

HETEROCYCLES, Vol. 102, No. 9, 2021, pp. 1803 - 1809. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 4th June, 2021, Accepted, 30th June, 2021, Published online, 2nd July, 2021
DOI: 10.3987/COM-21-14500

NEW SYNTHESIS OF ABEXINOSTAT

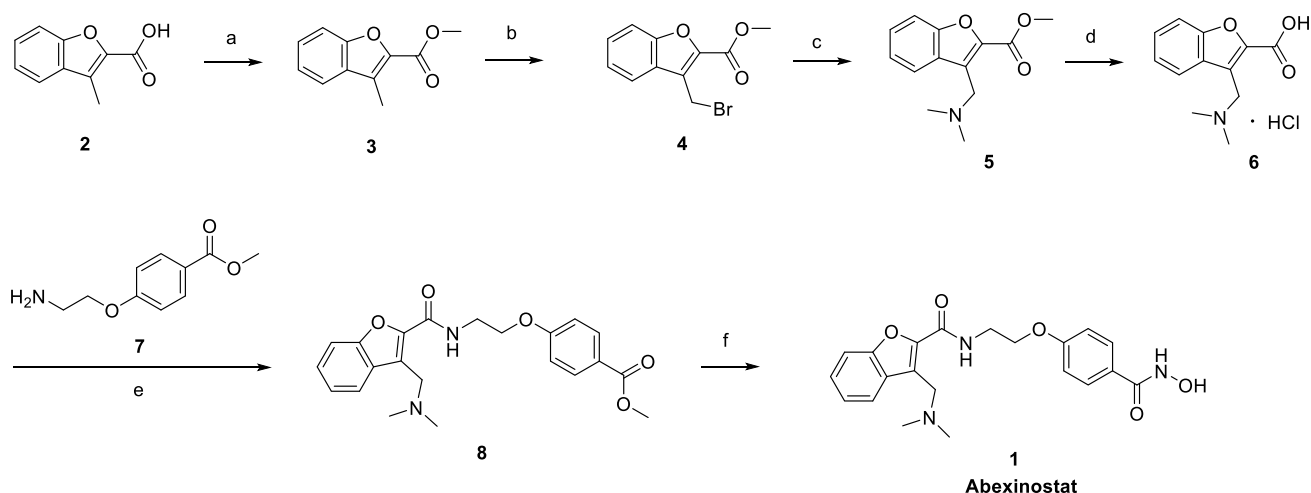
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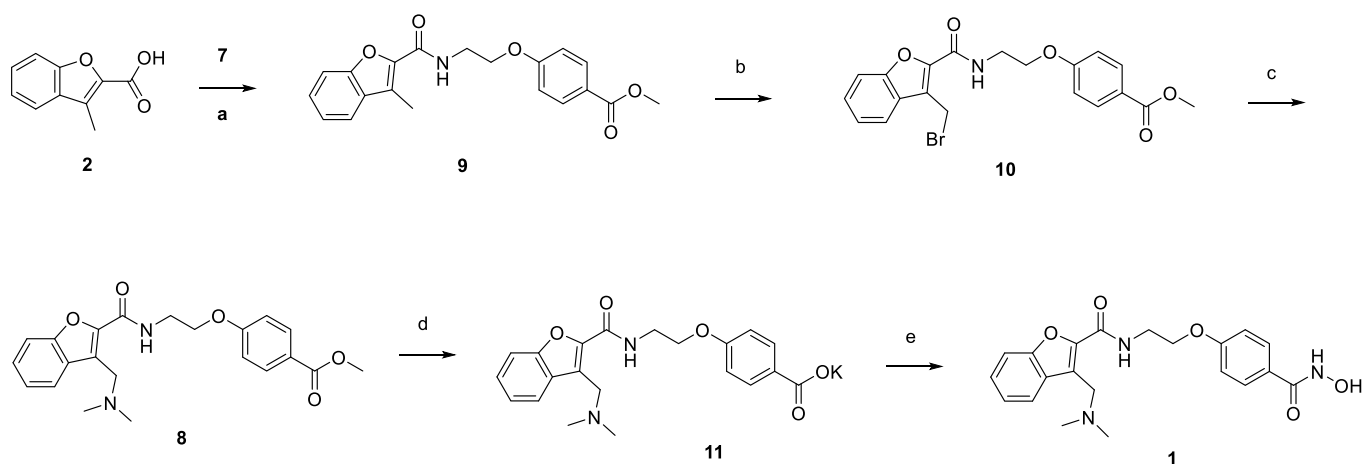
Abstract – A practical and convergent synthetic route of abexinostat was developed successfully. The key intermediate 3-((dimethylamino)methyl)-benzofuran-2-carboxylic acid (**16**) was prepared from 1-(2-hydroxyphenyl)ethan-1-one in 76.6% yield over 4 steps. Methyl 4-(2-aminoethoxy)benzoate hydrochloride (**20**) was synthesized from methyl 4-hydroxybenzoate in 97.0% yield over 2 steps. Abexinostat was obtained from **16** and **20** in 72.4% yield over 2 steps and 98.7% purity. Purification methods of the intermediates and the final product involved in the route were developed.

