Chapter 4

Synthesis, characterization and evaluation of pyrazolylpiperidine derivatives as anti-platelet agents.

Synthesis and Pharmacological Evaluation of Novel Pyrazolyl Piperidine Derivatives as Effective Antiplatelet Agents

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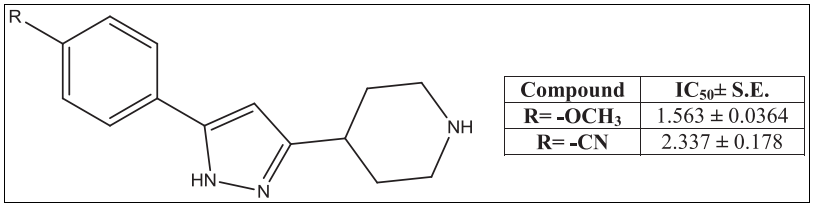

Compound	IC_{50}± S.E.
R= -OCH_3	1.563 ± 0.0364
R= -CN	2.337 ± 0.178

The synthesis and antiplatelet activity of substituted pyrazolyl piperidine derivatives (**3a–n**) are described. These compounds were synthesized by an improved ring opening reaction of 2-arylidene quinuclidinone using hydrazine hydrate under mild conditions. They were characterized and screened for their *in vitro* antiplatelet profile in human platelet aggregation using adenosine diphosphate as agonist. Investigation of structure activity relation revealed interesting results. Among these synthesized derivatives (**3a–n**), compounds **3a**, **3c**, **3j**, and **3l** exhibited excellent activity, while **3c** was the most potent one. Based on IC_{50} values, it was observed that most of the compounds possessed antiplatelet aggregation activity superior to the reference drug Aspirin.

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INTRODUCTION

Atherothrombotic coronary artery disease gives rise to a number of cardiocirculatory disorders such as myocardial infarction, unstable angina, or acute stroke associated with deep vein thrombosis [1]. Hence, it is a growing public health problem and one of the most common causes of death worldwide. The abnormal formation of intravascular occlusions is the main cause of these diseases. Hence, the prevention of thrombogenesis has become a vital target in the prophylaxis and therapy of cardiocirculatory disorders with thromboembolic complications [2]. According to World Health Organization, stroke is responsible for millions of deaths and stroke-related disability [3]. Thrombosis may occur when the hemostatic stimulus becomes unregulated. Important predisposing conditions to thrombosis are disturbed blood flow, hyper coagulation, and altered vessel wall [4]. Arterial thrombi are predominantly composed of platelets, a small amount of fibrin, and a few red blood cells. Because of this, the antiplatelet agents are successfully used in the treatment and prevention of arterial thrombosis. The relevance of antiplatelet drugs has been firmly established by clinical trials and experienced with drugs, such as aspirin, dipyridamole, and thienopyridines [5–7]. These drugs are the only oral antiplatelet agents currently approved by the Food and Drug Administration for use in patients. Recently, antiplatelet combination therapy using agents with different mechanisms of action seems to be an attractive preventive approach, because different signaling pathways contribute to platelet activation [8]. Three classes of antiplatelet agents are currently approved for clinical use and get specific recommendations from clinical guidelines for practical management of patients with acute coronary syndrome or those undergoing percutaneous coronary intervention (Fig. 1): (i) cyclooxygenase-1 inhibitors (aspirin); (ii) glycoprotein IIb/IIIa inhibitors (eptifibatide, abciximab, and tirofiban); and (iii) adenosine diphosphate (ADP) P2Y12 receptor antagonists (ticlopidine, clopidogrel, ticagrelor, and prasugrel) [9].

Clinical studies have demonstrated their efficacy in the minimizing ischemic recurrences and prevention of thromboembolic disease, but these are accompanied by side effects such as gastrointestinal toxicity because of aspirin including nausea, vomiting, dyspepsia, heartburn, gastrointestinal ulceration, and so on. In recent years, the issue of resistance to antiplatelet agents, in particular, aspirin and thienopyridines, has been highlighted in the medical literature [10–13].

In short, the treatment of acute coronary syndrome and its complications, especially the clinical management of aspirin and clopidogrel resistance, still demand much attention [14]. Novel effective antiplatelet agents with fast initiation of action and low risks of bleeding are still needed. In search of newer more potent antiplatelet agents with high activity and minimum side effects, some novel substituted

4.1 Introduction

Formation in a blood vessel of a thrombus that breaks and is carried by the blood stream to plug another vessel. Blood fluidity maintenance within the vascular systems is an important human physiological process.[1] In ideal conditions, there is an fine equilibrium between pathological states of hypocoagulability (thrombelastography) and hypercoagulability. Hypercoagulability is caused by, genetic deficiencies or autoimmune disorders. The clot may plug a vessel in the lungs, brain, gastrointestinal tract, kidneys, or leg. Thromboembolism is a significant cause of morbidity (disease) and mortality (death), especially in adults.[2] In the 1800s, Rudolf Virchow suggested a triad of causes for thrombosis formation: changes in the composition of blood, alterations in the vessel wall, and disruption of blood flow.[3] Abnormal blood clotting (thrombosis) is the major cause of death in the World and a leading cause of morbidity, with an annual incidence of about 1 case per 1,000 individuals. Nearly 1 million individuals die from thrombosis in the World each year, in contrast to 500,000 deaths from cancer. There are two distinct forms of thrombosis, Venous Thrombosis and Arterial Thrombosis, each of which can be presented by several subtypes. Venous thrombosis is the formation of a thrombus (blood clot) within a vein. There are several diseases which can be classified under this category: Deep vein thrombosis, Portal vein thrombosis, Renal vein thrombosis, Jugular vein thrombosis, Budd-Chiari syndrome, and Cavernous sinus thrombosis. Arterial thrombosis is the formation of a thrombus within an artery. In most cases, arterial thrombosis follows rupture of atheroma, and is therefore referred to as atherothrombosis. Another common cause of arterial occlusion is atrial fibrillation, which causes a blood stasis within the atria with

easy thrombus formation. Arterial thrombosis can embolize and is a major cause of arterial embolism, potentially causing infarction of almost any organ in the body.

In human physiological system maintenance of blood fluidity in the vasculature is essential. There is an equilibrium between pathological states of hypercoagulability and hypocoagulability and under common conditions.

Hemostasis is a process encompassing the various mechanisms that stop the bleeding when the vascular wall is ruptured.[1] A number of factors including the endothelial wall, the protein of coagulation cascade and fibrinolysis plays important roles in this function.[4] The coagulation phase also known as secondary hemostasis allows consolidation of the platelet clot by formation of fibrin clot.

Atherothrombotic coronary artery disease gives rise to a number of cardiocirculatory disorders such as myocardial infarction (MI), unstable angina (UA), or acute stroke associated with deep vein thrombosis (DVT).[5] Hence it is a growing public health problem and one of the most common causes of death worldwide. The abnormal formation of intravascular occlusions is the main cause of these diseases. Hence the prevention of thrombogenesis has become a vital target in the prophylaxis and therapy of cardiocirculatory disorders with thromboembolic complications.[6] According to World Health Organization (WHO) stroke is responsible for millions of deaths and stroke related disability.[7] Thrombosis may occur when the haemostatic stimulus becomes unregulated. Important predisposing conditions to thrombosis are disturbed blood flow, hyper coagulation and altered vessel wall.[8] Arterial thrombi are predominantly composed of platelets, a small amount of fibrin and a few red blood cells. Because of this, the antiplatelet agents are successfully used in the treatment and prevention of arterial thrombosis. The relevance of antiplatelet drugs has been firmly

established by clinical trials and experience with drugs, such as aspirin, dipyridamole and thienopyridines.[9,10,11] These drugs are the only oral antiplatelet agents currently approved by the FDA (Food and Drug Administration) for use in patients. Recently, antiplatelet combination therapy using agents with different mechanisms of action seems to be an attractive preventive approach, since different signaling pathways contribute to platelet activation.[12] Three classes of antiplatelet agents are currently approved for clinical use and get specific recommendations from clinical guidelines for practical management of patientswith acute coronary syndrome (ACS) or those undergoing PCI (Figure 1): (i) cyclooxygenase-1 (COX-1) inhibitors (aspirin); (ii) glycoprotein IIb/IIIa inhibitors (eptifibatide, abciximab, tirofiban); and (iii) adenosine diphosphate (ADP) P2Y12 receptor antagonists (ticlopidine, clopidogrel, ticagrelor, prasugrel)[13]

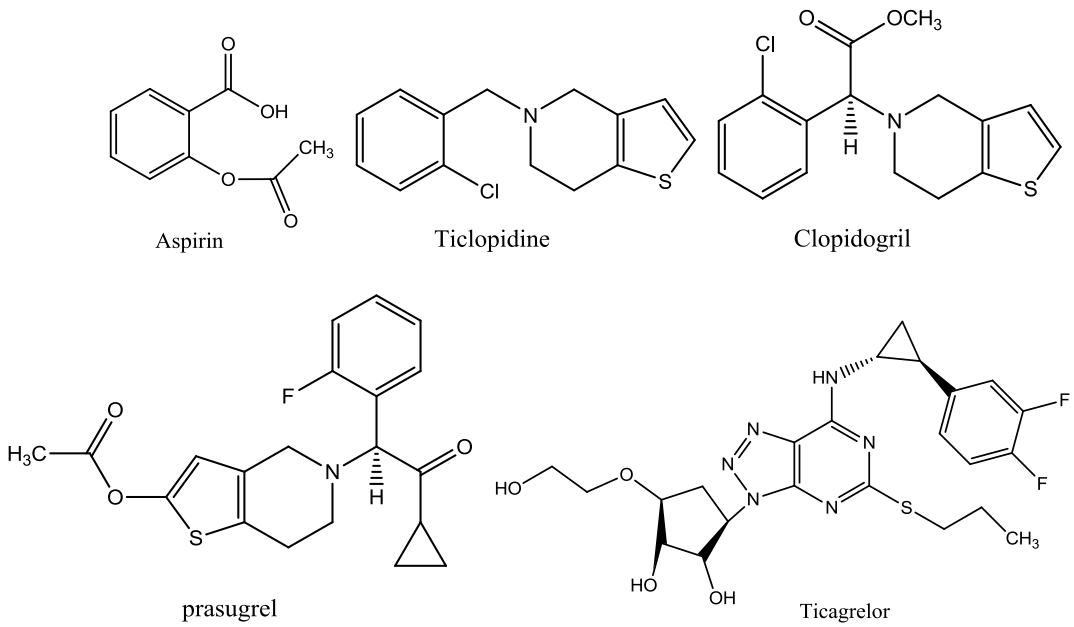


Figure 1: Structures of currently available antiplatelet drugs

Clinical studies have demonstrated their efficacy in the minimizing ischemic recurrences and prevention of thromboembolic disease, but these are accompanied by

side effects such as gastrointestinal toxicity due to aspirin including nausea, vomiting, dyspepsia, heartburn, gastrointestinal ulceration etc. In recent years, the issue of resistance to antiplatelet agents, in particular aspirin and thienopyridines, has been highlighted in the medical literature.[14,15,16,17]

In short, the treatment of ACS and its complications, especially the clinical management of aspirin and clopidogrel resistance still demand a lot of attention. Novel effective antiplatelet agents with fast initiation of action and low risks of bleeding are still needed. In search of newer more potent antiplatelet agents with high activity and minimum side effects, some novel substituted pyrazolyl piperidine derivatives were screened antiplatelet activity.[18]

Quinuclidinone Hydrochloride is important building block of many FDA approved drugs like Solifenacin, Azasetron, Quinupramine etc. and possesses wide spectrum of biological activity. Its analogues are known for α7 and α4β2 nicotinic receptors inhibitory activity,[19] Alzheimer's disease,[20] Antihistamine-Bronchodilating Agents.[21] Therefore, we focused our attention on the synthesis of some pyrazolyl piperidine derivatives from quinuclidinone hydrochloride with a view to evaluate their antiplatelet activity.

4.1.1 Platelet activation mechanism

Platelets play a critical role in the normal coagulation system by "preventing" bleeding after blood vessels are damaged. In addition they contribute to different phases of the atherosclerotic process.[22] Rupture of a previously formed atherosclerotic plaque exposes collagen, smooth muscle cells and von Willebrand factor (vWF) all of which trigger platelet activation and massive aggregation.[23] The result of this accumulation of platelets is thrombosis. Acute coronary syndrome (ACS) is a

consequence of the occlusion of an atherosclerotic vessel by the thrombotic process. As described before, collagen and vWF in addition to thromboxane A2 (TXa2), thrombin and adenosine diphosphate (ADP) are the most powerful platelet activators.[24] When a platelet is activated a conformational change occurs in a receptor located in the platelet membrane called glycoprotein IIb/IIIa which promotes platelet aggregation[25] Antiplatelet agents that target critical steps of the thrombotic mechanism described above have been developed in the last three decades. However, treatment with these agents can sometimes increase the risk of "undesirable" bleeding complications.[26]

4.1.2 Hemostasis

Hemostasis, the arrest of bleeding from an injured blood vessel, requires the combined activity of vascular, platelet, and plasma factors. Regulatory mechanisms counterbalance the tendency of clots to form. Hemostatic abnormalities can lead to excessive bleeding or thrombosis. Vascular factors reduce blood loss from trauma through local vasoconstriction (an immediate reaction to injury) and compression of injured vessels by extravasation of blood into surrounding tissues. Platelets provide surfaces for the assembly and activation of coagulation complexes and the generation of thrombin.[27] Thrombin converts fibrinogen to fibrin. Fibrin strands bind aggregated platelets to help secure the platelet-fibrin hemostatic plug. Plasma coagulation factors interact to produce thrombin, which converts fibrinogen to fibrin. Radiating from and anchoring the hemostatic plug, fibrin strengthens the clot.

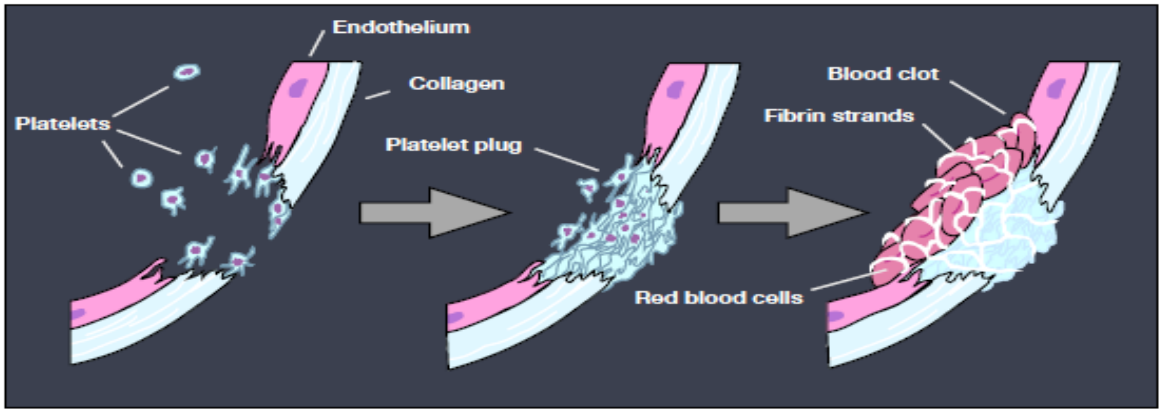


Figure 2: platelet forming platelet plug to stop blood flow.

4.1.3 Platelet activation

Platelets bud from mega karyocytes in the bone marrow, normally circulate about 10 days in the blood. Since they are fragments of cells, they have no nucleus. But platelets do have most other cellular organelles, including microtubules, which help hold the unactivated platelet in a nice, crisp discoid shape. When platelets are circulating through vessels with an intact, healthy endothelium, the platelets remain in their original, unactivated state. The absence of activating factors and the release of prostacyclin (prostaglandin I2) by the healthy endothelium supports this state. **(Figure 2)** However, when a platelet encounters a break in the endothelium, it encounters molecules that trigger its activation. One such molecule is collagen, which is characteristically found almost everywhere except inside a blood vessel. In addition, thromboxane A2, ADP and thrombin are other factors that trigger the same activation. The following are some of the main things that happen as a platelet is activated:

- Exocytosis of the dense granules and alpha granules.
- Activation of the membrane enzyme phospholipase A2. This leads to the formation of thromboxane A2 (TXA2)

- Change in shape to a more amorphous form with projecting fingers.
- Platelets adhere to one another and to collagen under the endothelium, forming a platelet plug. On the surface of the activated platelet are vWF receptors and glycoprotein IIb/IIIa. The latter is a receptor which binds fibrinogen. The binding of vWF by the vWF receptor causes the activated platelets to adhere to collagen under the broken endothelium. The binding of fibrinogen by glycoprotein IIb/IIa causes platelets to adhere to each other. The fibrinogen that connects two platelets via these receptors is found in the blood and also a little is released from the alpha granules.
- Coagulation reactions are promoted at the surface.

