A SIMPLE METHOD OF SYNTHESIZING THE DRUG COMPOUND BELINOSTAT

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Huynh Nhu Thao, Nguyen Cuong Quoc, Nguyen Trong Tuan,
Bui Thi Buu Hue, Tran Quang De
Department of Chemistry, College of Natural Sciences, Can Tho University

TÓM TẮT

PHƯƠNG PHÁP ĐƠN GIẢN TỔNG HỢP THUỐC BELINOSTAT

Một quy trình tiện lợi và thân thiện với môi trường được sử dụng để tổng hợp hoạt chất Belinostat, được sử dụng trong điều trị ung thư hạch tế bào T ngoại biên (PTCL). Trải qua một chuỗi gồm 6 bước bao gồm sử dụng nguyên liệu ban đầu đơn gian, có sẵn và rẻ tiền với hiệu suất tổng thể dao động từ 6.9% đến 13%. Một điểm nổi bật nữa là việc tinh chế các chất trung gian và sản phẩm đơn giản và nhanh chóng. Cấu trúc của các hợp chất tổng hợp đã được xác nhận dựa trên dữ liệu quang phổ IR, ^{1}H-NMR and ^{13}C-NMR.

Keywords: *Belinostat, Peripheral T-cell lymphoma (PTCL), HDAC inhibitors*

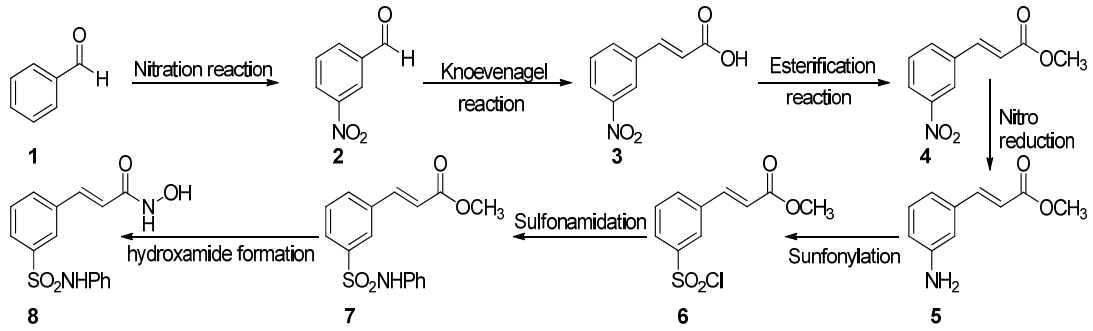
1. INTRODUCTION

Histone acetylation and deacetylation play an important role in the regulation of gene expression, influencing the transcription of many genes. Deregulation of histone results in abnormal gene expression profiles involved in controlling cell proliferation, differentiation and apoptosis of cancer cells, and is associated with malignancy [1-4]. Enzyme histone deacetylases (HDACs) are one of the main causes in the gene expression regulation network in cancer because of their repressive role on tumor suppressor genes [5]. Beyond cancer, there may be several novel therapeutic areas where HDACi may provide therapeutic benefits such as in inflammation, Polycythemia vera, Thrombocythemia, Myelofibrosisand Neurodegenerative diseases such as Alzheimer's disease and Huntington's disease [6].

Most HDAC inhibitors reported so far can be grouped into four chemical families (hydroxamic acids, benzamides, short-chain fatty acids, and macrocyclic peptides), and hydroxamic acids have been proven to be the most potent and the major class in clinical trials [7-8]. Hydroxamic acid derivatives that exert its activity by complexation of a zinc ion that is supposed to mediate the acetamide cleavage at the catalytic site [9]. There are several synthetic hydroxamic acids presenting good therapeutic utility in cancer. Among them, a compound worth mentioning is Beleodaq (Belinostat, PXD 101) which caught attention from the Medical Research Council from the very early of 1990s thanks to fundamental discoveries regarding its activity to control the mammalian cell cycle regulation [10]. At the beginning, there are many oppositions against the idea of working further on Belinostat as a potential inhibitor of HDAC enzymesbecause modulation of gene expression by such a blunt instrument was bound to be grossly toxic. In contrast to lingering doubts, a clinical trial confirmed the strong activity of belinostat with out-of-expected results. Specifically, in addition to responses in approximately 26% of patients,

nearly two thirds of patients experienced disease reduction when being treated by Beleodaq [11].