Abexinostat (**1**, Scheme 1), a new type of pan-histone deacetylase inhibitor (HDACI), is developed by Xu Nuo Pharmaceutical, a Sino-US multinational pharmaceutical company.¹ Abexinostat has good clinical effects in single or combined-medicine treatments. Since the extensive biological activity, abexinostat can effectively inhibit the growth of solid tumor cells.² It is currently in the phase II~III clinical research stage.

With regard to the synthesis of **1**, two methods had been reported so far, as shown in Scheme 1 to 2. The first route is shown in Scheme 1.³ 3-Methylbenzofuran-2-carboxylic acid (**2**) was chosen as starting material, methyl 3-((dimethylamino)methyl)benzofuran-2-carboxylate (**5**) was synthesized through esterification, bromination, and amine substitution in 53% over all yield at a 100 mg scale. The target product **1** was obtained from compound **5** and methyl 4-(2-aminoethoxy)benzoate (**7**) through the next 3 steps in 48% yield at a 100 mg scale, purified by preparative HPLC.

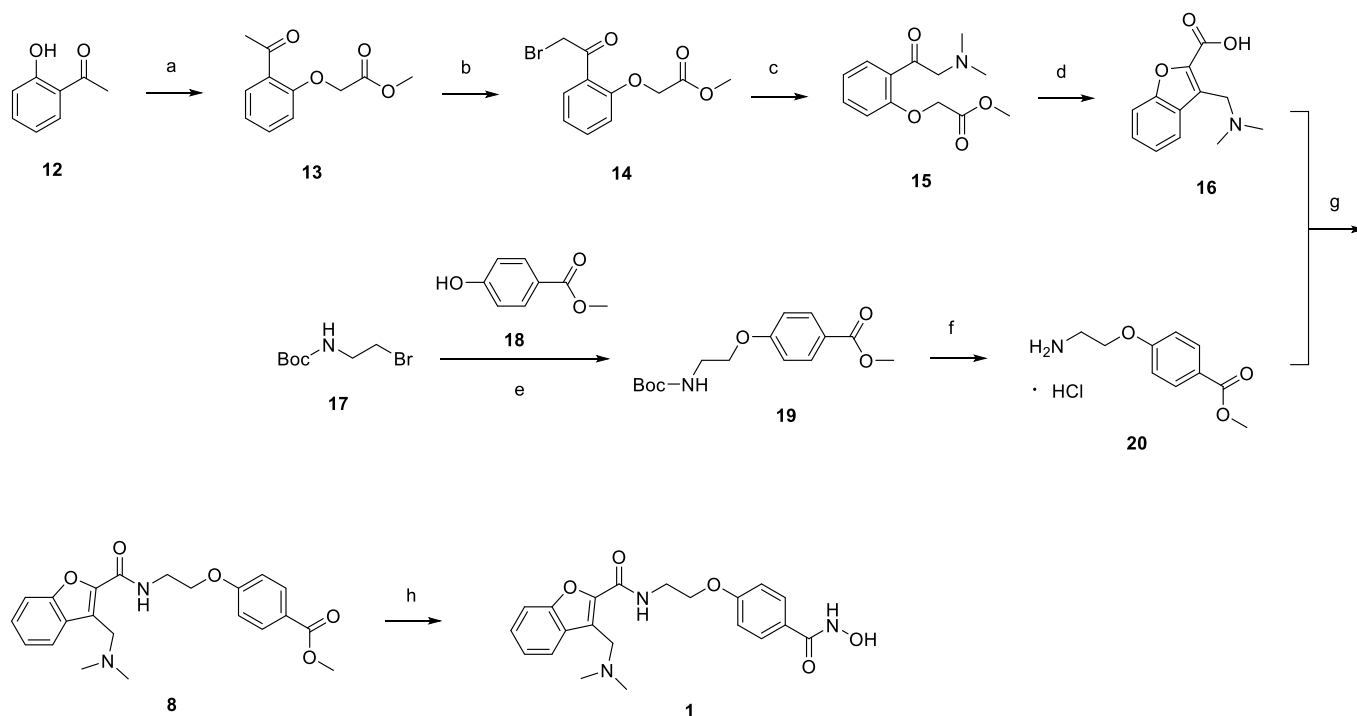


Scheme 1. Reagents and conditions: (a) oxalyl chloride, MeOH, TEA, DMF, rt, 12 h, 93%; (b) AIBN, NBS, CCl₄, reflux, 3 h; (c) dimethylamine, THF, DMF, rt, 2 h, 57%; (d) MeOH, NaOH, HCl; (e) DMF, EDCI, HOBt, TEA, rt, 12 h; (f) NH₂OH·HCl, NaOH, H₂O, THF, 48%.