4.1.4 Coagulation cascade

Coagulation is the process by which blood changes from a liquid to a gel. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The coagulation process that leads to haemostasis involves a complex set of protease reactions involving approximately 30 different proteins. These reactions convert fibrinogen, a soluble protein, to insoluble strands of fibrin, which, together with platelets, form a stable thrombus.[28]

The coagulation cascade of secondary hemostasis has two initial pathways including the intrinsic and extrinsic pathway model and the more recent cell-based model, which lead to fibrin formation. Two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its

glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin.

Damage to blood vessel walls exposes Tissue Factor-containing cells from underlying cell layers to the bloodstream. Tissue Factor (TF) is then able to bind in the presence of calcium to Factor VII (FVII), which circulates at low levels in the bloodstream, the calcium forming a bridge between TF and FVII.[28] This sets off an extracellular cascade involving sequential serine protease activations: TF/FVII is activated by auto-cleavage to TF/FVIIa. **(Figure 3)** Along with FVIIIa (cofactor) converts FIX to FIXa, which converts FX to FXa (although TF/FVIIa can also directly convert FX to FXa), which along with FVa (cofactor) converts FII (prothrombin) to FIIa (thrombin), which converts fibrinogen to fibrin, leading to fibrin deposition and the activation of platelets to form blood clots (the activation of FXIII to FXIIIa stabilises the fibrin clot by cross-linking it)[29]

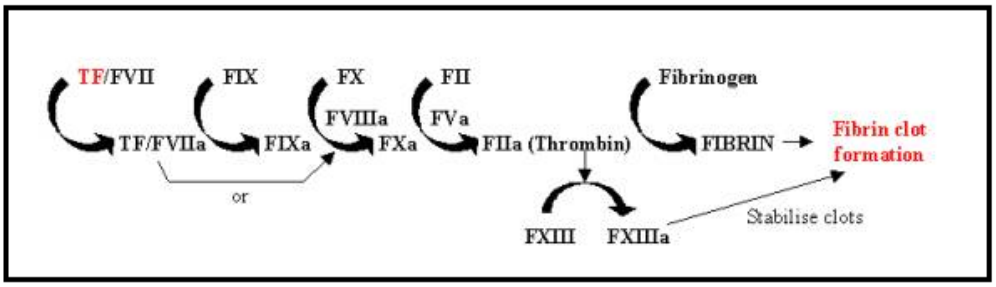


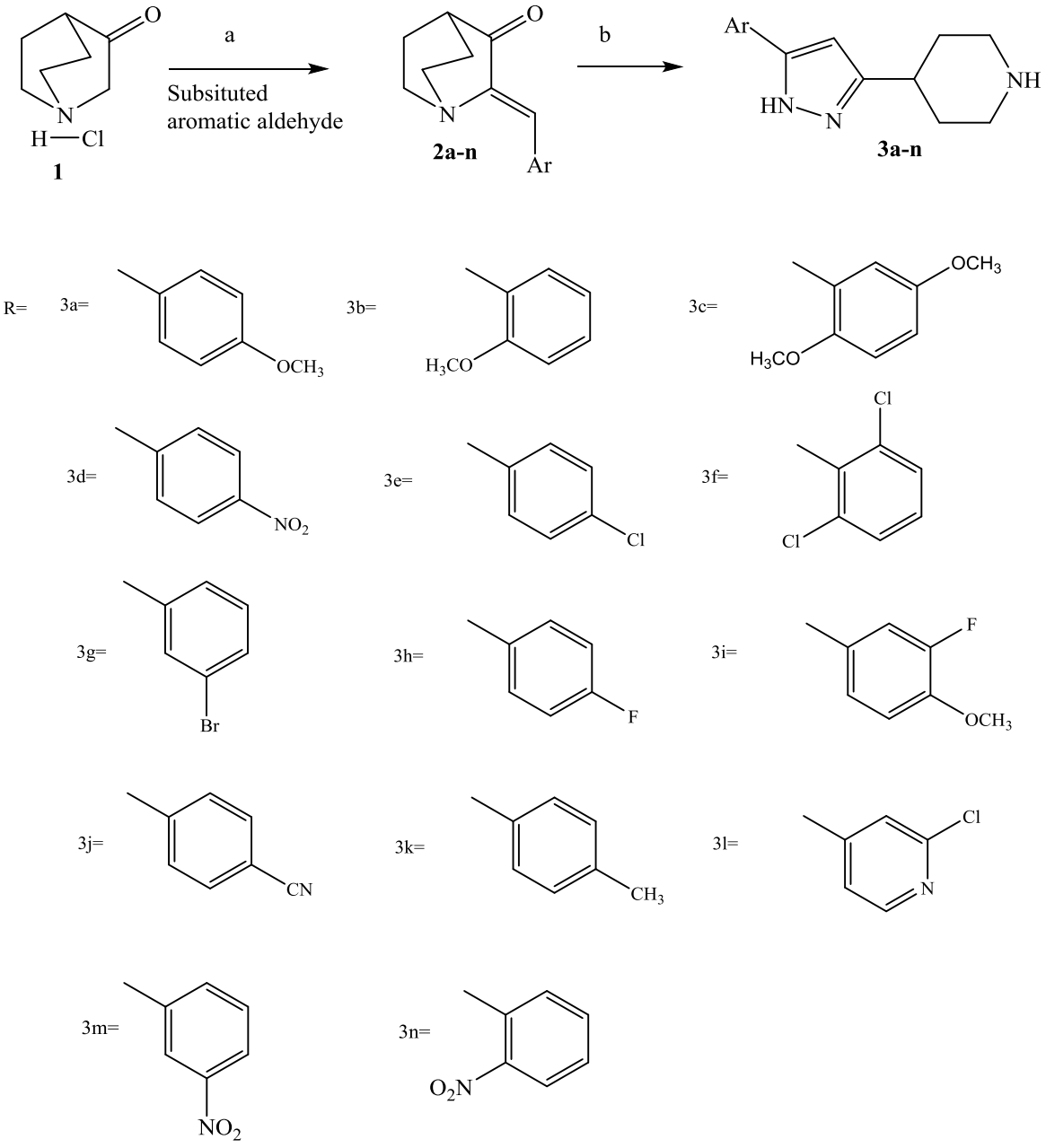
Figure 3: Coagulation cascade

4.2 Result and discussion

4.2.1 Chemistry

In the synthesis of designed compounds from 3-quinuclidinone hydrochloride **1** which was prepared as per the procedure described in our previous report.[30,31] It was converted into compound **2a-n** with various substituted aromatic aldehydes in the

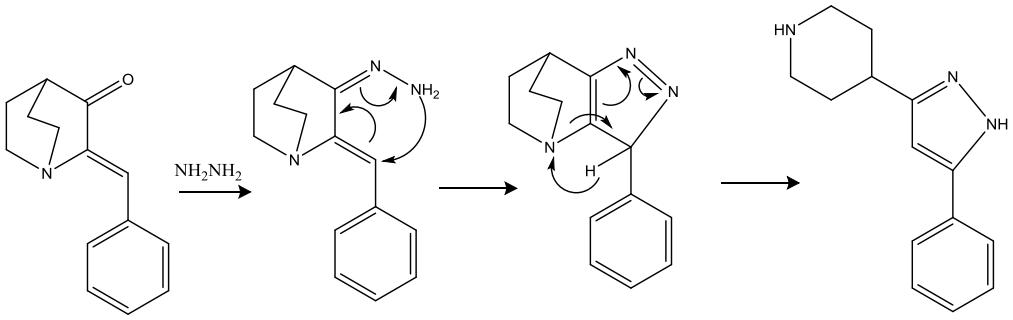
presence of sodium hydroxide in absolute ethanol as solvent as shown in **Scheme 1**. Finally, the new pyrazolyl piperidine compounds **3a-n** were prepared by improved method in good yields by refluxing **2a-n** with excess of hydrazine hydrate.



Scheme 1: Synthetic route for compounds 2a-n and 3a-n

Reagent and conditions: (a) substituted aromatic aldehyde, NaOH, EtOH, reflux (b) hydrazine hydrate, reflux.

This process is more efficient in terms of reaction time and conditions compared to the reported process.[32] The later uses KOH along with ethylene glycol and involves high temperature. The work up and isolation procedures are also very tedious involving prolonged heating at high temperatures. Instead, our method offers easy isolation of product with good yield. It is also more suitable for thermally labile compounds. The probable mechanism of ring opening with hydrazine hydrate has been shown in Scheme 2



Scheme 2: Plausible mechanism of ring opening in presence of hydrazine hydrate

The IR spectrum of compound **3a** (Figure 4) exhibited bands at 3071 and 3005 cm^{-1} indicated presence of methylene group respectively. In ^{1}H NMR of compound **3a** (Figure 5) multiplets at δ 1.66, 1.72, 2.73, 2.81 and 3.19 for two, two, two, one and two protons respectively confirmed the methylene protons of piperidine ring. Singlet at δ 3.85 for three protons indicated presence of methoxy group. All pyrazole and aromatic protons observed between δ 6.32 to 7.64 confirmed the formation of compound **3a**. The ^{13}C NMR spectrum of compound **3a** (Figure 6) showed 8 peaks is in accordance with structure of compound **3a**. The mass spectrum of compound **3a** (Figure 7) showed *m/z* value at 258.2 $[M+1]^{+}$ in ESI/MS confirmed formation of **3a**.

The IR spectrum of compound **3c** (Figure 9) exhibited bands at 3298, 2942 and 2833 cm^{-1} indicated presence of amine and methylene group respectively. In ^{1}H NMR of compound **3c** (Figure 10) multiplets at δ 1.66, 2.02, 2.76, 2.84 and 3.19 for two, two,

two, one and two protons respectively confirmed the methylene protons of piperidine ring. Two singlets at δ 3.84 and 3.94 for three protons each indicated presence of two methoxy group. All pyrazole and aromatic protons observed between δ 6.47 to 7.28 confirmed the formation of compound **3c**. The ^{13}C NMR spectrum of compound **3c** (Figure 11) showed 11 peaks is in accordance with structure of compound **3c**. The mass spectrum of compound **3c** (Figure 12) showed *m/z* value at 288.3 $[M+1]^+$ in ESI/MS confirmed formation of **3c**.

The IR spectrum of compound **3e** (Figure 14) exhibited bands at 3068, 1364 and 1265 cm^{-1} confirmed formation of product. In ^{1}H NMR of compound **3e** (Figure 15) multiplets at δ 1.67, 1.94, 2.67, 2.76 and 3.15 for two, two, two, one and two protons respectively confirmed the methylene protons of piperidine ring. All pyrazole and aromatic protons observed between δ 6.34 to 7.66 confirmed the formation of compound **3e**. The ^{13}C NMR spectrum of compound **3e** (Figure 16) showed 10 peaks is in accordance with structure of compound **3e**. The mass spectrum of compound **3e** (Figure 17) showed *m/z* value at 262.2 $[M+1]^+$ in ESI/MS confirmed formation of **3e**.

The IR spectrum of compound **3k** (Figure 19) exhibited bands at 3248, 3068, 1361 and 1265 cm^{-1} confirmed presence of amine and methylene groups. In ^{1}H NMR of compound **3k** (Figure 20) multiplets at δ 1.68, 1.97, 2.38, 2.69, 2.79 and 3.18 for two, two, three, one, two and two protons respectively confirmed the methylene protons of piperidine ring and methyl group of benzene ring. All pyrazole and aromatic protons observed between δ 6.34 to 7.59 confirmed the formation of compound **3k**. The ^{13}C NMR spectrum of compound **3k** (Figure 21) showed 10 peaks is in accordance with structure of compound **3k**. The mass spectrum of compound **3k** (Figure 22) showed *m/z* value at 242.2 $[M+1]^+$ in ESI/MS confirmed formation of **3k**.

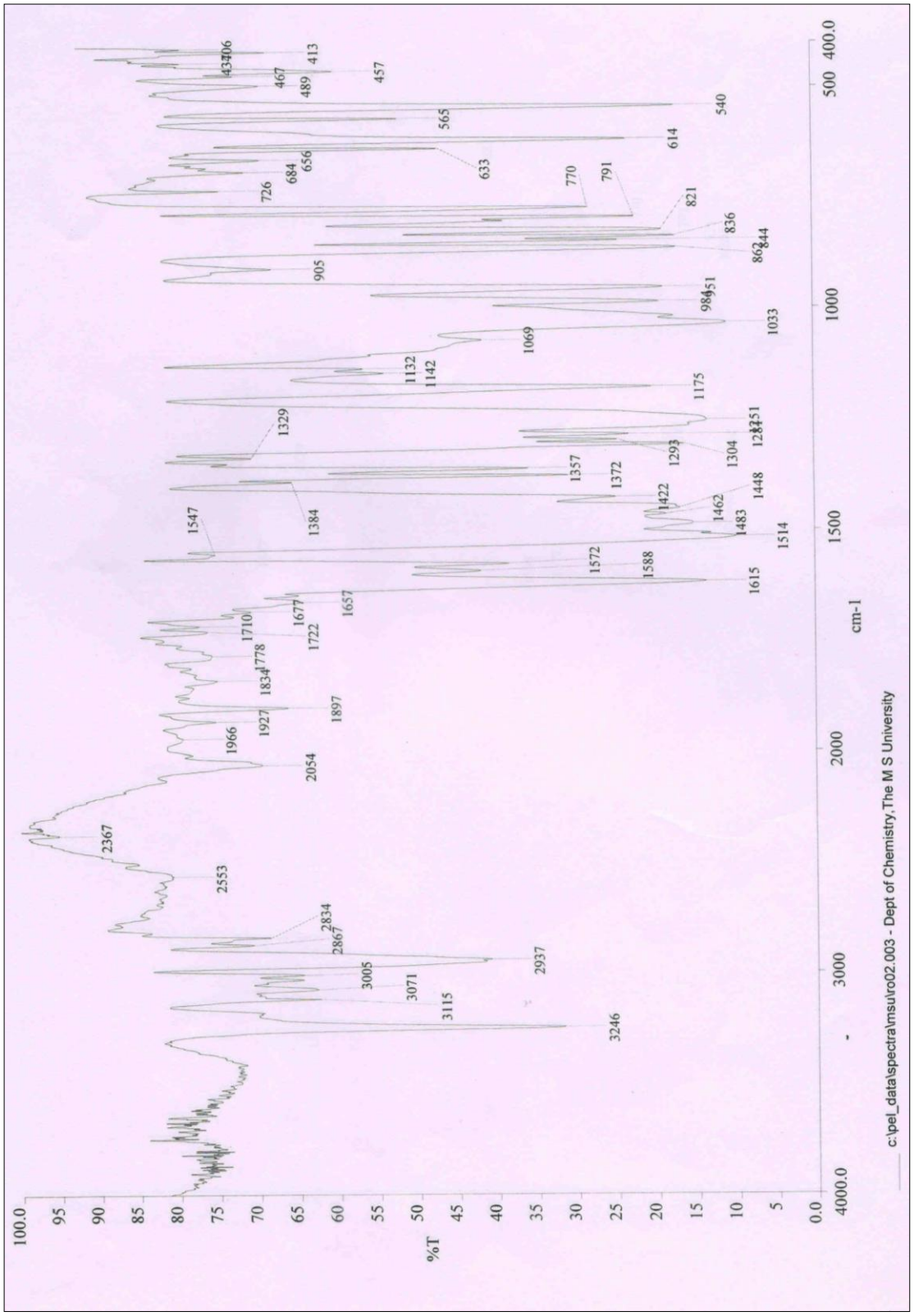


Figure 4: IR spectrum of 4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a

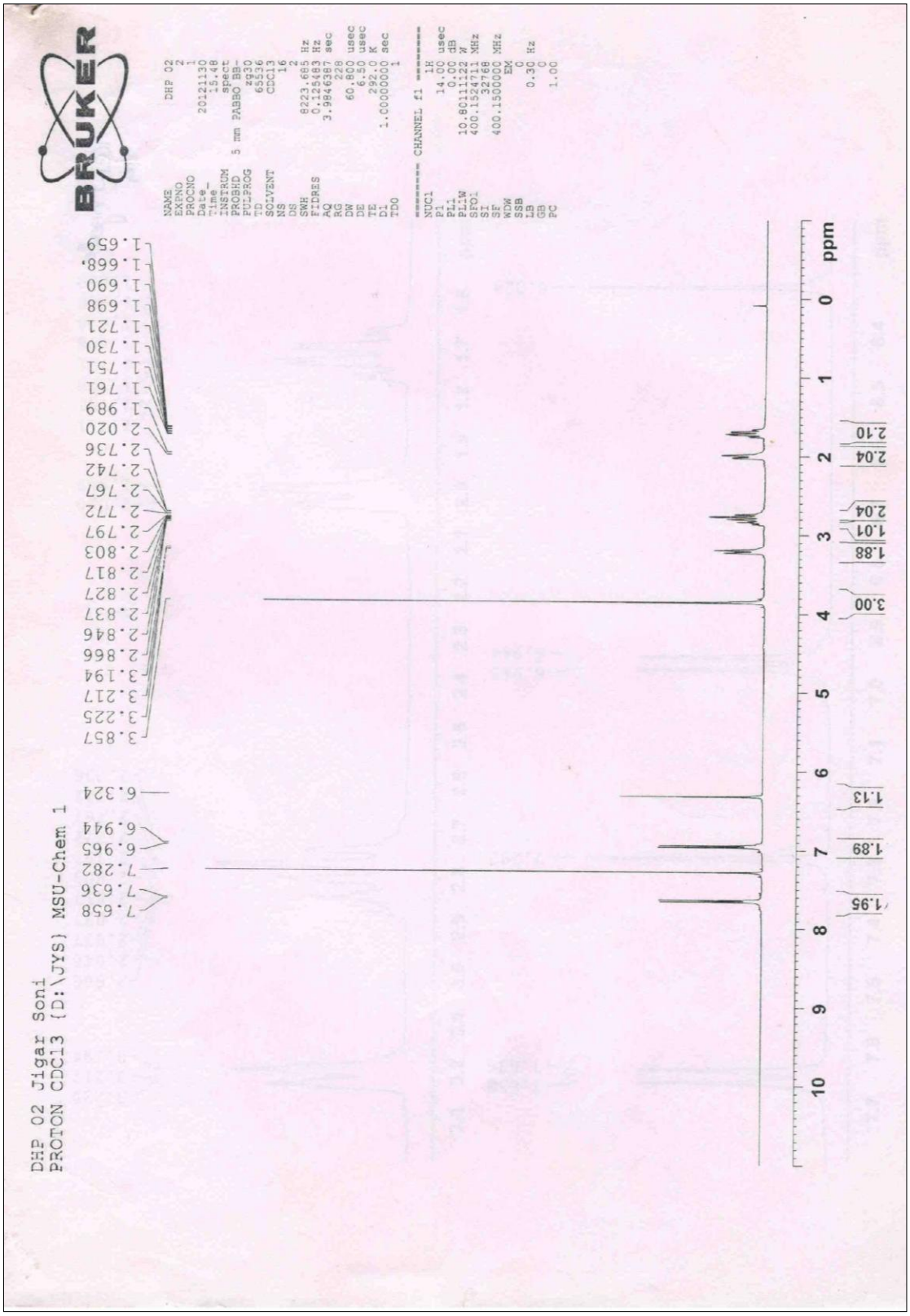


Figure 5: ^{1}H NMR spectrum of 4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a

