, 3H), 6.31 (s, 1H), 6.92 (t, *J*=7.6 Hz, 1H), 7.04 (t, *J*=7.6 Hz, 1H), 7.16 (d, *J*=7.6 Hz, 1H); 7.34 (d, *J*=8.4 Hz, 2H), 7.39 (m, 3H), 7.59 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 21.8, 28.8, 48.4, 49.3, 56.9, 105.8, 106.4, 108.9, 112.6, 116.7, 118.3, 120.0, 124.8, 127.7 (2C), 129.3 (2C), 133.9, 134.5, 136.4, 136.5, 140.5, 165.7, 190.6. MS (ESI): $[M+1]^+$= 436.2. Anal. calcd for $C_{24}H_{22}ClN_3OS$: C, 66.12; H, 5.09; N, 9.64; found: C, 65.93; H, 4.89; N, 9.42.

5.1.6.12. [2-Amino-4-((8-chloro-5-methyl-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-2-yl)methyl)thiophen-3-yl](4-chlorophenyl)methanone (3l). Following general procedure (E), derivative **3l** was purified by column chromatography (petroleum ether:EtOAc 1:9). Yield: 52%. Yellow solid, mp 60-62 °C. ^{1}H NMR (d_6-DMSO) δ: 2.24 (t, *J*=4.8 Hz, 2H), 2.56 (t, *J*=4.8 Hz, 2H), 2.94 (s, 2H), 3.10 (s, 2H), 3.57 (s, 3H), 6.30 (s, 1H), 7.06 (dd, *J*=8.8 and 2.4 Hz, 1H), 7.19 (d, *J*=2.4 Hz, 1H); 7.35 (d, *J*=8.8 Hz, 2H), 7.39 (m, 3H), 7.61 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 21.9, 29.1, 48.2, 49.2, 56.9, 105.9, 106.5, 110.6, 112.7, 116.3, 119.8, 123.1, 125.9, 127.8 (2C), 129.3 (2C), 134.6, 134.9, 136.1, 136.5, 140.6, 165.9, 190.7. MS (ESI): $[M+1]^+$= 470.4. Anal. calcd for $C_{24}H_{21}Cl_2N_3OS$: C, 61.28; H, 4.50; N, 8.93; found: C, 61.04; H, 4.33; N, 8.74.

5.1.6.13. (2-Amino-4-(((spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3m). Following general procedure (E), derivative **3m** was purified by column chromatography (dichloromethane:EtOAc 1:9). Yield: 34%. Yellow solid, mp 209-211 °C. ^{1}H NMR ($CDCl_3$) δ: 2.06 (t, *J*=6.0 Hz, 4H), 3.02 (t, *J*=6.0 Hz, 4H), 3.52 (s, 2H), 6.02 (bs, 2H), 6.76

(m, 5H), 7.42 (d, *J*=8.6 Hz, 2H), 7.57 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 441.1. Anal. calcd for $C_{23}H_{21}ClN_2O_3S$: C, 62.65; H, 4.80; N, 6.35; found: C, 62.44; H, 4.68; N, 6.18.

5.1.6.14. (2-Amino-4-(((4-fluoro-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3n). Following general procedure (E), derivative **3n** was purified by column chromatography (dichloromethane:EtOAc 9.9:0.1). Yield: 85%. Yellow solid, mp 80-81 °C. ^{1}H NMR (d_6-DMSO) δ: 1.75 (t, *J*=4.8 Hz, 4H), 1.95 (t, *J*=4.8 Hz, 4H), 2.94 (s, 2H), 6.25 (s, 1H), 6.72 (m, 1H), 6.78 (m, 2H), 7.51 (s, 4H), 7.61 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 33.9 (2C), 44.7 (2C), 56.5, 105.1, 105.9, 109.5, 112.4, 118.6, 121.6, 127.7 (2C), 129.3 (2C), 132.7, 134.,6, 136.0, 140.6, 144.5 (*J*=242 Hz), 148.9, 165.7, 190.6. MS (ESI): $[M+1]^+$= 459.1. Anal. calcd for $C_{23}H_{20}ClFN_2O_3S$: C, 60.19; H, 4.39; N, 6.10; found: C, 61.92; H, 4.23; N, 5.97.

5.1.6.15. (2-Amino-4-(((4-methyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3o). Following general procedure (E), derivative **3o** was purified by column chromatography (dichloromethane:EtOAc 7:3). Yield: 61%. Yellow solid, mp 184-186 °C. ^{1}H NMR ($CDCl_3$) δ: 1.93 (t, *J*=5.6 Hz, 4H), 2.20 (s, 3H), 3.14 (m, 4H), 3.46 (s, 2H), 5.84 (m, 2H), 6.64 (m, 3H), 6.88 (s, 1H), 7.42 (d, *J*=8.8 Hz, 2H), 7.53 (d, *J*=8.8 Hz, 2H). MS (ESI): $[M+1]^+$= 455.1. Anal. calcd for $C_{24}H_{23}ClN_2O_3S$: C, 63.36; H, 5.10; N, 6.16; found: C, 63.22; H, 4.98; N, 6.01.

5.1.6.16. (2-Amino-4-(((5-methyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3p). Following general procedure (E), derivative **3p** was purified by column chromatography (petroleum ether:EtOAc 2:8). Yield: 85%. White solid, mp 119-121 °C. ^{1}H NMR (d_6-DMSO) δ: 1.66 (m, 4H), 1.95 (m, 4H), 2.17 (s, 3H), 2.93 (s, 2H), 6.24 (s, 1H), 6.53 (d, *J*=7.6 Hz, 1H), 6.63 (s, 1H), 6.67 (d, *J*=7.6 Hz, 1H), 7.50 (m, 4H), 7.59 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 21.2, 34.6 (2C), 49.4 (2C), 57.2, 106.5, 108.3, 109.8, 112.9, 116.4, 121.4, 128.2 (2C), 129.8 (2C), 130.9, 135.1, 136.6, 141.1, 144.8, 146.9, 166.2, 191.2, MS (ESI): $[M+1]^+$= 455.1. Anal. calcd for $C_{24}H_{23}ClN_2O_3S$: C, 63.36; H, 5.10; N, 6.16; found: C, 63.18; H, 4.92; N, 6.03.

5.1.6.17. (2-Amino-4-(((5-tert-butyl-spiro-benzo[1,3-d]-dioxol-2-yl)-piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl) methanone (3q). Following general procedure (E), derivative **3q** was purified by column chromatography (dichloromethane:EtOAc 1:9). Yield: 38%. White solid, mp 100-102 °C. ^{1}H NMR ($CDCl_3$) δ: 1.27 (s, 9H), 1.96 (t, *J*=5.6 Hz, 2H), 2.06 (t, *J*=5.6 Hz, 2H), 3.01 (t, *J*=6.0 Hz, 4H), 3.67 (s, 2H), 6.15 (bs, 2H), 6.62 (d, *J*=8.2 Hz, 1H), 6.78 (m, 2H), 6.84 (s, 1H), 7.39 (d, *J*=8.4 Hz, 2H), 7.56 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 497.1. Anal. calcd for $C_{27}H_{29}ClN_2O_3S$: C, 65.24; H, 5.88; N, 5.64; found: C, 65.05; H, 5.69; N, 5.48.

5.1.6.18. (2-Amino-4-((N-methyl-N-phenyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl) methanone (3r). Following the general procedure (E), the product was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 92%. Yellow solid, mp 129-131°C. ^{1}H NMR (*d*$_6$-DMSO) δ: 2.77 (s, 3H), 3.70 (s, 2H), 5.83 (s, 1H), 6.64 (d, *J*=8.0 Hz, 2H), 6.56 (m, 1H), 7.06 (d, *J*=7.2 Hz, 1H), 7.09 (d, *J*=7.2 Hz, 1H), 7.51 (m, 4H), 7.94 (bs, 2H),. ^{13}C NMR (100 MHz, *d*$_6$-DMSO) δ: 38.0, 53.1, 103.8, 111.5, 112.3, 115.7 (2C), 128.3 (2C), 128.7 (2C), 128.9 (2C), 134.9 (2C), 140.5, 148.6, 167.4, 189.6. MS (ESI): $[M+1]^+$= 356.9. Anal. calcd for $C_{19}H_{17}ClN_2OS$: C, 63.95; H, 4.80; N, 7.85; found: C, 63.78; H, 4.63; N, 7.69.

5.1.6.19. (2-Amino-4-((N-ethyl-N-phenyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3s). Following general procedure (E), the product **3s** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 95%, yellow solid, mp 130-132°C. ^{1}H NMR (*d*$_6$-DMSO) δ: 0.90 (t, *J*=7.2 Hz, 3H), 3.20 (q, *J*=7.2 Hz, 2H), 3.63 (s, 2H), 5.89 (s, 1H), 6.42 (d, *J*=8.0 Hz, 2H), 6.53 (m, 1H), 7.04 (d, *J*=7.2 Hz, 1H), 7.08 (d, *J*=7.2 Hz, 1H), 7.47 (d, *J*=8.4 Hz, 2H), 7.53 (d, *J*=8.4 Hz, 2H), 7.94 (bs, 2H). ^{13}C NMR (100 MHz, *d*$_6$-DMSO) δ: 11.6, 44.0, 50.6, 103.9, 111.7 (2C), 112.2, 115.5, 128.3 (2C), 128.8 (2C), 128.9 (2C), 134.8, 135.1, 140.6, 147.5, 167.4, 189.7. MS (ESI): $[M+1]^+$= 371.0. Anal. calcd for $C_{20}H_{19}ClN_2OS$: C, 64.77; H, 5.16; N, 7.55; found: C, 64.59; H, 5.03; N, 7.41.

5.1.6.20. 2-Amino-4-((N-(4-fluorophenyl-N-methyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl) methanone (3t). Following general procedure C, the product **3t** was purified by column chromatography (EtOAc:petroleum ether 2:8 as eluent). Yield 95%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.74 (s, 3H), 3.75 (s, 2H), 5.97 (s, 1H), 6.40 (m, 4H), 6.84 (t, *J*=8.4 Hz, 2H), 7.36 (d, *J*=8.8 Hz, 2H), 7.49 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 374.9. Anal. calcd for $C_{19}H_{16}ClFN_2OS$: C, 60.88; H, 4.30; N, 7.47; found: C, 60.69; H, 4.12; N, 7.29.

5.1.6.21. 2-Amino-4-((N-4-Chlorophenyl-N-methyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl) methanone (3u). Following general procedure (E), the product **3u** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 90%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.79 (s, 3H), 3.77 (s, 2H), 5.91 (s, 1H), 6.41 (d, *J*=9.2 Hz, 2H), 6.46 (bs, 2H), 7.08 (d, *J*=9.2 Hz, 2H), 7.36 (d, *J*=8.8 Hz, 2H), 7.51 (d, *J*=8.8 Hz, 2H). MS (ESI): $[M+1]^+$= 391.3. Anal. calcd for $C_{19}H_{16}Cl_2N_2OS$: C, 58.32; H, 4.12; N, 7.16; found: C, 58.16; H, 4.01; N, 6.98.

5.1.6.22. 2-Amino-4-((N-methyl-N-4-(trifluoromethyl)phenyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3v). Following general procedure (E), the product **3v** was purified by column chromatography (EtOAc:petroleum ether 1.5:8.5 as eluent). Yield 73%. Yellow oil. ^{1}H NMR (d_6-DMSO) δ: 2.87 (s, 3H), 3.80 (s, 2H), 5.84 (s, 1H), 6.54 (d, *J*=8.8 Hz, 2H), 7.37 (d, *J*=8.8 Hz, 2H), 7.47 (d, *J*=8.4 Hz, 2H), 7.52 (d, *J*=8.4 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 38.0, 52.8, 103.9, 110.9 (2C), 112.2, 126.1 (2C), 128.2, 128.5 (2C), 129.0 (2C), 133.9, 135.1, 140.5, 151.1, 167.7, 189.7. MS (ESI): $[M+1]^+$= 425.0. Anal. calcd for $C_{20}H_{16}ClF_3N_2OS$: C, 56.54; H, 3.80; N, 6.59; found: C, 55.96; H, 3.67; N, 6.44.

5.1.6.23. 2-Amino-4-((N-Benzyl-N-methyl-amino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3w). Following general procedure (E), the product **3w** was purified by column chromatography (EtOAc:petroleum ether 1:1 as eluent). Yield 78%. Yellow solid, mp 112-114°C. ^{1}H NMR ($CDCl_3$) δ: 2.78 (s, 3H), 3.46 (s, 2H), 3.78 (s, 2H), 5.98 (s, 1H), 6.42 (bs, 2H), 6.60 (m, 1H), 7.02 (m, 2H),

7.08 (m Hz, 2H), 7.30 (d, *J*=8.6 Hz, 2H), 7.54 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 371.0. Anal. calcd for $C_{20}H_{19}ClN_2OS$: C, 64.77; H, 5.16; N, 7.55; found: C, 64.58; H, 4.98; N, 7.38.

5.1.6.24. (2-Amino-4-((N,N-diethylamino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3x). Following general procedure (E), the product **3x** was purified by column chromatography (EtOAc:MeOH 8:2 as eluent). Yield 78%. Yellow solid, mp 78-80°C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, *J*=7.0 Hz, 6H), 2.17 (q, *J*=7.0 Hz, 4H), 3.08 (s, 2H), 5.99 (bs, 2H), 6.25 (s, 1H), 7.36 (d, *J*=8.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 323.2. Anal. calcd for $C_{16}H_{19}ClN_2OS$: C, 59.52; H, 5.93; N, 8.68; found: C, 59.36; H, 5.77; N, 8.47.

5.1.6.25. (2-Amino-4-((N,N-dipropylamino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3y). Following general procedure (E), the product **3y** was purified by column chromatography (EtOAc:petroleum ether 9.5:0.5 as eluent). Yield 74%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.69 (t, *J*=7.0 Hz, 6H), 1.19 (m, 4H), 1.96 (t, *J*=7.0 Hz, 4H), 3.01 (s, 2H), 6.07 (bs, 2H), 6.19 (s, 1H), 7.34 (d, *J*=8.4 Hz, 2H), 7.51 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 350.9. Anal. calcd for $C_{18}H_{23}ClN_2OS$: C, 61.61; H, 6.61; N, 7.98; found: C, 61.48; H, 6.39; N, 7.78.

5.1.6.26. (2-Amino-4-((N,N-diisopropylamino)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3z). Following general procedure (E), the product **3z** was purified by column chromatography (EtOAc:petroleum ether 1:1 as eluent). Yield 68%. Brown oil. ^{1}H NMR ($CDCl_3$) δ: 0.86 (d, *J*=6.6 Hz, 12H), 1.66 (m, 2H), 3.66 (s, 2H), 5.28 (s, 1H), 6.25 (bs, 2H), 7.38 (d, *J*=8.4 Hz, 2H), 7.52 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M+1]^+$= 351.0. Anal. calcd for $C_{18}H_{23}ClN_2OS$: C, 61.61; H, 6.61; N, 7.98; found: C, 61.45; H, 6.40; N, 7.82.

5.1.6.27. (2-Amino-4-((N,N-dimethylamino)methyl)-5-phenylthiophen-3-yl)(4-chlorophenyl) methanone (3aa). Following general procedure (E), the product **3aa** was purified by column chromatography (EtOAc as eluent). Yield 48%. Yellow oil. ^{1}H NMR (d_6-DMSO) δ: 1.46 (s, 6H), 2.90 (s, 2H), 7.29 (bs, 2H), 7.36 (m, 5H), 7.49 (d, *J*=8.4 Hz, 2H), 7.54 (d, *J*=8.4 Hz, 2H). ^{13}C NMR

(100 MHz, d_6-DMSO) δ: 43.8 (2C), 55.7, 114.4, 121.2, 122.9, 127.2 (2C), 127.9 (2C), 128.6 (2C), 129.5 (2C), 132.7, 133.5, 134.8, 140.0, 161.8, 190.4. MS (ESI): $[M+1]^+$= 371.0. Anal. calcd for $C_{20}H_{19}ClN_2OS$: C, 64.77; H, 5.16; N, 7.55; found: C, 64.58; H, 5.02; N, 7.38.

5.1.6.28. (2-Amino-4-((N,N-diethylamino)methyl)-5-phenylthiophen-3-yl)(4-chlorophenyl) methanone (3ab). Following general procedure (E), the product **3ab** was purified by column chromatography (EtOAc:petroleum ether 7:3). Yield 72%. Yellow solid, mp 176-178 °C. ^{1}H NMR (d_6-DMSO) δ: 0.43 (t, *J*=7.2 Hz, 6H), 1.81 (q, *J*=7.2 Hz, 4H), 3.15 (s, 2H), 7.12 (bs, 2H), 7.38 (m, 5H), 7.52 (d, *J*=8.4 Hz, 2H), 7.62 (d, *J*=8.4 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 9.47 (2C), 43.7 (2C), 50.2, 114.6, 121.1, 127.0, 127.8 (2C), 128.5 (2C), 129.3 (2C), 129.7 (2C), 133.2, 133.6, 135.0, 139.5, 160.7, 190.0. MS (ESI): $[M+1]^+$= 399.2. Anal. calcd for $C_{22}H_{23}ClN_2OS$: C, 66.23; H, 5.81; N, 7.02; found: C, 66.03; H, 5.67; N, 6.88.

5.1.6.29. (2-Amino-4-((N,N-dipropylamino)methyl)- 5-phenylthiophen-3-yl)(4-chlorophenyl) methanone (3ac). Following general procedure (E), the product **3ac** was purified by column chromatography (EtOAc:petroleum ether 7:3). Yield 68%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.48 (t, *J*=7.2 Hz, 6H), 0.99 (m, 4H), 1.73 (t, *J*=7.2 Hz, 4H), 3.21 (s, 2H), 5.84 (bs, 2H), 7.34 (m, 5H), 7.43 (d, *J*=7.8 Hz, 2H), 7.67 (d, *J*=7.6 Hz, 2H). MS (ESI): $[M+1]^+$= 427.3. Anal. calcd for $C_{24}H_{27}ClN_2OS$: C, 67.51; H, 6.37; N, 6.56; found: C, 67.33; H, 6.22; N, 6.41.

5.1.6.30. (2-Amino-4-((N,N-diallylamino)methyl)-5-phenylthiophen-3-yl)(4-chlorophenyl) methanone (3ad). Following general procedure (E), the product **3ad** was purified by column chromatography (EtOAc:petroleum ether 8.5:1.5). Yield 82%. Yellow solid, mp 155-156 °C. ^{1}H NMR (d_6-DMSO) δ: 2.42 (d, *J*=6.8 Hz, 4H), 3.17 (s, 2H), 4.82 (m, 4H), 5.15 (m, 2H), 7.21 (bs, 2H), 7.32 (m, 2H), 7.41 (m, 3H), 7.53 (d, *J*=8.8 Hz, 2H), 7.65 (d, *J*=8.8 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 50.2, 54.2 (2C), 114.3, 117.3 (2C), 121.7, 127.1, 128.0 (2C), 128.5 (2C), 129.4 (2C), 129.9 (2C), 132.3, 133.5, 134.2 (2C), 135.3, 139.3, 161.2, 189.9. MS (ESI): $[M+1]^+$= 423.2. Anal. calcd for $C_{24}H_{23}ClN_2OS$: C, 68.15; H, 5.48; N, 6.62; found: C, 67.93; H, 5.35; N, 6.41.

5.1.6.31. (2-Amino-4-((N,N-diisopropylamino)methyl)-5-phenyl-thiophen-3-yl)(4-chlorophenyl) methanone (3ae). Following general procedure (E), the product **3ae** was purified by column chromatography (EtOAc:petroleum ether 7:3). Yield 88%. Yellow solid, mp 185-187 °C. ^{1}H NMR (d_6-DMSO) δ: 0.46 (d, *J*=6.4 Hz, 12H), 2.41 (m, 2H), 3.41 (s, 2H), 6.72 (bs, 2H), 7.31 (m, 2H), 7.41 (m, 3H), 7.55 (d, *J*=8.4 Hz, 2H), 7.77 (d, *J*=8.4 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 19.7 (4C), 42.7, 46.4 (2C), 115.7, 122.2, 127.0, 128.1 (2C), 128.3 (2C), 129.6 (2C), 130.8 (2C), 133.9, 134.2, 136.3, 137.9, 158.5, 189.7. MS (ESI): $[M+1]^+$= 427.1. Anal. calcd for $C_{24}H_{27}ClN_2OS$: C, 67.51; H, 6.37; N, 6.56; found: C, 67.35; H, 6.18; N, 6.27.

5.1.6.32. (2-Amino-4-((N-t-butyl-N-methylamino)methyl)-5-phenyl-thiophen-3-yl)(4-chlorophenyl) methanone (3af). Following general procedure (E), the product **3af** was purified by column chromatography (EtOAc:petroleum ether 6:4). Yield 87%. Yellow solid, mp 220-222 °C. ^{1}H NMR (d_6-DMSO) δ: 0.49 (s, 9H), 1.53 (s, 3H), 3.30 (s, 2H), 6.75 (bs, 2H), 7.29 (t, *J*=7.8 Hz, 1H), 7.38 (m, 4H), 7.53 (d, *J*=8.4 Hz, 2H), 7.72 (d, *J*=8.4 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 24.6 (3C), 33.5, 46.7, 53.5, 115.4, 121.3, 126.9, 128.2 (2C), 128.5 (2C), 129.3 (2C), 130.3 (2C), 133.8, 134.2, 136.0, 138.7, 158.5, 189.9. MS (ESI): $[M+1]^+$= 413.2. Anal. calcd for $C_{23}H_{25}ClN_2OS$: C, 66.89; H, 6.10; N, 6.78; found: C, 66.68; H, 5.96; N, 6.58.

5.1.6.33. (2-Amino-4-((N,N-dicyclohexylamino)methyl)-5-phenyl-thiophen-3-yl)(4-chlorophenyl) methanone (3ag). Following general procedure (E), the product **3ag** was purified by column chromatography (EtOAc:petroleum ether 8:2). Yield 67%. Yellow solid, mp 201-203 °C. ^{1}H NMR (d_6-DMSO) δ: 0.83 (m, 8H), 1.04 (m, 6H), 1.42 (m, 6H), 1.74 (m, 2H), 3.50 (s, 2H), 6.81 (bs, 2H), 7.31 (m, 1H), 7.39 (m, 4H), 7.55 (d, *J*=6.8 Hz, 2H), 7.74 (d, *J*=6.8 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 26.1 (2C), 26.6 (4C), 31.2 (4C), 44.4 (2C), 58.1, 116.1, 122.4, 127.6, 128.5 (2C), 128.9 (2C), 130.1 (2C), 131.4 (2C), 134.5, 135.4, 136.7, 138.5, 159.6, 190.3. MS (ESI): $[M+1]^+$= 507.3. Anal. calcd for $C_{30}H_{35}ClN_2OS$: C, 71.05; H, 6.96; N, 5.52; found: C, 70.87; H, 6.78; N, 5.38.