Fortunately, these discovery efforts led to the marketing approval of FDA for the treatment of patients with relapsed and refractory peripheral T-cell lympomas (PTCL) [12], a rare and fast-growing type of non-Hogkin lymphoma (NHL). Due to its outstanding biological and pharmacological properties, many total synthesis procedures of belinostat have been proposed, however, most of these face controversial issues such as environmental pollution [13], expensive catalysts and starting materials [14], lengthy and complex procedures [15]. Therefore, it is highly essential to research and optimize the Beleodaq synthetic pathway towards a simple, environmentally friendly and high yielding. In contrast to the urgency, not many Vietnamese researchers show interests in this promising compound. Specifically, there has not been any domestic report on the preparation of belinostat. Therefore, in this article, we propose a total synthetic pathway of Beleodaq with a 6-step process, starting with benzaldehyde; which is considered to be suitable for Vietnamese laboratories in particular and most basic organic chemistry laboratories in general.



Scheme 1. The general procedure of belinostat synthesis

2. MATERIALS AND METHODS

Experimental section:

General: All of the starting materials, reagents and solvents are commercially available and used without further purification. Analytical samples were obtained by column chromatography on silica gel. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 500 in Vietnam and a Bruker Ascend 300 in Taiwan. Electrospray ionization mass spectrometry (ESI-MS) analyses was recorded by an Agilent 1100 in Vietnam. The reactions were monitored by thin-layer chromatography (TLC) and compounds were visualized on TLC with UV-light.

Preparation of 3-Nitrobenzaldehyde (2)

1.1 g Potassium nitrate was dissolved in 5 mL concentrated sulfuric acid. This mixture was then cooled in the ice bath, adding 1.06 g Benzaldehyde **1** while stirring. Continuing stirring for 1 hour, the temperature of the vessle should be maintained from 5 to 10 °C. After the completion of the reaction, the crude product was poured slowly into the crushed ice while stirring vigorously. The yellow precipitate will be obtained. Washing the precipitate with solution of Na_2CO_3 until pH>7, with 100 mLof water twice and then use the vaccum filter to get the pure 3-Nitrobenzaldehyde. FT–IR (KBr) ν (cm^{-1}): 3064, 2924, 2879, 1704, 1613, 1533, 1351, 1200, 1080, 934, 814, 728, 672. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 10.12 (s, 1H, –C**H**O), 8.71 (dd, *J*=1.8 Hz, 1H, =C**H**–), 8.47-8.71 (m, 1H, =C**H**–), 8.21-8.25 (m, 1H, =C**H**–), 7.76 (t, *J*=7.95 Hz, 1H, =C**H**–).

Preparation of (*E*)-3-(3-Nitrophenyl)acrylic acid (3)

0.52 g Malonic acid and 0.25 mL Pyridine were added into a round bottom flask and then mixed well to dissolve malonic acid. Then 0.775 g of *m*-Nitrobenzaldehyde**2** was introduced into the flask. The mixture was stirred and heated under reflux for 2 hours. After the reaction was complete, the excess acid was neutralized by saturated ammonium chloride solution and 1N HCl solution, and a fine white precipitate will be obtained. The mixture was cooled for 1 hour for complete crystallization and filtered to get **3** in while powder. FT-IR (KBr) ν (cm^{-1}): 2978, 1690, 1634.4, 1442, 1421.9, 1278.4, 1227.8, 976.6, 713.5. ^{1}H-NMR (300 MHz, $CDCl_3$, δppm*)* (Phụlục 2.)*:* 12.63 (*s*, 1H, –COOH), 8.5 (*t*, *J*=1.8, 1H, =C**H**–), 8.24 (*q*, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 1H, =C**H**–), 8.2 (*q*, J_1= 6.9, J_2 = 0.6 Hz, 1H, =C**H**–), 7.7 (*q*, J_1 = 8.4 Hz, J_2 = 15.6 Hz, 2H, =C**H**–), 6.74 (*d*, *J* = 16.2, 1H, =C**H**–).

