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A Novel Synthesis of the NAMPT Inhibitor FK866

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Abstract: In this article, we report a safe and high-yield synthesis method for the NAMPT inhibitor FK866, which avoids the use of flammable lithium aluminum hydride and highly toxic sodium azide, and the synthesis of key intermediates by the Gabrielle synthesis method to construct the amino group in 33% total reaction yield, which is a twofold increase in yield compared with existing synthesis methods, and enhances the safety of the synthesis method. Nicotinamide adenine dinucleotide (NAD) is an important cofactor in life's energy metabolism, regulating redox-related proteins such as cellular respiration, glycolysis, citric acid cycle, cell communication, transcriptional regulation, post-translational protein modification, and oxidative phosphorylation in cellular respiration. NAD is a core coenzyme of metabolism, mainly involved in redox reactions, but also in post-translational modifications to regulate DNA damage responses or gene expression, as a substrate for poly ADP ribose polymerase (PARP) and deacetylating Sirtuins. In cells, a specific set of synthases regulates the three major biosynthetic pathways of NAD, including the quinolinic acid phosphotransferase (QAPRT)-mediated ab initio synthesis, the nicotinic acid phosphoribosyltransferase (NAPRT)-mediated preprocessor (PH) synthesis pathway, and the NAMPT-mediated salvage pathway. Therefore, enzymes involved in NAD metabolism are attractive targets for drug discovery.

1. Introduction

In mammals, NAMPT is the rate-limiting enzyme in the NAD salvage synthesis pathway and is involved in the nicotinamide cycle to maintain sufficient intracellular NAD levels to convert nicotinamide (NAM) to nicotinamide mononucleotide (NMN)[1-5]. NMN is converted to NAD in the adenosine triphosphate (ATP) hydrolysis coupling reaction catalyzed by NMNAT. NAMPT plays a key role in a variety of biochemical processes, including energy supply, metabolism, cell survival, proliferation and apoptosis, DNA damage repair, and inflammation[6-11]. NAMPT promotes tumor cell proliferation and dedifferentiation[12-15]. Both intracellular and extracellular levels of NAMPT are significantly elevated in certain tumor models. NAMPT plays an important role in tumor cell biosynthesis and may be an important target for anti-tumor proliferation studies with promising applications in the development of new tumor treatment modalities. Antagonizing NAMPT is essential for the treatment of related tumor diseases. NAMPT is a promising metabolic target for cancer therapy.

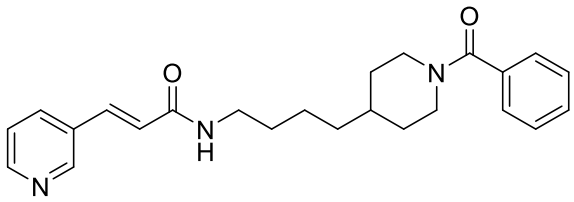

Figure 1: The Structural of FK866 (1)

The NAMPT inhibitor FK866 (**1**) has been reported in the related literatures[16,17] (Figure 1). However, the synthesis method of FK866 was poorly reported, and the only available synthesis method required the use of flammable lithium aluminum hydride and highly toxic sodium azide (Figure 2), and the combined yield of the multi-step reaction was only 12%. To prepare compound FK866 in large quantities, the original synthetic route could not meet the demand for bulk synthesis due to the low yield. Here, we report a safe and high-yield synthetic route for FK866 that avoids the use of sodium azide and lithium aluminum hydride, improves the safety of the synthetic method, and achieves a combined multi-step yield of 33%.

