



NOTE

Allergy-preventive effects of linarinic acid and its tetrahydropyrrolo[2,1-b]quinazoline derivatives isolated from *Linaria vulgaris*

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Received: 15 December 2017 / Accepted: 2 February 2018
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Abstract

Linarinic acid, (–)-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (**4a**), was isolated from the ethanol extract of *Linaria vulgaris* Mill. In our previous study, a series of tetrahydropyrrolo[2,1-b]quinazoline derivatives **4b**, **4c**, **5a**, **5b**, **6a** and **6b** that were structurally related to **4a** and evaluated as neuroprotective agents were synthesized. The aim of the present study was to investigate the novel features of these compounds. We examined their allergy-preventive effects using an in vivo assay system we developed previously, that monitors a decrease in blood flow in the tail vein of mice subjected to sensitization with hen egg-white lysozyme. We observed that **4a** and its three derivatives, amide (**6a**), ester (**5a**), bromine (**4b**), and alcohol substituent (**6b**), showed significant allergy-preventive activities. The study confirmed the allergy-preventive activity of tetrahydropyrrolo[2,1-b]quinazoline derivatives by comprehensively monitoring the specific blood flow decrease occurring in the induction phase of allergy. This finding may aid in the development of new agents for the treatment of allergic diseases such as atopic dermatitis, allergic asthma, and hay fever.

Keywords *Linaria vulgaris* · Tetrahydropyrrolo[2,1-b]quinazoline · Allergy-preventive activity · Hen egg-white lysozyme · Linarinic acid · Blood flow

Introduction

(–)-Linarinic acid, (–)-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (**4a**), is an optimally active tricyclic quinazoline alkaloid that was isolated from *Linaria vulgaris* Mill., which is distributed in the northeastern and Inner Mongolia regions of China. *L. vulgaris* is a traditional Chinese medicine plant used for treating scattered stasis,

detumescence, headache, jaundice, and dermatosis, and as antitussive, anti-asthmatic, diuretic, and laxative agents [1]. We accomplished the total asymmetric synthesis and assignment of absolute configuration for this natural product in 2006 [2]. In our previous study, various derivatives were designed and synthesized, which were evaluated as neuroprotective agents against ischemia-induced cell injury in an in vitro oxygen–glucose deprivation model of ischemic stroke in SH-SY5Y cells. Most of the target compounds exhibited moderate to excellent protective potency [3].

In a continuing effort to determine the new features of **4a**, we examined its preventive effect against allergy using the system we developed previously. This assay system monitors the decrease in blood flow (BF) in the tail vein of mice subjected to hen egg-white lysozyme (HEL) sensitization alone without HEL challenge as a guide [4–6]. We termed this phenomenon ‘the induction phase of the allergy’ which progressed with xenobiotics. HEL-sensitized mice showed various immunological responses including production of anti-HEL immunoglobulin E (IgE) antibody during this phase, and re-administration of HEL (challenge with HEL) induced

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11418-018-1187-9>) contains supplementary material, which is available to authorized users.

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antigen-specific anaphylaxis [7]. The BF of HEL-sensitized mice (control group) gradually and significantly decreased to 70–80% of the BF of normal mice at day 9, without changes. BF decrease is extremely complicated and involves various factors such as nitric oxide (NO), thromboxane A₂ (TXA₂), prostacyclin (PGI₂), and endothelin-1 (ET-1) together with granulocytic elastase (GE), cyclooxygenase (COX)-1 and -2, inducible nitric oxide synthase (iNOS), and constitutive nitric oxide synthase (cNOS) [4]. The reduction in BF occurs via iNOS-independent and -dependent pathways [5], and this decrease is similar to ‘oketsu’ (the state of blood stagnation) in Oriental medicine [6]. This monitoring system would be useful when searching for substances that can prevent complicated inflammatory allergies involving NO, TXA₂, PGI₂, ET-1, GE, COX-1, COX-2, and oketsu.

In this study, we evaluated the allergy-preventive effects of **4a** and its derivatives, which were designed and synthesized for obtaining further knowledge on structure–activity relationships (SAR), to investigate the novel features of quinazoline alkaloids from *L. vulgaris*, and develop a novel agent for the treatment of allergic diseases.

Materials and methods

Materials

Complete Freund’s adjuvant (CFA), HEL, and epinastine hydrochloride were purchased from Difco (MI, USA), Sigma Co., Ltd. (St. Louis, MO, USA), and Tokyo Chemical

Industry Co., Ltd. (Tokyo, Japan), respectively. The synthesis of tetrahydropyrrolo[2,1-b]quinazoline derivatives **4a**, **4b**, **4c**, **5a**, **5b**, **6a**, and **6b** containing different substituents on the tricyclic quinazoline ring has been reported previously (Fig. 1). In this study, we synthesized the derivatives according to the reported methods. The detailed preparation method and the spectral and elemental analysis data of their compounds have been reported elsewhere [3].

Animals

Four-week-old male ddY mice (SPF grade) were obtained from Japan SLC, Inc. (Shizuoka, Japan) and housed at 26 ± 2 °C. The mice were provided ad libitum access to food and water. All animal experiments were performed in accordance with the Guidelines for Animal Experiments of Mukogawa Women’s University.

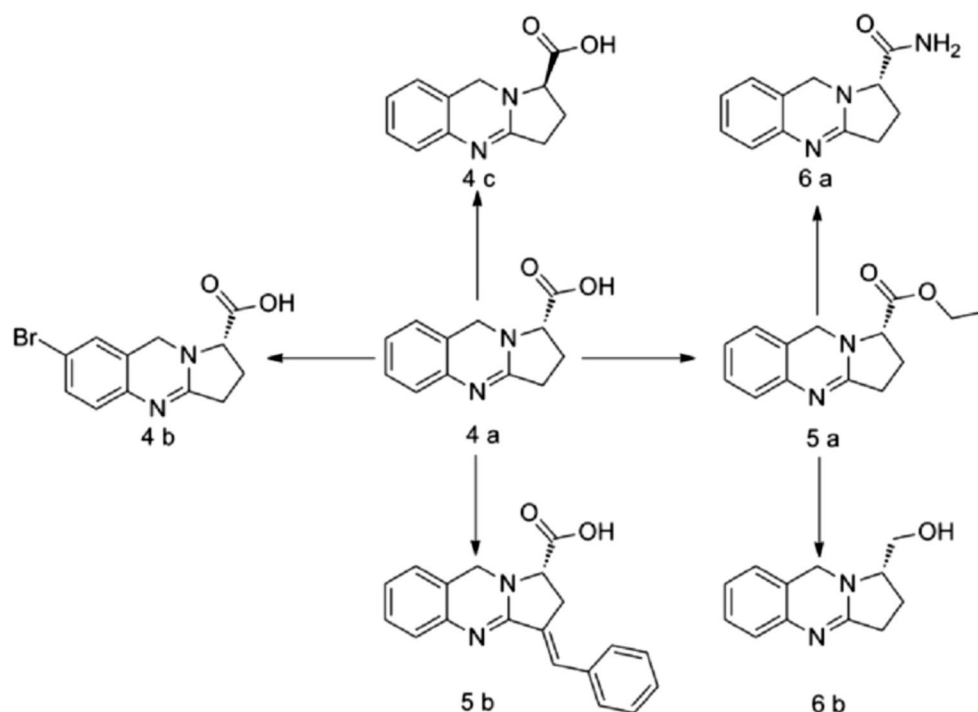
HEL sensitization

Sensitization with HEL was performed as previously described [4]. On day 0, 5-week-old male ddY mice were injected intraperitoneally with 50 µg of HEL in 50% CFA.

BF measurement

Subcutaneous BF in mice tail veins was monitored using a laser Doppler blood flow meter (FLO-C1; Neuroscience, Tokyo, Japan) as reported previously [5]. Each mouse was warmed for 15 min at 36 °C prior to the experiment, and

Fig. 1 Structures of seven tetrahydropyrrolo[2,1-b]quinazoline derivatives examined in this study



then placed on a holder (Muromachi Kikai Co., Ltd., Tokyo, Japan) in a measuring chamber kept at 36 °C throughout the measurement. The systolic microcirculatory BF in the tail veins of unanesthetized mice was measured ($n = 5$). Normal BF was measured for 10 min 1 day before initiation of the study. The BF of sensitized mice was measured for 10 min every other day up to 7 days after sensitization. The results were expressed as the relative percent of normal BF for each mouse.

In vivo assay method of allergy-preventive activity

Compound **4a** at 10 and 20 mg/kg, and each derivative at 50 $\mu\text{mol/kg}$ were dissolved in water and administered (10 μL of test solution/g body weight) orally to HEL-sensitized mice at days 0 (1 h before sensitization), 3 and 6. The control group was administered water on the same schedule.

Statistical analysis

Data are presented as mean $\pm$ SE and analysis was performed using GraphPad Prism 6. ANOVA was applied for between-group comparisons. When significant differences ($p < 0.05$) were identified, the data were further analyzed by Dunnett's hoc multiple comparison test coupled with Bonferroni inequality for significant differences between each test group and the control group. For the Bonferroni test, 4 points were used 3 days after HEL sensitization because a significant difference was observed between the BF of untreated and sensitized mice after 3 days. The results were considered significant at $p < 0.05$.

Results

Synthesis and identification of compounds

Tetrahydropyrrolo[2,1-*b*]quinazoline derivatives **4a**, **4b**, **4c**, **5a**, **5b**, **6a**, and **6b** containing different substituents on the tricyclic quinazoline ring were synthesized according to previously reported methods (Fig. 1). The detailed preparation method and the spectral and elemental analysis data of their compounds have been reported elsewhere. The structures of these compounds were identified by comparison of their physical and spectroscopic data (UV, MS, IR, ^1H -, and ^{13}C -NMR) with the literature values (Supplementary data). These compounds were subjected to bioassay.

Effects of **4a** on HEL-induced BF decrease in the allergic induction phase

The BF in the tail vein of HEL-sensitized mice (control group) gradually and significantly decreased to

approximately 70% of the BF of normal mice at day 9 (Fig. 2). However, oral administration of the lead compound **4a** (20 mg/kg) significantly ($p < 0.05$) inhibited the BF decrease compared with the control group after day 7 of HEL sensitization. Compound **4a** at a dose of 10 mg/kg (46 $\mu\text{mol/kg}$, p.o.) showed significant effectiveness. Therefore, we examined the activity of other compounds at 50 $\mu\text{mol/kg}$ (p.o.) on HEL-induced BF decrease.