5.1.6.34. (2-Amino-5-phenyl-4-((piperidin-1-yl)methyl)-thiophen-3-yl)(4-chlorophenyl)methanone (3ah). Following general procedure (E), the product **3ah** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield 72%. Yellow solid, mp 185-187 °C. ^{1}H NMR (d_6-DMSO) δ: 1.13 (m, 6H), 1.56 (m, 4H), 2.95 (s, 2H), 7.21 (bs, 2H), 7.32 (m, 3H), 7.39 (m, 2H), 7.53 (d, *J*=8.4 Hz, 2H), 7.63 (d, *J*=8.4 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 23.6, 24.9 (2C), 52.6 (2C), 55.2, 114.3, 120.8, 127.0, 127.6 (2C), 128.5 (2C), 129.3 (2C), 129.6 (2C), 132.5, 133.5, 134.7, 139.8, 161.1, 190.3. MS (ESI): $[M+1]^+$= 411.2. Anal. calcd for $C_{23}H_{23}ClN_2OS$: C, 67.22; H, 5.64; N, 6.82; found: C, 67.03; H, 5.48; N, 6.63.

5.1.6.35. (2-Amino-4-((N-n-butyl-N-methyl-amino)methyl)-5-phenyl-thiophen-3-yl)(4-chlorophenyl) methanone (3ai). Following general procedure (E), the product **3ai** was purified by column chromatography (EtOAc:petroleum ether 7:3). Yield 45%. Yellow solid, mp 84-86 °C. ^{1}H NMR ($CDCl_3$) δ: 0.78 (t, *J*=7.2 Hz, 3H), 0.89 (m, 4H), 1.82 (m, 3H), 2.03 (t, *J*=7.2 Hz, 2H), 3.75 (s, 2H), 5.68 (bs, 2H), 7.29 (m, 3H), 7.39 (m 2H), 7.49 (m, 4H). MS (ESI): $[M+1]^+$= 413.2. Anal. calcd for $C_{23}H_{25}ClN_2OS$: C, 66.89; H, 6.10; N, 6.78; found: C, 66.73; H, 5.96; N, 6.59.

5.1.6.36. (2-Amino-4-((N-benzyl-N-methyl-amino)methyl)-5-phenyl-thiophen-3-yl)(4-chlorophenyl) methanone (3aj). Following general procedure (E), the product **3aj** was purified by column chromatography (EtOAc:petroleum ether 4:6). Yield: 56%. Yellow solid, mp 86-88 °C. ^{1}H NMR ($CDCl_3$) δ: 1.74 (s, 3H), 3.23 (m, 2H), 4.34 (m, 2H), 5.78 (bs, 2H), 7.19 (m, 2H), 7.25 (m, 7H), 7.47 (m, 5H). MS (ESI): $[M+1]^+$= 447.4. Anal. calcd for $C_{26}H_{23}ClN_2OS$: C, 69.86; H, 5.19; N, 6.27; found: C, 69.68; H, 5.02; N, 6.03.

5.1.6.37. (2-Amino-5-bromo-4-((N,N-diallylamino)methyl)-thiophen-3-yl)(4-chlorophenyl) methanone (3ak). Following general procedure (E), the product **3ak** was purified by column chromatography (EtOAc:petroleum ether 3:7). Yield: 31%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 2.73 (d *J*=6.6 Hz, 4H), 3.64 (s, 2H), 4.92 (m, 4H), 5.34 (s, 2H), 5.89 (bs, 2H), 7.25 (d, *J*=8.6 Hz, 2H), 7.42

(d, *J*=8.6 Hz, 2H). MS (ESI): $[M]^+$= 424.0, $[M+2]^+$= 426.1. Anal. calcd for $C_{18}H_{18}BrClN_2OS$: C, 50.78; H, 4.26; N, 6.58; found: C, 50.61; H, 4.08; N, 6.38.

5.1.6.38. (2-Amino-5-bromo-4-((N,N-diisopropylamino)methyl)thiophen-3-yl)(4-chlorophenyl) methanone (3al). Following general procedure (E), derivative **3al** was purified by column chromatography (EtOAc:petroleum ether 1:1). Yield: 27%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.03 (d, *J*=6.6 Hz, 12H), 1.63 (m, 2H), 3.49 (s, 2H), 6.34 (bs, 2H), 7.43 (d, *J*=8.4 Hz, 2H), 7.60 (d, *J*=8.4 Hz, 2H). MS (ESI): $[M]^+$= 428.0, $[M+2]^+$= 430.2. Anal. calcd for $C_{18}H_{22}BrClN_2OS$: C, 50.30; H, 5.16; N, 6.52; found: C, 50.02; H, 4.98; N, 6.34.

5.1.6.39. (2-Amino-4-(phenyloxy)methyl-thiophen-3-yl)(4-chlorophenyl)methanone (3am). Following general procedure (E), the product **3am** was purified by column chromatography (EtOAc-petroleum ether 1.5-8.5 as eluent). Yield 78%. Yellow solid, mp 136-138°C. ^{1}H NMR ($CDCl_3$) δ: 4.33 (s, 2H), 6.31 (s, 1H), 6.50 (d, *J*=8.6 Hz, 2H), 6.58 (bs, 2H), 7.11 (d, *J*=8.6 Hz, 2H), 7.42 (m, 5H). MS (ESI): $[M+1]^+$= 344.1. Anal. calcd for $C_{18}H_{14}ClNO_2S$: C, 62.88; H, 4.10; N, 4.07; found: C, 62.68; H, 3.97; N, 3.92.

5.1.6.40. (2-Amino-4-(4-methyl-phenyloxy)methyl-thiophen-3-yl)(4-chlorophenyl)methanone (3an). Following general procedure (E), the product **3an** was purified by column chromatography (EtOAc-petroleum ether 1:9 as eluent). Yield 85%. Yellow solid, mp 128-130 °C. ^{1}H NMR ($CDCl_3$) δ: 2.25 (s, 3H), 4.33 (s, 2H), 6.32 (s, 1H), 6.45 (d, *J*=8.4 Hz, 2H), 6.60 (bs, 2H), 6.96 (d, *J*=8.6 Hz, 2H), 7.31 (d, *J*=8.4 Hz, 2H), 7.47 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 357.9. Anal. calcd for $C_{19}H_{16}ClNO_2S$: C, 63.77; H, 4.51; N, 3.91; found: C, 63.58; H, 4.25; N, 3.74.

5.1.6.41. [2-Amino-4-(4-isopropyl-phenyloxy)methyl-thiophen-3-yl](4-chlorophenyl)methanone (3ao). Following general procedure (E), the product **3ao** was purified by column chromatography (EtOAc-petroleum ether 0.5:9.5 as eluent). Yield 74%. Yellow solid, mp 114-116 °C. ^{1}H NMR ($CDCl_3$) δ: 1.18 (d, *J*=6.8 Hz, 6H), 2.81 (m, 1H), 4.34 (s, 2H), 6.33 (s, 1H), 6.48 (d, *J*=8.6 Hz, 2H),

6.62 (bs, 2H), 7.02 (d, *J*=8.6 Hz, 2H), 7.27 (d, *J*=8.6 Hz, 2H), 7.46 (d, *J*=8.6 Hz, 2H). MS (ESI): $[M+1]^+$= 386.1. Anal. calcd for $C_{21}H_{20}ClNO_2S$: C, 65.36; H, 5.22; N, 3.63; found: C, 65.19; H, 5.07; N, 3.49.

5.1.6.42. [2-Amino-4-(4-fluorophenyloxy)methyl-thiophen-3-yl](4-chlorophenyl)methanone (3ap). Following general procedure (E), the product **3ap** was purified by column chromatography (EtOAc-petroleum ether 2:8 as eluent). Yield 91%. Yellow solid, mp 138-140 °C. ^{1}H NMR (d_6-DMSO) δ: 4.35 (s, 2H), 6.45 (s, 1H), 6.56 (m, 2H), 6.98 (m, 2H), 7.28 (d, *J*=8.8 Hz, 2H), 7.40 (d, *J*=8.8 Hz, 2H), 8.01 (bs, 2H),. ^{13}C NMR (100 MHz, d_6-DMSO) δ: 65.6, 107.6, 111.8, 115.1 , 115.2 , 115.4 (2C), 127.8 (2C), 128.6 (2C), 133.5, 134.5, 140.0, 153.8, 155.1 (*J*=235 Hz), 167.2, 189.9. MS (ESI): $[M+1]^+$= 361.9. Anal. calcd for $C_{18}H_{13}ClFNO_2S$: C, 59.75; H, 3.62; N, 3.87; found: C, 59.58; H, 3.47; N, 3.59.

5.1.6.43. (2-Amino-4-(phenylthio)methyl-thiophen-3-yl)(4-chlorophenyl)methanone (3aq). Following general procedure (E), the product **3aq** was purified by column chromatography (EtOAc:petroleum ether 1:1 as eluent). Yield 88%. Yellow solid, mp 168-170°C. ^{1}H NMR (d_6-DMSO) δ: 3.61 (s, 2H), 6.26 (s, 1H), 6.99 (m, 2H), 7.15 (m, 1H), 7.22 (m, 2H), 7.44 (d, *J*=7.6 Hz, 2H), 7.50 (d, *J*=7.6 Hz, 2H), 7.76 (bs, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 33.7, 106.9, 112.0, 115.5, 125.9, 128.1 (2C), 128.4 (2C), 128.7 (2C), 129.5, 133.3, 135.2, 135.7, 139.7, 166.4, 189.8. MS (ESI): $[M+1]^+$= 360.2. Anal. calcd for $C_{18}H_{13}ClNOS_2$: C, 60.07; H, 3.92; N, 3.89; found: C, 59.88; H, 3.67; N, 3.72.

5.2. Biology Experiments

5.2.1. 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-cyclopentyladenosine; 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 CPA (N^6-cyclopentyladenosine) were obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and obtained from commercial sources.

5.2.2. 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 x g and the supernatant was then centrifuged for 30 min at 100,000 x 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 [20].

5.2.3. Binding Experiments in hA_1CHO membranes

5.2.3.1. [^{3}H]CCPA Binding Experiments.

Saturation binding experiments of [^{3}H]CCPA (0.05 to 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 [18a]. Non-specific binding was defined as binding in the presence of 1 μM R-PIA.

5.2.3.2. [^{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.

5.2.3.3. Assay of the Antagonist Activity versus A_1, A_{2A} and A_3 ARs.

A_1, A_{2A} and A_3AR 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).

5.2.4. 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 activity. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2,000 *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 [23]. 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 2,000 *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).

5.2.5. 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, Ki, were also calculated from the IC_{50} values according to the Cheng and Prusoff equation $Ki = IC_{50}/(1+[C^*]/K_D^*)$, 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. ^{1}H and ^{13}C spectra of compounds **3a**, **3c**, **3d**, **3n**, **3p**, **3r**, **3s**, **3v**, **3ab**, **3ad**, **3ae**, **3ag**, **3ah** and **3ap**.

References and notes

[1]. K. A. Jacobson, Z.-G. Gao. Nature Rev. Drug Discovery. 5 (2006) 247-254.

[2]. D. M. Van Calker, M. Muller, B. Hamprecht. J. Neurochem. 33 (1999) 999-1005.

[3]. M. P. De Ninno. Ann. Rep. Med. Chem. 33 (1998) 111-120.

[4]. a) A. K. Dhalla, J. C. Shryock, R. Shreeniwas, L. Belardinelli. Curr. Top. Med. Chem.3 (2003) 369-385; b) B. B. Fredholm Cell. Death Differ. 14 (2007) 1315-1323.

[5]. Z. -G. Gao, K. A. Jacobson. Expert. Opin. Emerging Drugs 12 (2007) 479-492.

[6]. a) L. T. May, V. A. Avlani, P. M. Sexton, A. Christopoulos. Curr. Pharm. Des. 10 (2004) 2003-2013; b) L. T. May, K. Leach, P. M. Sexton, A. Christopoulos. Annu. Rev. Pharmacol. Toxicol. 47 (2007) 1-51.

[7]. A. Gobylos, A.P. Ijzerman. Biochem. Biophys. Acta. 1808 (2011) 1309-1318.

[8]. C. Valant, L. Aurelio, V. B. Urmaliya, P. White, P. J. Scammells, P. M. Sexton, A. Christopoulos. Mol. Pharmacol. 78 (2010) 444−455.

[9]. T. W. Schwartz, B. Holst. Trends Pharmacol. Sci. 28 (2007) 366-373.

[10]. a) R. F. Bruns, J. H. Fergus. Mol. Pharmacol. 38 (1990) 939-949, b) R. F. Bruns, J. H. Fergus, L. L. Coughenour, G. G. Courtland, T. A. Pugsley, J. H. Dodd, F. J. Tinney. Mol. Pharmacol. 38 (1990) 950-958.

[11]. a) Z.-G. Gao, S.-K. Kim, A. P. Ijzerman, K. A. Jacobson. Mini-Rev. Med. Chem. 5 (2005) 545-553; b) P. G. Baraldi, M. A. Iaconinoto, A. R. Moorman, M. D. Carrion, C. Lopez-Cara, D. Preti, O. C. López, F. Fruttarolo, M. Aghazadeh Tabrizi, R. Romagnoli. Mini-Rev. Med. Chem. 7 (2007) 559-569; c) R. Romagnoli, P. G. Baraldi, M. Aghazadeh Tabrizi, S. Gessi, P. A. Borea, S. Merighi. Curr. Med. Chem. 17 (2010) 3488-3502, d) A. Göblyös, A. P. IJzerman. Purinergic Signalling 5 (2009) 51-61, e) C. E. Tranberg, A. Zickgraf, B. N. Giunta, H. Luetjens, H. Figler, L. J. Murphree, R. Falke, H. Fleischer, J. Linden, P. J. Scammells, R. A. Olsson. J. Med. Chem. 45 (2002) 382-389; f) H. Luetjens, A. Zickgraf, H. Figler, J. Linden, R. A. Olsson, P. J. Scammells. J. Med. Chem. 46 (2003) 1870-1877, g) L.

Aurelio, C. Valant, P. M. Sexton, A. Christopoulos, P. J. Scammells. J. Med. Chem. 52 (2009) 4543-4547.

[12]. S. R. Childers, X. Li, R. Xiao, J. C. Eisanach. J. Neurochem. 93 (2005) 715-723.

[13]. a) R. Romagnoli, P. G. Baraldi, M. D. Carrion, C. Lopez-Cara, O. Cruz-Lopez, M. A. Iaconinoto, D. Preti**,** J. C. Shryock, A. R. Moorman, F. Vincenzi, K. Varani, P. A. Borea. J. Med. Chem. 51 (2008) 5875-5879; b) R. Romagnoli, P. G. Baraldi, M. D. Carrion, C. Lopez-Cara, O. Cruz-Lopez, M. Kimatrai Salvador, D. Preti, M. Aghazadeh Tabrizi, J. C. Shryock, A. R. Moorman, F. Vincenzi, K. Varani, P. A. Borea, Bioorg. Med. Chem. 20 (2012) 996-1007.

[14]. a) S. B. Rajur; A. Y. Merwade, S. B. Hendi, L. D. Basanagoudar. Indian J. Chem. Sect. B 12 (1989) 1065-1068; b) R. Romagnoli, P. G. Baraldi, M. D. Carrion, O. Cruz-Lopez, C. Lopez Cara, D. Preti, M. Aghazadeh Tabrizi, J. Balzarini, E. Hamel, E. Fabbri, R. Gambari. Lett. Drug Design Discov. 6 (2009) 298-303.

[15]. N. Krogsgaard-Larsen, M. Begtrup, M. M. Herth, J. Kehler. Synthesis (2010) 4287-4299.

[16]. N. Khrorana, A. Purohit, K. Herrick-Davis, M. Teitler, R. A. Glennon. Bioorg. Med. Chem. 11 (2003) 717-722.

[17]. E. K. Moltzen, J. Perregaard, E. Meier. J. Med. Chem. 38 (1995) 2009-2017.

[18]. R. Romagnoli, P. G. Baraldi, M. D. Carrion, C. Lopez-Cara, O. Cruz-Lopez, M. Kimatrai Salvador, D. Preti, M. Aghazadeh Tabrizi, A. R. Moorman, F. Vincenzi, P. A. Borea, K. Varani. J. Med. Chem. 55 (2012) 7719-7735.

[19]. In our experiments, the reference compound PD 81,723 (at a concentration of 10 μM) did not inhibit [^{3}H]DPCPX binding to human A_1 receptors transfected in CHO cells. For the same reference compound, Bruns (Reference 10) showed a K_i 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 (reference 12) 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.

[20]. M. M. Bradford. Anal Biochem. 72 (1976) 248-254.

[21]. P. A. Borea, A. Dalpiaz, K. Varani, S. Gessi, G. Gilli. Life Sciences 59 (1996) 1373-1388.

[22]. P. A. Borea, A. Dalpiaz, K. Varani, S. Gessi, G. Gilli. Biochem. Pharmacol. 49 (1995) 461-469.

[23]. K. Varani, S. Merighi, S. Gessi, K. N. Klotz, E. Leung, P. G. Baraldi, B. Cacciari, R. Romagnoli, G. Spallato, P. A. Borea. Mol. Pharmacol. 57 (2000) 968-975.

[24]. P. J. Munson, D. Rodbard. Anal. Biochem. 107 (1980) 220-239.

[25]. Y. Cheng, W. H. Prusoff**.** Biochem. Pharmacol. 1 (1973) 3099-3108.

Figure, Chart and Scheme Captions

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 Experimental Section.

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 Bmax was 534 ± 47 fmol/mg protein. In the presence of novel enhancers (10 μM), K_D values were similar to those obtained in controls and Bmax values were as reported in Table 2. Values are the means and vertical lines are the SEM of three separate experiments, as described in Experimental Section. Scatchard plots of the same experimental data (B).

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

Chart 2. Chemical structures of 2-amino-3-(4-chlorobenzoyl)thiophene derivatives **3r-aq**.

Scheme 1. Reagents. **a**: Amine **5a-q** or **5w-z**, K_2CO_3,CH_2Cl_2, +4 °C for 30 min then rt for 2h; **b**: amine **5r-v**, K_2CO_3, MeCN, 60 °C; **c**: H_2, 10% Pd/C, DMF, rt; **d**: NH_2NH_2, EtOH, reflux.

Scheme 2. **Reagents**. **a**: Appropriate amine, TEA, CH_2Cl_2; **b**: NH_2NH_2, EtOH, reflux.

Scheme 3. **Reagents**. **a**: (CH_2=$CHCH_2$)NH or (*i*-C_3H_7)NH, TEA, CH_2Cl_2; **b**: NH_2NH_2, EtOH, reflux; **c**: NaH, DMF; **c**: appropriate phenol or thiophenol, K_2CO_3, MeCN, 60 °C.

Table 1. Effect of the novel allosteric enhancers **3a-aq** and **PD 81,723** in cAMP assay in hA_1 CHO cells.

Compound	% inhibition of cAMP production[a]	% inhibition of cAMP production + CCPA[b]
PD 81,723 (1)	18±1	20±2
3a	66±6	62±6
3b	69±7	65±6
3c	64±6	60±5
3d	43±4	41±4
3e	71±7	72±6
3f	7±1	11±1
3g	3±1	13±1
3h	41±4	43±4
3i	3±1	11±1
3j	8±1	13±1
3k	28±2	27±2
3l	24±2	28±2
3m	45±4	43±4
3n	56±5	52±5
3o	46±4	47±4
3p	48±4	45±4
3q	51±4	55±5
3r	18±1	16±1
3s	19±1	18±1
3t	28±2	26±2
3u	22±2	21±2
3v	29±3	31±3
3w	42±4	40±3
3x	5±1	12±1
3y	9±1	10±1
3z	4±1	14±1

(continued)

Compound	% inhibition of cAMP production[a]	% inhibition of cAMP production + CCPA[b]
3aa	6±1	10±1
3ab	25±2	27±2
3ac	28±2	29±2
3ad	36±3	34±3
3ae	9±1	12±1
3af	10±1	13±1
3ag	31±3	28±2
3ah	24±2	22±2
3ai	5±1	12±1
3aj	4±1	11±1
3ak	8±1	13±1
3al	12±1	14±1
3am	9±1	11±1
3an	11±1	16±1
3ao	15±1	18±1
3ap	10±1	15±1
3aq	18±2	21±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.

Table 2. Antagonist activity of the novel allosteric enhancers **3a-aq** and of **PD 81,723**

compound	% inhibition A_1[a]	% inhibition A_{2A}[b]	% inhibition A_3[c]
PD 81,723	0±0	0±0	21±2
3a	3±1	0±0	3±1
3b	0±0	2±1	5±1
3c	4±1	0±0	4±1
3d	1±1	0±0	6±1
3e	0±0	3±1	5±1
3f	1±1	4±1	1±1
3g	6±1	0±0	7±1
3h	0±0	3±1	1±1
3i	0±0	1±1	1±1
3j	2±1	1±1	1±1
3k	2±1	1±1	2±1
3l	1±1	0±0	1±1
3m	1±1	1±1	5±1
3n	1±1	1±1	7±1
3o	2±1	1±1	5±1
3p	0±0	3±1	6±1
3q	1±1	0±0	12±1
3r	1±1	1±1	9±1
3s	0±0	0±0	11±1
3t	3±1	2±1	4±1
3u	8±1	0±0	12±1
3v	3±1	0±0	4±1
3w	1±1	1±1	7±1
3x	0±0	1±1	2±1
3y	4±1	0±0	5±1
3z	1±1	0±0	3±1

(continued)

Compound	% inhibition A_1[a]	% inhibition A_{2A}[b]	% inhibition A_3[c]
3aa	1±1	1±1	2±1
3ab	1±1	5±1	9±1
3ac	1±1	1±1	11±1
3ad	3±1	1±1	5±1
3ae	5±1	6±1	11±1
3af	5±1	1±1	10±1
3ag	6±1	1±1	7±1
3ah	1±1	1±1	7±1
3ai	1±1	1±1	5±1
3aj	1±1	3±1	6±1
3ak	6±1	1±1	8±1
3al	1±1	1±1	3±1
3am	1±1	0±0	2±1
3an	5±1	1±1	11±1
3ao	3±1	1±1	9±1
3ap	1±1	1±1	8±1
3aq	1±1	1±1	5±1

Inhibition activity is expressed as percent displacement value (±SEM, n=3) of 1 nM [^{3}H]DPCPX (a), of 2 nM [^{3}H]ZM 241385 (b) and of 2 nM [^{3}H]MRE 3008F20 (c) by the novel allosteric enhancers (10 μM).

