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Novel and efficient process for the synthesis of 1,3,4 Oxadiazole containing MBX-4132

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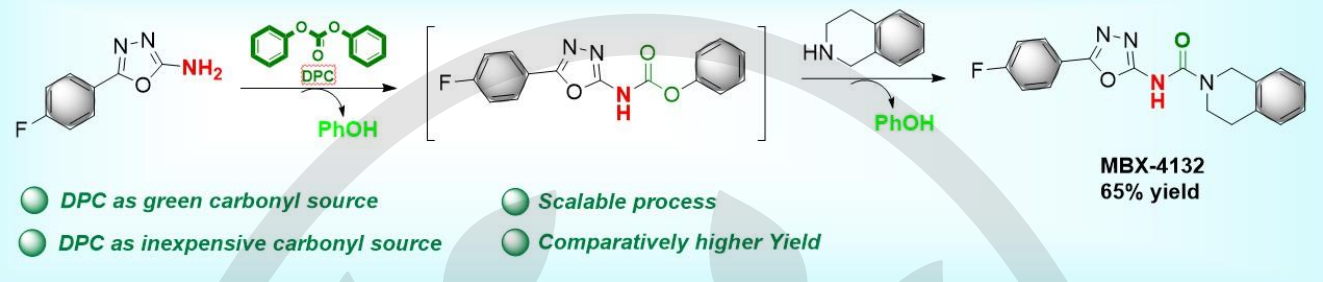
Novel and efficient process for the synthesis of 1,3,4 Oxadiazole containing MBX-4132



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Abstract A novel and efficient approach to the synthesis of MBX-4132 has been reported. Having 1,3,4-oxadiazole containing compound that inhibits trans translation process by binding to the bacterial ribosome and act as an antimicrobial agent. It involved the reaction of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine, with diphenyl carbonate to yield the corresponding carbamates, which *in-situ* reacted with 1,2,3,4-tetrahydroisoquinoline to produce MBX-4132 with a comparatively higher yield (65%). The above process involves mild reaction conditions and uses non-toxic, non-hazardous and cheaper reagents such as diphenyl carbonate as carbonyl source thereby making the process economical and environment friendly.
Keywords Diphenyl carbonate, Aryl carbamate, Unsymmetrical urea, Anti-microbial, 1,3,4-Oxadiazole, MBX-4132.

Introduction

Antibacterial resistance is a natural evolutionary mechanism for microorganisms to survive in adverse conditions. However, the resistant is accelerated by excessive use and misuse of antibacterial agents in human and animals all over the globe. World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS) study[1], concluded that antibiotic resistance is on the rise, particularly in developing countries that may cause significant death and morbidity[2]. In many nations, antibiotic-resistant diseases disproportionately impact, mostly, children and newborns with pneumonia and bloodstream infections (BSIs) and this is one of the leading causes of childhood mortality below five years of age. Bacterial infections resistant to first-line medicines kill approximately 30% of neonates with sepsis.[3] If new antibiotic are not discovered and delivered in near future, antibiotic-resistant bacterial infections would become a significant threat to human health, and approximately with up to 10 million deaths per year are predicted by 2050.[4]

One approach to addressing the anti-microbial resistance (AMR) problem is to create new therapeutic compounds that are sensitive to bacteria. Researchers all over the world are developing novel compounds to prevent the development of antibacterial resistance.[2] To accomplish this, researchers all over the world are searching novel molecular processes that are capable of being targeted. Amongst others, trans-translation, the main quality control mechanism for releasing trapped ribosomes in bacteria, seems to be an ideal choice, providing us to tackle this important cellular function in a completely novel manner.[5]

The majority of newly produced compounds are of heterocyclic nature and motifs containing 1,3,4-oxadiazole ring derivatives may work as useful and promising antimicrobials.[2] The five-membered heterocyclic compounds known as oxadiazoles comprise one oxygen atom, two nitrogen atoms, in their structure. Numerous medications, including anticancer-zibotentan[6], antibacterial-furamizole[7], antiviral-raltegravir[8], ataluren-for Duchenne muscular dystrophy[9], and others (Figure 1&2), contain the isomeric form of oxadiazole. The first synthesis of 1,3,4-oxadiazole derivatives dates back to the end of the nineteenth century. The reactions of suitable hydrazides and phosgene[10], thermal cyclization of 1-acylsemicarbazides[11], or cyclization of 1,2-diacylhydrazines using dehydrating agents[12] were utilized to create the novel 1,3,4-oxadiazole structures.

The number of studies on these molecule increased dramatically from the 1950s to twentieth century.[13, 14] At the moment, scientists are making use of a variety of techniques to produce 1,3,4-oxadiazole derivatives[15], some of which are improved versions of older synthetic methods e.g. cyclization oxidative reactions of *N*-acylhydrazones, cyclodehydration reactions of diacylhydrazines, or hydrazide reactions.[16] There has been a tremendous rise in study on the 1,3,4-oxadiazole ring over the last two decades.[17]

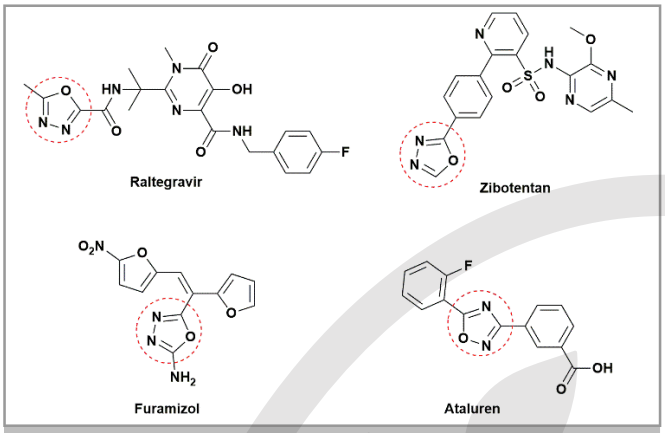


Figure 1 Oxadiazole containing drug molecules

Due to the considerable study on 1,3,4 -oxadiazole derivatives antibacterial properties, numerous oxadiazole derivatives have been investigated for their ability to inhibit the bacterial ribosomes trans-translation process. Keiler's team discovered the most promising oxadiazole-containing compound, KKL-35 from a high-throughput screening test using luciferase assay on a library of many molecules in 2013.[18] This compound with broad-spectrum antibiotic activity served as the initial lead for the development of small molecules that inhibit transtranslation.[19]

