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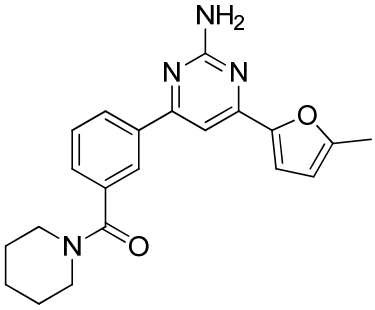
Graphical abstract



$A_{2A}Ki$ = 6.34 nM
A_1Ki = 9.54 nM

2-Aminopyrimidines as dual adenosine A_1/A_{2A} antagonists

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Abstract

In this study thirteen 2-aminopyrimidine derivatives were synthesised and screened as potential antagonists of adenosine A_1 and A_{2A} receptors in order to further investigate the structure activity relationships of this class of compounds. 4-(5-Methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**) was identified as a compound with high affinities for both receptors, with an A_{2A}*K*i value of 6.34 nM and an A_1*K*i value of 9.54 nM. The effect of selected compounds on the viability of cultured cells was assessed and preliminary results indicate low cytotoxicity. *In vivo* efficacy at A_{2A} receptors was illustrated for compounds **8k** and **8m** since these compounds attenuated haloperidol-induced catalepsy in rats. A molecular docking study revealed that the interactions between the synthesised compounds and the adenosine A_{2A} binding site most likely involve Phe168 and Asn253, interactions which are similar for structurally related adenosine A_{2A} receptor antagonists.

Keywords: Adenosine A_{2A} antagonist, Adenosine A_1 antagonist, 2-aminopyrimidine, Parkinson's disease

1. Introduction

Parkinson's disease is a complex, chronic neurodegenerative disorder, mainly characterised by a decline in motor function, but also associated with non-motor manifestations such as cognitive deficits (e.g. dementia) and neuropsychiatric symptoms, such as depression [1]. Management of the non-motor symptoms in particular is challenging and represents an important unmet medical need [2,3,4]. The neuropathology of the disease is hallmarked by the selective degeneration of dopaminergic neurons of the nigrostriatal pathway and the resulting deficiency of dopamine in the

basal ganglia [5]. However, the importance of other neurotransmitter systems and organs in Parkinson's disease pathogenesis are increasingly recognised [6,7,8].

Nevertheless, current treatments are still focused on enhancing dopaminergic neurotransmission and include the use of levodopa, (which is still the gold standard nearly 50 years since its introduction), dopamine agonists, catechol-*O*-methyltransferase and monoamine oxidase inhibitors. Since the chronic nature of the disease requires long-term use of medication, the occurrence of side-effects is unavoidable, and levodopa especially is associated with the development of debilitating dyskinesias [9, 10]. Limited progress has been made in altering the progress of neurodegeneration, and to date, no agent has been established as neuroprotective or disease modifying [11,12]. Non-dopaminergic targets for the disease are thus particularly appealing; especially if these would also improve non-motor symptoms and provide neuroprotection [13]. In recent years, antagonists of the adenosine A_{2A} receptor in particular has received attention as a promising non-dopaminergic alternative (for examples of reviews see 3, 4, 14-17] and several of these agents such as istradefylline (KW-6002), preladenant and tozadenant have been investigated clinically with promising results. Istradefylline for example has been approved for use as adjunctive treatment for Parkinson's disease in Japan [18-22].

Adenosine receptors are G-protein coupled receptors and consist of four subtypes, namely A_1, A_{2A}, A_3 and A_{2B} [23,24]. Of particular importance to Parkinson's disease are adenosine A_{2A} receptors which are concentrated in the indirect striatopallidal GABAergic pathway. This pathway also expresses the D_2 dopamine receptor and enkephalin [25-27]. Antagonism of A_{2A} receptors potentiate dopamine mediated responses and partly restores the imbalance between the hypoactive direct striatonigral and hyperactive indirect striatopallidal pathways that develops in Parkinson's disease, thus relieving motor symptoms [14,16,28,29]. Both epidemiological and experimental data have shown that adenosine A_{2A} antagonists exert a neuroprotective effect [16]. Furthermore, it has been reported that the adenosine A_{2A} antagonist KW-6002, exhibit antidepressant properties in animal models of depression [30,31] and that adenosine A_{2A} antagonists have potential in the management of dyskinesias [29,32]. This illustrates the promise of these agents as multifactorial non-dopaminergic treatment of Parkinson's disease.

Adenosine A_1 receptors on the other hand, are expressed throughout the brain, including the cortex, hippocampus and striatum [23]. Since A_1 receptor antagonism also results in motor activation in animals [33,34] it has been suggested that dual antagonism of A_1 and A_{2A} receptors would act synergistically in improving motor deficits in Parkinson's disease [4]. Furthermore, since the A_1 receptor also occurs in systems that are important for cognitive function, adenosine A_1 antagonism may improve cognitive deficits experienced in Parkinson's disease, as illustrated in animals [35-37]. Several dual adenosine A_1/A_{2A} antagonists have in fact been investigated in animals and has not only

shown effectiveness in improving motor disabilities [4,38-43], but has also illustrated effectiveness in enhancing cognition [38]. In summary, dual adenosine A_1 and A_{2A} antagonists would thus not only treat the motor symptoms of Parkinson's disease and potentially be neuroprotective, but may also improve non-motor symptoms.

The 2-aminopyrimidine motif frequently occurs in compounds (e.g. **1- 6**) that exhibit potent adenosine A_{2A} and/or adenosine A_1 affinity (Figure 1) and indicates that this scaffold is privileged for antagonism of these receptors [4,15,17,40-48]. Of particular relevance to this paper are the findings of Van Veldhoven, Matasi and Shook and co-workers [40-42,46,47], who synthesised a number of 2-aminopyrimidines (e.g. **3**), indenopyrimidones (e.g. **4**, **5**) and indenopyrimidines (e.g. **6**), respectively.

Our research group has been interested in the design, synthesis and evaluation of heterocycles as antagonists of adenosine receptors. Based on the aforementioned findings, the aim of the present study was to explore the necessity of the methylene bridge as present in the indenopyrimidine or indenopyrimidone scaffolds previously synthesised [40-42,46] and to further investigate the structure-activity relationships of the 2-aminopyrimidine scaffold for dual antagonism of adenosine A_1 and A_{2A} receptors. We thus set out to synthesise firstly, a set of 2-aminopyrimidines substituted with simple electron withdrawing and donating groups (**8a** - **h**), and secondly an amide substituted series (**8j** - **n**), related to the indenopyrimidones (e.g. **5**) as synthesised by Shook and co-workers [41]. We report herein their affinities for adenosine A_{2A} and A_1 receptors, the results of docking selected compounds into the adenosine A_{2A} receptor's binding site, as well as the *in vivo* activities of selected compounds.

2. Chemistry

As shown in scheme 1, the 2-aminopyrimidines were readily synthesised in two or three steps, albeit in low yields. Firstly, a Claisen-Schmidt condensation, using commercially available ketones and aldehydes under basic conditions [49] yielded the desired intermediate chalcones (**7a** - **i**). For the amide derivatives (**8j** - **n**), the condensation reaction was followed by an amide coupling reaction mediated by 1,1′-carbonyldiimidazole (CDI) resulting in chalcones **7j** - **n**. All chalcones were cyclised with guanidine hydrochloride in the presence of sodium hydride [50] to yield the desired 2-aminopyrimidines (**8a** - **n**), since the use of sodium hydroxide in ethanol resulted in complicated mixtures of products.

3. Results and discussion

The affinities of the 2-aminopyrimidines for the adenosine A_{2A} and A_1 receptors were determined by radioligand binding and are expressed as the receptor-ligand dissociation constants (*K*i, nM) (Tables 1 and 2). Adenosine A_{2A} receptor affinity was determined using the non-selective adenosine antagonist, [^{3}H]5'-N-ethylcarboxamide-adenosine ([^{3}H]NECA) in the presence of N^6-cyclopentyladenosine

(CPA), and A_1 receptor affinity was determined using 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) [51-53].

The 2-aminopyrimidines of series 1 (**8a - h**) exhibited moderate to weak affinities for the adenosine A_{2A} receptor and moderate to good affinities for A_1 receptors with $A_{2A}K_i$ values ranging from approximately 3 µM (**8f**, **8g**) to approximately 250 nM (**8b**, **8d**, **8h**) and A_1*K*i values ranging from 23 nM (**8a**) to 650 nM (**8f**). These compounds all have higher affinities for the adenosine A_1 receptor than for the adenosine A_{2A} receptor, with compound **8a** being the most selective (selectivity index of 42). For adenosine A_{2A} receptor affinity, the replacement of the phenyl substituent (R^3) with either a furan or methyl furan group, resulted in improved affinity (compound **8a** vs. compounds **8b** and **8c**), whereas the opposite was true for A_1 affinity, where methyl furan substitution in particular proved to be detrimental. When the affinities of compounds **8b** and **8c**, and compounds **8d** and **8e** are compared, it is clear that methyl substitution of the furan ring results in decreased affinity, especially for the adenosine A_1 receptor. Based on these results, it thus appears that for R^3, furan substitution is preferable for A_{2A} affinity, while phenyl substitution is optimal for A_1 receptor affinity. These results are in agreement with literature since the preference of the adenosine A_{2A} receptor for furan substitution is well documented [e.g. 46]. The affinity is apparently not significantly affected by the electronic effects of the substituent on the phenyl ring (R^2) as compounds **8b** and **8d** ($A_{2A}K_i$ values approx. 250 nM, A_1K_i values 40 - 60 nM) as well as compounds **8c** and **8e** ($A_{2A}K_i$ values approx. 400 nM, A_1K_i values 130- 140 nM) had similar affinities for A_{2A} and A_1 receptors, respectively.
On the other hand, the position of the substituent on the phenyl ring seems to have a significant effect on both A_1 and A_{2A} affinity, as substitution on the *meta* position (**8c**, R^1 = H, R^2 = Cl) is superior to substitution on the *para* position (**8g**, R^1 = Cl, R^2 = H). A similar observation is made when the affinities of compounds **8e** and **8f** are compared, where addition of a second methoxy group, in the *para* position, results in much weaker A_1 and A_{2A} affinity. However, since only a limited number of derivatives have been synthesised, these structure-activity relationships should be seen as preliminary.

For compounds **8j** - **n**, the 5-methyl-2-furanyl group was selected as the R^3 substituent, since it was synthetically easier to work with than furan and is also less likely to present with metabolic liabilities [40]. Gratifyingly, this amide series showed improved adenosine A_{2A} affinity, while still retaining good A_1 affinity. The most promising candidate was compound **8m**, with dual affinity for both A_{2A} and A_1 receptors with K_i values of 6.34 nM and 9.54 nM, respectively. For adenosine A_{2A} affinity, the amine groups yielded the following order of affinity: piperidine > methyl piperazine > morpholine = ethyl piperazine > pyrrolidine. Piperidine substitution was also most advantageous for A_1 affinity, while methyl piperazine substitution was least favourable. The affinities of this series of compounds are quite similar to those reported for related arylindenopyrimidones [41], and indicate that the

presence of the five-membered indenyl ring (e.g as present in **5** and **6**) is not an absolute requirement for dual affinity.

To gain an indication of potential cytotoxicity of the amide derivatives, the effect of these compounds on the viability of cultured HeLa cells were measured. For this purpose, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was used [54]. Cell viability was generally above 60% when exposed for 24 h to either 1 µM or 10 µM of test compound (Table 3). These concentrations are almost 1000-fold higher than the reported *K*i values of these compounds. Cytotoxicity at the doses required to obtain adenosine A_1 and A_{2A} affinity should thus not be problematic.

Selected representatives **8m** (the compound with best dual affinity, $A_{2A}K_i$ = 6.34 nM, A_1K_i = 9.54 nM) and **8k** ($A_{2A}K_i$ = 16.3 nM), were subjected to *in vivo* studies to determine the effectiveness of these compounds as dual adenosine A_1/A_{2A} antagonists. For these studies the effect of the test compounds on haloperidol-induced catalepsy in rats was investigated [33].

As depicted in figure 2, a significant reduction in catalepsy time was observed for **8m** at all intraperitoneal (ip) administered doses while a significant reduction in catalepsy time was only observed for the highest two doses (1 and 2 mg/kg) for compound **8k** (Figures 2a and b).

The results obtained with compounds **8m** and **8k** are thus similar to those obtained with other adenosine A_{2A} antagonists where catalepsy is reversed in the presence of A_{2A} antagonists or dual adenosine A_{2A}/A_1 antagonists [33,34,43], and provides evidence of *in vivo* efficacy of these compounds as antagonists.

In order to rationalise the results obtained in the radioligand binding studies, a docking study was performed using CDOCKER (Discovery Studio 3.1). The synthesised compounds were docked into a model of the binding site of the adenosine A_{2A} receptor (PDB code: 3PWH). Visual inspection of the docked poses with most favourable CDOCKER interaction energy firstly revealed that the three-membered ring system of all derivatives (**8a** - **n**), undergo π-π interactions with Phe168. Hydrogen bonding interactions also occur between the exocyclic amino-group and Asn253 for most derivatives. These interactions most likely anchor the aminopyrimidine in the binding site and are also important binding interactions predicted for other 2-aminopyrimidine antagonists [55] (Figure 3 and 4). Additionally, hydrogen bonding interactions were also observed with His250, Ser67, Glu169, Phe168 and Ala63 for some compounds. Interestingly, for compounds **8a** - **h** there were two different orientations, one where the C-6 substituent was orientated towards His250 and another where the C-4

substituent was orientated towards His250 (Figure 3). The CDOCKER interaction energies of these two poses were in all cases very similar. The docking results gave no clear explanation for the observed higher affinity of the amide derivatives (**8j - n**) compared to the series 1 compounds (**8a - h**). However, when ranked according to CDOCKER interaction energy, most of the series 2 compounds ranked above the series 1 compounds, except for compound **8f**, which ranked above compound **8n**. It would thus appear that the interaction between the amide derivatives (**8j - n**) and the receptor is generally more favourable than for the "smaller" derivatives and provides some explanation for the superior affinity of compounds **8j - n**.

4. Conclusion

This series of 2-aminopyrimidines, particularly the amide derivatives (**8j - n**), which are related to previously synthesised arylindenopyrimidines, retain affinity for both adenosine A_1 and A_{2A} receptors. The 2-aminopyrimidine scaffold can thus be optimised and the presence of a five-membered "linker ring" is not an absolute requirement for affinity. *In vivo* activity is indicative of adenosine antagonism rather than agonism and has been illustrated for compounds **8k** and **8m** with A_{2A}*K*i values of 16.3 nM and 6.34 nM and A_1*K*i values of 136 nM and 9.54 nM, respectively. Docking results indicate that these compounds are predicted to bind in a fashion similar to that illustrated for other compounds of the 2-aminopyrimidine class.

5. Experimental section

5.1 Chemistry

Chemical reagents were purchased from Sigma-Aldrich, and used without further purification. Reactions were routinely monitored on TLC using precoated Kieselgel 60 F254 sheets with ethyl acetate: petroleum ether (1:4) as mobile phase for series 1 (**8a - h**) and dichloromethane: methanol (9:1) as mobile phase for series 2 compounds (**8j - n**). Melting points were determined using a Buchi B-545 apparatus, and are uncorrected. Mass spectra were obtained on a dual focusing DFS magnetic sector mass spectrometer in EI+ mode. The mass spectrum for compound **7n** was obtained with a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Samples were dissolved in either deuterochloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-*d6*). ^{1}H NMR data are reported in parts per million (ppm) and the following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual solvent signal ($CDCl_3$ 7.26 and 77.0 ppm for ^{1}H and ^{13}C respectively; DMSO-*d*6: 2.5 and 39.5 ppm for ^{1}H and ^{13}C, respectively). Assignments were based on data obtained from 1D (^{1}H, ^{13}C, DEPT) and 2D (HSQC,

HMBC, COSY) NMR experiments. HPLC analyses were conducted with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used with a solvent gradient program (30% acetonitrile and 70% MilliQ water initially) at a flow rate of 1 ml/min. The concentration of acetonitrile in the mobile phase was linearly increased up to 85% over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. The test compound was injected (20 µl, 1 mM) into the HPLC system and the eluent was monitored at a wavelength of 254 nm.

5.1.1 Procedure for the synthesis of 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**)

A solution of 4% (w/v) sodium hydroxide (34 mmol) was added to a suspension of 3-formylbenzoic acid (17 mmol) and 1-(5-methyl-2-furyl)ethanone (17 mmol) in methanol (100 ml). The mixture was stirred at room temperature for 24 h and acidified with concentrated hydrochloric acid to a pH of 1-2. The precipitate that formed was filtered, rinsed with water and recrystallised from methanol to afford **7i**.

5.1.1.1 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**)

Yield 68%; Pale yellow crystals; mp 191.7-194.1 °C (methanol); ^{1}H NMR (600 MHz, DMSO-*d6*) δ 13.20 (br s, 1H, O<u>H</u>), 8.33 (br s, 1H, H-2′), 8.07 (br d, *J* = 7.8 Hz, 1H, H-6′), 7.98 (dt, *J* = 1.4, 7.7 Hz, 1H, H-4′), 7.82 (d, *J* = 3.5 Hz, 1H, H-3′′), 7.75 (d, *J* = 16.0 Hz, 1H, H-7 or H-8), 7.71 (d, *J* = 16.0 Hz, 1H, H-7 or H-8), 7.57 (t, *J* = 7.7 Hz, 1H, H-5′), 6.42 (dd, *J* = 1.1, 3.5 Hz, 1H, H-4′′), 2.40 (s, 3H, C<u>H</u>$_3$).^{13}C NMR (151 MHz, DMSO-*d6*) δ 175.6 (C-1), 167.0 (acid C=O), 158.8 (C-5′′), 151.8 (C-2′′), 141.2 (C-3), 135.0 (C-1′), 132.8 (C-6′), 131.6 (C-3′), 131.0 (C-4′), 129.2 (C-2′, C-5′), 123.1 (C-2), 121.9 (C-3′′), 109.6 (C-4′′), 13.8 (<u>C</u>H$_3$). EI-HRMS m/z: calcd for $C_{15}H_{12}O_4$, 256.07356, found 256.07292; Purity (HPLC): 100%.

5.1.2 General procedure for the synthesis of chalcones (**7j** – **7n**)

1,1'-Carbonyldiimidazole (CDI) (7.0 mmol) was added to a suspension of the acid (**7i**) (5.8 mmol) in dichloromethane (70 ml). The reaction mixture was stirred under nitrogen at room temperature for 2 h and the appropriate amine (7.0 mmol) was added. The mixture was then stirred for a further 3 h. The reaction was quenched by the addition of brine and the aqueous phase extracted with dichloromethane (2 × 20 ml). The combined organic fractions were washed once with saturated sodium hydrogen carbonate and twice with brine. The organic fraction was concentrated (*in vacuo*), purified with column chromatography [dichloromethane: methanol (98:2)] and recrystallised from methanol.

5.1.2.1 (*2E)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one* (**7j**)

The title compound was prepared from *(1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid* (**7i**) and morpholine in a yield of 54%: mp 149.1-149.9 °C (methanol), pale yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 15.8 Hz, 1H, H-3), 7.69 (br t, *J* = 1.6 Hz, 1H, H-2′), 7.65 (dt, *J* = 1.6, 7.7 Hz, 1H, H-6′), 7.47 – 7.36 (m, 3H, H-2, H-5′, H-4′), 7.25 (br d, *J* = 3.4 Hz, 1H, H-3′′), 6.21 (dd, *J* = 1.0, 3.4 Hz, 1H, H-4′′), 3.93 – 3.31 (m, 8H, 4 x morpholine C$\underline{H}_2$), 2.43 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8 (C-1), 169.6 (amide C=O), 158.4 (C-5′′), 152.3 (C-2′′), 141.8 (C-3), 136.0 (C-1′ or C-3′), 135.4 (C-1′ or C-3′), 129.9 (C-6′), 129.1, 128.5 (C-4′, C-5′)*, 126.6 (C-2′), 122.4 (C-2), 119.8 (C-3′′), 109.4 (C-4′′), 66.8, 48.2, 42.5 (morpholine $\underline{C}H_2$). EI-HRMS m/z: calcd for $C_{19}H_{19}NO_4$, 325.13141, found 325.13031; Purity (HPLC): 100%. * In no particular order.

5.1.2.2 (2*E*)-1-*(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one* (**7k**)

The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-methylpiperazine in a yield of 67 %: mp 131.0-132.2 °C (methanol), yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 15.8 Hz, 1H, H-3), 7.68 (br t, *J* = 1.6 Hz, 1H, H-2′), 7.63 (dt, *J* = 1.6, 7.7 Hz, 1H, H-6′), 7.45 – 7.35 (m, 3H, H-4′, H-5′, H-2), 7.25 (d, *J* = 3.5 Hz, 1H, H-3′′), 6.20 (d, *J* = 3.5 Hz, 1H, H-4′′), 3.81 (br s, 2H, CONC$\underline{H}_2$), 3.44 (br s, 2H, CONC$\underline{H}_2$), 2.50-2.27 (m, 10H, 2 x $\underline{C}H_2NCH_3$, 2 x C$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8 (C-1), 169.5 (amide C=O), 158.3 (C-5′′), 152.3 (C-2′′), 141.9 (C-3), 136.5 (C-1′ or C-3′), 135.3 (C-1′ or C-3′), 129.7 (C-6′), 129.0 (C-4′ or C-5′), 128.5 (C-4′ or C-5′), 126.6 (C-2′), 122.3 (C-2), 119.8 (C-3′′), 109.4 (C-4′′), 55.2 ($\underline{C}H_2NCH_3$), 54.6 ($\underline{C}H_2NCH_3$), 47.6 (CON$\underline{C}H_2$), 45.9 (piperazine $\underline{C}H_3$,), 42.0 (CON$\underline{C}H_2$), 14.1 (furan $\underline{C}H_3$,). EI-HRMS m/z: calcd for $C_{20}H_{22}N_2O_3$, 338.16304, found 338.16270; Purity (HPLC): 97%.

5.1.2.3 *(2E)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one* (**7l**)

The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-ethylpiperazine in a yield of 63%: mp 98.5-98.8 °C (methanol), orange solid.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 15.9 Hz, 1H, H-3), 7.68 (br s, 1H, H-2′), 7.63 (br d, *J* = 7.6 Hz, 1H, H-6′), 7.45 – 7.36 (m, 3H, H-4′, H-5′, H-2), 7.25 (d, *J* = 3.5 Hz, 1H, H-3′′), 6.21 (d, *J* = 3.5 Hz, 1H, H-4′′), 3.81 (br s, 2H, CONC$\underline{H}_2$), 3.44 (br s, 2H, CONC$\underline{H}_2$), 2.55 – 2.33 (m, 9H, 2 x C$\underline{H}_2$NCH$_3$, NC$\underline{H}_2$CH$_3$, furan C$\underline{H}_3$), 1.08 (t, *J* = 7.2 Hz, 3H, NCH$_2$C$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8 (C-1), 169.4 (amide C=O), 158.3 (C-5′′), 152.3 (C-2′′), 141.9 (C-3), 136.5 (C-1′ or C-3′), 135.3 (C-1′ or C-3′), 129.8 (C-6′), 129.0 (C-4′ or C-5′), 128.5 (C-4′ or C-5′), 126.6 (C-2′), 122.3 (C-2), 119.8 (C-3′′), 109.4 (C-4′′), 53.1 ($\underline{C}H_2NCH_2$), 52.3 ($\underline{C}H_2NCH_2$), 52.2 (N$\underline{C}H_2CH_3$), 47.7 (CON$\underline{C}H_2$), 42.1

(CON<u>C</u>H$_2$), 14.1 (furan <u>C</u>H$_3$), 11.8 (piperazine <u>C</u>H$_3$). EI-HRMS m/z: calcd for $C_{21}H_{24}N_2O_3$, 352.17869, found 352.17790; Purity (HPLC): 98%.

5.1.2.4 *(2E)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one* (**7m**)

The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and piperidine in a yield of 78%: mp 144.7 – 145.1 ~~144.2-144.4~~ °C (methanol), pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, *J* = 15.8 Hz, 1H, H-3), 7.68 (br s, 1H, H-2′), 7.63 (br d, *J* = 7.8 Hz, 1H, H-6′), 7.45 – 7.35 (m, 3H, H-4′, H-5′, H-2), 7.25 (d, 1H, *J* = 3.4 Hz, H-3″), 6.21 (d, *J* = 3.4 Hz, 1H, H-4″), 3.71 (s, 2H, CONC<u>H</u>$_2$), 3.33 (s, 2H, CONC<u>H</u>$_2$), 2.43 (s, 3H, C<u>H</u>$_3$), 1.71 – 1.42 (m, 6H, 3 x piperidine C<u>H</u>$_2$).^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9 (C-9), 169.5 (amide C=O), 158.3 (C-5″), 152.3 (C-2″), 142.1 (C-3), 137.2 (C-3′), 135.2 (C-1′), 129.5 (C-6′), 128.9 (C-4′ or C-5′), 128.3 (C-4′ or C-5′), 126.4 (C-2′), 122.2 (C-2), 119.8 (C-3″), 109.4 (C-4″), 48.7 (CON<u>C</u>H$_2$), 43.1 (CON<u>C</u>H$_2$), 26.5, 25.5, 24.5 (3 x piperidine <u>C</u>H$_2$), 14.2 (<u>C</u>H$_3$). EI-HRMS m/z: calcd for $C_{20}H_{21}NO_3$, 323.15214, found 323.15116; Purity (HPLC): 96%.