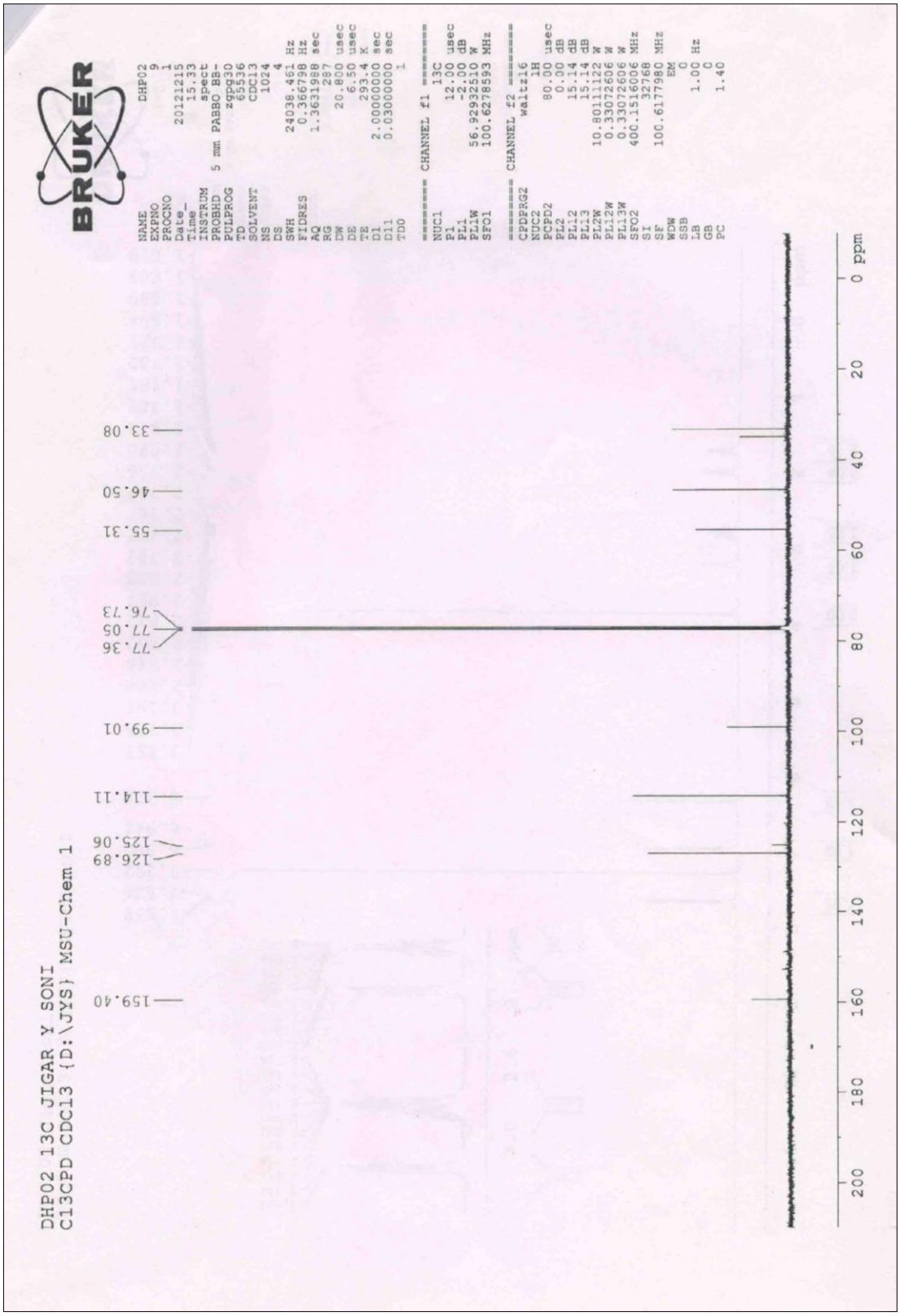


Figure 6: ^{13}C NMR spectrum of 4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a

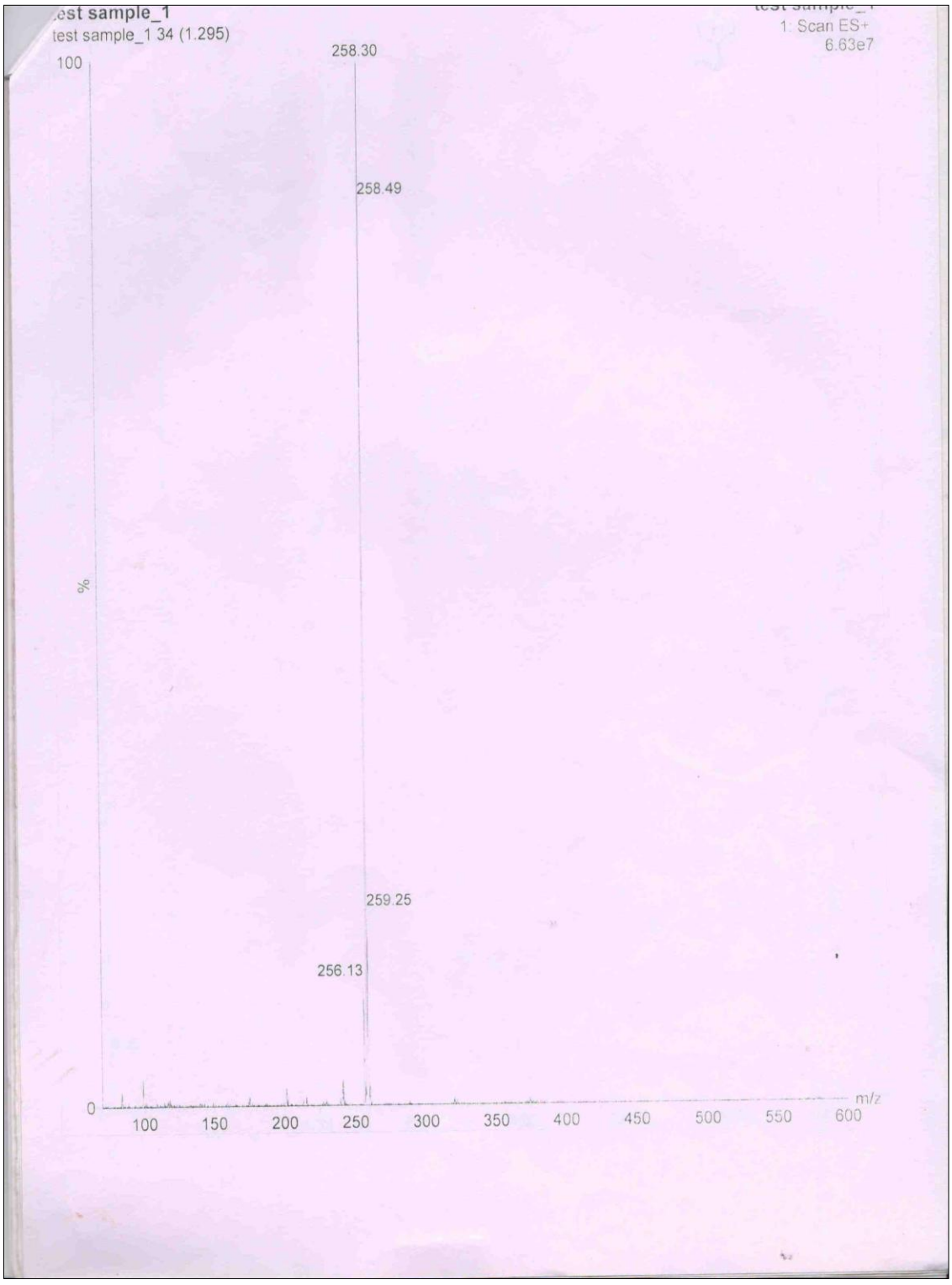


Figure 7: Mass spectrum of 4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a

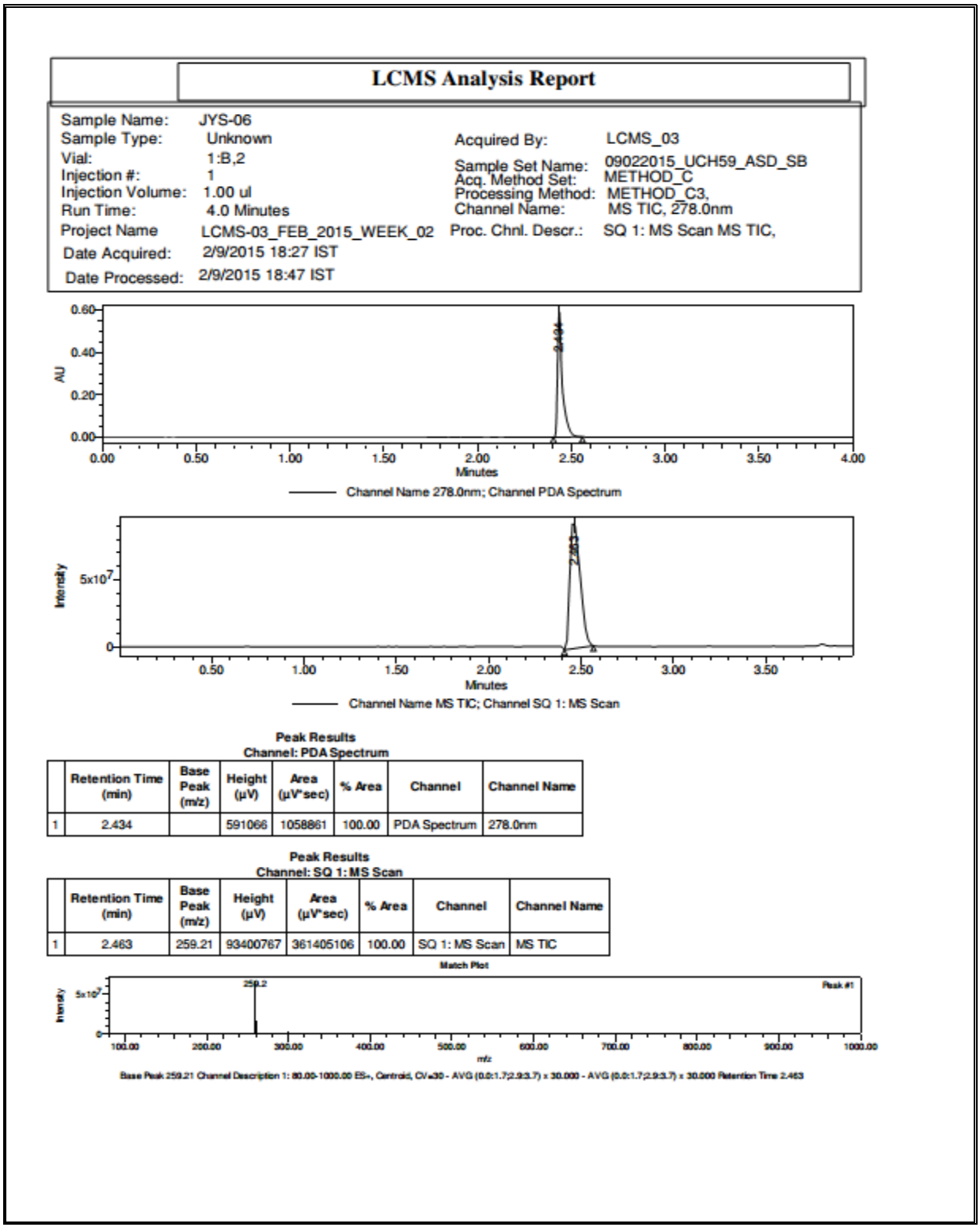


Figure 8: LCMS spectrum of 4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a