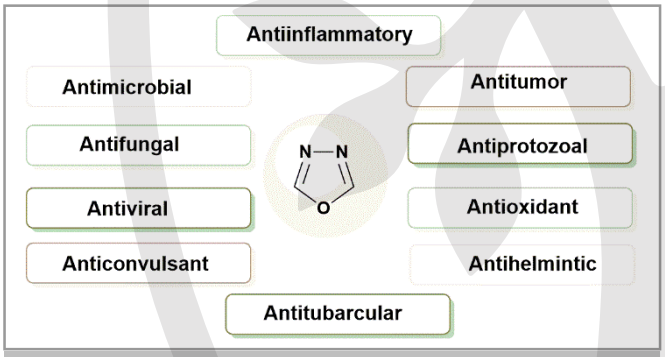


Figure 2 Therapeutic activity of oxadiazole

In KKL-35, substituting α-chloro pyridiyl group for a α-chloro phenyl yielded a significantly stronger inhibitor of trans-translation (compound CT1-83, Figure 3). However, it was found unsuitable for animal use as the amide bond of KKL-35 got rapidly hydrolyzed in liver microsomes.[20,21] As a result, a novel uriedo-oxadiazole derivate, MBX-4132, was developed as an outcome of a recent structure-activity relationship (SAR) programme (Figure 3).[5]

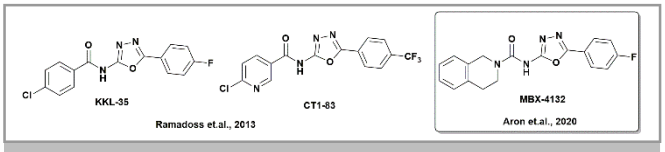


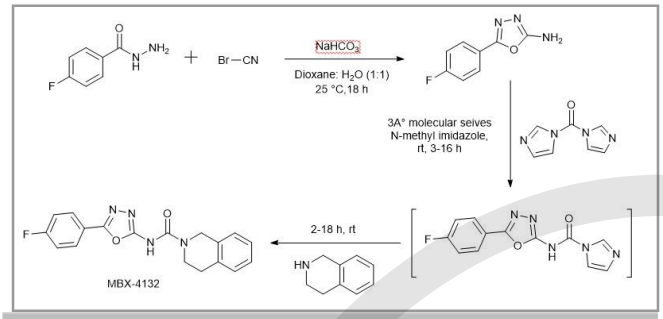
Figure 3 Chemical structure of experimental compounds containing 1,3,4-oxadiazole

When MBX-4132 was tested against multidrug-resistant *Neisseria Gonorrhoeae,* it was 80% eliminated in 6 days from infected mice in a single dose study indicating that the compound was efficacious under an ideal clinical dosing regimen.[19] And, MBX-4132 is substantially more stable than KKL-35 and CT1-83 with almost similar efficacy, and can inhibit trans-translation both in vitro and in vivo.[19] Further, studies showed that the molecules have low toxicity against human cells, enzymes, and receptors making MBX-4132 for further clinical development as an anti-bacterial agent for use against drug-resistant *N. gonorrhoea* in humans.[19]

So, based on important and effective use of MBX-4132 against multi-drug resistance bacteria, particularly against *N. Gonorrhoea,* it was decided to develop a novel, simple, straightforward, and efficient synthesis of this molecule with goal to reduce the cost of synthesis. Mainly because MBX-4132 is presently supplied by Medchemexpress, GLPBIO, biorbyt, MedKoo biosciences, Cambridge biosciences etc. for research and clinical use purpose at high price, and therefore a cost-effective synthesis will be of high importance to chemical and biological scientists.

A review of literature revealed that MBX-4132 synthesis[19,22] involved a three step (Scheme1). 4-fluorobenzhydrazide reacts with cyanogen bromide in the presence of a mild base to yield 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine, which further reacted with carbonyldiimidazole (CDI) to generate a reactive intermediate. similar kind of intermediate prepared using phenyl chloroformates are reported.[23, 24] which in a subsequent displacement reaction with 1,2,3,4-tetrahydroisoquinoline afforded MBX-4132 in a low yield of 40%.

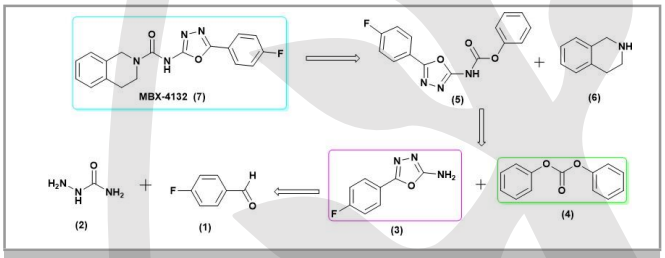
Further, the above reported process has serious drawbacks such as the use of carbonyldiimidazole (CDI) as a coupling reagent which is also an expensive and a moisture sensitive reagent, process require longer reaction time and overall yield of the process was on low side. Herein, a novel, an efficient, three-step synthetic route to MBX-4132 is being reported[25] that involves benign reagents and facile processing and isolation of the final product with at least >60% increased yield for the final step of the synthesis.



Scheme 1 Reported synthesis of MBX-4132

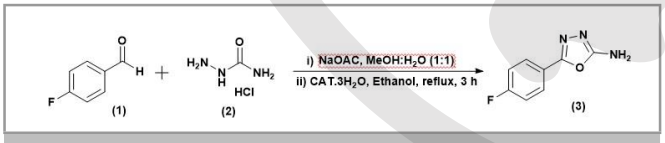
Results and Discussion

It was envisaged that a proposed synthesis of MBX-4132, ((*N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide) (**7**) could be a three-step process. That involved *in-situ* synthesis of phenyl (5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) carbamate (**5**) from, its corresponding amine (**3**), followed by its reaction with (**4**) (DPC) to yield (**5**) which upon *in-situ* reaction with (**6**) gave MBX-4132 (Scheme 2).