Table 3. A_1AR density expressed as Bmax values (A) obtained by [^{3}H]CCPA binding assays in hA_1 CHO membranes in the presence of **3a-aq** and reference compounds PD81,723 (10 μM).. Modulation of the novel allosteric enhancers (10 μM) on the CCPA affinity (CCPA Ki shift) in [^{3}H]DPCPX competition binding experiments (B).

Compound	(A)		(B)	
	Bmax (fmol/ mg protein)	Bmax shift (fold of increase)	CCPA Ki (nM)	CCPA Ki shift (fold of increase)
PD 81,723	675±65	1.3±0.1	9.8±0.8	1.6±0.1
3a	4021±395	7.5±0.7	2.3±0.2	6.6±0.6
3b	4356±412	8.2±0.8	2.1±0.2	7.2±0.7
3c	3948±357	7.4±0.7	2.2±0.2	6.9±0.6
3d	2588±232	4.8±0.4	4.1±0.4	3.7±0.3
3e	4326±396	8.1±0.8	2.0±0.2	7.6±0.6
3f	635±55	1.2±0.1	13.7±1.2	1.1±0.1
3g	541±51	1.0±0.1	15.2±1.4	1.0±0.1
3h	2456±216	4.6±0.4	3.7±0.3	4.1±0.4
3i	546±52	1.0±0.1	14.5±1.4	1.0±0.1
3j	618±58	1.2±0.1	13.6±1.3	1.1±0.1
3k	1674±154	3.1±0.3	5.2±0.4	2.9±0.2
3l	1485±133	2.8±0.3	5.7±0.5	2.6±0.2
3m	2734±248	5.1±0.4	3.2±0.3	4.7±0.4
3n	3462±318	6.5±0.7	2.4±0.2	6.3±0.6
3o	2684±244	5.0±0.5	3.1±0.3	4.9±0.4
3p	2739±256	5.1±0.5	3.6±0.3	4.2±0.4
3q	3342±322	6.3±0.6	2.5±0.2	6.0±0.5
3r	1274±108	2.4±0.1	7.0±0.7	2.2±0.1
3s	1223±117	2.3±0.2	7.8±0.8	1.9±0.1
3t	1731±149	3.2±0.3	4.4±0.3	3.4±0.3
3u	1321±111	2.5±0.2	6.8±0.7	2.2±0.2
3v	1722±163	3.2±0.3	5.3±0.5	2.8±0.2
3w	2366±224	4.4±0.4	3.8±0.3	4.0±0.4

3x	538±43	1.0±0.1	14.9±1.4	1.0±0.1
3y	666±59	1.2±0.1	13.4±1.2	1.1±0.1
3z	552±48	1.0±0.1	14.7±1.3	1.0±0.1
3aa	581±50	1.1±0.1	14.6±1.3	1.0±0.1
3ab	1505±132	2.8±0.2	6.2±0.6	2.4±0.2
3ac	1647±141	3.1±0.3	6.1±0.5	2.5±0.2
3ad	2258±213	4.2±0.4	3.9±0.3	3.9±0.3
3ae	657±62	1.2±0.1	11.8±1.1	1.3±0.1
3af	698±62	1.3±0.1	11.4±1.2	1.3±0.1
3ag	1876±177	3.5±0.3	4.7±0.4	3.2±0.3
3ah	1563±136	2.9±0.2	5.5±0.5	2.7±0.2
3ai	587±49	1.1±0.1	13.5±1.2	1.1±0.1
3aj	573±46	1.1±0.1	13.8±1.3	1.1±0.1
3ak	639±51	1.2±0.1	12.7±1.1	1.2±0.1
3al	797±63	1.5±0.1	10.9±1.1	1.4±0.1
3am	688±57	1.3±0.1	12.6±1.1	1.2±0.1
3an	674±52	1.3±0.1	10.8±1.0	1.4±0.1
3ao	952±88	1.8±0.2	10.1±0.9	1.5±0.1
3ap	655±60	1.2±0.1	12.5±1.1	1.2±0.1
3aq	1158±98	2.2±0.2	7.5±0.6	2.0±0.2

The values are expressed as the mean ± SEM, n=3 independent experiments.
(A) = Bmax (fmol/mg protein) and Bmax shift obtained in [^{3}H]CCPA saturation binding experiments performed in the absence (Bmax = 534 ± 37 fmol/mg protein) or in the presence of 10 μM enhancer s.
(B) = Ki values of CCPA in the presence of 10 μM tested compounds and CCPA shift = Ki(CCPA)/Ki(CCPA+10 μM enhancers) where the Ki of CCPA was 15.2 ± 1.3 nM.

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Figure 1

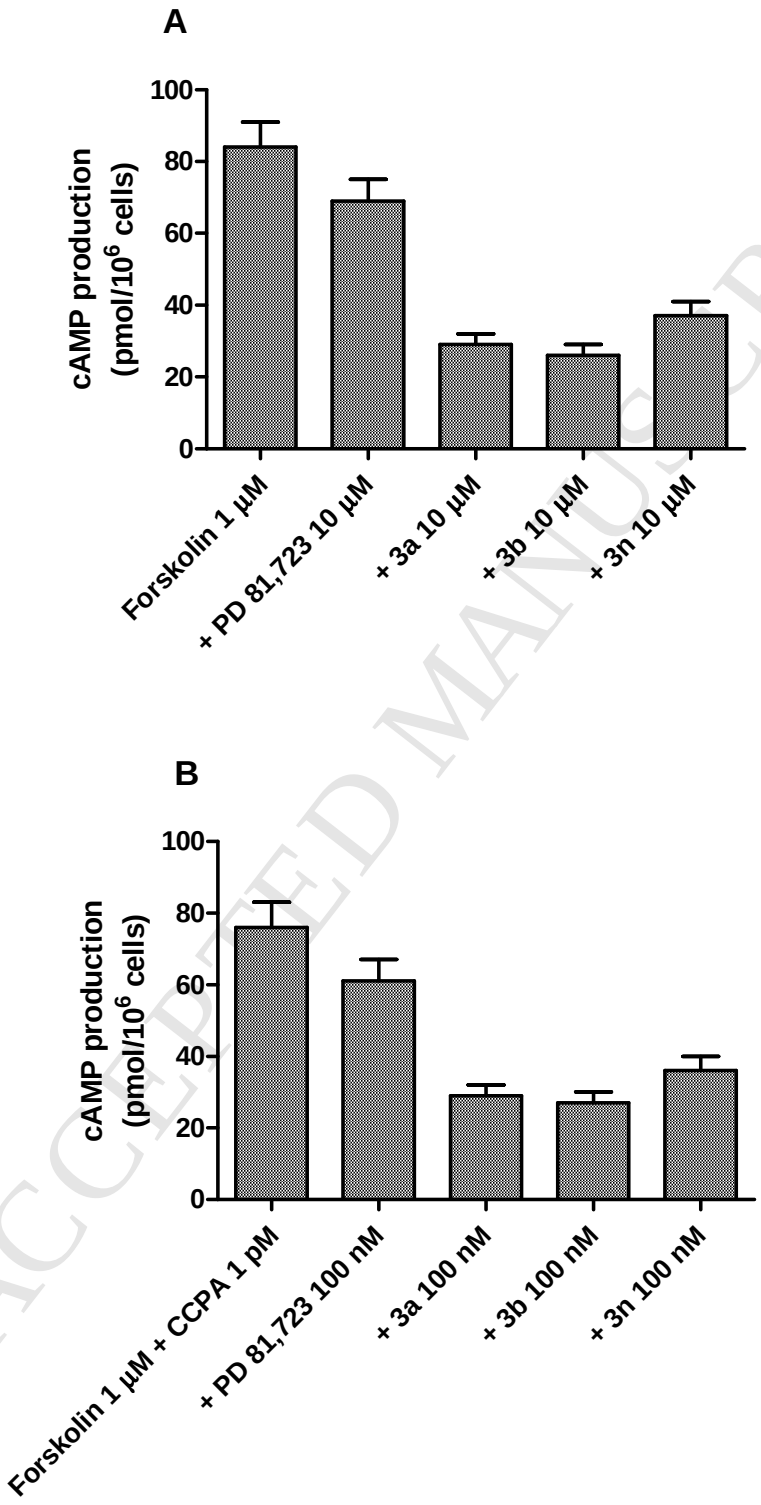


Figure 2

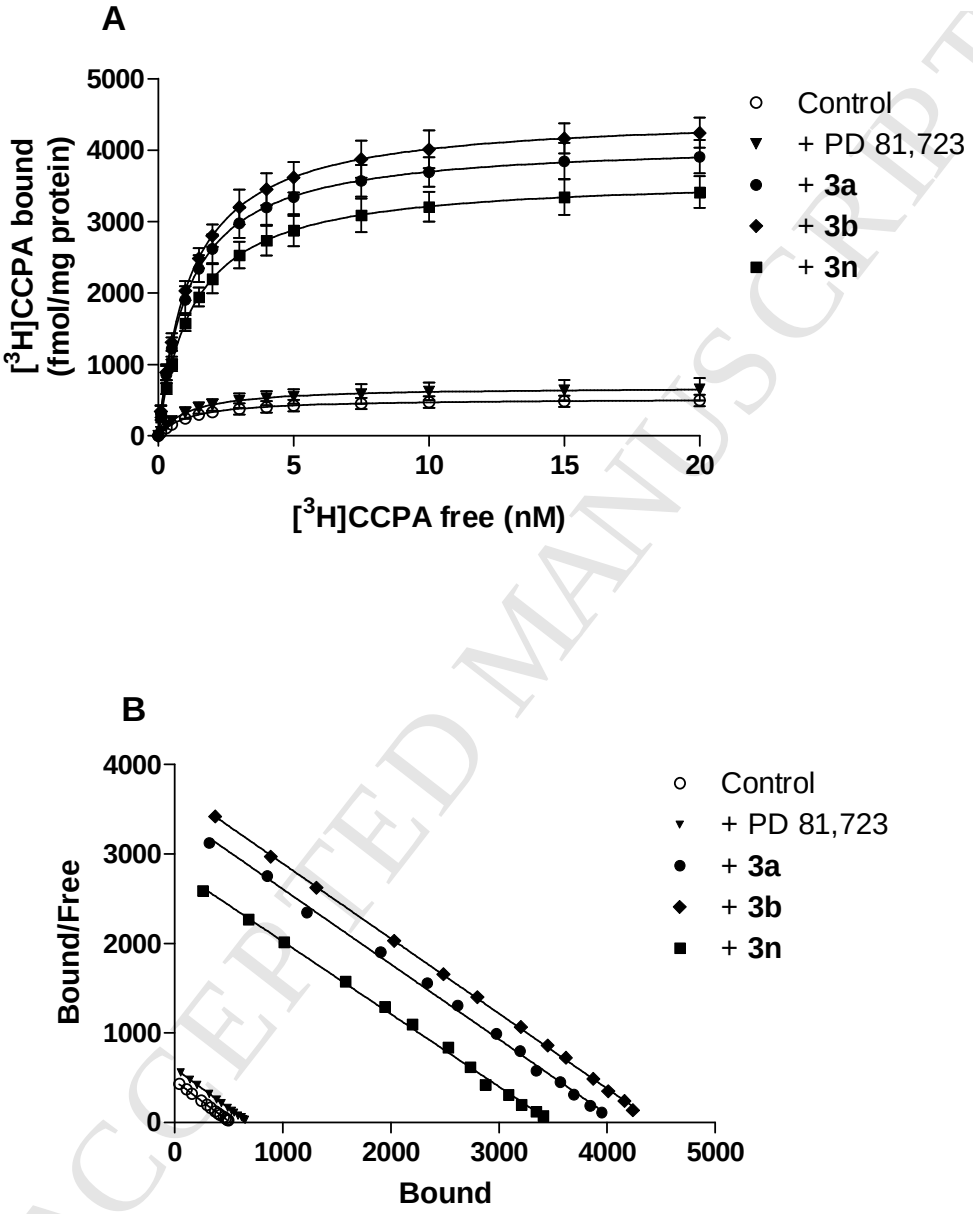
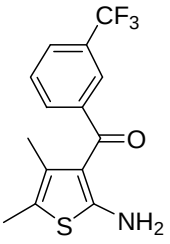
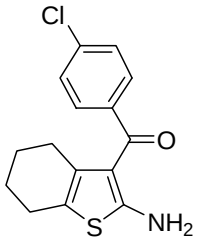


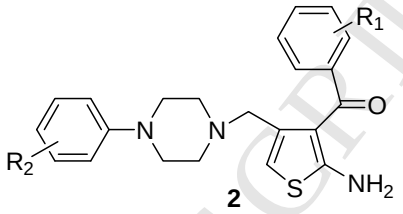
Chart 1. Chemical structures of 2-amino-3-aroyl thiophene derivatives **1a, b**, **2** and **3a-q**, evaluated as allosteric modulators for the A_1 adenosine receptor



1a, PD 81,723

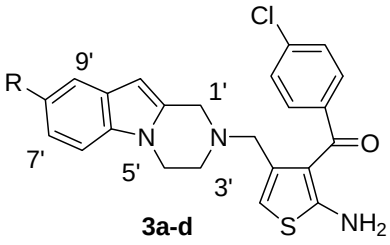


1b, T 62



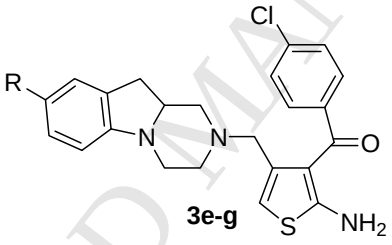
2

R_1=4-Cl or 3-CF_3
R_2= EWG (F, Cl, CF_3, NO_2) or ERG (Me or OMe



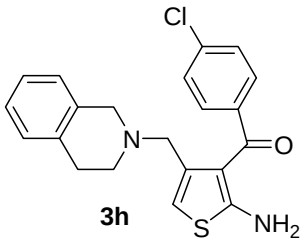
3a-d

3a; R=H
3b; R=F
3c; R=Cl
3d; R=OCH_3

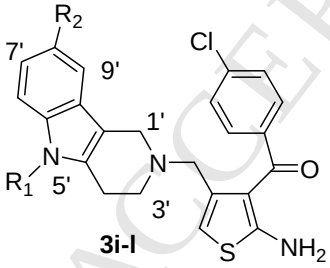


3e-g

3e; R=H
3f; R=Cl
3g; R=Me

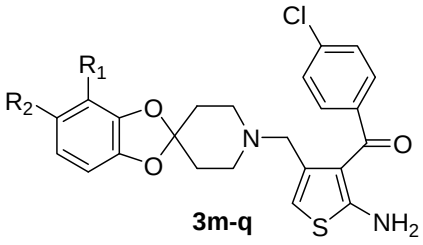


3h



3i-l

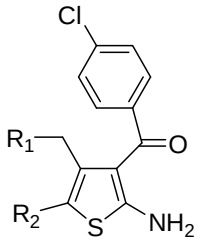
3i; $R_{1,2}$=H
3j; R_1=H, R_2=Cl
3k; R_1=CH_3, R_2=H
3l; R_1=CH_3, R_2=Cl



3m-q

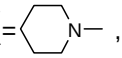
3m; $R_{1,2}$=H
3n; R_1=F, R_2=H
3o; R_1=CH_3, R_2=H
3p; R_1=H, R_2=CH_3
3q; R_1=H, R_2=$C(CH_3)_3$

Chart 2. Chemical structures of 2-amino-3-(4-chlorobenzoyl)thiophene derivatives **3r-aq**.



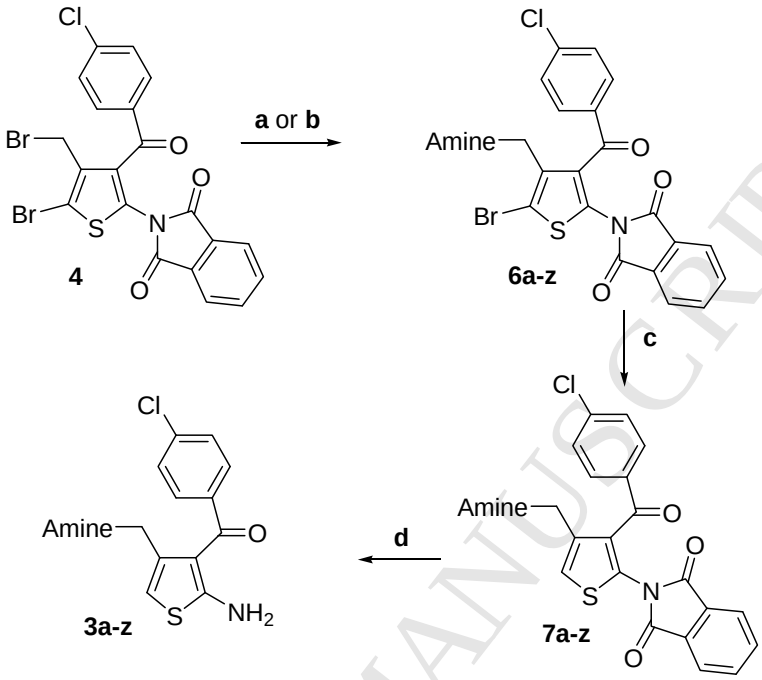
3r-aq

3r; $R_1=C_6H_5N(CH_3)$, $R_2=H$
3s; $R_1=C_6H_5N(C_2H_5)$, $R_2=H$
3t; $R_1=p\text{-}F\text{-}C_6H_4N(CH_3)$, $R_2=H$
3u; $R_1=p\text{-}Cl\text{-}C_6H_4N(CH_3)$, $R_2=H$
3v; $R_1=p\text{-}CF_3\text{-}C_6H_4N(CH_3)$, $R_2=H$
3w; $R_1=C_6H_5CH_2N(CH_3)$, $R_2=H$
3x; $R_1=(C_2H_5)_2N$, $R_2=H$
3y; $R_1=(n\text{-}C_3H_7)_2N$, $R_2=H$
3z; $R_1=(i\text{-}C_3H_7)_2N$, $R_2=H$
3aa; $R_1=(CH_3)_2N$, $R_2=C_6H_5$
3ab; $R_1=(C_2H_5)_2N$, $R_2=C_6H_5$
3ac, $R_1=(n\text{-}C_3H_7)_2N$, $R_2=C_6H_5$
3ad, $R_1=(CH_2=CHCH_2)_2N$, $R_2=C_6H_5$
3ae; $R_1=(i\text{-}C_3H_7)_2N$, $R_2=C_6H_5$
3af; $R_1=((CH_3)_3C)N\ (CH_3)$, $R_2=C_6H_5$
3ag; $R_1=(C_6H_{11})_2N$, $R_2=C_6H_5$

3ah; $R_1=$  , $R_2=C_6H_5$

3ai; $R_1=(n\text{-}C_4H_9)N(CH_3)$, $R_2=C_6H_5$
3aj; $R_1=C_6H_5CH_2N(CH_3)$, $R_2=C_6H_5$
3ak, $R_1=(CH_2=CHCH_2)_2N$, $R_2=Br$
3al; $R_1=(i\text{-}C_3H_7)_2N$, $R_2=Br$
3am; $R_1=C_6H_5O$, $R_2=H$
3an; $R_1=p\text{-}CH_3\text{-}C_6H_4O$, $R_2=H$
3ao; $R_1=p\text{-}(CH_3)_2CH\text{-}C_6H_4O$, $R_2=H$
3ap; $R_1=p\text{-}F\text{-}C_6H_4O$, $R_2=H$
3aq; $R_1=C_6H_5S$, $R_2=H$

Scheme 1



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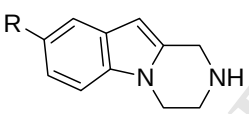
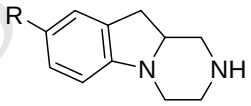
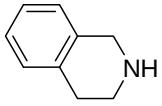


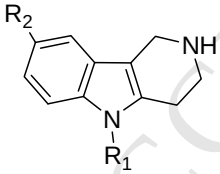
5a; R=H
5b; R=F
5c; R=Cl
5d; R=OCH_3



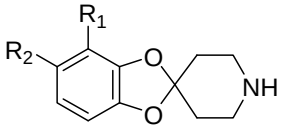
5e; R=H
5f; R=Cl
5g; R=Me



5h



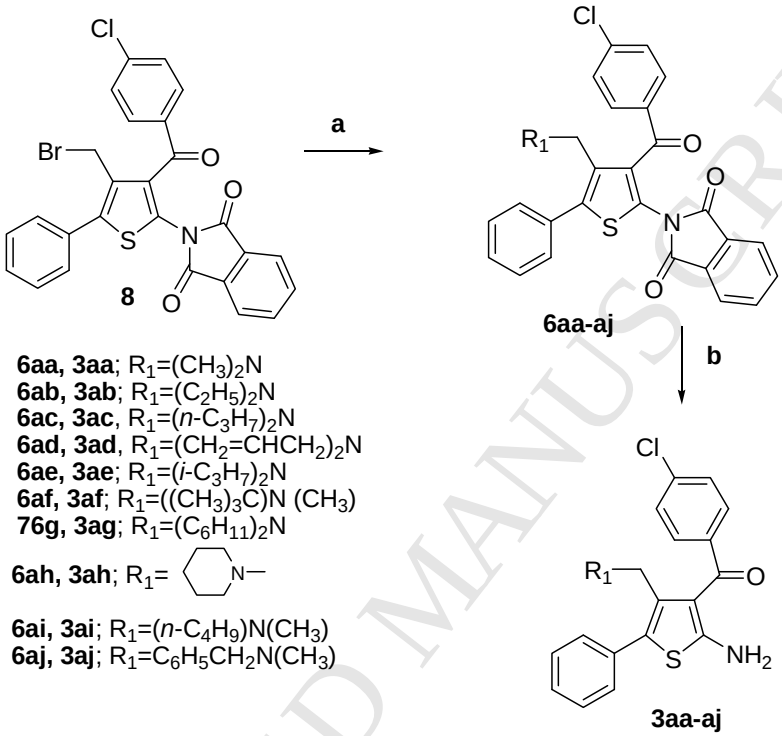
5i; $R_{1,2}$=H
5j; R_1=H, R_2=Cl
5k; R_1=CH_3, R_2=H
5l; R_1=CH_3, R_2=Cl