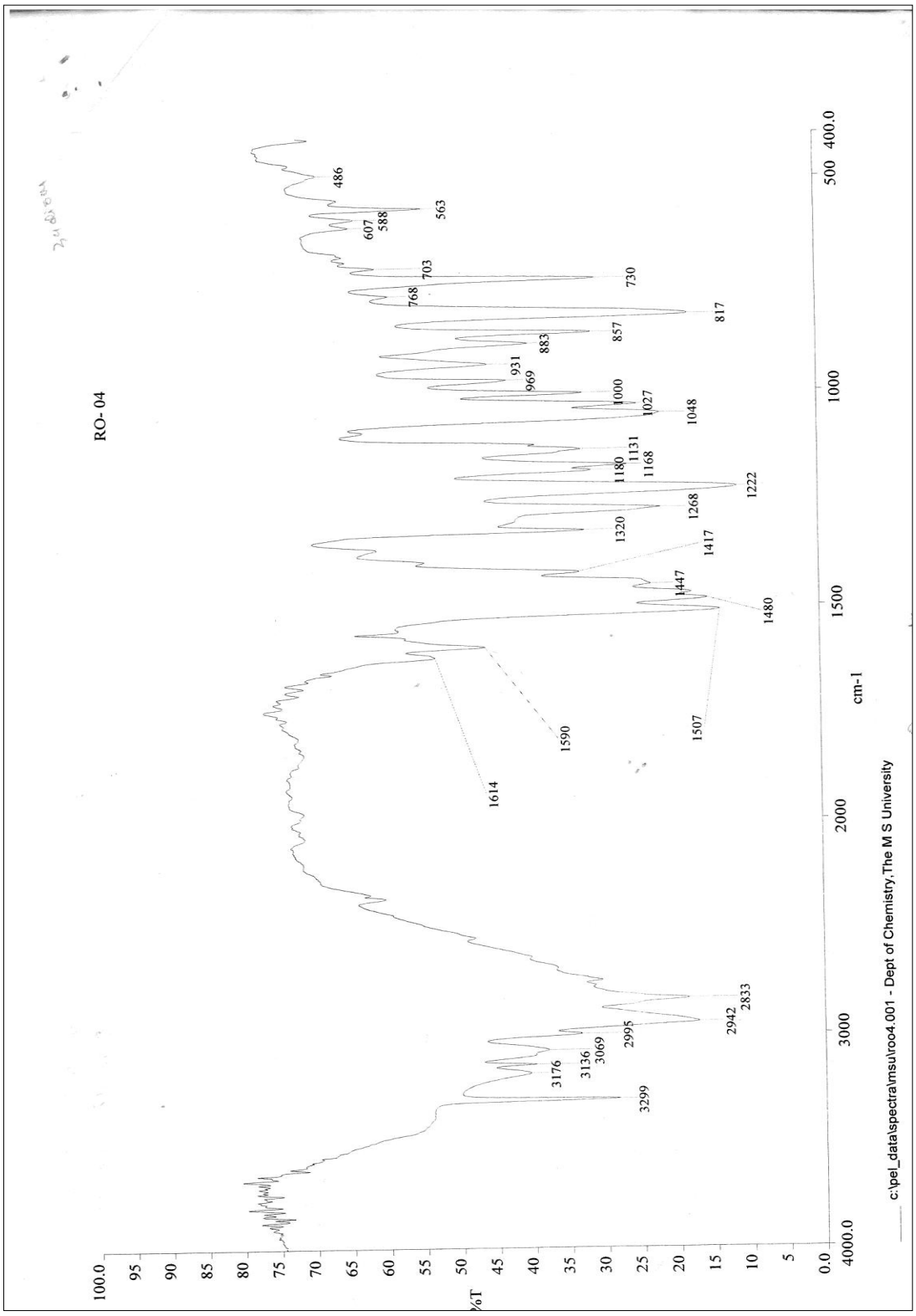


Figure 9: IR spectrum of 4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c

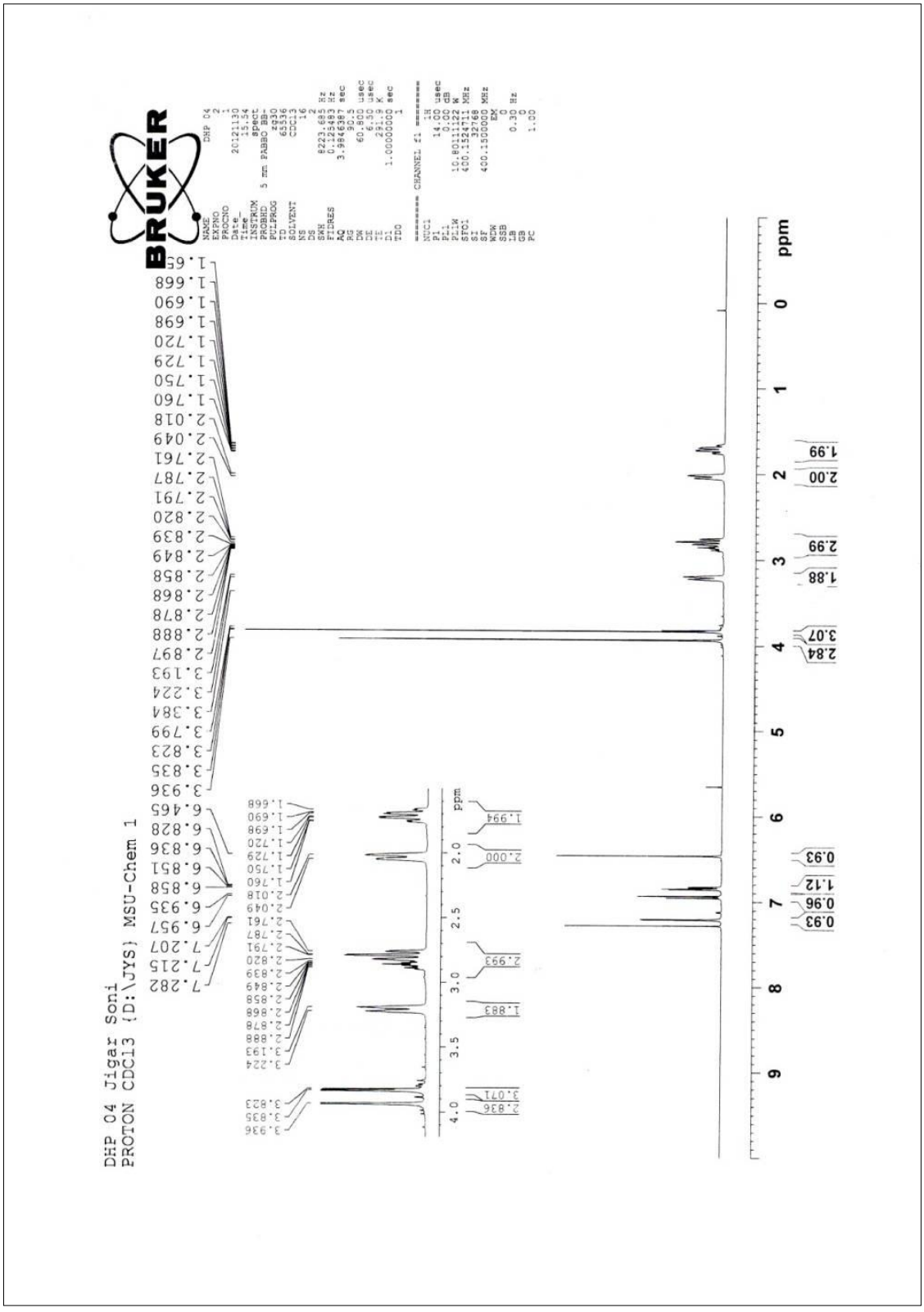


Figure 10: ^{1}H NMR spectrum of 4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c

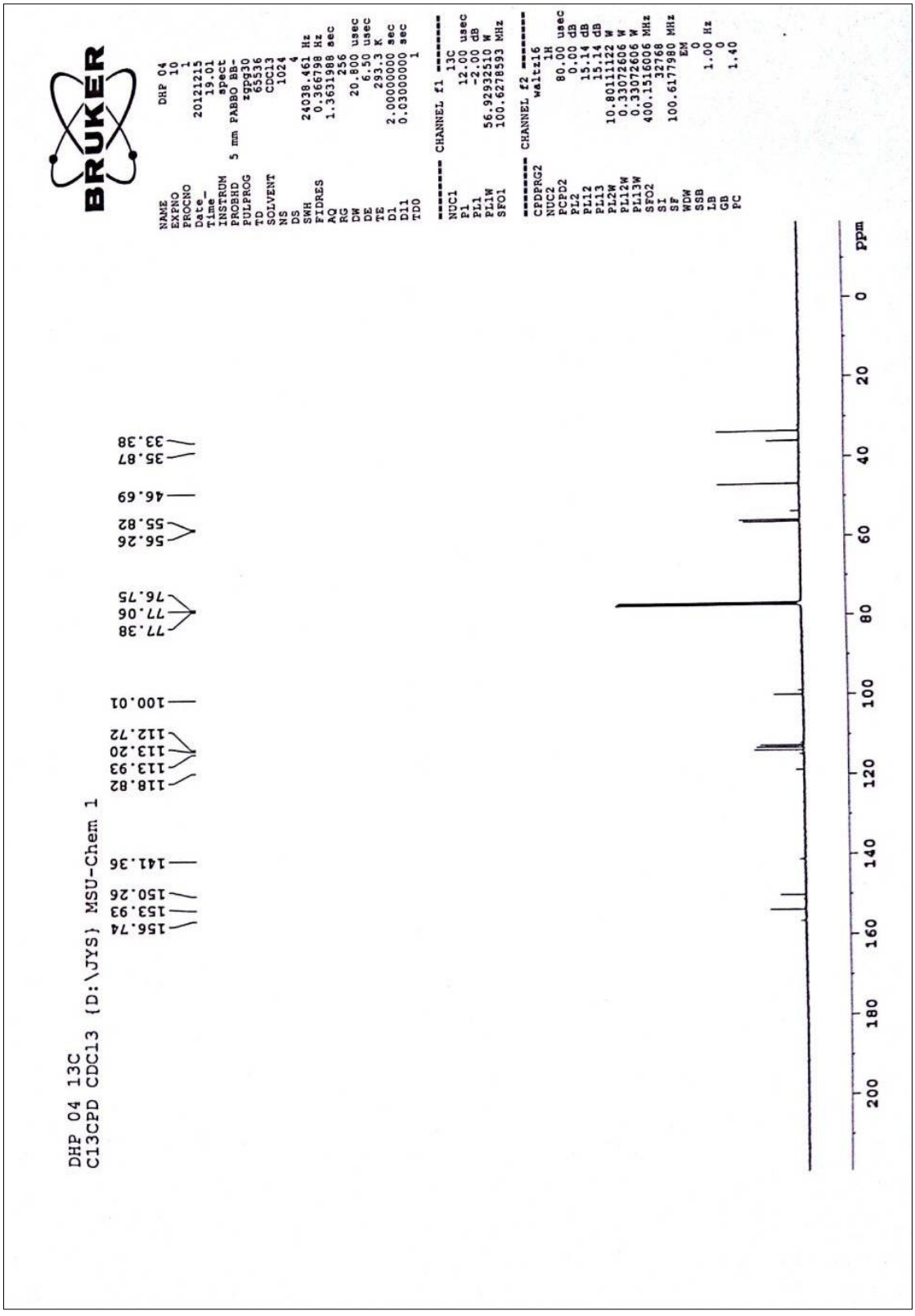


Figure 11: ^{13}C NMR spectrum of 4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c

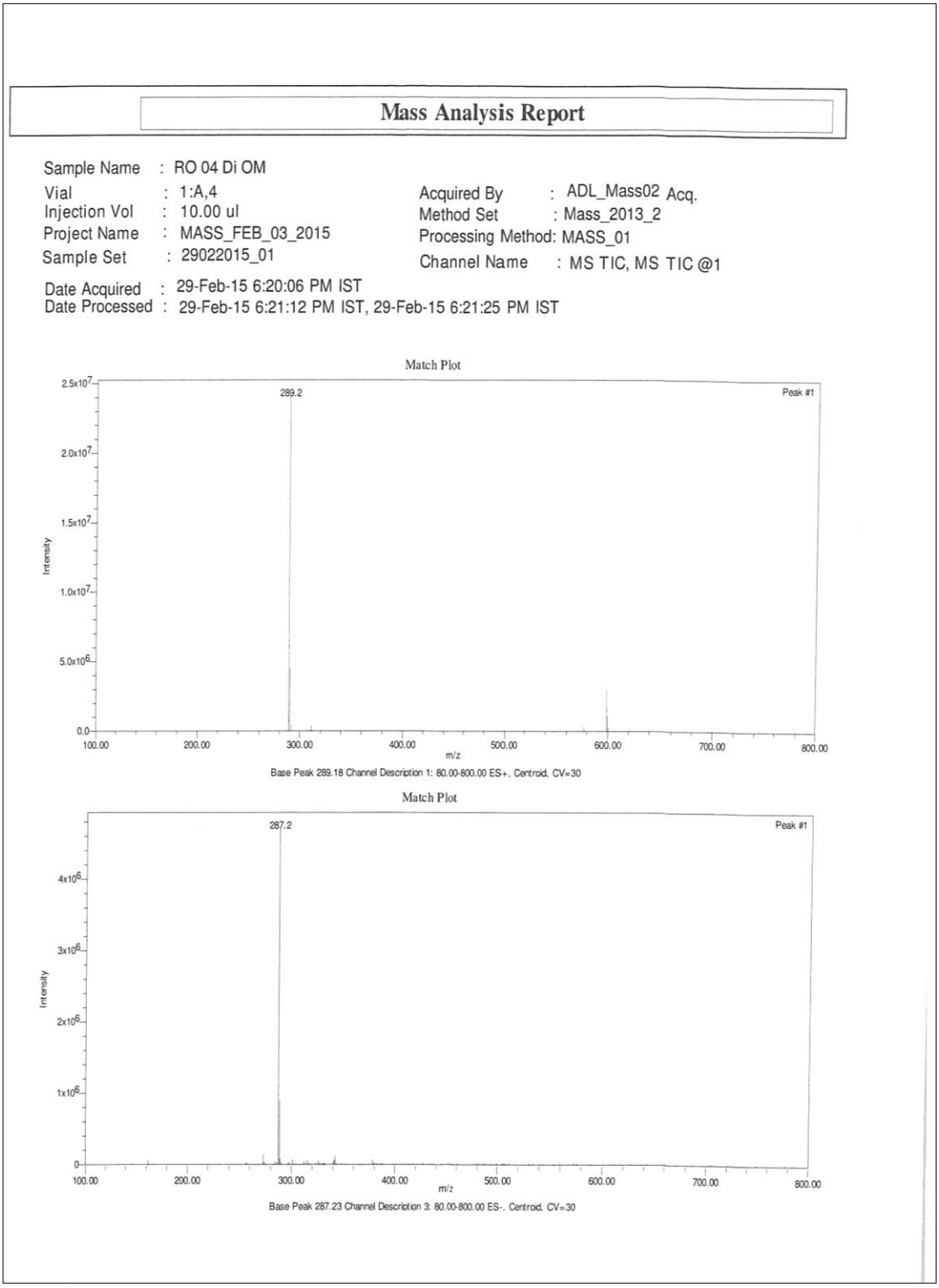


Figure 12: Mass spectrum of 4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c

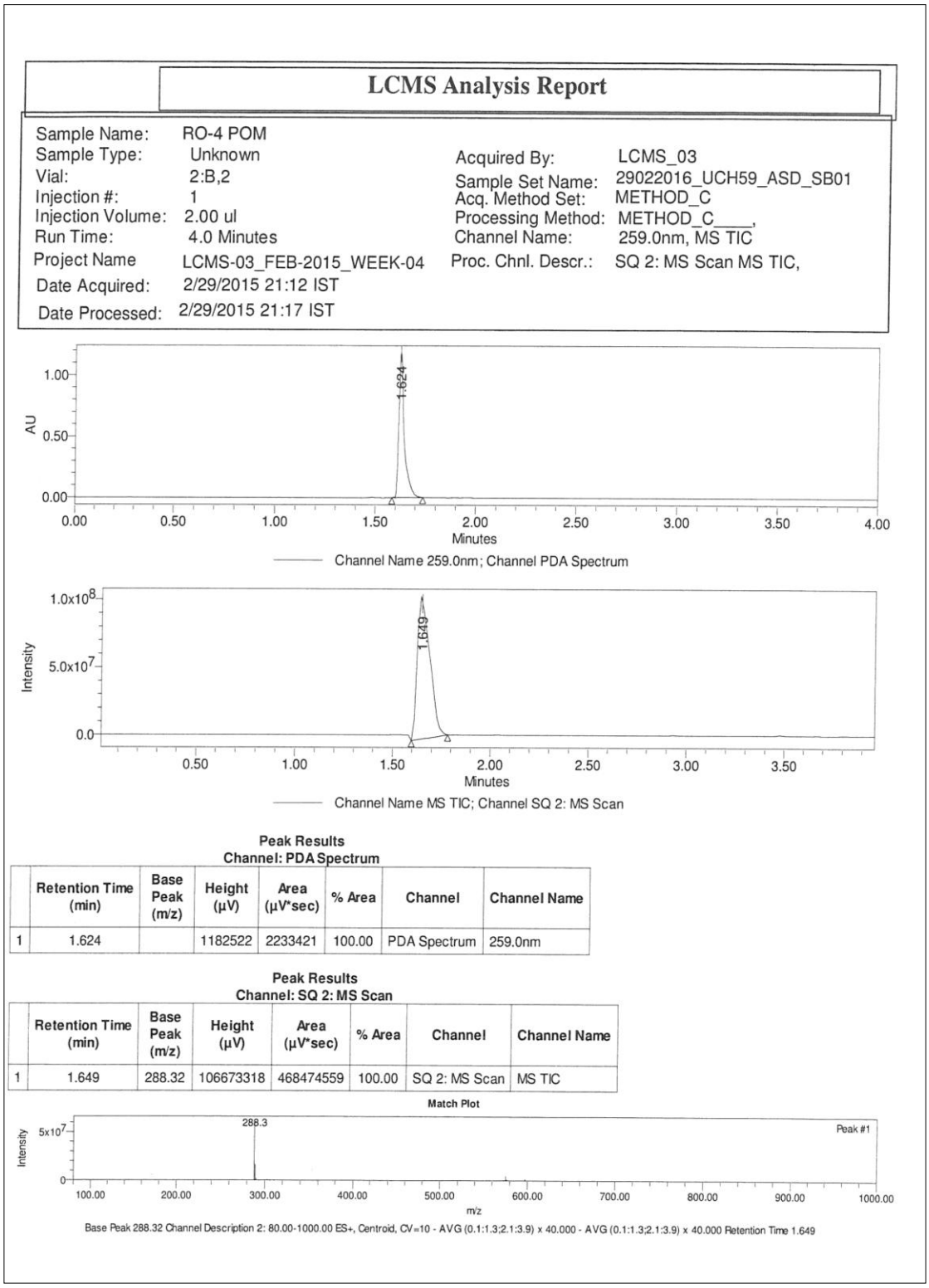


Figure 13: LCMS spectrum of 4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c