5.1.2.5 *(2E)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one* (**7n**)

The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and pyrrolidine in a yield of 74%: mp 120.8-122.6 °C (methanol), yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 – 7.76 (m, 2H, H-2′, H-3), 7.62 (br d, *J* = 7.7 Hz, 1H, H-6′), 7.49 (br d, *J* = 7.7 Hz, 1H, H-4′), 7.44 – 7.37 (m, 2H, H-5′, H-2), 7.24 (d, *J* = 3.5 Hz, 1H, H-3″), 6.2 (d, *J* = 3.5 Hz, 1H, H-4″), 3.63 (t, *J* = 7.1 Hz, 2H, CONC<u>H</u>$_2$), 3.41 (t, *J* = 6.6 Hz, 2H, CONC<u>H</u>$_2$), 2.41 (s, 3H, CH_3), 1.95 (p, *J* = 6.9 Hz, 2H, CH_2C<u>H</u>$_2CH_2$), 1.86 (p, *J* = 6.7 Hz, 2H, CH_2C<u>H</u>$_2CH_2$).^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9 (C-1), 168.9 (amide C=O), 158.3 (C-5″), 152.3 (C-2″), 142.1 (C-3), 137.8 (C-3′), 135.0 (C-1′), 129.9 (C-6′), 128.8 (C-4′ or C-5′), 128.6 (C-4′ or C-5′), 126.6 (C-2′), 122.1 (C-2), 119.8 (C-3″), 109.4 (C-4″), 49.5 (CON<u>C</u>H$_2$), 46.2 (CON<u>C</u>H$_2$), 26.3 (CH_2<u>C</u>H$_2CH_2$), 24.3 (CH_2<u>C</u>H$_2CH_2$), 14.1 (<u>C</u>H$_3$). APCI-HRMS m/z: calcd for $C_{19}H_{19}NO_3$ $(M + H)^+$, 310.1438, found 310.1448; Purity (HPLC): 93%.

5.1.3 General procedure for the synthesis of 2-aminopyrimidines

Guanidine hydrochloride (4.6 mmol) was dissolved in a small amount of DMF (15 ml), and the appropriate chalcone (3.1 mmol) and sodium hydride (9.2 mmol) were added while stirring. The reaction mixture was heated (110 ˚C) for 24 h under nitrogen, allowed to cool to room temperature and then diluted with equal volumes of ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (twice) and the organic layers were combined. All traces of DMF were removed by washing the combined organic layers with water (4 times). The organic layer was concentrated *in*

vacuo and the crude product was purified with column chromatography [petroleum ether: ethyl acetate (4:1)] and recrystallised from ethanol.

5.1.3.1 *4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine* (**8a**)

The title compound was prepared from (2*E*)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (**7a**) in a yield of 15%: mp 128.6-131.4 °C (ethanol), (lit. 132 – 133 °C) [56] white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (m, 3H, Ar-H), 7.92 (dt, *J* = 1.5, 7.6 Hz, 1H, H-4'/6'), 7.53 – 7.39 (m, 6H, Ar-H, H-5), 5.42 (s, 2H, N$\underline{H}_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.5, 164.64, 163.6 (C-2, C-4, C-6)*, 139.5, 137.4, 134.8 (C-1′, C-1″, C-3″)*, 130.6, 130.3, 130.0, 128.8 (2C), 127.2, 127.1 (2C), 125.1 (Ar-C), 104.1 (C-5). EI-HRMS m/z: calcd for $C_{16}H_{12}ClN_3$, 281.07198, found 281.07167; Purity (HPLC): 99%.* In no particular order.

5.1.3.2 *4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine* (**8b**)

The title compound was prepared from (2*E*)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (**7b**) in a yield of 22%: mp 144.1-144.4 °C (ethanol), light yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (br t, *J* = 1.9 Hz, 1H, H-2'), 7.91 (dt, *J* = 1.45, 7.6 Hz, 1H, H-6'), 7.59 (dd, *J* = 0.8, 1.8 Hz, 1H, H-5"), 7.42 (ddd, *J* = 8.0, 2.1, 1.2 Hz, 1H, H-4'), 7.38 (br t, *J* = 7.8 Hz, 1H, H-5'), 7.34 (s, 1H, H-5), 7.20 (dd, *J* = 0.8, 3.5 Hz, 1H, H-3"), 6.56 (dd, *J* = 1.8, 3.5 Hz, 1H, H-4"), 5.41 (s, 2H, N$\underline{H}_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.6 (C-2 or C-4), 163.4 (C-2 or C-4), 157.24 (C-6), 152.0 (C-2"), 144.6 (C-5"), 139.3 (C-1'), 134.8 (C-3'), 130.4 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 112.3 (C-3" or C-4"), 111.80 (C-3" or C-4″), 101.94 (C-5). EI-HRMS m/z: calcd for $C_{14}H_{10}ClN_3O$, 271.05124, found 271.05049; Purity (HPLC): 98%.

5.1.3.3 *4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine* (**8c**)

The title compound was prepared from (2*E*)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7c**) in a yield of 45%: mp 170.5-172.1°C (ethanol), yellow solid.^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (br s, 1H, H-2'), 7.92 (d, *J* = 7.6 Hz, 1H, H-6'), 7.46 – 7.37 (m, 2H, H-4', H-5'), 7.31 (s, 1H, H-5), 7.11 (br s, 1H, H-3"), 6.17 (br s, 1H, H-4"), 5.26 (s, 2H, N$\underline{H}_2$), 2.43 (s, 3H, C$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 164.3 (C-2 or C-4), 163.3 (C-2 or C-4), 157.3 (C-6), 155.4 (C-5"), 150.5 (C-2"), 139.4 (C-1'), 134.8 (C-3'), 130.3 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 113.3 (C-3"), 108.8 (C-4"), 101.6 (C-5), 14.0 (C$\underline{H}_3$). EI-HRMS m/z: calcd for $C_{15}H_{12}ON_3Cl$, 285.06690, found 285.06556; Purity (HPLC): 100%.

5.1.3.4 *4-(3-methoxyphenyl)-6-(furan-2-yl)pyrimidin-2-amine* (**8d**)

The title compound was prepared from (2*E*)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**7d**) in a yield of 19%: mp 113.8-116.8 °C (ethanol), dark brown crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ

7.64 – 7.57 (m, 3H, H-2',H-4',H-5"), 7.40 – 7.37 (m, 2H, H-5, H-5'), 7.19 (dd, *J* = 0.82, 3.4 Hz, 1H, H-3"), 7.03 (ddd, *J* = 0.96, 2.59, 8.1 Hz, 1H, H-6'), 6.55 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4"), 5.41 (s, 2H, N$\underline{H}_2$), 3.89 (s, 3H, OC$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 165.92 (C-4), 163.35 (C-2), 159.96 (C-3'), 156.43 (C-6), 152.15 (C-2"), 144.51 (C-5"), 138.89 (C-1'), 129.68 (C-5'), 119.47 (C-4'), 116.54 (C-6'), 112.21 (C-2'/C-3"/C-4"), 112.21 (C-2'/C-3"/C-4"), 112.07 (C-2'/C-3"/C-4"), 102.18 (C-5), 55.37 (O$\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{15}H_1N_3O_2$, 267.10078, found 267.09949; Purity (HPLC): 92%.

5.1.3.5 *4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine* (**8e**)

The title compound was prepared from (2*E*)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7e**) in a yield of 43%: mp 149.0 -150.6 °C (ethanol), light orange crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 – 7.58 (m, 2H, H-2', H-4'), 7.38 (br t, *J* = 7.9 Hz, 1H, H-5'), 7.33 (s, 1H, H-5), 7.09 (d, *J* = 3.3 Hz, 1H, H-3"), 7.02 (dd, *J* = 2.6, 8.2 Hz, 1H, H-6'), 6.15 (dd, *J* = 0.9, 3.3 Hz, 1H, H-4"), 5.44 (s, 2H, N$\underline{H}_2$), 3.88 (s, 3H, OC$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 165.6 (C-4), 163.4 (C-2), 159.9 (C-3'), 157.0 (C-6), 155.1 (C-5"), 150.6 (C-2"), 139.1 (C-1'), 129.6 (C-5'), 119.4 (C-4'), 116.3 (C-6'), 113.0 (C-3"), 112.1 (C-2'), 108.7 (C-4"), 101.7 (C-5), 55.4 (O$\underline{C}H_3$), 14.0 ($\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{16}H_{15}N_3O_2$, 281.11643, found 281.11556 ; Purity (HPLC): 100%.

5.1.3.6 *4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine* (**8f**)

The title compound was prepared from (2*E*)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7f**) in a yield of 27%: mp 167.4-168.1 °C (ethanol), dark yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68 (d, *J* = 2.0 Hz, 1H, H-2′), 7.61 (dd, *J* = 2.1, 8.4 Hz, 1H, H-6'), 7.29 (s, 1H, H-5), 7.08 (d, *J* = 3.3 Hz, 1H, H-3"), 6.93 (d, *J* = 8.4 Hz, 1H, H-5'), 6.15 (dd, *J* = 0.8, 3.3 Hz, 1H, H-4"), 5.35 (s, 2H, N$\underline{H}_2$), 3.98 (s, 3H, OC$\underline{H}_3$), 3.93 (s, 3H, OC$\underline{H}_3$), 2.42 (s, 3H, C$\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 165.2 (C-4), 163.3 (C-2), 156.7 (C-6), 155.0 (C-5"), 151.1, 150.6 (C-4' or C-3' and C-2′′)*, 149.0 (C-3′ or C-4′), 130.2 (C-1'), 120.1 (C-6'), 112.8 (C-3"), 110.7 (C-5'), 109.8 (C-2'), 108.7 (C-4"), 100.8 (C-5), 56.0 (O$\underline{C}H_3$), 55.9 (O$\underline{C}H_3$), 14.00 ($\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{17}H_{17}N_3O_3$, 311.12699, found 311.12618; Purity (HPLC): 100%* In no particular order.

5.1.3.7 *4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine* (**8g**)

The title compound was prepared from (2*E*)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7g**) in a yield of 30%: mp 204.6 – 205.8 ~~205-205.1~~°C (ethanol), dark yellow powder. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 8.14 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.56 (d, *J* = 8.6 Hz, 2H, H-3', H-5'), 7.39 (s, 1H, H-5), 7.21 (d, *J* = 3.3 Hz, 1H, H-3"), 6.76 (s, 2H, N$\underline{H}_2$), 6.32 (dd, *J* = 1.0, 3.3 Hz, 1H, H-4"), 2.37 (s, 3H, C$\underline{H}_3$).^{13}C NMR (151 MHz, DMSO-*d6*) δ 163.9 (C-2 or C-4), 163.1 (C-2 or C-4), 156.7 (C-6), 154.7 (C-5"), 150.4 (C-2"), 136.0 (C-1'), 135.2 (C-4'), 128.8 (C-2', C-6' or C-3', C-5'), 128.6 (C-

2', C-6' or C-3', C-5'), 113.3 (C-3"), 108.9 (C-4"), 99.4 (C-5), 13.7 (<u>C</u>H_3). EI-HRMS m/z: calcd for $C_{15}H_{12}ClN_3O$, 285.06689, found 285.06633; Purity (HPLC): 99%.

5.1.3.8 *4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine* (**8h**)

The title compound was prepared from (2*E*)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (**7h**) in a yield of 41%: mp 162.3-162.5 °C (lit. 170 °C) [57] (ethanol), faded yellow crystals.^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 – 8.03 (m, 2H, H-2',H-6'), 7.59 (dd, *J* = 0.8, 1.8 Hz, 1H, H-5"), 7.37 (s, 1H, H-5), 7.19 – 7.13 (m, 3H, H-3", H-3', H-5'), 6.57 (dd, *J* = 1.8, 3.4 Hz, 1H, H-4"), 5.28 (s, 2H, N<u>H</u>$_2$).^{13}C NMR (151 MHz, $CDCl_3$) δ 165.0 (C-2 or C-4), 164.4 (d, J_{C-F} = 250.5 Hz, C-4′), 163.3 (C-2 or C-4), 157.1 (C-6), 152.2 (C-2"), 144.6 (C-5"), 133.5 (d, J_{C-F} = 3.5 Hz, C-1'), 129.1 (d, J_{C-F} = 8.5 Hz C-2', C-6'), 115.7 (d, J_{C-F} = 21.3 Hz, C-3′, C-5′), 112.3 (C-3"), 111.6 (C-4"), 101.7 (C-5). EI-HRMS m/z: calcd for $C_{14}H_{10}FN_3O$, 255.08079, found 255.07982; Purity (HPLC): 100%.

5.1.3.9 *4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine* (**8j**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (**7j**) in a yield of 20%: mp 205.9 – 206.4 ~~205-205.1~~ °C (methanol), yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (m, 2H, H-2', H-6'), 7.55 – 7.46 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3"), 6.16 (br d, *J* = 3.4 Hz, 1H, H-4"), 5.32 (s, 2H, N<u>H</u>$_2$), 3.87 – 3.43 (m, 8H, morpholine C<u>H</u>$_2$), 2.42 (s, 3H, C<u>H</u>$_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 (C=O), 164.6 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5"), 150.4 (C-2"), 138.1 (C-1'), 135.7 (C-3'), 128.8, 128.7, 128.4, 125.9 (C-2′, C-4′, C-5', C-6')*, 113.2 (C-3"), 108.7 (C-4"), 101.5 (C-5), 66.8, 48.2, 42.6 (morpholine <u>C</u>H_2) 14.0 (<u>C</u>H_3). EI-HRMS m/z: calcd for $C_{20}H_{20}N_4O_3$, 364.15354, found 364.15292; Purity (HPLC): 92%. * In no particular order.

5.1.3.10 *4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine* (**8k**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (**7k**) in a yield of 57%: 194.3-194.4 °C (methanol), cream solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 – 8.07 (m, 2H, H-2', H-6'), 7.53 – 7.45 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3"), 6.15 (br d, *J* = 3.4 Hz, 1H, H-4"), 5.30 (s, 2H, N<u>H</u>$_2$), 3.83 (br s, 2H, CONC<u>H</u>$_2$), 3.46 (br s, 2H, CONC<u>H</u>$_2$), 2.60 – 2.19 (m, 10H, 2 x <u>C</u>H_2NCH_3, 2 x C<u>H</u>$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.8 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5"), 150.5 (C-2"), 138.1 (C-1'), 136.2 (C-3'), 128.8, 128.7, 128.2, 125.9 (C-2', C-4′, C-5′, C-6′)*, 113.2 (C-3"), 108.8 (C-4"), 101.6 (C-5), 55.2 (<u>C</u>H_2NCH_3), 54.6 (<u>C</u>H_2NCH_3), 47.7 (CON<u>C</u>H_2), 46.0 (piperazine <u>C</u>H_3), 42.1 (CON<u>C</u>H_2), 14.0 (furan <u>C</u>H_3). EI-HRMS m/z: calcd for $C_{21}H_{23}N_5O_2$, 377.18518, found 377.18422; Purity (HPLC): 85%. *In no particular order.

5.1.3.11 *4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine* (**8l**)

The title compound was prepared from (2*E*)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7l**) in a yield of 16%: 178.1-178.4 °C (methanol), light orange solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 – 8.07 (m, 2H, H-2', H-6'), 7.52 – 7.44 (m, 2H, H-4', H-5'), 7.34 (s, 1H, H-5), 7.08 (d, *J* = 3.4 Hz, 1H, H-3"), 6.14 (dd, *J* = 1.0, 3.4 Hz, 1H, H-4"), 5.38 (s, 2H, NH_2), 3.83 (br s, 2H, CONCH_2), 3.46 (s, 2H, CONCH_2), 2.62 – 2.21 (m, 9H, 2 x CH_2NCH_3, NCH_2CH_3, furan CH_3), 1.07 (t, *J* = 7.4 Hz, 3H, NCH_2CH_3).^{13}C NMR (151 MHz, $CDCl_3$) δ 169.7 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5"), 150.4 (C-2"), 138.0 (C-1'), 136.2 (C-3'), 128.7, 128.7, 128.2, 125.9 (C-2', C-4', C-5', C-6')*, 113.1 (C-3"), 108.7 (C-4"), 101.5 (C-5), 53.0 (CH_2NCH_2), 52.3 (CH_2NCH_2), 52.2 (NCH_2CH_3), 47.7 (CONCH_2), 42.1 (CONCH_2), 14.0 (furan CH_3), 11.8 (NCH_2CH_3). EI-HRMS m/z: calcd for $C_{22}H_{25}N_5O_2$, 391.20083, found 391.20052; Purity (HPLC): 100%. *In no particular order.

5.1.3.12 *4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine* (**8m**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (**7m**) in a yield of 56%: 179.2-180.5 °C (methanol), orange crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 – 8.05 (m, 2H, H-2', H-6'), 7.52 – 7.44 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3"), 6.15 (dd, *J* = 1.1, 3.4 Hz, 1H, H-4"), 5.35 (s, 2H, NH_2), 3.73 (br s, 2H, CONCH_2), 3.36 (br s, 2H, CONCH_2), 2.42 (s, 3H, CH_3), 1.77 – 1.39 (m, 6H, 3 x piperidine CH_2).^{13}C NMR (151 MHz, $CDCl_3$) δ 169.8 (C=O), 164.9 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5"), 150.4 (C-2"), 137.9 (C-1'), 136.8 (C-3'), 128.7, 128.5, 127.9 125.7 (C-2', C-4', C-5′, C-6′), 113.1 (C-3"), 108.7 (C-4"), 101.6 (C-5), 48.8 (CONCH_2), 43.1 (CONCH_2), 26.5, 25.5, 24.5 (3 x piperidine CH_2), 14.00 (CH_3). EI-HRMS m/z: calcd for $C_{21}H_{22}N_4O_2$, 362.17428, found 362.17292; Purity (HPLC): 97%.

5.1.3.13 *4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine* (**8n**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (**7n**) in a yield of 27%: 212.7 – 213.4 ~~213.7 213.8~~ °C (methanol), yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (t, *J* = 1.8 Hz, 1H, H-2'), 8.10 (dt, *J* = 1.5, 7.8 Hz, 1H, H-6'), 7.59 (dt, *J* = 1.4, 7.7 Hz, 1H, H-4'), 7.49 (t, *J* = 7.7 Hz, 1H, H-5'), 7.36 (s, 1H, H-5), 7.08 (d, *J* = 3.4 Hz, 1H, H-3"), 6.14 (dd, *J* = 1.2, 3.3 Hz, 1H, H-4"), 5.37 (s, 2H, NH_2), 3.66 (t, *J* = 7.0 Hz, 2H, CONCH_2), 3.44 (t, *J* = 6.7 Hz, 2H, CONCH_2), 2.41 (s, 3H, CH_3), 1.95 (p, *J* = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.86 (p, *J* = 6.7 Hz, 2H, $CH_2CH_2CH_2$).^{13}C NMR (151 MHz, $CDCl_3$) δ 169.2 (C=O), 164.8 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-4"), 150.5 (C-1"), 137.7 (C-1′ or C-3′), 137.6 (C-1' or C-3'), 128.8, 128.5, 128.3, (C-4', C-5', C-6')*, 125.9 (C-2'), 113.1 (C-3"), 108.7 (C-4"), 101.5 (C-5), 49.6 (CONCH_2), 46.2 (CONCH_2), 26.3 ($CH_2CH_2CH_2$), 24.4 ($CH_2CH_2CH_2$), 14.0 (CH_3).

EI-HRMS m/z: calcd for $C_{20}H_{20}N_4O_2$, 348.15863, found 348.15730; Purity (HPLC): 95%. *In no particular order.

5.2 Biological methods

5.2.1.Materials

Adenosine deaminase (type X from calf spleen), N^6-cyclopentyladenosine (CPA), anhydrous magnesium chloride, Trizma® Base, Trizma® Hydrochloride, silicone solution (Sigma-cote), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), phosphate-buffered saline (PBS) and isopropanol were obtained from Sigma Aldrich. Dimethyl sulfoxide (DMSO), Whatman® GF/B 25 mm diameter filters and formic acid were obtained from Merck. Filtercount scintillation fluid, radioligands, [^{3}H]5'-N-ethylcarboxamide-adenosine {[^{3}H]NECA, 25 Ci/mmol (250 µCi)} and 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX, 120 Ci/mmol) were obtained from Separation Scientific SA. Cell culture media (Dulbecco's Modified Eagle Medium (DMEM)), fungizone, trypsin/EDTA, streptomycin, fetal bovine serum and penicillin were obtained from Gibco and Merck. Well-plates (24 and 96) and culture flasks were obtained from Corning. Sterile syringe filters (0.22 µM) were obtained from Pall Corporation Life Sciences.

5.2.2 Radioligand binding studies

Tissue preparation for binding studies

Radioligand binding studies were carried out as reported in literature [51-53]. The Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5) approved the collection of animal tissue required for these assays. Adult male Sprague Dawley rats were obtained from the Vivarium of the North-West University, Potchefstroom campus. Striata (for the adenosine A_{2A} assays) and whole brains (for the adenosine A_1 assay) were dissected on ice and immediately snap frozen with liquid nitrogen and then stored at -70 ˚C until required. The frozen striata as well as the whole brain tissue were suspended in ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C) and homogenised using a Polytron PT-10 homogeniser (Brinkman) to yield final suspensions of 1 g/5 ml, which were aliquoted and stored at -70 ˚C until required. Test compounds were dissolved (10 mM) and further diluted in DMSO, with the final concentration of DMSO in the incubations being 1%. All pipette tips as well as the 4 ml polypropylene tubes used for the incubations were coated with Sigmacote®. The incubations were prepared using 50 mM Tris buffer (pH 7.7 at 25 °C). For the adenosine A_{2A} assay, the final volume of the incubations was 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding ~10 mg of original tissue weight of rat striata, 10 mM $MgCl_2$, 0.2 units of adenosine deaminase, 50 nM CPA and 4 nM [^{3}H]NECA. The $MgCl_2$ (A_{2A} assay) and adenosine deaminase (A_{2A} and A_1 assays) were firstly added to the membrane suspension, and this mixture was subsequently added to the incubations. The order of addition was test compound, membrane

suspension, CPA and [^{3}H]NECA. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). For the adenosine A_1 assay, the final volume of the incubations was also 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding 5 mg of original tissue weight of rat whole brain, 0.1 units of adenosine deaminase and 0.1 nM [^{3}H]DPCPX. The order of addition was test compound, membrane suspension and [^{3}H]DPCPX. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). After incubation for 1 h (with vortexing after 30 minutes), the incubations were rapidly filtered through Whatman® GF/B 25 filters (25 mm diameter) fitted on a Hoffeler vacuum system. The damp filters were placed into scintillation vials and scintillation fluid (4 ml) was added. The vials were shaken thoroughly and left for 2 h. A Packard Tri-CARB 2100 TR scintillation counter was used to count the radioactivity retained on the filters. Specific binding was defined as total binding minus nonspecific binding, and was expressed as counts per minute (CPM).

Data analysis

Using the one site competition model of the Prism 5 software package (GraphPad) the CPM values were plotted against the logarithm of the ligand concentration to give a sigmoidal dose-response curve, from which the IC_{50} values were determined. For adenosine A_1 binding, K_i values were calculated from the IC_{50} values by using the Cheng-Prusoff equation [58], as applicable to radioligand binding assays [51]. K_d, the equilibrium dissociation constant for the radioligand [^{3}H]DPCPX, was taken as 0.36 nM [52]. Since adenosine A_{2A} binding was performed in the presence of CPA, an adapted version of the Cheng-Prusoff equation was used. The K_d of the radioligand, [^{3}H]NECA, was taken as 15.3 nM and a K_c value of 685 nM was used for CPA [51]. The binding affinities of the known adenosine A_1 agonist, CPA, and the A_{2A} antagonists, KW-6002 and ZM241385 were also determined as controls. The results of the radioligand binding studies are reported as the mean ± standard error of the mean (SEM) of duplicate determinations.

5.2.3 MTT cell viability assay

Cell culture

HeLa cells were maintained in 250 cm^2 flasks in DMEM media (30 ml) containing 10% fetal bovine serum, 1% penicillin (10 000 units/ml)/streptomycin (10 mg/ml), and fungizone (250 µg/ml). The cells were incubated at 37 °C in an atmosphere of 10 % CO_2. The media was replaced once a week and cells were allowed to reach confluency before use in assays.

Preparation of compounds

Stock solutions of test compounds were prepared in DMSO (10 mM) and further diluted in DMSO to concentrations of 1 μM and 10 μM. These solutions were filtered via a syringe filter before addition to the cell cultures.

MTT Assay

Once confluent, cells were detached with 3 ml trypsin/EDTA (0.25%/0.02%) and seeded in 24-well plates at 500 000 cells/ well. Plates were then incubated for 24 h and the wells subsequently rinsed with 0.5 ml DMEM free from fetal bovine serum. A volume of 0.99 ml DMEM (free from fetal bovine serum) was subsequently added to each well followed by 10 μl of the test compound. In each 24-well plate, wells were reserved as either positive control (100% cell death via lyses with 0.03% formic acid) or negative controls (100% cell viability as a result of no drug treatment). The plates were then incubated at 37 °C for a further 24 h where after media was aspirated from each well. The wells were then washed twice with 0.5 ml/well PBS and 200 μl of 0.5% MTT (prepared in PBS) was added to each well. The well plates were incubated at 37 °C for 2 h in the dark, where after the residual MTT was aspirated and 250 μl isopropanol was added to dissolve the formed formazan crystals. The well-plates were then incubated at room temperature for 5 min to allow dissolution of the blue formazan crystals, where after 100 μl of the isopropanol solution of each well plate was transferred to a corresponding well in a 96-well plate. The absorbance was measured spectrophotometrically at 560 nM (using a Labsystems Multiscan RC UV/V spectrophotometer), with the absorbance of the negative control signifying 100 % viability and the absorbance of the positive control signifying 0% viability. The effects of the test compounds were evaluated in triplicate and the residual cell viabilities reported as the mean ± SD of the percentage viable cells compared to the negative control (100%).

5.2.4 *In vivo* assays

Animals

Sprague-Dawley rats were given free access to standard laboratory food and water until the required weight was obtained (240 g - 300 g). All efforts were made to minimise animal suffering as experiments were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the North-West University ethical committee (NWU-00035-10-A5).

Compounds

To induce catalepsy a dose of 5 mg/kg haloperidol (Serenace Injection; 5 mg/ml) was administered intraperitoneally (ip) [33]. A vehicle solution was prepared by mixing a 1:1:4 solution of DMSO, Tween 80 and saline. Compounds (**8k** and **8m**) were dissolved in a required amount of vehicle solution to yield concentrations of 0.1, 0.4, 1 and 2 mg/ml. A suitable volume of these solutions was

injected depending on the weight of the rat, resulting in final dose concentrations of 0.1, 0.4, 1 and 2 mg/kg. All injections were administered ip.

Catalepsy test

The experiments were carried out between 8:00 and 15:30 in a lit room with a controlled temperature. All the rats were drug naïve and were only used once. Haloperidol-induced catalepsy was measured with the standard bar test, in a Perspex chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal plastic bar (diameter, 1 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 7 cm from the back of the box.

