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THE SIMPLE SYNTHETIC ROUTE OF BELINOSTAT-A GREEN APPROACH

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ABSTRACT

Several synthetic methods have been reported. In this report, Belinostat was easily prepared from 3-nitrobenzaldehyde as starting material via a five-step process in 14.16 % total yield and including the Knoevenagel condensation of aldehydes with malonic acid in the presence of organic bases, esterification, reduction of nitro group to amino group, sulfochlorination by thionyl chloride ($SOCl_2$ in H_2O order to provide SO_2) via diazotization in 26.81 % acceptable overall yield, sulfonamidation with aniline and the final amidation with hydroxylamine. The main advantages of the route include inexpensive starting materials, simple methods, safer and more robust and significant environmental benefits.

1. INTRODUCTION

Belinostat (PXD101, **1**, figure 1), with the name (*E*)-*N*-hydroxy-3-(3-(*N*-phenylsulfamoyl) phenyl) acrylamide, a new generation histone deacetylase (HDAC) inhibitor of molecular weight <500 dvC) with a sulfonamide-hydroxamide structure developed by CuraGen/ TopoTarget to treat hematologic and solid tumours (Steele, N. L., *et al.*, 2008; Jane A. Plumb *et al.*, 2003).

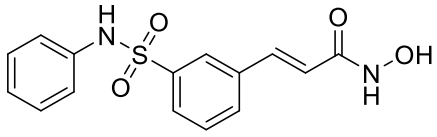


Figure 1. The chemical structure of Belinostat (1)

And has been approved in the US for the treatment of relapsed or refractory peripheral T-cell lymphoma (PTCL) in July 2014 with the trade name is Beleodaq. Although its structure is rather simple, the reported synthetic methods provided belinostat with a 12-33% total yield and are some disadvantages: the use of extremely corrosive oleum required tedious separation and caused a large amount of wastewater, the employment of environmentally unfriendly SO_2, expensive palladium acetate and oxidant 1-hydroxy-1-oxide-1,2-Benziodoxol-3(1*H*)-one were used, and no industrial-scale 3-bromobenzenesulfonyl chloride was commercially available, complicated purification process (Wang, Q., *et al.*, 2016; Reisch, H., *et al.*, 2009; Luo, J., *et al.*, 2015; Xuefei Bao *et al.*, 2016).

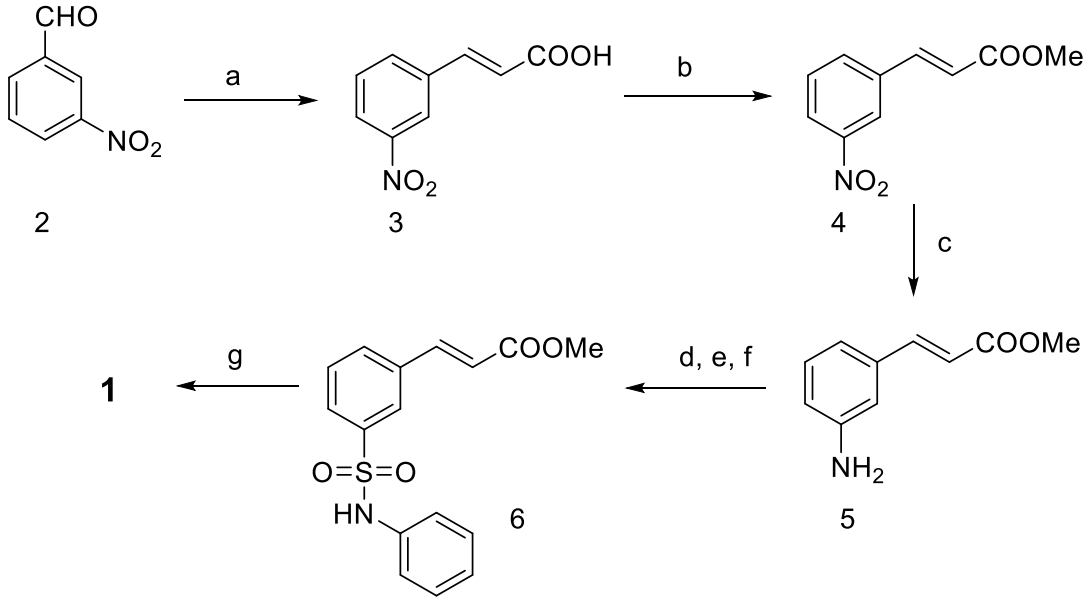
The empirical approach based on the success, as well as the limitations of the published research domestically and internationally serves as a theoretical basis for the Synthesis of belinostat applications in pharmaceutical chemistry. From there, to improve a simpler and more efficient synthesis process, using less toxic reaction agents for the

environment/healthy and inexpensive materials under laboratory conditions.