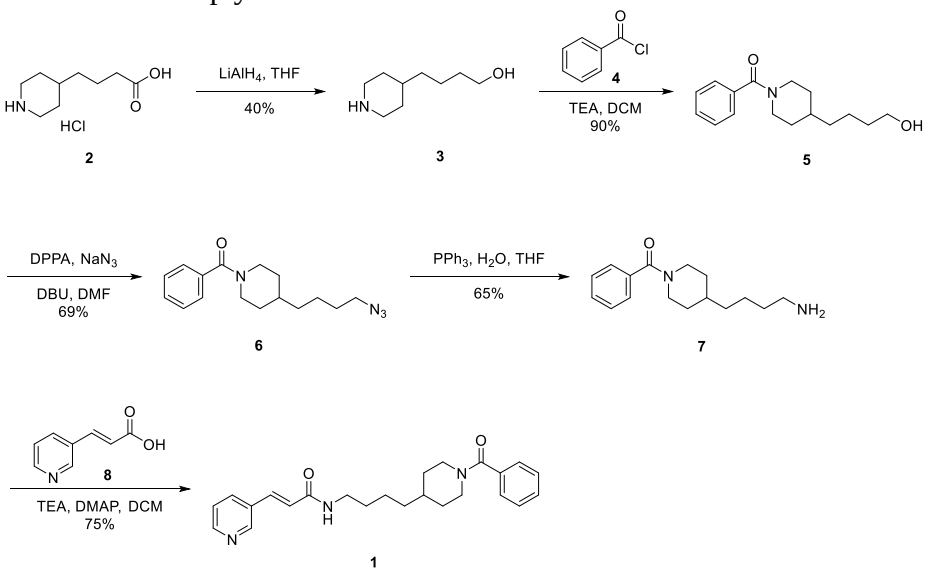

Figure 2: Reported synthesis routes of FK866

2. Results and discussion

Using tert-butyl 4-(4-hydroxybutyl) piperidine-1-carboxylate (**9**) as the starting material, we needed to convert the hydroxyl group to an amino group. In Scheme 1, an azide reduction method was used, and to avoid using the highly toxic compound sodium azide, we decided to use the Gabriel synthesis method to convert the hydroxyl group to an amino group. The hydroxyl group of compound **9**, which was easily dissociated by linking the Ts groups, was then substituted with the potassium salt of phthalimide to give the intermediate compound **12**. Compound **12** was subjected to hydrazinolysis to give the amino intermediate **13**, and the total yield of the three-step reaction to convert the hydroxyl group to amino was 72.5%. Compound **13** was coupled with compound **8** to give intermediate **14**, with a yield of 67% in this step. Intermediate **14** was then deBoc-protected and coupled with benzoic acid to give the target compound FK866, with a total yield of 33% for the five-step reaction (Figure 3).

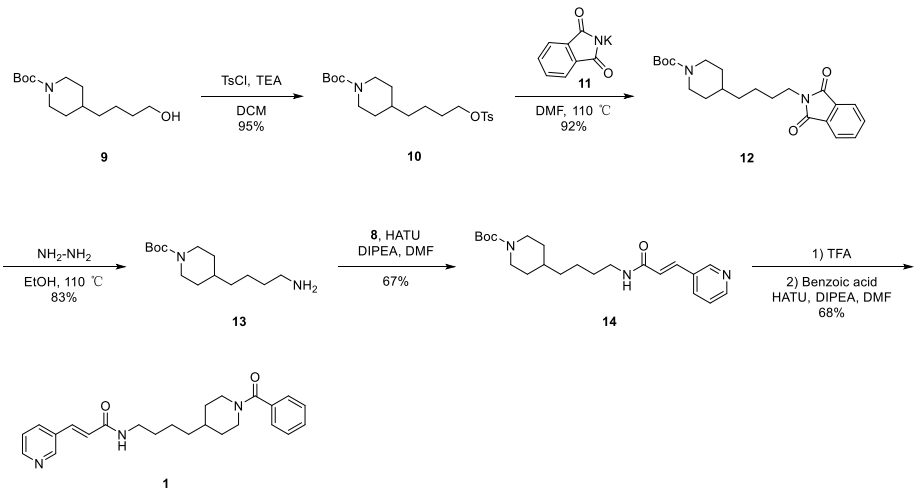

Figure 3: Reported synthesis routes of FK866

In this five-step reaction, the yield of most of the reactions was above 80%. However, only the last two steps of the condensation-coupling reactions showed yields below 70%. Although the overall yield is more than two times higher than reported synthetic routes, the condensation-coupling reactions still need to be optimized to potentially achieve higher yields.

In conclusion, we have developed a safer and higher yielding method for the synthesis of the NAMPT inhibitor FK866 that avoids the use of the flammable and highly toxic compounds lithium aluminum hydride and sodium azide. During the synthesis process, the purification and separation method was also simpler, using simple silica gel column chromatography to separate and purify the intermediate from the final compound. This synthetic method is also applicable to the synthesis of FK866 derivatives.