Scheme 2 Proposed synthesis of MBX-4132 (**7**)

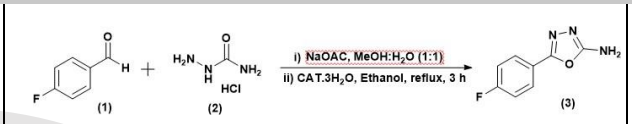
Our quest started with the preparation of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (**3**) using semicarbazide hydrochloride (**2**) and 4-fluoro benzaldehyde (**1**) (Scheme 3) under different reaction conditions.[26-28] The use of molecular iodine[29] for cyclization reaction of above reagent in 1,4-dioxane either at 80 ˚C or at 80 ˚C -120 ˚C for 24 hours (entry 1, Table 1) gave incomplete consumption of the reagents. And when chloramines-T trihydrate was used as the cyclising agent in ethanol 80 ˚C for 3 hours the desired oxadiazole amine was isolated in 80% yield.[30] (entry 2, Table 1)



Scheme 3 Synthesis of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine

After successful synthesis of (**3**), a carbamate synthesis was carried out *i.e.* phenyl (5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) carbamate (**5**) was synthesized using (**4**) as a green carbonyl source.

Table 1 Optimization of reaction condition for preparation of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (3)



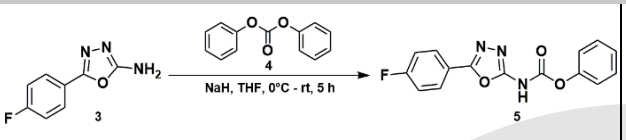
Entry	Reaction conditions	Observation
2	NaOAc, I_2, K_2CO_3, 1,4-dioxane rt to 80 °C-120 °C, 24 h	Complete conversion was not observed
3	**NaOAc, CAT-3H$_2$O, EtOH, rt to 80 ˚C, 3 h**	**80%**

In earlier synthesis of MBX-4132, carbonyldiimidazole (CDI) has been used as a coupling reagent/carbonyl source. CDI is a costly and moisture sensitive reagent, and gave very poor yield of the final product which ultimately lowered the overall yield of the process. Other reagents, such as isocyanates, chloroformates, phosgene etc., used for the synthesis of unsymmetrical ureas[24] - MBX-4132 also contains an unsymmetrical urea in its scaffold – are generally hazardous, costly and difficult to handle on-scale.

Our earlier work explored diphenyl carbonate chemistry and it has led to the synthesis of both alkyl and aryl carbamates, synthesis of various sulfonylureas,[31] monosubstituted ureas,[32] hydantoins[33] and semicarbazides.[34] Apart from this diphenyl carbonate has been used to synthesize *N*-aryl carbamates or *N*-alkyl carbamates using metal catalysts[35] and also involved in the preparation of urazole[36] synthesis. Therefore, diphenyl carbonate (**4**) was the reagent of choice to prepare phenyl 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) carbamate (**5**) – a much desired intermediate in the synthesis of MBX-4132 (**7**).

For carbamate synthesis, our earlier optimized reaction conditions were applied[31] but without any success (entry 1 Table 2), possibly due to lower basicity (pKa <3) of the amine. Then, other reaction conditions using different bases and solvents e.g. DBU, DMAP, *t*-BuOk, Cs_2CO_3 in acetonitrile, DMF, THF (entries 2-5, Table 2) were also found futile as no carbamate product was detected. However, when a strong base, NaH in THF was used to generate the amine anion, the desired product was formed, as shown by thin layer chromatography along with a little of unreacted starting materials. The complete conversion to carbamate was observed when NaH concentration was increase to 4 equivalents, but at 2 equivalents unreacted starting materials were still present.

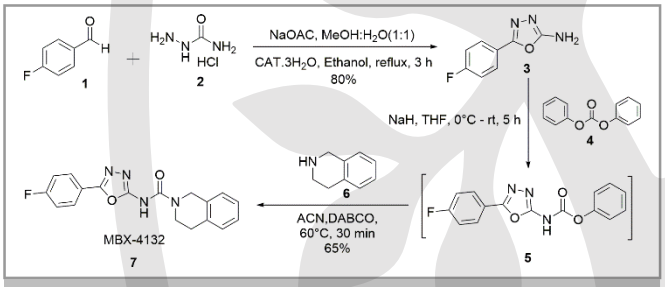
Table 2 Optimization of reaction condition for synthesis of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) carbamate (**5**)



Entry	Reaction conditions	Observation
1	H_2O: THF, rt, 24 h	nd
2	DBU, Acetonitrile, 80 ˚C, 24 h	nd
3	DMAP, THF, 80 ˚C, 24 h	nd
4	Cs_2CO_3, THF, 80 ˚C, 24 h	nd
5	*t*-BuOk, DMF, 80-130 ˚C, 24 h	nd
6	NaH, THF, 0 ˚C to rt, 5 h	Used as such (crude)

[a]nd=not detected

The crude (**5**), without any purification, was reacted with 1,2,3,4-Tetrahydroisoquinoline (**6**) (Scheme 4) in acetonitrile and catalytic amount of DABCO at 65 ˚C for 30 min. to yield (**7**) in 65% yield. The overall yield of the process is 52%.



Scheme 1 Synthesis of MBX-4132 (*N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide)

Conclusions

MBX-4132, *N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (**7**), has been synthesized in a three-step process on gram scale with highest overall yield (52%) to date. This novel process avoids the use of hazardous, toxic and expensive reagents; hence it is suitable for scale-up synthesis.