Effects of derivatives of **4a** on HEL-induced BF decrease in the allergic induction phase

Figure 3 shows the effects of epinastine hydrochloride of the positive control and **4a** at 50 $\mu\text{mol/kg}$ (p.o.). Epinastine hydrochloride and **4a** significantly inhibited HEL-induced BF decrease after day 7 of HEL sensitization. Figure 4 shows the effects of **4a** and its derivatives at 50 $\mu\text{mol/kg}$ (p.o.). The bromo derivative **4b** and ethyl ester derivative **5a** significantly inhibited HEL-induced BF decrease after day 5 of HEL sensitization. However, **4c** showed no significant inhibition on HEL-induced BF decrease after day 9 of HEL sensitization (Fig. 4a). Compounds **4a** and **6a** significantly inhibited HEL-induced BF decrease. **6b** showed a significant suppressant effect, which was weaker than that of **4a** and **6a**, while **5b** showed no significant inhibition (Fig. 4b). Compounds **4a**, **4b**, **4c**, **5a**, **5b**, **6a**,

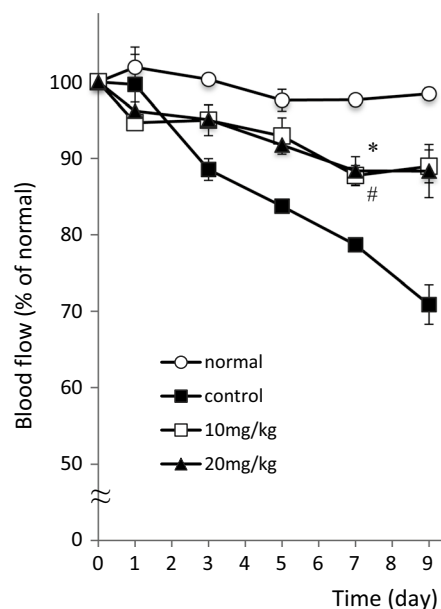


Fig. 2 Inhibitory effect of compound **4a** on HEL-induced blood flow decrease in male ddY mice. Open circle: normal (untreated mice); filled square: control (blood flow of HEL-sensitized mice); open square and filled triangle: treatment with compound **4a** at days 0 (starting day), 3 and 6 from sensitization. Each value presents the mean $\pm$ SE ($n = 5$). * and # $p < 0.05$ compared with the control (Dunnett's test with Bonferroni)

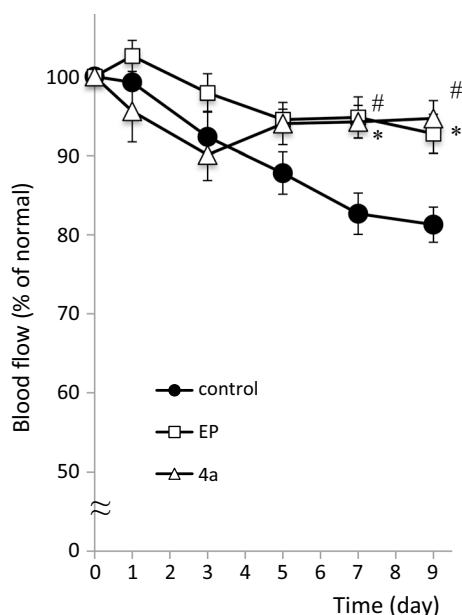


Fig. 3 Inhibitory effect of compounds **4a** and epinastine hydrochloride on HEL-induced blood flow decrease in male ddY mice. Filled circle: control (blood flow of HEL-sensitized mice); open triangle: pretreatment with 50 $\mu\text{mol/kg}$ **4a** or epinastine hydrochloride at days 0 (starting day), 3 and 6 from sensitization. Each value presents the mean $\pm$ SE ($n = 5$). * and # $p < 0.05$ compared with the control (Dunnett's test with Bonferroni)

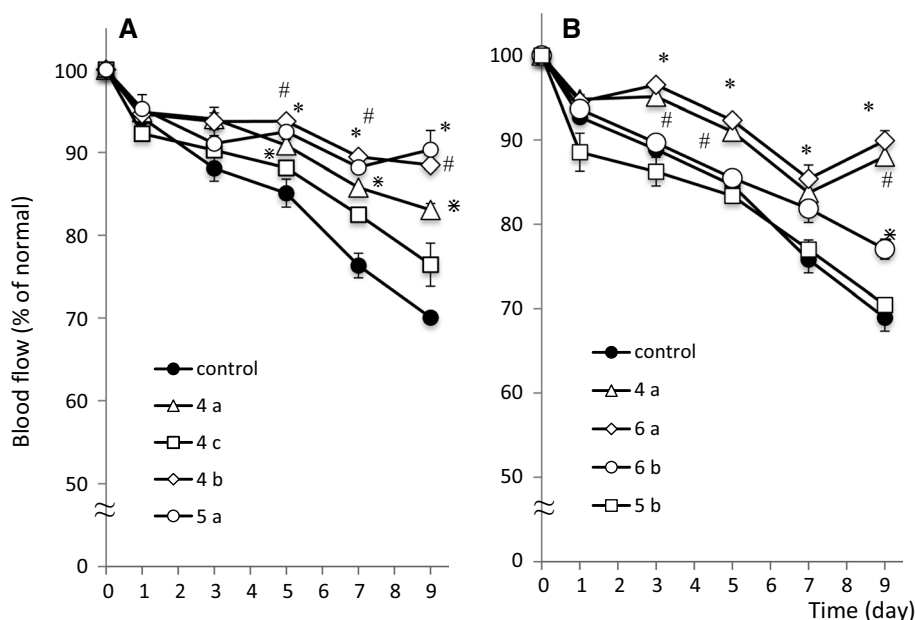
and **6b** when treated individually did not affect the normal BF (data not shown). Therefore, it can be suggested that compounds **4a**, **4b**, **5a**, **6a** and **6b** improved the HEL-induced BF decrease in the allergy induction phase, but did not increase the normal BF.

Discussion

A series of tetrahydropyrrolo[2,1-*b*]quinazoline derivatives **4b**, **4c**, **5a**, **5b**, **6a**, and **6b** that were structurally related to **4a** and evaluated as neuroprotective agents were synthesized in our previous study [3].

In this study, we investigated the allergy-preventive effects of **4a** and its related compounds using an in vivo assay method to identify substances that can prevent complicated inflammatory allergy. This assay system monitors the decrease in BF of mice during 'the induction phase of the allergy' induced by HEL [4–6]. HEL-sensitized mice showed various immunological responses including production of anti-HEL IgE antibody during this phase. The re-administration of HEL (challenge with HEL) induced antigen-specific anaphylaxis after day 9 as previously reported [7]. The lead compound **4a** significantly inhibited BF decrease (Figs. 2, 3) in the HEL-induced allergy induction phase compared to the control group. The other three derivatives **4b**, **5a**, and **6a** also significantly inhibited BF decrease. **6b** showed a significant suppressant effect, which was weaker than that of **4b**, **5a**, and **6a**. **4c** and **5b** showed no significant inhibition (Fig. 4a, b). The presence of a free carboxylic acid is not essential for allergy-preventive activity as opposed to the neuroprotective effects reported previously [3]. By comparing the experimental activity of the compounds reported in this study to their structures, the following SAR was postulated. The addition of a bromine substituent at the C-7 position of **4a** resulted in slightly increased activity (compound **4b**) (Fig. 1). The ester derivative **5a** and the amide derivative **6a** showed improved activities. Furthermore, the alcohol derivative **6b** showed decreased activity

Fig. 4 Inhibitory effect of compounds **4a**, **4b**, **4c**, **5a**, **5b**, **6a**, and **6b** on HEL-induced blood flow decrease in male ddY mice. Filled circle: control (blood flow of HEL-sensitized mice); **a**, **b** open triangle, open diamond, open circle, open square: pretreatment with compounds at days 0 (starting day), 3 and 6 from sensitization. Each value presents the mean $\pm$ SE ($n = 5$). *, #, and * $p < 0.05$ compared with the control (Dunnett's test with Bonferroni)



compared to the corresponding carboxylic acid derivatives. We also found that changing the configuration of the chiral carbon of **4a** resulted in slightly decreased activity (compound **4c**), suggesting that S configuration of the chiral carbon might favor the inhibitory activity. The introduction of a phenylmethylidene substituent at the C-3 position of **4a** led to considerable loss of activity (compound **5b**). This could be due to low hydrophilicity and lipophilicity of compound **5b**. None of the derivative compounds showed an effect stronger than that of the lead compound. These findings will aid in the development of stronger pharmaceutical products. Further results will be reported elsewhere. In previous studies, we demonstrated that flavonoids, chalcones, and iridoids significantly inhibited HEL-induced BF decrease. The inhibitory mechanisms involved inhibition of platelet aggregation and production of COX-2, iNOS, and NO. We suggest that the inhibition of BF decrease by **4a** and its derivatives may occur in a similar manner [8–11]. The detailed mechanisms of action of **4a** and its active derivatives are under investigation. Tricyclic quinazolines and their derivatives have been reported to possess a broad spectrum of biological activities, such as antimicrobial, bronchodilator, and hypotensive actions [12–15]. A previous study demonstrated that quinazoline alkaloids showed potential anti-inflammatory and anti-allergic activities, such as an inhibitory effect on pro-inflammatory cytokines [16]. To our knowledge, this is the first study to confirm the allergy-preventive activity of tetrahydropyrrolo[2,1-b]quinazoline derivatives by comprehensively monitoring specific BF decrease occurring in the induction phase of allergy.

Conclusion

In summary, we designed and synthesized a series of tetrahydropyrrolo[2,1-b]quinazolines and most of these compounds exhibited moderate to good activities against HEL-induced BF decrease. Compound **5a** showed the best activity among all the synthesized analogs. The synthesized compounds certainly hold great promise in the pursuit to discover novel agents for the treatment of allergic diseases such as atopic dermatitis, allergic asthma, and hay fever. Our study is a preliminary step towards developing newly synthesized preventive agents against BF decrease. Further studies are being conducted to acquire more information on SAR of the novel synthesized compounds to clarify their mechanisms of action. Modifications to improve the potency of the synthesized derivatives by structural diversification are currently under progress in our laboratory.

Acknowledgements We gratefully acknowledge the financial support from the National Natural Science Foundation of China (Grant 81502931), Fund for Career Development Support Plan for Young

and Middle Aged Teachers in Shenyang Pharmaceutical University (Grant ZQN2015024), Student's Platform for Innovation and Entrepreneurship Training Program (Grant 201710163000175), Program for Innovative Research Team of the Ministry of Education, and Program for Liaoning Innovative Research Team in University. The work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Supplementary Material

Journal of Natural Medicines

(Note)

**Allergy-preventive Effects of Linarinic Acid and Its
Tetrahydropyrrolo[2,1-b]quinazoline Derivatives Isolated from *Linaria vulgaris***

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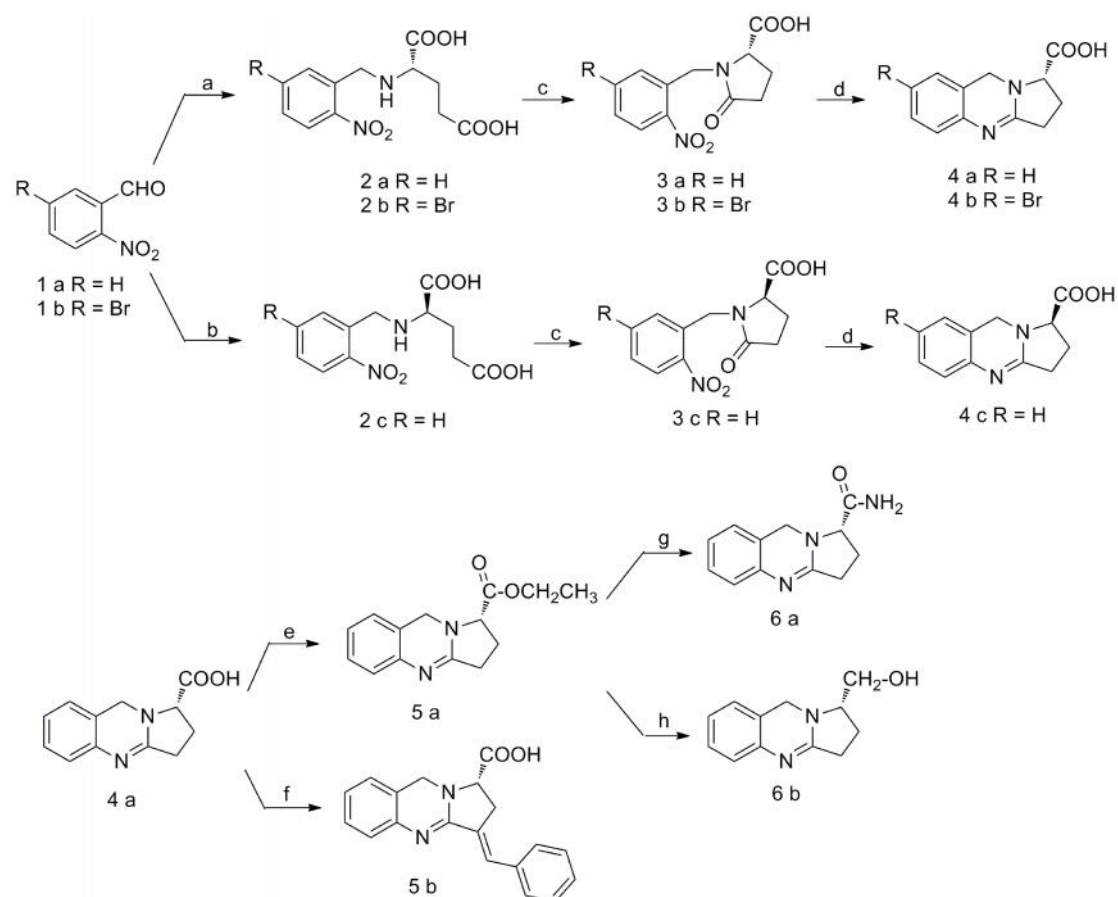
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Chemistry