Animals were divided into 5 groups, each group containing 6 rats. The 5 groups were treated with 0, 0.1, 0.4, 1 and 2 mg/kg of the test compound, respectively. All rats received ip injections of haloperidol (5.0 mg/kg) to induce catalepsy. 30 Min. later, the rats in each group received ip injections of compound. The vehicle solution was administered to rats in the control group. Catalepsy was measured 60 min. after the haloperidol injections by placing the rats in the Perspex box with their front paws on the horizontal bar. Catalepsy was measured as the time the animal maintained its position on the bar. Time was recorded until one or both of the rat's front paws were removed from the bar, or up to 120 seconds.

The results of the animal experiments are reported as the mean ± standard error of the mean (SEM). Data were analysed by means of one-way analysis of variance (ANOVA) across all groups, and were subsequently subjected to Dunnett's post-test to determine if statistical differences exist between mean values. A p value < 0.05 is judged as being statistical significant. These analyses were carried out with the Prism software package.

5.3 Molecular docking

Molecular docking studies were carried out with the Windows based Accelrys® Discovery Studio 3.1 software. The crystal structure of the adenosine A_{2A} receptor [Protein Data Bank (PDB) code 3PWH] co-crystalised with the known A_{2A} antagonist ZM241385 was used. This receptor was prepared with the 'Clean protein' function to correct problems such as incomplete amino acid side chains and typed with the CHARMm forcefield. A fixed atom constraint was applied to the backbone and a minimisation was then carried out using the Generalised Born approximation with Molecular Volume (GBMV) as the solvent model to obtain a receptor at energetic minimum. A binding sphere with a radius of 5 Å was defined using the existing ligand (ZM241385) before it was removed from the receptor. Selected inhibitors were cleaned and prepared for docking with the 'Prepare ligand' protocol to correct valences and remove duplicates whereafter ligands were visually inspected and remaining errors corrected. The CDOCKER protocol was used for the docking of ligands. The orientations,

CDOCKER and CDOCKER interaction energies of the ten different conformers of each ligand were considered and the best conformation for each ligand selected. An *in situ* ligand minimisation was then performed on the selected conformers and minimised conformers visually inspected and compared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at..... These data include the NMR data of the intermediates (**7a** – **7h**) and examples of sigmoidal dose response curves obtained during the radioligand binding assays.

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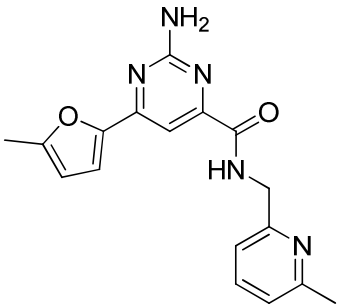
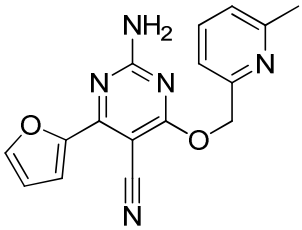


1

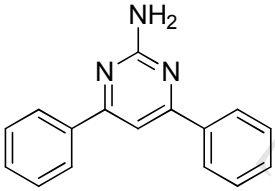
$A_{2A}Ki$ = 2.5 nM

A_1Ki = 133 nM



2

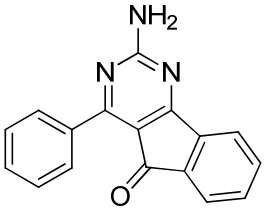
$A_{2A}Ki$ = 0.6 nM



3

$A_{2A}Ki$ = 405 nM

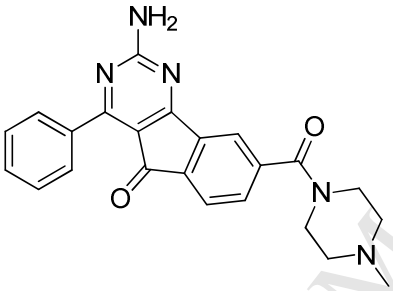
A_1Ki = 17.7 nM



4

$A_{2A}Ki$ 0.1 nM

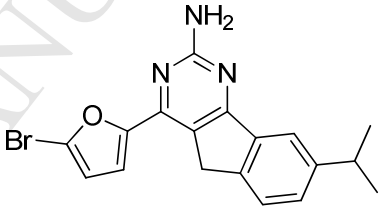
A_1Ki = 1.1 nM



5

$A_{2A}Ki$ = 8.2 nM

A_1Ki = 58.4 nM



6

$A_{2A}Ki$ = 0.8 nM

Figure 1: Adenosine A_{2A} antagonists containing the 2-aminopyrimidine moiety.

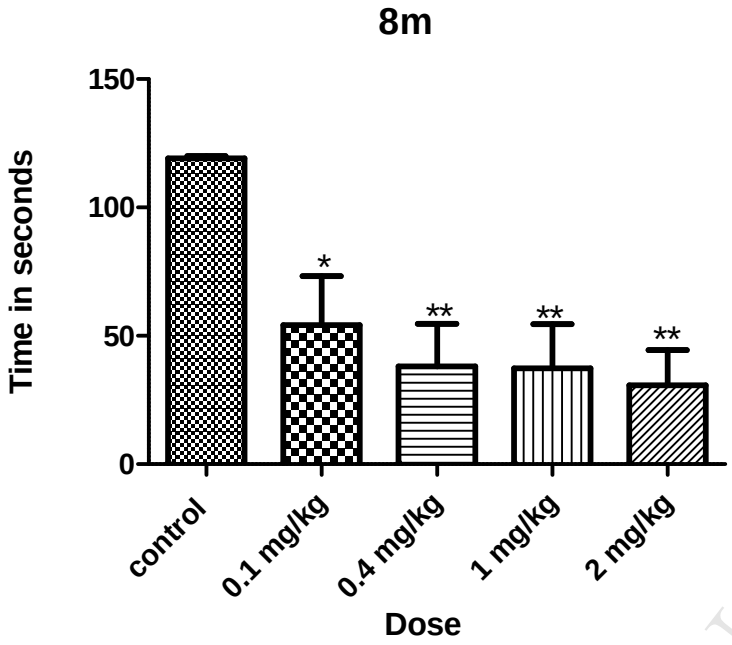


Figure 2a. Reduction in haloperidol induced-catalepsy in male Sprague-Dawley rats by compound **8m**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8m** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (± SEM) of (n = 6) rats in the cataleptic position (*,**indicate significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [F (4, 25) = 5.893; P < 0.005) followed by Dunnet's post test with *P = 0.01 - 0.05 and **P = 0.001 – 0.01).

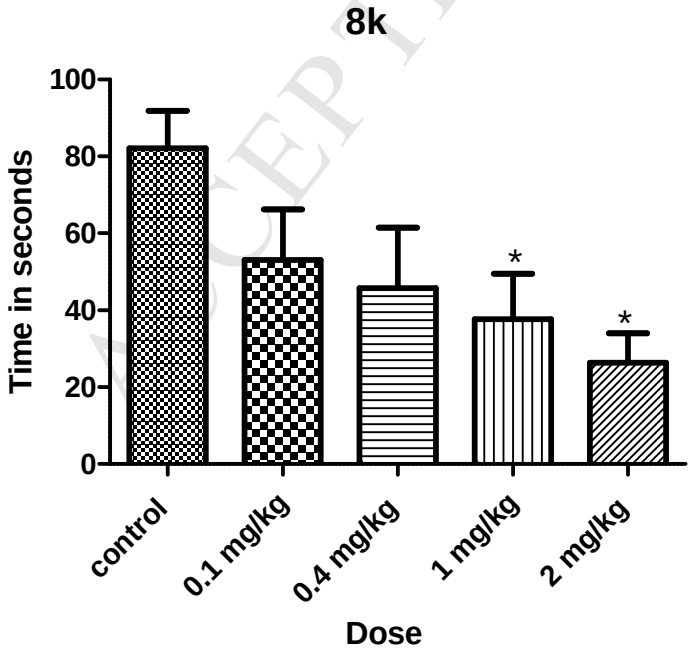
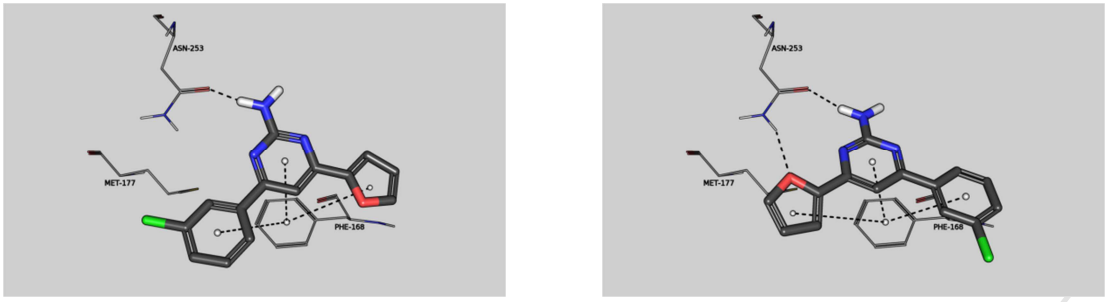


Figure 2b. Reduction in haloperidol induced-catalepsy in male Sprague-Dawley rats by compound **8k**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8k** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (± SEM) of (n = 6) rats in the cataleptic position (* indicates significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [$F(4, 25) = 3.103$; $P < 0.05$) followed by Dunnet's post test with $P = 0.01 – 0.05$).



A **B**

Figure 3: Illustration of the different orientations of compound **8b** in the binding site of the A_{2A} receptor. Intermolecular hydrogen bond interactions are observed between the exocyclic amino group and Asn253, while π=π stacking occurs between the three ring systems and Phe168 (**A**). In orientation **B**, an additional hydrogen bonding interaction is observed between the furan oxygen and Asn253 (Figure generated using Pymol).

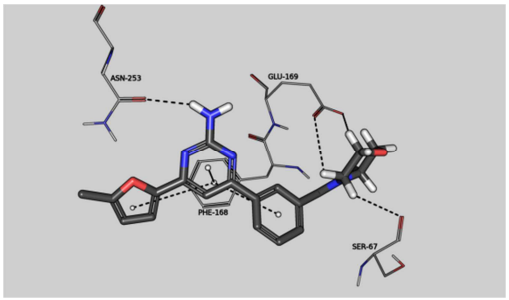
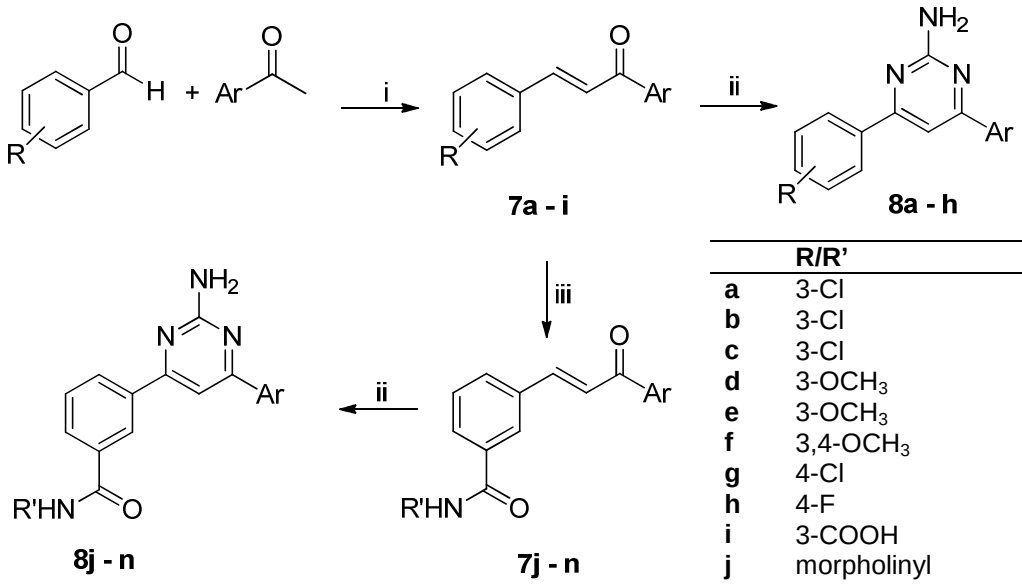


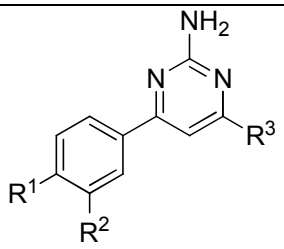
Figure 4: Docking orientation of compound **8j** in the active site of the adenosine A_{2A} receptor. An intermolecular hydrogen bond interaction is observed between the exocyclic amino group and Asn253, while π=π stacking occurs between the three ring systems and Phe168. Additional hydrogen bond interactions occur between the morpholine in the side chain and Glu169 and Ser67 (Figure generated using Pymol).

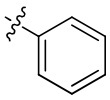
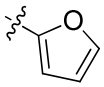
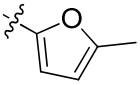
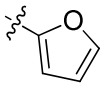
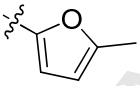
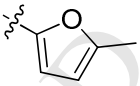
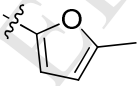
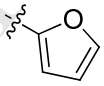
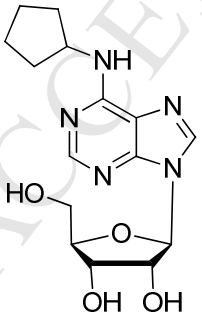
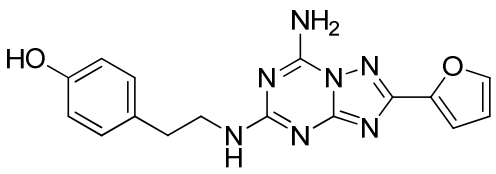


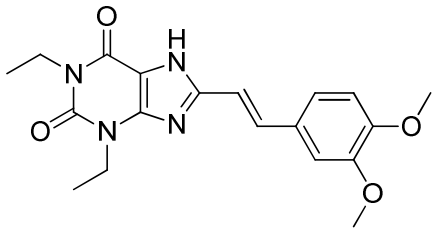
	R/R'	Ar
a	3-Cl	Phenyl
b	3-Cl	2-furanyl
c	3-Cl	5-methyl-2-furanyl
d	3-OCH_3	2-furanyl
e	3-OCH_3	5-methyl-2-furanyl
f	3,4-OCH_3	5-methyl-2-furanyl
g	4-Cl	5-methyl-2-furanyl
h	4-F	2-furanyl
i	3-COOH	5-methyl-2-furanyl
j	morpholinyl	5-methyl-2-furanyl
k	4-methyl-piperazinyl	5-methyl-2-furanyl
l	4-ethyl-piperazinyl	5-methyl-2-furanyl
m	1-piperidinyl	5-methyl-2-furanyl
n	1-pyrrolidinyl	5-methyl-2-furanyl

Scheme 1. Synthesis of 2-aminopyrimidine derivatives **8**. Reagents and Conditions: (i) NaOH, EtOH/MeOH, rt, 3 h; (ii) Guanidine hydrochloride, NaH, DMF, 110 °C, 24 h. (iii) CDI, CH_2Cl_2, NHR, rt, 5 h.

Table 1: Adenosine receptor affinities (*K*i) of compounds **8a - h**.



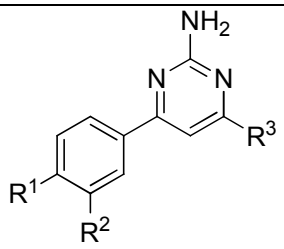
Comp.	R^1	R^2	R^3	$A_{2A}Ki$ (nM)	A_1Ki (nM)	SI $A_{2A}Ki/A_1Ki$
8a	-H	-Cl		948 ± 141	22.8 ± 5.51	42
8b	-H	-Cl		245 ± 21.1	39.1 ± 3.64	6.3
8c	-H	-Cl		399 ± 151	129 ± 12.1	3.1
8d	-H	$-OCH_3$		249 ± 79.6	61.4 ± 0.435	4.1
8e	-H	$-OCH_3$		409 ± 217	145 ± 19.6	2.8
8f	$-OCH_3$	$-OCH_3$		2778 ± 375	650 ± 63.0	4.3
8g	-Cl	-H		3320 ± 484	434 ± 13.0	7.6
8h	-F	-H		257 ± 36.4	158 ± 12.1	1.6
CPA					10.4 ± 1.57	
				2.88 ± 0.670		

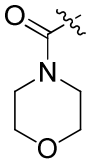
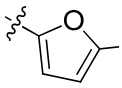
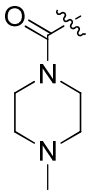
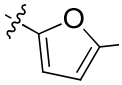
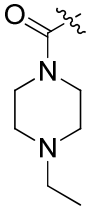
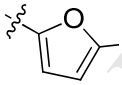
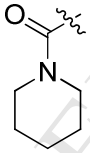
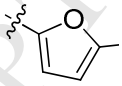
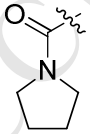
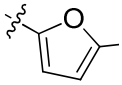
ZM241385  **KW-6002**		11.1 ± 3.97

[a]Values are given as mean ± SEM of duplicate determinations

[b]Selectivity index

Table 2: Adenosine receptor affinities (*K*i) of compounds **8j** - **n**.



Comp.	R^1	R^2	R^3	$A_{2A}Ki$ (nM)[a]	A_1Ki (nM)[a]	SI[b] $A_{2A}Ki/A_1Ki$
8j	-H			29.3 ± 0.978	52.1 ± 16.9[c]	0.6
8k	-H			16.3 ± 2.18	136 ± 1.88	0.1
8l	-H			30.9 ± 3.10	37.5 ± 0.857	0.8
8m	-H			6.34 ± 0.532	9.54 ± 1.34	0.7
8n	-H			58.2 ± 19.9	36.8 ± 5.42	1.6
	CPA				10.4 ± 1.57	
	ZM241385			2.88 ± 0.670		
	KW-6002			11.1 ± 3.97		

[a]Values are given as mean ± SEM of duplicate determinations

[b]Selectivity index

[c]n= 4

Table 3: The percentage viable cells remaining after treatment with amide derivatives (**8j - 8n**), as compared to untreated cells (100%).

	% Viable cells	
Comp.	**1 µM[a]**	**10 µM[a]**
8j	98.6 ± 4.45	81.7 ± 5.97
8k	106.6 ± 9.76	76.8 ± 18.1
8l	65.7 ± 4.95	60.1 ± 0.00
8m	64.3 ± 4.74	61.6 ± 4.46
8n	101 ± 6.22	67.9 ± 7.09

[a]Values are given as mean ± SD of triplicate determinations

Research highlights

- 2-Aminopyrimidines were synthesised and screened for dual adenosine A_1 and A_{2A} receptor affinity
- Compound **8m** was the most potent compound with A_{2A} and A_1 *K*i values of 6.34 nM and 9.54 nM respectively.
- Two of the amide derivatives (**8k** and **8m**) exhibited *in vivo* activity in the haloperidol induced catalepsy assay performed in rats

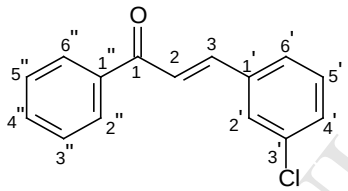
Supporting information:

Synthetic procedures

*General procedure for the synthesis of chalcones (***7a** – **7h***)*

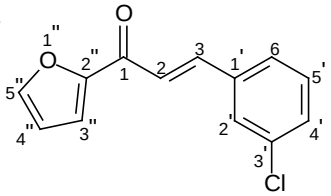
Ketone (8.57 mmol, 1 equiv) and benzaldehyde (8.57 mmol, 1 equiv) were dissolved in ethanol, and stirred at room temperature. To this mixture, a solution of 40% (w/v) sodium hydroxide (0.5 equiv) was added drop wise. After the reaction mixture was stirred at room temperature for 3 hours, the residue that formed was filtered and washed with cold ethanol. The resulting solid was recrystallised from ethanol.

(2*E*)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (**7a**)



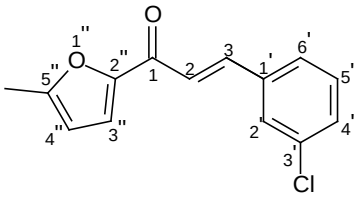
The title compound was prepared from acetophenone and 3-chlorobenzaldehyde in a yield of 34%: mp 74.7-75.1 °C (ethanol), pale yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 – 8.00 (m, 2H, H-2″, H-6″), 7.73 (d, *J* = 15.7 Hz, 1H, H-3), 7.65 – 7.57 (m, 2H, H-2′, H-4″), 7.56 – 7.47 (m, 4H, H-2, H-3″, H-5″, H-6′), 7.40 – 7.32 (m, 2H, H-4′, H-5′); ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.0 (C-1), 143.0 (C-3), 137.8 (C-1″), 136.7 (C-1′), 134.9 (C-3′), 133.0 (C-4″), 130.3, 130.2 (C-4′, C-5′), 128.7, 128.5 (C-2″, C-6″ and C-3″, C-5″), 127.9 (C-2′), 126.8 (C-6′), 123.2 (C-2). EI-HRMS m/z: calcd for $C_{15}H_{11}ClO$, 242.04984, found 242.04878; Purity (HPLC): 93%.

(2*E*)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (**7b**)



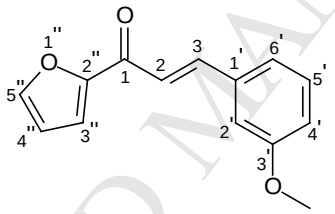
The title compound was prepared from 2-acetylfuran and 3-chlorobenzaldehyde in a yield of 17%: mp 76.2-77.1 °C (ethanol), light yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 15.8 Hz, 1H, H-3), 7.67 – 7.65 (m, 1H, H-5″), 7.62 (br t, *J* = 1.9 Hz, 1H, H-2′) 7.49 (br dt, *J* = 1.6, 7.3 Hz, 1H, H-6′), 7.43 (d, *J* = 15.8 Hz, 1H, H-2), 7.39 – 7.31 (m, 3H, H-4′, H-5′, H-3″), 6.60 (dd, *J* = 1.7, 3.6 Hz, 1H, H-4″); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.5 (C-1), 153.5 (C-2″), 146.7 (C-5″), 142.2 (C-3), 136.5 (C-1′), 134.9 (C-3′), 130.4, 130.1 (C-4′, C-5′), 127.9 (C-2′), 126.9 (C-6′), 122.3 (C-2), 117.8 (C-3″), 112.6 (C-4″). EI-HRMS m/z: calcd for $C_{13}H_9ClO_2$, 232.02911, found 232.02796; Purity (HPLC): 92%.

(2*E*)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7c**)



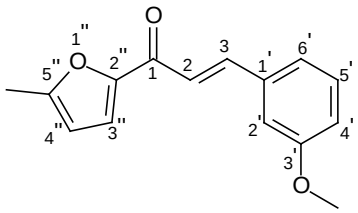
The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3-chlorobenzaldehyde in a yield of 12%: mp 91.4-92.3 °C (ethanol), orange crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 15.7 Hz, 1H, H-3), 7.65 – 7.61 (m, 1H, H-2'), 7.52 – 7.47 (m, 1H, H-6'), 7.41 – 7.32 (m, 3H, H-4', H-5', H-2), 7.31 – 7.27 (m, 1H, H-3''), 6.24 (m, 1H, H-4''), 2.46 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.7 (C-1), 158.4 (C-2'' or C-5''), 152.3 (C-2'' or C-5''), 141.4 (C-3), 136.6 (C-1'), 134.8 (C-3'), 130.2, 130.1 (C-4' and C-5'), 127.7 (C-2'), 126.8 (C-6'), 122.5 (C-2), 119.9 (C-3''), 109.4 (C-4''), 14.2 (C$\underline{H}_3$). EI-HRMS m/z: calcd for $C_{14}H_{11}ClO_2$, 246.04476, found 246.04435; Purity (HPLC): 100%.

(2*E*)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**7d**)



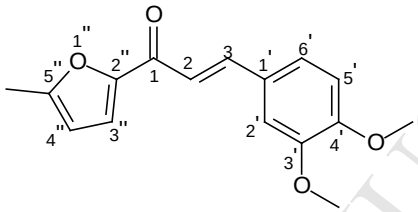
The title compound was prepared from 2-acetylfuran and 3-methoxybenzaldehyde in a yield of 22%: mp 65.4-66.4 °C (ethanol), amber crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, *J* = 15.8 Hz, 1H, H-3), 7.65 (dd, *J* = 0.8, 1.7 Hz, 1H, H-5''), 7.42 (d, *J* = 15.8 Hz, 1H, H-2), 7.35 – 7.29 (m, 2H, H-5', H-3''), 7.25 – 7.22 (br d, 7.5 Hz, 1H, H-6'), 7.15 (br t, *J* = 2.0 Hz, 1H, H-2'), 6.95 (ddd, *J* = 0.9, 2.6, 8.2 Hz, 1H, H-4'), 6.59 (dd, *J* = 1.7, 3.6 Hz, 1H, H-4''), 3.84 (s, 3H, OC$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.9 (C-1), 159.8 (C-3'), 153.6 (C-2''), 146.5 (C-5''), 143.9 (C-3), 136.0 (C-1'), 129.9 (C-5'), 121.3 (C-6' or C-2), 121.1 (C-6' or C-2), 117.6 (C-3''), 116.3 (C-4'), 113.4 (C-2'), 112.5 (C-4''), 55.3 (O$\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{14}H_{12}O_3$, 228.07864, found 228.07796; Purity (HPLC): 94%.

(2*E*)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7e**)



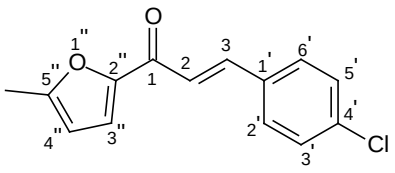
The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3-methoxybenzaldehyde in a yield of 46%: mp 96.9-97.5 °C (ethanol), yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 16.1 Hz, 1H, H-3), 7.35 (d, *J* = 16.1 Hz, 1H, H-2), 7.31 (t, *J* = 8.0 Hz, 1H, H-5'), 7.24 (d, 1H, *J* = 3.5 Hz, H-3''), 7.22 (br d, 1H, *J* = 7.7 Hz, H-6'), 7.13 (br s, 1H, H-2'), 6.94 (dd, *J* = 2.6, 8.2 Hz, 1H, H-4'), 6.20 (dd, *J* = 0.8, 3.4 Hz, 1H, H-4''), 3.83 (s, 3H, $OC\underline{H}_3$), 2.43 (s, 3H, $C\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 177.1 (C-1), 159.8 (C-3'), 158.2 (C-5''), 152.4 (C-2''), 143.1 (C-3), 136.1 (C-1'), 129.8 (C-5'), 121.5 (C-2), 121.0 (C-6'), 119.6 (C-3''), 116.0 (C-4'), 113.4 (C-2'), 109.3 (C-4''), 55.3 ($O\underline{C}H_3$), 14.1 ($\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{15}H_{14}O_3$, 242.09429, found 242.09396; Purity (HPLC): 100%.