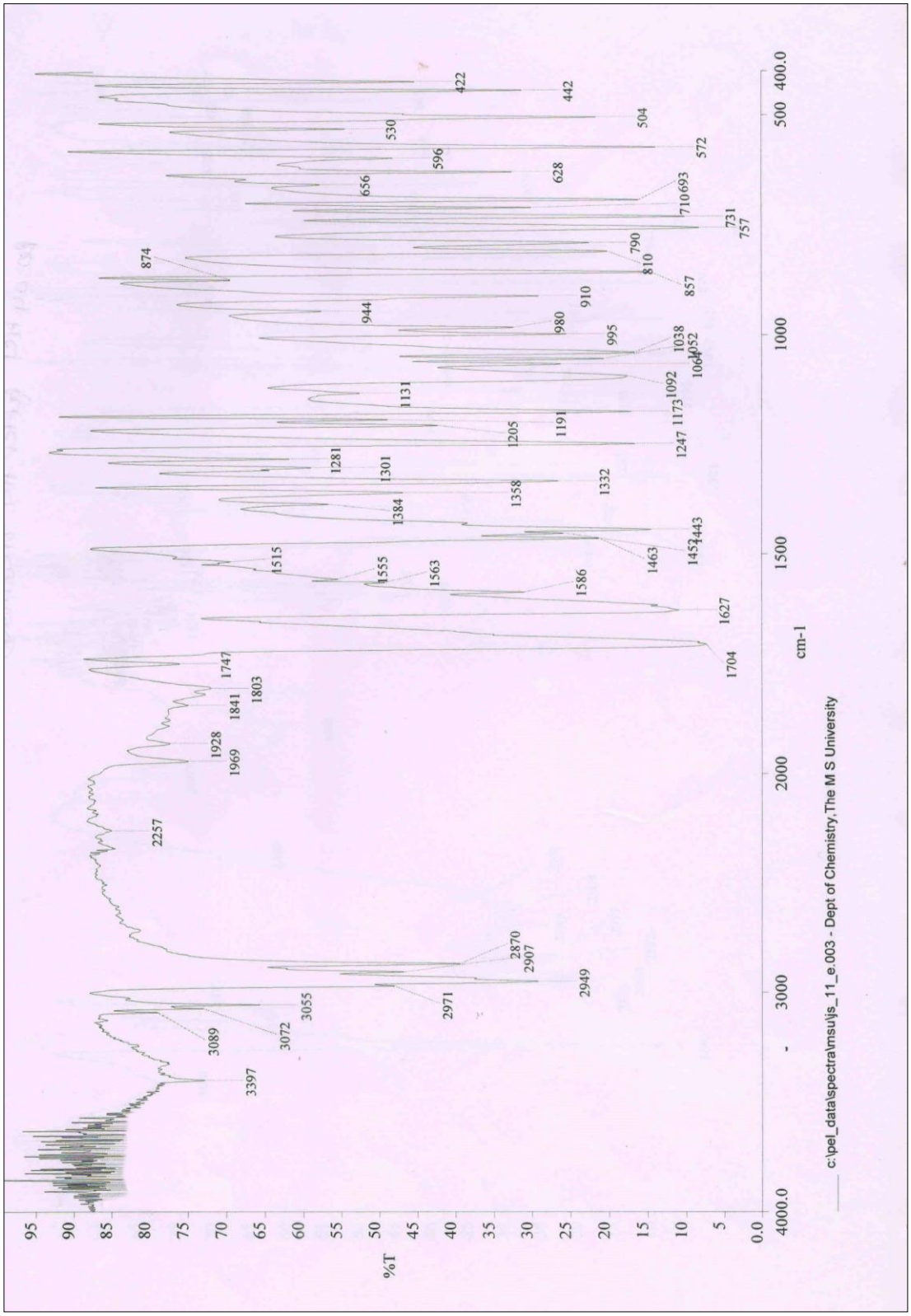


Figure 14: IR spectrum of 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e

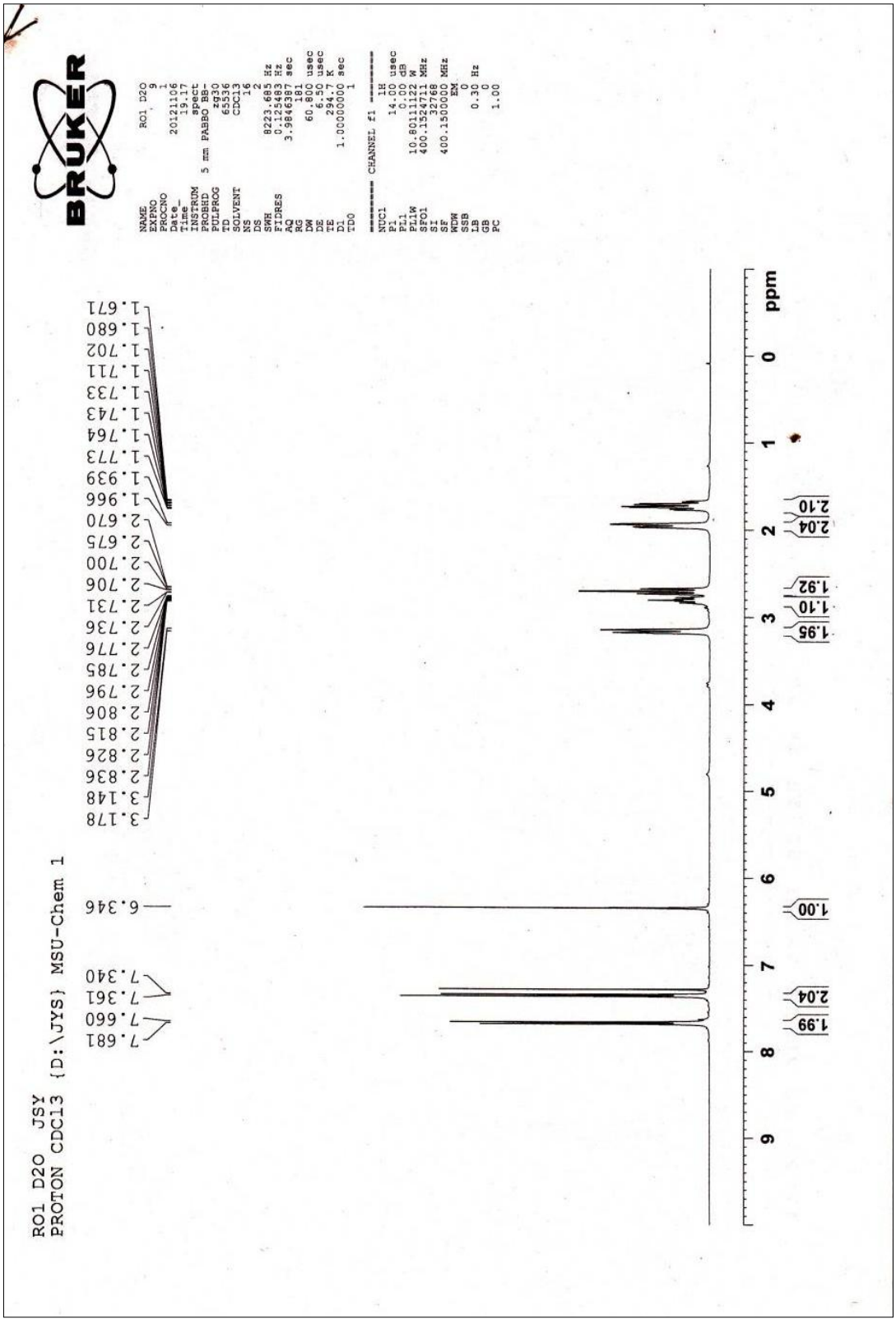


Figure 15: ^{1}H NMR spectrum of 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e

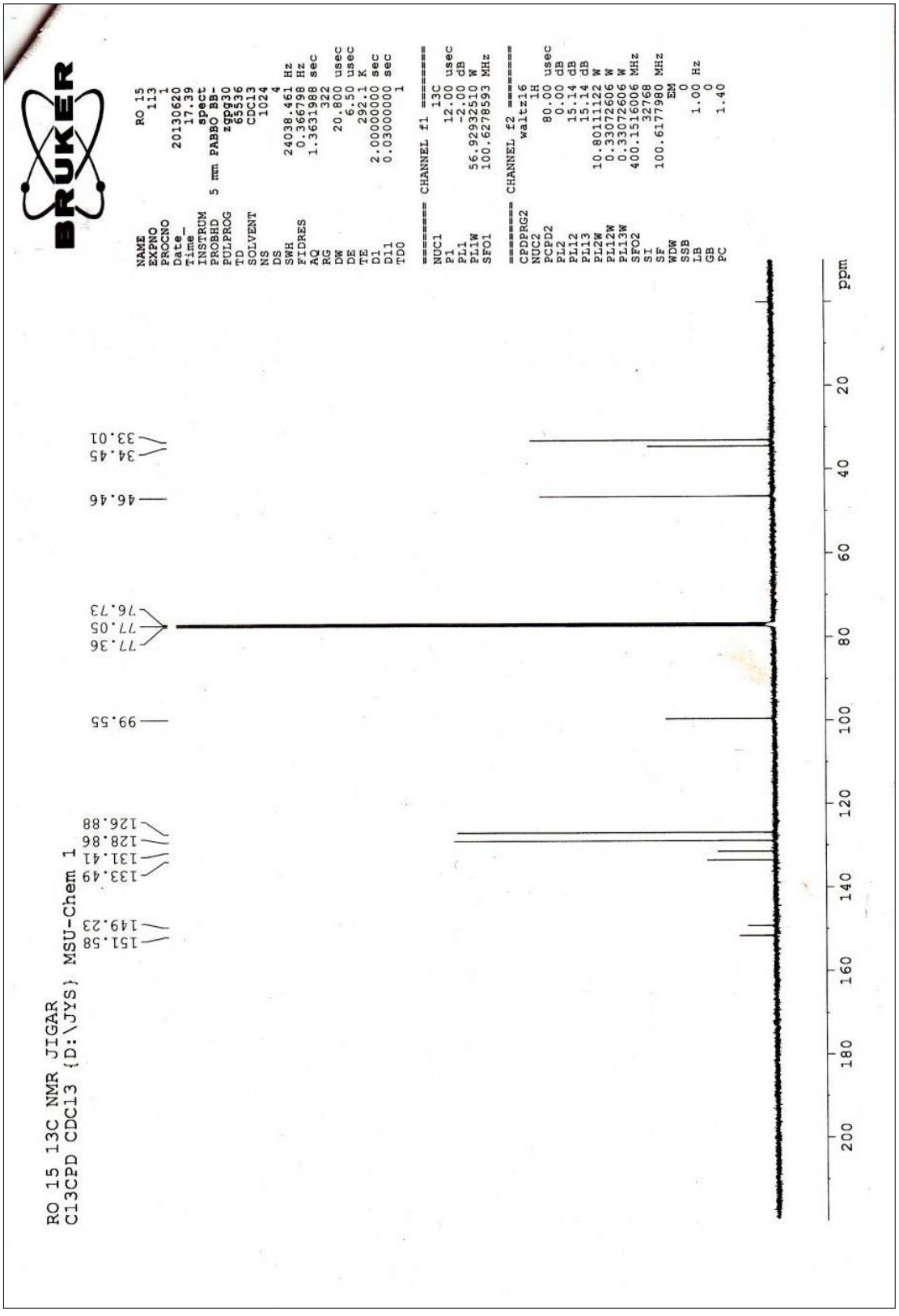


Figure 16: ^{13}C NMR spectrum of 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e

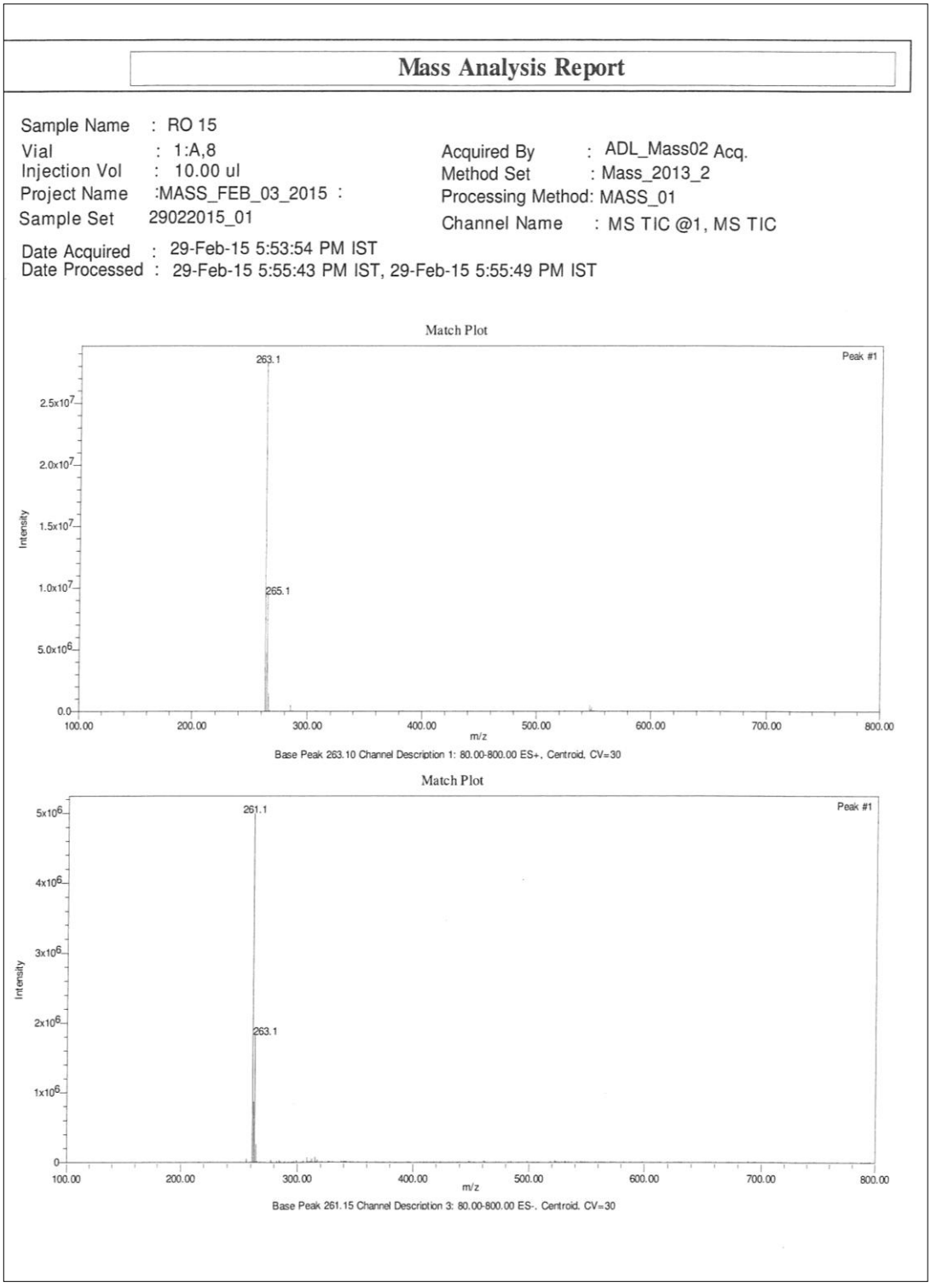


Figure 17: Mass spectrum of 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e

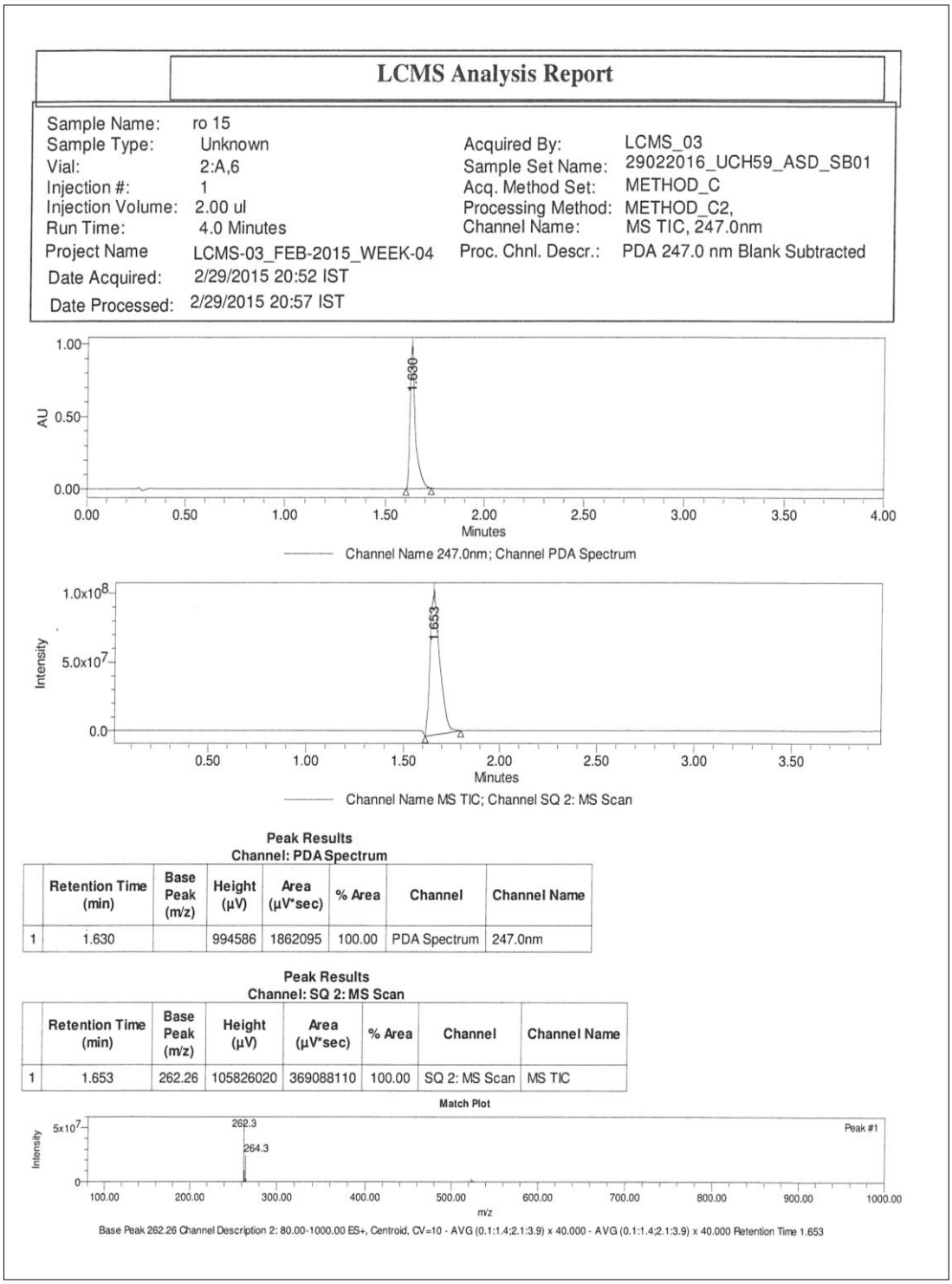


Figure 18: LCMS spectrum of 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e