Preparation of Methyl (*E*)-3-(3-nitrophenyl)acrylate (4)

To a solution of **2** (0.03 mol, 5.79 g) and CH_3OH (150 mL) in a 250 mL flask were added concentrated sulfuric acid (0,00075 mol, 0.0735 g). The mixture in the flask was heated under reflux . After 12 hours, the solvent was evaporated partially under reduced pressure, and then neutralized with solution of $NaHCO_3$ 10% to get a white precipitate. It was then filtered by a vacuum, rinsed with 100 mL of H_2O to obtain a fine white crystal **4** (5.5 g, 88.5%). FT–IR (KBr) ν (cm^{-1}): 3091, 2953, 2925, 1708, 1637, 1524, 1436, 1355, 1318, 1291, 1202, 1167, 1096, 986, 815, 742, 663, 579, 544. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.37 (d, *J*= 1.8 Hz, 1H, =C**H**–), 8.24 (dd, J_1= 1.5 Hz, J_2= 1.2 Hz, 1H, =C**H**–), 7.80 (d, *J*= 7.8 Hz, 1H, =C**H**–), 7.72 (d, *J*= 15.9 Hz, 1H, =C**H**–), 7.58 (t, *J*= 7.95 Hz, 1H, =CH-), 6.55 (d, *J*= 15.9 Hz, 1H, =C**H**–), 3.83 (s, 3H, –C**H**$_3$).

Preparation of Methyl (*E*)-3-(3-aminophenyl)acrylate (5) using $SnCl_2.2H_2O$

To a stirred solution of $SnCl_2.2H_2O$ (1.7 mmol, 0.3842 g) in 5 mL of EtOH in a 25 mL round bottom flask was added **4** (0.5 mmol, 0.1035 g), then the mixture was heated at 80 °C for 3.5 hours. The mixture was allowed to cool to room temperature, then evaporate the solvent. The residue is neutralized to pH = 7 with saturated Na_2CO_3 solution. The resulting mixture is extracted with EtOAc. The organic extract is washed again with brine, then dried by anhydrous Na_2SO_4, evaporated, and then a orange solid is obtained. Purifying the product by chromatography of the silica gel column to get **5** in luminous green (0.085 g, 95.6%). FT–IR (KBr) ν (cm^{-1}): 3448, 3357, 3218, 2950, 1703, 1634, 1601, 1581, 1460, 1334, 1307, 1258, 1177, 983, 922, 791, 683. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J*= 15.9 Hz, 1H, =C**H**–), 7.17 (t, *J*= 7.65 Hz, 1H, =C**H**–), 6.92 (d, *J*= 7.8 Hz, 1H, =C**H**–) 6.81 (t, *J*= 1.8 Hz, 1H, =C**H**–), 6.68-6.72 (m, 1H, =C**H**–), 6.37 (d, *J*= 15.9 Hz, 1H, =C**H**–), 3.79 (s, 1H,–C**H**$_3$), 3.73 (s, 2H, –N**H**$_2$).

Preparation of Methyl (*E*)-3-(3-aminophenyl)acrylate (5) using Zn

To a solution of comound **4** (221 mg, 1 mmol) in MeOH were added NH_4OAc (3 mmol, 231 mg) and Zn (5 mmol, 325 mg). The mixture was stirred at room temperature for 15 minutes. It should be noted that zinc powder should be added slowly to avoid unwanted by-products. After the completion of the reaction, zinc was removed, the desired product was extracted by ethyl acetate and then purified by column chromatography to afford the luminous green solid **5** (140 mg, 72%).