(2*E*)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7f**)



The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3,4-dimethoxybenzaldehyde in a yield of 35%: mp 116.3-117 °C (ethanol), yellow crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (br d, *J* = 15.7 Hz, 1H, H-3), 7.26 – 7.16 (m, 3H, H-6', H-2, H-3''), 7.11 (br s, 1H, H-2'), 6.85 (br d, *J* = 8.4 Hz, 1H, H-5'), 6.19 (br s, 1H, H-4''), 3.93 (s, 3H, $OC\underline{H}_3$), 3.90 (s, 3H, $OC\underline{H}_3$), 2.40 (s, 3H, $C\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 177.2 (C-1), 157.8 (C-5''), 152.5 (C-2''), 151.2 (C-3' or C-4'), 149.1 (C-3' or C-4'), 143.3 (C-3), 127.7 (C-1'), 123.0 (C-2), 119.1 (C-6' or C-3''), 119.2 (C-6' or C-3''), 111.0 (C-5'), 110.1 (C-2'), 109.2 (C-4''), 55.9 ($O\underline{C}H_3$), 55.9 ($O\underline{C}H_3$), 14.1 ($\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{16}H_{16}O_4$, 272.10486, found 272.10447; Purity (HPLC): 90%.

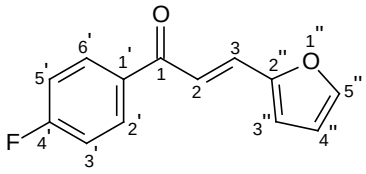
(2*E*)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7g**)



The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 4-chlorobenzaldehyde in a yield of 41%: mp 151.4-151.9 °C (ethanol), pale yellow solid.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, *J* = 15.7 Hz, 1H, H-3), 7.49 (m, 2H, H-2', H-6'), 7.29 – 7.24 (m, 3H, H-2, H-3', H-5'), 7.18 (d, *J* = 3.4 Hz, 1H, H-3''), 6.14 (dd, *J* = 0.9, 3.4 Hz, 1H, H-4''), 2.36 (s, 3H, $C\underline{H}_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9 (C-1), 158.3 (C-5''), 152.4 (C-2''), 141.7 (C-3), 136.2 (C-4'), 133.3 (C-1'), 129.5 (C-2', C-6' or

C-3', C-5'), 129.1(C-2', C-6' or C-3', C-5'), 121.7 (C-2), 119.7 (C-3''), 109.4 (C-4''), 14.2 ($\underline{C}H_3$). EI-HRMS m/z: calcd for $C_{14}H_{11}ClO_2$, 246.04476, found 246.04409; Purity (HPLC): 96%.

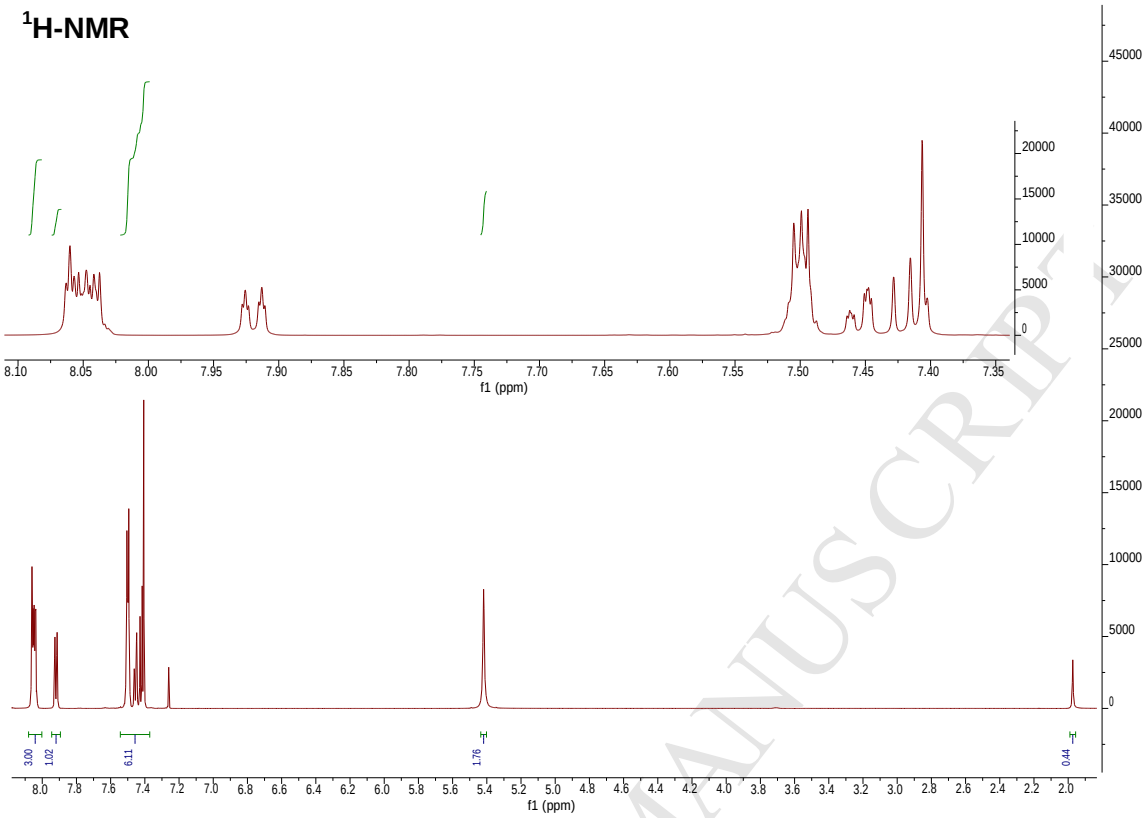
(2*E*)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (**7h**)



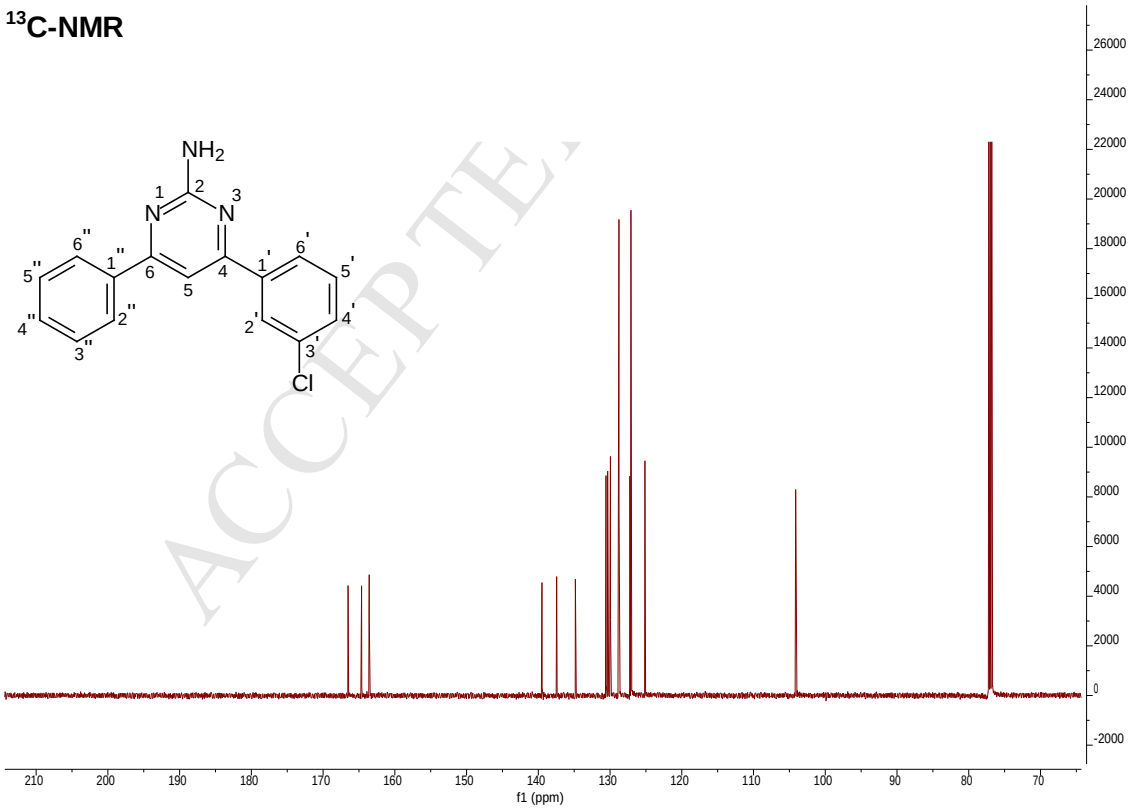
The title compound was prepared from 1-(4-fluorophenyl)ethanone and furan-2-carbaldehyde in a yield of 40%: mp 59.9-60.5 °C (ethanol), dark orange solid.^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 – 8.02 (m, 2H, H-2′, H-6′), 7.59 (d, *J* = 15.3 Hz, 1H, H-3), 7.52 (d, *J* = 1.7 Hz, 1H, H-5″), 7.42 (d, *J* = 15.3 Hz, 1H, H-2), 7.19 – 7.12 (m, 2H, H-3′, H-5′), 6.72 (d, *J* = 3.4 Hz, 1H, H-3″), 6.51 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4″).^{13}C NMR (151 MHz, $CDCl_3$) δ 188.0 (C-1), 165.5 (d, $J_{C\text{-}F}$ = 253.5 Hz, C-4′), 151.5 (C-2″), 145.0 (C-5″), 134.4 (d, $J_{C\text{-}F}$ = 3.2 Hz, C-1′), 130.96 (d, $J_{C\text{-}F}$ = 9.6 Hz, C-2′, C-6′), 130.8 (C-3), 118.7 (C-2), 116.4 (C-3″), 115.6 (d, $J_{C\text{-}F}$ = 21.9 Hz, C-3′, C-5′), 112.7 (C-4″). EI-HRMS m/z: calcd for $C_{13}H_9FO_2$, 216.05866, found 216.05805; Purity (HPLC): 91%.

4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (**8a**)

^{1}H-NMR

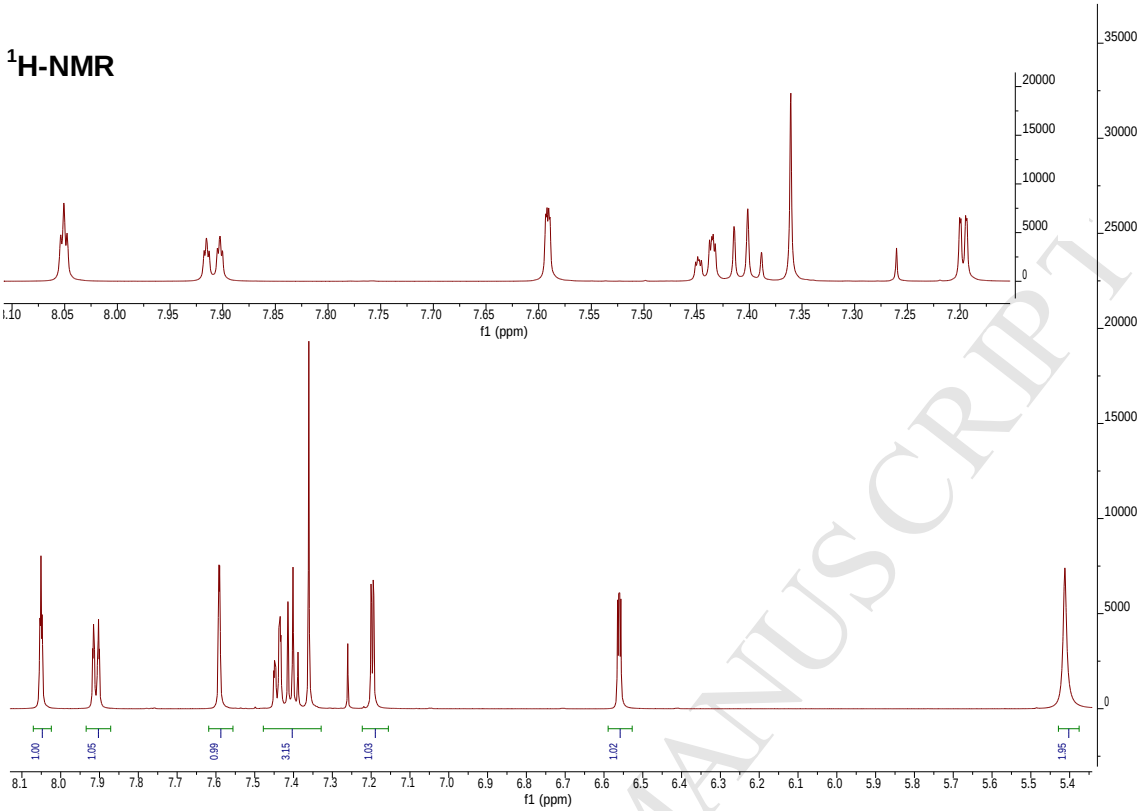


^{13}C-NMR

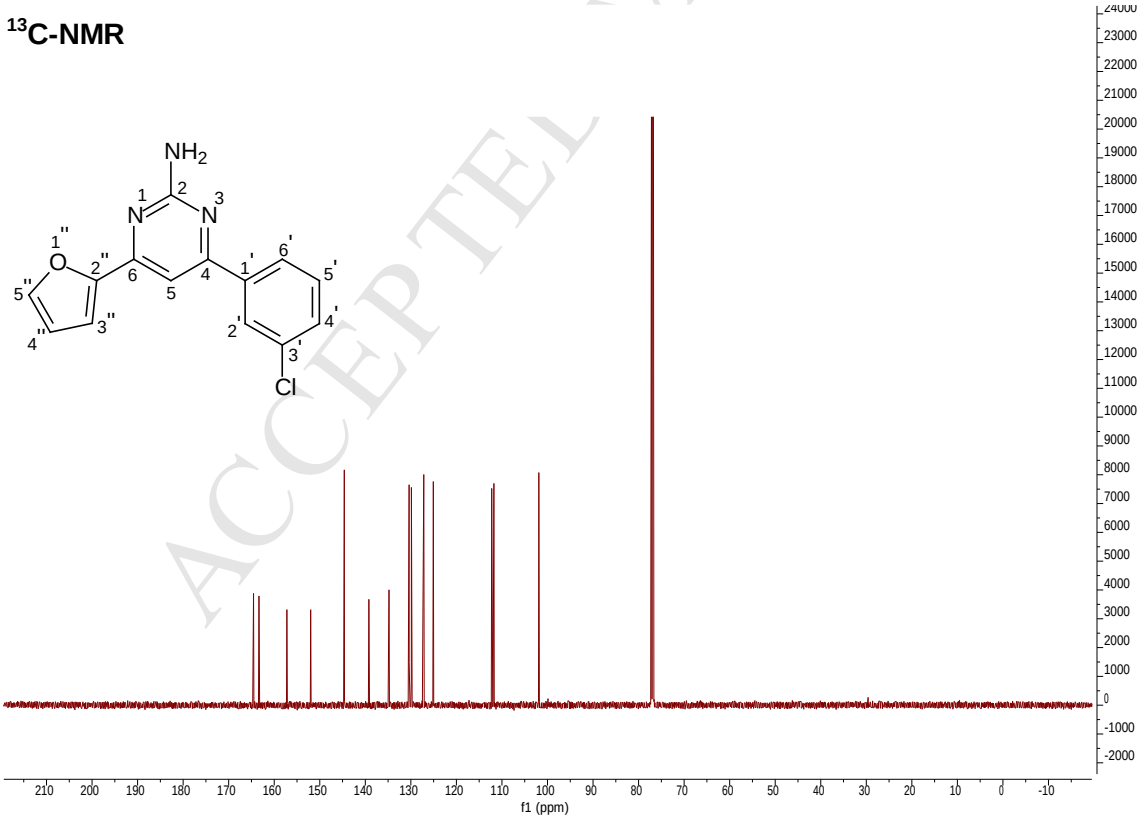


4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8b**)

^{1}H-NMR

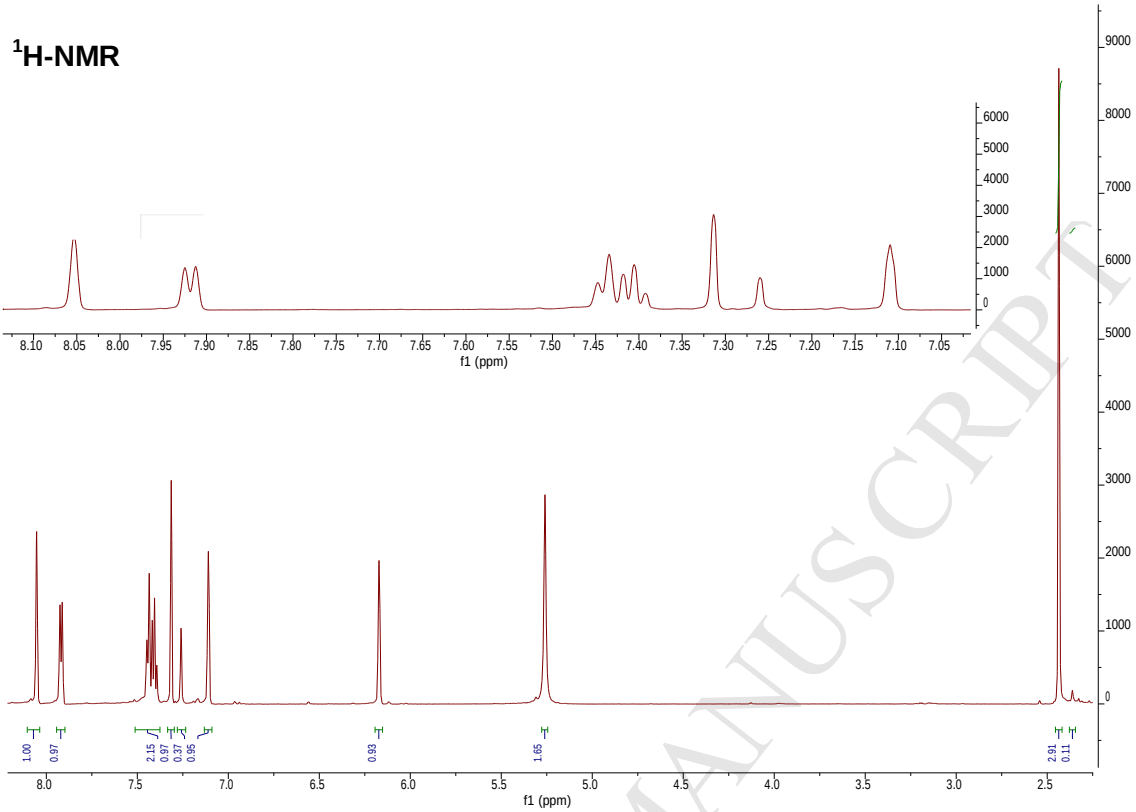


^{13}C-NMR

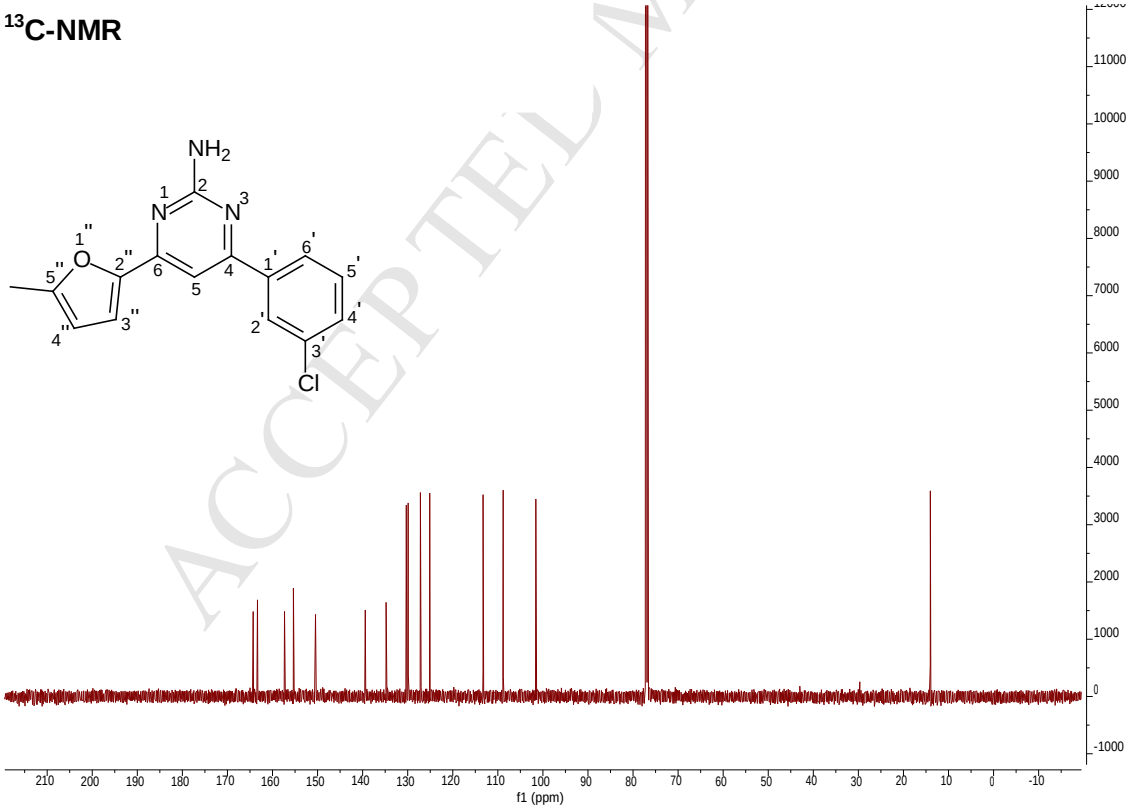


4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8c**)

^{1}H-NMR

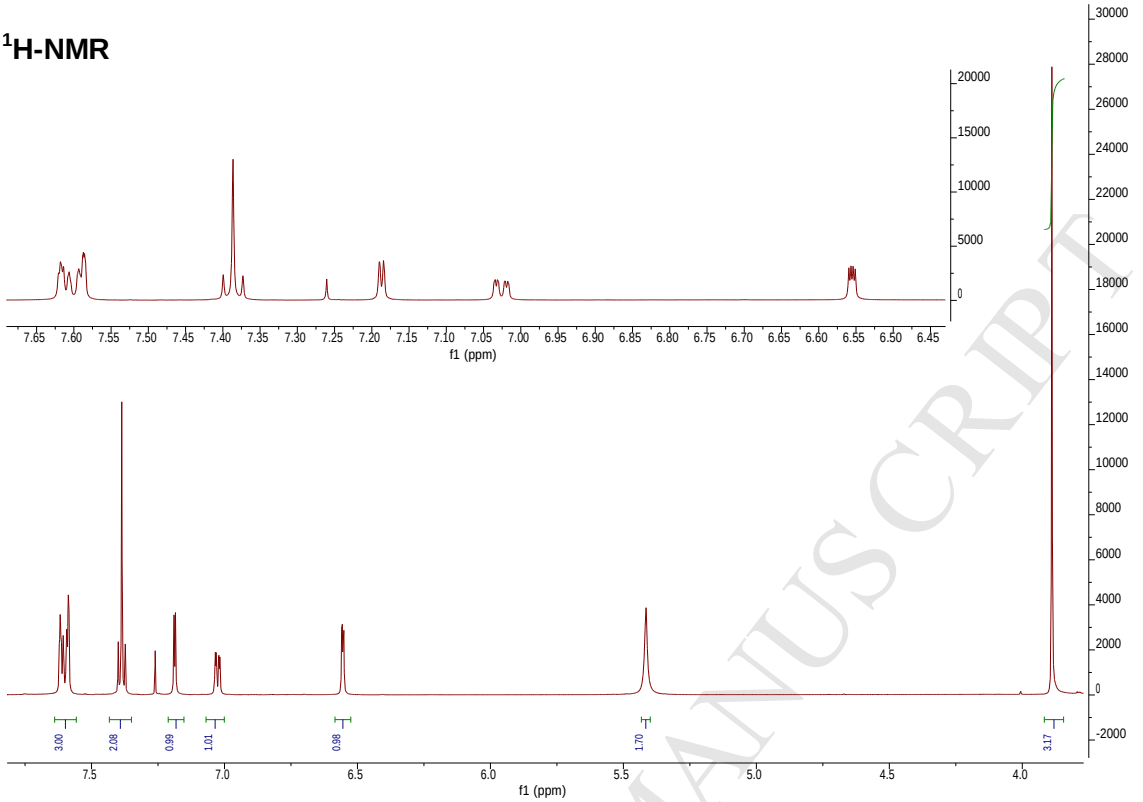


^{13}C-NMR

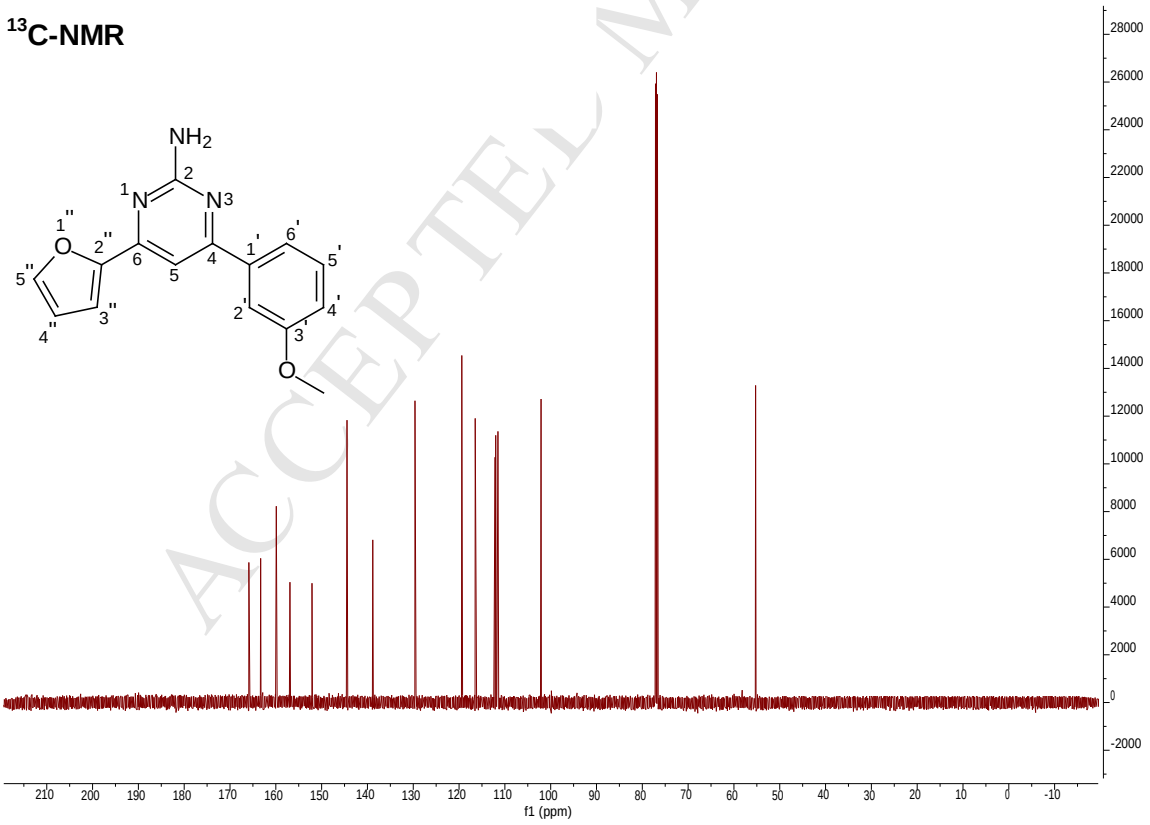


4-(furan-2-yl)-6-(3-methoxyphenyl)pyrimidin-2-amine (**8d**)