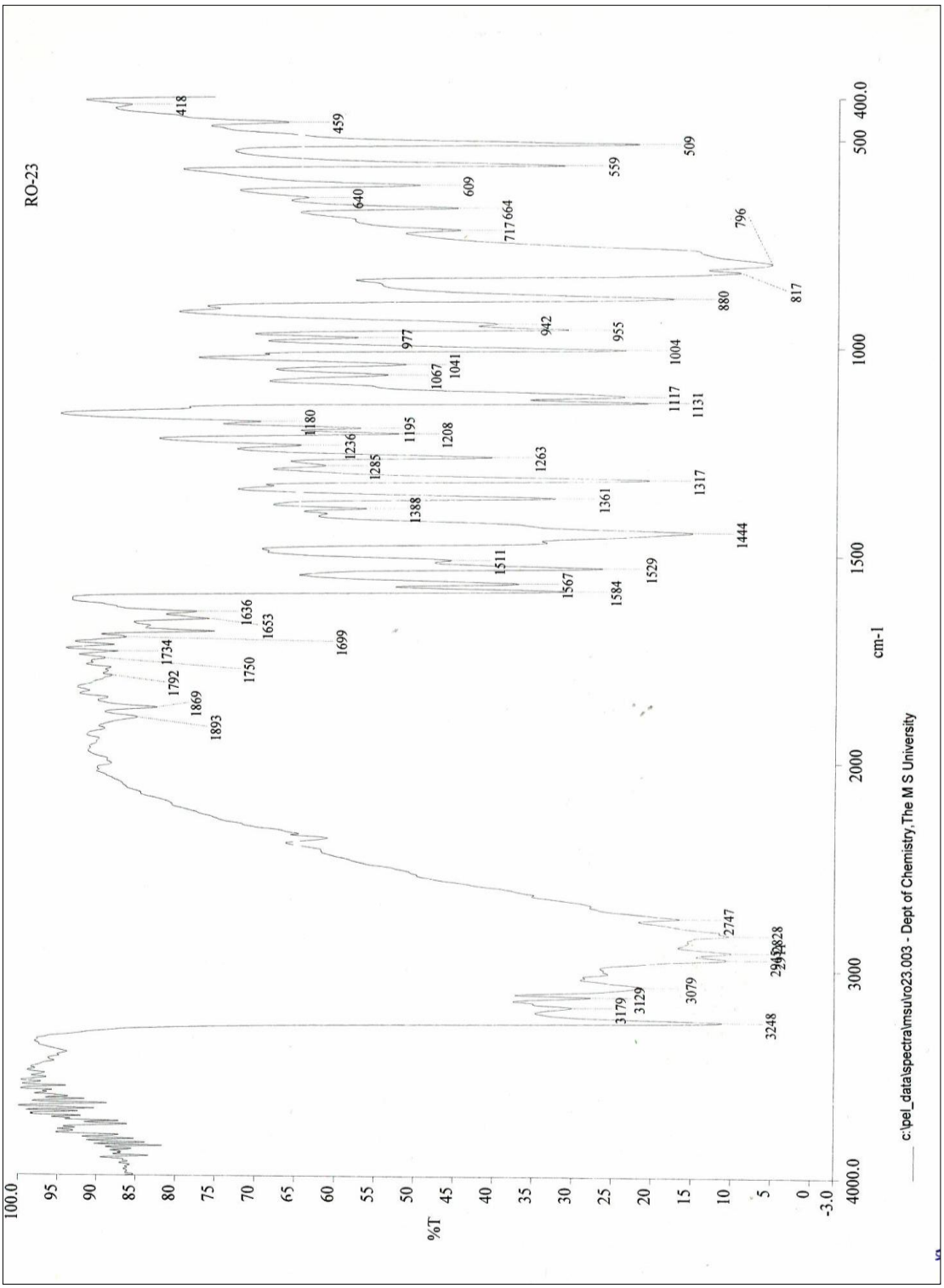


Figure 19: IR spectrum of 4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k

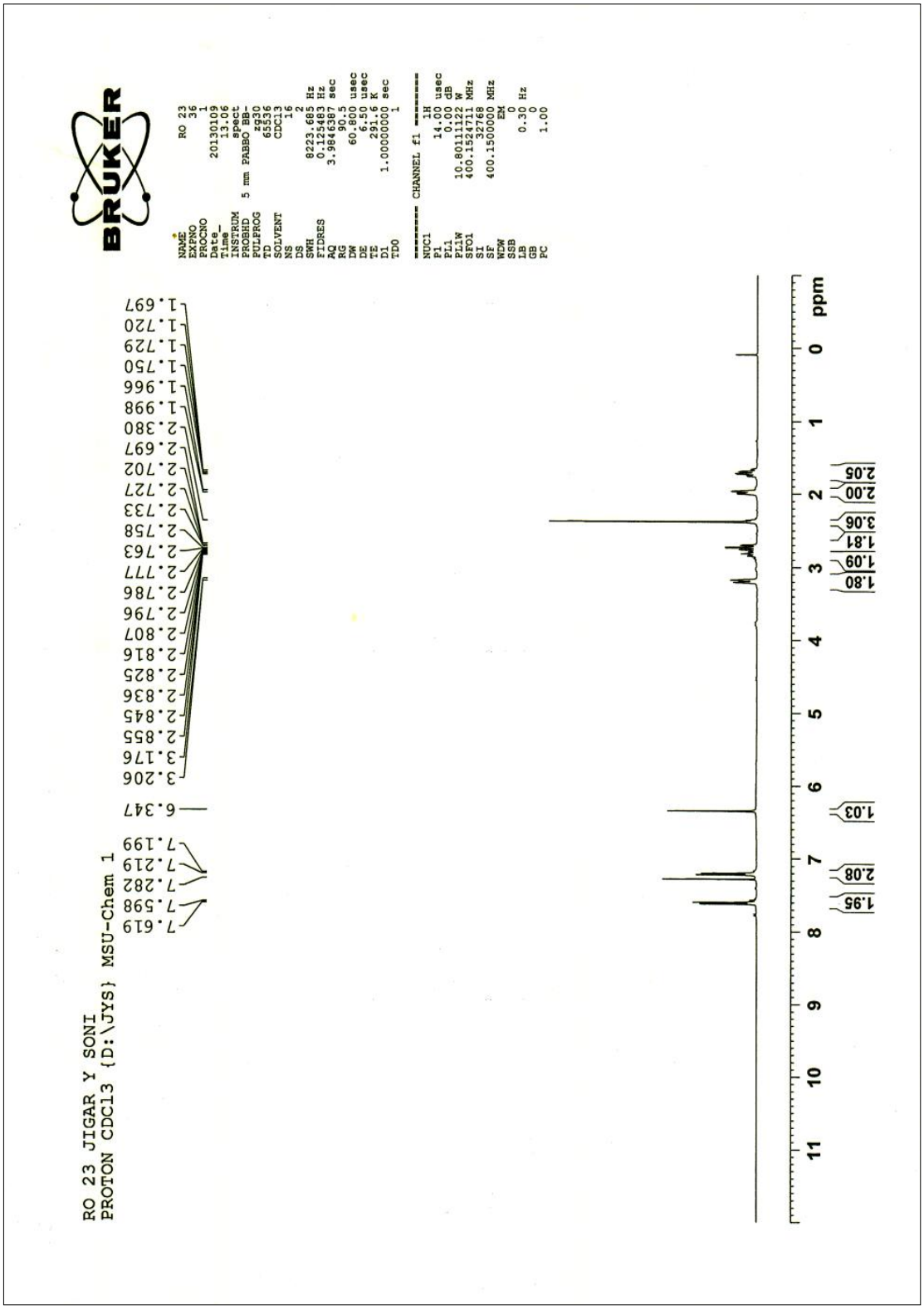


Figure 20: ^{1}H NMR spectrum of 4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k

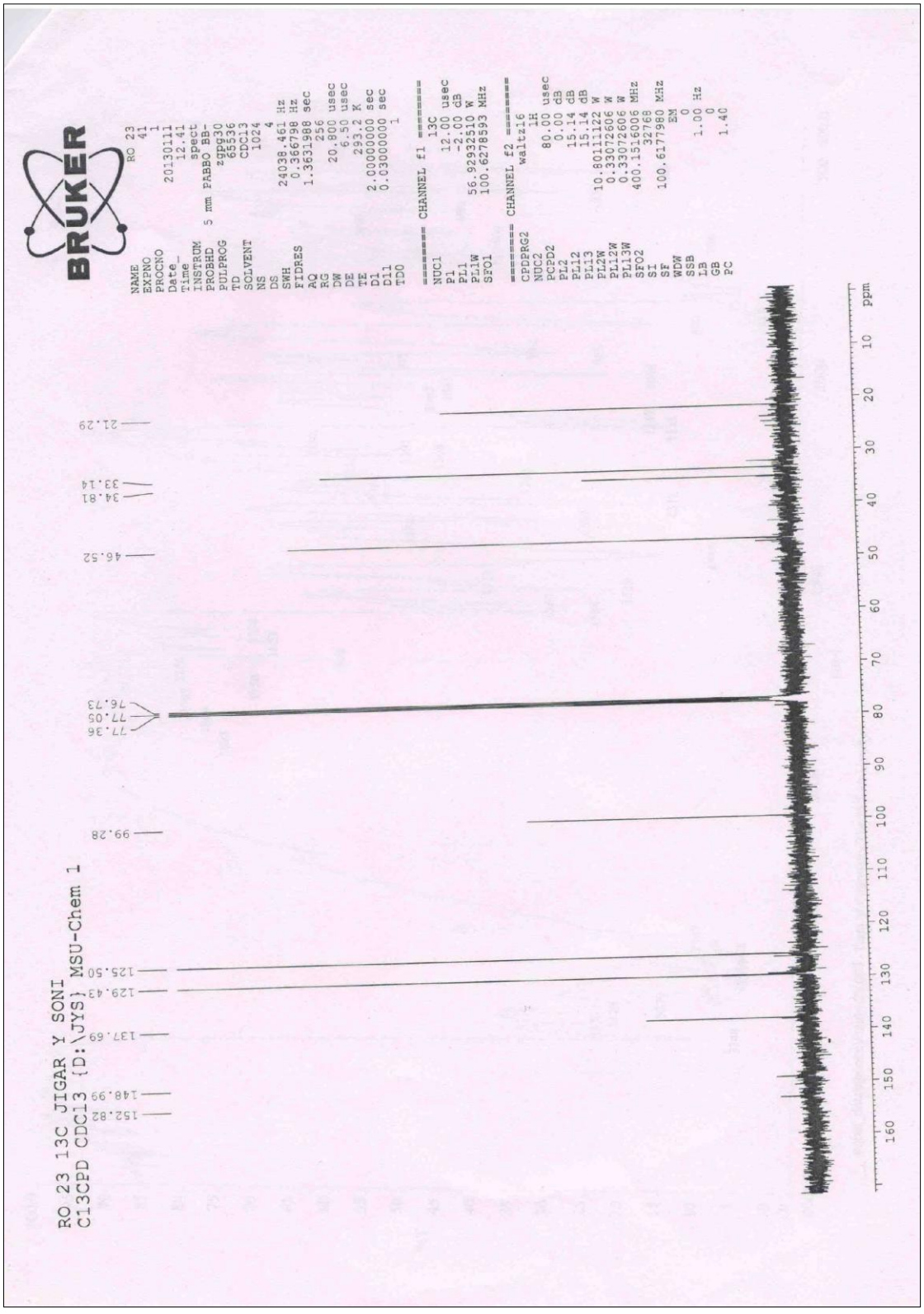


Figure 21: ^{13}C NMR spectrum of 4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k

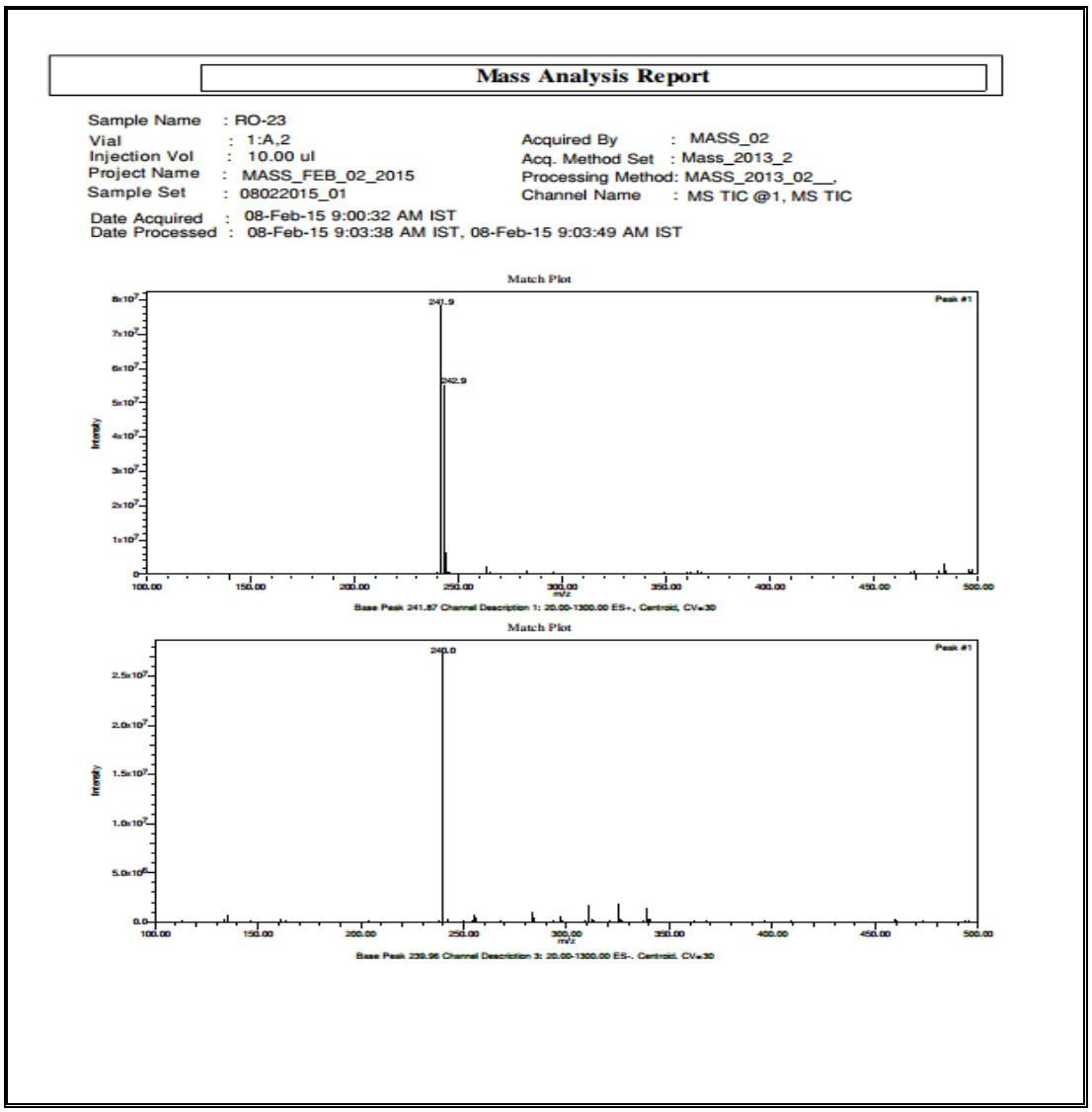


Figure 22: Mass spectrum of 4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k