3. Experimental Section

Unless otherwise stated, all chemical reagents were purchased from commercial suppliers and were ready for use without further purification. Thin layer chromatography was performed using silica gel GF254 glass plates (Purchased from Qingdao Haiyang Chemical Co.) and using a 254 nm UV lamp for visualized detection. The target products were purified and separated using an automated flash chromatography system with silica gel columns (provided by Changzhou Santai Technology). Evaporation was carried out using a Heidolph rotary evaporator. NMR spectra were recorded on Bruker AVANCE 600 MHz spectrometer in DMSO-d6 solvent and the spectra were processed using MestNova (v14.0) with reference to the solvent peaks.

tert-butyl 4-(4-(tosyloxy)butyl)piperidine-1-carboxylate (10). Raw material **9** (1.00 g, 3.89 mmol) was dissolved in DCM (20 mL), TEA (0.59 g, 5.83 mmol) and TsCl (1.11 g, 5.83 mmol) were added under ice bath and then the reaction mixture was brought to room temperature and stirred for 20 h. The DCM was removed under reduced pressure and the residue was purified by silica gel column chromatography to give compound **10** (1.52 g, 95%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.78 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 4.01 (t, J = 6.3 Hz, 2H), 3.88 (s, 2H), 2.61 (s, 2H), 2.42 (s, 3H), 1.56 – 1.48 (m, 4H), 1.38 (s, 9H), 1.27 – 1.22 (m, 1H), 1.22 – 1.16 (m, 2H), 1.08 – 1.02 (m, 2H), 0.89 – 0.80 (m, 2H).

tert-butyl 4-(4-(1,3-dioxoisoindolin-2-yl)butyl)piperidine-1-carboxylate (12). Compound **10** (1.52 g, 3.69 mmol) and the potassium salt of phthalimide (0.89 g, 4.80 mmol) were dissolved in DMF (15 mL) and the reaction was stirred at 110 ℃ for 12 h. The reaction was monitored by TLC. The reaction solution was poured into water (150 mL), EA (70 mL) extracted three times, and the

organic phase was dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to isolate compound **12** (1.31 g, 92%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 – 7.85 (m, 2H), 7.84 – 7.83 (m, 2H), 3.89 (s, 2H), 3.56 (t, *J* = 7.1 Hz, 2H), 2.89 (s, 1H), 2.73 (d, *J* = 0.7 Hz, 1H), 1.60 – 1.54 (m, 4H), 1.37 (s, 9H), 1.36 – 1.31 (m, 1H), 1.30 – 1.24 (m, 2H), 1.24 – 1.19 (m, 2H), 0.96 – 0.88 (m, 2H).

tert-butyl 4-(4-aminobutyl)piperidine-1-carboxylate (13). Compound **12** (1.31 g, 3.39 mmol) was dissolved in EtOH (10 mL), 85% hydrazine hydrate (10 mL) was added and the reaction was heated to 110 ℃ and stirred for 12 h. After cooling the reaction mixture to room temperature, the solid formed in the reaction was removed by filtration. The filtrate was concentrated under reduced pressure to give compound **13** (0.72 g, 83%).

tert-butyl (*E*)-4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carboxylate (14). Compound **8** (0.50 g, 3.37 mmol) and HATU (1.28 g, 3.37 mmol) were dissolved in DMF (10 mL), DIPEA (1.09 g, 8.42 mmol) was added and stirred for 5-10 min, then compound **13** (0.72 g, 2.81 mmol) was added and stirred overnight at room temperature. After monitoring the reaction by TLC, the reaction solution was poured into water, extracted three times with ethyl acetate, combined with the ethyl acetate phase, washed once with saturated saline, dried with anhydrous sodium sulfate, extracted and filtered. The residue was purified by silica gel column chromatography to isolate compound **14** (0.73 g, 67%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.75 (d, *J* = 2.2 Hz, 1H), 8.54 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.14 (t, *J* = 5.7 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.46 – 7.41 (m, 2H), 6.72 (d, *J* = 15.9 Hz, 1H), 3.90 (s, 2H), 3.62 – 3.57 (m, 2H), 3.17 (q, *J* = 6.8 Hz, 2H), 2.65 (s, 2H), 1.79 – 1.73 (m, 2H), 1.64 – 1.58 (m, 2H), 1.46 – 1.41 (m, 2H), 1.38 (s, 9H), 1.31 – 1.29 (m, 1H), 0.93 (qd, *J* = 12.5, 4.3 Hz, 2H).