2. MATERIALS AND METHODS

All of the starting materials, reagents (Merk) and solvents (Chemsol) are commercially available and used without further purification (except the ethanol and methanol solvent is redistilled and stored in Arlene containing dry Na_2SO_4 and sealed with a septum). Analytical samples were obtained by column chromatography on silica gel (230-400 mesh, Merk). The reactions were monitored by thin-layer chromatography (TLC) on 0.2 mm pre-coated silica-gel 60 F_{254} aluminum plates (Merck) and compounds were visualized on TLC with UV-light (254 nm). The structures of the compounds were determined by the spectroscopic methods: FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI/HR-MS and related documents to identify the chemical structure of the isolated substances. The ^{1}H- and ^{13}C-NMR spectra were measured by Bruker 500 MHz equipment and the chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard, ESI-MS and HR-MS were recorded with a VG 7070 Mass Spectrometer operating at 70 eV and SCIEX X500 QTOF Mass Spectrometer system, respectively at the Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi.

3. RESULTS AND DISCUSSION



Scheme 1: Reagents and conditions for the synthesis of belinostat: (a) Malonic acid/pyridine, 105 ^{o}C to 110 ^{o}C, 4 hours; (b) MeOH, 80 ^{o}C, 12 hours; (c) $SnCl_2.2H_2O$/ dry EtOH, reflux, 3 hours; (d) $NaNO_2$/*p*-TsOH/H_2O, room temperature; (e) $SOCl_2$/CuCl/H_2O, 2 hours; (f) aniline, -5 ^{o}C to 0 ^{o}C, 2 hours; (g) $NH_2OH.HCl$/KOH, dry EtOH, 1 hour.

The synthetic route of belinostat **1**, from commercially available 3-nitrobenzaldehyde **2**, involved Knoevenagel condensation reaction with malonic acid in the presence of an organic base, preferably, pyridine for the chemical transformation of benzaldehydes into their corresponding *α,β*-unsaturated acids to afford adduct (*E*)-3-(3-nitrophenyl) acrylic acid **3** in 80.67% yield (K. C. Pandya and Toquir Ahmad Vahidy, 1936). One advantage of the Knoevenagel condensation is that it is highly stereoscopic selective. The esterification reaction is applied to protect the carboxylic acid

group of conjugated acid 3 to methyl (*E*)-3-(3-nitrophenyl) acrylate **4** in 88.86% yield. The reaction conditions for both two steps are quite simple, and the result is the solid product (in high yield) without undergoing purification by column chromatography. This compound **4** was reduced with $SnCl_2$ in anhydrous ethanol to provide methyl (2*E*)-3-(3-aminophenyl) prop-2-enoate 5 with the isolated yield was 91.41 % (Lei Yang *et al.*, 2010). With refined product in hand, a small three-step was carried out successively to transform from the amino group to the sulfonamide group. Diazotization of amine **5** with $NaNO_2$ in dilute *p*-TsOH (Ksenia V. Kutonova *et al.*, 2013), followed by substitution with $SOCl_2$/CuCl in H_2O (sulfonylation) (Philip J. Hogan and Brian G. Cox, 2009), formed the key intermediate methyl (2*E*)-3-(3-chlorosulfonylphenyl) prop-2-enoate. Amination of the sulfonyl chloride with aniline in the presence of pyridine in ethyl acetate afforded methyl (*E*)-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylate **6** with an overall yield of 26.81 % for three steps.

Belinostat was finally obtained by the oximation of sulfonamide **6** with $NH_2OH.HCl$/KOH in anhydrous ethanol in 80.63% yield after purified by column chromatography (Xuefei Bao, 2016).