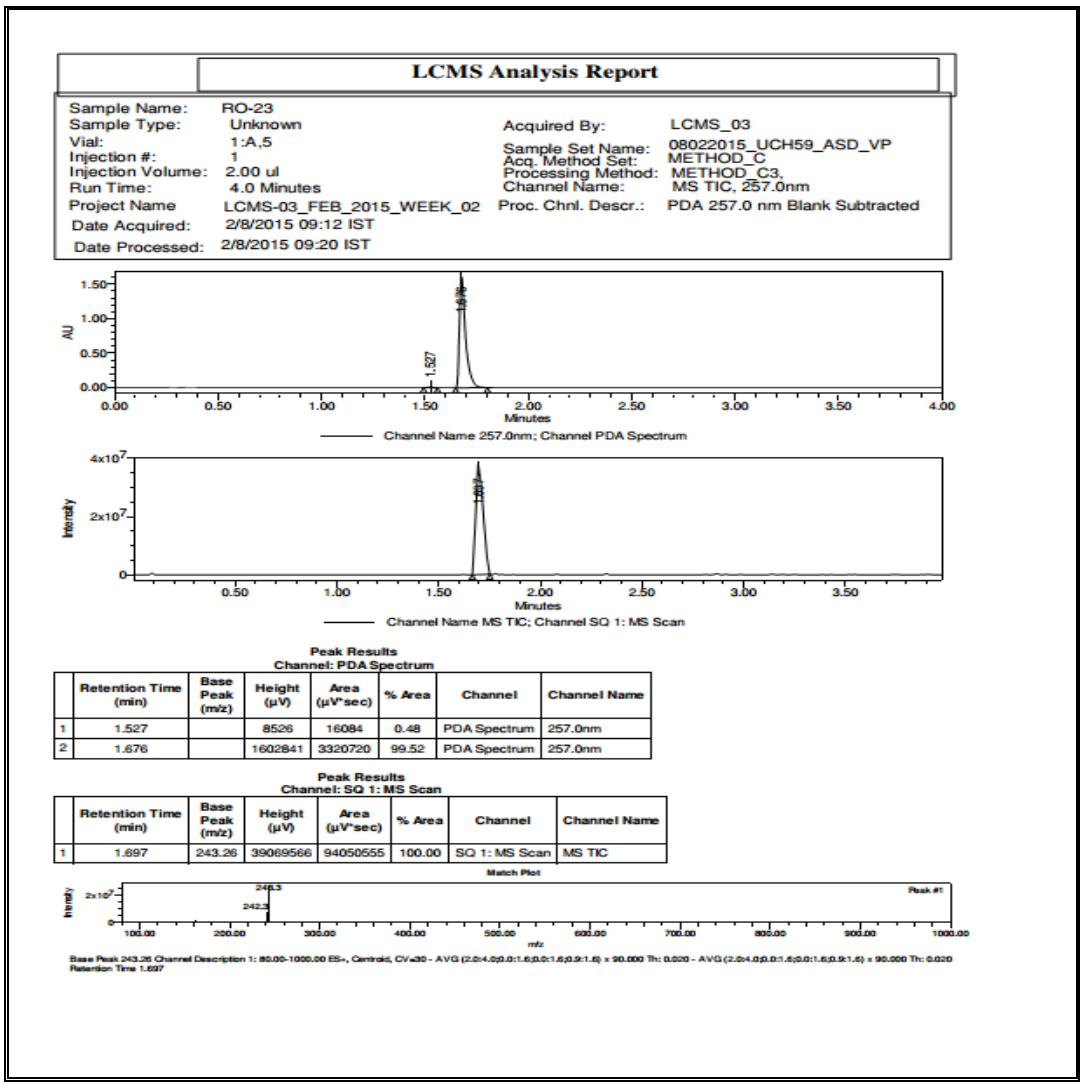


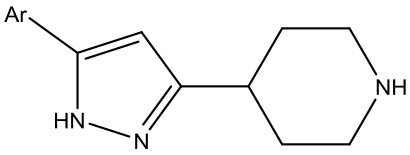
Figure 23: LCMS spectrum of 4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k

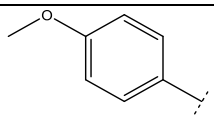
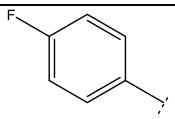
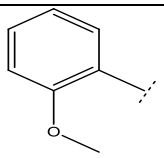
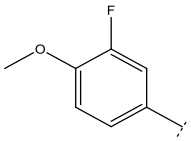
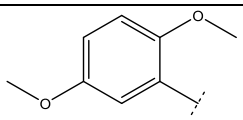
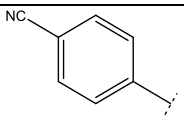
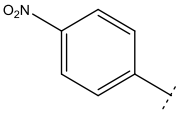
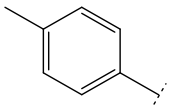
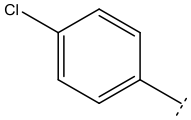
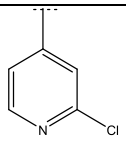
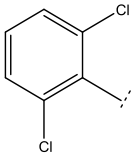
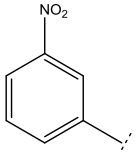
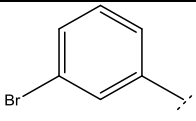
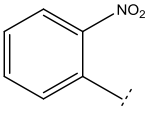
4.2.2 Biological evolution

To confirm the potentiality of the pyrazolyl piperidine moiety for designing new antiplatelet agents, the pyrazolyl piperidine derivatives (3a–n) were screened as our previous protocol described in Literature.[16] Initially target compounds were screened at 100 µM for inhibitory effects on human platelets aggregation using ADP as agonist. Interestingly, almost all compounds presented a significant inhibitory profile at this concentration. All the compounds were screened thrice at 5, 10 and 15 µM

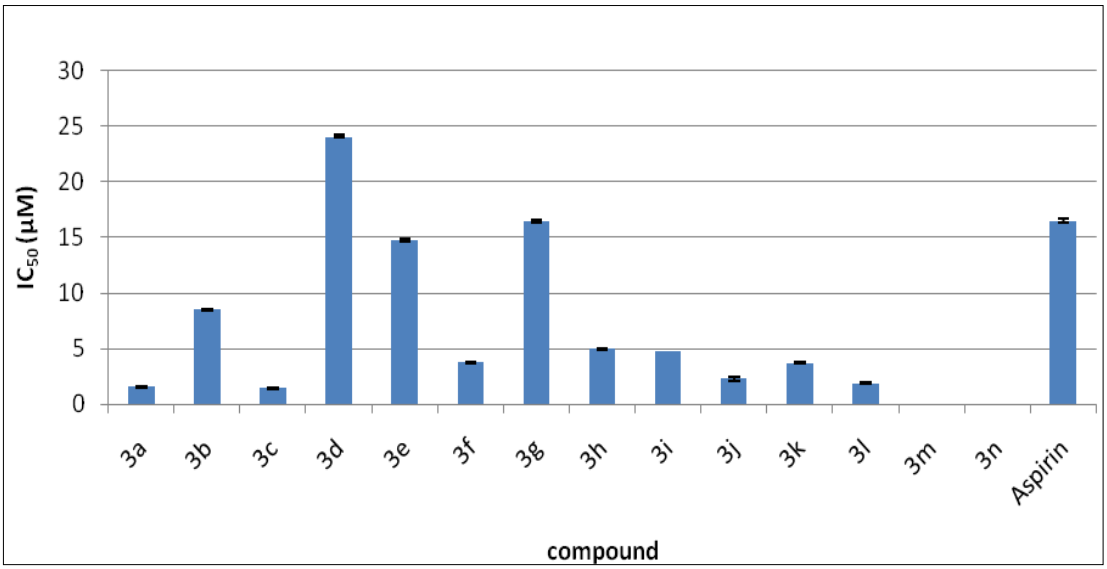
concentrations and experimental half-maximal inhibitory concentration (IC_{50}) was calculated **(Table: 1).**

Table 1 Structure and IC_{50} values of target compounds (3a-n)



Compound	Ar	IC_{50}(µM) ± S.E.	Compound	Ar	IC_{50}(µM) ± S.E.
3a		1.563± 0.0364	3h		4.988 ± 0.076
3b		8.495 ± 0.053	3i		4.771 ± 0.048
3c		1.435 ± 0.053	3j		2.337 ± 0.178
3d		24.07 ± 0.119	3k		3.717 ± 0.055
3e		14.78 ± 0.145	3l		1.884 ± 0.070
3f		3.786 ± 0.06	3m		-
3g		16.46 ± 0.143	3n		-

For the synthesized compounds (3a-n), the IC_{50} of all derivatives were comparable with aspirin; the most used antiplatelet drug currently in the market (Figure 1).



The determination of IC_{50} on ADP-induced platelet aggregation assays showed two different levels of antiplatelet activity that included values lower (1.53–14.78 µM) as well as similar (16.5-24.07µM) to aspirin (16.5 ± 0.2 µM).The correlation of structure with activity for our compounds revealed interesting results. The derivatives with methoxy group **3a-c** were most potent while those with nitro group showed least activity. Except *p*-nitro compound **3d** all other nitro-derivatives did not show any activity. methoxy group at *p*-position **3a** increases the activity while that at *o*-position **3b** decreases the activity, But when an additional methoxy group was introduced as in compound **3c**, the activity was improved. *p*-Chloro group position **3e** showed least activity which further improved when both the *ortho* position were substituted by chloro group **3f**. Substitution by various halogens affected the activity significantly. Among halogens, fluoro **3** gives better activity than chloro. An additional methoxy group **3i** does have much impact on the activity of fluoro compound. The cyano group

3j and methyl group at *p*-position **3a** did not have any enhancement in the activity significantly. Instead of chlorophenyl **3e**, chloro pyridyl derivative **3l** showed much higher activity almost comparable to methoxy phenyl derivative **3a**. The best profile (1.53–14.78 μM) was observed for compounds **3a, 3c, 3l** and **3j**. On the basis of SAR, the order of activity of different compounds can be summarized as 3c> 3a> 3l >3j > 3k >3f >3i > 3h > 3b > 3e > 3g > 3d. The reaction of equimolar proportion of compound **1** and aromatic substituted aldehyde in presence of 1 equlient of sodium hydroxide in absolute ethanol refluxed. The progress of the reaction was monitored by TLC. After completion, the solvent was evaporated in vacuum. The residue was dissolved in ethyl acetate (50 ml) and washed with water. Organic layer was dried with sodium sulfate and solvent evaporated in vacuum. The yellow solid obtained was recrystallized from IPA/H_2O (1:1) (compounds 2d, 2i-2k) or absolute methanol (compound 2e).

The product of step one **2a-n** was taken with excess of base hydrazine hydrate and refluxed to give title compound **3a-n**. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature. Crystalline solid precipitated which was filtered and washed with water. Drying under *vacuum* afforded pure desired compound **3a-n.** Calculations and statistics were performed using graph pad Prism 3.02 software. Data was expressed as mean ± standard error.

4.3 Experimental

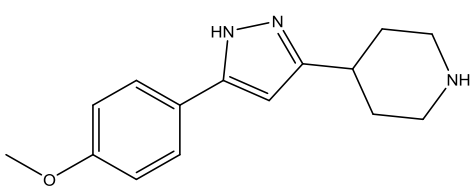
4.3.1 Chemistry

Melting points were measured using a (Buchi B-545) melting point apparatus and are uncorrected. Infrared spectra were recorded on Perkin-Elmer RX 1 and Perkin-Elmer

580B spectrometer. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. ^{1}H and ^{13}C NMR spectra were recorded on Advance Bruker (400 MHz) spectrometer in suitable deuterated solvents. ^{1}HNMR data were recorded as follow: chemical shift measured in parts per million (ppm) downfield from TMS (d), multiplicity, observed coupling constant (*J*) in Hertz (Hz), proton count. Multiplicities are reported as singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q) and multiplet (m). ^{13}C NMR chemical shifts are reported in ppm downfield from TMS and identifiable carbons are given. Solvents and reagents were purified by literature methods. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020. The reaction progress was monitored by TLC in ultra violet light as well as in iodine vapour. Platelet aggregation study was done by using Chrono Log model 592VS dual channel whole blood aggregometer from Chrono-Log Corporation (Havertown, PA, USA).

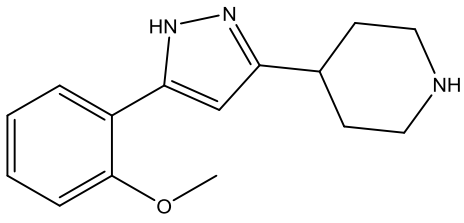
General procedure for the preparation of compounds 3a-n

The product of step one (2a-n) (0.5g) was taken in excess of hydrazine hydrate and refluxed to give title compound (3a-n). The progress of the reaction was monitored by TLC. After completion the reaction mixture was allowed to cool to room temperature. A crystalline solid precipitated which was filtered and washed with water. Drying under *vacuum* afforded pure desired compound 3a-n.

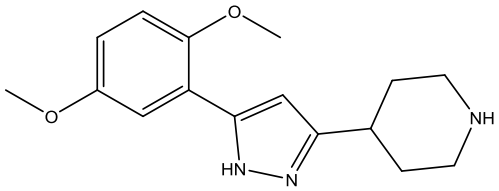


4-(5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3a: This compound was obtained as White solid. Yield: 74%; mp: 180-182ºC (rep. 181-182 ºC). [33] IR (KBr, cm^{-1}) (Figure 4): 3071, 3005, 1304, 1251, 984. ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 5): δ 1.66-1.76 (2H, m, CH_2); 1.72-1.76 (2H, m,

CH_2); 2.73-2.79 (2H, m, CH); 2.81-2.86 (1H, m, CH) 3.19-3.22 (2H, m, CH_2); 3.85 (3H, s, OCH_3) 6.32 (1H, s, pyrazol proton); 6.95 (2H, d, *J*= 8.4 Hz) 7.65 (2H, d, ArH, J= 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 6): δ 33.0, 46.5, 55.31, 99.1, 114.1, 125.0, 126.8, 159.4. ESI/MS (Figure 7) 258.2 $[M+1]^+$ calculated for $C_{15}H_{19}N_3O$. Anal. Calcd. for $C_{15}H_{19}N_3O$: C, 70.01; H, 7.44; N, 16.33; Found: C, 69.80; H, 7.69; N, 16.56.

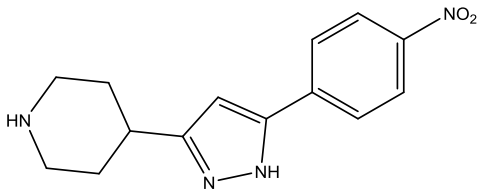


4-(5-(2-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3b: This compound was obtained as White solid. Yield: 64%; mp: 160-162ºC. IR (KBr, cm^{-1}): 3059, 3003, 2942, 2828, 1036. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.76-1.77 (2H, m, CH_2); 1.99- 2.02 (2H, m, CH_2); 2.74-2.89 (3H, m, CH, CH_2); 3.21-3.24 (2H, d, CH_2); 3.86 (3H, s, OCH_3) 6.38 (1H, s, pyrazol proton); 6.89 (1H, dd, ArH, *J*= 4.8, 2.4 Hz,); 7.29-7.33 (3H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 32.9, 34.6, 44.4, 55.3, 99.8, 110.8, 113.7, 118.1, 129.8, 159.9. ESI/MS 258.2 $[M+1]^+$ calculated for $C_{15}H_{19}N_3O$. Anal. Calcd. for $C_{15}H_{19}N_3O$: C, 70.01; H, 7.44; N, 16.33; Found: C, 70.34; H, 7.28; N, 16.19.

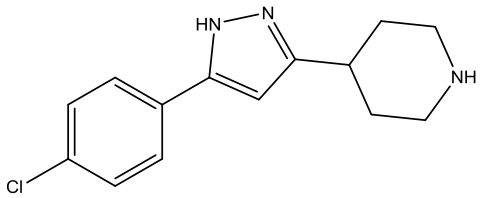


4-(5-(2, 5-dimethoxyphenyl)-1*H*-pyrazol-3-yl)piperidine 3c: This compound was obtained as White solid. Yield: 89%; mp: 158-160ºC. IR (KBr, cm^{-1}) (Figure 9): 3298, 2942, 2833, 1048, 1000. ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 10): δ 1.66-1.76 (2H, m, CH_2); 2.02-2.05 (2H, m, CH_2); 2.76-2.82 (2H, m, CH_2); 2.84-2.90 (1H, m, CH_2); 3.19-3.22 (2H, d, CH_2); 3.84 (3H, s, OCH_3), 3.94 (3H, s, OCH_3); 6.47 (1H, s, pyrazol proton); 6.84 (1H, dd, *J*= 8.8, 2.8 Hz); 6.93 (1H, d, *J*= 8.8 Hz); 7.24 (1H, d, ArH, *J*= 2.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$)

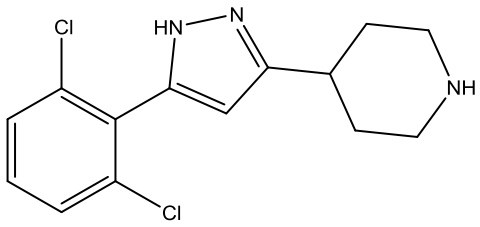
(Figure 11): δ 33.3, 35.9, 46.7, 55.8, 56.3, 100.0, 112.7, 113.2, 113.9, 150.3, 153.9. ESI/MS (Figure 12) 288.3 $[M+1]^+$ calculated for $C_{16}H_{21}N_3O_2$. Anal. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62; Found: C, 67.03; H, 7.26; N, 14.74.