Scheme 2. Reagents and conditions: (a) MeCN, pyridine, POCl₃, 0~5 °C; (b) AIBN, NBS, PhCl, 80 °C, 1 h; (c) dimethylamine, THF, 0~5 °C, rt, 2 h, 57%; (d) KOH, MeOH, 60 °C, 96%; (e) HCl-dioxane, DMF, HOBt, EDCI, NH₂OH·HCl, TEA, MeOH, rt, 0~5 °C, rt, 6 h, 51%.

Another synthetic route of **1** is shown in Scheme 2.⁴ Compounds **2** and **7** were used as the starting materials, followed by condensation, bromination, and amine substitution, to give the intermediate methyl 4-(2-(3-((dimethylamino)methyl)benzofuran-2-carboxamido)ethoxy)benzoate (**8**) in 57% over all yield at a 70 g scale. The final product **1** was obtained through the next 2 steps in 51% yield at a 10 g scale, purified by recrystallization from DMF/EtOH. The starting material **2** is commercially available but expensive. The preparation of **2** requires 2~5 steps in around 20% overall yield.⁵⁻⁷



Scheme 3. Reagents and conditions: (a) methyl 2-chloroacetate, K_2CO_3 , DMF, $rt\sim 70\text{ }^\circ C$, 1.5 h, 98%; (b) Br_2 , $AlCl_3$ (cat.), MTBE, rt , 1 h, 97%; (c) dimethylamine, THF, DMF, $-5\sim 0\text{ }^\circ C$, 89%; (d) NaOMe, MeOH, $60\text{ }^\circ C$, 5 h, 89%; (e) K_2CO_3 , DMF, $90\text{ }^\circ C$, 5 h; (f) HCl-EtOAc, $40\text{ }^\circ C$, 5 h, 97% (steps e and f); (g) HATU, DMF, TEA, $40\sim 45\text{ }^\circ C$, 5 h; (h) $NH_2OH\cdot MeOH$, $35\sim 40\text{ }^\circ C$, 2 h, 72% (steps g and h).