All commercially available reagents and solvents were used without further purification. Melting points were determined in a Büchi Melting Point B-540 apparatus and were uncorrected. Infrared spectra were recorded in a Bruker IFS-55 spectrophotometer. Optical rotations were measured on a Perkin Elmer 241MC spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded in a Bruker ARX 300 MHz. Chemical shift values were reported in δ (ppm), and coupling constants were reported in Hertz. High-resolution mass spectrometry (HRMS) data were obtained using a Bruker microTOF-Q time of flight mass spectrometer or a Bruker APEXIII 7.0 Tesla FT-MS. Column chromatography was performed on silica gel H, and analytical TLC data was obtained using silica gel HF254.

The synthetic procedures adopted to obtain the test compounds are depicted in Charts 1. The synthetic strategy began with the reductive amination of 2-nitrobenzaldehydes **1a–b** using commercially available L-glutamic acid or D-glutamic acid followed by an intramolecular cyclization and dehydration in refluxing ethanol to produce the desired series of significant intermediates **3a–c**. Next, compounds **3a–c** were treated with an excess of 80% hydrazine hydrate, ferric chloride, and activated carbon in refluxing ethanol for 72 h, yielding three of the target compounds **4a–c**. Compounds **4a** were then reacted with thionyl chloride and ethanol to afford compounds **5a** via the esterification. In addition, treatment of compound **5a** with an aqueous solution of ammonia at 60 $^\circ\text{C}$ afforded the corresponding amide derivative **6a**. On the other hand, reduction of **5a** with lithium aluminum hydride in tetrahydrofuran afforded **6b**. The structures of all target compounds were identified by ^1H NMR, ^{13}C NMR, IR, and HRMS.



Reagents and condition: (a) L-glutamic acid, NaOH, NaBH₄, and HCl/H₂O; (b) D-glutamic acid, NaOH, NaBH₄, and HCl/H₂O; (c) CH₃CH₂OH, reflux; (d) 80% NH₂NH₂ H₂O, FeCl₃, active carbon, CH₃CH₂OH, reflux; (e) SOCl₂, CH₃CH₂OH; (f) Benzaldehyde, reflux; (g) NH₃ H₂O, reflux; (h) LiAlH₄, THF

Chart 1. Synthetic Scheme for Compounds

(S)-(-)-1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (4a)

White solid; yield 37.3%; mp: 229.4–232.5 °C; $[\alpha]_{\text{D}}^{25}$: -222.2 (c 0.25, MeOH); ¹H NMR (300 MHz, CD₃OD) δ: 2.22–2.33 (m, 1H), 2.53–2.66 (m, 1H), 2.93–3.32 (m, 2H), 4.32 (dd, 1H, *J* = 4.1, 9.3 Hz), 4.76 (d, 1H, *J* = 15.4 Hz), 4.98 (d, 1H, *J* = 15.4 Hz), 7.00–7.02 (m, 1H), 7.19–7.27 (m, 2H), 7.29–7.35 (m, 1H); ¹³C NMR (75 MHz, CD₃OD) δ: 25.6, 30.2, 46.5, 70.2, 117.7, 118.4, 128.1, 128.3, 130.3, 132.2, 165.0, 174.6; IR (KBr): 3419.7, 2921.7, 1667.3, 1613.4, 1584.2, 1499.5, 1385.3, 1291.4, 764.2 cm⁻¹; ESI-HRMS: calcd for C₁₂H₁₃N₂O₂, 217.0972; found, 217.0969. [M+H]⁺.

(S)-(-)-7-Bromo-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (4b)

White solid; yield 30.8%; mp: 282.5–284.9 °C; $[\alpha]_{\text{D}}^{25}$: -224.0 (c 0.25, MeOH); ¹H

NMR (300 MHz, CD₃OD) δ : 2.22–2.33 (m, 1H), 2.53–2.66 (m, 1H), 2.92–3.13 (m, 2H), 4.32 (dd, 1H, J = 3.9, 9.3 Hz), 4.75 (d, 1H, J = 15.7 Hz), 4.97 (d, 1H, J = 15.8 Hz), 6.94 (d, 1H, J = 8.5 Hz), 7.43 (d, 1H, J = 1.7 Hz), 7.48 (dd, 1H, J = 1.7, 8.5 Hz); ¹³C NMR (75 MHz, CD₃OD) δ : 25.6, 30.3, 46.1, 70.3, 119.4, 120.5, 120.8, 131.2, 131.7, 133.3, 165.2, 174.7; IR (KBr): 3424.7, 2926.1, 1657.7, 1577.2, 1497.1, 1421.1, 1373.5, 1296.3, 819.0 cm⁻¹; ESI-HRMS: calcd for C₁₂H₁₂BrN₂O₂, 295.0077; found, 295.0079. [M+H]⁺.

(R)-(+)-1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (4c)

White solid; yield 28.5%; mp: 279.7–282.8°C; [α]_D²⁵: +212.2 (c 0.25, MeOH); ¹H NMR (CD₃OD, 300MHz) δ : 2.23~2.30 (m, 1H), 2.58~2.62(m, 1H), 2.98~3.07 (m, 2H), 4.31 (dd, 1H, J = 4.1, 9.3 Hz), 4.73~4.79 (d, 1H, J = 15.4 Hz), 4.95~5.00 (d, 1H, J = 15.3 Hz), 6.98~7.01 (m,1H), 7.18~7.34 (m, 3H); ¹³C NMR (75 MHz, CD₃OD) δ : 24.2, 28.8, 45.1, 68.8, 116.4, 117.0, 126.7, 126.9, 128.9, 130.9, 163.6, 173.5; IR (KBr): 3412.7, 2917.8, 1664.9, 1614.3, 1585.4, 1499.9, 1384.8, 1290.7, 764.6 cm⁻¹; ESI-HRMS: calcd for C₁₂H₁₂N₂O₂, 216.0899; found, 217.0984. [M+H]⁺.

(S)-(-)-ethyl 1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylate (5a)

Thionyl chloride (10 mL) was added dropwise to a stirred solution of **4a** (9.26 mmol) in anhydrous ethanol (30 mL) at 0–5°C. The mixture was stirred at this temperature for 6 h and then concentrated under vacuum. The residue was alkalified to pH 8 with a 5% aqueous solution of potassium carbonate and then dissolved in ethyl acetate (300 mL). After being washed with water and brine, the organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using ethyl acetate to give compound **5a** (1.38 g, 61.2%) as a white solid. mp: 103.7-106.0°C; [α]_D²⁵: -117.2 (c 0.25, MeOH); ¹H NMR (CDCl₃, 300MHz) δ : 1.31 (3H, t, J = 7.1, 7.2 Hz), 2.07-2.17 (m, 1H), 2.31 -2.44 (m, 1H), 2.59-2.70 (m, 1H), 2.74-2.85 (m, 1H), 4.05 (dd, 1H, J = 4.2, 8.7 Hz), 4.26 (dd, 2H, J = 7.1, 15.6 Hz), 4.50 (d, 1H, J = 15.1 Hz), 4.72 (d, 1H, J = 13.0 Hz), 6.88 (d, 1H, J = 7.3 Hz), 6.99 (t, 1H, J = 7.3, 7.4 Hz), 7.06 (d, 1H, J = 7.1 Hz), 7.17 (t, 1H, J = 7.2, 7.4 Hz); ¹³C NMR (75 MHz, CD₃OD) δ : 14.4, 23.8,

29.5, 45.4, 61.4, 63.2, 120.1, 123.8, 124.1, 126.4, 128.3, 143.2, 162.4, 171.5; IR (KBr): 3447.3, 2973.5, 1733.9, 1622.9, 1591.5, 1572.5, 1492.0, 1446.0, 1290.6, 776.0 cm^{-1} ; ESI-HRMS: calcd for $\text{C}_{14}\text{H}_{17}\text{N}_2\text{O}_2$, 245.1285; found, 245.1293. $[\text{M}+\text{H}]^+$.

(S)-(-)-3-phenylmethyldene-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-1-carboxylic acid (5b)

Compound **4a** (40 mmol) was added into a solution of benzaldehyde (40 ml), the mixture was heated at reflux for approximately 1h and cooled to the room temperature. The precipitate was filtered off, washed with ethyl ether, dried, and crystallized from methanol to furnish compound **5b** (8.19 g, 67.3%) as a light yellow solid. mp: 255.5–256.0 °C; $[\alpha]_{\text{D}}^{25}$: -104.2 (c 0.25, MeOH); ^1H -NMR(300MHz, DMSO, δ ppm) δ 3.25~ 3.31(m, 1H), 3.64~3.73(m, 1H), 4.85~4.89(m, 1H), 4.93~5.00(m, 2H), 7.25~7.69(m, 9H), 8.59(s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ : 31.7, 45.4, 48.4, 66.8, 116.6, 116.8, 126.4, 126.6, 126.8, 128.9, 129.0, 130.0, 130.2, 131.0, 133.9, 134.3, 158.1, 173.0; IR (KBr): 3428.7, 2920.5, 1652.6, 1571.5, 1497.8, 1455.1, 1372.1, 1289.6, 1196.6, 1136.4, 937.6, 760.0 cm^{-1} ; ESI-HRMS: calcd for $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_2\text{Na}$, 327.1104; found, 327.1113. $[\text{M}+\text{Na}]^+$.

(S)-(-)-1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazoline-1-carboxamide (6a)

White solid. mp: 261.0–261.4 °C; $[\alpha]_{\text{D}}^{25}$: 192.2 (c 0.25, MeOH); ^1H NMR (300 MHz, DMSO- d_6) δ : 1.78–1.88 (m, 1H), 2.15–2.28 (m, 1H), 2.40–2.60 (m, 2H), 3.93 (dd, 1H, J = 5.0, 8.3 Hz), 4.42 (s, 2H), 6.81–6.83 (m, 1H), 6.90–6.94 (m, 2H), 7.05–7.10 (m, 1H), 7.25 (s, 1H), 7.69 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 24.0, 29.6, 44.9, 63.8, 119.8, 123.3, 123.4, 126.1, 127.9, 143.2, 162.7, 172.2; IR (KBr): 3357.5, 3193.5, 2922.5, 2852.1, 1680.5, 1621.2, 1596.5, 1573.4, 1490.2, 1291.2, 763.3 cm^{-1} ; ESI-HRMS: calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}$, 216.1131; found, 216.1134. $[\text{M}+\text{H}]^+$.