Preparation of Methyl (*E*)-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylate (7) using $SOCl_2$

Preparation of diazonium salt **5a**: Concentrated hydrochloric acid (5 mL) was slowly added to **5** (5 mmol, 0.885 g). The reaction vessel should be kept under 5 °C during the addition. The resulting mixture is then cooled to 0 °C. A solution of $NaNO_2$ (5 mmol, 0.345 g in 1.48 mL H_2O) was added very slowly to the

mixture. After that, the stirring was continued for 10 minutes to obtain diazonium salt of **5a**.

Preparation of sulfonyl chloride **6**: $SOCl_2$ (0.02 mol, 2.38 g) was slowly added to 10 mL of H_2O. The mixture was cooled to 0-5 °C followed by the addition of CuCl (0.1 mmol, 0.00995 g). Diazonium salt **5a** was added dropwise (making sure that the reaction temperature does not exceed 5 °C). After the addition is complete, the mixture continues to be stirred at 0 °C for 75 minutes. Then the mixture was neutralized with 10% $NaHCO_3$ solution, extracted with EtOAc, dried by anhydrous Na_2SO_4, evaporated the solvent to obtain compound **6** as a brown liquid (R_f = 0.42 Hex:EtOAc = 5:1). Compound **6** is used directly for subsequent reactions without purification. (Note: **6** decomposes at over 40 °C).

Preparation of sulfonamide Sulfonamide reaction: Pyridine (0.48 mL, 7.5 mmol) is added to aniline (0.59 mL, 6.5 mmol) in 3 mL EtOAc, which is continuously stirred at a temperature of 0-5 °C. Compound **6** obtained from the above reaction is dissolved in 2 mL EtOAc and added slowly to the mixture, keeping the reaction vessel temperature not exceeding 5 °C for 1 hour. After the reaction was complete, the mixture was washed with HCl, then neutralized with $NaHCO_3$, extracted with EtOAc, washed with brine and dried by anhydrous Na_2SO_4. The residue was purified with column chromatography to obtain colorless crystals **7** (0.36 g, the overall yield is 22.6%). FT-IR (KBr) ν (cm^{-1}): 3172, 3081, 2953, 1697, 1643, 1437, 1345, 1331, 1305, 1218, 1157, 1090, 996, 867, 772, 713. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 7.87 (s, 1H,=C**H**–), 7.74 (d, *J*= 8.1 Hz, 1H, =C**H**–), 7.65 (d, *J*= 6 Hz, 1H, =C**H**–), 7.61 (d, *J*= 15.9 Hz, 1H, =C**H**–), 7.45 (t, *J*= 7.8 Hz, 1H, =C**H**–), 7.23-7.28 (m, 2H, =C**H**–), 7.05-7.16 (m, 3H, =C**H**-), 6.69 (br, 1H, –N**H**–), 6.41 (d, *J*= 15.5 Hz, 1H, =C**H**–), 3.81 (s, 3H, –C**H**$_3$)

Preparation of Methyl (*E*)-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylate (7) using SO_2

Preparation of diazonium salt **5a**: Diazonium salt **5a** was prepared as mentioned above.

Preparation of sulfonyl chloride **6**: In another flask, SO_2 gas is introduced into 50 mL AcOH, the temperature of the reaction vessel should be lower than 5 °C until getting saturation. CuCl (2.5 mmol, 250 mg) was added to the reaction vessel, then continue adding SO_2 until the solution changing from green to yellow-green. Diazonium salt was added slowly to the mixture and then the mixture was stirred at a temperature not exceeding 5 °C. After 2 hours, the reaction mixture was extracted with EtOAc, the organic extract was washed again with 5% $NaHCO_3$ solution and dried by anhydrous Na_2SO_4 and evaporated to obtain **6** in black oil. The product is used directly for the next step without further purification.