5m; $R_{1,2}$=H
5n; R_1=F, R_2=H
5o; R_1=CH_3, R_2=H
5p; R_1=H, R_2=CH_3
5q; R_1=H, R_2=$C(CH_3)_3$

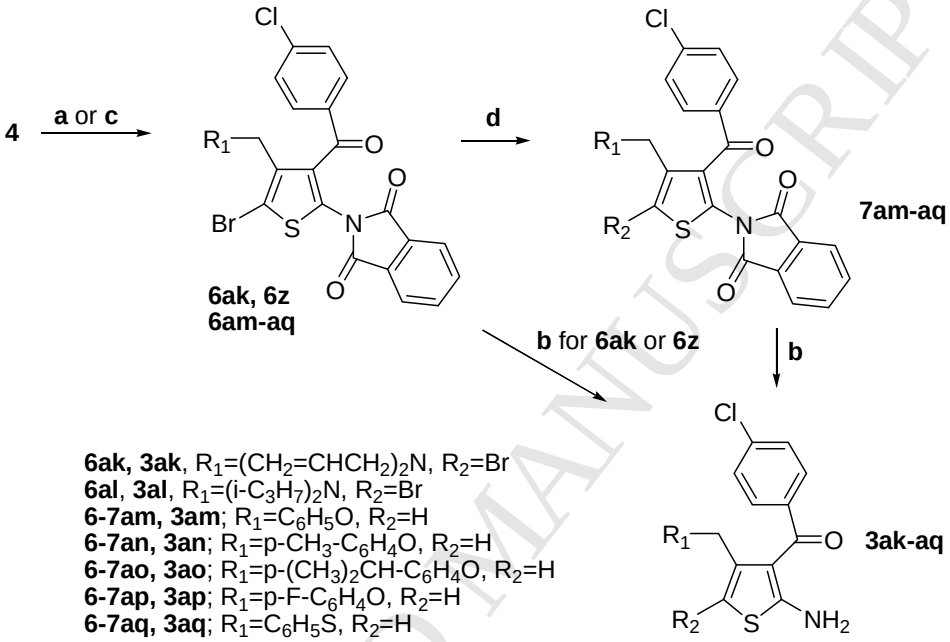
5r; $C_6H_5NH(CH_3)$
5s; $C_6H_5N(NC_2H_5)$
5t; p-F-$C_6H_4NH(CH_3)$
5u; p-Cl-$C_6H_4NH(CH_3)$
5v; p-CF_3-$C_6H_4NH(CH_3)$
5w; $C_6H_5CH_2NH(CH_3)$
5x, $(C_2H_5)_2NH$
5y; $(n\text{-}C_3H_7)_2NH$
5z; $(i\text{-}C_3H_7)_2NH$

Scheme 2



6aa, 3aa; $R_1=(CH_3)_2N$
6ab, 3ab; $R_1=(C_2H_5)_2N$
6ac, 3ac, $R_1=(n\text{-}C_3H_7)_2N$
6ad, 3ad, $R_1=(CH_2=CHCH_2)_2N$
6ae, 3ae; $R_1=(i\text{-}C_3H_7)_2N$
6af, 3af; $R_1=((CH_3)_3C)N\ (CH_3)$
76g, 3ag; $R_1=(C_6H_{11})_2N$
6ah, 3ah; $R_1=$
6ai, 3ai; $R_1=(n\text{-}C_4H_9)N(CH_3)$
6aj, 3aj; $R_1=C_6H_5CH_2N(CH_3)$

Scheme 3



6ak, 3ak, $R_1=(CH_2=CHCH_2)_2N$, $R_2=Br$
6al, **3al**, $R_1=(i\text{-}C_3H_7)_2N$, $R_2=Br$
6-7am, 3am; $R_1=C_6H_5O$, $R_2=H$
6-7an, 3an; $R_1=p\text{-}CH_3\text{-}C_6H_4O$, $R_2=H$
6-7ao, 3ao; $R_1=p\text{-}(CH_3)_2CH\text{-}C_6H_4O$, $R_2=H$
6-7ap, 3ap; $R_1=p\text{-}F\text{-}C_6H_4O$, $R_2=H$
6-7aq, 3aq; $R_1=C_6H_5S$, $R_2=H$

Highlights

- Compounds **3a-d** appeared to be more active than PD 81,723 in the functional assay.
- None of compounds **3a-aq** inhibited antagonist binding at the hA_1AR, hA_2AR, or hA_3AR.
- Derivatives **3a-c**, **3e** and **3n** were the most active compounds in binding experiments.

Synthesis and Biological Effects of Novel 2-Amino-3-(4-Chlorobenzoyl)-4-Substituted Thiophenes as Allosteric Enhancers of the A_1 Adenosine Receptor

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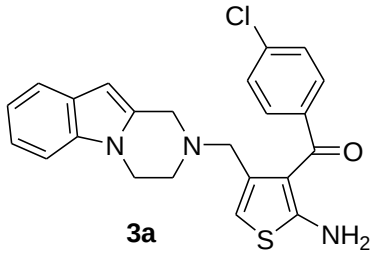
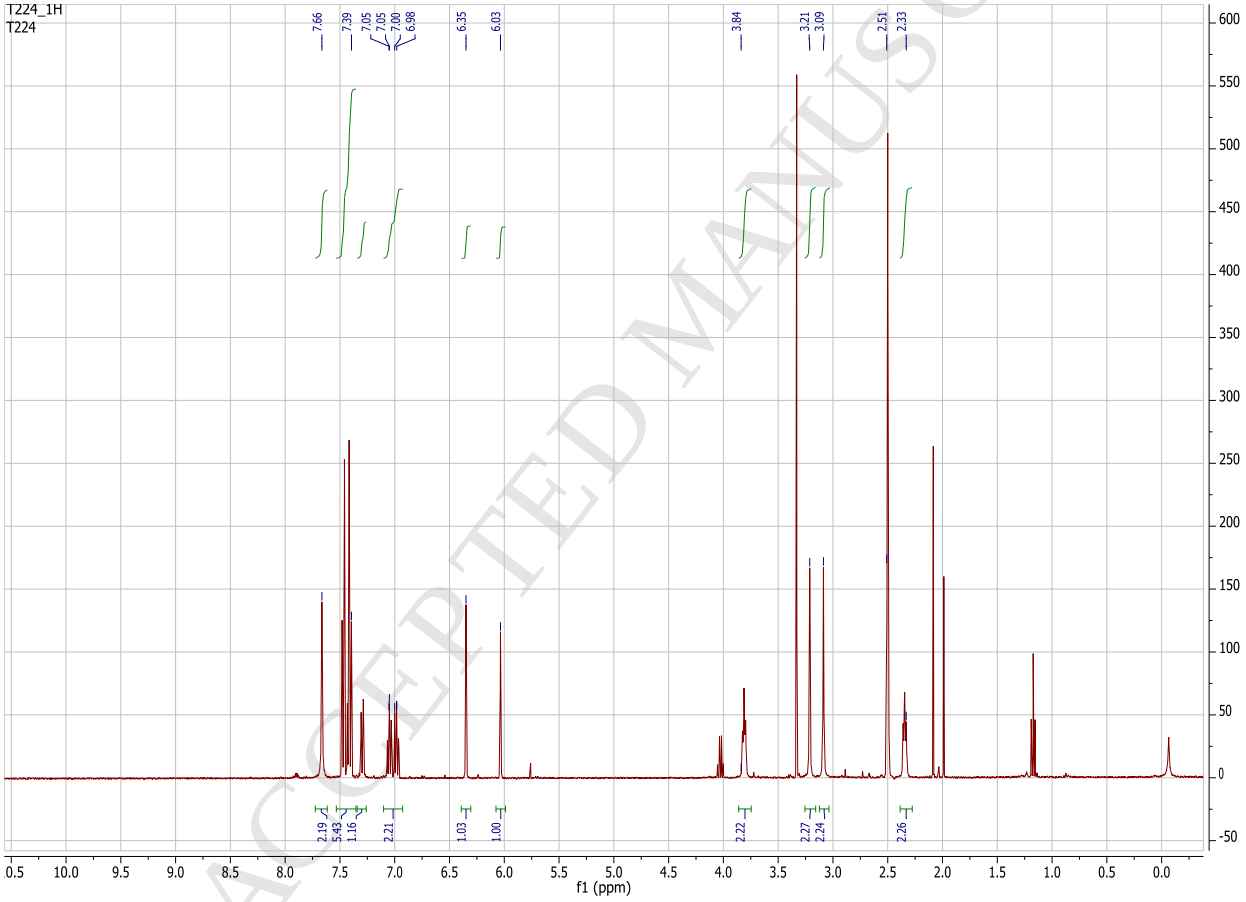
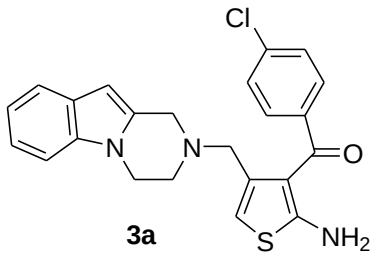
Supplementary data

^{1}H and ^{13}C spectra of compounds **3a**, **3c**, **3d**, **3n**, **3p**, **3r**, **3s**, **3v**, **3ab**, **3ad**, **3ae**, **3ag**, **3ah** and **3ap**