We report an improved synthesis of belinostat **1** with the following advantages in our laboratory conditions. This route emphasized the introduction of a chlorosulfonyl group into the benzene ring via diazotization and sulfonylation by using *p*-TsOH/$NaNO_2$ in H_2O as solvent at room temperature and $SOCl_2$/CuCl in H_2O at 0 ^oC. Firstly, it is well known that traditional diazonium salts have certain disadvantages-poor thermal stability and explosive properties (it is necessary to avoid temperatures above 5 ^oC in order to prevent uncontrolled decomposition of the intermediate diazonium salt) (Ksenia V. Kutonova *et al.*, 2013). The preparation of aryldiazonium tosylates ($ArN2^+TsO^-$) at room temperature, which possesses high diazonium reactivity but in contrast to usual diazonium salts, has good thermal and storage stabilities and is soluble in water and many organic solvents (John A. Murphy, 2000). Furthermore, using *p*-TsOH/$NaNO_2$ in H_2O is safer and more simple than using acetic acid as solvent, concentrated aq HCl.

Secondly, the preparation of arylsulfonyl chlorides from diazonium salts in the presence of copper (I) salts (CuCl), together with the *situ* reaction of thionyl chloride with H_20 as the sulfur dioxide source instead of introducing SO_2 in glacial acetic acid at 0 ^oC until saturation, has considerable advantages: the product precipitated from the reaction mixture and collected by vacuum filtration, washed with water and dried under vacuum without extracting with organic solvent, thus being protected arylsulfonyl chlorides from hydrolysis. During reaction time, additional benefits arose from the increased thermal stability of the diazonium salt, compared with that in acetic acid as solvent, and the avoidance neutralizing the acetic acid and organic extracts with aqueous solution (Philip J. Hogan and Brian G. Cox, 2009).

So, the aqueous process chemistry for the preparation of aryl sulfonyl chloride by using *p*-TsOH/$NaNO_2$ and $SOCl_2$/CuCl in H_2O as the solvent, which is additionally safer and more robust, can be readily scaled up and has significant environmental benefits.

4. EXPERIMENTAL

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

(*E*)-3-(3-nitrophenyl) acrylic acid was prepared by a modified literature method. 3-nitrobenzaldehyde (1.511 g, 10 mmol) was added to a mixture of malonic acid (1.041 g, 10

mmol) and pyridine (3 mL). The formed mixture was stirred at 105-110 ºC for 4 hours. After the reaction was complete, the mixture was dissolved into NaOH solution until pH= 8-9, then neutralized 4N HCl (pH= 4-5), and a fine white precipitate will appear and filter (by a vacuum) to get **3** (1.557 g, approximately 80.67 %) in while powder. **FT-IR** (KBr, ν (cm^{-1})): 3108-2852 (O–H stretching of carboxylic acid and C–H stretching of methylene and methyl), 1693 (C=O stretching), 1635 (C=C stretching), 1421 (O–H i.p. bending), 1357 and 1301 (N–O stretching), 1280 (C–O stretching), 978 (O–H o.p. bending). **^{1}H-NMR** (500 MHz, $CDCl_3$, δ ppm): 8.39 (*t*, *J*= 2 Hz, 1H, =CH–), 8.22-8.24 (*m*, 1H, =CH–), 7.85 (*d*, *J*= 8 Hz, 1H, =CH–), 7.72 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene), 7.60 (*t*, *J*= 8 Hz, 1H, =CH–), 6.56 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene). **ESI-MS (positive)**: $[M]^+$ = 192.9 *m/z*, **ESI-MS (negative)**: $[M–H]^-$ = 191.7 *m/z*.

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

(*E*)-3-(3-nitrophenyl)acrylic acid (3.860 g, 20 mmol) in anhydrous MeOH (100 mL) and followed by the addition of concentrated sulfuric acid (0.0005 mol, 0.049 g), the mixture was heated at 80 ºC for 12 hours. The mixture was half evaporated under reduced pressure and neutralized with 10 % $NaHCO_3$ solution to get a white precipitate. After filtered vacuum and washed with cooled ethanol (10 mL) and H_2O to obtain a white power **4** (3.682 g, 88.86 %). **FT–IR** (KBr, ν (cm^{-1})): 3091 (=C-H stretching of alkene), 2953 and 2925 (C-H stretching of methylene), 1708 (C=O stretching), 1637 (C=C stretching), 1355 and 1318 (N–O stretching), 1291 (C–O stretching). **^{1}H-NMR** (500 MHz, $CDCl_3$, δ ppm): 8.38 (*t*, *J*= 1.5 Hz, 1H, =CH–), 8.22-8.25 (*m*, 1H, =CH–), 7.82 (*d*, *J*= 8 Hz, 1H, =CH–), 7.73 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene), 7.59 (*t*, *J*= 8 Hz, 1H, =CH–), 6.57 (*d*, *J*= 16 Hz, 1H, =CH–), 3.84 (*s*, 3H, $–OCH_3$). **ESI-MS (positive)**: $[M+H]^+$= 207.9 *m/z*, **ESI-MS (negative)** $[M–CH3]^-$= 192.6 *m/z*.

