De Novo Design of Multi-target Ligands with an Iterative Fragment-growing Strategy

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Supporting Information

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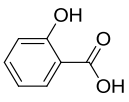
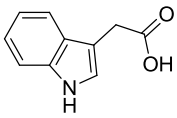
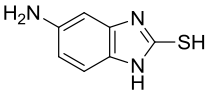
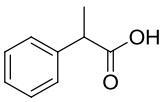
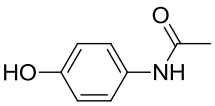
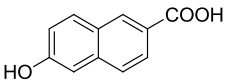
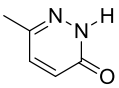
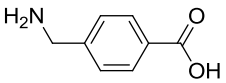
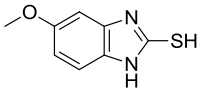
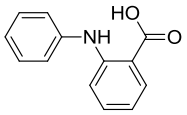
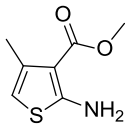
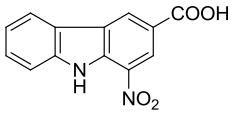
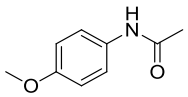
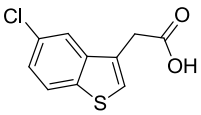
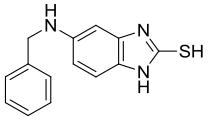
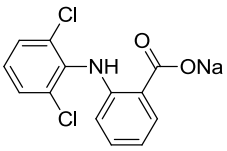
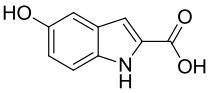
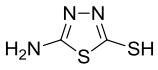
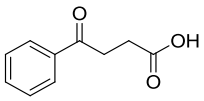
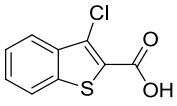
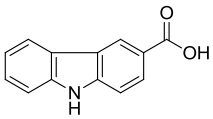
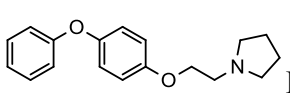
Starting Fragments

Table S-1. 21 fragments selected from the extracted fragment library of known inhibitors of COX and LTA_4H as starting fragments[a]

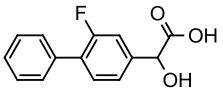
No.	Structure	No.	Structure	No.	Structure
SH-1		SH-8		*SH-15*	
SH-2		**SH-9**		SH-16	
SH-3		*SH-10*		***SH-17***	
SH-4		**SH-11**		**SH-18**	
SH-5		SH-12		***SH-19***	
SH-6		SH-13		**SH-20**	
SH-7		SH-14		**SH-21**	

[a]Fragments SH-5, SH-10, SH-15, SH-17, and SH-19 are from inhibitors of LTA_4H; The other fragments are from COX inhibitors; Fragments in bold are found as dual-target seeds.