^{1}H-NMR

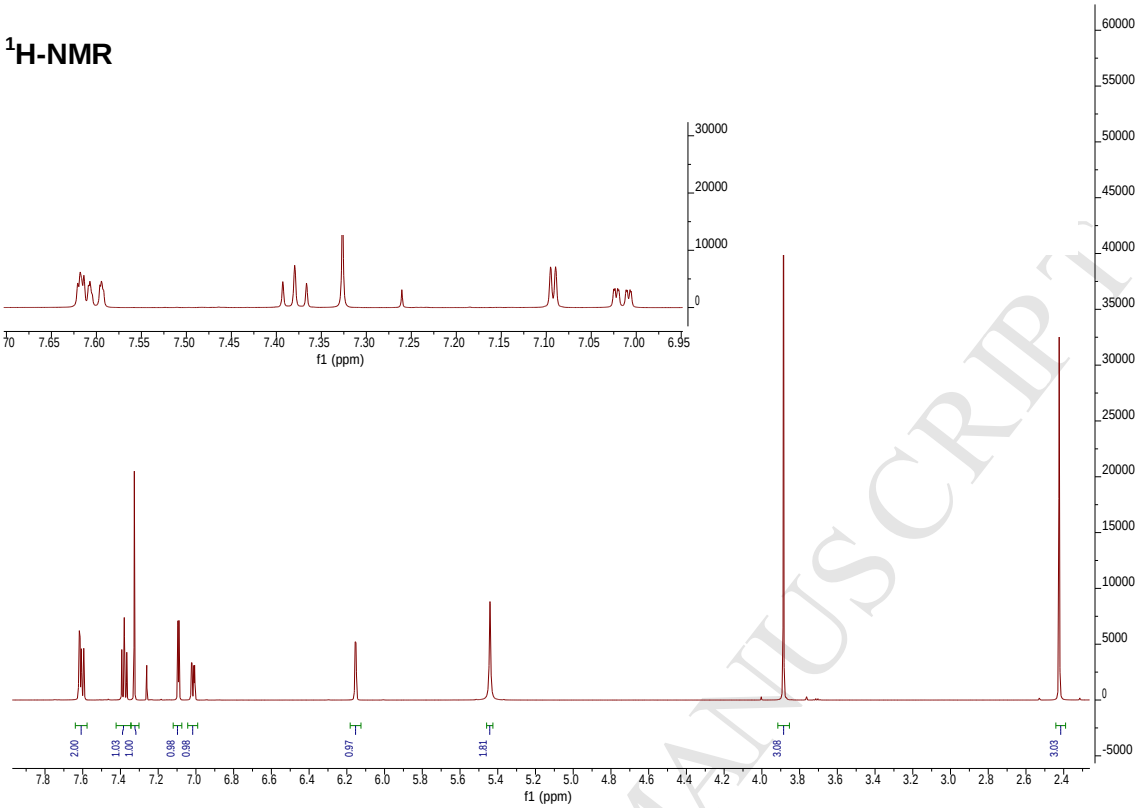


^{13}C-NMR

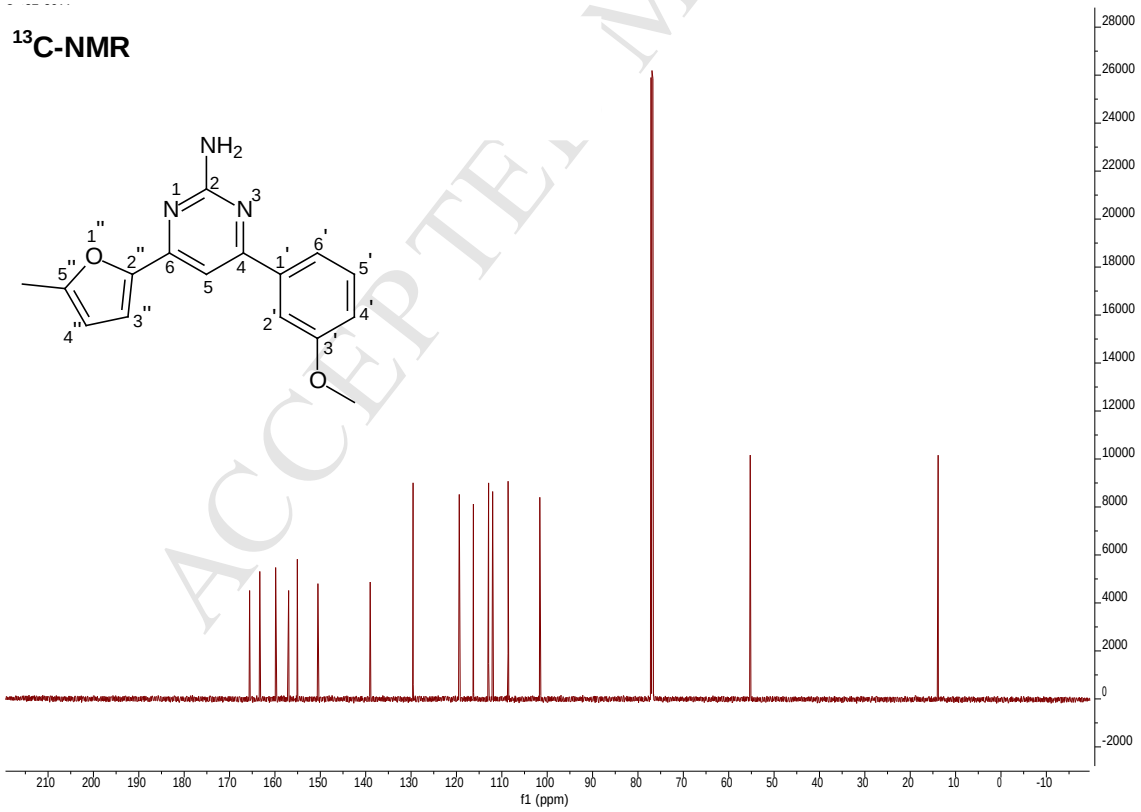


4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8e**)

^{1}H-NMR

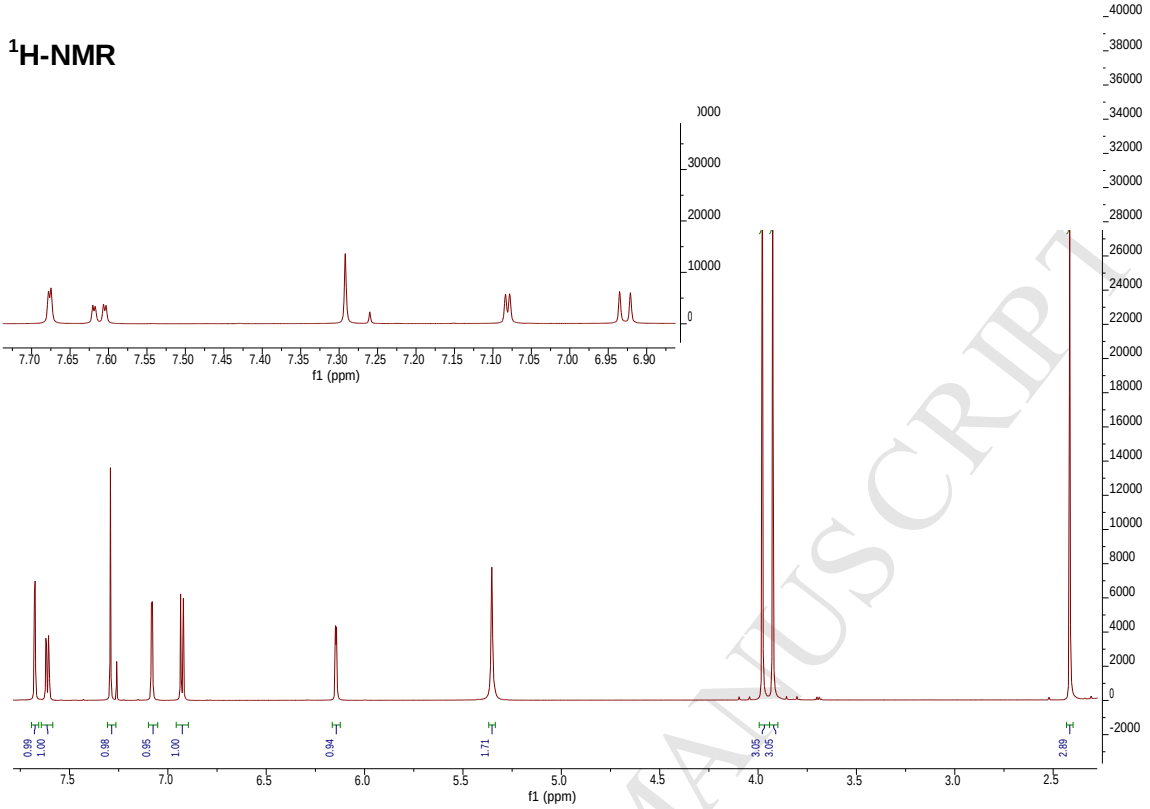


^{13}C-NMR

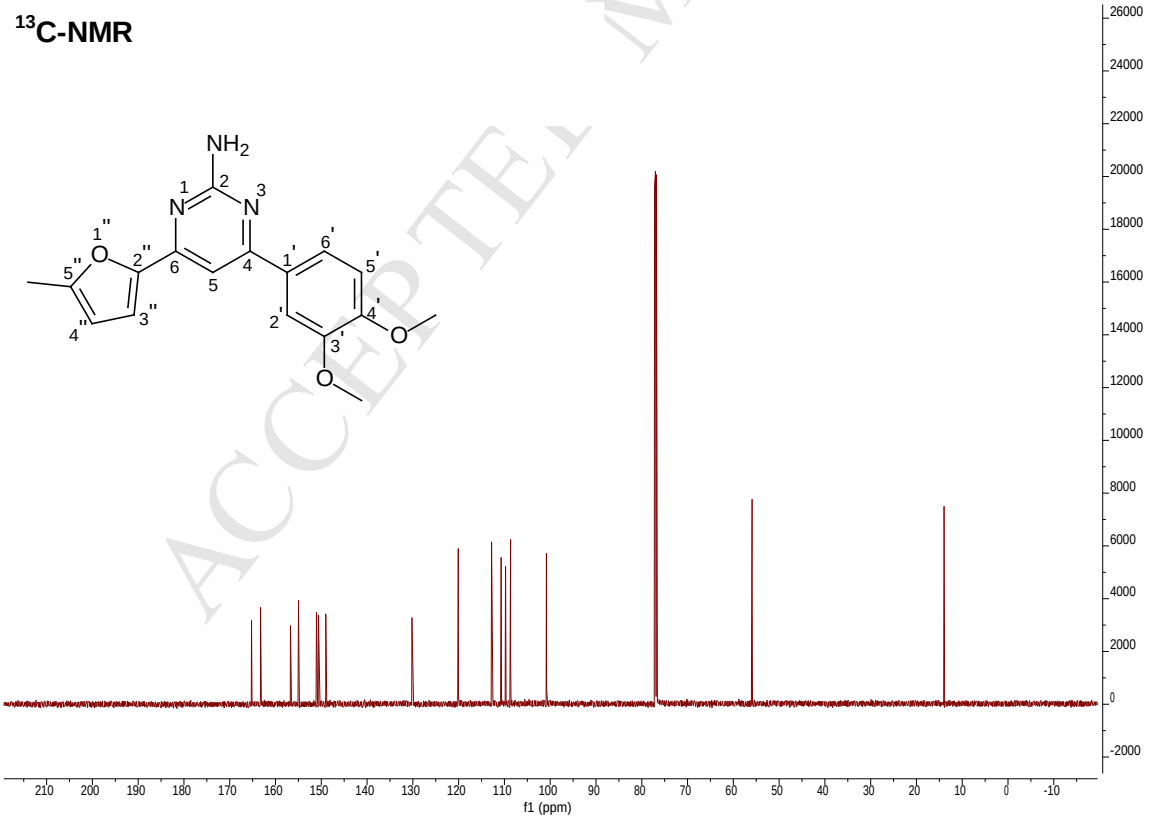


4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8f**)

^{1}H-NMR

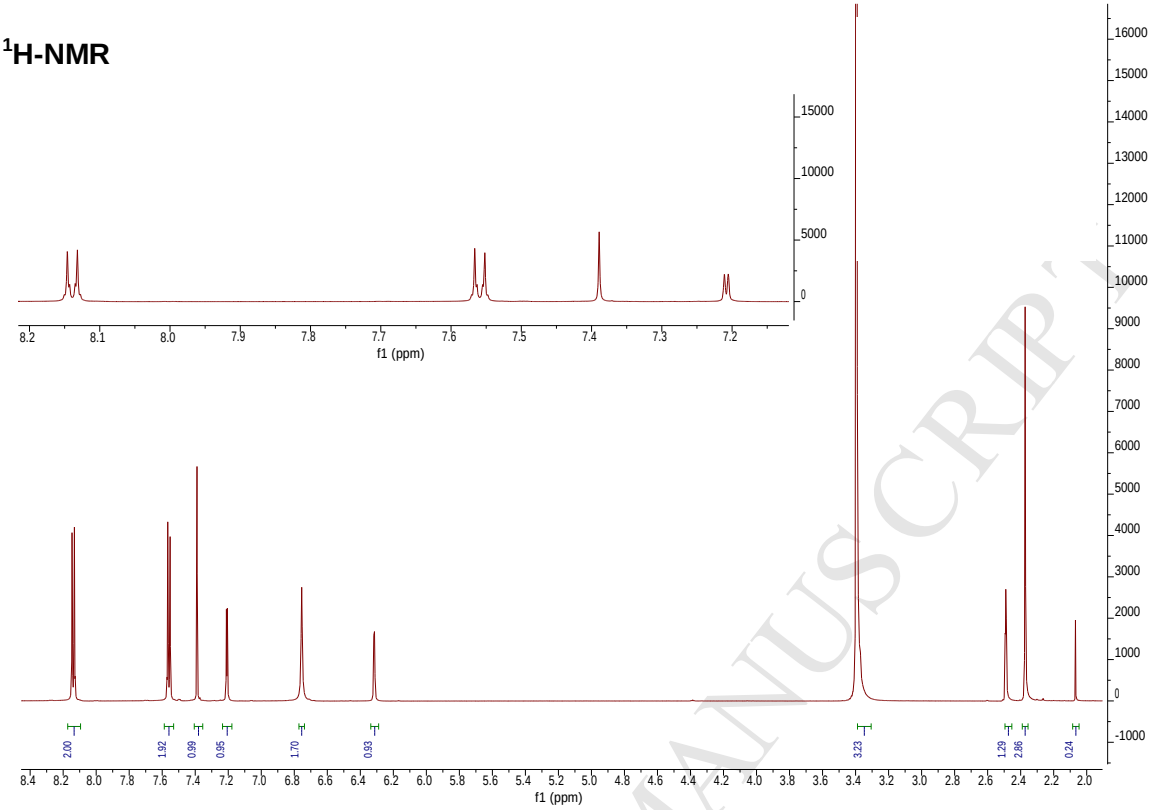


^{13}C-NMR

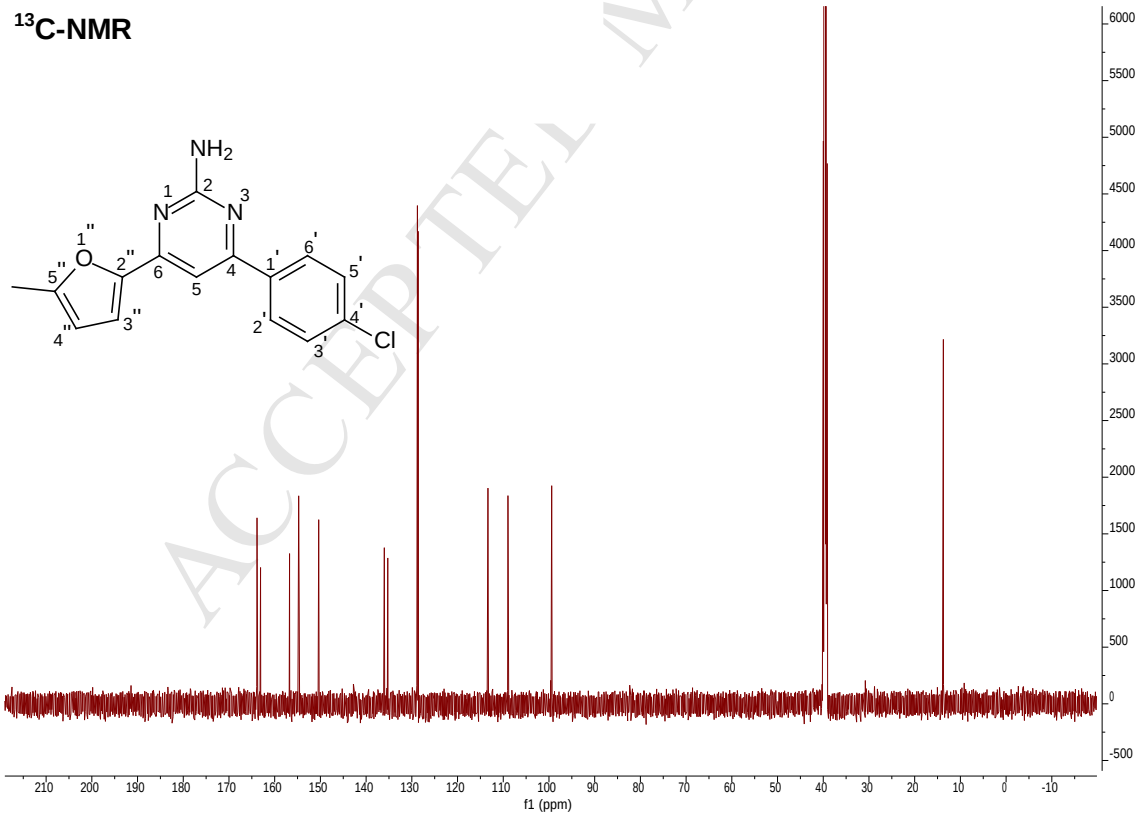


4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8g**)

^{1}H-NMR

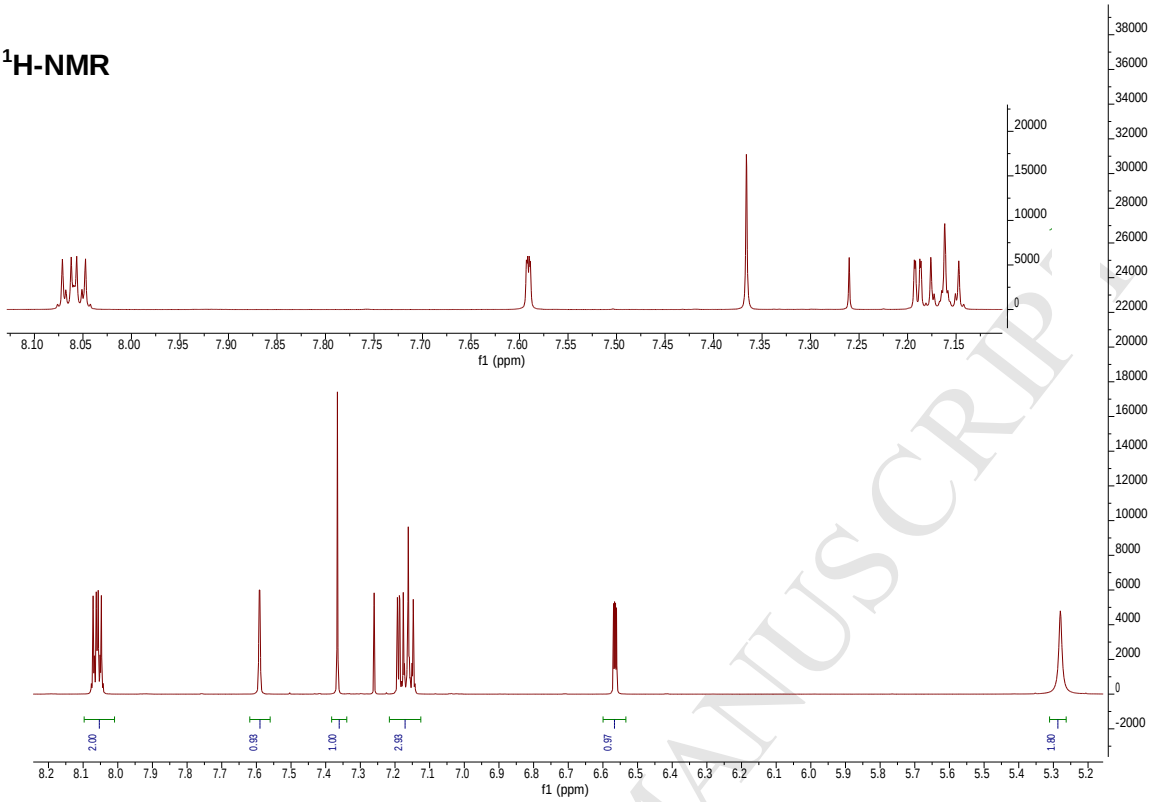


^{13}C-NMR

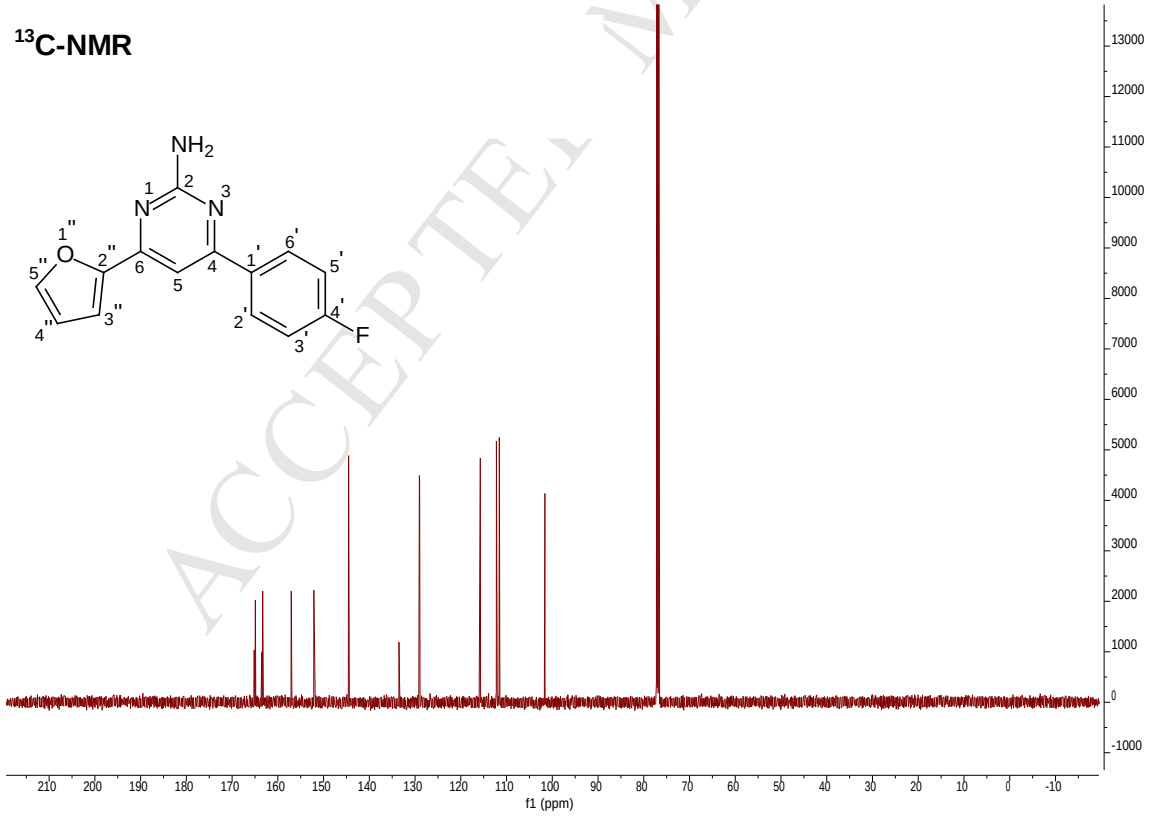


4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8h**)