Preparation of methyl (*E*)-3-(3-aminophenyl)acrylate (5)

Methyl (2*E*)-3-(3-nitrophenyl)prop-2-enoate **4** (2.072 g, 10 mmol) and $SnCl_2.2H_2O$ (7.910 g, 35 mmol) in anhydrous ethanol (25 mL) was heated at 80 ºC for 3 hours. The mixture was allowed to cool, and then the solvent was half evaporated under reduced pressure. The residue was poured into ice water and neutralized with a saturated Na_2CO_3 solution, and the resulting mixture was extracted with ethyl acetate. The organic extract was washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was then evaporated under reduced pressure to provide an orange solid. Purify the product by chromatography of the silica gel column, yielding amin **5** in white powder (1.618 g, 91.41 %). **FT–IR** (KBr, ν (cm^{-1})): 3448 and 3357 (N–H stretching of primary amine), 3218 (shoulder band of primary amine), 2950 (C–H stretching of methylene), 1703 (C=O stretching), 1634 (C=C stretching), 1601 (N–H bending of primary amine), 1258 (C–N stretching), 791 (N–H wagging of primary amine). **^{1}H-NMR** (500 MHz, $CDCl_3$, δ ppm): 7.60 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene), 7.17 (*t*, *J*= 8 Hz, 1H, =CH–), 6.93 (*d*, *J*= 7.5 Hz, 1H, =CH–) 6.82 (*t*, *J*= 2 Hz, 1H, =CH–), 6.70-6.72 (*m*, 1H, =CH–), 6.38 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene), 3.80 (*s*, 1H, $–OCH_3$). **ESI-MS (positive)**: $[M]^+$= 177.8 *m/z*, **ESI-MS (negative)** $[M–H]^-$= 175.7 *m/z*.

Preparation of methyl (*E*)-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylate (6)

(**a**) Thionyl chloride (1.56 mL) was added dropwise over 10 min to water (9 mL), was cooled to -3 ºC using an acetone/ice bath.

Copper (I) chloride (0.006g) was added to the mixture and the resultant yellow-green solution.

(**b**) To a solution of *p*-TsOH.H_2O (8.559 g, 45 mmol) in H_2O (45 mL) was added methyl (2*E*)-3-(3-aminophenyl) prop-2-enoate (0.885 g, 5 mmol) under vigorous stirring at room temperature. After stirring for 20 minutes, anhydrous $NaNO_2$ (3.1 g, 45 mmol) was slowly added for 5 minutes. The results solution was then stirred until the starting methyl (2*E*)-3-(3-aminophenyl) prop-2-enoate **5** disappeared as monitored by TLC.

(**c**) The diazonium solution from step **b** was added dropwise to the yellow-green solution from step **a**, maintaining the temperature of the reaction mixture between -3 to 0 ºC. As the reaction proceeded, a solid began to precipitate. When the addition was complete, the reaction mixture was cooled at 0 °C for 2 hours. The solid was collected by vacuum filtration, washed with water and dried under vacuum at below 35 °C. **FT-IR** (KBr, ν (cm^{-1})): 2952 (=C–H stretching of alkene), 2924 (C–H stretching of methylene), 2853 (C–H stretching of methyl), 1719 (C=O stretching), 1640 (C=C stretching), 1437 (S=O asymmetrical stretching), 1277 (C–O stretching), 1172 (S=O symmetrical stretching).