In order to develop a practical method for preparing of abexinostat (**1**), a new, efficient and convergent synthetic route was developed successfully, as shown in Scheme 3. Using 1-(2-hydroxyphenyl)ethan-1-one (**12**) as the starting material, methyl 2-(2-(2-acetylphenoxy)-acetate (**13**) was synthesized through a Williamson etherification reaction in 98% yield and >99% purity.⁶ Methyl 2-(2-(dimethylglycyl)phenoxy)acetate (**15**) was obtained by the following nucleophilic substitution occurred by bromine (yield 97%) and dimethylamine (yield 89%) respectively.^{8,9} The further intramolecular ring closure was completed under the basic NaOMe/MeOH condition to give the key intermediate 3-((dimethylamino)methyl)benzofuran-2-carboxylic acid (**16**) in 89% yield.¹⁰ Methyl 4-(2-aminoethoxy)benzoate hydrochloride (**20**) was prepared from *t*-butyl (2-bromoethyl)carbamate (**17**)¹¹ and methyl 4-hydroxybenzoate (**18**) through Williamson etherification reaction. The protection group was further removed under the action of hydrochloride acid, to give **20** in 97% yield over steps e and f.¹² Compound **8** was obtained by condensation of intermediates **16** and **20** under HATU and triethylamine, which was directly treated with hydroxylamine hydrochloride to give the final product **1**, in 72% yield over steps g and h, and 98.8% purity.^{13,14}

EXPERIMENTAL

All commercially available chemicals and solvents were used as received without any further purification. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker UltraShield 400 Plus spectrometer using TMS as an internal standard. Mass spectra were obtained from a Finnigan MAT-95/711 spectrometer. Melting points were measured on a Shenguang WRS-1B melting point apparatus and uncorrected. The HPLC data were acquired using a Waters 2487 UV/Visible Detector and Waters 515 Binary HPLC Pump. The purity of the compounds was based on the areas of HPLC UV.

Methyl (2-acetylphenoxy)acetate (13). A mixture of K_2CO_3 (101.5 g, 0.734 mol) and 1-(2-hydroxyphenyl)ethan-1-one (**12**) (50 g, 0.367 mol) in DMF (500 mL) was stirred for 30 min at room temperature.⁷ The mixture was heated to 70 °C and methyl 2-chloroacetate (59.8 g, 0.551 mol) was added over 30 min and stirred for another 1 h, and cooled to 30 °C. Water (1.5 L) was added to the mixture and was stirred for 30 min. The resulting solid was obtained by suction filtration, washed with water (60 mL \times 2), dried at 50 °C for 8 h to give **13** (75.1 g, 98.3%) as a white solid. mp 72~74 °C. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.59 (dd, $J = 7.6, 1.7$ Hz, 1H), 7.55 – 7.47 (m, 1H), 7.19 – 6.98 (m, 2H), 4.97 (s, 2H), 3.73 (s, 3H), 2.62 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 199.42, 169.30, 157.14, 134.05, 130.01, 128.77, 121.64, 113.74, 65.51, 52.34, 32.11. HRMS (ESI) calcd for: $\text{C}_{11}\text{H}_{12}\text{O}_4\text{K}$ [$\text{M} + \text{K}$]⁺ 247.03727, Found: 247.03708.

HPLC Conditions: Column: Agilent InertSustain C18 (250 mm \times 4.6 mm \times 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30 °C; Injection load: 1 μL ; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 40 min; Mobile phase A: water; Mobile phase B: MeOH/ $\text{HCO}_2\text{H} = 100/0.1$; Gradient program: Mobile phase A/Mobile phase B = 10/90; $t_{\text{R}} = 2.964$ min, purity: 99.9%.