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All reagents and starting materials were supplied from commercial sources. And used as such without purification otherwise mentioned. All reactions performed in round bottom flask with reflux condition as well as in a screw-capped vial. The progress of reaction was monitored by thin layer chromatography (TLC). TLC plates were visualized in UV light and iodine chamber. The 1H and 13C NMR spectra were obtained in DMSO-d_6 as a solvent using 500, 600 and 125, 151 MHz spectrometer respectively with internal reference standard SiMe4. High resolution mass spectra (HRMS) were obtained under electron spray ionisation technique (ESI) and LC-MS/LTQ was obtained in APCI mode. Chemical shifts *(δ)* are reported in parts per million *(ppm)*. Coupling constant *(J)* were reported in Hz. The abbreviation used to characterize the signal are as follows *s* = singlet, *d* = doublet, *dd* = double of doublet, *t* = triplet, *m* = multiplet.

General procedure for the preparation of **Synthesis of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (3)**[19, 37]

In a 100 mL round-bottom flask equipped with magnetic stirrer bar, semicarbazide hydrochloride (2.78 g, 25.0 mmol) and sodium acetate (2.05 g, 25.0 mmol) in H_2O (10 mL) at room temperature was added a solution of *p*-fluoro-benzaldehyde (3.10 g, 25.0 mmol) in MeOH (10 mL), and the resultant mixture was stirred at room temperature for 10 minutes. After that, it was concentrated under reduced pressure, and the residue obtained was dissolved in ethanol and treated with chloramine-T (6.82 g, 30.0 mmol) under reflux for 3 h. After completion of the reaction, sodium chloride formed in the reaction was filtered off, and residue was washed with ethanol. The filtrate and ethanol washings were combined and concentrated under reduced pressure to get crude residue which was then extracted with 10% HCl (15.0 ml). The aqueous acidic layer was washed with dichloromethane (2x30 ml), then neutralized with 10% NaOH (15.0 ml) to obtain 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine as a white solid in (80% 1.29 g) isolated yield.

^{1}H NMR (600 MHz, DMSO-d_6) δ = *(ppm)* 7.80 (dd), 7.41 (dd), 7.02 (s). ^{13}C NMR (151 MHz, DMSO-*d6*) δ = *(ppm)* 164.43, 162.81, 157.14, 128.02, 121.58, 116.93. ^{19}F NMR (500 MHz, DMSO-d_6) δ = *(ppm)* -109-73 (F). HRMS (ESI): m/z calcd. for $C_8H_6FN_3O$: 179.0495; found, [M+1]: 180.0576.

Synthesis of MBX-4132, *N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (7)[19, 37]

To a slurry of NaH (0.19 g, 8.0 mmol) in anhydrous THF at 0˚C was added 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (0.35 g, 2.0 mmol) and the mixture was allowed to warm to room temperature over the period of 1.5 h. To above mixture was added a solution of diphenyl carbonate (0.85 g, 4.0 mmol), in anhydrous THF (5.0 ml) and the reaction mixture was kept for stirring at room temperature for 4-5 h to get phenyl- (5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) carbamate as the reactive intermediate. This was followed by removal of the solvent and *in-situ* treatment with 1,2,3,4-tetrahydroisoquinoline (0.46 g, 3.5 mmol) and DABCO (0.2 equivalent) in acetonitrile under reflux for 30 minutes to yield MBX-4132. The crude product was filtered off and washed with hexane to obtain off-white amorphous solid in an isolated yield of (65% 0.8556 g).

^{1}HNMR (600 MHz, DMSO-d_6) δ = *(ppm)* 7.92 (d, *J* = 3.3 Hz), 7.39 (t, *J* = 8.8 Hz), 7.15 (m), 4.65 (s), 3.71 (t, *J* = 5.0 Hz), 2.82 (t, *J* = 5.7 Hz).^{13}C NMR (151 MHz, DMSO-d_6) δ = *(ppm)* 169.72, 163.93, 162.15, 155.02, 135.73, 129.18, 127.4, 126.73, 126.24, 116.63, 49.13, 47.35, 29.23.^{19}F NMR (500 MHz, DMSO-d_6) δ = *(ppm)* -108.02 to -109-70 (F). HRMS (ESI): m/z calcd. for $C_{18}H_{15}FN_4O_2$: 338.1179; found, [M + Na$^+$] $C_{18}H_{15}FN_4NaO_2$: 361.1076; 361.1078. FTIR (KBr): υ (cm^{-1}) = 3150 (NH), 1648 (C=O); mp 185-195 °C (Lit; ≥190 °C).

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Conflict of Interest

"There are no conflicts to declare".

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Supporting Information

Novel and efficient process for the synthesis of 1,3,4 Oxadiazole containing MBX-4132

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Contents

General Consideration

All reagents and starting materials were supplied from commercial sources. And used as such without purification otherwise mentioned. All reactions performed in round bottom flask with reflux condition as well as in a screw-capped vial. The progress of reaction was monitored by thin layer chromatography (TLC). TLC plates were visualized in UV light and iodine chamber. The 1H and 13C NMR spectra were obtained in DMSO-d_6 as a solvent using 500, 600 and 125, 151 MHz spectrometer respectively with internal reference standard MeSi4. High resolution mass spectra (HRMS) were obtained under electron spray ionisation technique (ESI) and LC-MS/LTQ was obtained in APCI mode. Chemical shifts *(δ)* are reported in parts per million *(ppm).* Coupling constant *(J)* were reported in Hz. The abbreviation used to characterize the signal are as follows *s* = singlet, *d* = doublet, *dd* = double of doublet, *t* = triplet, *m* = multiplet.

Synthesis of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (1)[1, 2]

In a 50 mL round-bottom flask equipped with magnetic stirrer bar, semicarbazide hydrochloride (2.78 g, 25.0 mmol) and sodium acetate (2.05 g, 25.0 mmol) in H_2O (10 mL) at room temperature was added a solution of *p*-fluorobenzaldehyde (3.10 g, 25.0 mmol) in MeOH (10 mL), and the resultant mixture was stirred at room temperature for 10 minutes. After that, it was concentrated under reduced pressure, and the residue obtained was dissolved in ethanol and treated with chloramine-T (6.82 g, 30.0 mmol) under reflux for 3 h. After completion of the reaction, sodium chloride formed in the reaction was filtered off, and residue was washed with ethanol. The filtrate and ethanol washings were combined and concentrated under reduced pressure to get crude residue which was then extracted with 10% HCl (15.0 ml). The aqueous acidic layer was washed with dichloromethane (2x30 ml), then neutralized with 10% NaOH (15.0 ml) to obtain 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine as a white solid in (80% 1.29 g) isolated yield.