Structure of Ref-1 and Flur



Ref-1

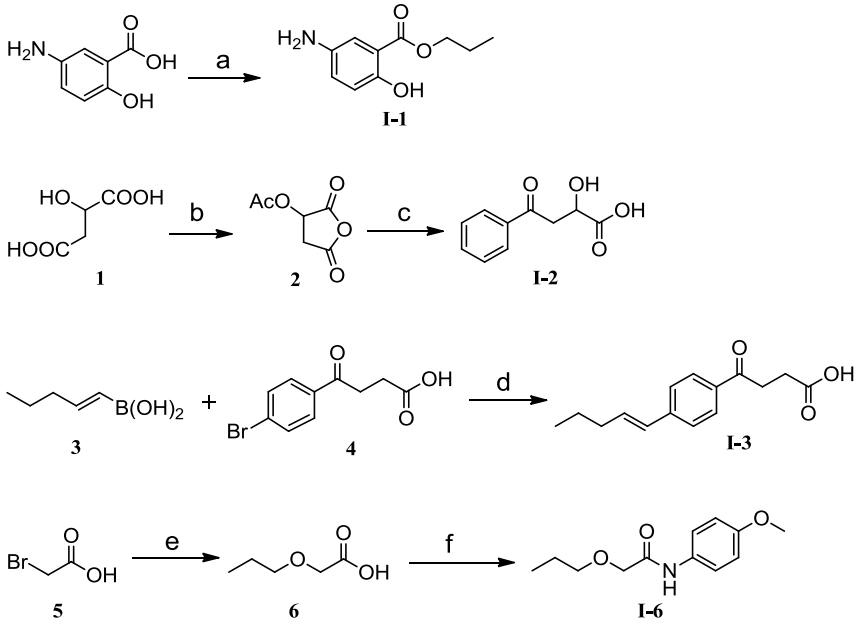


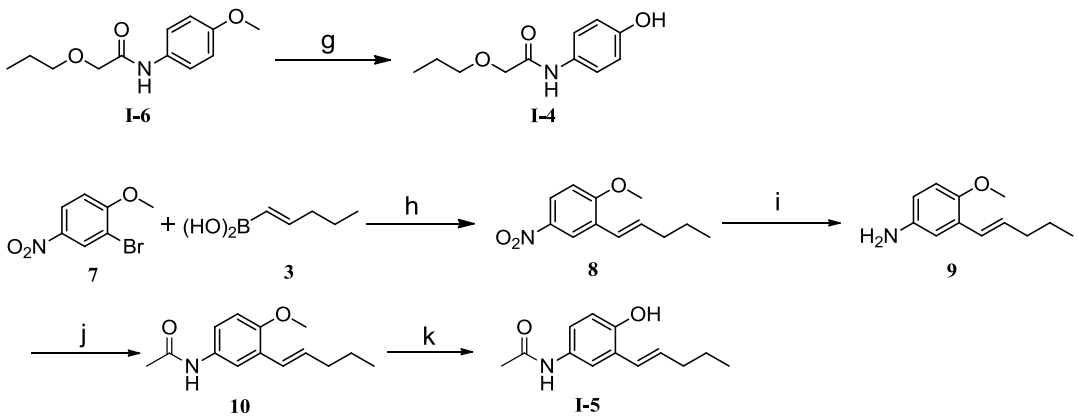
Flur

Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend 300/400 MHz spectrometer. High resolution mass spectra were recorded on a Bruker Apex IV FTMS mass spectrometer using ESI (electrospray ionization). Tetrahydrofuran and toluene were distilled from Na and benzophenone. Dichloromethane were dried over 4Å molecular sieves. All other reagents were purchased from commercial sources and used as received.

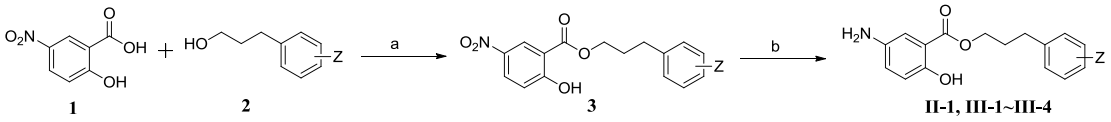
Scheme S-1[a]. Synthesis of the first round compounds





[a] Reagents and conditions: (a) 50% H_2SO_4/H_2O, 1-propanol, reflux, 4h, 64%; (b) AcCl, reflux, 5h, 98%; (c) (i)$AlCl_3$, benzene, 0 ºC~rt, 4h; (ii) HCl/H_2O, 69%; (d) $Pd(PPh_3)_4$, K_2CO_3, EtOH/toluene/H_2O, reflux, 20h, 93%; (e) (i) Na/1-propanol, reflux, 2h; (ii) HCl/H_2O, 96%; (f) (i)$SOCl_2$, reflux, 4h; (ii) p-methoxyaniline, THF, rt, 5h, 64%; (g) EtSNa, DMF, 100 ºC, 5h, 86%; (h) $Pd(PPh_3)_4$, K_2CO_3, EtOH/toluene/H_2O, reflux, 20h, 93%; (i) $Na_2S\cdot 9H_2O$, EtOH, reflux, 6h, 52%; (j) AcCl, Et_3N, THF, rt, 6h, 96%; (k) BBr_3, DCM, -78ºC~rt, 5h, 41%.

Scheme S-2[a]. Synthesis of 2nd round and **II-1** derivatives



[a]Reagent and conditions: (a) EDCI, DMAP, THF, rt; (b) H_2, Pd/C, MeOH or AcOEt.

The scheme S-1 illustrates the synthetic route of first round designed compounds. The propyl 5-amino-2-hydroxybenzoate **I-1** and 2-hydroxy-4-oxo-4-phenylbutanoic acid **I-2** were obtained by the known literature procedures starting from 5-aminosalicylic acid and malic acid respectively.[1, 2]

(E)-4-oxo-4-(4-(pent-1-en-1-yl)phenyl) butanoic acid **I-3** was prepared from the Suzuki-Miyaura cross-coupling reaction between boronic acid **3** and bromide **4** in the presence of a palladium catalyst. The reaction of bromoacetic acid with propanol provided the intermediate **6**. Acid chloride formation of **6** with thionyl chloride followed by the amidation gave the target compound N-(4-methoxyphenyl)-2-propoxyacetamide **I-6**, which was further deprotected to obtain target compound N-(4-hydroxyphenyl)-2-propoxyacetamide**I-4**. Suzuki-Miyaura cross-coupling between bromide **7** and boronic acid **3** afforded the (E)-1-methoxy-4-nitro-2-(pent-1-en-1-yl)benzene **8**. Subsequent reduction of the nitro group with H_2 gave the corresponding amino product **9**, which was then reacted with acetyl chloride to provide (E)-N-(4-methoxy-3-(pent-1-en-1-yl)phenyl) acetamide **10**. In the final step, phenolic methyl ether was cleaved with tribromoborane to obtain the target compound (E)-N-(4-hydroxy-3-(pent-1-en-1-yl)phenyl) acetamide **I-5**.