Methyl 2-(2-(2-bromoacetyl)phenoxy)acetate (14). AlCl_3 (3.5 g, 26.42 mmol) was added into the solution of compound **13** (50 g, 0.24 mol) in 300 mL MTBE at room temperature. A solution of Br_2 (34.5 g, 0.216 mol) in 100 mL MTBE was added to the reaction solution over 1 h and stirred at room temperature for another 1 h.⁸ The resulting solid was filtered, washed with MTBE (30 mL \times 2), dried at 40 °C for 4 h to obtain **14** (67.1 g, 97.2%) as a white solid. mp 89~93 °C. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.73 – 7.52 (m, 2H), 7.23 – 7.07 (m, 2H), 5.00 (s, 2H), 4.95 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 192.68, 169.25, 157.11, 135.10, 130.90, 125.58, 122.10, 114.15, 65.89, 52.48, 39.14.

Methyl 2-(2-(dimethylglycyl)phenoxy)acetate (15). To a cooled solution of compound **14** (30 g, 0.104 mol) in THF (150 mL) was added 2 M dimethylamine in THF (150 mL, 0.30 mol) slowly over 1 h at $-5\sim 0$ °C and stirred at the temperature for another 1 h.⁹ The resulting solid was filtered, washed with THF

(10 mL × 2). The combined filtrate was concentrated under reduced pressure to obtain **15** (23.5 g, 89.5%) as a light-yellow oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.57 – 7.46 (m, 2H), 7.12 – 7.04 (m, 2H), 4.93 (s, 2H), 3.78 (s, 2H), 3.74 (s, 3H), 2.23 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 199.99, 169.25, 156.66, 133.85, 129.98, 128.08, 121.78, 113.64, 69.20, 65.67, 52.36, 45.46.

3-((Dimethylamino)methyl)benzofuran-2-carboxylic acid (16). A mixture of compound **15** (20 g, 79.59 mmol) and 1 M NaOMe in MeOH (167 mL, 0.167 mol) was heated and stirred at 60 °C for 5 h. The reaction solution was cooled to room temperature.¹⁰ The resulting solid was filtered, washed with MeOH (10 mL × 2). The combined filtrate was concentrated under reduced pressure to obtain compound **16** (15.5 g, 88.6%), which was used directly at the next step. ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.37 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.3 Hz, 1H), 4.63 (s, 2H), 2.87 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 161.84, 153.26, 150.00, 127.98, 126.93, 123.66, 120.83, 114.06, 112.31, 50.81, 41.97. MS (ESI): *m/z* = 220.1 [M + H]⁺.

Methyl 4-(2-((*t*-butoxycarbonyl)amino)ethoxy)benzoate (19). A mixture of K₂CO₃ (36.3 g, 0.262 mol), methyl 4-hydroxybenzoate **18** (20 g, 0.131 mol) in DMF (100 mL) was stirred at room temperature for 30 min and then heated to 90 °C.¹¹ Then *t*-butyl (2-bromoethyl)carbamate (**17**) (58.9 g, 0.262 mol) was added slowly into the mixture over 1 h and stirred at 90~100 °C for another 4 h. After cooled to room temperature, the reaction mixture was added into water (300 mL). The resulting solid was collected by suction filtration, washed with water (60 mL × 2), dried at 50 °C for 8 h to afford **19** (38.3 g, 98.7%) as a light-yellow solid. mp 98~103 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.88 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 8.1 Hz, 3H), 4.03 (s, 2H), 3.79 (s, 3H), 3.32 (d, *J* = 4.3 Hz, 2H), 1.36 (s, 9H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.32, 162.80, 156.15, 131.67, 122.38, 114.91, 78.27, 67.19, 52.22, 28.65. HRMS (ESI) calcd for: C₁₅H₂₁NO₅Na [M + Na]⁺ 318.13181, Found: 318.13174.