^{1}H NMR (600 MHz, DMSO-d_6) δ = *(ppm)* 7.86-7.80 (dd, 2H), 7.46-7.41 (dd, 2H), 7.02 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ = *(ppm)* 164.43, 162.81, 157.14, 128.02, 121.58, 116.93. ^{19}F NMR (500 MHz, DMSO-d_6) δ = *(ppm)* -109-73 (F). HRMS (ESI): m/z calcd. for $C_8H_6FN_3O$: 179.0495; found, [M+1]: 180.0576.

Synthesis of MBX-4132, *N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (7)[1, 2]

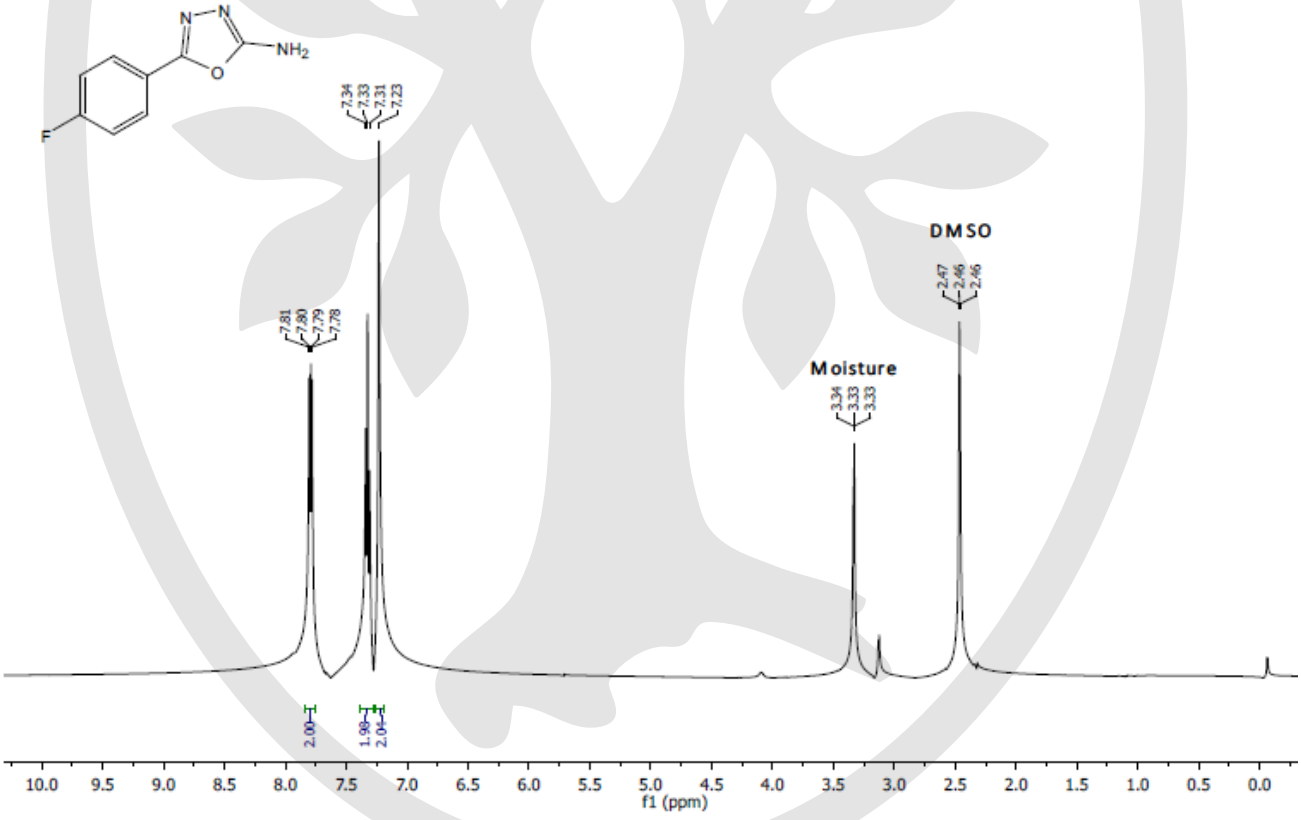
To a slurry of NaH (0.19 g, 8.0 mmol) in anhydrous THF at 0˚C was added 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (0.35 g, 2.0 mmol) and the mixture was allowed to warm to room temperature over the period of 1.5 h. To above mixture was added a solution of diphenyl carbonate (0.85 g, 4.0 mmol), in anhydrous THF and the reaction mixture was kept for stirring at room temperature for 4-5 h to get phenyl- (5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)carbamate as the reactive intermediate. This was followed by removal of the solvent and *in-situ* treatment with 1,2,3,4-tetrahydroisoquinoline (0.46 g, 3.5 mmol) and DABCO (0.2 equivalent) in acetonitrile

under reflux for 30 minutes to yield MBX-4132. The crude product was filtered off and washed with hexane to obtain off-white amorphous solid in an isolated yield of (65% 0.8556g).