(*E*)-*N*-(4-(1-benzoylpiperidin-4-yl) butyl)-3-(pyridin-3-yl)acrylamide (1). Compound **14** (0.73 g, 1.88 mmol) was dissolved in DCM (10 mL), added with TFA (5 mL) and stirred at room temperature for 4 h. DCM and TFA were removed under reduced pressure, DMF (10 mL), DIPEA (0.73 g, 5.65 mmol), benzoic acid (0.28 g, 2.26 mmol) and HATU (0.86 g, 2.26 mmol) were added and stirred at room temperature overnight, the reaction was monitored by TLC and the reaction solution was poured into water, extracted three times with ethyl acetate, combined with the ethyl acetate phase, washed once with saturated saline, dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to isolate compound **1** (0.50 g, 68%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.74 (d, *J* = 2.3 Hz, 1H), 8.54 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.13 (t, *J* = 5.7 Hz, 1H), 7.96 (dt, *J* = 8.1, 2.0 Hz, 1H), 7.46 – 7.40 (m, 5H), 7.36 – 7.33 (m, 2H), 6.72 (d, *J* = 15.9 Hz, 1H), 4.45 (s, 1H), 3.54 (s, 1H), 3.20 – 3.15 (m, 2H), 2.98 (s, 1H), 2.73 (s, 1H), 1.67 (d, *J* = 87.2 Hz, 2H), 1.53 – 1.41 (m, 3H), 1.35 – 1.28 (m, 2H), 1.28 – 1.22 (m, 2H), 1.05 (s, 2H).