HPLC Conditions: Column: Agilent InertSustain C18 (250 mm × 4.6 mm × 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30 °C; Injection load: 1 μL; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 25 min; Mobile phase A: water; Mobile phase B: MeOH/HCO₂H = 100/0.1; Gradient program: Mobile phase A/Mobile phase B = 10/90; *t*_R = 3.852 min, purity: 98.25%.

Methyl 4-(2-aminoethoxy)benzoate hydrochloride (20). A 10% HCl-EtOAc solution (237 g, 0.65 mol) was added slowly into the mixture compound **19** (38 g, 0.128 mol) in EtOAc (100 mL) at 45~55 °C. The reaction mixture was stirred at 45~55 °C for another 3 h.¹² After cooled to room temperature, the resulting solid was collected by suction filtration, washed with chilled EtOAc (30 mL × 3), dried at 40 °C for 6 h to afford **20** (25 g, 98.4%) as a light-tan solid. mp 148~152 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (s,

3H), 7.92 (d, $J = 8.7$ Hz, 2H), 7.08 (d, $J = 8.7$ Hz, 2H), 4.28 (t, $J = 4.8$ Hz, 2H), 3.80 (s, 3H), 3.20 (t, $J = 4.9$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.29, 162.17, 131.70, 122.85, 115.14, 65.01, 52.32, 38.57. HRMS (ESI) calcd for: $\text{C}_{10}\text{H}_{13}\text{NO}_3\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 218.07931, Found: 218.07906.

Abexinostat (1). A mixture of compound **16** (10 g, 45.6 mmol) and HATU (17.3 g, 45.61 mmol) in THF (60 mL) was stirred at $-5\sim 0$ °C. TEA (9.2 g, 91.22 mmol) was then added slowly into the mixture and stirred at $0\sim 5$ °C for 30 min.¹³ Compound **20** (9 g, 46.1 mmol) was added slowly into the mixture and then heated and stirred at $40\sim 45$ °C for 5 h.¹⁴ The resulting solid was removed by suction filtration, the filtrate was concentrated under reduced pressure to give **8** (19.6 g) as a light-brown viscous liquid, which was dissolved in 50 mL MeOH.

KOH (129.03 g, 2.3 mol) was added slowly to the mixture of hydroxylamine hydrochloride (107.4 g, 1.55 mol) in 570 mL anhydrous MeOH at 0 °C, and stirred for 30 min. The resulting solid was removed by suction filtration, and the filtrate was dried with anhydrous sodium sulfate (50 g) over 4 h. The salt was removed by suction filtration to give the 10% hydroxylamine in MeOH solution.

A 10% hydroxylamine in MeOH solution (75.9 g, 0.23 mol) was added to the above solution of **8** (45.6 mmol) in MeOH, and the mixture was heated and stirred at $35\sim 40$ °C for 2 h and cooled to room temperature. Then the reaction solution was acidified to pH ~ 7 with AcOH (~ 90 mL) to give a brown solid mixture. The reaction solution was concentrated under reduced pressure to obtain crude product **1** as a tan solid (22 g). The crude **1** and active carbon (2 g) were suspended in MeOH (80 mL), heated to reflux for 30 min. The insoluble solid was filtered through a celite pad in hot. Water 20 mL was added to the filtrate and the solution was stirred at room temperature for 2 h and at $0\sim 5$ °C for 1 h. The resulting solid was isolated by filtration, washed with 80% MeOH- H_2O (10 mL \times 2), dried at 40 °C for 4 h to give **1** (13.1 g, 72.4%) as an off-white solid. mp $175\sim 180$ °C (decomp.). ^1H NMR (400 MHz, DMSO- d_6) δ 11.08 (s, 1H), 10.06 (t, $J = 4.8$ Hz, 1H), 8.92 (s, 1H), 7.86 (d, $J = 7.8$ Hz, 1H), 7.75 (d, $J = 8.7$ Hz, 2H), 7.64 (d, $J = 8.3$ Hz, 1H), 7.46 (t, $J = 7.7$ Hz, 1H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.04 (d, $J = 8.7$ Hz, 2H), 4.22 (t, $J = 5.2$ Hz, 2H), 3.82 (s, 2H), 3.74 – 3.68 (m, 2H), 2.19 (s, 6H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 161.11, 159.67, 153.42, 146.28, 129.17, 128.88, 127.31, 125.67, 123.81, 122.01, 120.52, 114.58, 112.11, 66.87, 51.73, 44.80, 38.94. HRMS (ESI) calcd for: $\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_5$ [$\text{M} + \text{Na}$] $^+$ 420.15354, Found: 420.15298.

