Supporting Information

Determination of benvitimod in cosmetics by HPLC with pre-column fluorescence derivatization of 2- ((7-Nitrobenzene [c] [1,2,5] oxadiazole -4- yl) oxy) benzoic acid (NBD-SA)

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Content

1.Synthesis method of intermediates

*compound **NBD-SA methyl ester***: Methyl salicylate (2.28 g, 15 mmol) and 4-chloro-7-nitrobenzofurazan (1.99 g, 10 mmol) were dissolved in 50 mL DMF and add 15 mL triethylamine was added to the above solution. The mixture was stirred at room temperature for 2 hours and the progress of reaction was monitored by TLC. When the reaction is complete, water is added and stirred to produce solid precipitation. After filtration, the filter cake was washed with water and dried to give a pure brown solid **NBD-SA methyl ester**.

*compound **NBD-SA***: NBD-SA methyl ester (3.15 g, 10 mmol) was put into a 100 mL round-bottomed flask and 20 mL 95% ethanol and 20 mL 10% NaOH solution were added to the above reaction solution. The mixture was stirred overnight at room temperature. When the reaction was complete (indicated by TLC), the excess (10 mL) of 2 mol l-1 hydrochloric acid solution was added to the reaction mixture. The precipitate was filtered. A yellow-brown solid was dried under vacuum dried to obtain **NBD-SA**.

*compound **NBD-B***: Add 50 μl Benvitimod standard solution to 2 ml ampoules, and sequentially add 300 μl DCM, 90 μl EDC (0.1 mol/l), 90 μl DMAP (0.2 mol/l), and 50 μl NBD-SA and 200 μl acetonitrile. After sealing, place the ampoule in a 45 ℃ water bath for 30 minutes. After the reaction, the derivative was cooled to room temperature, and then the solution was diluted to 10 ml with acetonitrile and stored in the freezer before HPLC/FLD analysis.

To a 50 ml round-bottomed flask, Benvitimod (0.25 g, 1 mmol) and 20 ml

dichloromethane were added, and stirred at 45 ℃. Then, EDC (0.05 g, 0.5 mmol), DMAP (0.06 g, 0.5 mmol), and fluorescence derivatization reagent NBD-SA (0.45 g, 1.5 mmol) were added to the above solution in turn. After the reaction is c omplete, it was extracted with NaOH solution and dichloromethane (3 × 30 ml), combined with organic phase, dried with anhydrous sodium sulfate, and separated and purified by DCM/MeOH (15:1) silica gel column chromatography to obtain a yellow solid.

2.Structural characterization data of compounds

Characterization data of compound NBD-SA:

2-((7-nitrobenzo [c] [1,2,5] oxadiazol-4-yl) oxy) benzoic acid : Yellow-brown solid, Yield 64%.^{1}H NMR (500 MHz, DMSO) δ 8.73 (t, J = 10.3 Hz, 1H), 7.78 (d, J = 7.8 Hz, 2H), 7.50 (dd, J = 11.1, 4.3 Hz, 2H), 7.13 – 6.77 (m, 4H), 4.50 (q, J = 6.9 Hz, 2H), 2.50 (s, 3H), 1.49 (t, J = 6.9 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 171.83, 161.05, 154.09, 145.31, 143.98, 136.12, 135.58, 130.19, 119.11, 117.02, 112.83, 106.08, 66.96, 13.95. MS (EI) m/z: 301.03 (M+H) +. Anal. calcd for C13H7N3O6: C, 51.84; H, 2.34; N, 13.95; O, 31.87.Found C, 51.82; N, 13.97; O, 31.86.