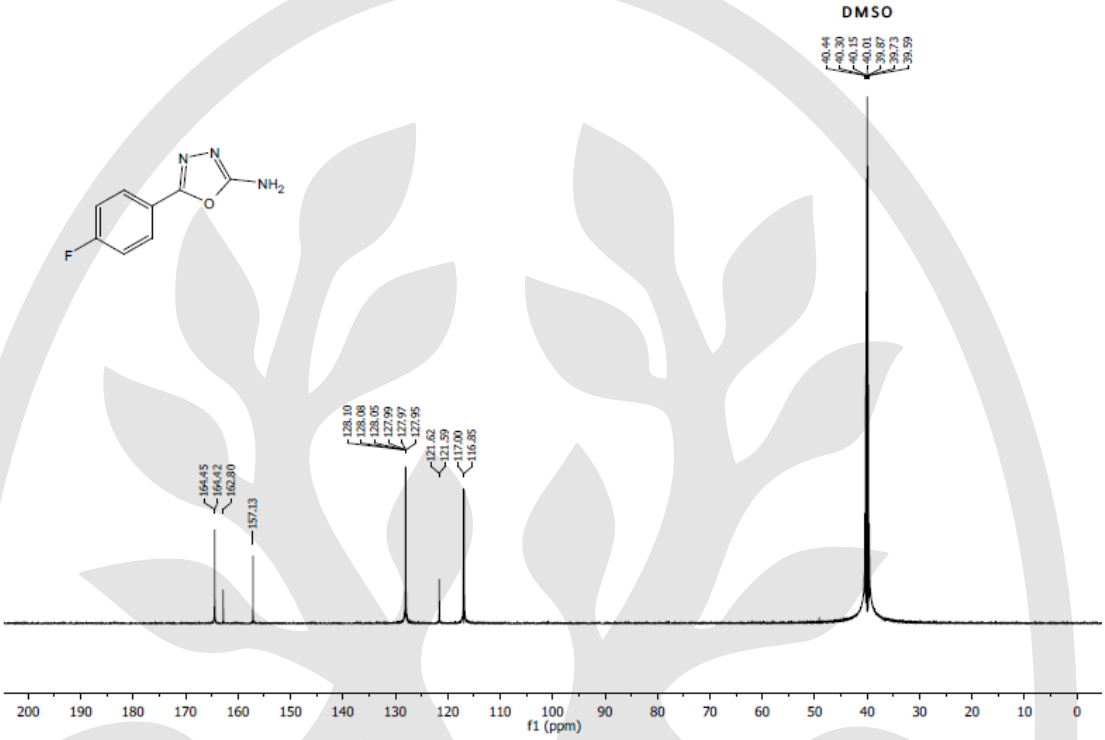
^{1}H NMR (600 MHz, DMSO-d_6)[1] δ = *(ppm)* 7.90-7.92 (d, *J* = 3.3 Hz, 2H), 7.45-7.39 (t, *J* = 8.8 Hz, 2H), 7.18-7.15 (m, 4H), 4.65 (s, 2H), 3.71 (t, *J* = 5.0 Hz, 2H), 2.82 (t, *J* = 5.7 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ = *(ppm)* 169.72, 163.93, 162.15, 155.02, 135.73, 129.18, 127.4, 126.73, 126.24, 116.63, 49.13, 47.35, 29.23.^{19}F NMR (500 MHz, DMSO-d_6) δ = *(ppm)* -108.02 to -109-70 (F). HRMS (ESI): m/z calcd. for $C_{18}H_{15}FN_4O_2$: 338.84; found, [M+Na$^+$] $C_{18}H_{15}FN_4NaO_2$: 361.1076; found: 361.1078. IR (KBr): υ (cm^{-1}) = 3150 (NH), 1648 (C=O); mp 185-195 °C; (Lit; ≥190 °C).

^{1}H and ^{13}C, ^{19}F NMR Data

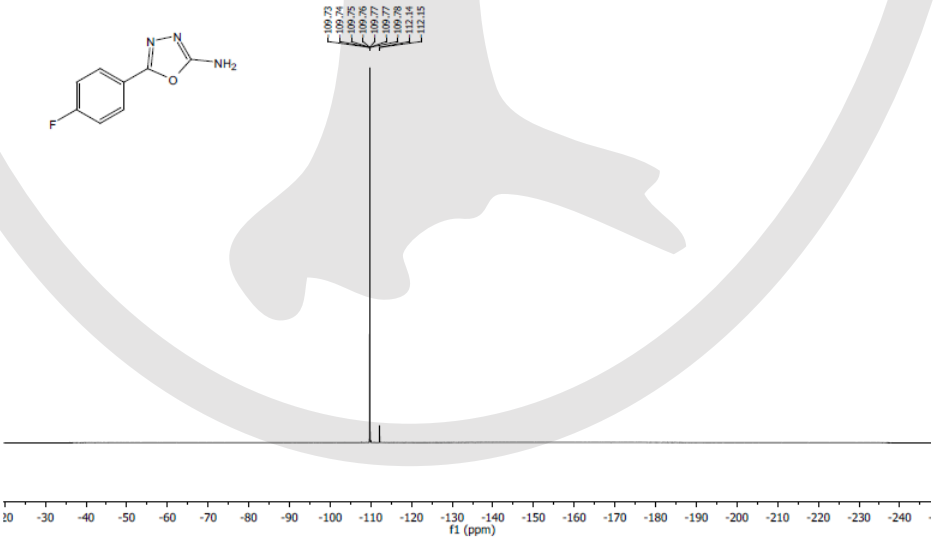
^{1}H NMR Spectra of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (3)[1, 2]



^{13}C NMR Spectra of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (3)[1, 2]



^{19}F NMR Spectra of 5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (3)



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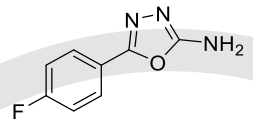

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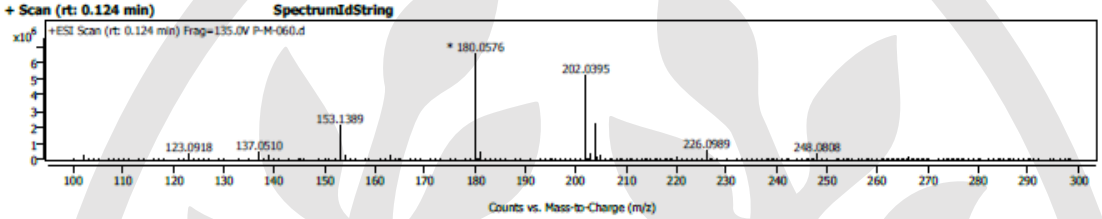