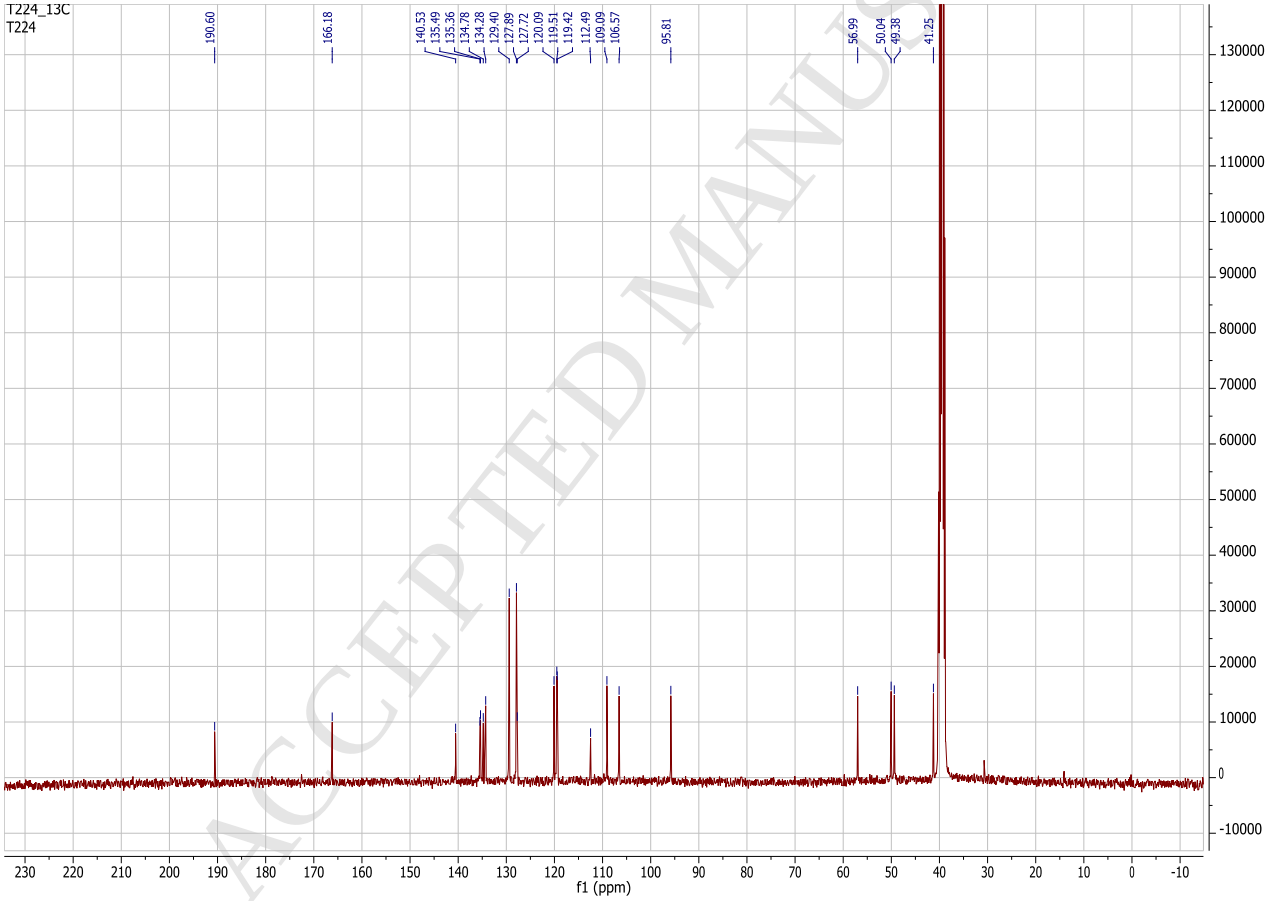


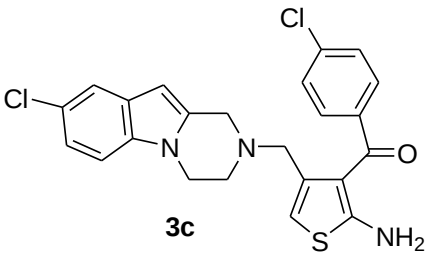
3a



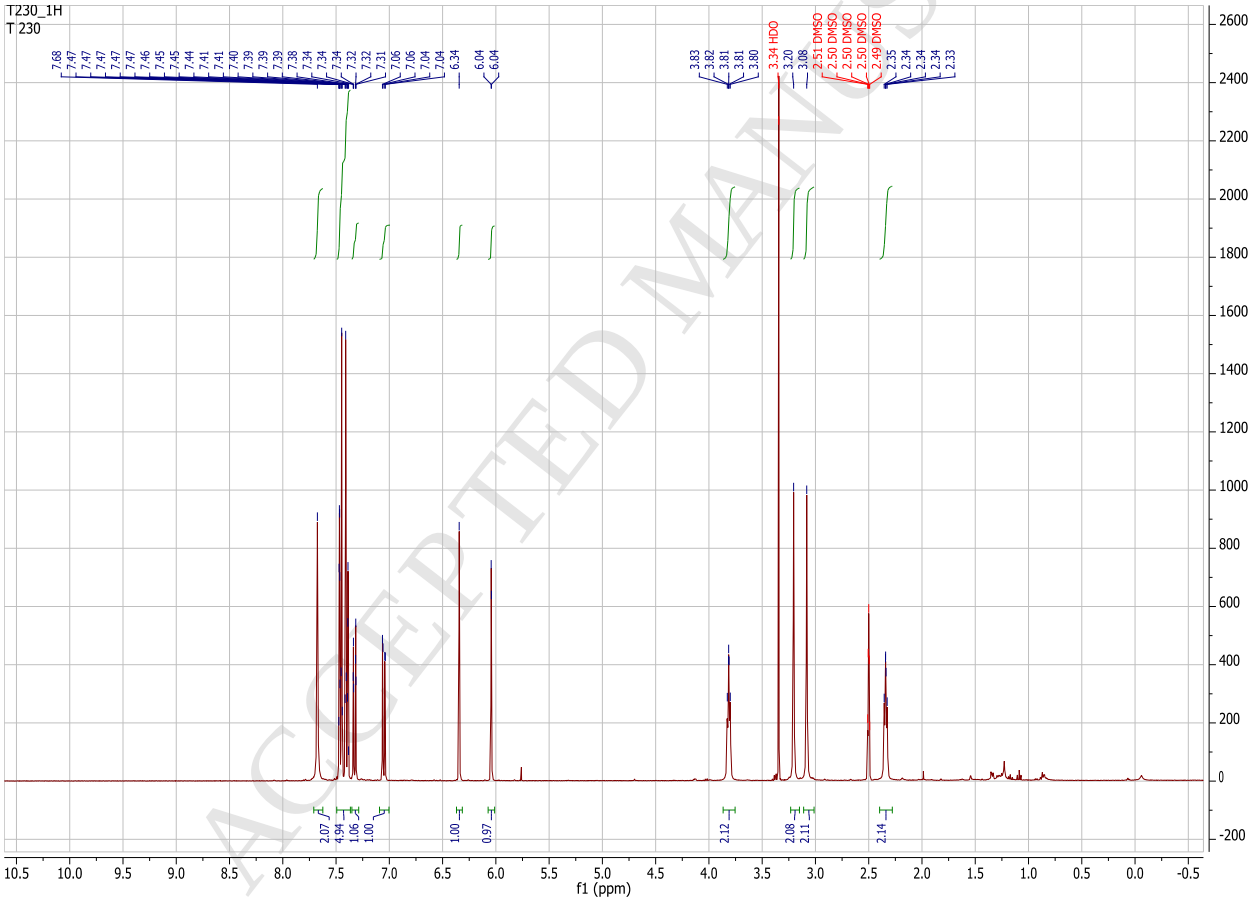


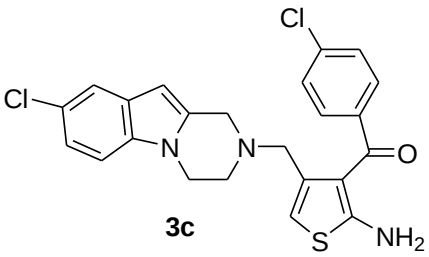
3a



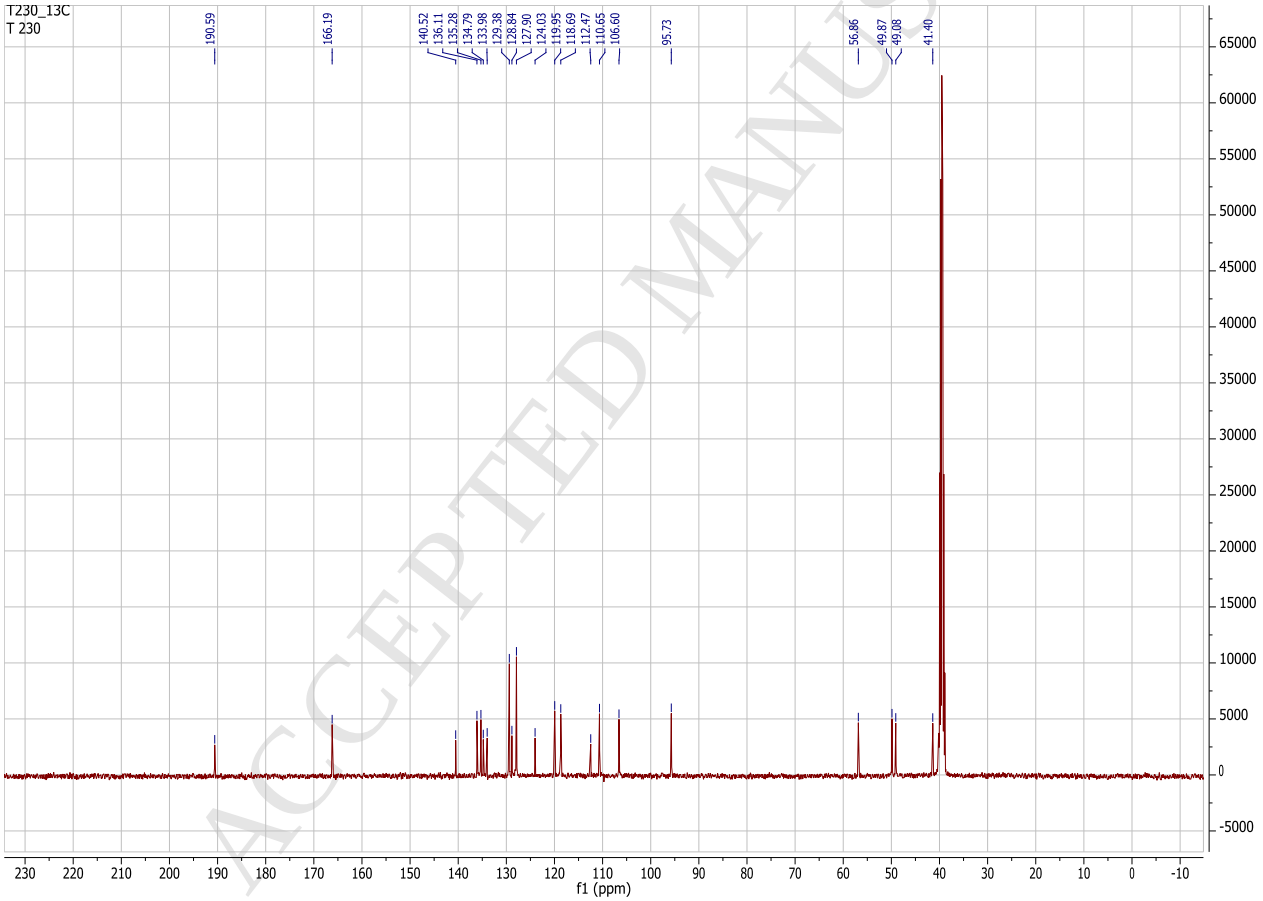


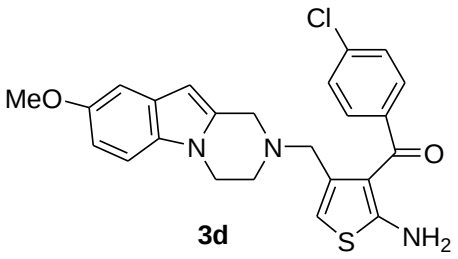
3c



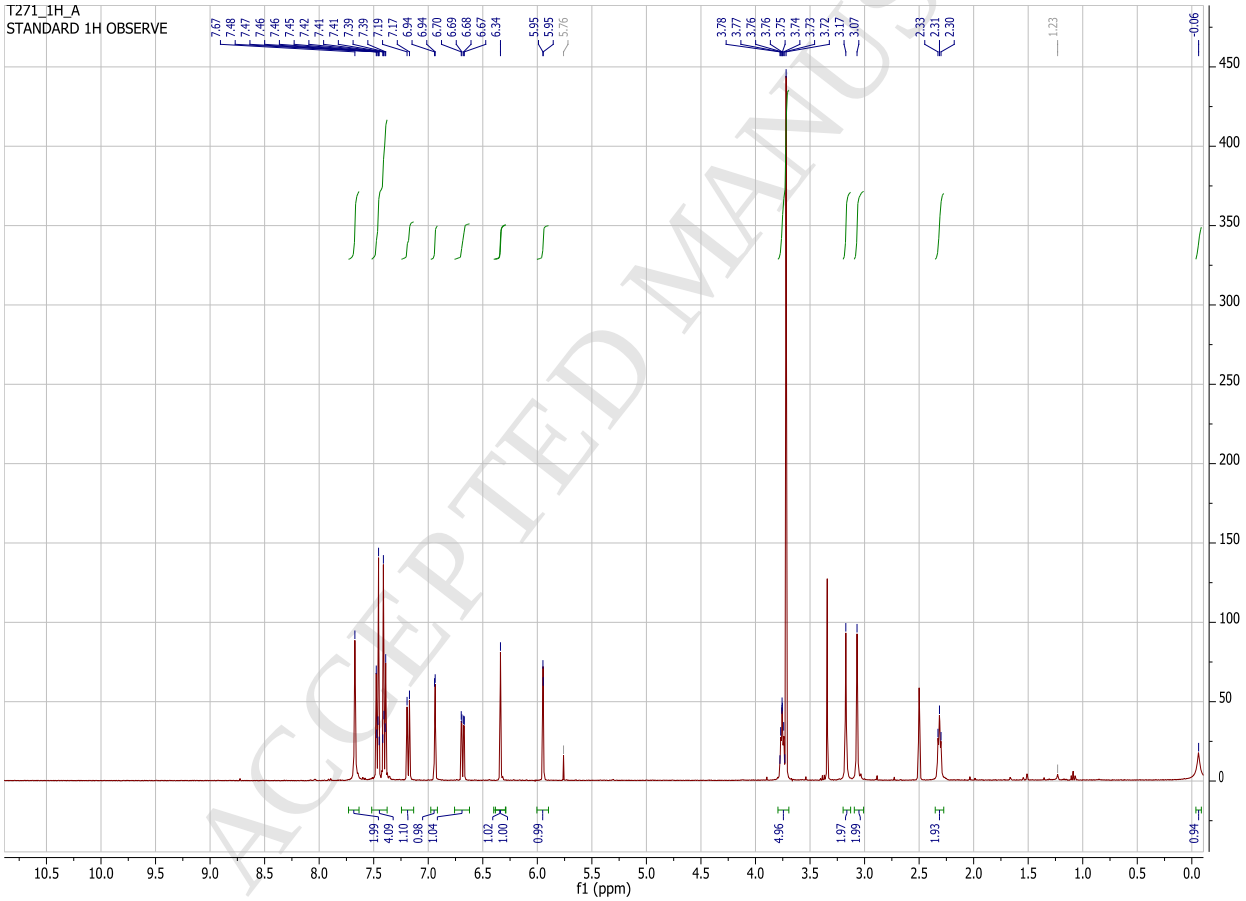


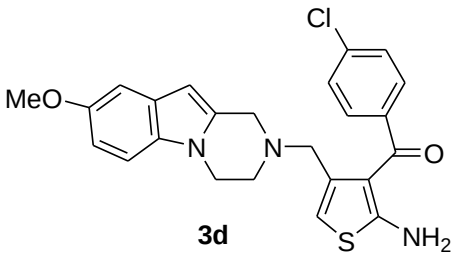
3c



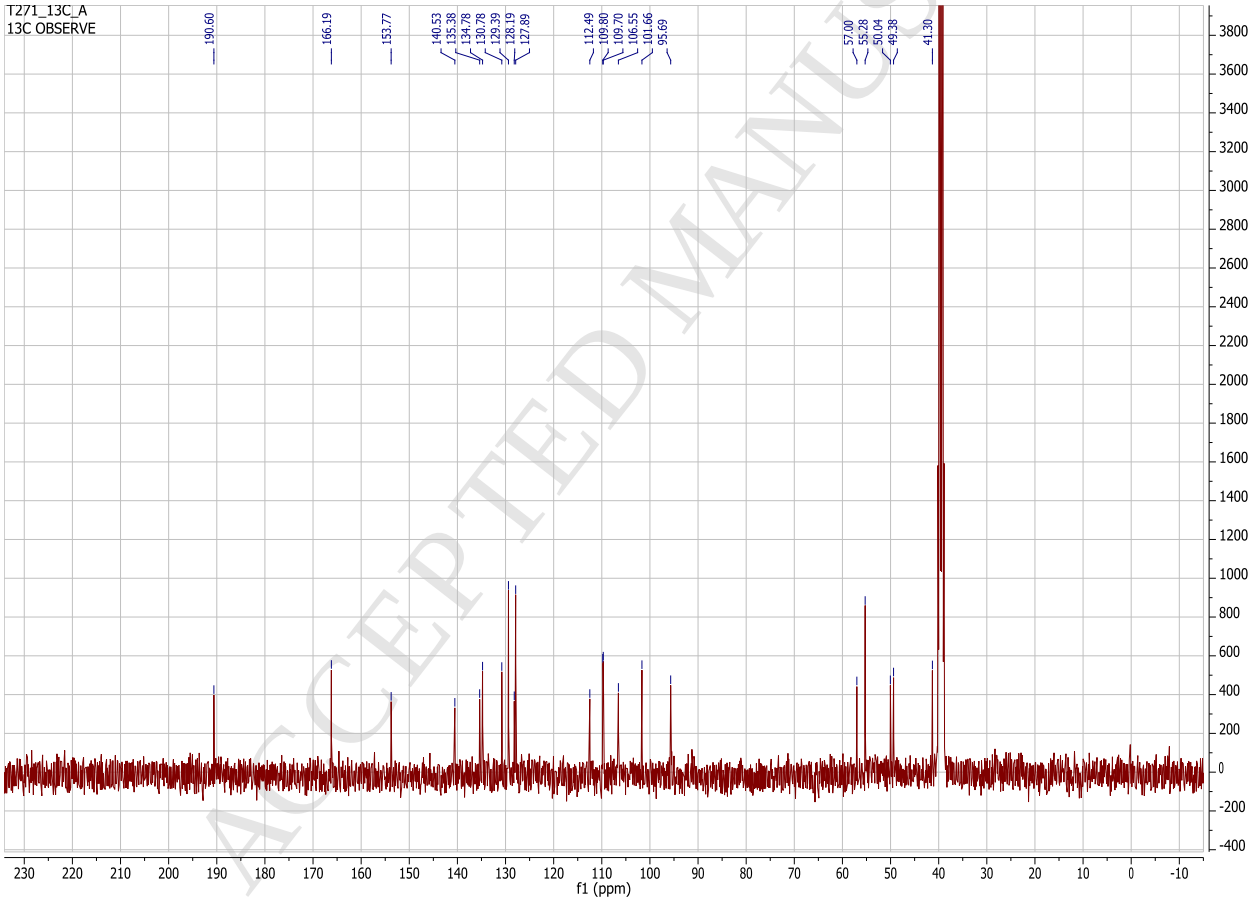


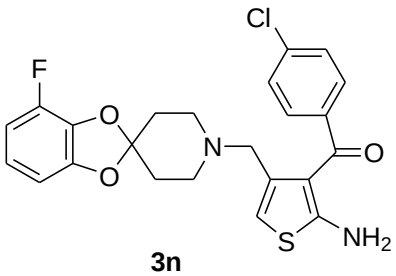
3d



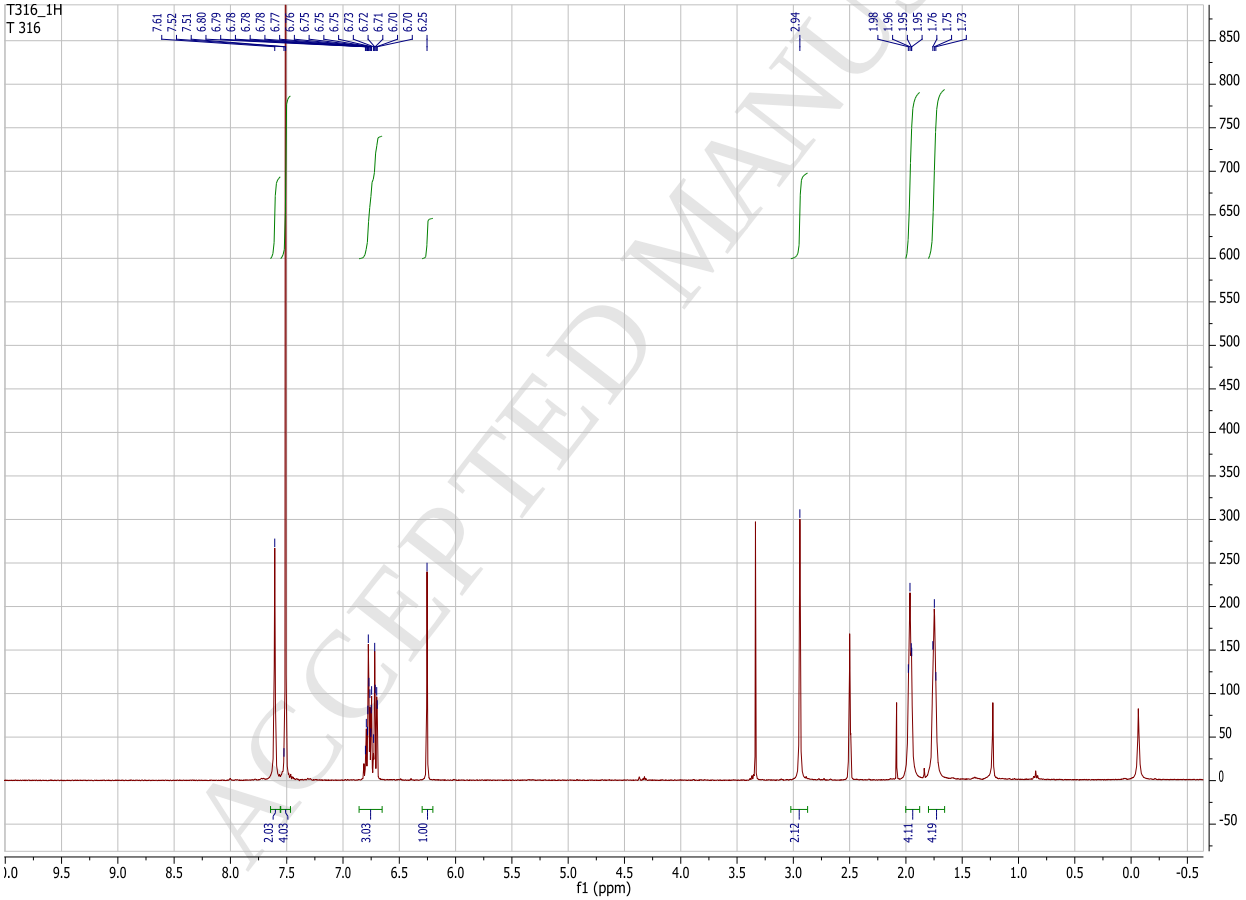


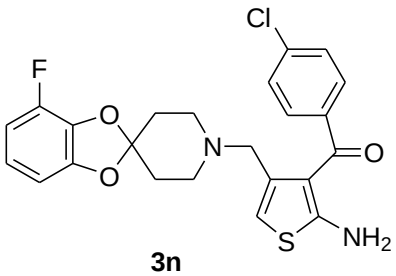
3d



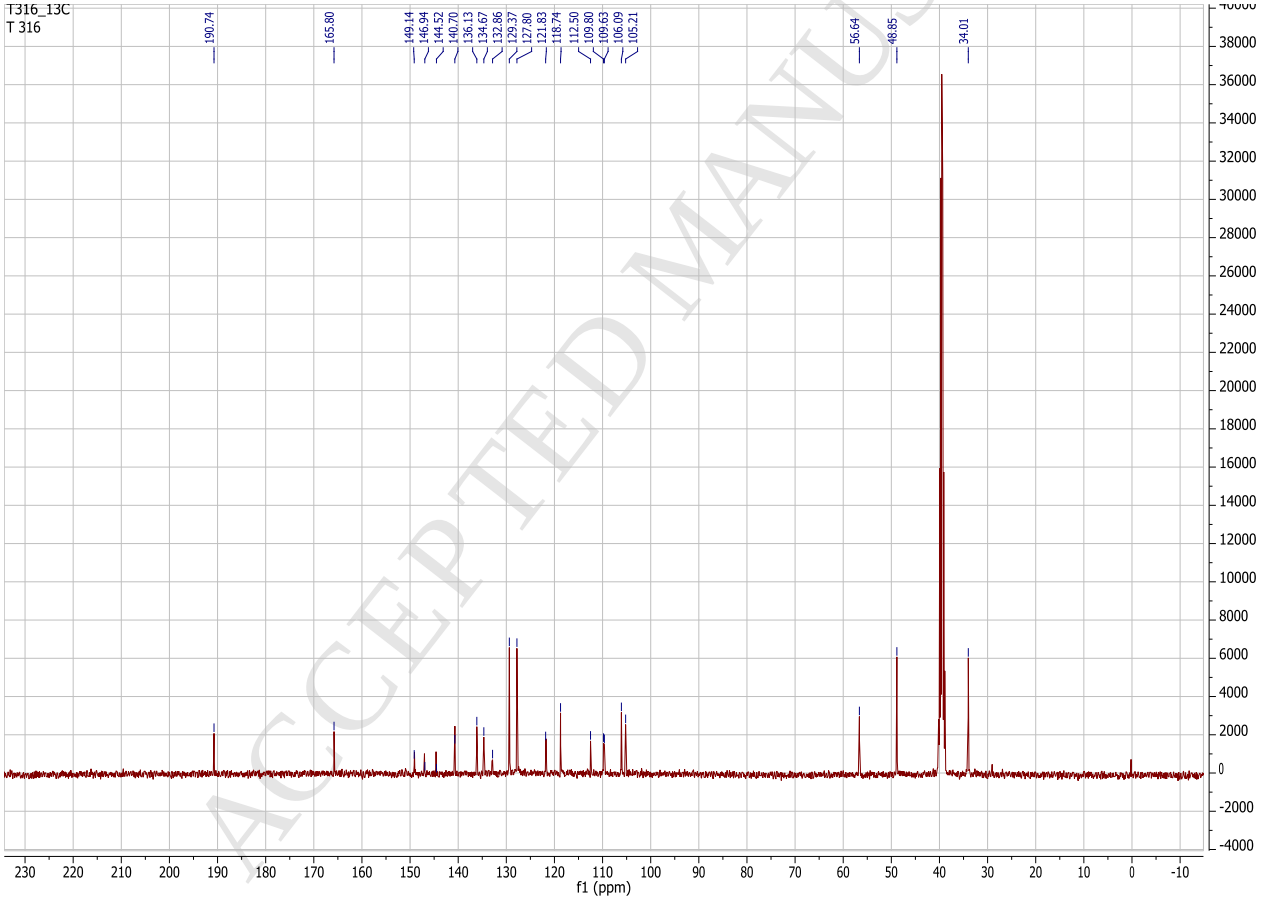


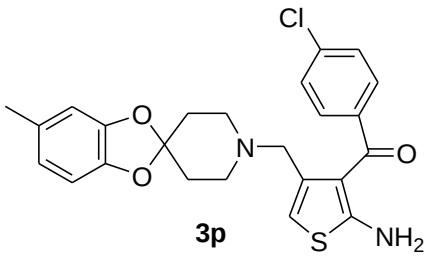
3n



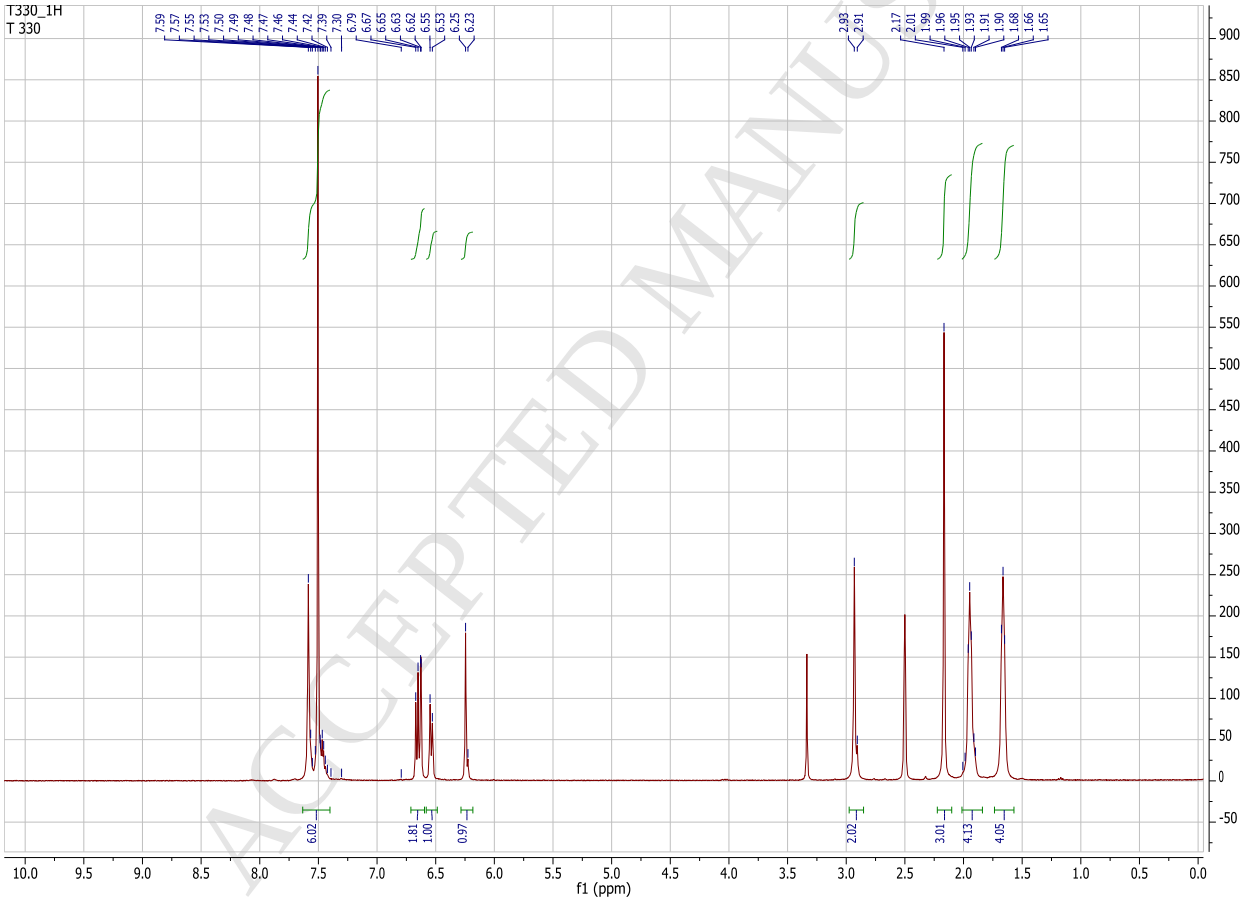


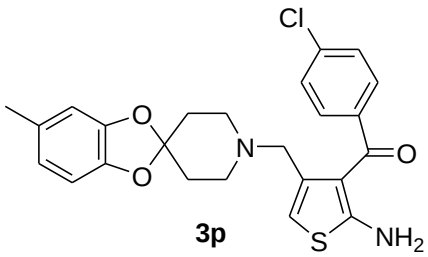
3n





3p





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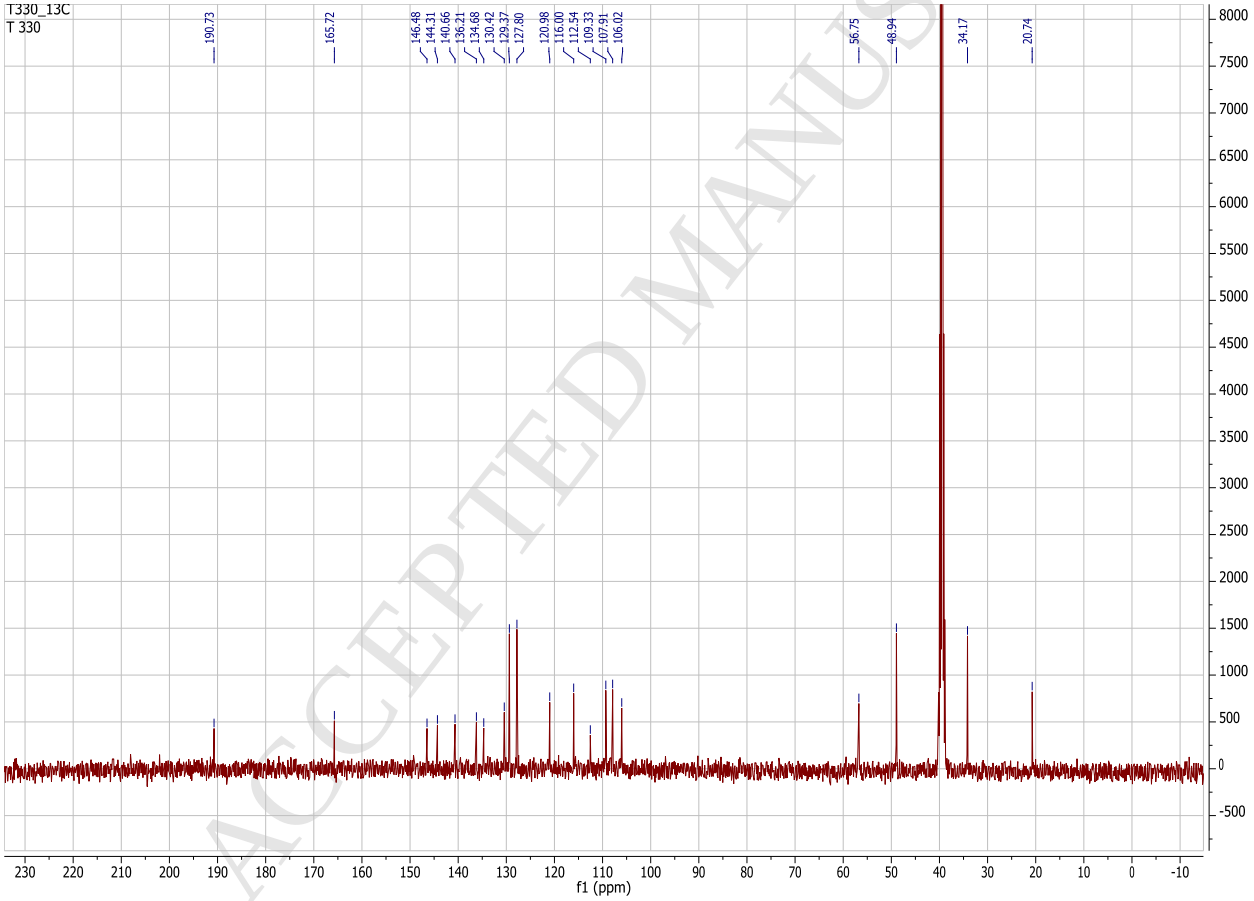