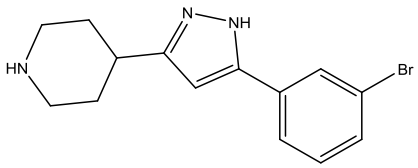
4-(5-(4-nitrophenyl)-1*H*-pyrazol-3-yl) piperidine 3d: This compound was obtained as Yellow solid. Yield: 78%; mp: 168-170ºC. IR (KBr, cm^{-1}): 3317, 1602, 1508, 1336, 853. ^1H NMR (400 MHz, $CDCl_3$): δ 1.73-1.83 (2H, m, CH_2); 2.00-2.04 (2H, m, CH_2); 2.76-2.79 (2H, m, CH_2); 2.82-2.89 (1H, m, CH); 3.23-3.26 (2H, m, CH_2); 6.50 (1H, s, pyrazol proton); 7.95 (2H, d, ArH, *J*= 8.8 Hz,); 8.28 (2H, d, ArH, *J*= 8.8 Hz). ^{13}C NMR (100 MHz, DMSO + $CDCl_3$): δ 37.7, 38.9, 34.2, 51.2, 104.4, 128.6, 130.4, 139.0, 144.4, 151.2. ESI/MS 273.0 $[M+1]^+$ calculated for $C_{14}H_{16}N_4O_2$. Anal. Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58; Found: C, 62.01; H, 5.74; N, 20.41.



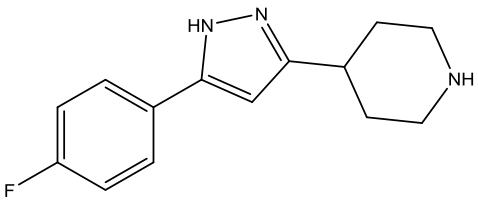
4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3e: This compound was obtained as White solid. Yield: 83%; mp: 185-187ºC (rep 186-187 ºC)[24] IR (KBr, cm^{-1}) (Figure 14): 3068, 1364, 1265, 1004, 907. ^1H NMR (400 MHz, $CDCl_3$) (Figure 15): δ 1.67-1.77 (2H, m, CH_2); 1.94-1.97 (2H, m, CH_2); 2.67-2.73 (2H, m, CH); 2.76-2.84 (1H, m, CH) 3.15-3.18 (2H, m, CH_2); 6.34 (1H, s, pyrazol proton); 7.35 (2H, m, ArH, *J*= 8.4 Hz); 7.67 (2H, d, *J*= 8.6 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 16): δ 33.0, 34.5, 46.5, 99.6, 126.9, 128.9, 131.4, 133.5, 149.2, 151.6. ESI/MS (Figure 17) 262.2 $[M]^+$, 264.2 $[M+2]^+$ calculated for $C_{14}H_{16}N_3Cl$. Anal. Calcd. for $C_{14}H_{16}N_3Cl$: C, 64.24; H, 6.16; N, 16.05; Found: C, 64.04; H, 6.38; N, 16.24.



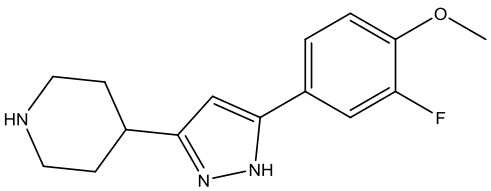
4-(5-(2,6-dichlorophenyl)-1*H*-pyrazol-3-yl) piperidine 3f: This compound was obtained as White solid. Yield: 84%; mp: 148-152ºC. IR (KBr, cm^{-1}): 3237, 3236, 1646, 1320, 1295, 1079. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.66-1.76 (2H, m, CH_2); 1.97-2.01 (2H, m, CH_2); 2.79-2.87 (3H, m, CH, CH_2); 3.18-3.21 (2H, m, CH_2); 6.40 (1H, s, pyrazol proton); 7.34 (1H, m, ArH, *J*= 8.4 Hz,); 7.40 (1H, d, *J*= 8.4 Hz, ArH); 7.73-7.75 (1H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.1, 34.7, 46.5, 99.5, 125.6, 127.8, 128.7, 132.4, 149.3, 152.5. ESI/MS 296.1 $[M]^+$, 298.2 $[M+2]^+$, 300.1 $[M+4]^+$ calculated for $C_{14}H_{15}N_3Cl_2$. Anal. Calcd. for $C_{14}H_{15}N_3Cl_2$: C, 56.77; H, 5.10; N, 14.19; Found: C, 56.63; H, 5.45; N, 14.35.



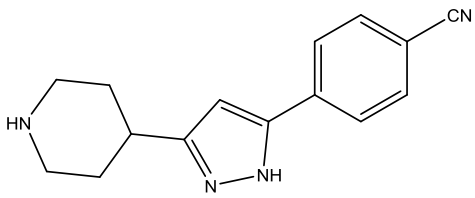
4-(5-(3-bromophenyl)-1*H*-pyrazol-3-yl) piperidine 3g: This compound was obtained as White solid. Yield: 62%; mp: 152-156ºC. IR (KBr, cm^{-1}): 3336, 3079, 2996, 2953, 2817, 1360, 1287, 1119. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69-1.79 (2H, m, CH_2); 1.99-2.02 (2H, m, CH_2); 2.74-2.80 (2H, m, CH); 2.82-2.88 (1H, m, CH); 3.20-3.23 (2H, d, CH_2); 6.39 (1H, s, pyrazol proton); 6.99-7.04 (1H, m, ArH *J*= 8.4 Hz) 7.36-7.40 (1H, m, ArH, *J*= 8.4 Hz,); 7.45-7.48 (1H, m, *J*= 8.4 Hz, ArH); 7.52-7.54 (1H, m , ArH,). ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.1, 34.4, 46.4, 99.8, 112.3, 112.6, 114.5, 114.7, 121.2, 130.1, 130.2, 130.3, 135.1. ESI/MS 306.1 $[M]^+$, 308.1 $[M+2]^+$calculated for $C_{14}H_{16}N_3Br$. Anal. Calcd. for $C_{14}H_{16}N_3Br$: C, 54.91; H, 5.27; N, 26.10; Found: C, 55.00; H, 5.38; N, 26.64.



4-(5-(4-fluorophenyl)-1*H*-pyrazol-3-yl)piperidine 3h: This compound was obtained as White solid. Yield: 67%; mp: 181-184ºC. IR (KBr, cm^{-1}): 3246, 2950, 2830, 1363, 1264, 1095, 1006. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68-1.79 (2H, m, CH_2); 1.99-2.02 (2H, m, CH_2); 2.73-2.81 (2H, m, CH); 2.82-2.89 (1H, m, CH); 3.20-3.23 (2H, m, CH_2); 6.34 (1H, s, pyrazol proton); 7.07-7.12 (2H, m, ArH); 7.70-7.73 (2H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.0, 34.5, 46.4, 99.41, 115.5, 115.7, 127.2, 127.3, 151.7, 161.3, 163.7. ESI/MS 246.2 $[M+1]^+$calculated for $C_{14}H_{16}N_3F$. Anal. Calcd. for $C_{14}H_{16}N_3F$: C, 68.55; H, 6.57; N, 17.13; Found: C, 68.79; H, 6.73; N, 17.25.

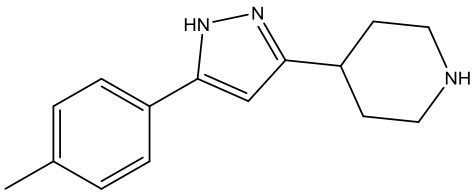


4-(5-(3-fluoro-4-methoxyphenyl)-1*H*-pyrazol-3-yl) piperidine 3i: This compound was obtained as White solid. Yield: 74%; mp: 142-144ºC. IR (KBr, cm^{-1}): 3335, 2945, 2842, 1364, 1270. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69-1.77 (2H, m, CH_2); 1.96-2.00 (2H, m, CH_2); 2.71-2.75 (2H, m, CH_2); 2.77-2.85 (1H, m, CH); 3.18-3.21 (2H, m, CH_2); 3.92 (3H, S, OCH_3); 6.30 (1H, s, pyrazol proton); 6.96-7.00 (1H, t, ArH, *J*= 8.8, 8.4 Hz,); 7.45-7.50 (2H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 32.9, 34.5, 46.5, 56.3, 99.3, 105.4, 113.4, 113.6, 121.3, 121.4, 147.3, 151.2, 153.7. ESI/MS 276.1 $[M+1]^+$ calculated for $C_{15}H_{18}N_3FO$. Anal. Calcd. for $C_{15}H_{18}N_3FO$: C, 65.44; H, 6.59; N, 15.26; Found: C, 65.57; H, 6.51; N, 15.42.

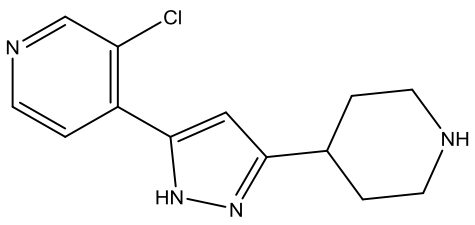


4-(3-(piperidin-4-yl)-1*H*-pyrazol-5-yl) benzonitrile 3j: This compound was obtained as White solid. Yield: 76%; mp: 168-170ºC.

IR (KBr, cm^{-1}): 3355, 3068, 2265, 1364, 1265, 1004, 907. ^1H NMR (400 MHz, $CDCl_3$): δ 1.71-1.79 (2H, m, CH_2); 2.00-2.02 (2H, m, CH_2); 2.75-2.90 (3H, m, CH, CH_2); 3.21-3.24 (2H, m, CH_2); 6.46 (1H, s, pyrazol proton); 7.69-7.71 (2H, m, ArH, *J*= 8.4 Hz,); 7.89 (2H, d, *J*= 8.4 Hz, ArH). ^{13}C NMR (100 MHz, DMSO): δ 33.0, 34.2, 46.3, 100.2, 109.7, 119.5, 125.9, 133.1, 138.4, 147.9, 151.4. ESI/MS 253.2 $[M+1]^+$ calculated for$C_{15}H_{16}N_4$. Anal. Calcd. for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.21; Found: C, 71.17; H, 6.55; N, 22.28.

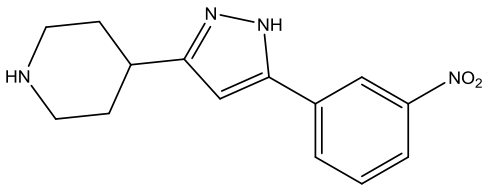


4-(5-(4-tolyl)-1*H*-pyrazol-3-yl) piperidine 3k: This compound was obtained as White solid. Yield: 62%; mp: 158-161ºC. IR (KBr, cm^{-1}) (Figure 16): 3248, 3068, 1361, 1265, 1004, 907. ^1H NMR (400 MHz, $CDCl_3$) (Figure 19): δ 1.68-1.76 (2H, m, CH_2); 1.97-2.00 (2H, m, CH_2); 2.38 (3H, s, CH_3) 2.69-2.77 (1H, m, CH); 2.79-2.85 (2H, m, CH_2); 3.18-3.02 (2H, m, CH_2); 6.34 (1H, s, pyrazol proton); 7.20-7.22 (2H, m, ArH, *J*= 8.4 Hz,); 7.60 (2H, d, *J*= 8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 21): δ 21.3, 33.1, 34.8, 46.5, 99.3, 125.5, 129.4, 137.7, 148.9, 152.8. ESI/MS (Figure 22) 242.2 $[M+1]^+$ calculated for $C_{15}H_{19}N_3$. Anal. Calcd. for $C_{15}H_{19}N_3$: C, 74.65; H, 7.94; N, 17.41; Found: C, 74.93; H, 7.76; N, 17.31.

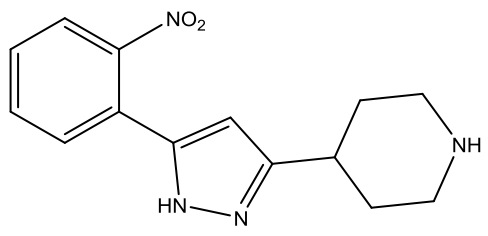


3-chloro-4-(3-(piperidin-4-yl)-1*H*-pyrazol-5-yl) pyridine 3l: This compound was obtained as White solid. Yield: 48%; mp: 176-179ºC. IR (KBr, cm^{-1}): 3339, 3068, 1364, 1265, 1004, 907. ^1H NMR (400 MHz, DMSO): δ 1.47-1.57 (2H, m, CH_2); 1.84-1.87 (2H, m, CH_2); 2.54-2.60 (2H, m, CH_2); 2.74-2.80 (1H, m, CH); 2.98-3.01 (2H, m, CH_2); 6.72 (1H, s, pyrazol proton); 7.84 (1H, d, ArH, *J*= 6 Hz,); 8.50 (1H, d, *J*= 6.4 Hz,

ArH); 8.65 (1H, s, ArH); 13.1 (1H, b, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.0, 34.2, 46.3, 100.2, 119.8, 141.2, 141.6, 147.0, 147.5, 150.4. ESI/MS 263.0 $[M]^+$, 265.1 $[M+2]^+$ calculated for $C_{13}H_{15}ClN_4$. Anal. Calcd. for $C_{13}H_{15}ClN_4$: C, 59.43; H, 5.75; N, 21.32; Found: C, 59.19; H, 5.97; N, 21.19.



4-(5-(3-nitrophenyl)-1*H*-pyrazol-3-yl) piperidine 3m: This compound was obtained as Yellow solid. Yield: 65%; mp: 158-161ºC. IR (KBr, cm^{-1}): 3326, 3002, 2267, 1531, 2217, 1348. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75-1.82 (2H, m, CH_2); 1.99-2.03 (2H, m, CH_2); 2.73-2.77 (2H, m, CH_2); 2.79-2.80 (1H, m, CH); 3.20-3.23 (2H, m, CH_2); 6.43 (1H, s, pyrazol proton); 7.59-7.55 (1H, m, ArH); 8.05 (1H, d, *J*= 8.4 Hz, ArH); 8.22-8.24 (1H, m, ArH, *J*= 8.4 Hz,); 8.56-8.61 (1H, m, ArH, *J*= 8.4 Hz). ^{13}C NMR (100 MHz, DMSO): δ 33.1, 34.7, 46.5, 99.5, 125.6, 127.8, 128.7, 132.4, 149.3, 152.5. ESI/MS 273.0 $[M+1]^+$ calculated for$C_{14}H_{16}N_4O_2$. Anal. Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58; Found: C, 61.59; H, 6.04; N, 20.51.



4-(5-(2-nitrophenyl)-1*H*-pyrazol-3-yl) piperidine 3n: This compound was obtained as Yellow solid. Yield: 67%; mp: 140-142ºC. IR (KBr, cm^{-1}): 3320, 3006, 2258, 2223, 1534, 1344. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.74-1.80 (2H, m, CH_2); 2.00-2.03 (2H, m, CH_2); 2.74-2.78 (2H, m, CH_2); 2.80-2.83 (1H, m, CH); 3.20-3.23 (2H, m, CH_2); 6.48 (1H, s, pyrazol proton); 7.60-7.68 (1H, m, ArH); 7.89-7.92 (1H, m, ArH,) 8.00-8.20 (2H, m, ArH). ^{13}C NMR (100 MHz, DMSO): δ 21.2, 29.0, 35.1, 101.2, 124.4, 125.3, 129.6, 132.4, 135.3, 148.3, 157.0 152.5. ESI/MS 273.0 $[M+1]^+$ calculated for

$C_{14}H_{16}N_4O_2$. Anal. Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58; Found: C, 61.65; H, 6.09; N, 20.79.

4.3.2 Biology

Platelet aggregation study was done as per our previous protocol[16] by using Chrono-Log model 592VS dual channel whole Blood Aggregometer from Chrono-Log Corporation (Havertown, PA, USA). Electrical impedance method was used with 450 µl of whole human blood withdrawn from healthy human volunteers. It was diluted with 450 µl PBS and then incubated at 37°C. Blood sample was equilibrated for 2 min for getting stable baseline. 10 µl of test sample was added followed by addition of 10 µl of ADP while stirring at 1000 rpm. The maximum impedance value was determined and results were analyzed using Aggrolink version 4.75 software up to 6 min. The IC_{50} values were calculated by using three individual experiments at three different concentrations (*viz.* 5, 10 and 15 µM).

4.4 Conclusion

In summary, based on our biological results we identified some of the synthesized pyrazolo-piperidine derivatives as potential lead compounds and significant inhibitors (IC_{50}< 20 µM) **3a, 3c, 3l, 3j** for further *in vitro* and *in vivo* investigation. The compounds were synthesized by an improved process which is more efficient in terms of reaction time and conditions compared to the reported process.[28] Our method offers easy isolation of product with good yield. It is also more suitable for thermally labile compounds. Interestingly, the antiplatelet profile of these compounds offered promising results when compared to standard aspirin.

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