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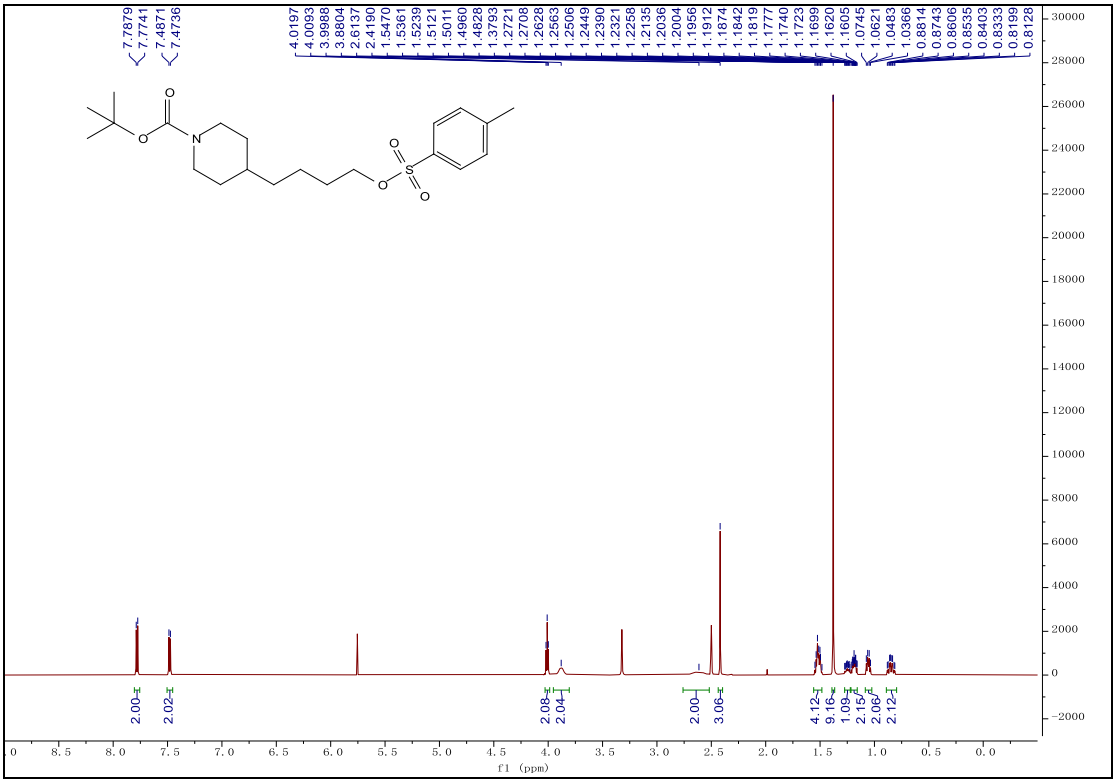
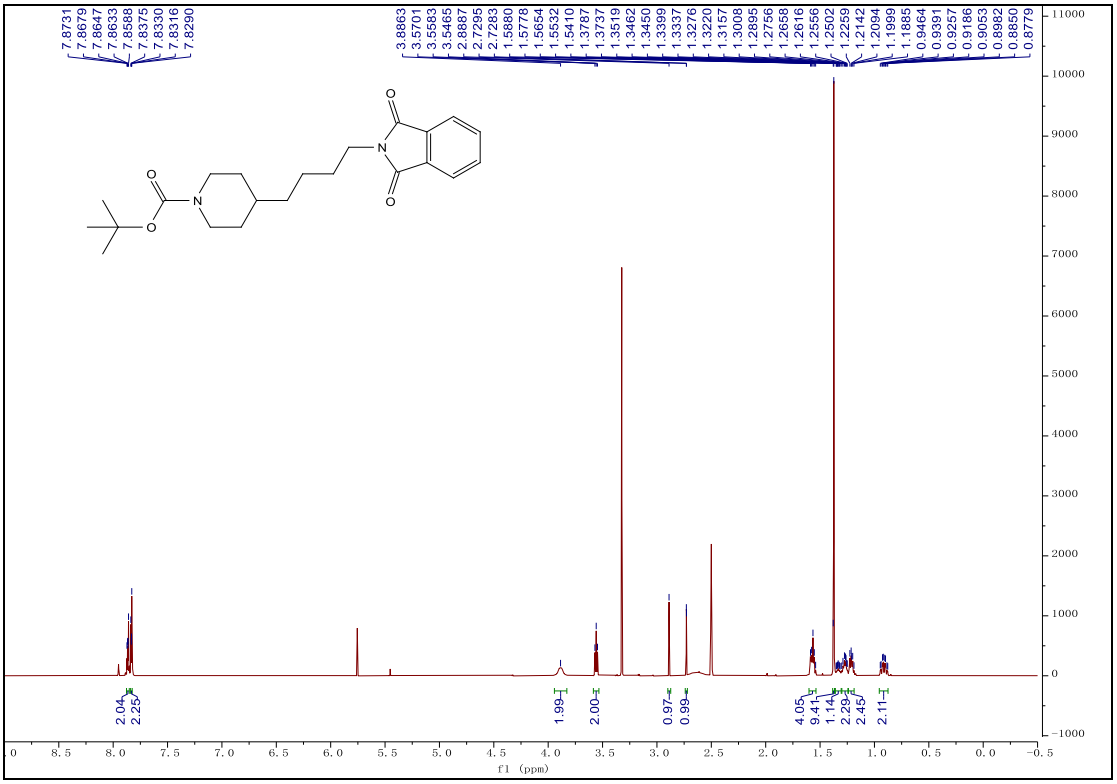
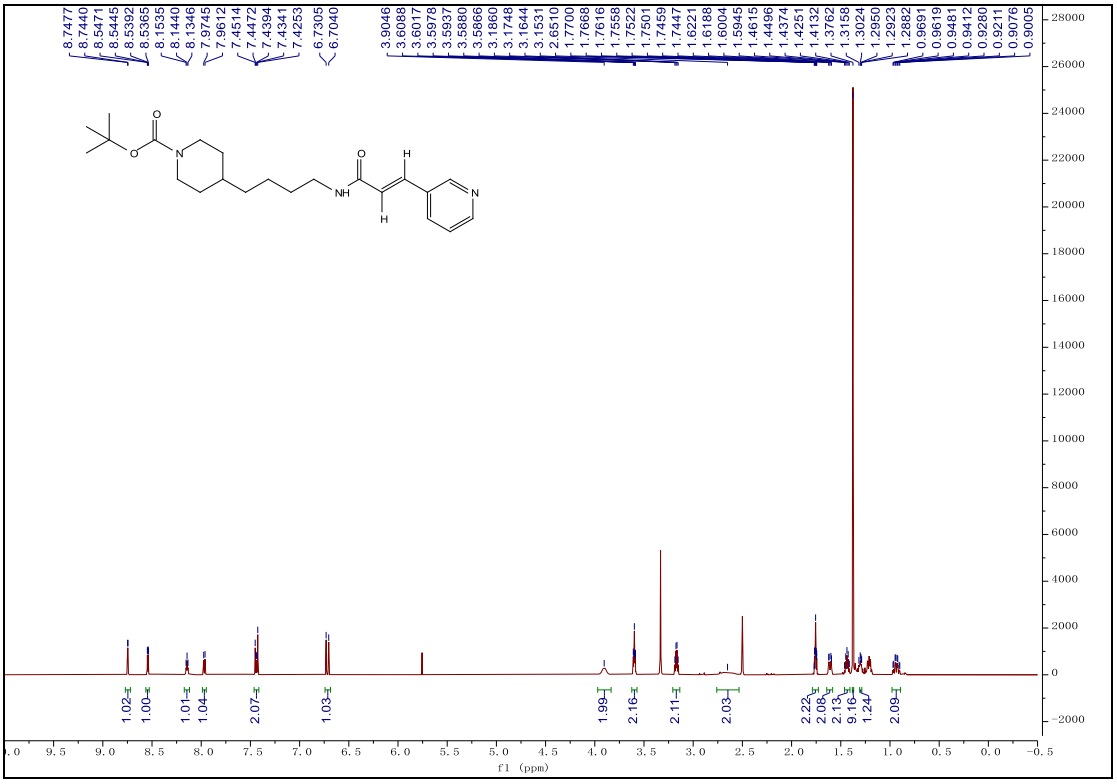
Appendix