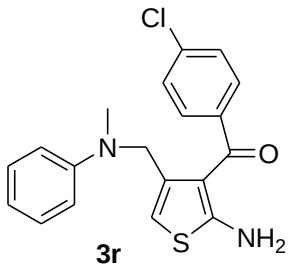

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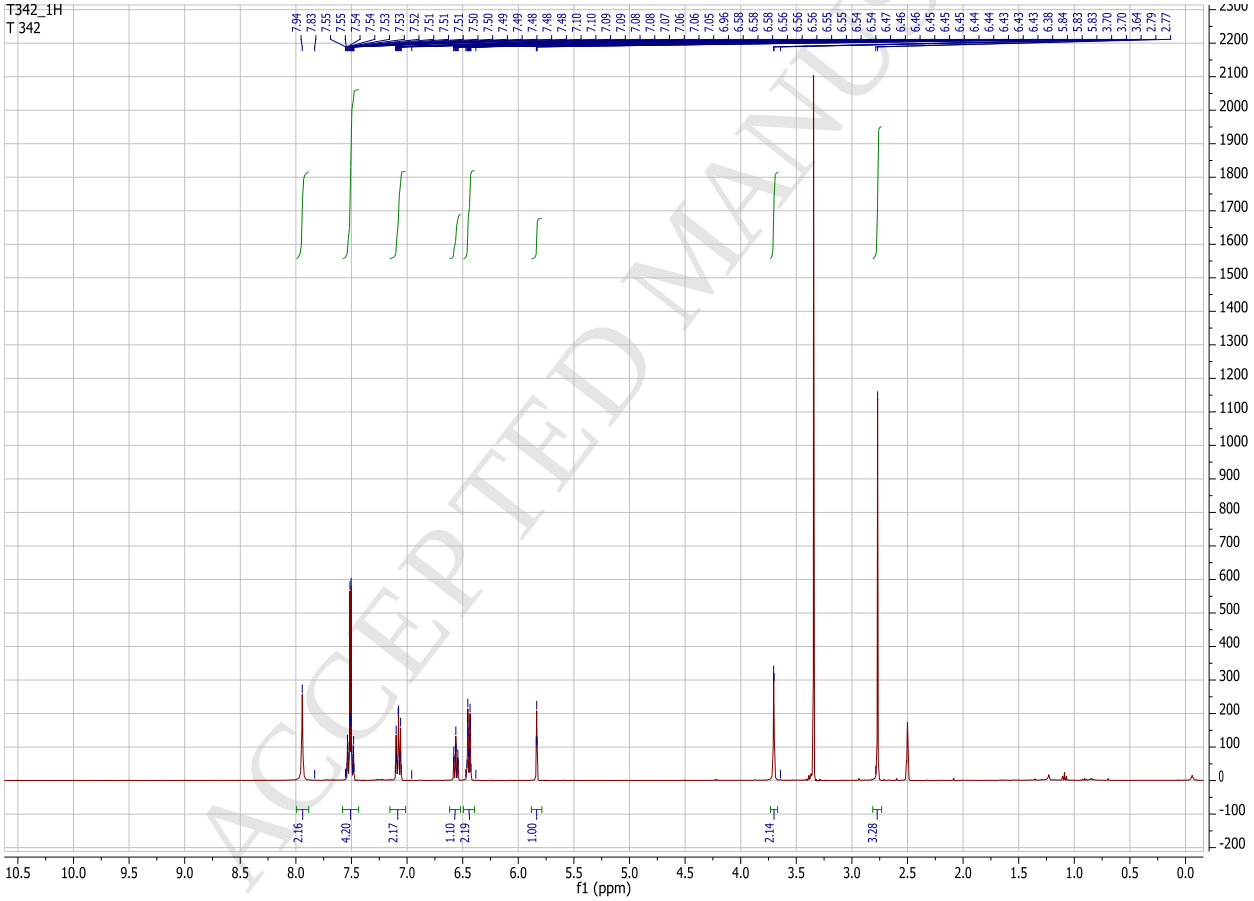

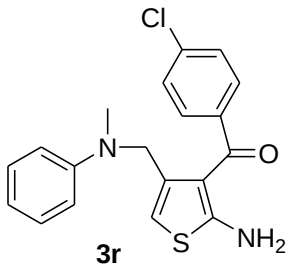

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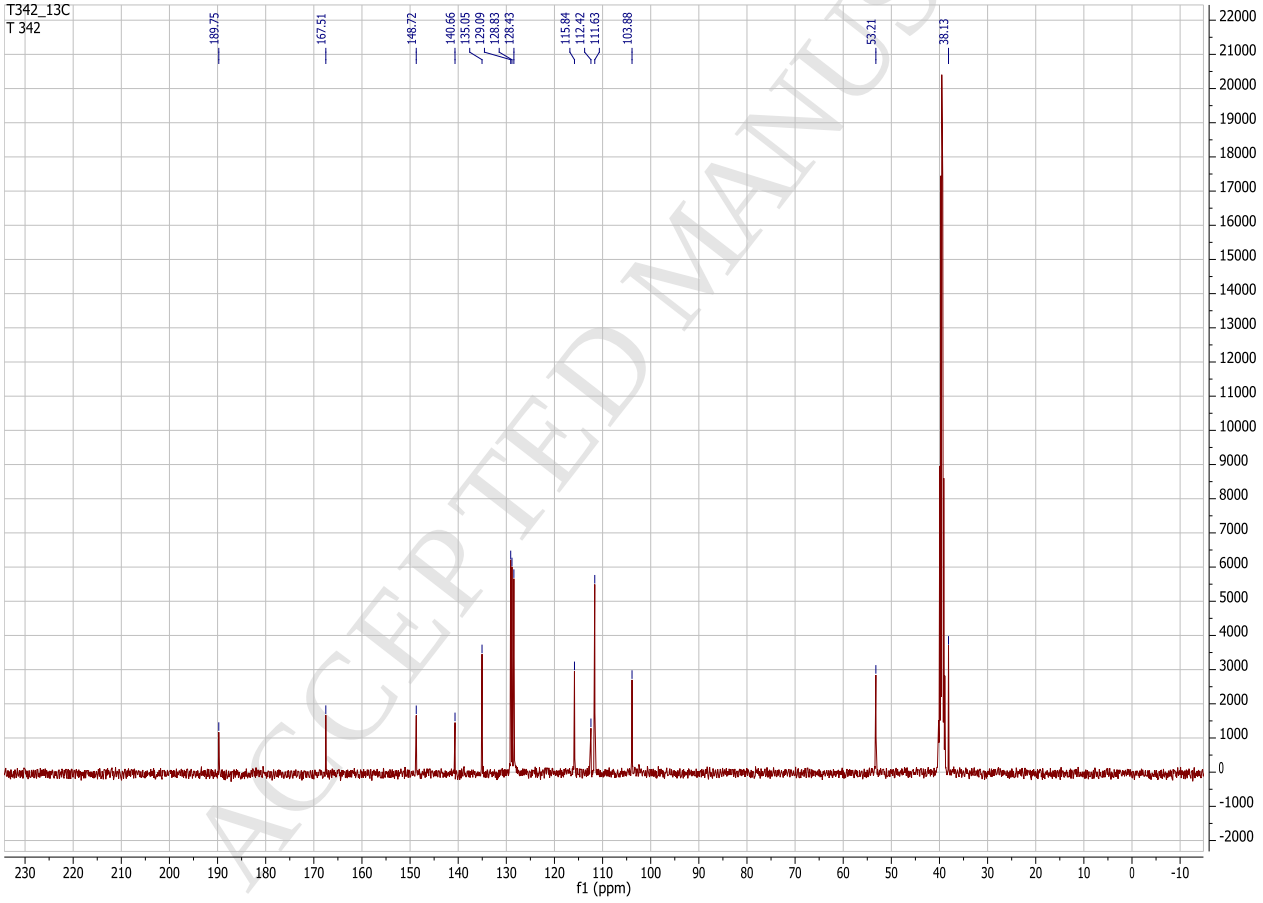
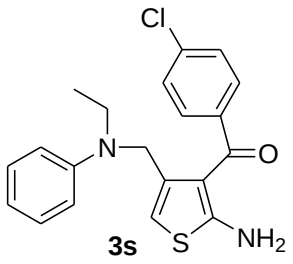
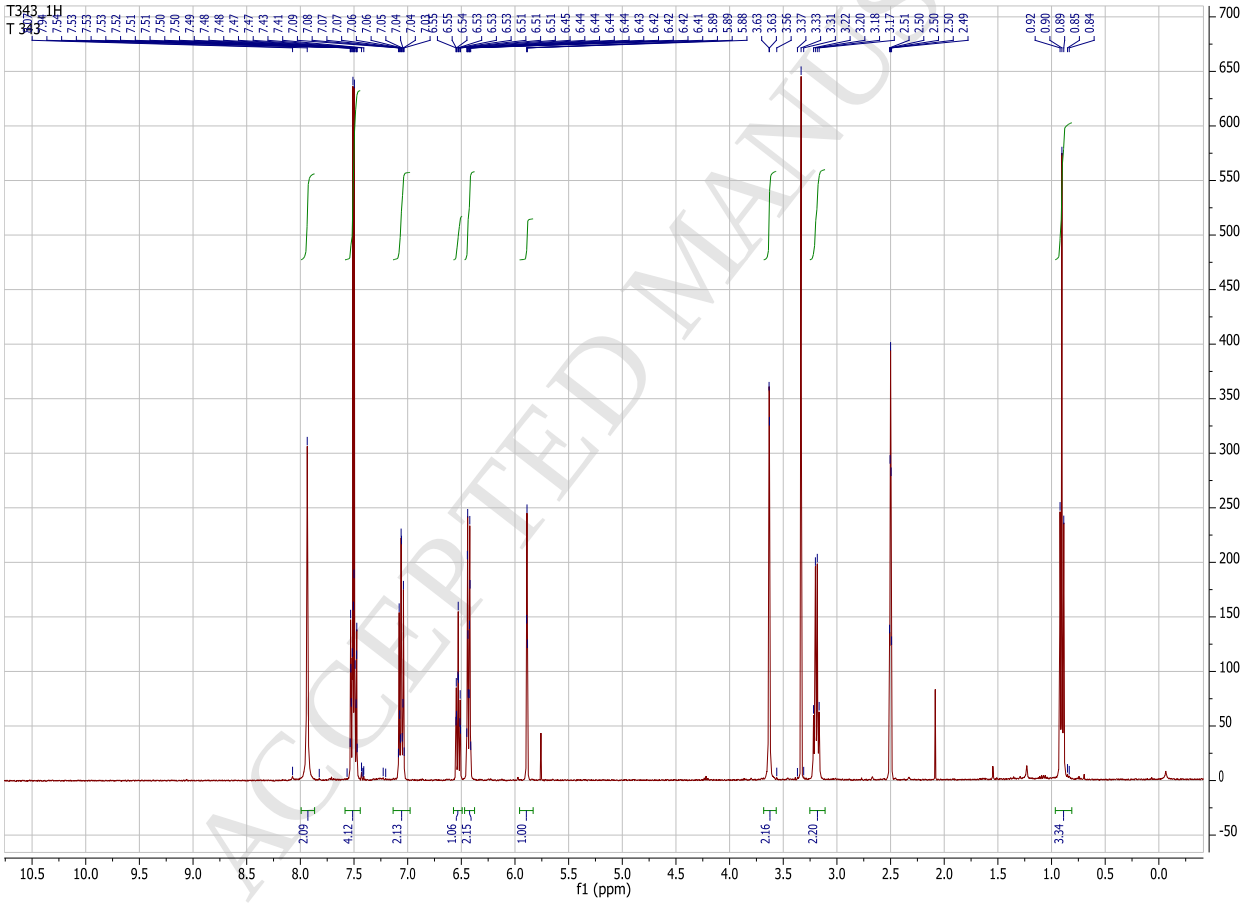
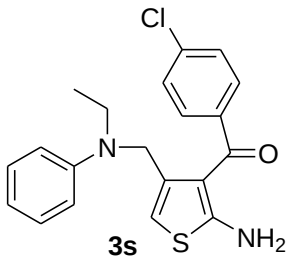




3s





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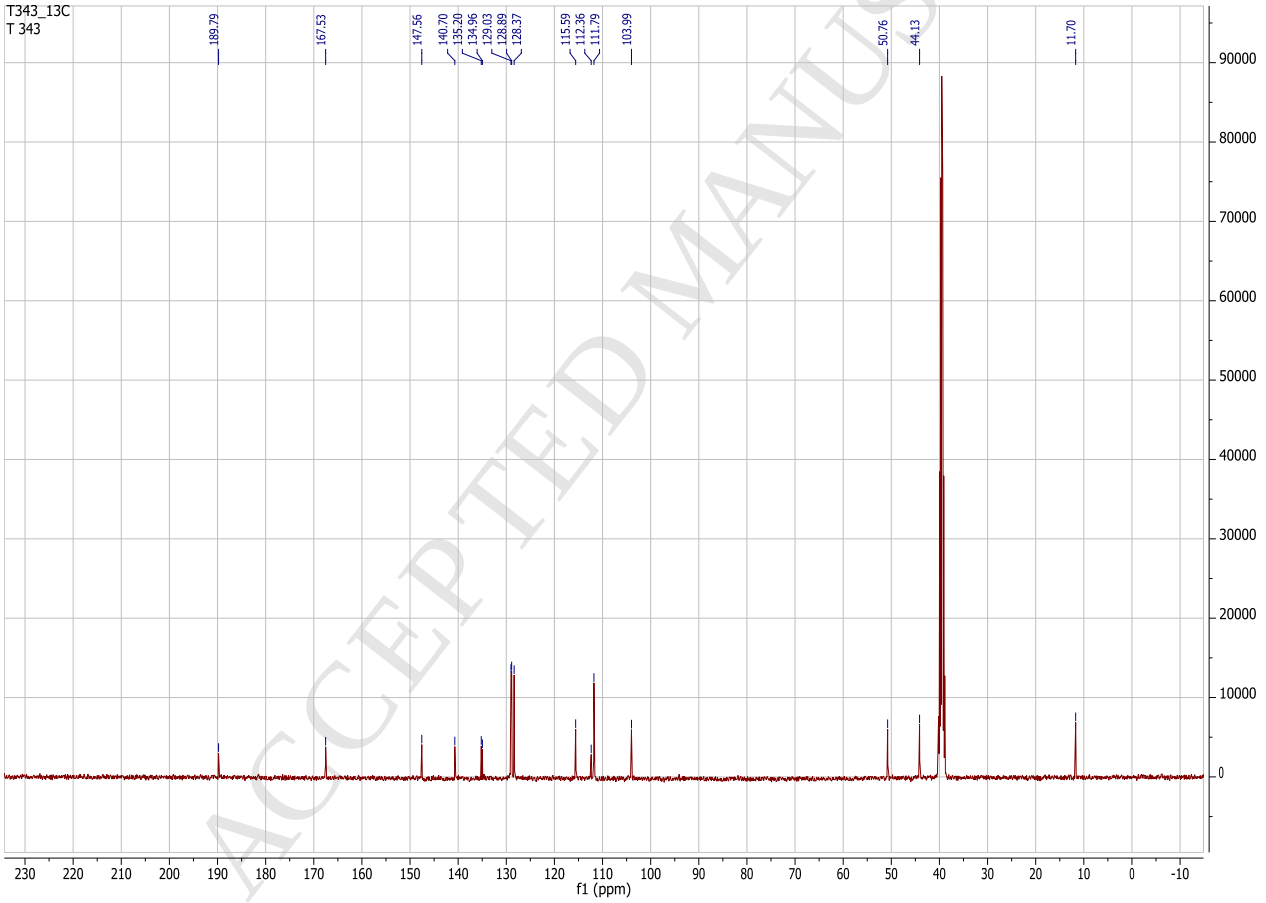
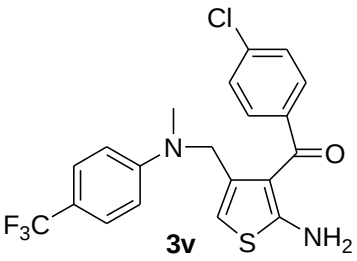
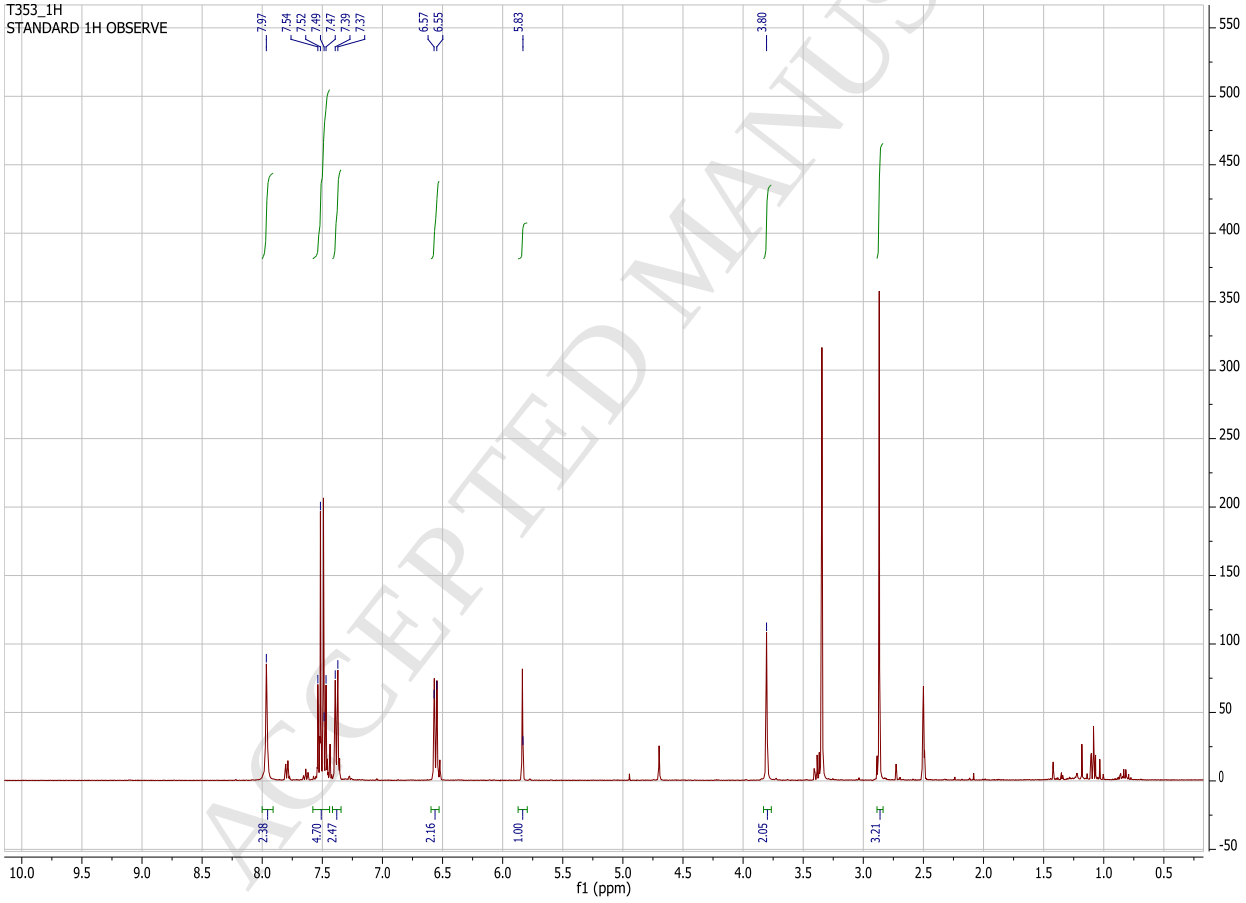
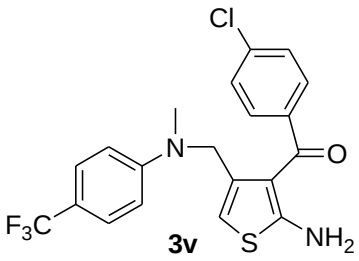