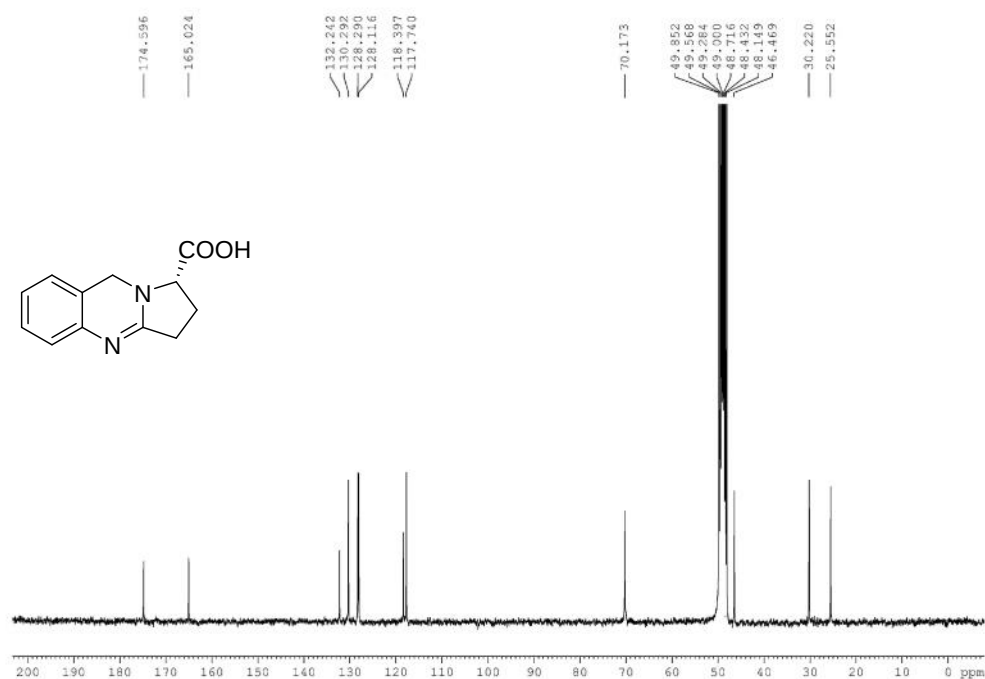
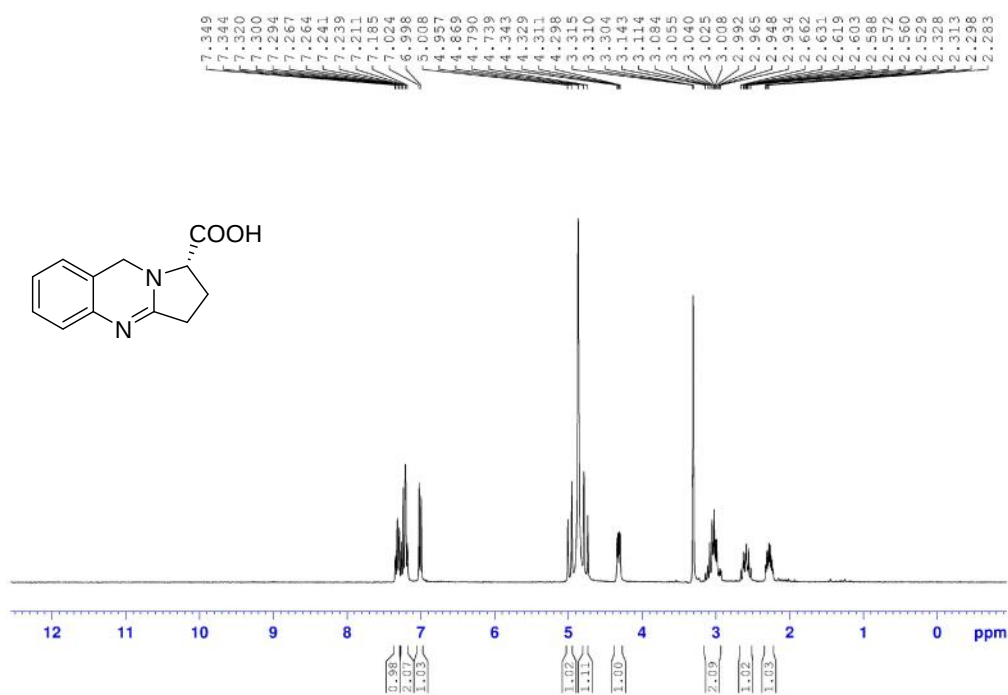
(S)-(-)-1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazoline-1-methanol 6b

A suspension of **5a** (7.32 g, 30.0 mmol) in an anhydrous solution of tetrahydrofuran (40 mL) was cooled to 0 °C, lithium aluminum hydride (2.28 g, 60 mmol) was added in small portions at 0–5 °C, the mixture was stirred at this temperature for 0.5 h. The water (5 ml) was added dropwise, at this point, the mixture was filtered and the

solvent was removed by vacuum evaporation. The precipitate was filtered off, washed with ethyl ether, dried, and crystallized from ethyl acetate to furnish compound **6b** (4.65 g, 76.7%) as a white solid. mp: 179.9–181.0 °C; $[\alpha]_D^{25}$: -132.2 (c 0.25, MeOH); ^1H -NMR (DMSO-*d*₆, 300MHz) δ : 6.91-6.86 (m, 1H), 6.75-6.68 (m, 2H), 6.61 (d, 1H, $J = 7,7$ Hz), 4.80 (s, 1H), 4.50 (d, 1H, $J = 13.9$ Hz), 4.25 (d, 1H, $J = 13.9$ Hz), 3.49-3.46 (m, 1H), 3.37-3.27 (m, 2H), 2.38-2.17 (m, 2H), 1.85-1.78 (m, 1H), 1.61-1.58 (m, 1H); ^{13}C NMR (75 MHz, DMSO-*d*₆) δ : 21.9, 30.2, 45.0, 60.9, 63.6, 120.4, 123.4, 123.5, 126.4, 128.2, 144.4, 172.2; IR (KBr): 3357.5, 3193.5, 2922.5, 2852.1, 1680.5, 1621.2, 1596.5, 1573.4, 1490.2, 1291.2, 763.3 cm^{-1} ; ESI-HRMS: calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{O}$, 203.1179; found, 203.1193. $[\text{M}+\text{H}]^+$.

Spectra data for part of target compounds

^1H NMR, ^{13}C NMR, HRMS and IR spectra of **4a**

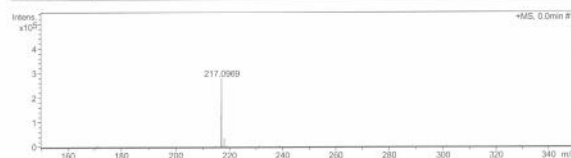


Mass Spectrum Molecular Formula Report

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 Instrument / Ser#: microTOF-Q 125

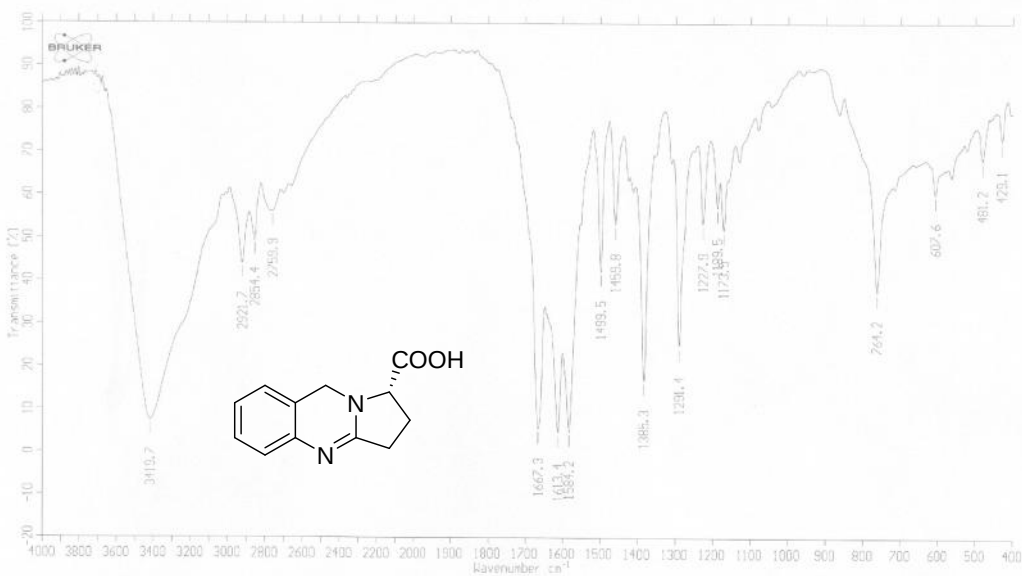
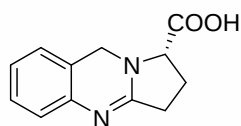
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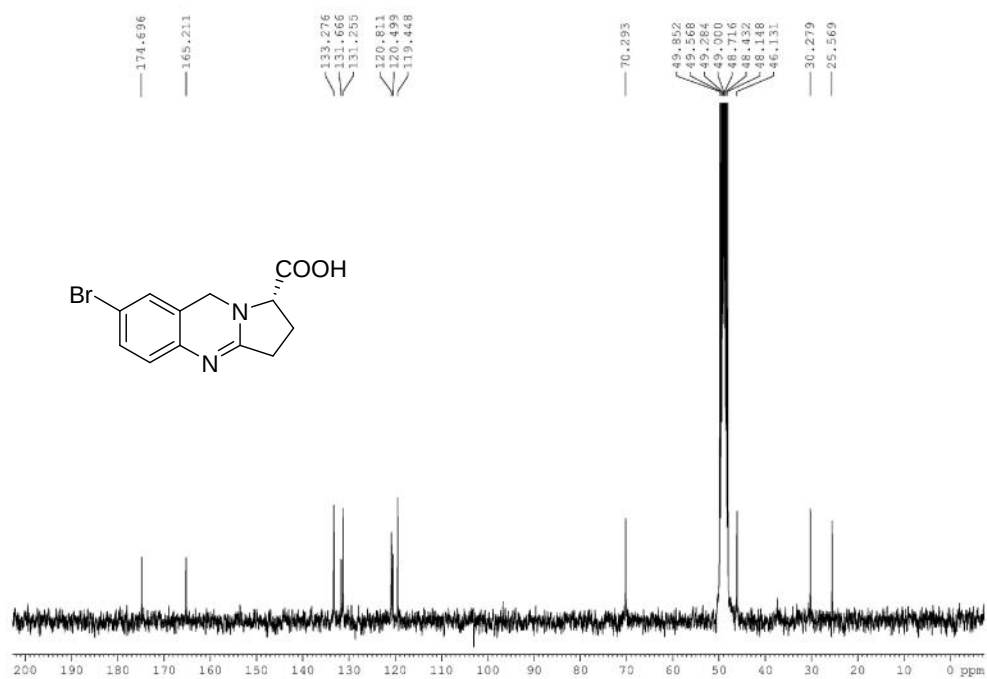
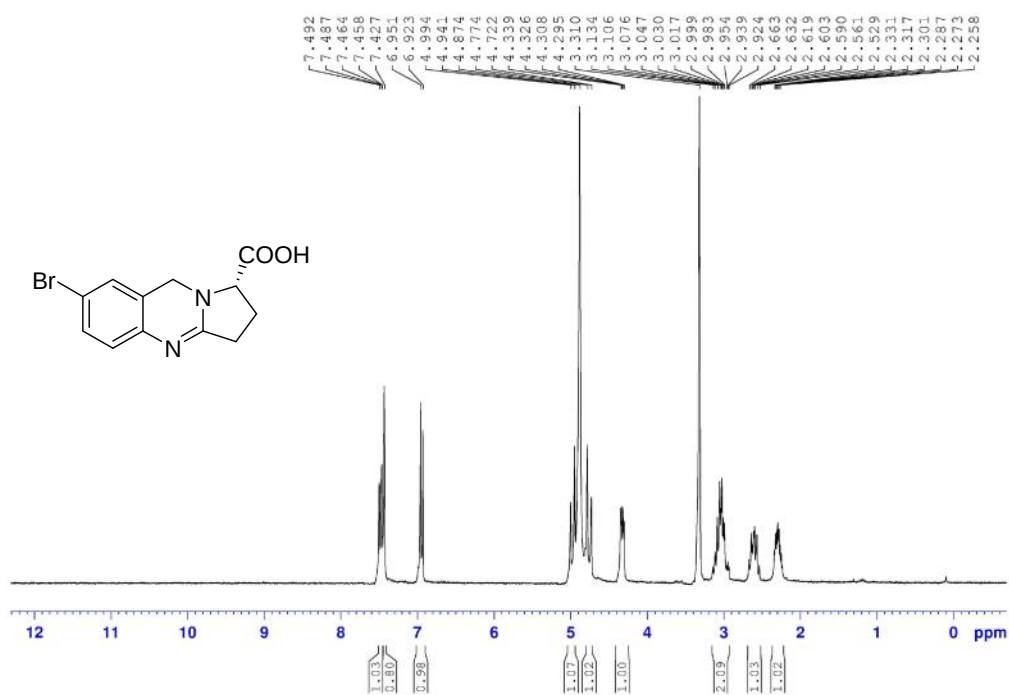