Preparation of sulfonamide Sulfonamide reaction: The sulfonamide formation reaction is similar to the above description with product **6** obtained (0.5 g, the overall yield is 32%).

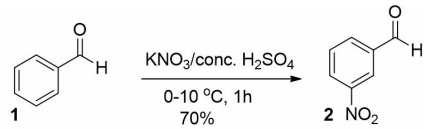
Preparation of (*E*)-*N*-hydroxy-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylamide (Beleodaq 8)

KOH (2.2 g, 39 mmol) was added to the hydroxylamine hydrochloride (2.7 g, 39 mmol) in anhydrous EtOH (10 mL). The mixture was stirred and then cooled to 0 °C and filtered. The filtrate, KOH (0.35 g, 6.39 mmol) and **7** (0.37 g, 1.17 mmol) were added to a round bottom flask under agitation at 0 °C for 1 hour. After that, 10 mL H_2O was added to quench the reaction. Then the neutralization was carried out by concentrated HCl until pH = 7, followed by the extraction with EtOAc, washed with brine and the removal of solvent. Purification of the product was carried out by silica gel column chromatography to obtain an off white solid **8** (0.31 g, 84%). FT-IR (KBr) ν (cm^{-1}): 3225, 3020, 2881, 1661, 1601, 1491, 1422, 1337, 1304, 1215, 1156, 1097, 1061, 1003, 975, 929, 885, 710, 673. MS (ESI) *m/z* 316.8[M–H]$^-$. ^{1}H-NMR (500 MHz, DMSO, δ

ppm): 7.89 (s, 1H,=C**H**–), 7.74 (d, *J*= 8 Hz, 1H, =C**H**–), 7.69 (d, *J*= 8 Hz, 1H, =C**H**–), 7.54 (t, *J*= 7.75 Hz, 1H, =C**H**–), 7.43 (d, *J*= 16 Hz, 1H, =C**H**–), 7.19-7.21 (m, 2H, =C**H**–), 6.98 (t, *J*= 7.25 Hz, 1H, =C**H**–), 6.51 (d, *J*= 16 Hz, 1H, =C**H**–). ^{13}C-NMR (125 MHz, DMSO, δ ppm): 140.85, 138.40, 136.43, 135.78, 131.64, 129.88, 127.04, 124.72, 123.73, 121.26, 120.38.

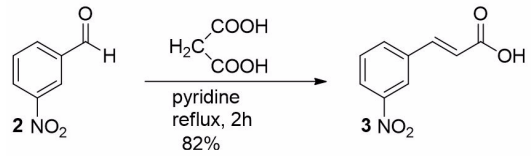
3. RESULTS AND DISCUSSION

The first reaction of the procedure is nitration reaction employing available commercial materials including benzaldehyde and the mixture of potassium nitrate and concentrated sulfuric acid. Conventionally, the nitration reaction is set up between aromatic aldehyde and the acidic mixture of concentrated sulfuric acid and nitric acid. However, with the existence of carbonyl group as an electron withdrawing group, a milder solution is required to form nitronium ion. In this report, we use potassium nitrate as the main source of nitronium ion, with the yield of 70% (**Scheme 2**).



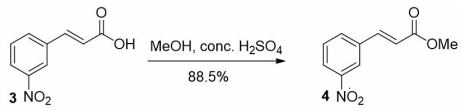
*Scheme 2. Nitration reaction of benzaldehyde **1***

The nitration reaction is followed by Knoevenagel condensation between **2**, and malonic acid as a nucleophile (**Scheme 3**). Pyridine is also added as a basic reagent. One advantage of the Knoevenagelemployment is that it is highly stereoselective. This is highly beneficial because only the *E* isomer possesses therapeutic activity while the isomer with *Z*-double bond configuration leads to inactive compound.



*Scheme 3. Knoevenagel condensation between **2** and malonic acid*

Before a chain of reactions occurring on nitro group, the esterification reaction is applied to protect the carboxylic acid group of the Knoevenagel adduct (**Scheme 4**).