3v





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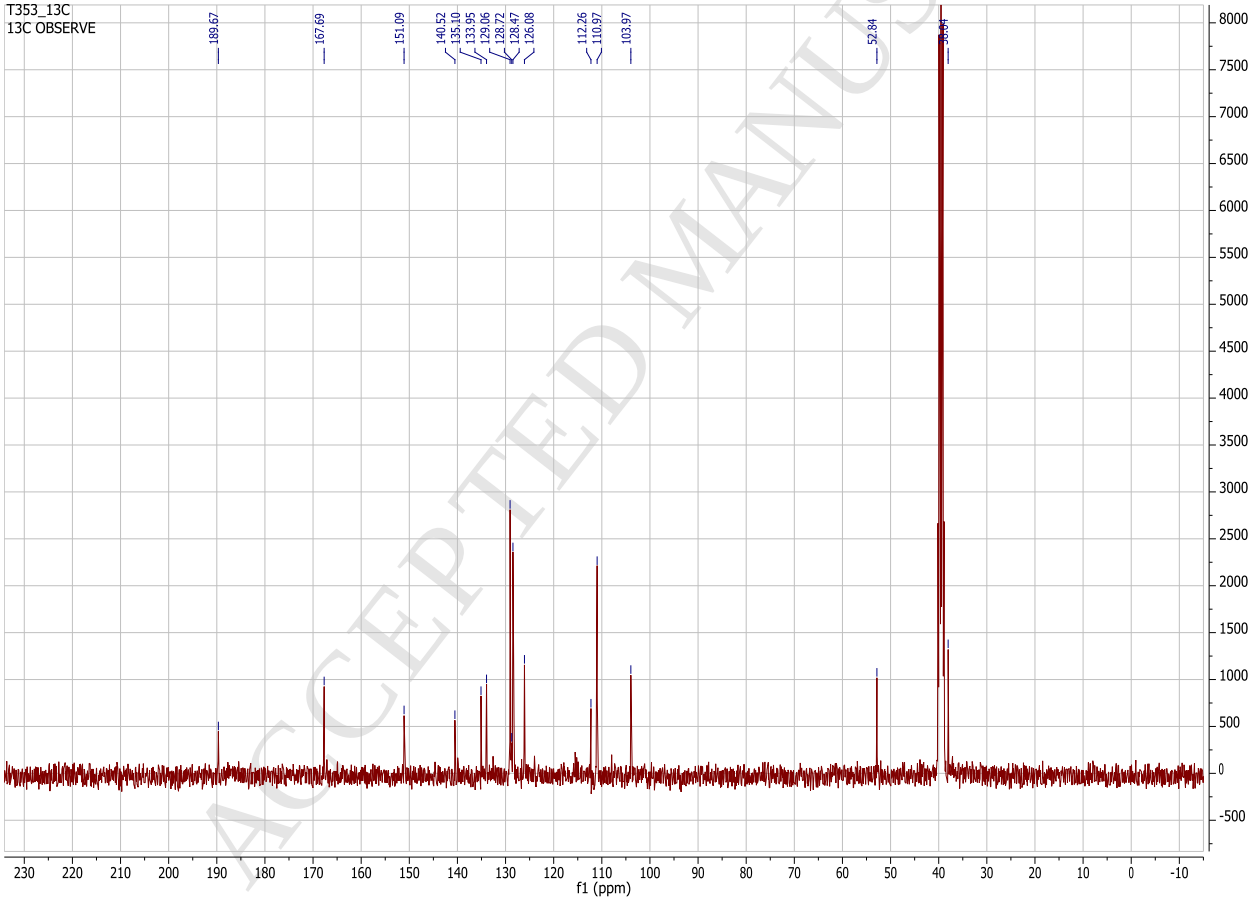
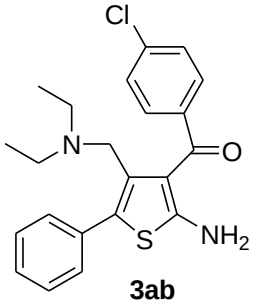
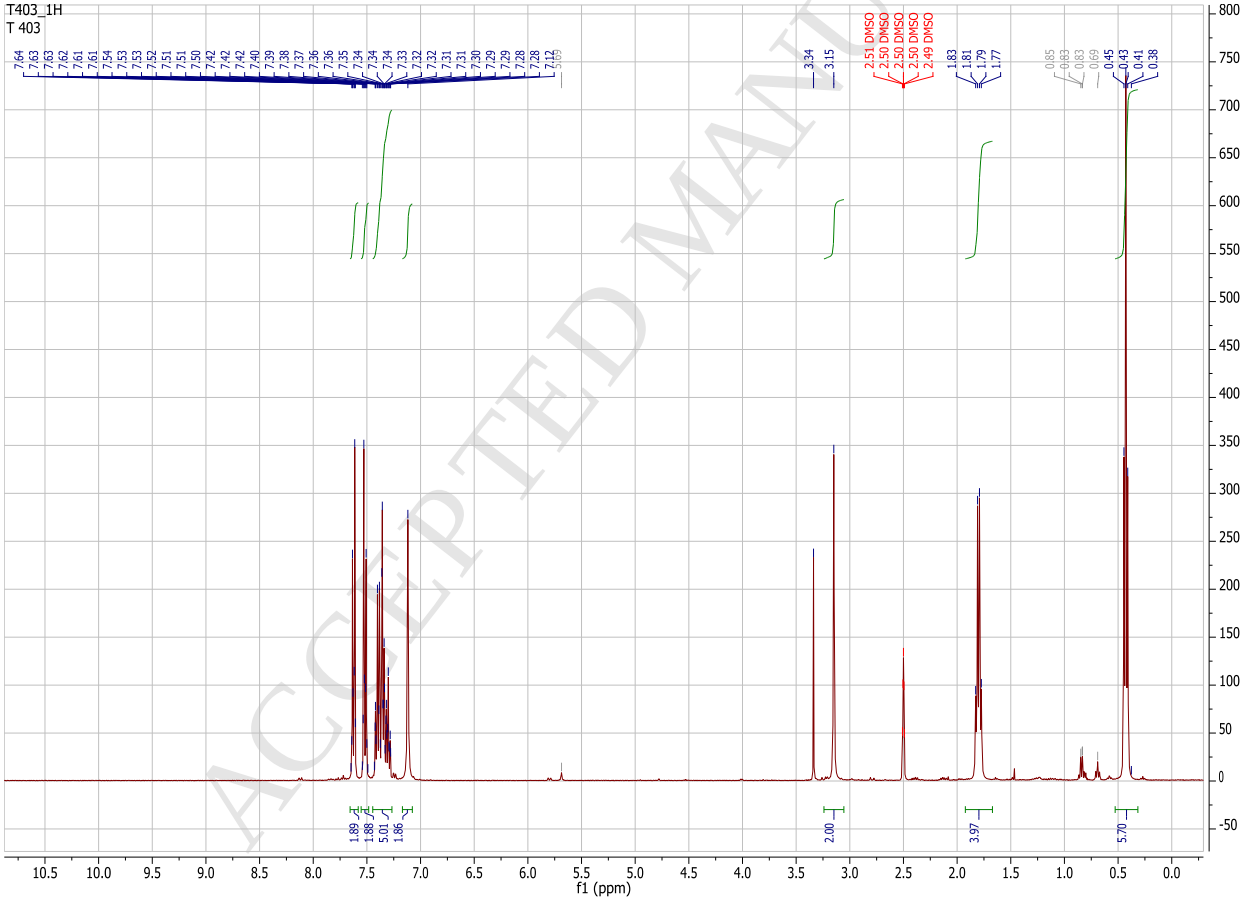
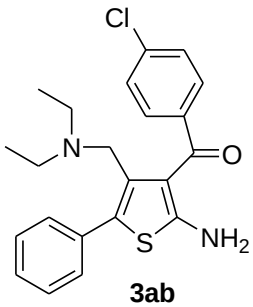




3ab





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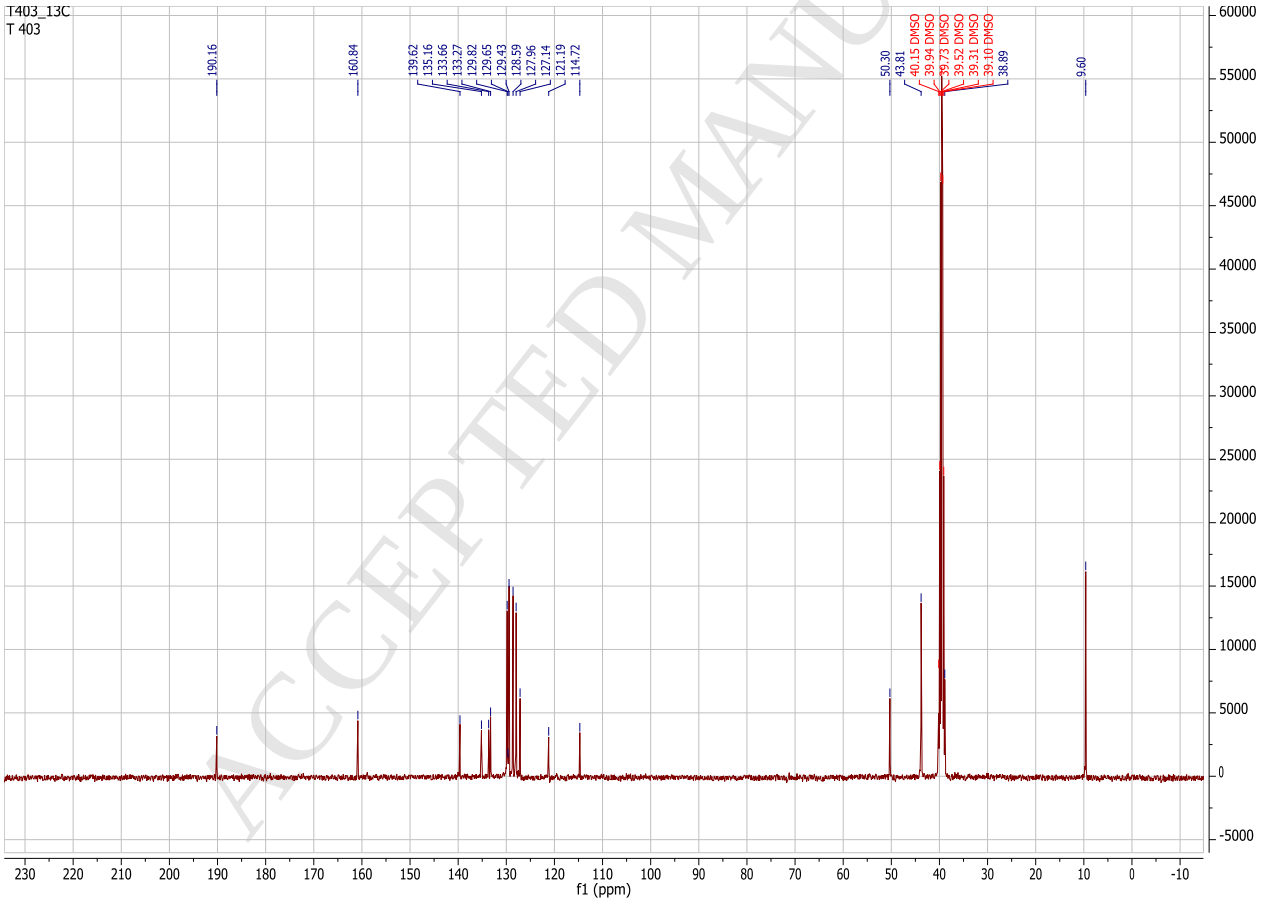
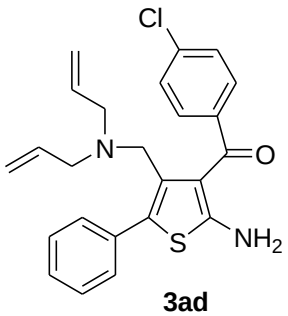
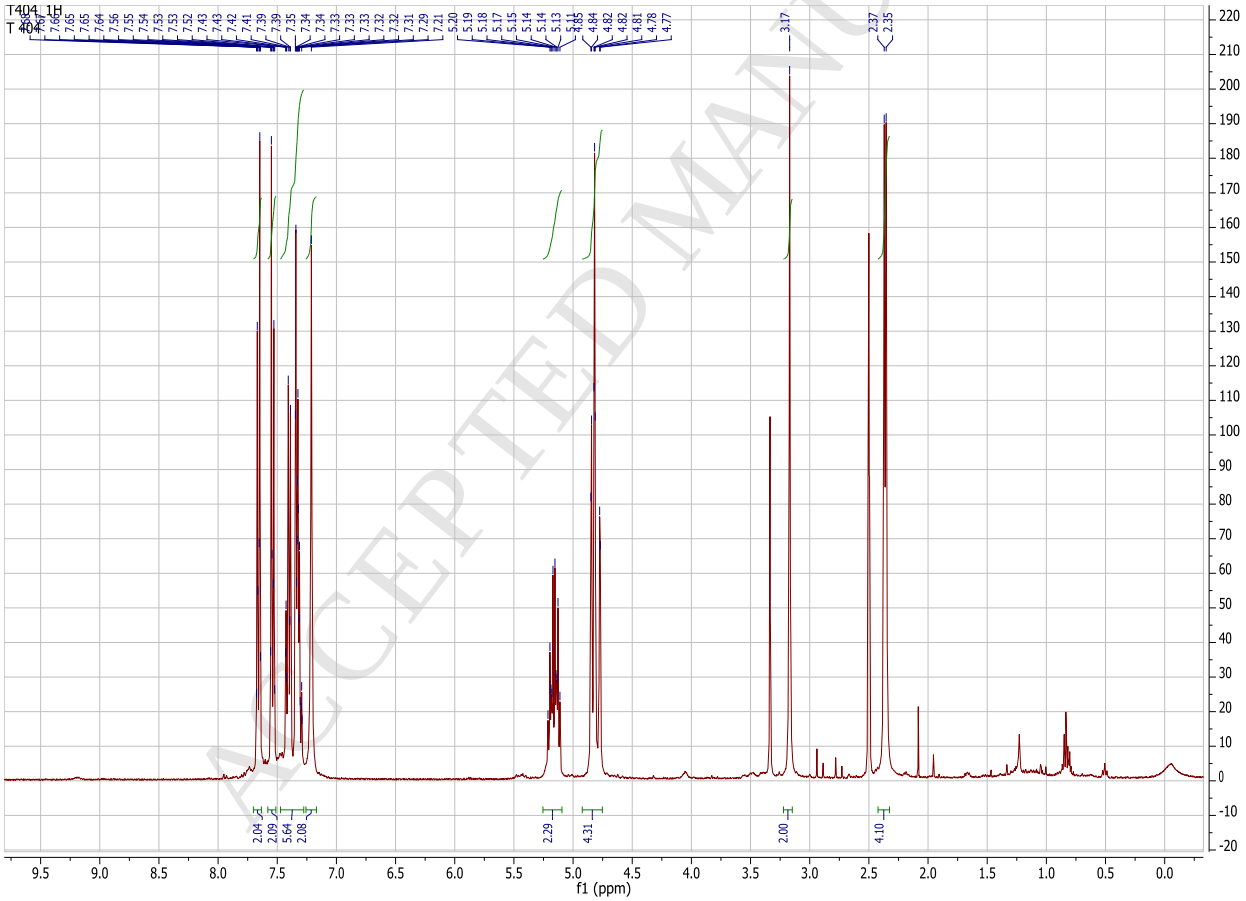
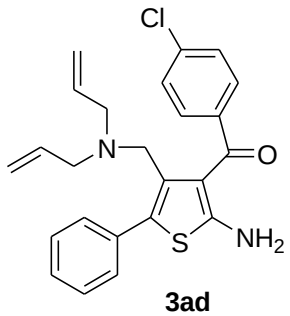




3ad





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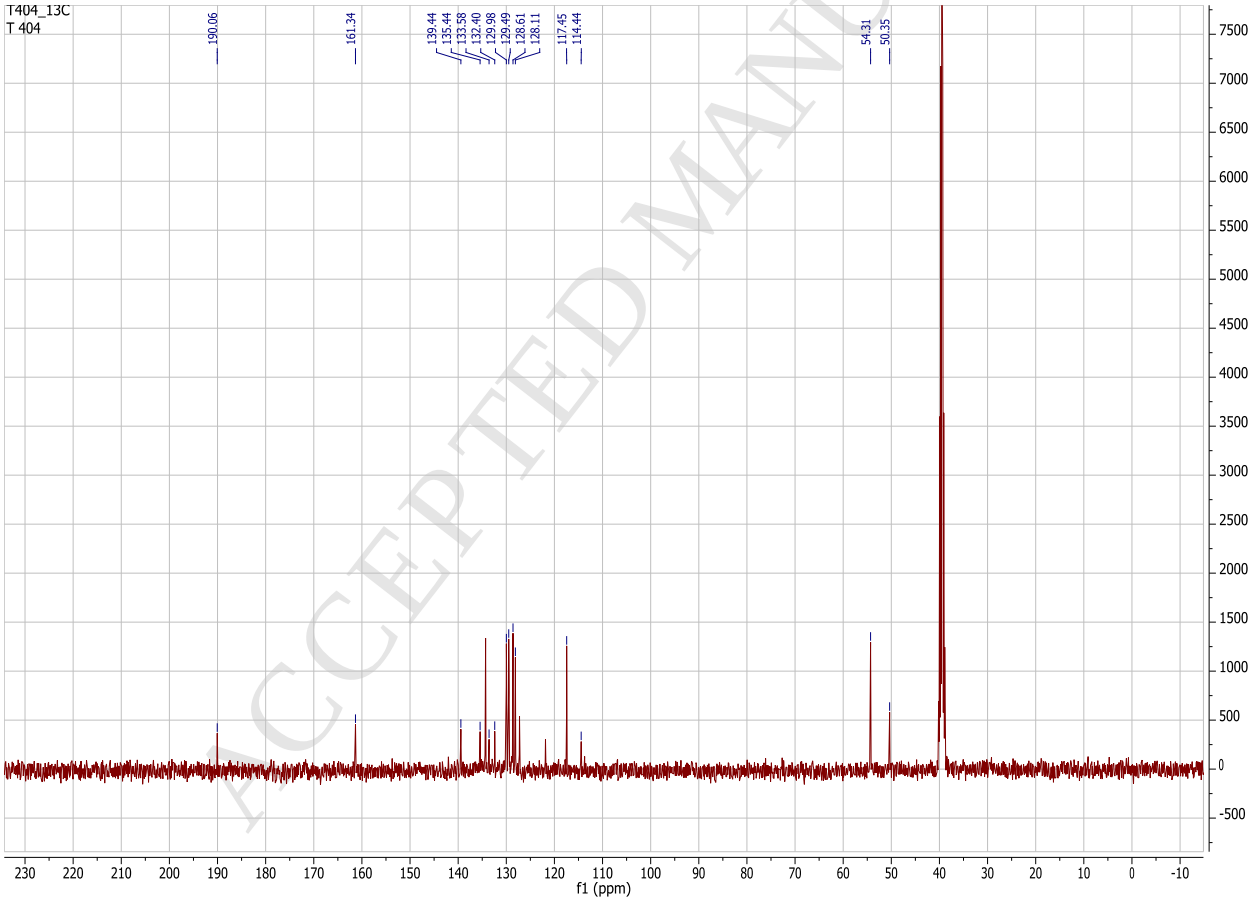

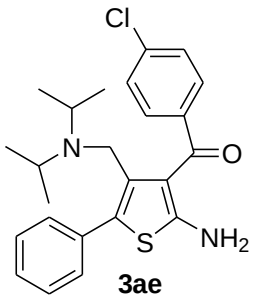

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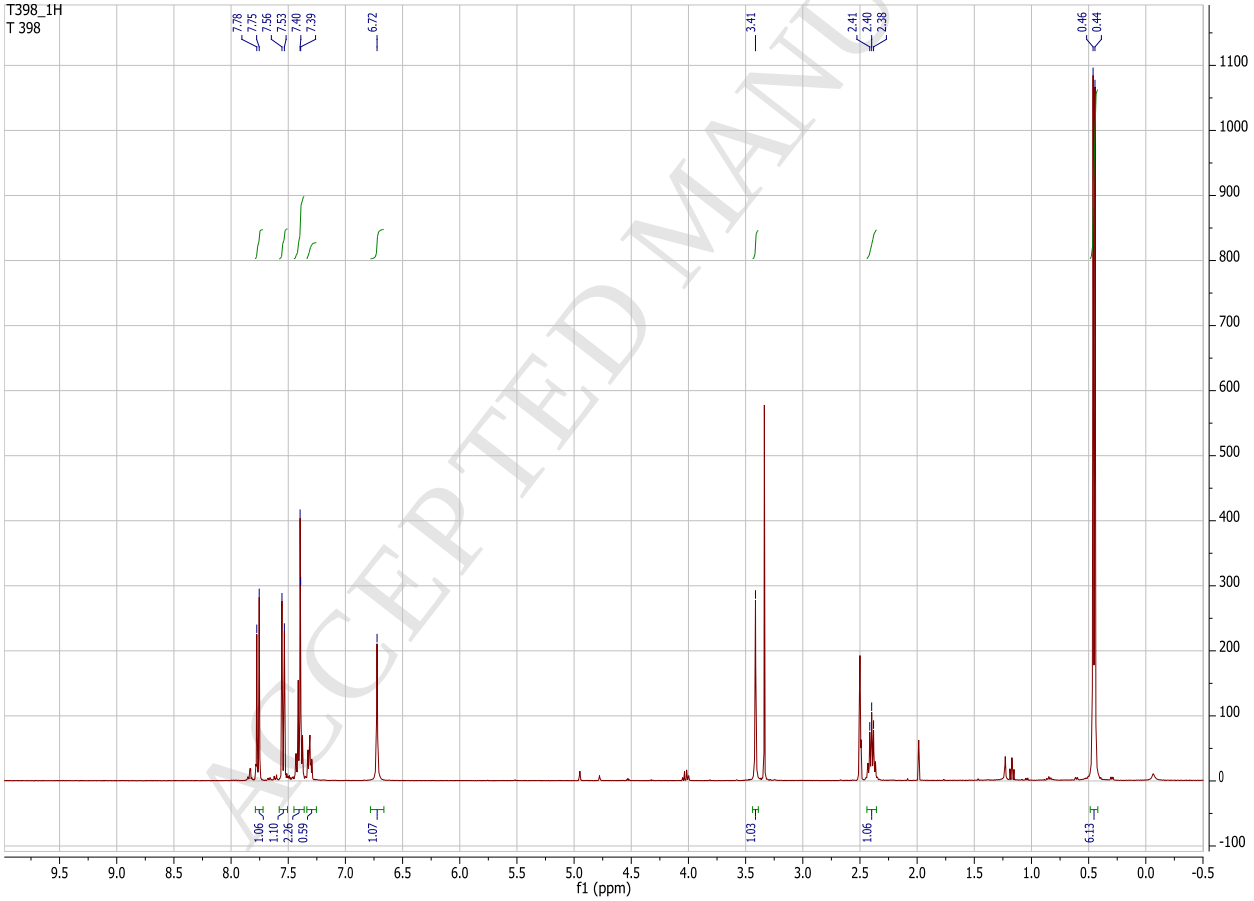