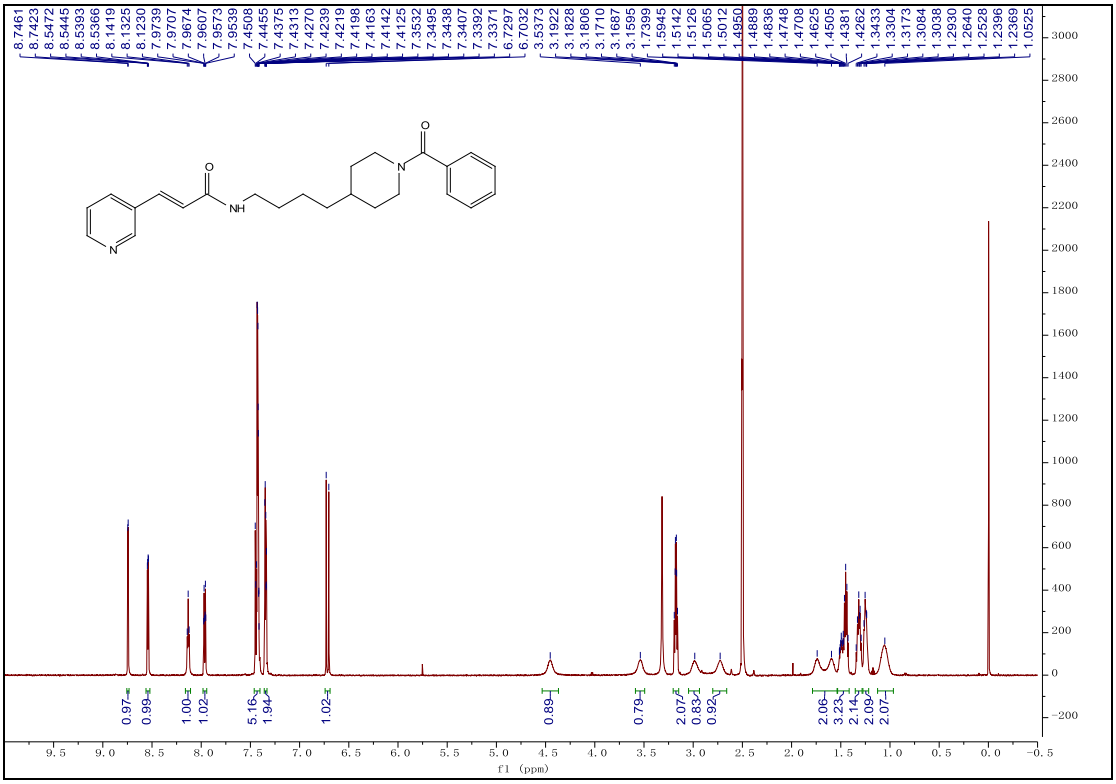
^{1}H NMR spectrogram of compound **10**



^{1}H NMR spectrogram of compound **12**



^{1}H NMR spectrogram of compound **14**



^{1}H NMR spectrogram of compound **1**