*Scheme 4. Esterification of **3***

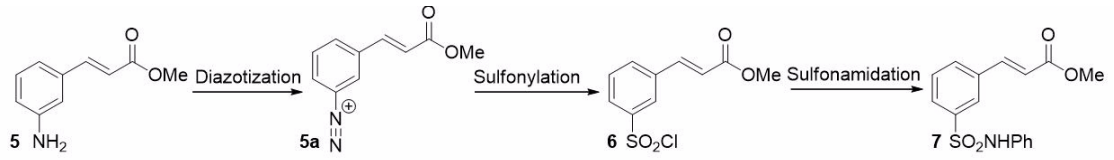
The nitro group reduction is carried out by two different reducing agents namely $SnCl_2$ and Zn (**Scheme 5**). As shown in **Table 1**, the formation of **5** through SnCl2 and Zn shows different strong points and weaknesses.

*Scheme 5. Nitro reduction of **4***

Table 1. The comparison between the use of $SnCl_2$ and Zn as reducing agents

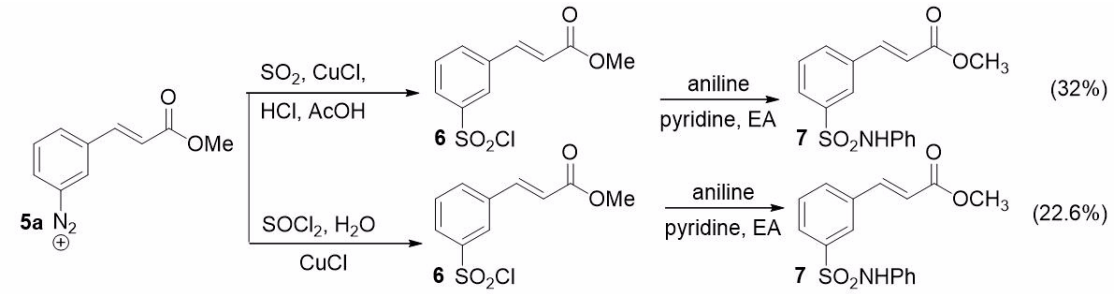
Reductant	Yield	Advantages	Disadvantages
$SnCl_2$. $2H_2O$	95.6%	High yield	Long reaction time (3.5h)
Zn/ NH_4OAc	72%	Short reaction time (15 min)	Lower yield

The next step is the transformation from amine group to sulfonamide group, including three smaller steps including diazotination, sulfonylation and sulfonamidation (**Scheme 6**).



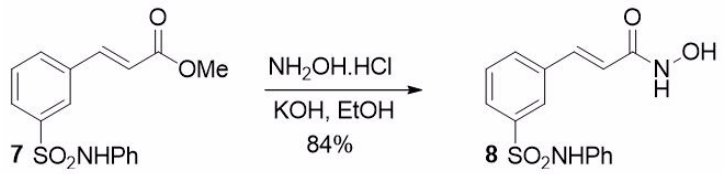
Scheme 6. The transformation from amine to sulfonamide

It should be noted that the introduction of sulfonyl chloride group into the aromatic ring is considered the most challenging but vital task of the whole procedure. Although previous reports proposed effective starting materials namely oleumandchlorosulfonic acid by only one step but expensive and inconvenient to store. Therefore, an efficient method is postulated in this report. At first, the conversion from the amine **5** to azo compound **5a**is required. With **5a** in hands, two different ways of sulfonyl chloride addition, sulfur dioxide and thionyl chloride, are tested (**Scheme 7**). The sulfonylation was then followed by the sulfonamidation of **6** in the presence of pyridine to afford **7** with the yield of 32% and 22.6% for the use of SO_2 and $SOCl_2$ respectively. Either of $SOCl_2$ or SO_2 has its own benefits and drawbacks. While the reaction carried by SO_2 is more selective and efficient with higher yield, it then leads to environmental concerns and the need for complex apparatus. In contrast, the use of $SOCl_2$ as the sulfur dioxide source is more direct and simple but produces lower yield and by-products.