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137.0510		469838	7.28					
153.1389		2067764	32.03					
180.0576	1	6455338	100.00					
181.0602	1	463814	7.18					
202.0395	1	5114423	79.23					
203.0422	1	358364	5.55					
204.0547		2144520	33.22					
226.0989		524389	8.12					
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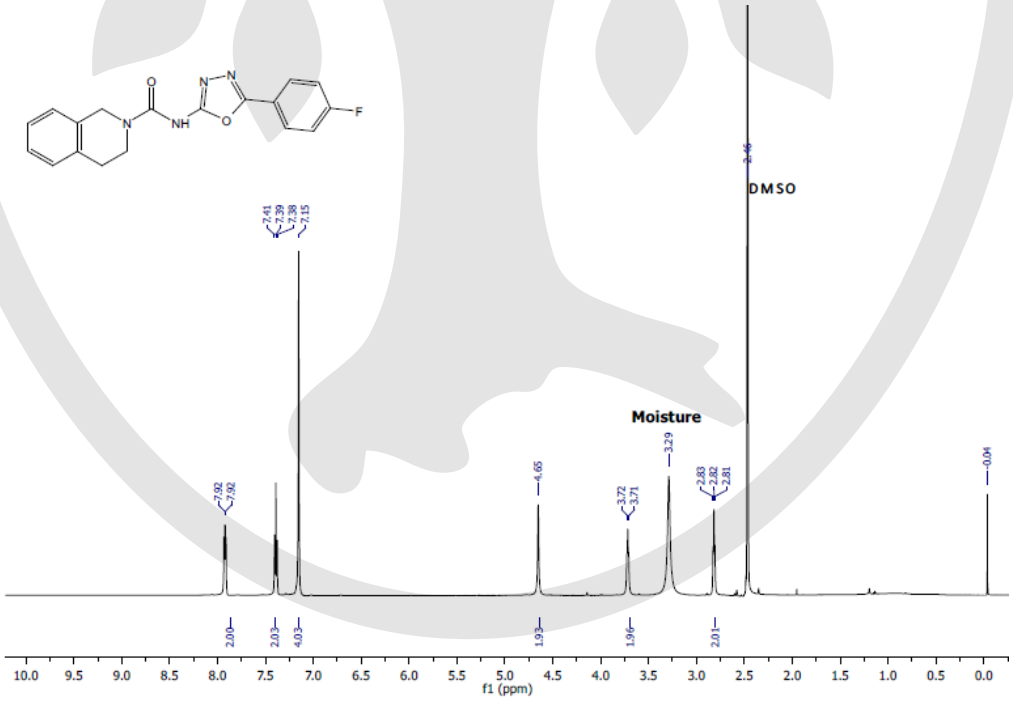
MassHunter Qual 10.0
(End of Report)

^{1}H NMR Spectra of MBX-4132 (7)[2]



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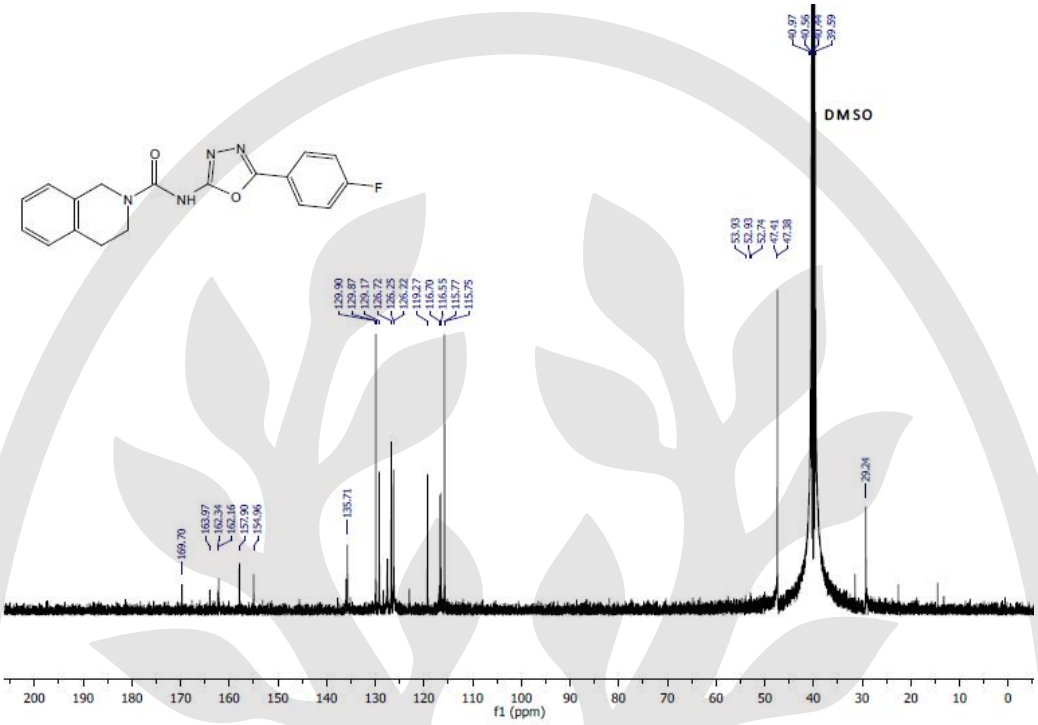
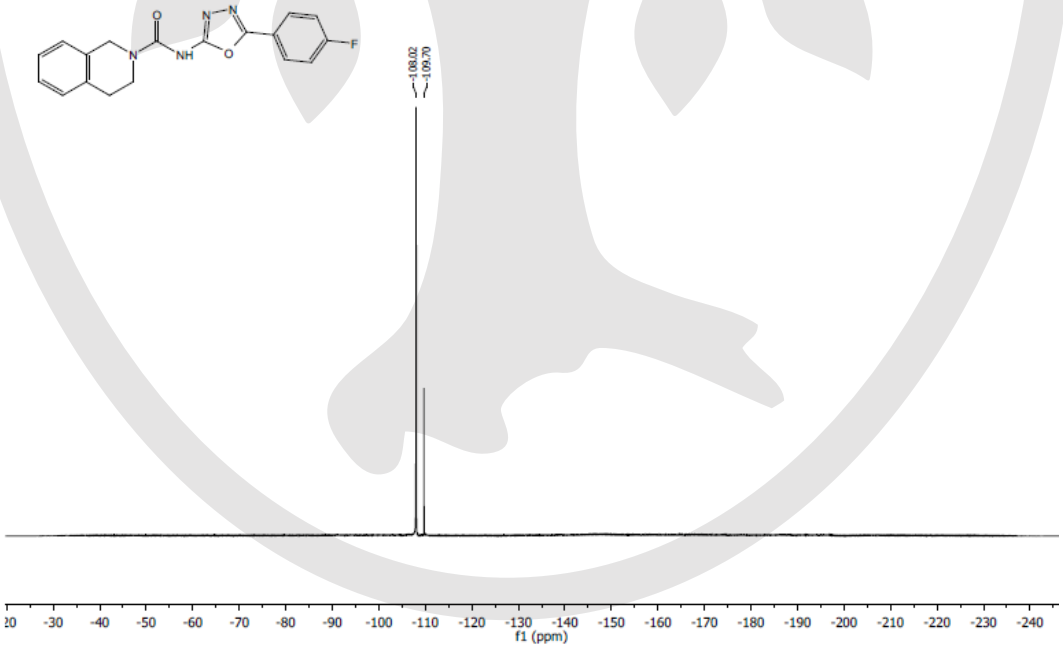