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^1H NMR, ^{13}C NMR, HRMS and IR spectra of **4b**

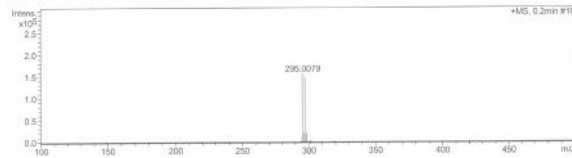


Mass Spectrum Molecular Formula Report

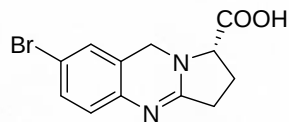
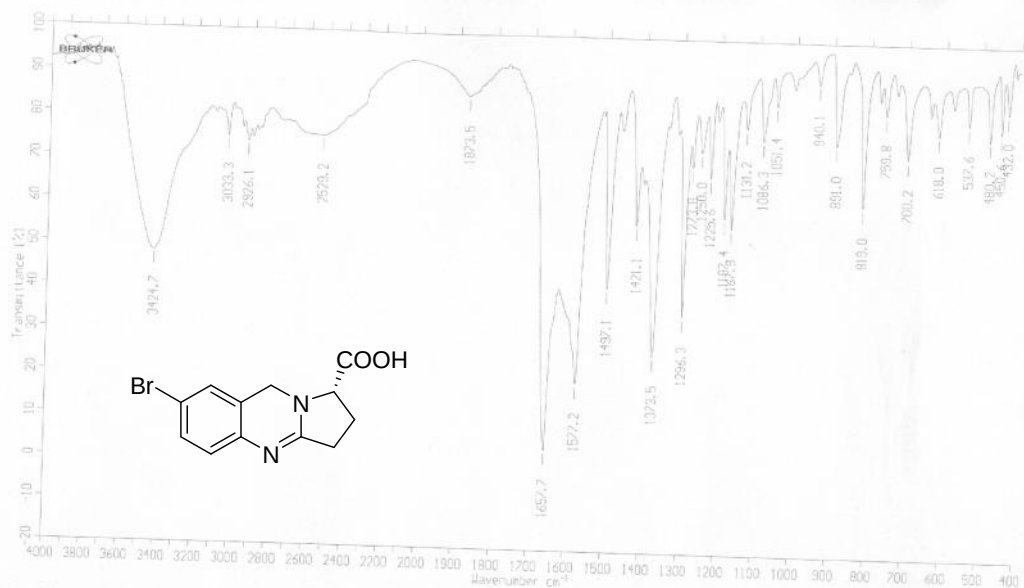
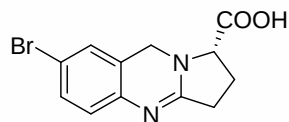
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 Instrument / Ser#: microTOF-Q 125

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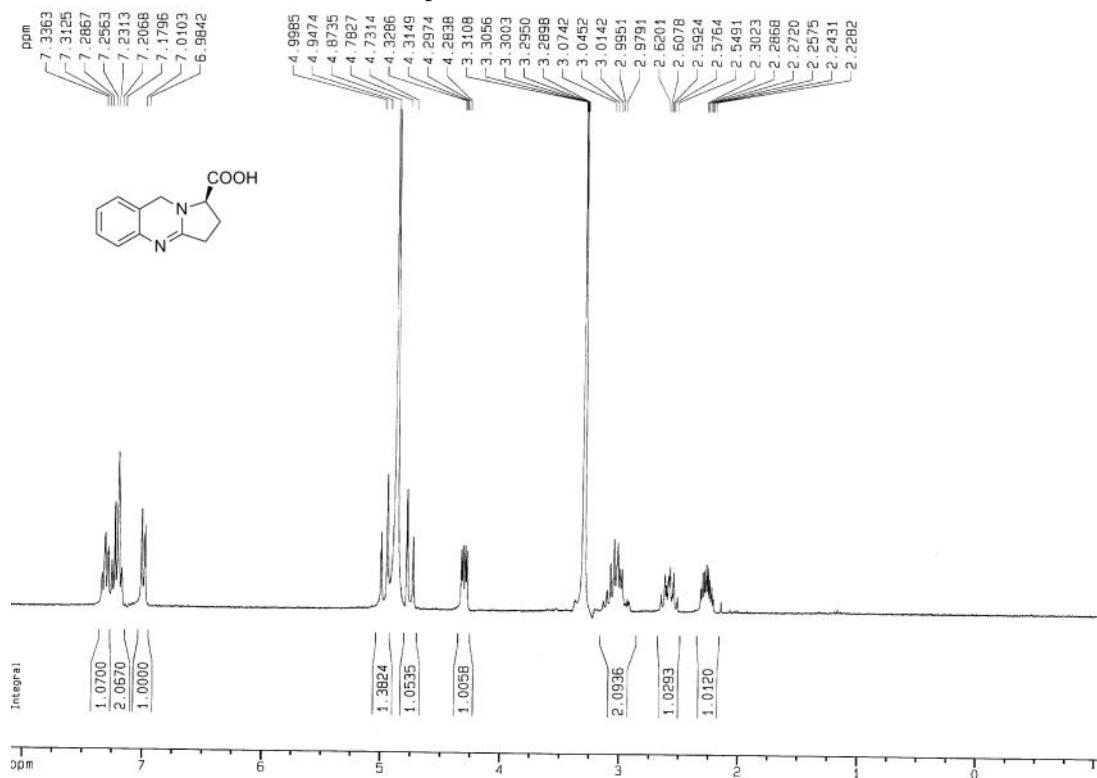
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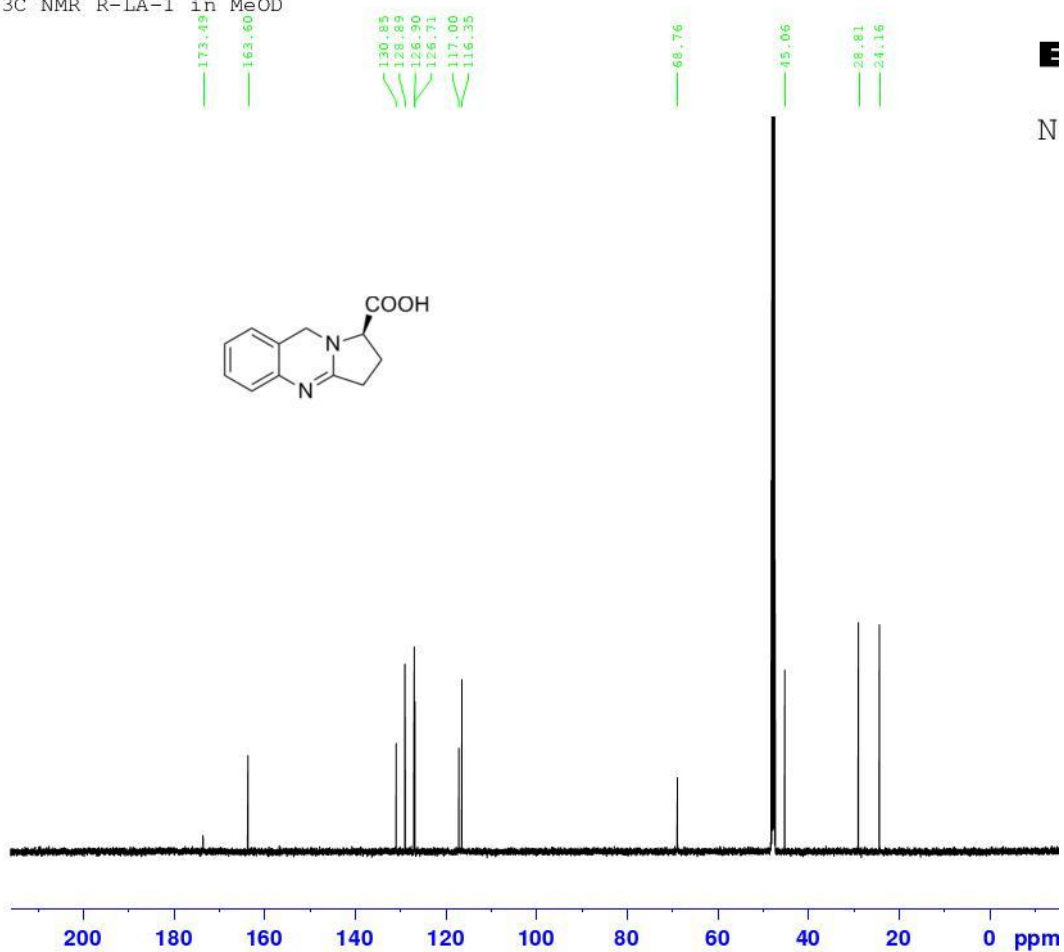
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^1H NMR, ^{13}C NMR, HRMS and IR spectra of **4c**



^{13}C NMR R-LA-1 in MeOD

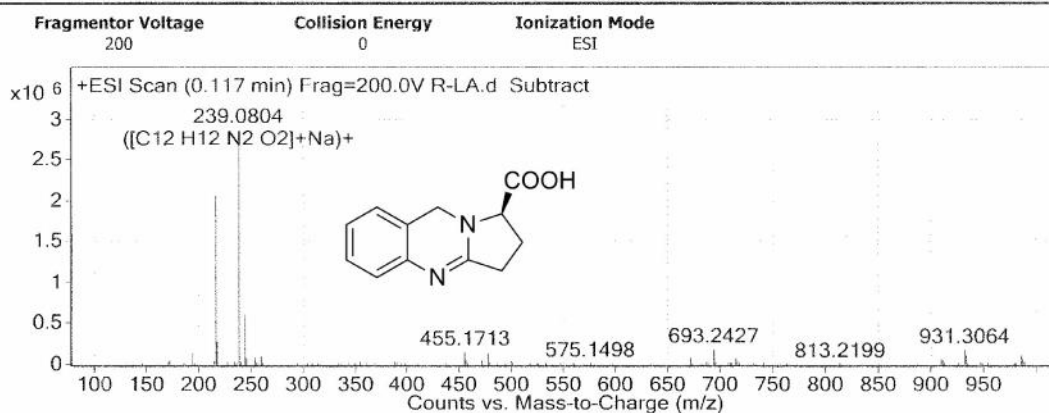


Qualitative Analysis Report

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Acq Method	ZHENG100-1000.m	Acquired Time	5/15/2017 3:28:44 PM
IRM Calibration Status	Success	DA Method	20170311.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