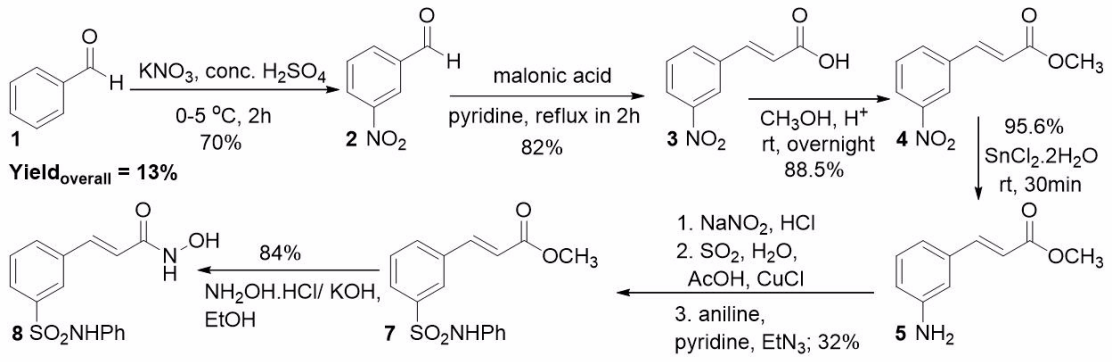
Scheme 7. Sulfonamide formation using SO_2 and $SOCl_2$

Belinostat was finally obtained by hydroxamide formation reaction between**7**and $NH_2OH.HCl$ in 84% yield.

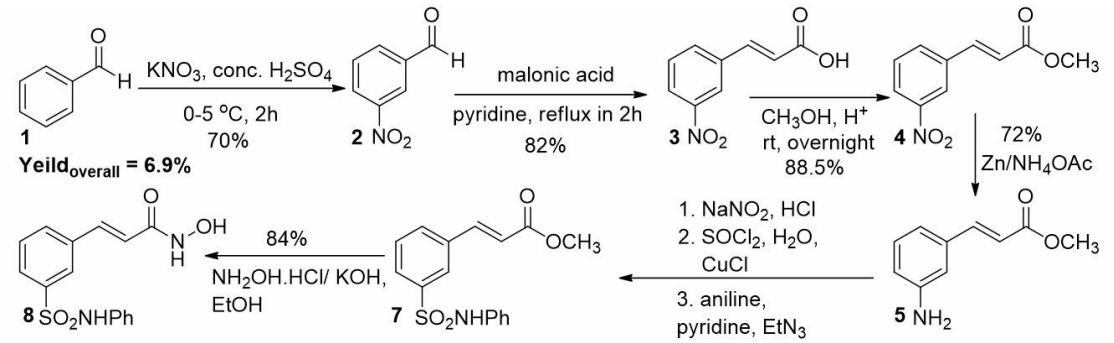


Scheme 8. Hydroxamic acid formation employing $NH_2OH.HCl$

After all experiments conducted, we come to conclude the conditions for the total synthesis of Beleodaq as shown in **Scheme 9** and **Scheme 10** with the yield ranging from 6.9% to 13%. The lowest yield goes to the conditions employed from **Scheme 9** with reducing agents being Zn and sulfonylation agent being $SOCl_2$. However, the strong point from this pathway is simple apparatus and time saving. The opposite can be seen for **Scheme 10**.



Scheme 9. The first pathway of Belinostat synthesis



Scheme 10. The second pathway of Belinostat synthesis

4. CONCLUSION

In conclusion, our proposal of new methods of Belinostat synthesis seems promising with a handful of advantages ranging from inexpensive materials to environmentally benign conditions. The procedure proceeds through 6 steps with the highest yield being 13% and the lowest being 6.9% in total, and the key of the reaction is in charge of sulfonylation.

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