3.Spectrums of compounds

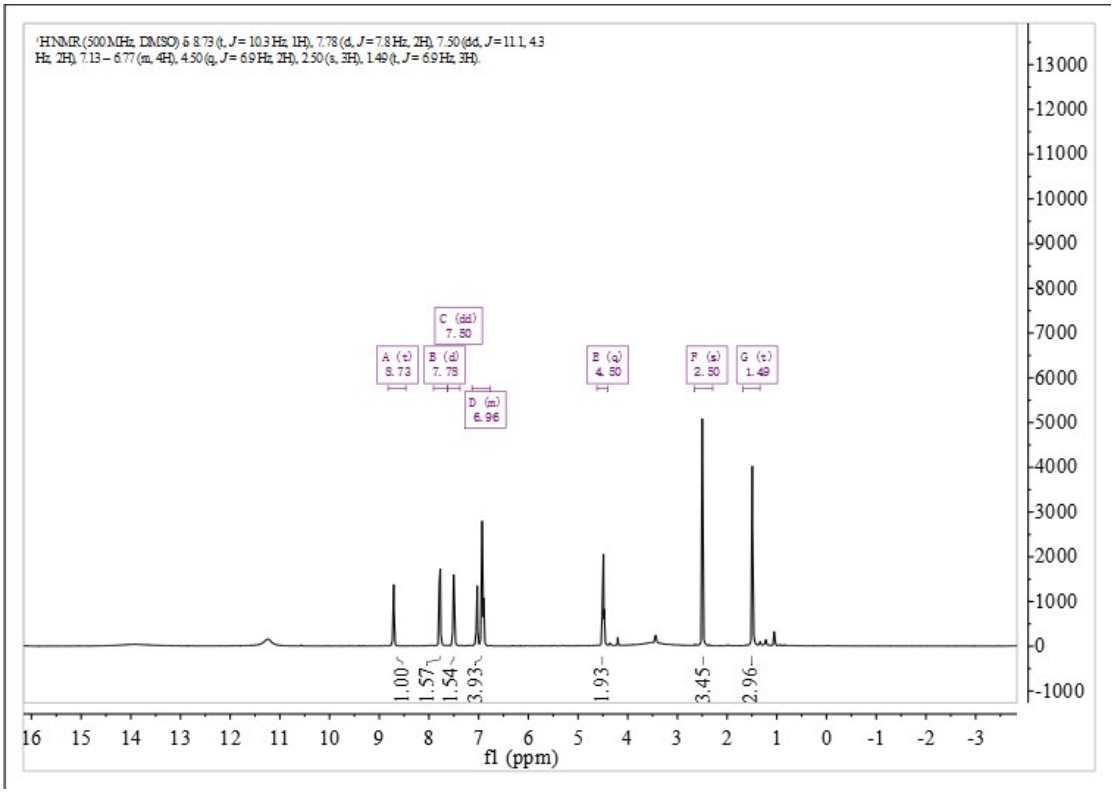

Fig. S1. The ^{1}H NMR spectra of Compound NBD-SA

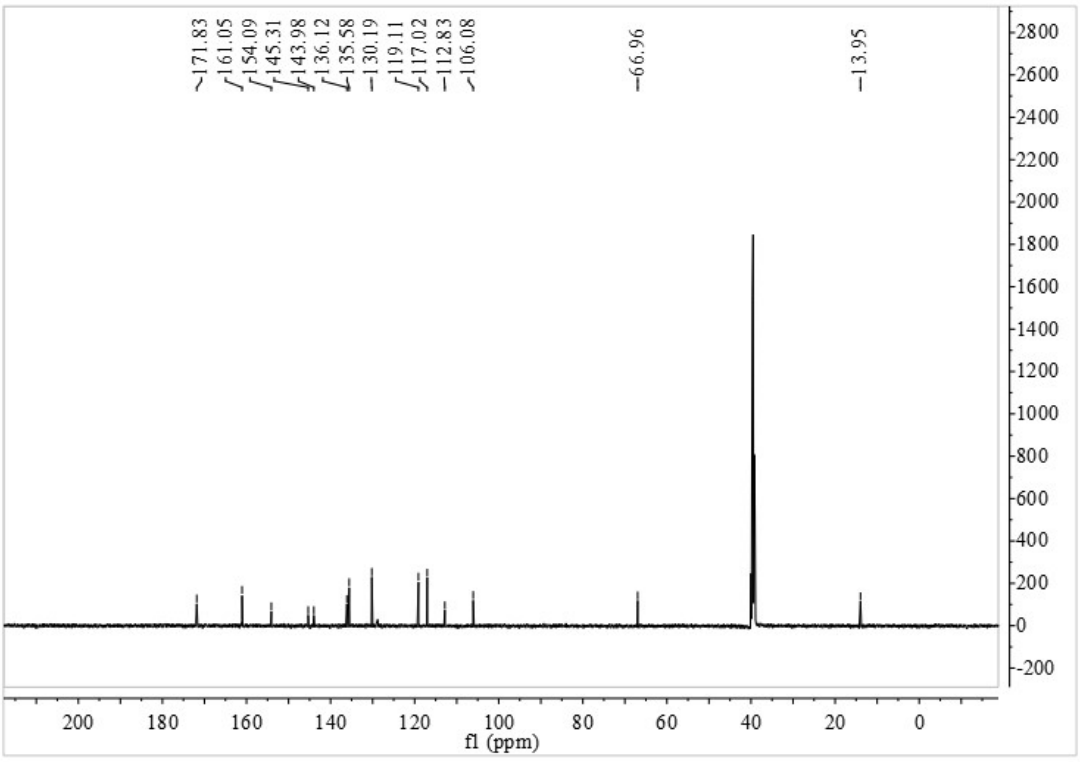

Fig. S2. The ^{13}C NMR spectra of Compound NBD-SA