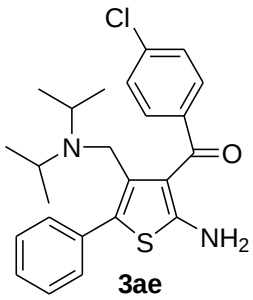

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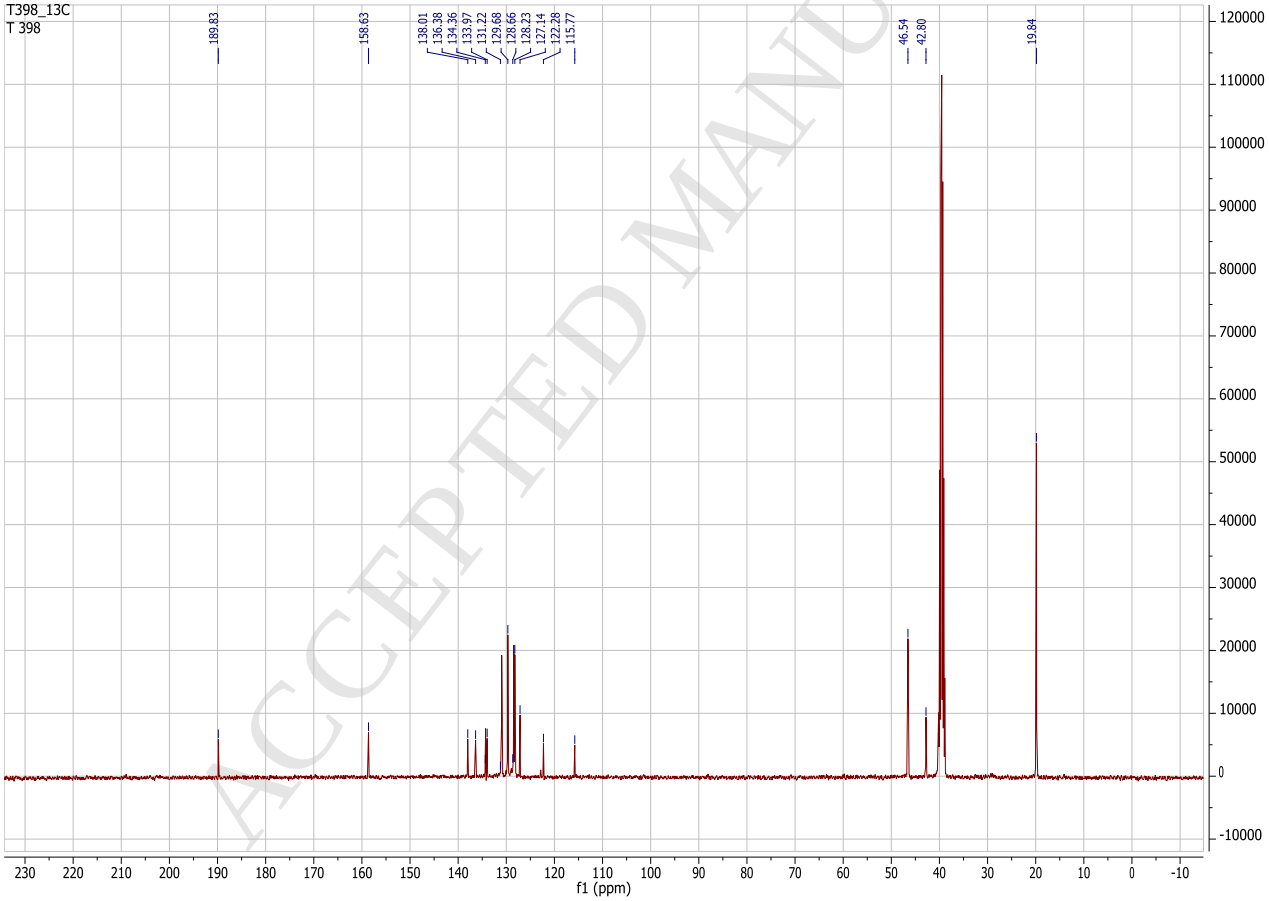

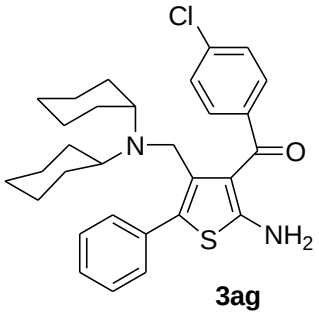

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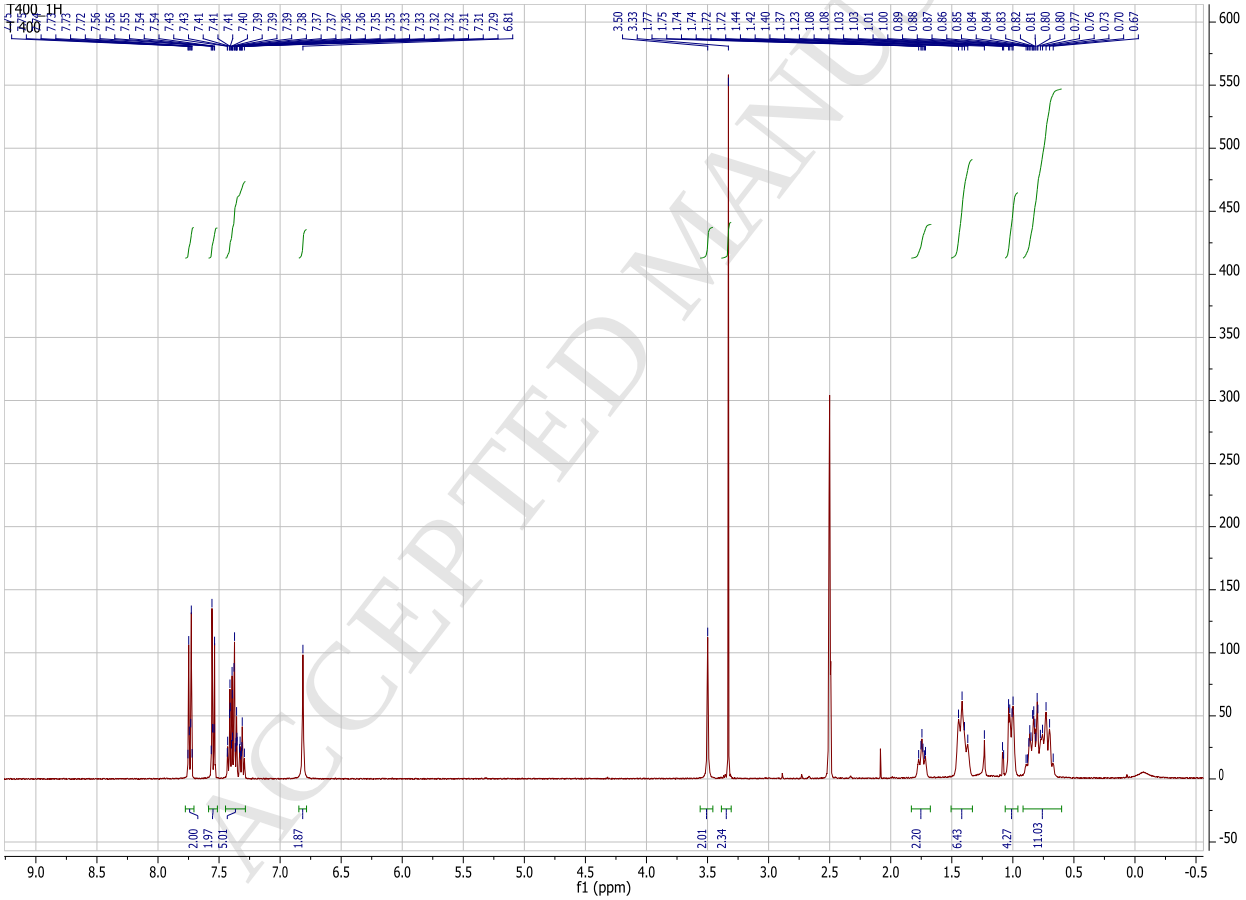

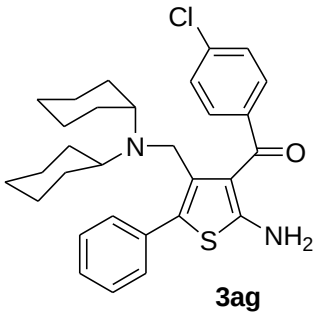

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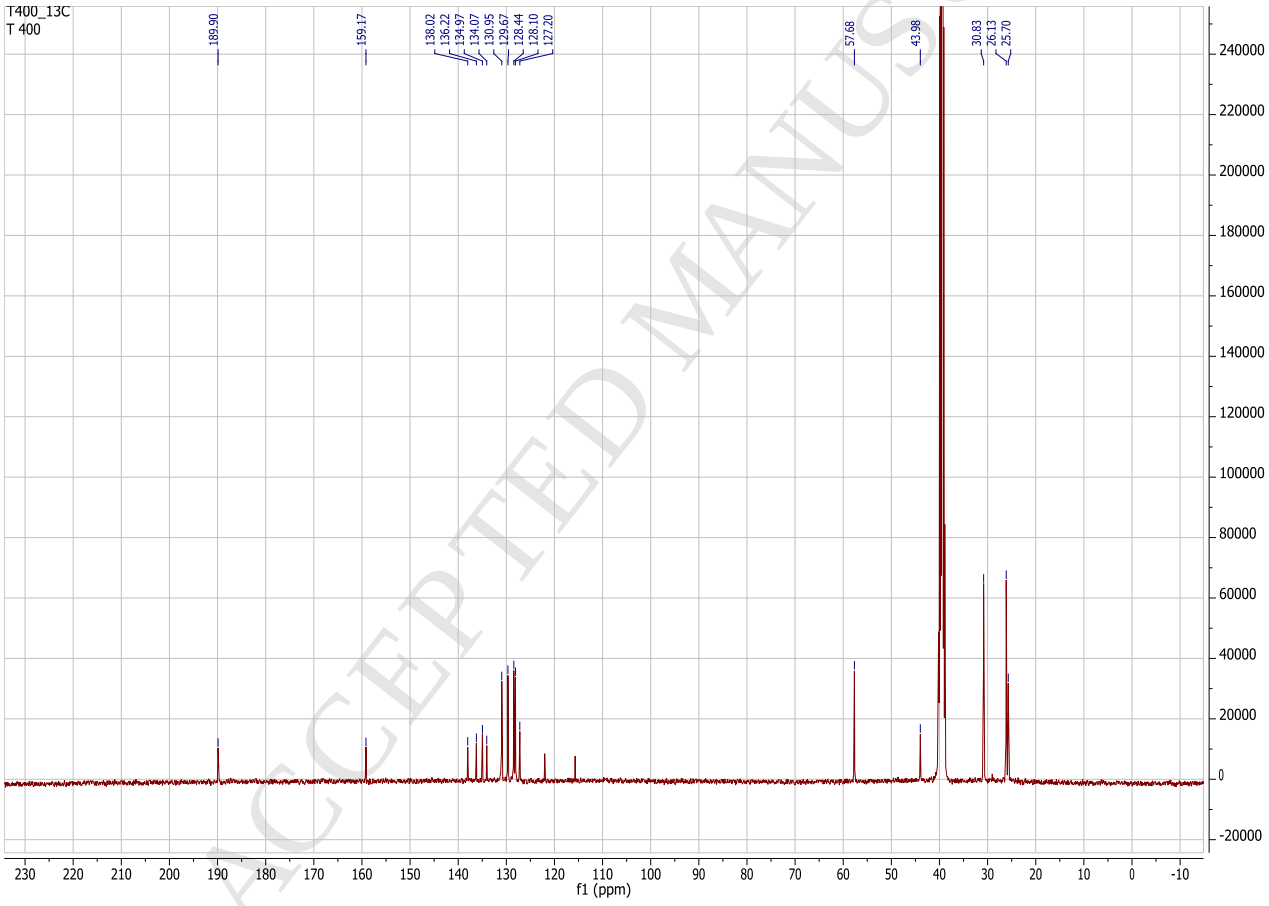

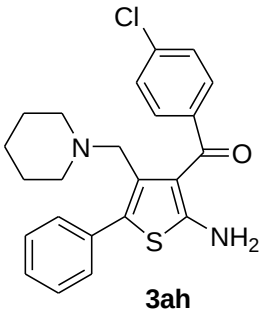

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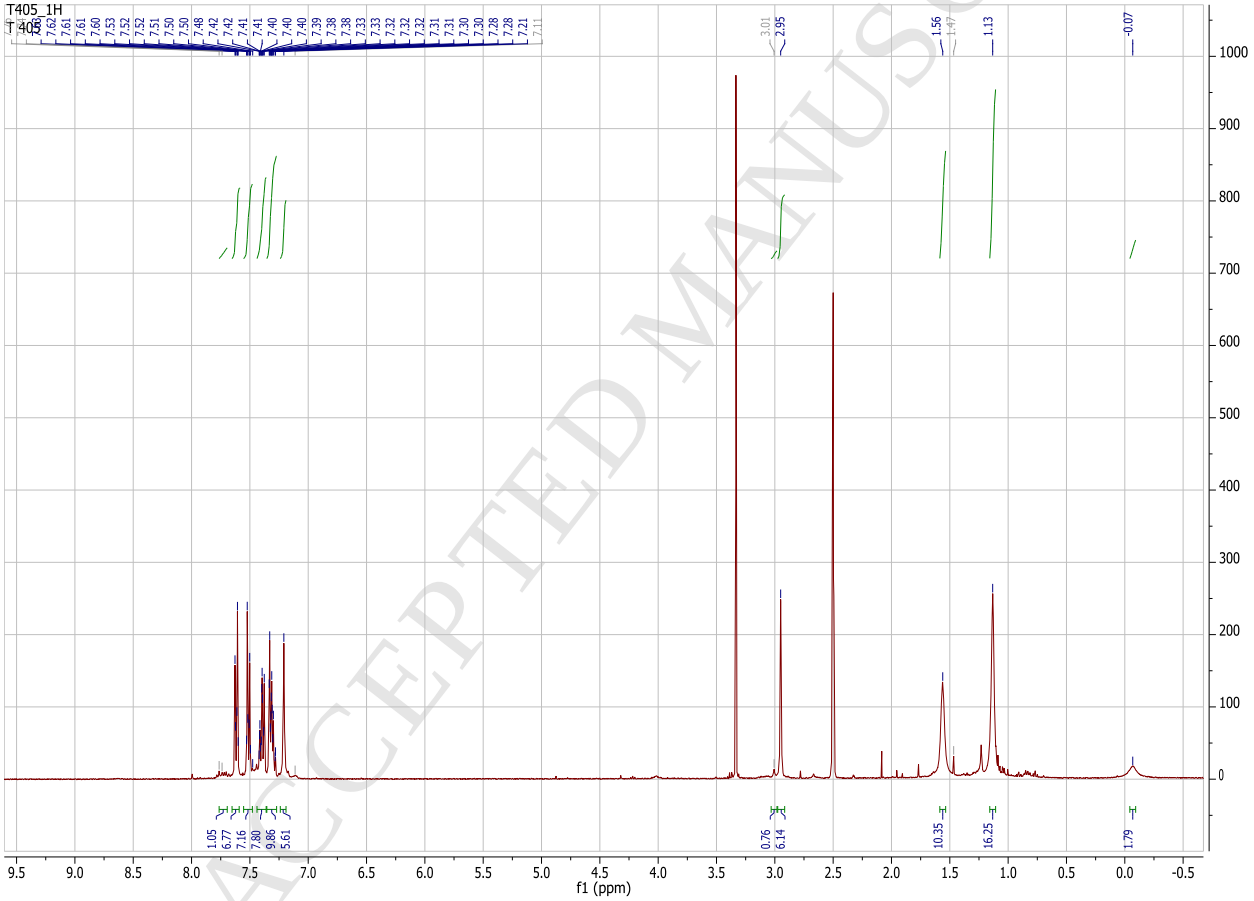

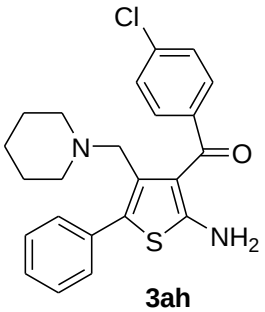

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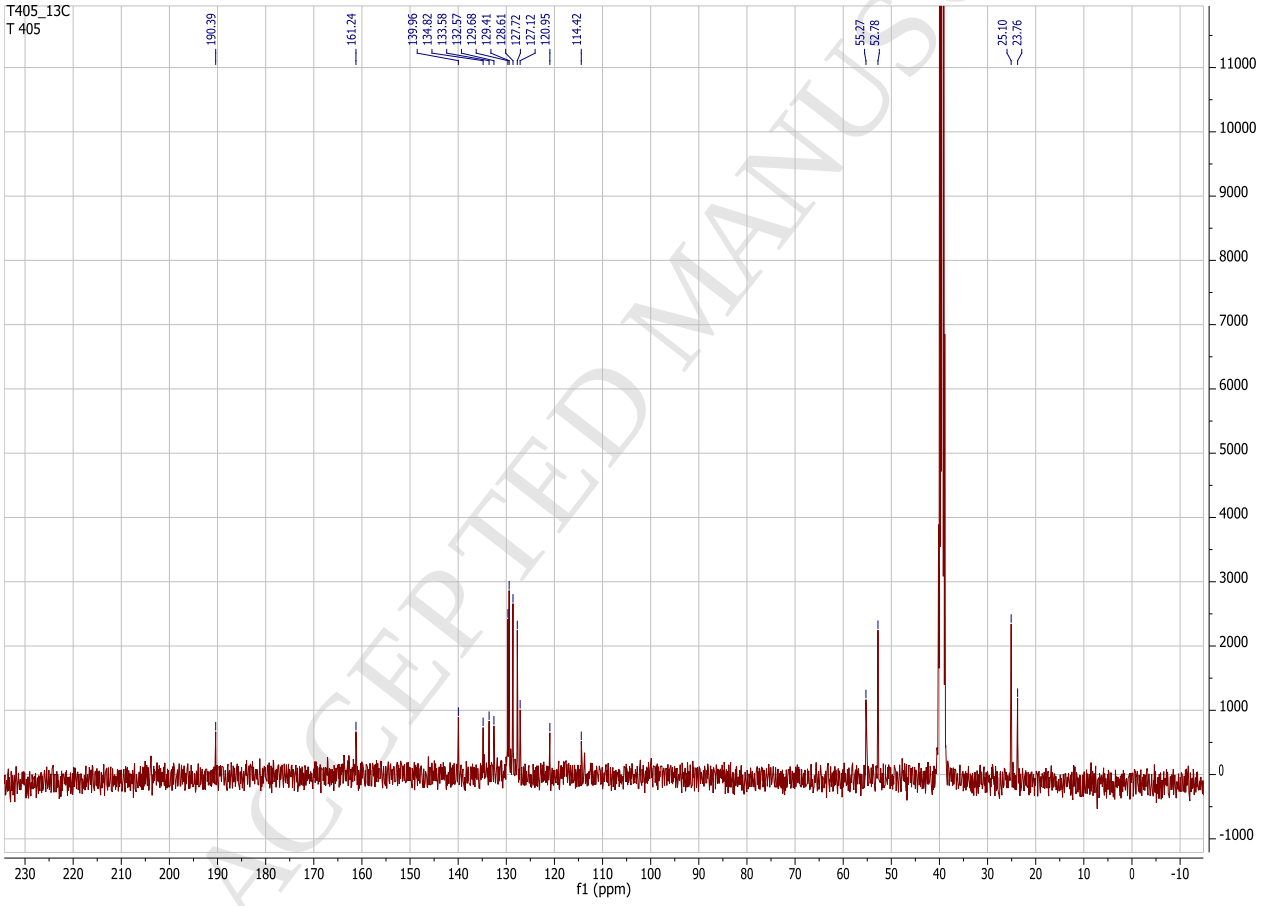

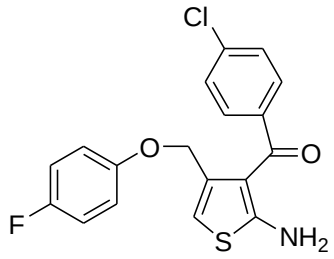

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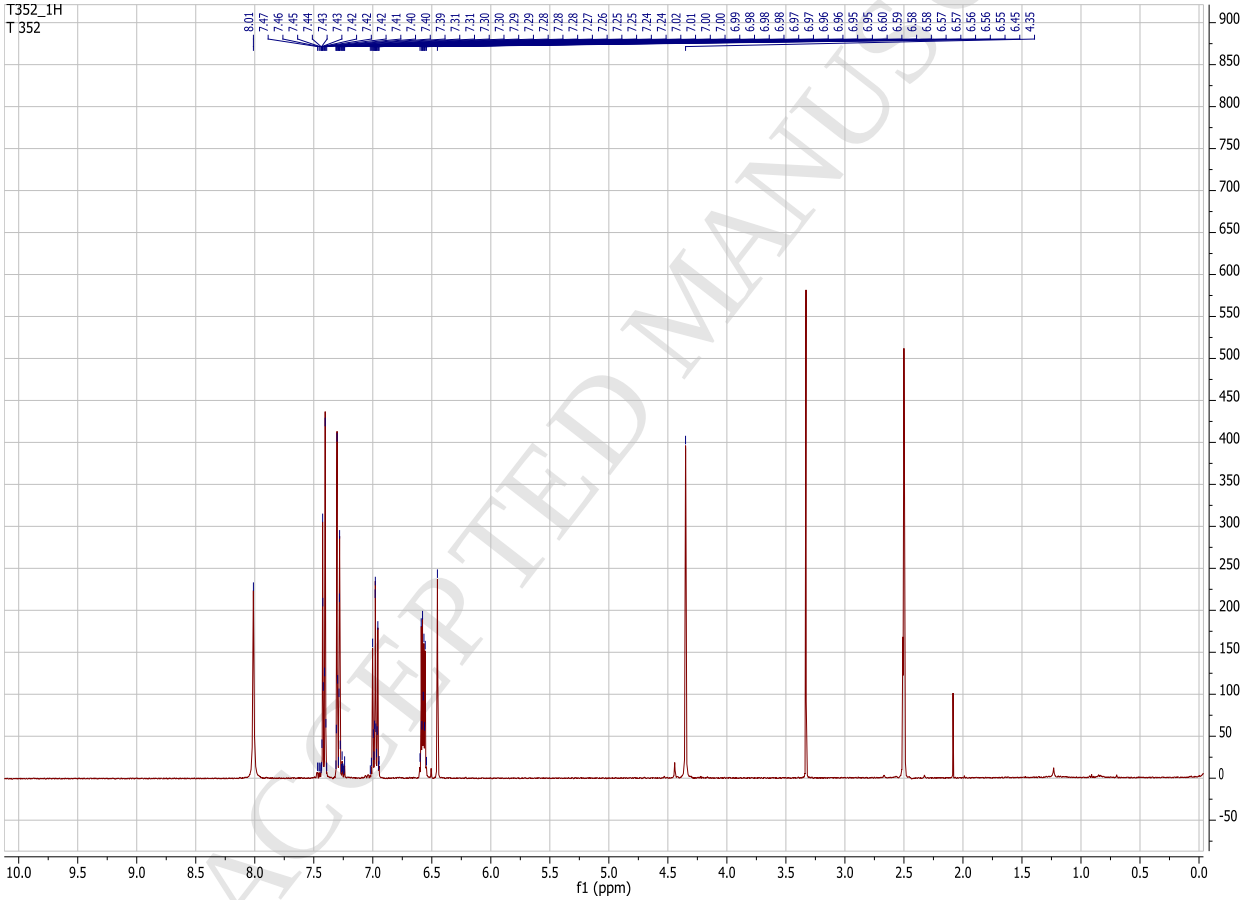

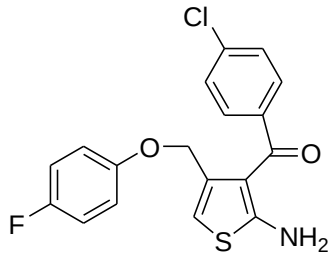

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