^{19}F NMR Spectra of MBX-4132 (7)



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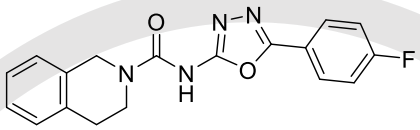

Molecular Weight: 338.34

Peak Spec

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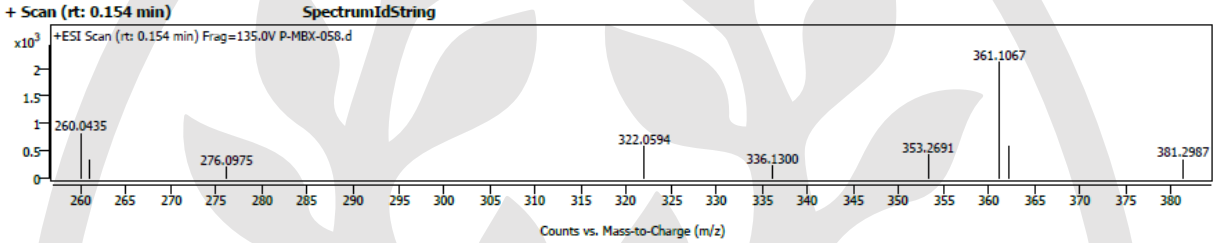

SpectrumIdString

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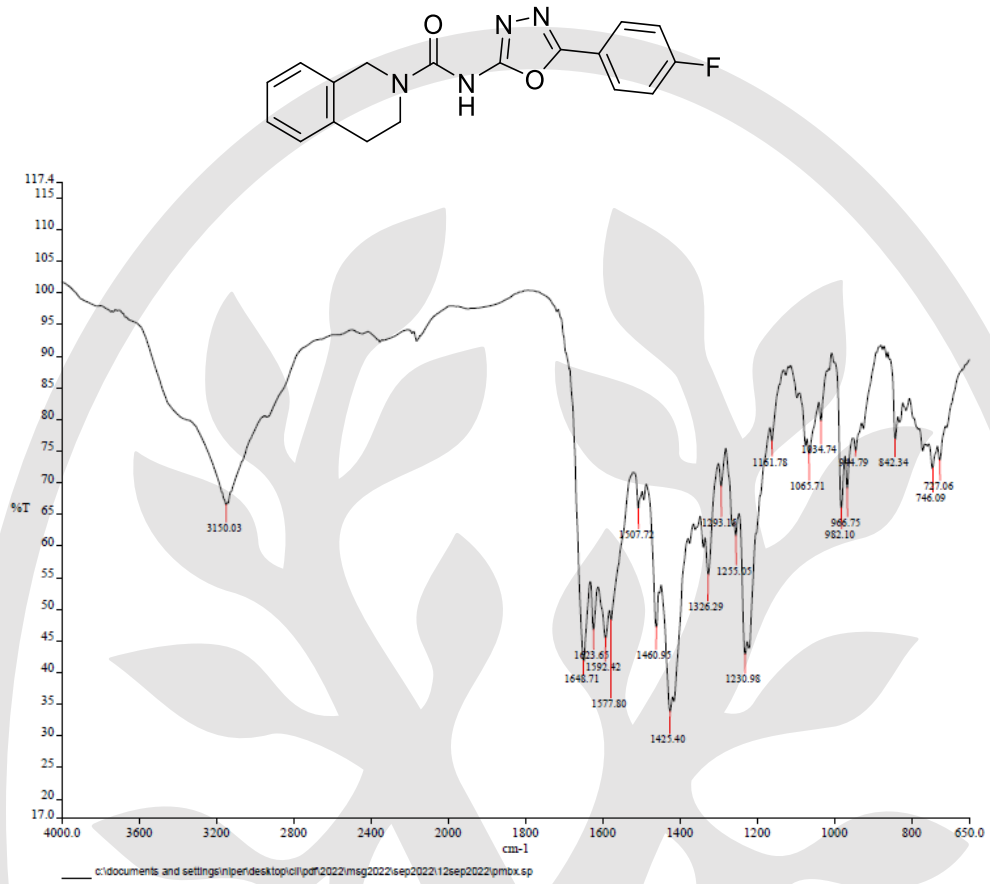
260.0539		233	11.13					
261.0489		316	15.11					
276.0975		200	9.57					
322.0594		565	27.03					
336.1300		224	10.70					
353.2691		428	20.44					
361.1067	1	2092	100.00					
362.1099	1	578	27.64					
381.2987		337	16.11					

MassHunter Qual 10.0
(End of Report)

FTIR spectra of MBX-4132 (7)



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