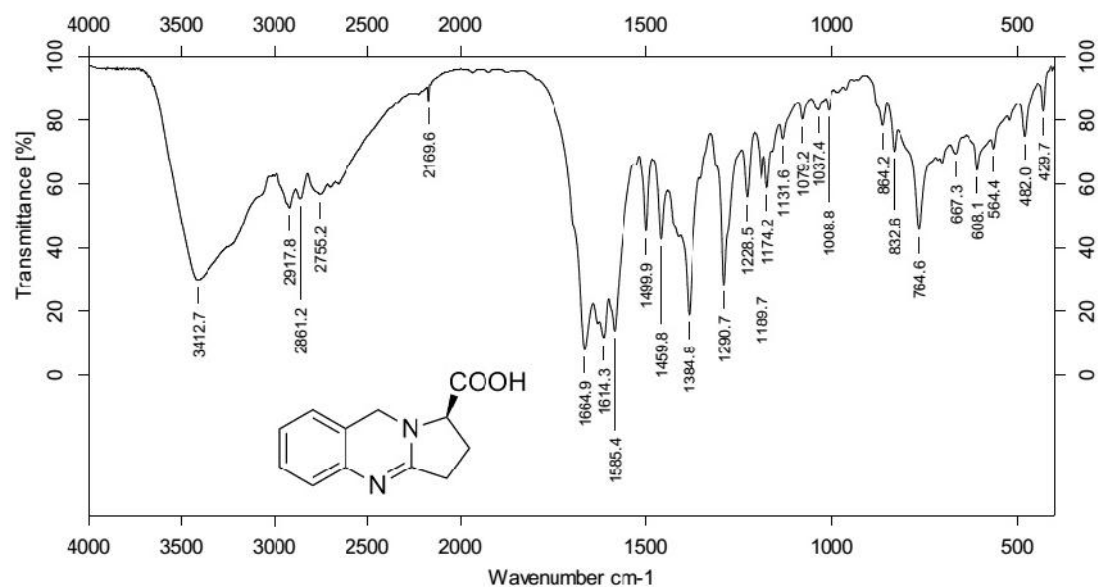
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239.0804	1	2739297	C12 H12 N2 O2	(M+Na)+
239.2111		158697.95		
240.084	1	385670.31	C12 H12 N2 O2	(M+Na)+
245.1304	1	605265.31		
455.1713	1	153064.2		
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Formula Calculator Element Limits

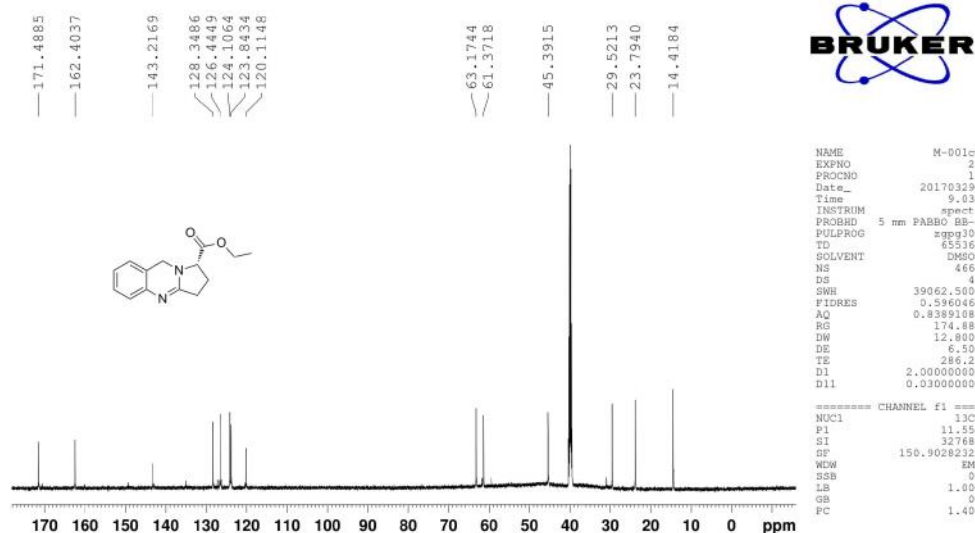
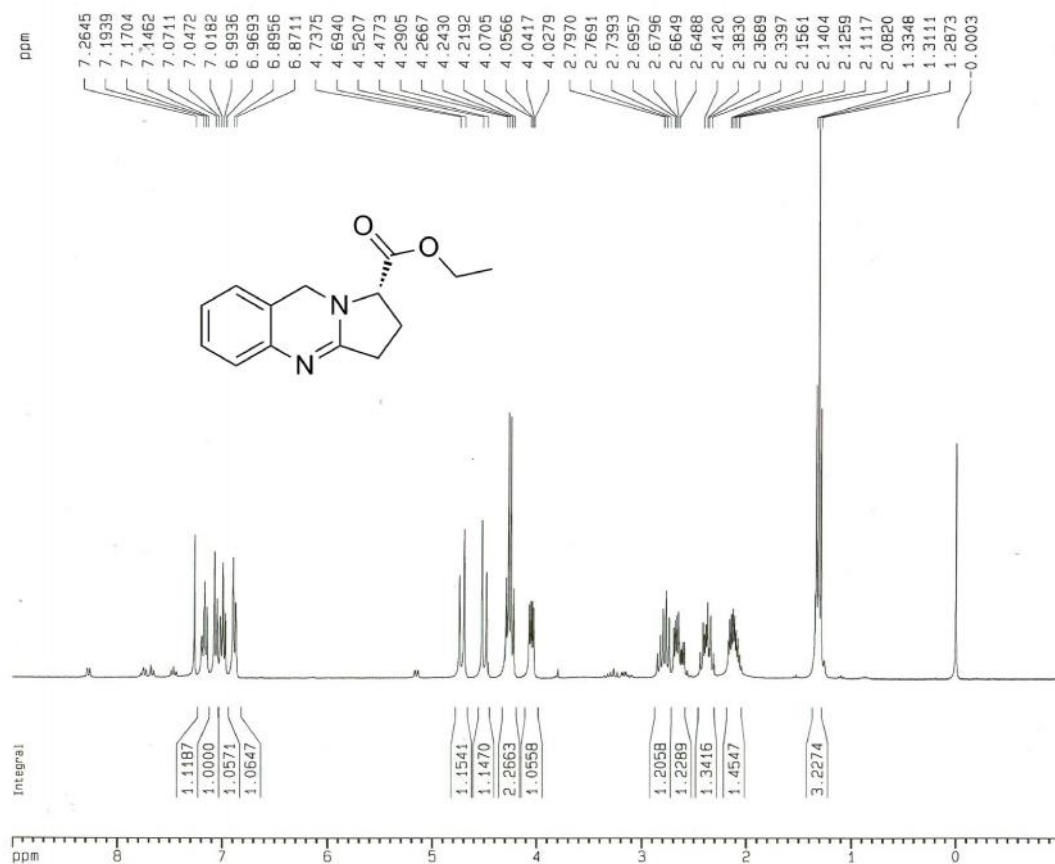
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H	0	120
O	0	30
N	0	30

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C12 H12 N2 O2	TRUE	216.0913	216.0899	-6.42	C12 H12 N2 Na O2	91.9



^1H NMR, ^{13}C NMR, HRMS and IR spectra of **5a**



Mass Spectrum SmartFormula Report

Analysis Info

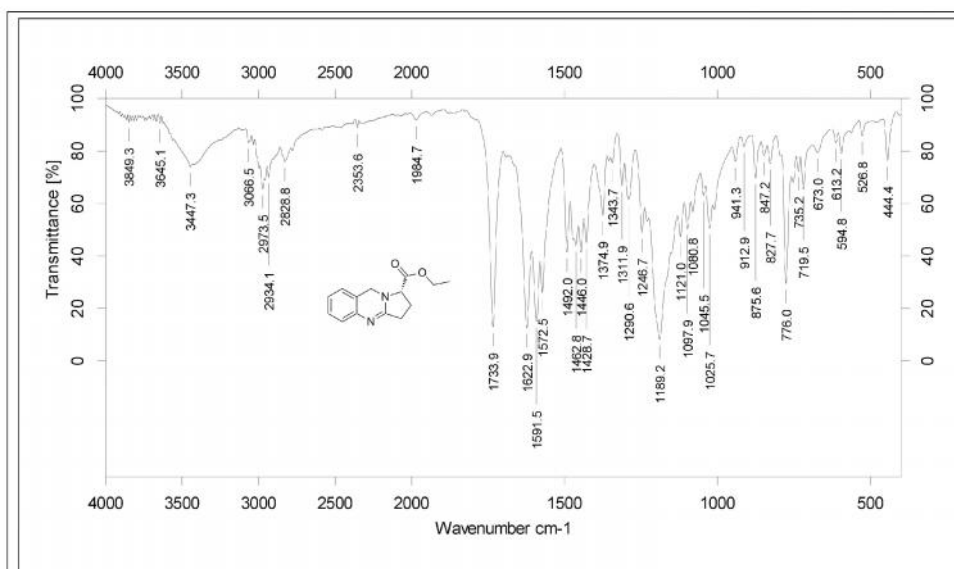
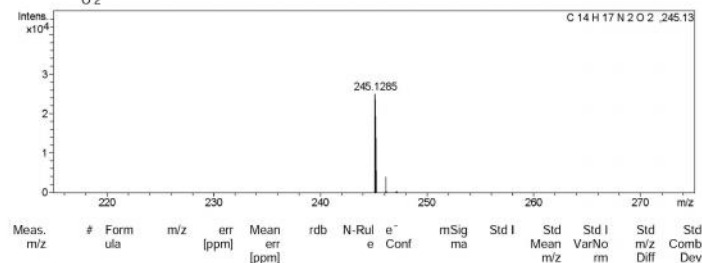
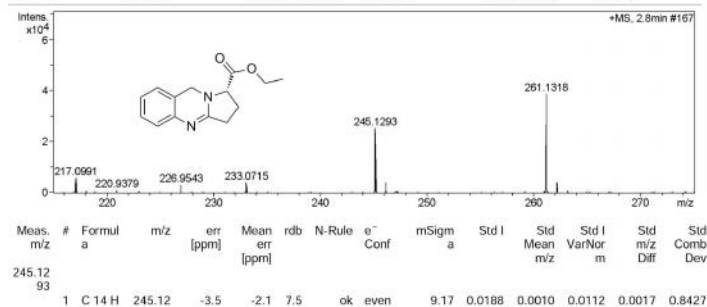
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 Sample Name: M-001
 Comment:

Acquisition Date: 3/23/2017 2:54:12 PM

Operator: Bruker Customer
 Instrument / Ser#: microTOF-Q 125

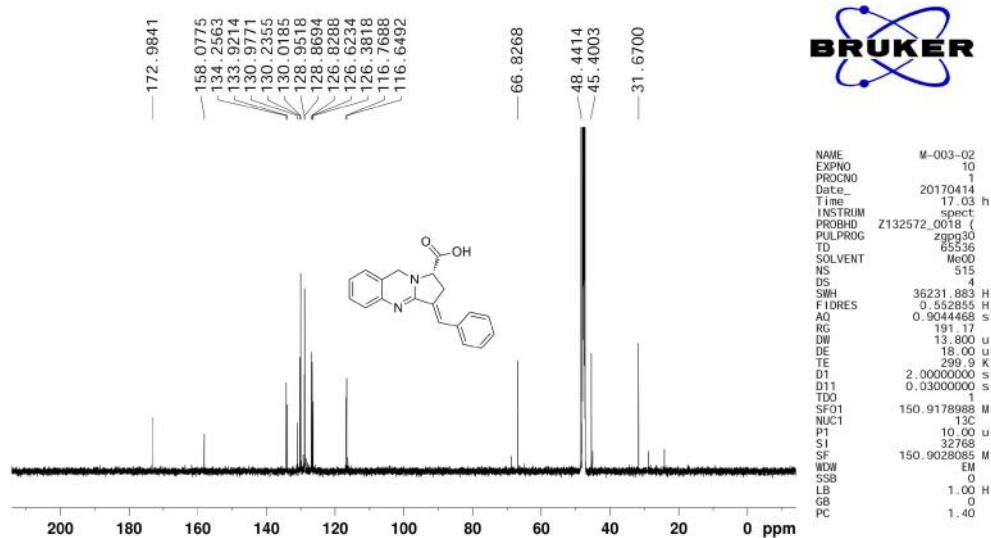
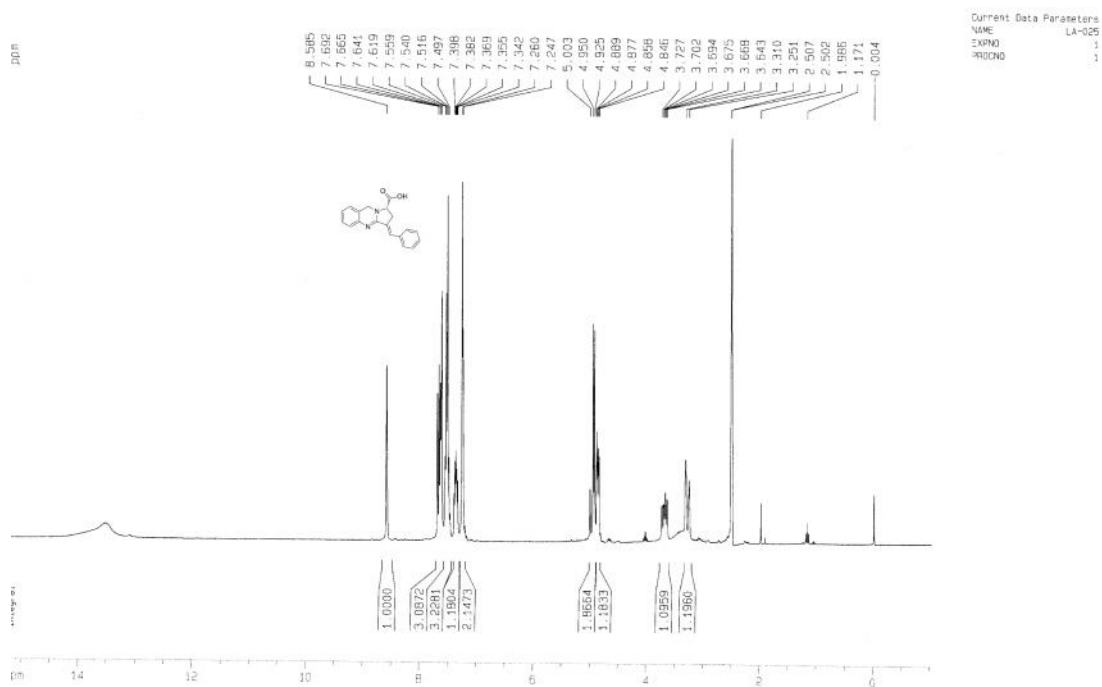
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Sample : M-001	Frequency Range : 3999.64 - 400.157	Measured on : 2017-3-21
Technique : Sample form	Resolution : 2	Instrument : EQUINOX55
Customer : Default	ZeroFilling : 2	Sample Scans : 16
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^1H NMR, ^{13}C NMR, HRMS and IR spectra of **5b**



Mass Spectrum SmartFormula Report

Analysis Info

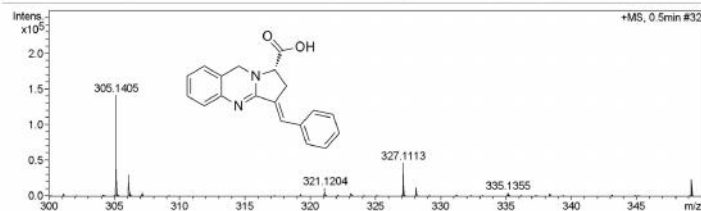
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Acquisition Date: 3/23/2017 2:19:11 PM

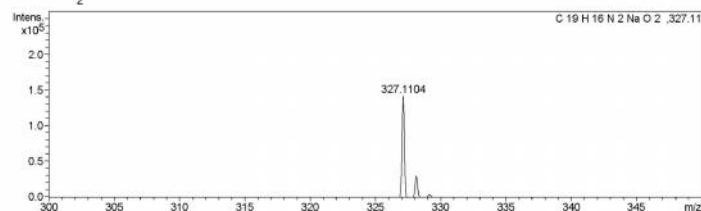
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 Instrument / Ser#: micrOTOF-Q 125

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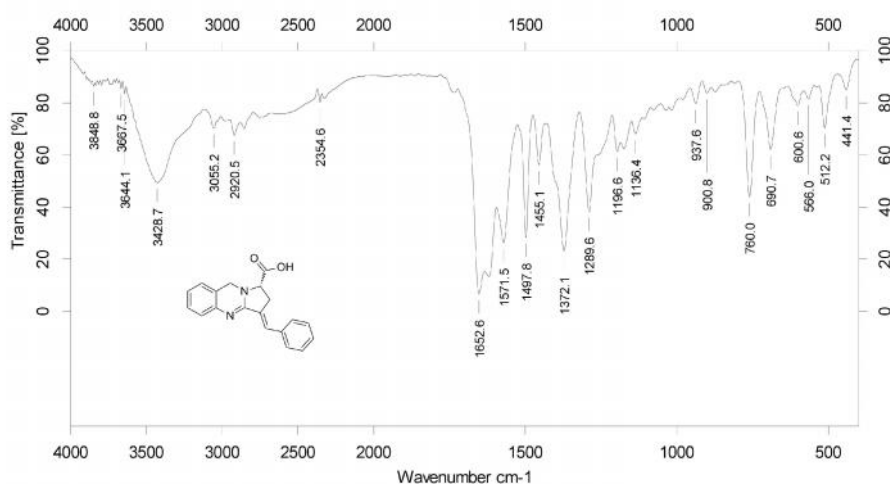
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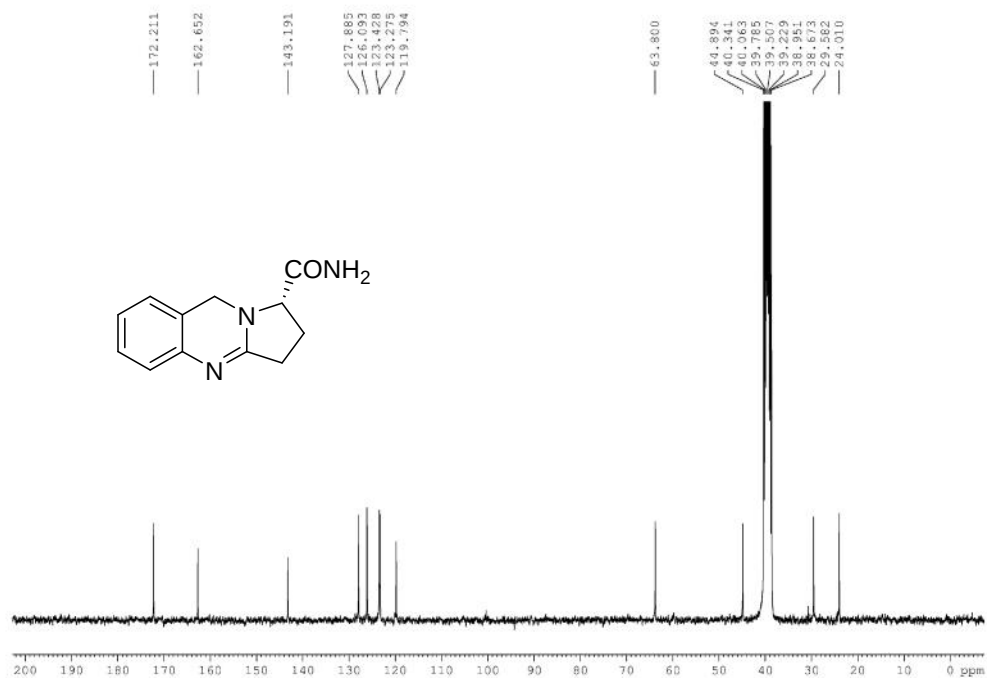
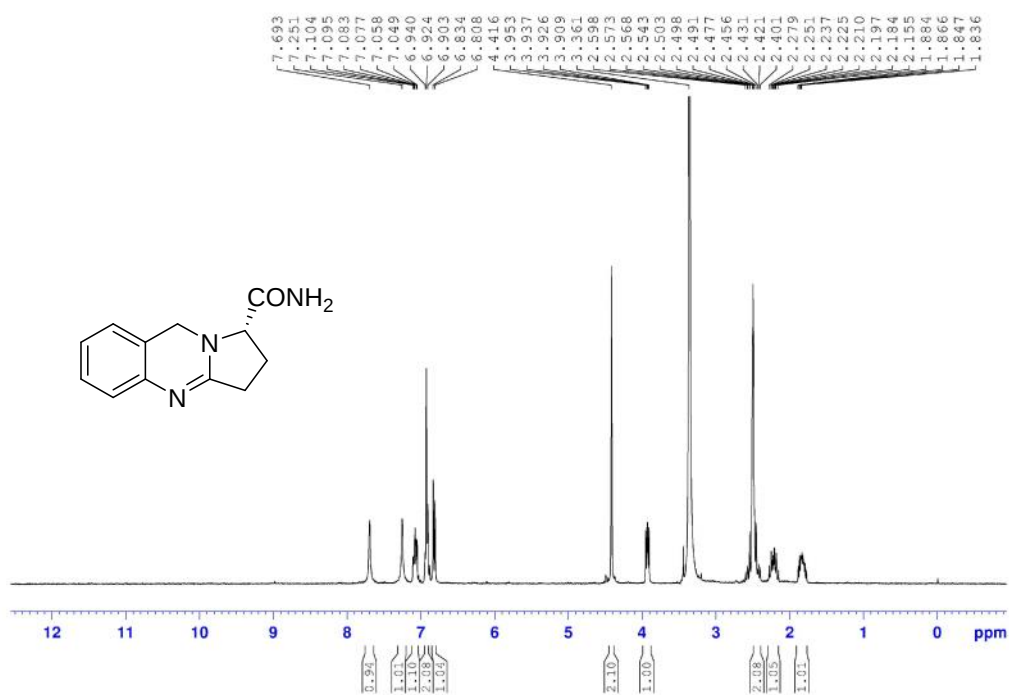


Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rd	N-Rule	e ⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNo	Std m/z Diff	Std Comb Dev
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Sample : M-003		Frequency Range : 3999.64 - 400.157		Measured on : 2017-3-21	
Technique : Sample form	Resolution : 2	Instrument : EQUINOX55		Sample Scans : 16	
Customer : Default	Zerofilling : 2	Acquisition : Double Sided For			

^1H NMR, ^{13}C NMR, HRMS and IR spectra of **6a**



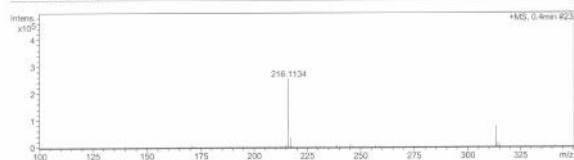
Mass Spectrum Molecular Formula Report

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 Instrument / Ser#: micrOTOF-Q 125

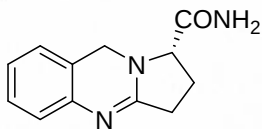
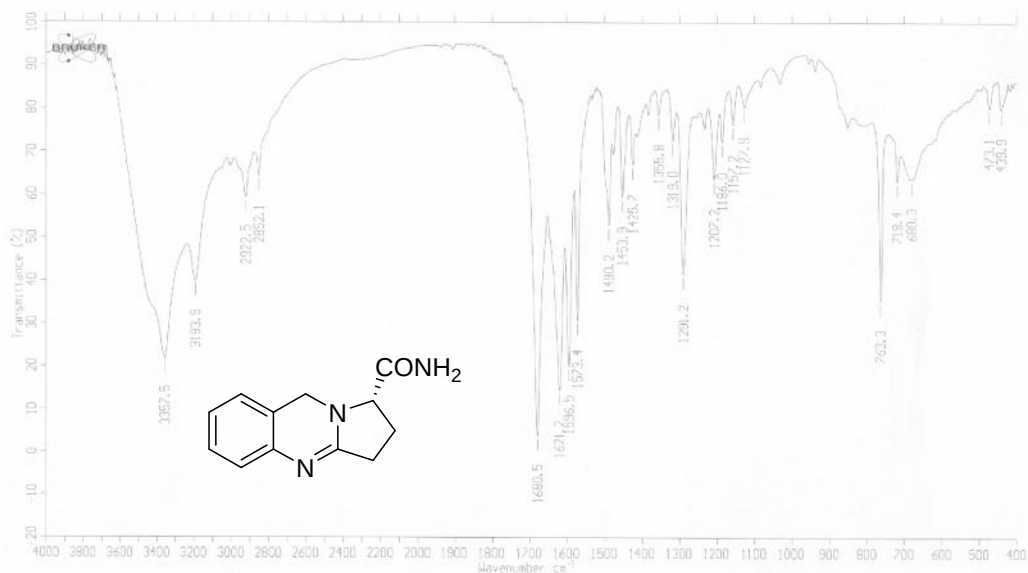
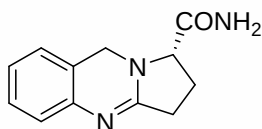
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 Set Diverter Valve: Source

Generate Molecular Formula Parameter

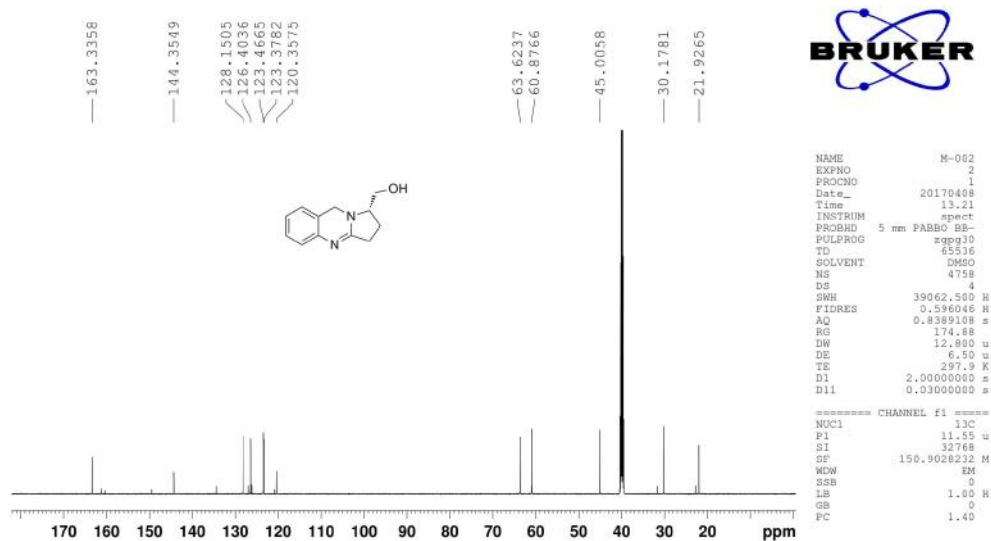
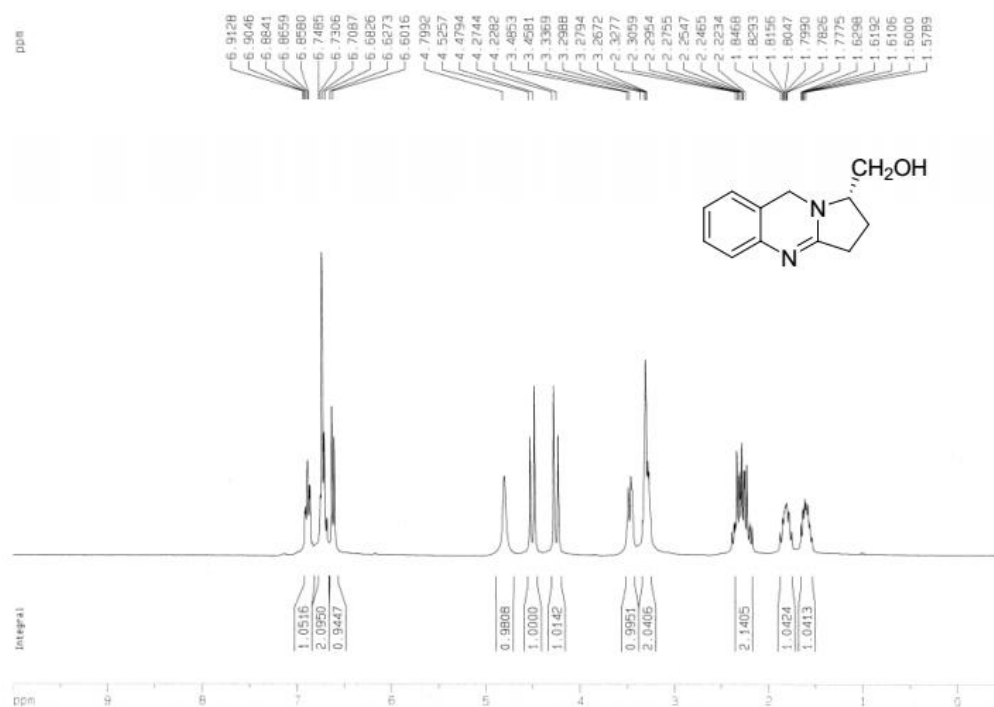
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even		e



^1H NMR, ^{13}C NMR, HRMS and IR spectra of **6b**



Mass Spectrum SmartFormula Report

Analysis Info

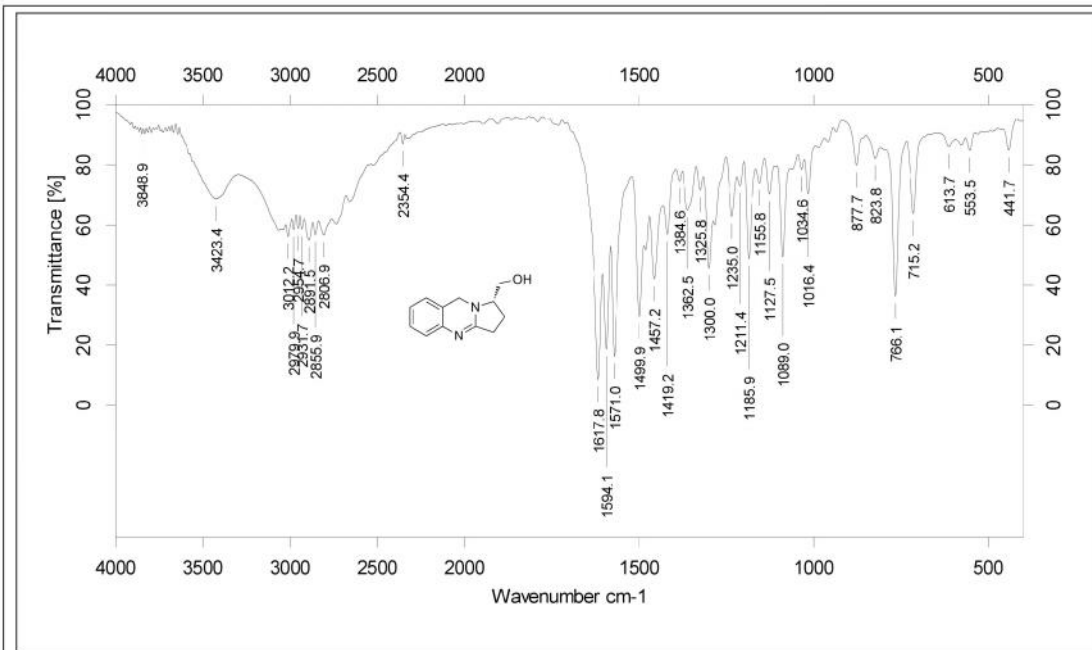
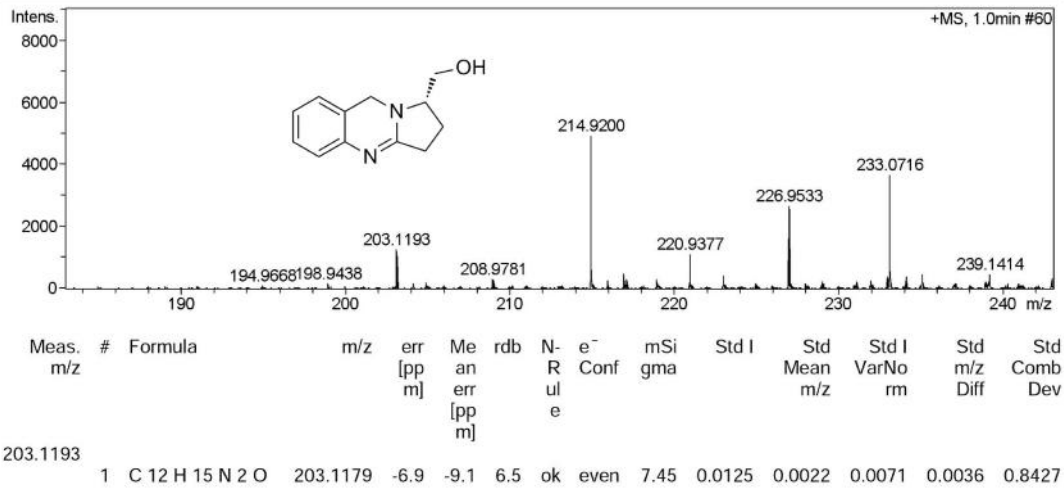
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Acquisition Date 3/23/2017 2:50:28 PM

Operator Bruker Customer
 Instrument / Ser# micrOTOF-Q 125

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Sample : M-002		Frequency Range : 3999.64 - 400.157		Measured on : 2017-3-21	
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Customer : Default	Zerofilling : 2	Acquisition : Double Sided,For			