(**d**) The solid from step **c** was dissolved in ethyl acetate (20 mL) and cooled at 0 °C. Then, this solution was added dropwise to the solution of aniline (4 mmol, 0.365 ml) and pyridine (4.5 mmol, 0.363 mL) in ethyl acetate (16 mL) at 0 °C (the solution of step c was maintained at -5 °C throughout the addition). The mixture was stirred and kept the reaction vessel temperature not increased by 5 °C for 2 hours. The mixture after the reaction was washed with 10% HCl and extracted with ethyl acetate, brined and dried by anhydrous Na_2SO_4. Purify the product by silica gel column chromatography to obtain sulfonamide **6** colorless solid forms (0.425 g, the whole process efficiency is 26.81 %). **FT-IR** (KBr, ν (cm^{-1})): 3172 (N–H stretching of –SO_2NH–), 3081 (=C–H stretching of alkene), 2953 (C–H stretching of methylene), 1697 (C=O stretching), 1643 (C=C stretching), 1438 (C–N stretching), 1345 (S=O asymmetrical stretching), 1291 (C–O stretching), 1157 (S=O symmetrical stretching). **^{1}H-NMR** (500 MHz, $CDCl_3$, δ ppm): 7.87 (*t*, *J*= 1.5 Hz, 1H, =CH–), 7.73 (*m*, 1H, =CH–), 7.65 (*d*, *J*= 8 Hz, 1H, =CH–), 7.62 (*d*, *J*= 16.5 Hz, 1H, =CH–, *trans*-alkene), 7.46 (*t*, *J*= 8 Hz, 1H, =CH–), 7.25-7.28 (*m*, 2H, =CH–), 7.14-7.17 (*m*, 1H, =CH–), 7.06-7.07 (*m*, 2H), 6.52 (*s*, 1H, –NH–), 6.41 (*d*, *J*= 16 Hz, 1H, =CH–, *trans*-alkene), 3.81 (*s*, 3H, $–OCH_3$). **ESI-MS (positive)**: $[M]^+$= 317.8 *m/z*, **ESI-MS (negative)** $[M–H]^-$= 315.8 *m/z*.

Preparation of (*E*)-*N*-hydroxy-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylamide (1)

Belinostat 1 was prepared by a modified literature method. Potassium hydroxide (KOH, 2.2 g, 39.0 mmol) was added to a solution of hydroxylamine hydrochloride ($NH_2OH.HCl$, 2.70 g, 39.0 mmol) in anhydrous ethanol (10 mL) under agitation at room temperature, and the resulting mixture was cooled to 0 °C using an ice bath and filtered. Potassium hydroxide (0.35 g, 6.39 mmol) and sulfonamide 6 (0.405 g, 1.27 mmol) were added to the filtered solution, and the mixture was stirred at 0 °C for 1 h. Then 30 ml of water were added to quench the reaction, and the mixture was neutralized with 4N HCl solution, followed by the extraction with ethyl acetate, brine and dried by anhydrous Na_2SO_4. Purify the product by silica gel column chromatography to provide belinostat **1** as a white solid (0.326 g, 80.63 % yield). **FT-IR** (KBr, ν (cm^{-1})): 3225 (N–H stretching), 3020 (=C–H stretching of alkene), 2882, 1661 (C=O stretching), 1601 (C=C stretching), 1491-1422 (C–N stretching), 1337 (S=O asymmetrical stretching), 1215 (C–O stretching), 1156 (S=O symmetrical stretching).

^{1}H-NMR (500 MHz, MeOD, δ ppm): 7.90 (*s*, 1H, =CH–), 7.72-7.75 (*m*, 2H, =CH–), 7.55 (*d*, *J*= 15.5 Hz, 1H, =CH–, *trans*-alkene), 7.51 (*t*, *J*= 7.5 Hz, 1H, =CH–), 7.23 (*m*, 2H, =CH–), 7.09 (*m*, 3H, =CH–), 6.49 (*d*, *J*= 15.5 Hz, 1H, =CH–, *trans*-alkene), 6.51 (*d*, *J*= 16 Hz, 1H, =CH–). **^{13}C-NMR** (125 MHz, MeOD, δ ppm): 165.5 (C=O); 141.9, 139.54, 138.7, 137.24, 132.6, 130.7, 130.2, 128.9, 127.0, 126.1, 122.7, 120.8 (=CH_2–). **HR-MS**: $[M+H]^+$= 319.0747 *m/z*.

5. CONCLUSIONS

In summary, Belinostat was prepared successfully from easily available 3-nitrobenzaldehyde with a total yield of 14.16 % via the five-step process in our laboratory conditions. This route has convenient and economical by using inexpensive reagents/materials and less toxic with the environment, classical organic synthesis method (heating combined reflux, cooling in salt/ice bath), easy purification process.

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