^{1}H-NMR

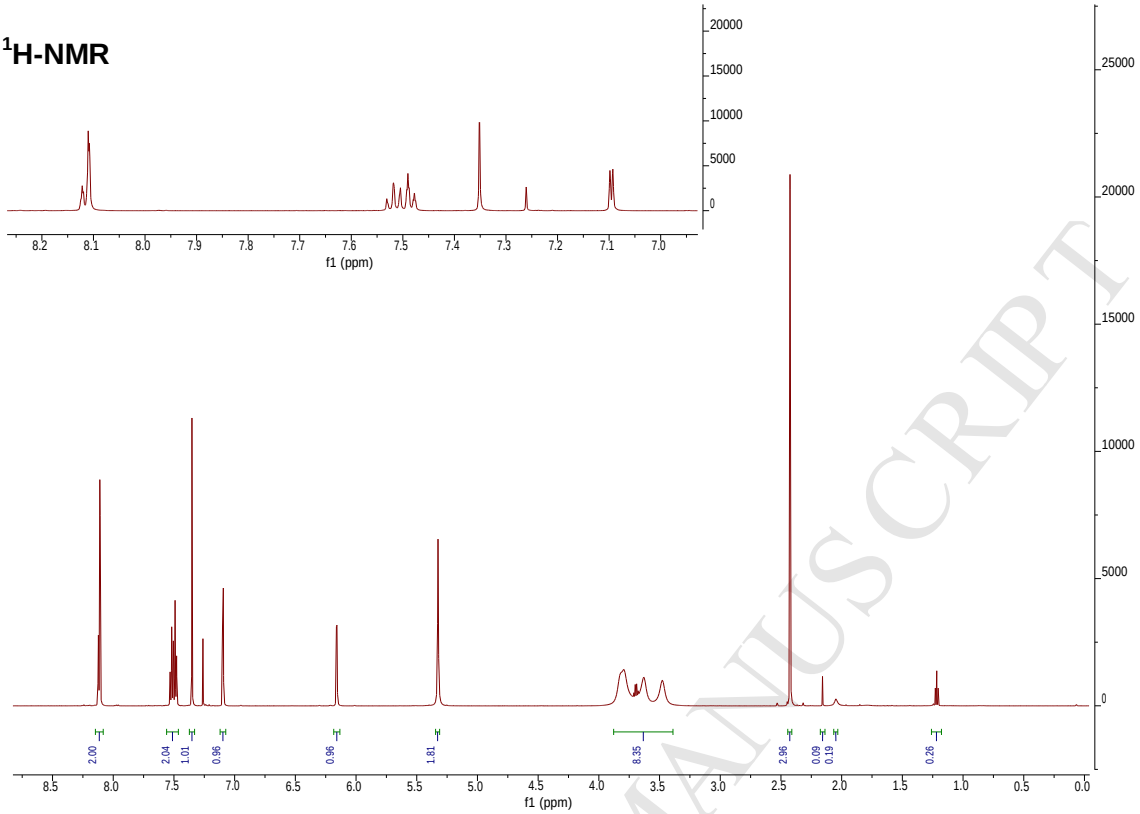


^{13}C-NMR

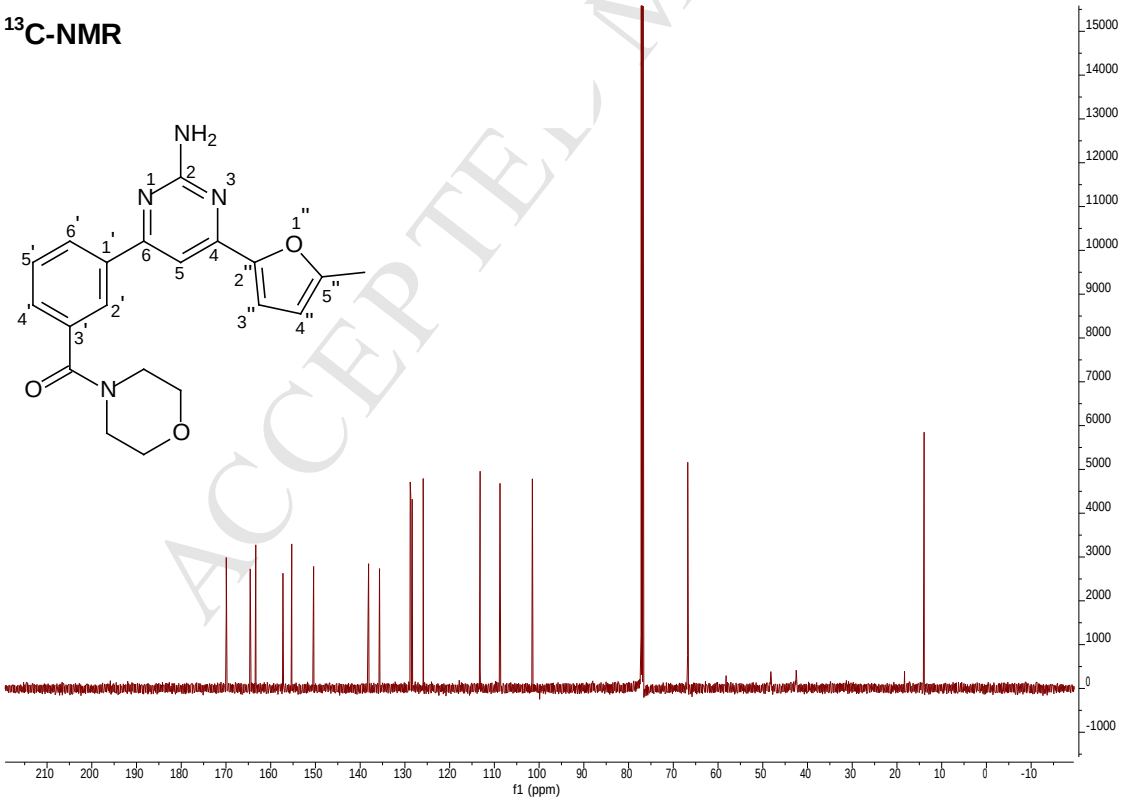


4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (**8j**)

^{1}H-NMR

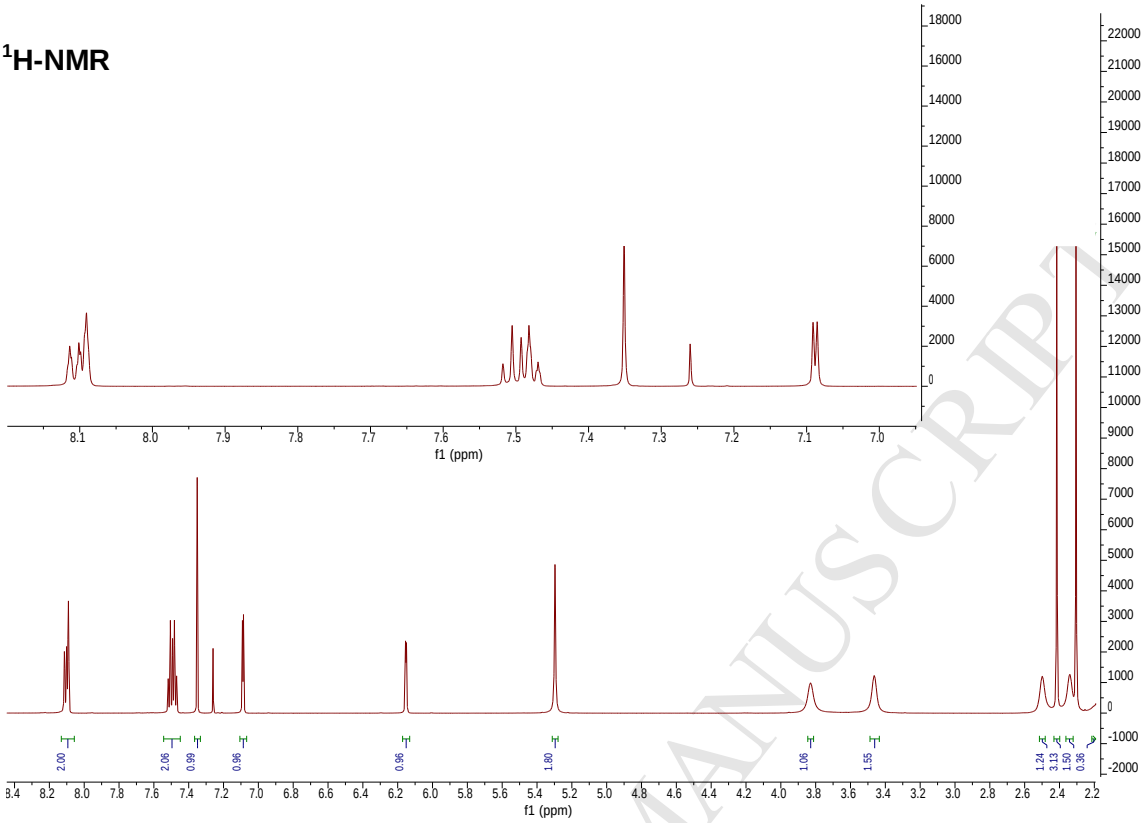


^{13}C-NMR

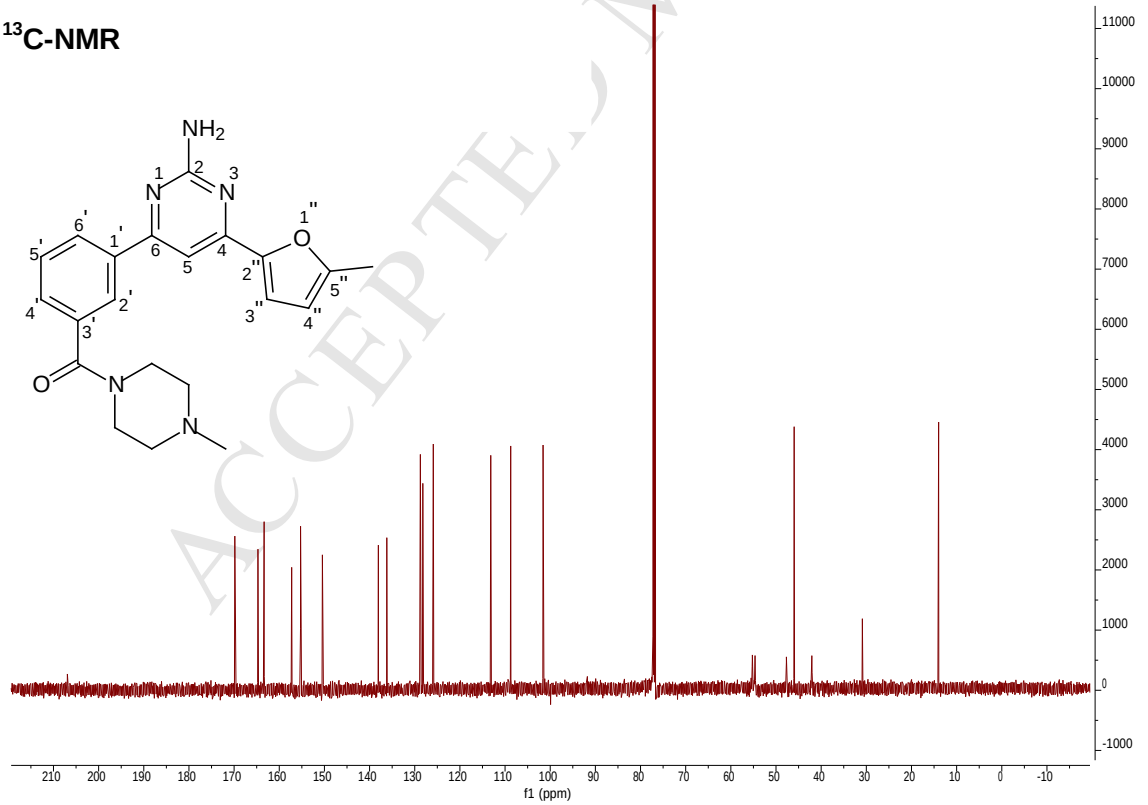


4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8k**)

^{1}H-NMR

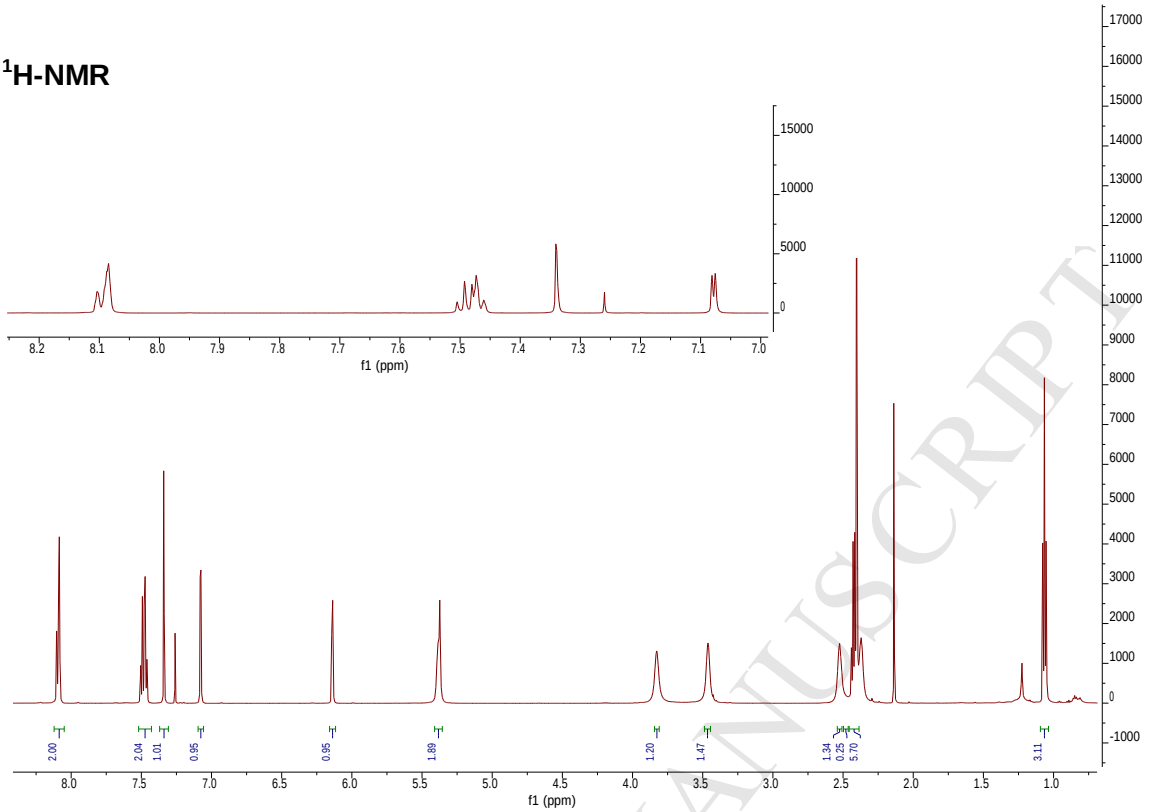


^{13}C-NMR

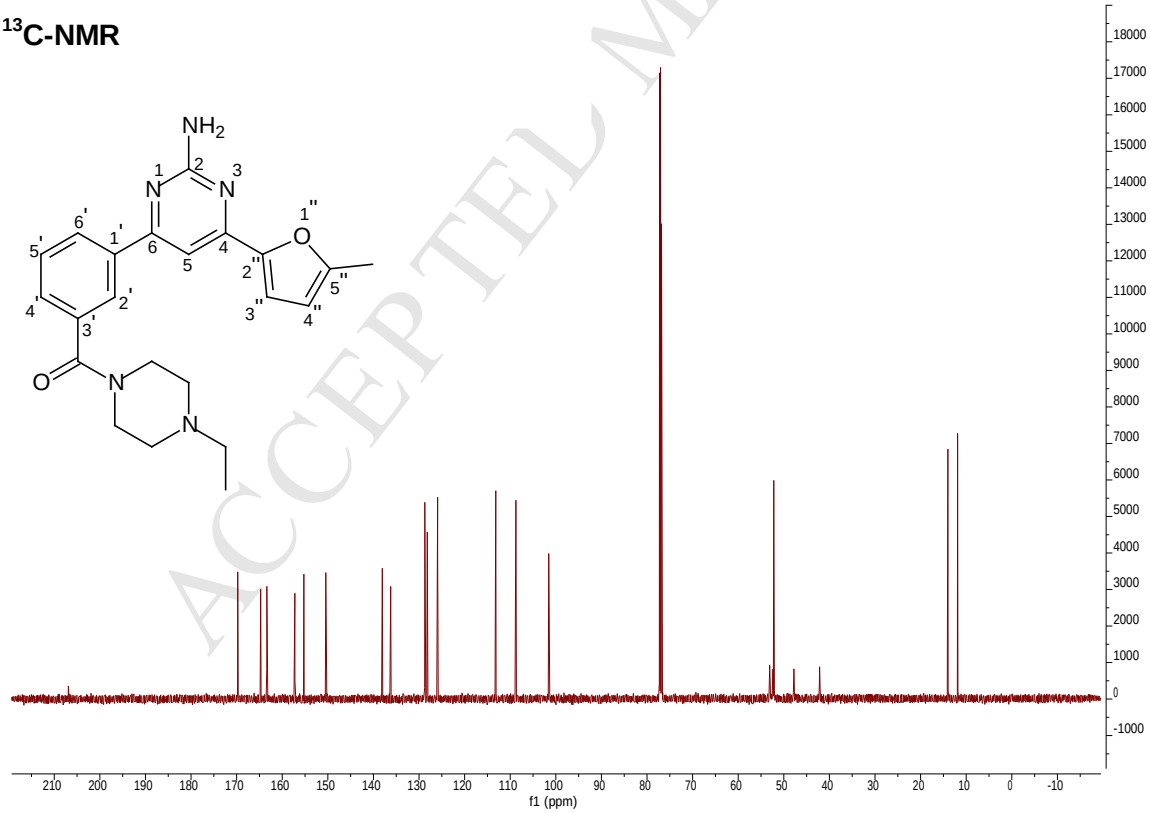


4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8l**)

^{1}H-NMR

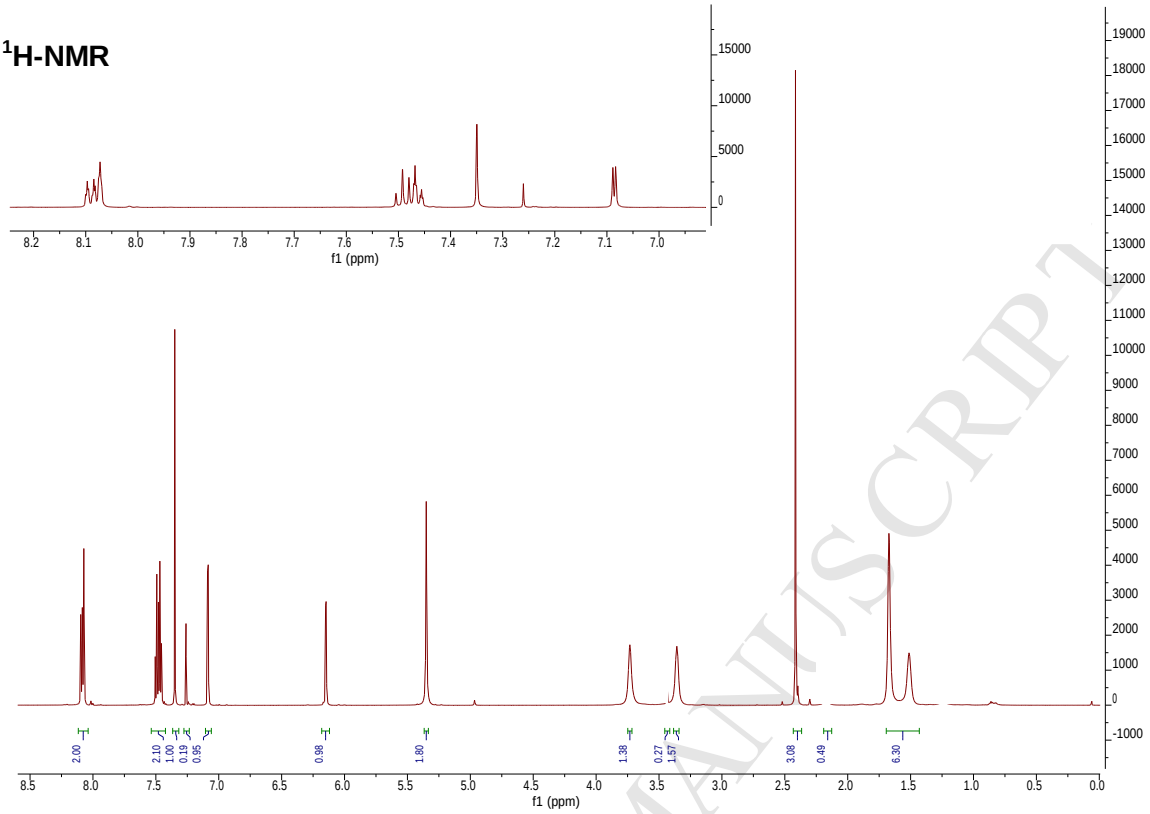


^{13}C-NMR

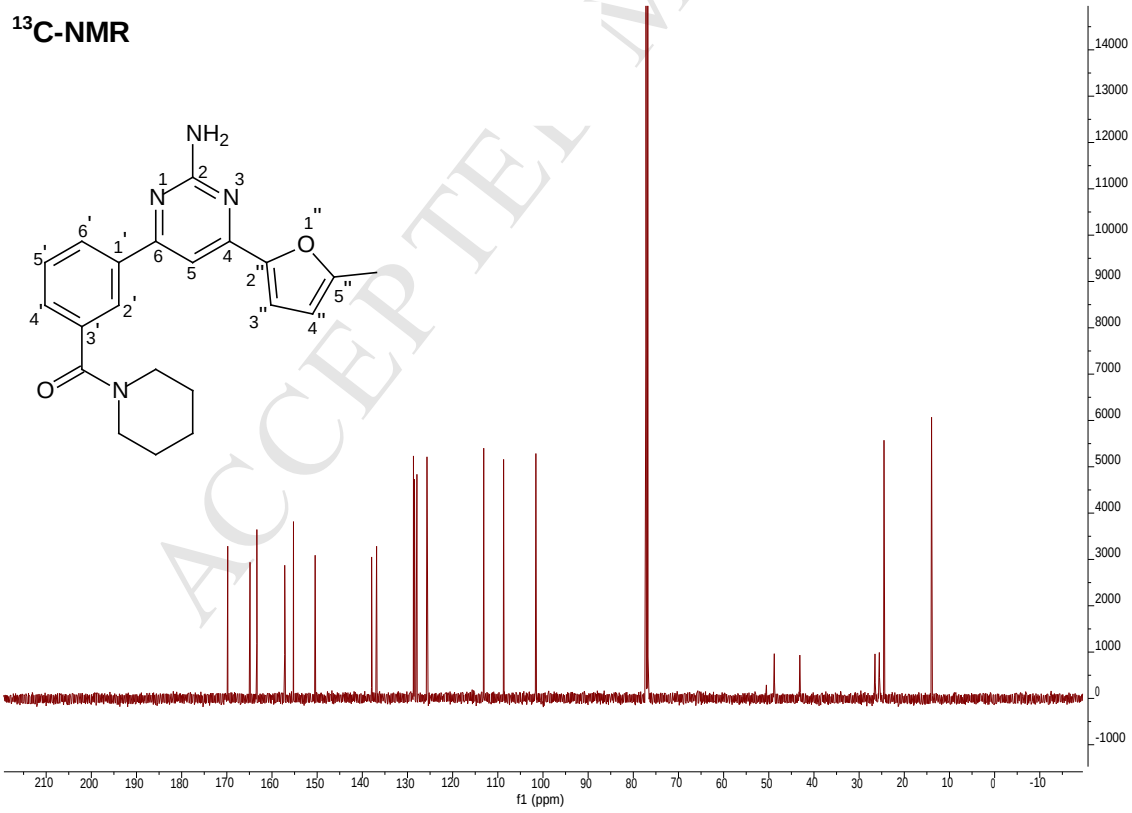


4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**)