HPLC Conditions: Column: Acclaim C18 (150 mm \times 2.1 mm \times 5 μm); Detection: 210 nm; Flow rate: 0.8 mL/min; Temperature: 45 °C; Injection load: 1 μL ; Solvent: in MeOH; Concentration: 0.2 mg/mL; Run time: 30 min; Mobile phase: in MeOH/water = 80/20, t_{R} : 7.385 min, purity: 98.77%.

REFERENCES

1. R. Aggarwal, S. Thomas, N. Pawlowska, I. Bartelink, J. Grabowsky, T. Jahan, A. Cripps, A. Harb, J. Leng, A. Reinert, I. Mastroserio, T. G. Truong, C. J. Ryan, and P. N. Munster, *J. Clin. Oncol.*, 2017, **35**, 1231.
2. A. M. Evens, S. Balasubramanian, J. M. Vose, W. Harb, L. I. Gordon, R. Langdon, J. Sprague, M. Sirisawad, C. Mani, J. Yue, Y. Luan, S. Horton, T. Graef, and N. L. Bartlett, *Clin. Cancer Res.*, 2016, **22**, 1059.
3. E. J. Verner, M. Sendzik, C. Baskaran, J. J. Buggy, and J. Robinson, WO 2004092115, 2004.
4. K. E. Bass, S. E. Broder, and P. R. Young, WO 2006042035, 2006.
5. Y. Rao, Z. Xu, Y. T. Hu, C. Li, Y. H. Xu, Q. Q. Song, H. Yu, B. B. Song, S. B. Chen, Q. J. Li, S. L. Huang, J. H. Tan, T. M. Ou, H. G. Wang, G. P. Zhong, J. M. Ye, and Z. S. Huang, *Eur. J. Med. Chem.*, 2020, **192**, 112172.
6. P. W. Tseng, S. W. Yeh, and C. H. Chou, *J. Org. Chem.*, 2008, **73**, 3481.
7. T. Suzuki, K. Tanemura, T. Horaguchi, T. Shimizu, and T. Sakakibara, *J. Heterocycl. Chem.*, 1992, **29**, 423.
8. L. S. Melvin, WO 2009079391, 2009.
9. K. E. Bass, S. E. Broder, and P. R. Young, WO 2006042035, 2006.
10. M. Delor, J. Dai, T. D. Roberts, J. R. Rogers, S. M. Hamed, J. B. Neaton, P. L. Geissler, M. B. Francis, and N. S. Ginsberg, *J. Am. Chem. Soc.*, 2018, **140**, 6278.
11. G. S. Singh, *Tetrahedron*, 2003, **59**, 7631.
12. B. H. Tian, M. Z. He, S. X. Tang, I. Hewlett, Z. Tan, J. Li, Y. Jin, and M. Yang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2162.
13. H. Charville, D. Jackson, G. Hodges, and A. Whiting, *Chem. Commun.*, 2010, **46**, 1813.
14. H. Suzuki, A. Kanai, T. Uehara, F. L. G. Gomez, H. Hanaoka, and Y. Arano, *Bioorg. Med. Chem.*, 2012, **20**, 978.