^{1}H-NMR

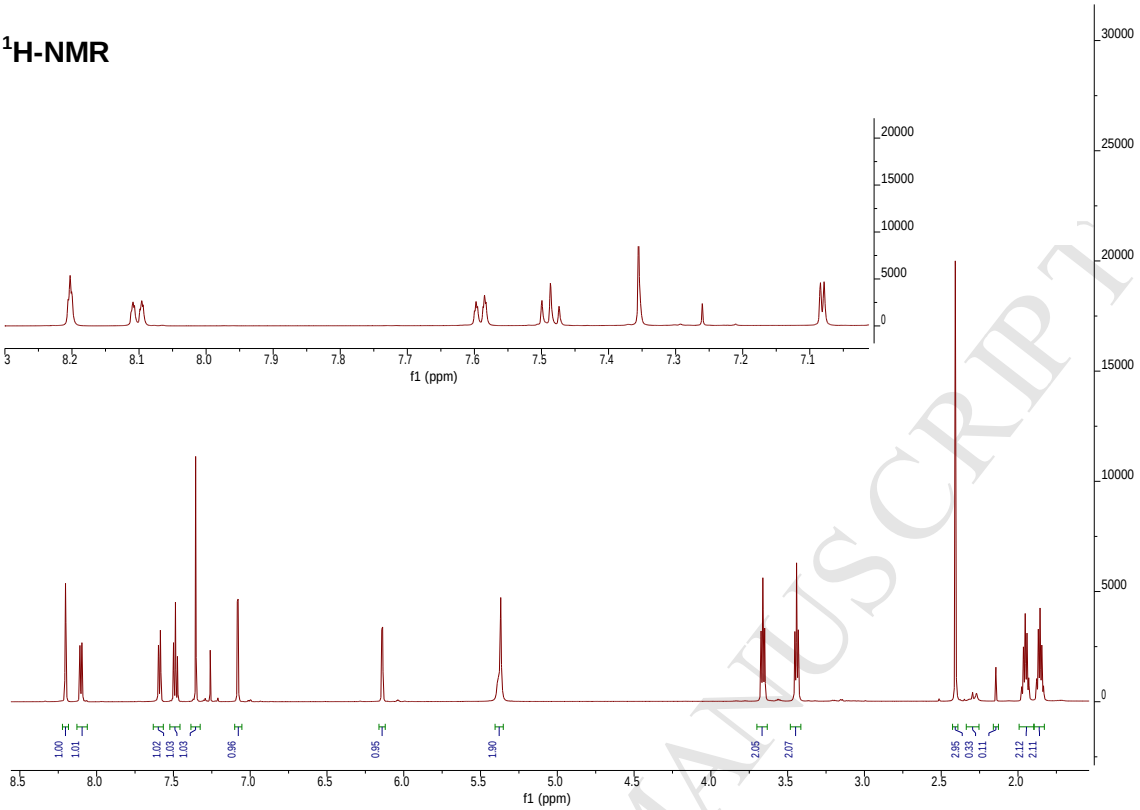


^{13}C-NMR

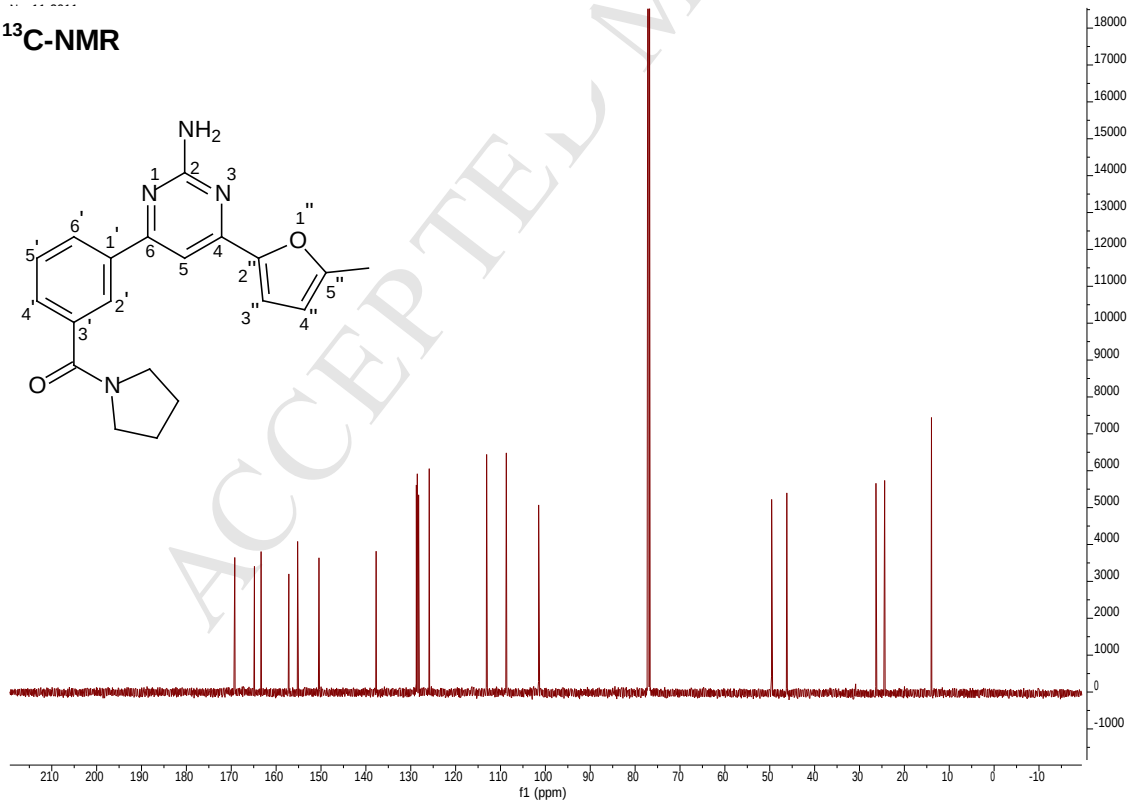


4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8n**)

^{1}H-NMR



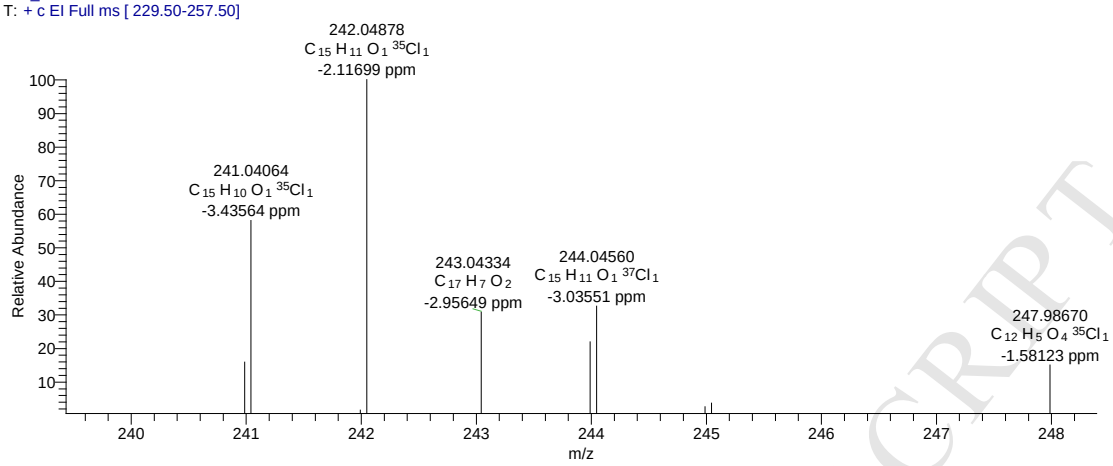
^{13}C-NMR



Mass spectra

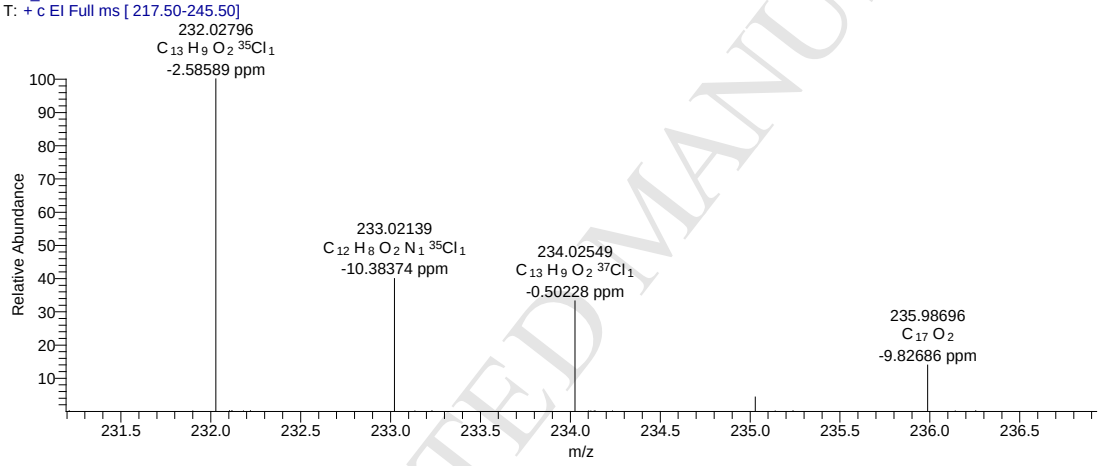
(2*E*)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (**7a**)

SJ2_HR-c1 #29 RT: 0.77 AV: 1 NL: 1.72E5
T: + c EI Full ms [229.50-257.50]



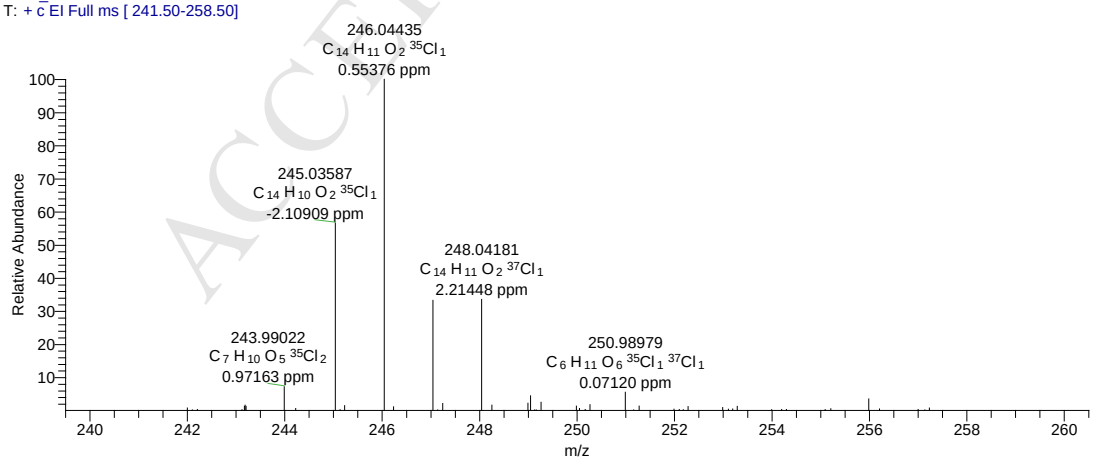
(2*E*)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (7**b**)

SJ8_HR-c1 #91 RT: 1.65 AV: 1 NL: 2.70E6
T: + c EI Full ms [217.50-245.50]

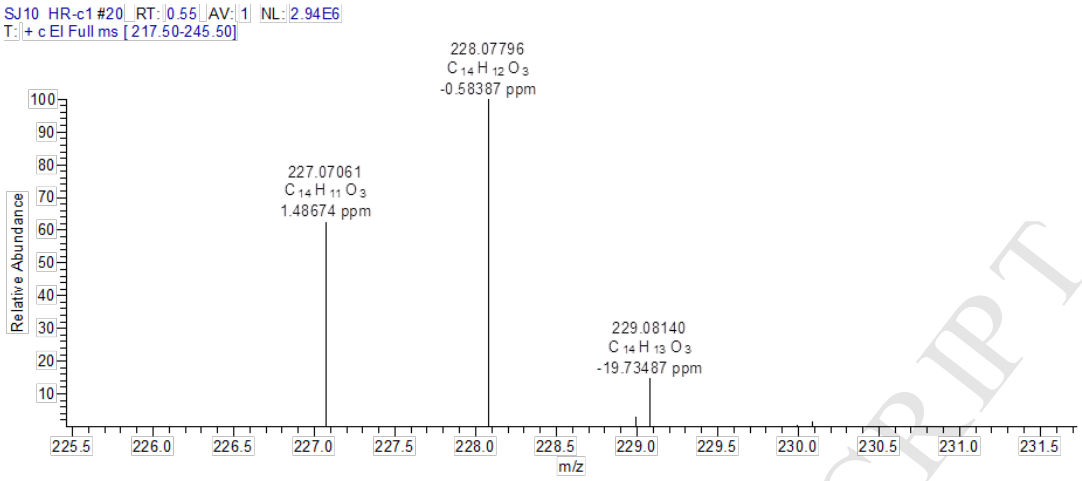


(2*E*)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7c**)

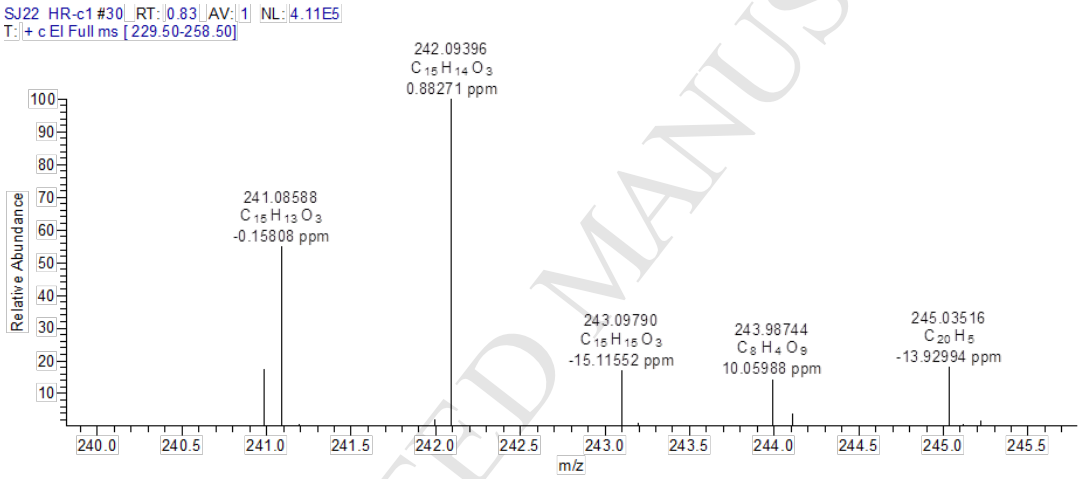
SJ20_HR-c1 #40 RT: 0.69 AV: 1 NL: 8.70E5
T: + c EI Full ms [241.50-258.50]



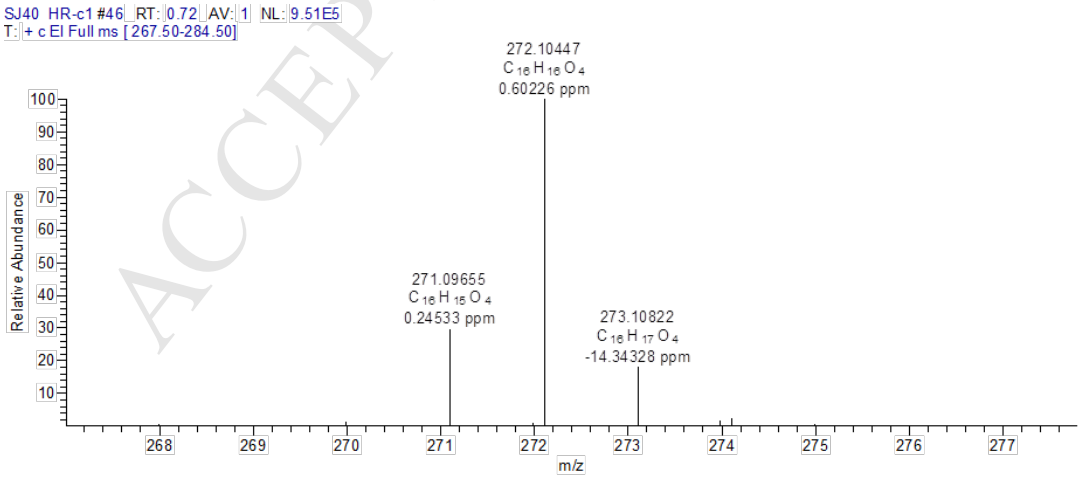
(2*E*)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**7d**)



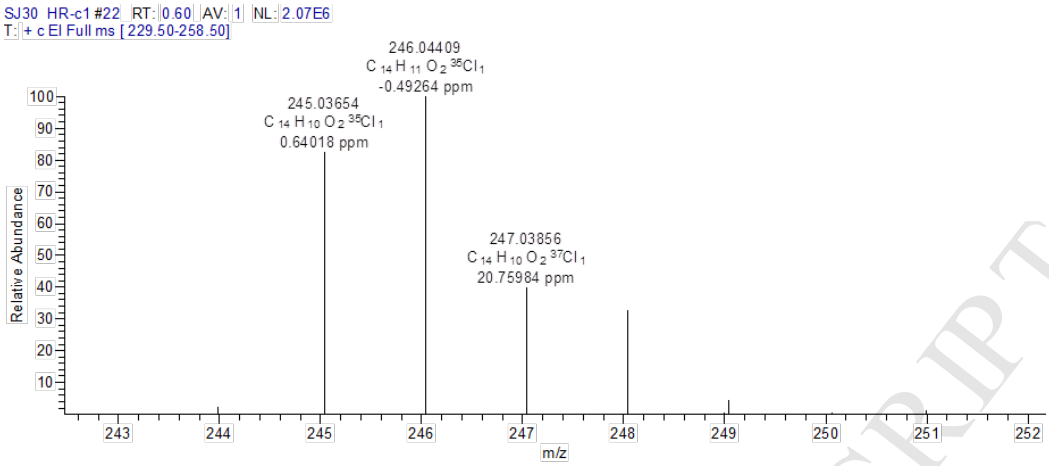
(2*E*)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7e**)



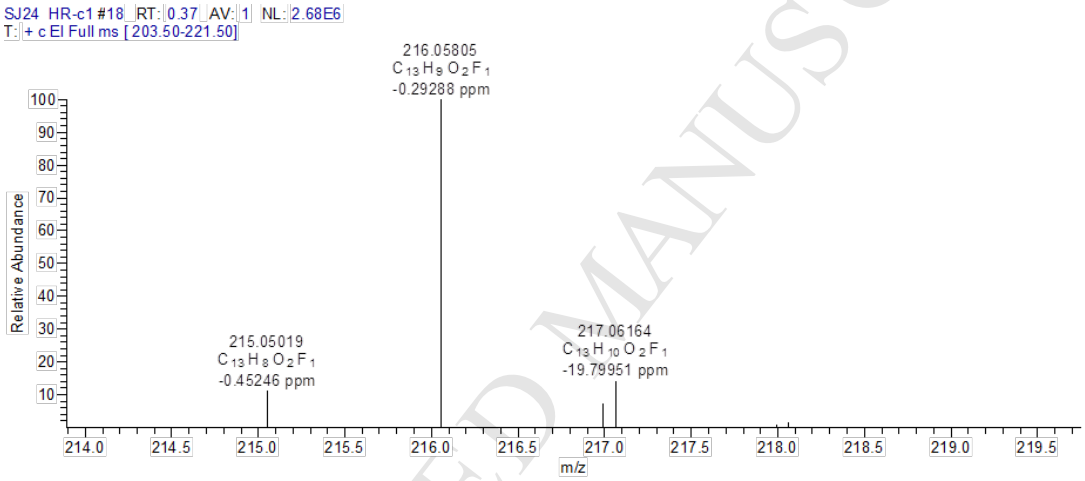
(2*E*)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7f**)



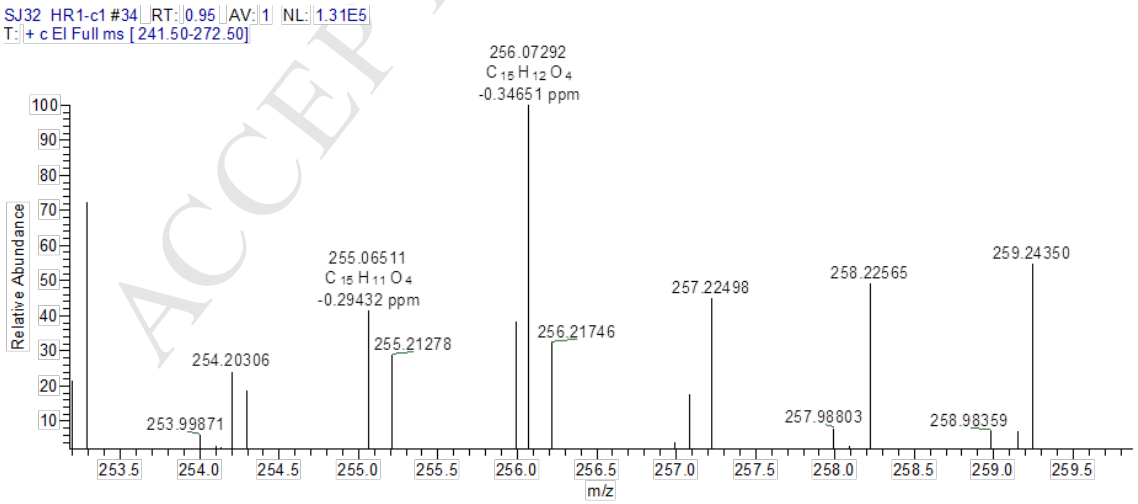
(2*E*)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7g**)



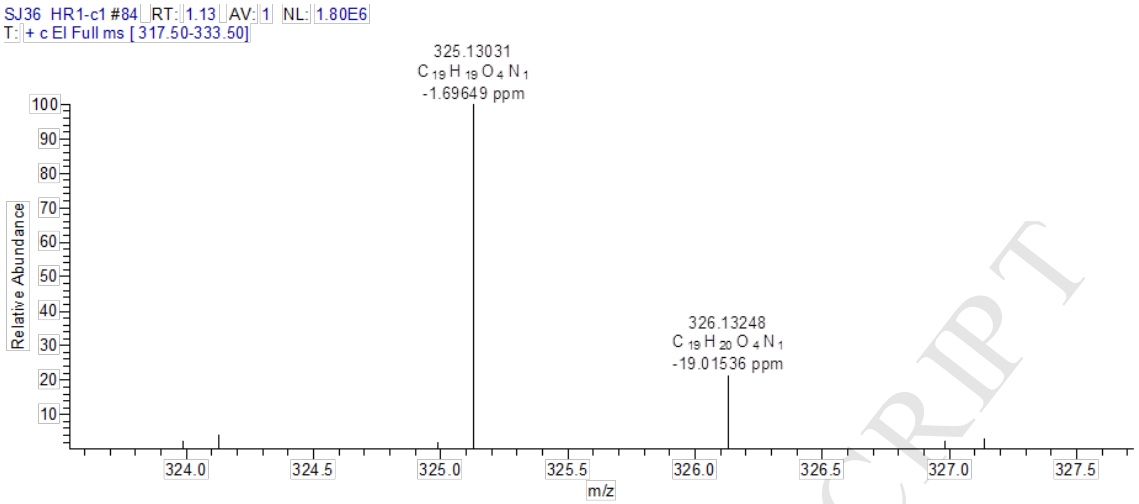
(2*E*)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (**7h**)



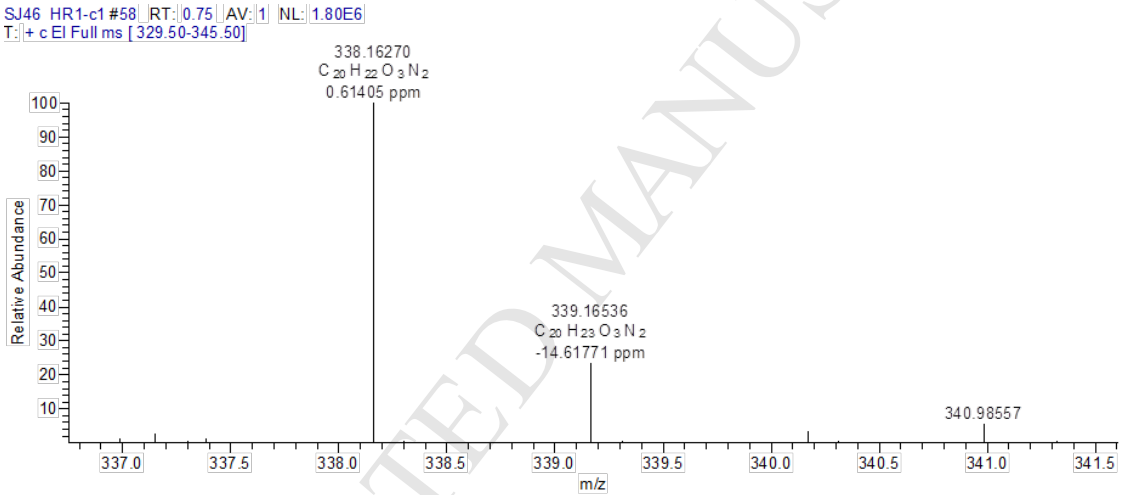
3-[(1*E*)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (7**i**)



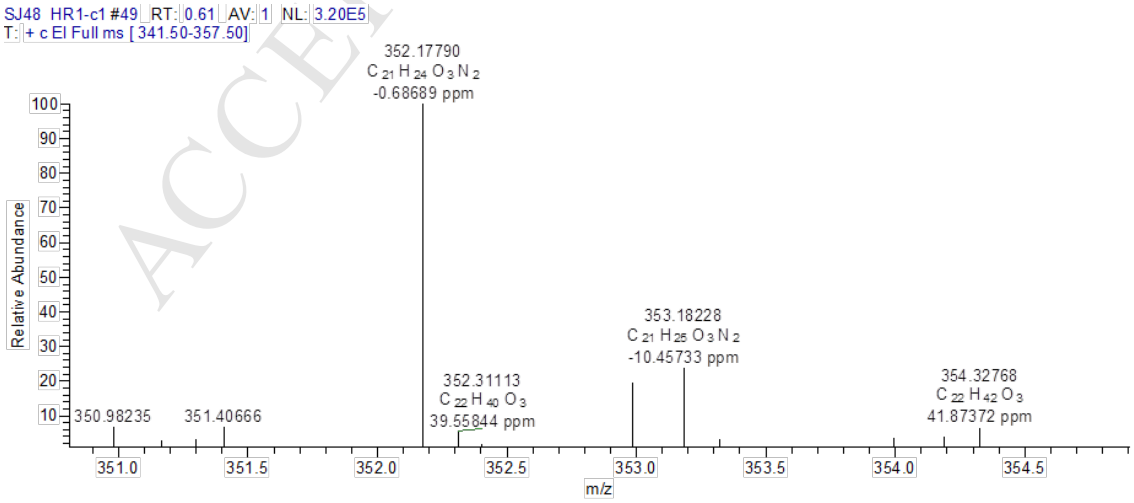
(2*E*)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (**7j**)



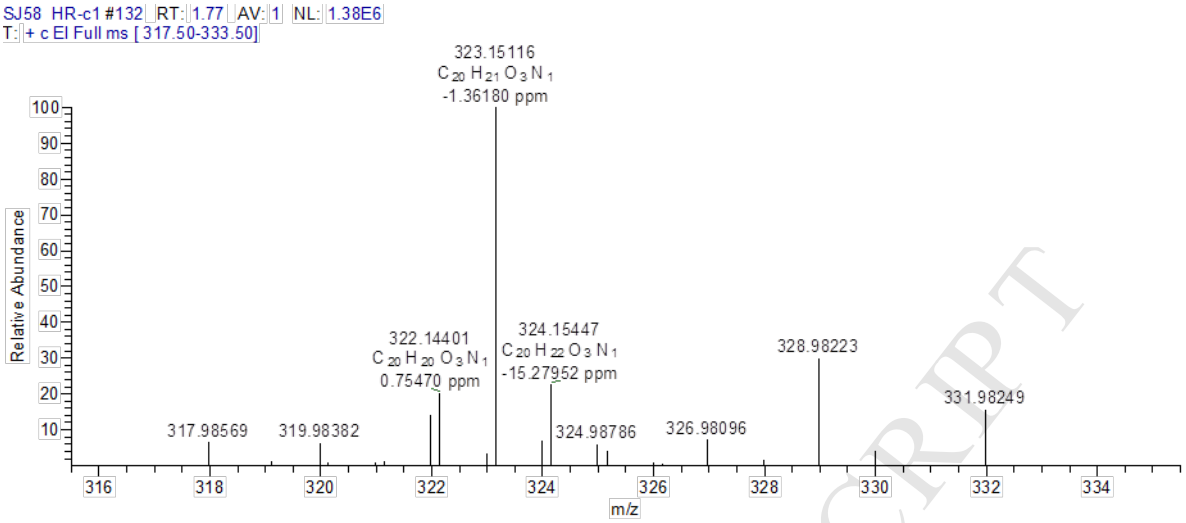
(2*E*)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (**7k**)



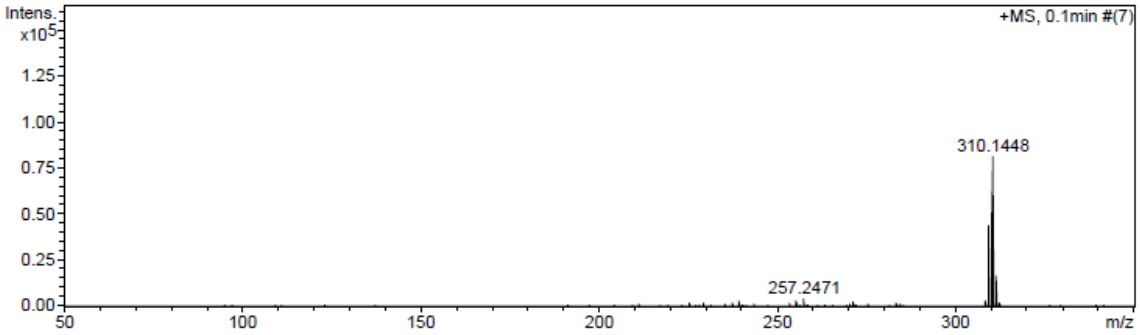
(2*E*)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (7**l**)



(2*E*)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (**7m**)

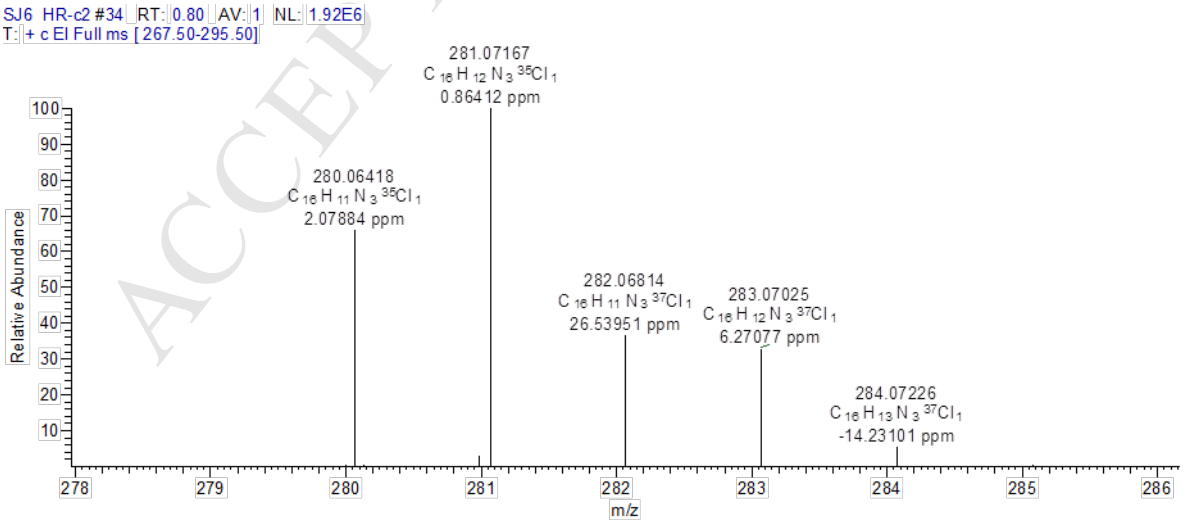


(2*E*)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (**7n**)

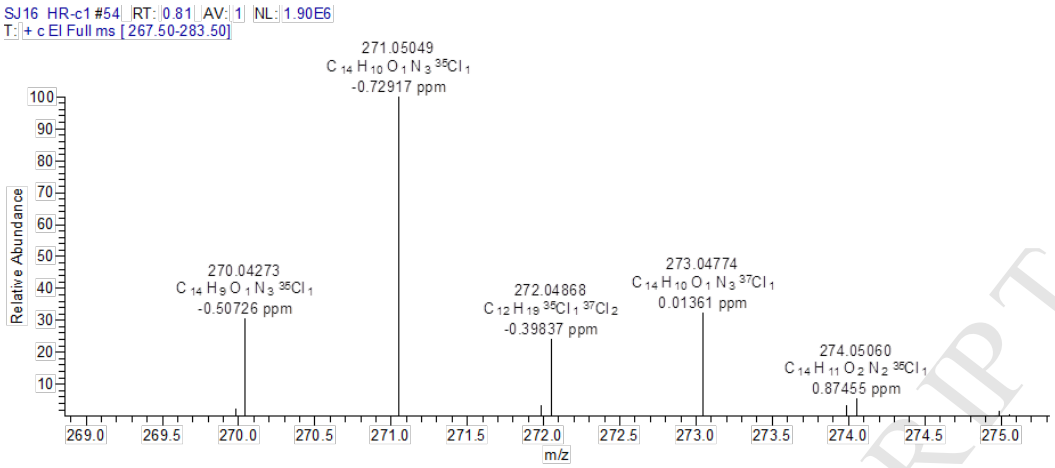


Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
310.1448	1	C 19 H 20 N O 3	100.00	310.1438	-1.0	-3.4	4.9	10.5	even	ok
	2	C 15 H 16 N 7 O	9.62	310.1411	-3.7	-12.0	8.8	11.5	even	ok

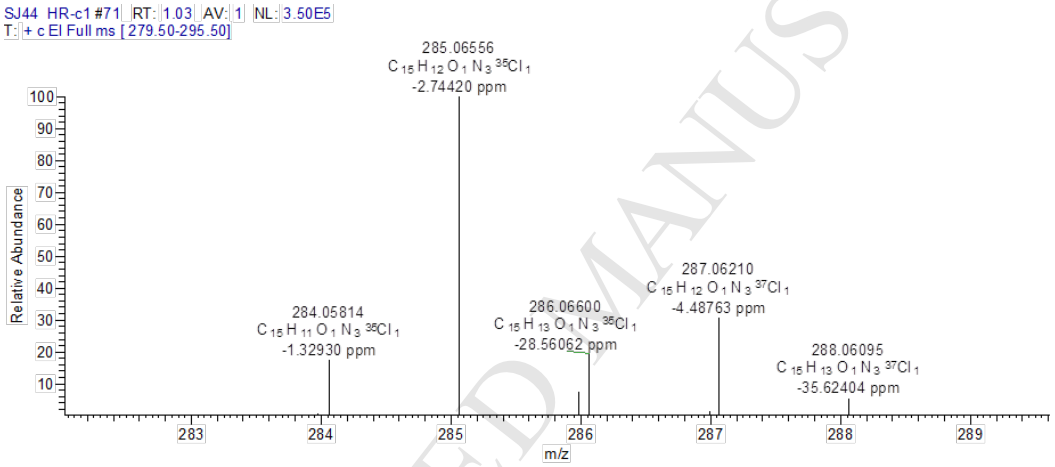
4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (**8a**)



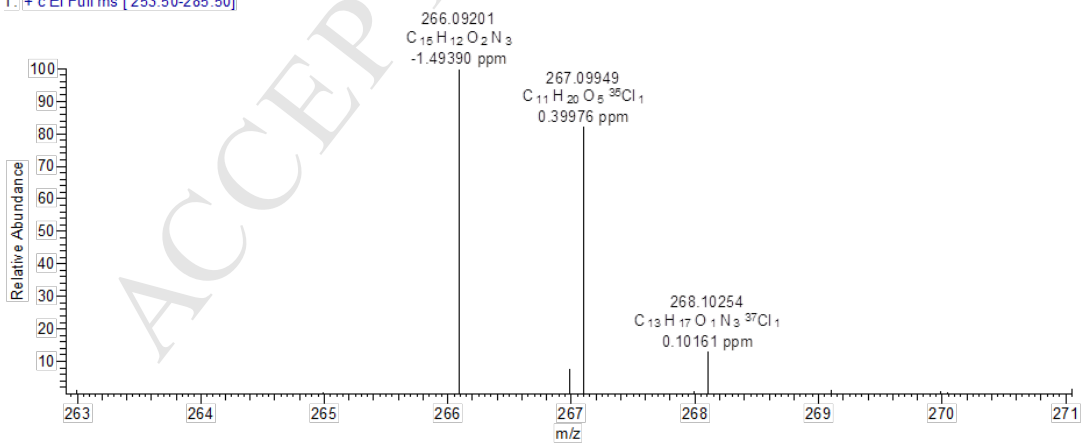
4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8b**)



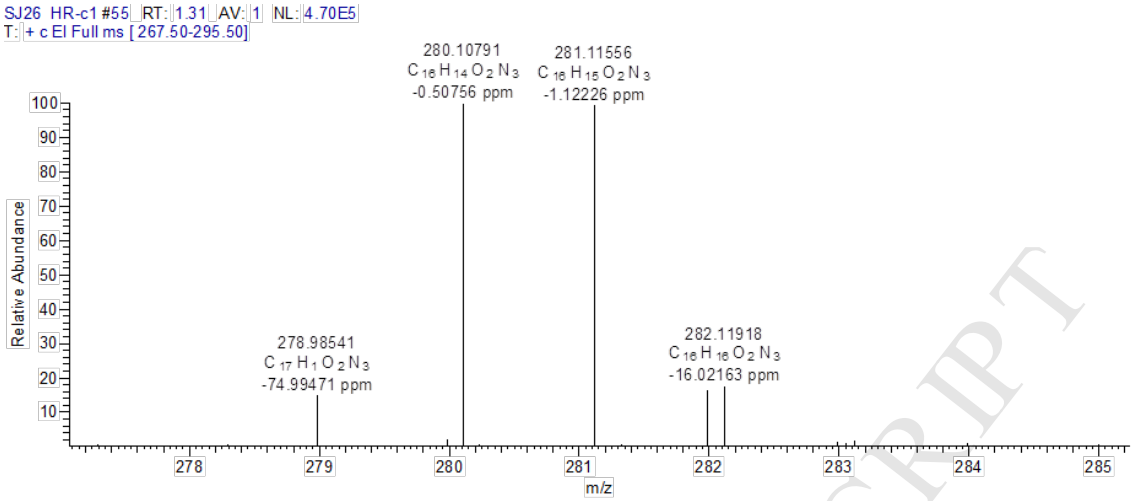
4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8c**)



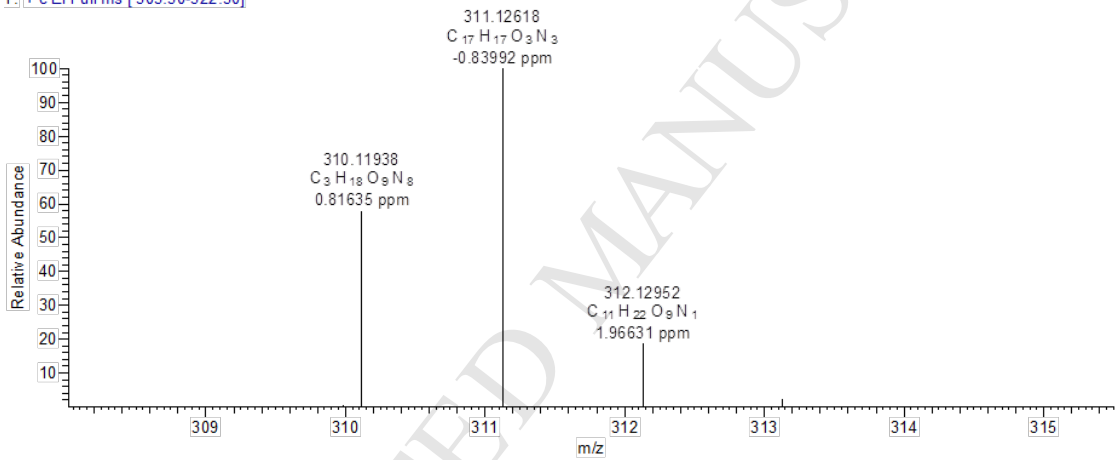
4-(3-methoxyphenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8d**)



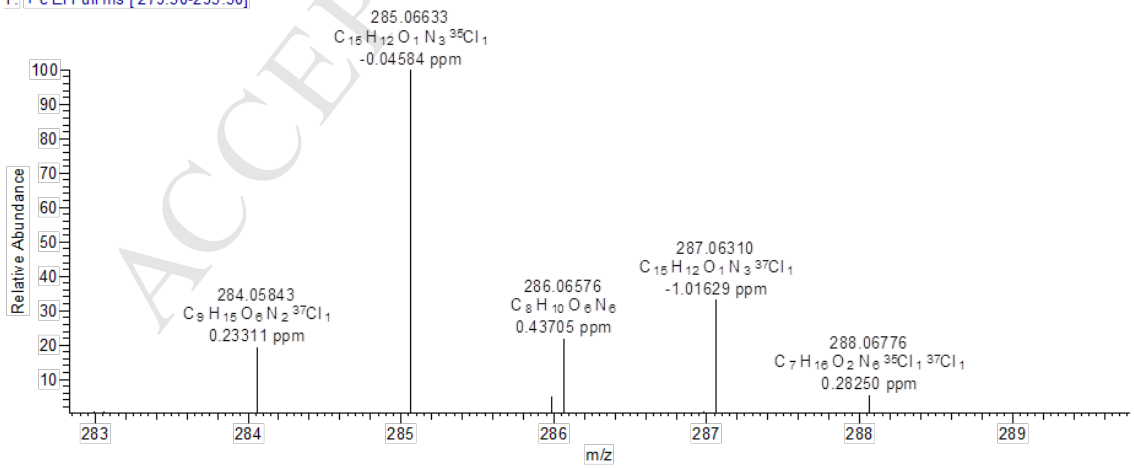
4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8e**)



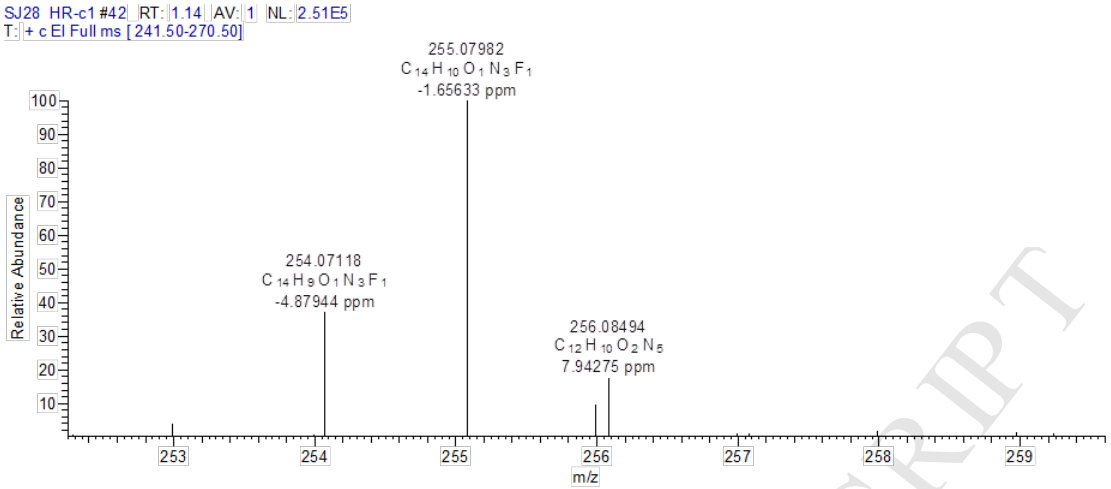
4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8f**)



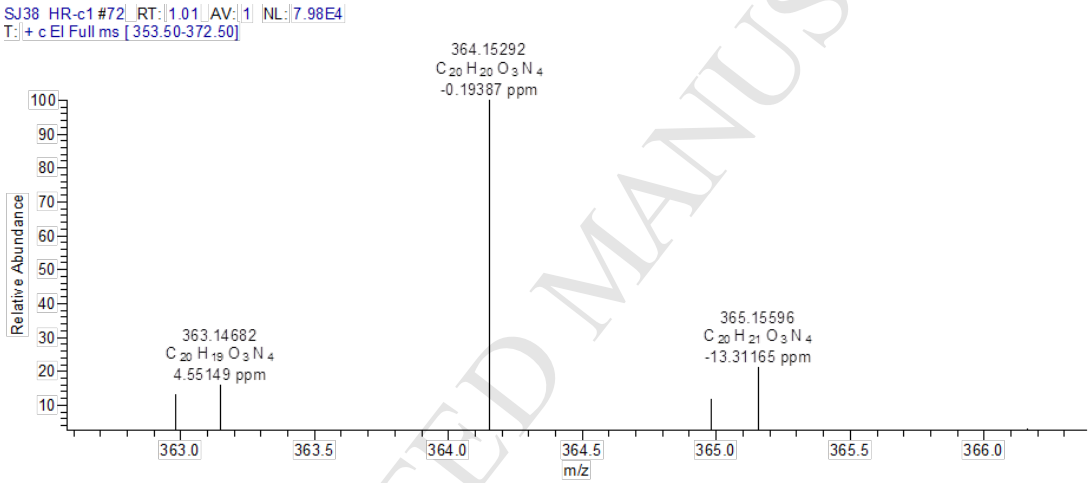
4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8g**)



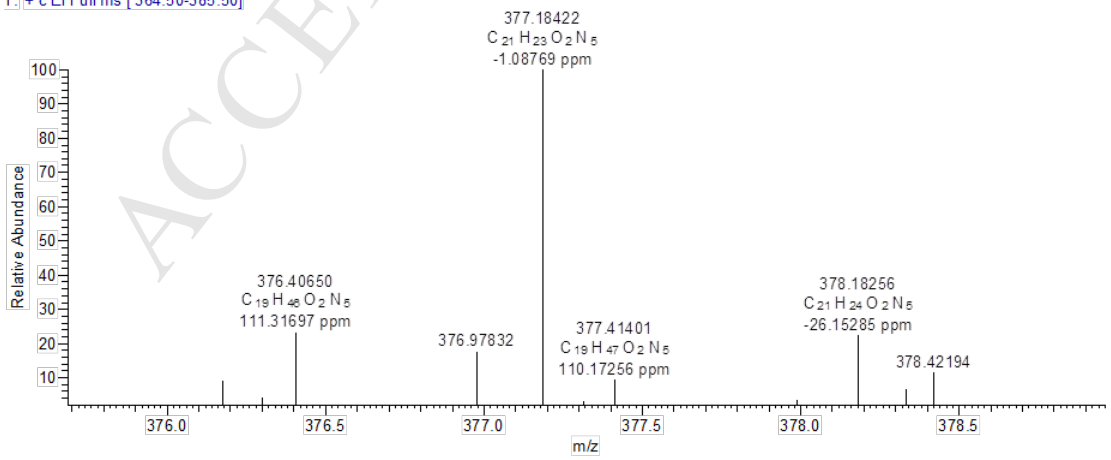
4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8h**)



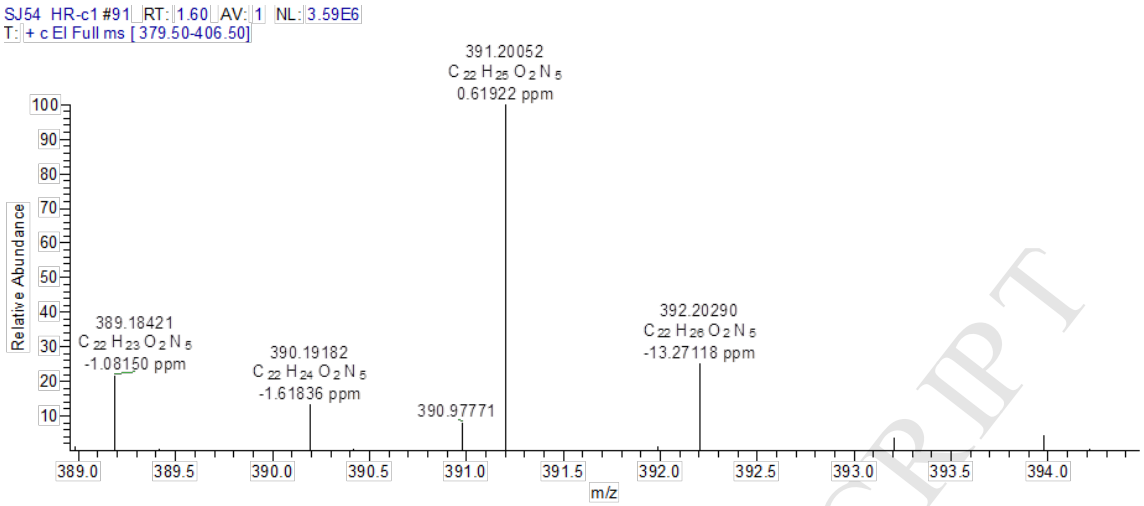
4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (**8j**)



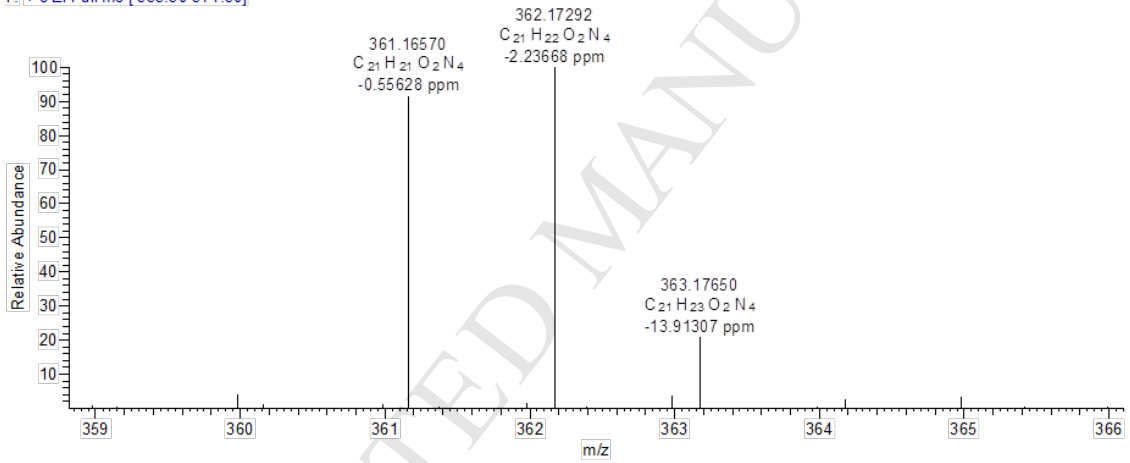
4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8k**)



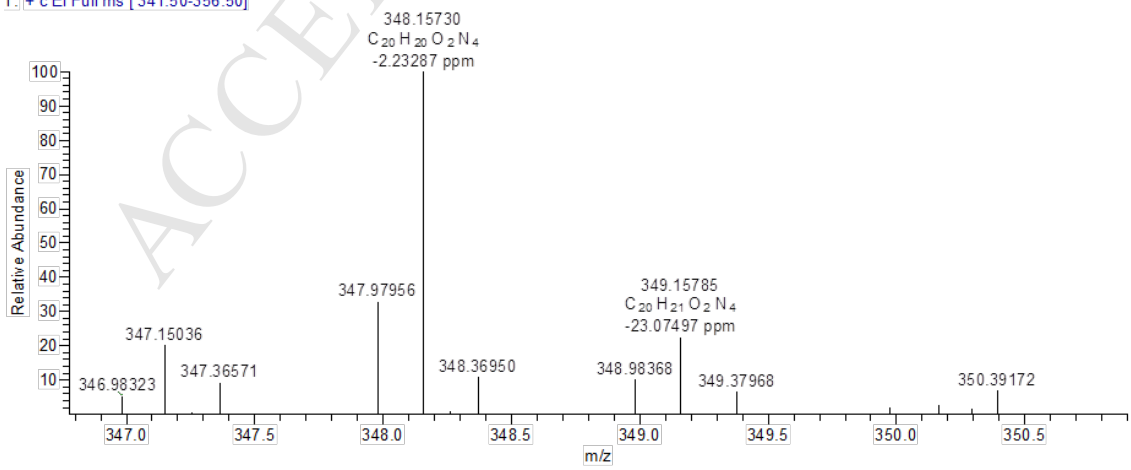
4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8l**)



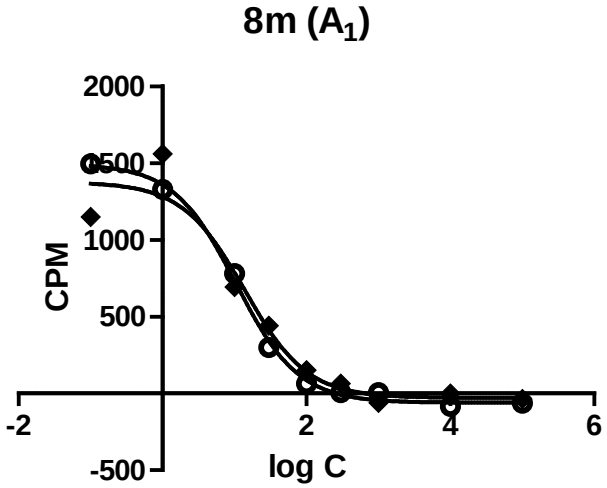
4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**)



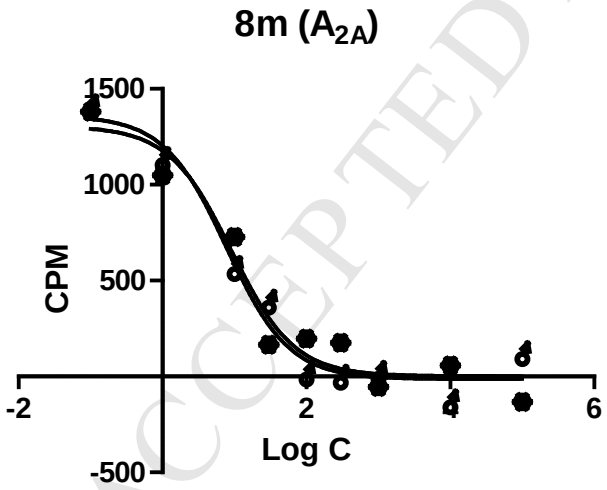
4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8n**)



Examples of sigmoidal dose response curves



Sigmoidal dose response curve obtained during the determination of A_1 affinity of compound **8m** in the radioligand binding assay .



Sigmoidal dose response curve obtained during the determination of A_{2A} affinity of compound **8m** in the radioligand binding assay.