Propyl 5-amino-2-hydroxybenzoate **(I-1).** 5-Aminosalicylic acid (0.153 g, 1.0 mmol) was added to a solution of H_2SO_4 (50%, 2 mL) in 1-propanol (10mL) and the mixture was stirred and refluxed for 4 h. Water (15 mL) was added to the cooled mixture and extracted with ethyl acetate (20 mL × 3). The combined organic phase was washed with saturated $NaHCO_3$aq, water, saturated NaCl aq. The organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure to provide a brown residue of **I-1** (0.124 g, 64%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 10.29 (s, 1H), 7.17 (d, 1H), 6.85 (m, 2H), 4.28 (t, 2H), 3.44 (br, 2H), 1.80 (m, 2H), 1.06 (t, 3H).

(±) 2-hydroxy-4-oxo-4-phenylbutanoic acid **(I-2).** The solution of (±)-malic acid (5.0 g, 37 mmol) in acetyl chloride (180 mL) was heat to reflux for 5 h. The excess of acetyl chloride and acetic acid formed were eliminated in vacuo to obtain a colorless oil of **2** (5.8g, 98%). It was used in the next step without further purification.

To a solution of **2** (0.60 g, 5.3 mmol) in dried benzene (10 mL) at 0 °C, aluminiumtrichloride (2.0 g, 15 mmol) was added in one portion. The mixture was stirred and refluxed for 4 h. The cooled reaction solution was poured into ice-cold 50% HCl and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with saturated NaCl aq and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residual was recrystallized from ethyl acetate/petroleum ether to furnish **I-2** as white solid (0.70 g, 69%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.98 (m, 2H), 7.65 (t, 1H), 7.51 (t, 2H), 4.73 (q, 1H), 3.60 (q, 2H).

(E)-4-oxo-4-(4-(pent-1-en-1-yl)phenyl)butanoic acid **(I-3).** To a solution of **4**(0.26 g,1.0 mmol), K_2CO_3 (1.6 g,11.6 mmol)and $Pd(PPh_3)_4$ (0.036 g, 0.03 mmol) in 10 mL of toluene/H_2O (3:2), the solution of **3** (0.13 g,1.15 mmol) in EtOH was added. The mixture was degassed and purged with argon and then stirred and refluxed for 20 h. After cooling to room temperature, water (20 mL) was added to the reaction solution. The mixture was brought to pH = 2 with 1 M HCl and extracted with EtOAc (20 mL × 3). The combined organic phases were washed with

saturated NaCl aq and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residual was recrystallised from ethyl acetate/petroleum ether to furnish **I-3** as white solid (0.23 g, 93%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.91 (d, 2H), 7.42 (d, 2H), 6.40 (m, 2H), 3.28 (t, 2H), 2.80 (t, 2H), 1.51 (q, 2H), 1.25 (t, 2H), 0.96 (t, 3H).

N-(4-methoxyphenyl)-2-propoxyacetamide **(I-6).** Na (0.58 g, 25 mmol) was added to 8 mL of 1-propanol in portions and the resulting suspension was heated to reflux. After disappearing of solid, the mixture was cooled to room temperature. A solution of bromoacetic acid (1.40 g, 10 mmol) in 1-propanol (10 mL) was added and the mixture was stirred and refluxed for 2 h. The solvent was evaporated under reduced pressure and the residual was dissolved with 10 mL water. After washing with Et_2O (10 mL × 2), the aqueous solution was cooled with ice bath and brought to pH = 1~2 with 5 M HCl. The resulting solution was extracted with Et_2O (10 mL × 4) and then the combined organic layers were dried over Na_2SO_4. The solvent was evaporated under reduced pressure to obtain **6** (1.1 g, 96%). It was used in the next step without further purification.

A solution of **6** (1.0 g, 8.5 mmol) in $SOCl_2$ (10 mL) was stirred and refluxed for 4 h. The solvent was evaporated under reduced pressure and added a solution of Et_3N (2.58 g, 26 mmol) in THF (10 mL) to the resulting residual. After stirring for several minutes, a solution of p-anisilidine (1.0 g, 8.5 mmol) in THF (5 mL) was added. The mixture was allowed to stir at room temperature for 5 h and then 30 mL

of water was added. The aqueous layer was extracted with EtOAc (20 mL × 3), and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting crude residual was purified by chromatography on silica gel (EtOAc/petroleum ether) to provide **I-6** (1.1 g, 64% yield for two steps). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.21 (br, 1H), 7.47 (d, 2H), 6.88 (d, 2H), 4.04 (s, 2H), 3.80 (s, 3H), 3.55 (t, 2H), 1.71 (m, 2H), 1.00 (t, 3H).

N-(4-hydroxyphenyl)-2-propoxyacetamide **(I-4).** To a stirred solution of NaH (0.06 g, 1.3 mmol) in DMF (5 mL) cooled to 0 °C in an ice bath, was added EtSH (96 μL, 1.3 mmol). The mixture was heated to 100 °C for 5 h, cooled to room temperature, diluted with EtOAc (50 mL) and quenched with saturated NH_4Cl aq (30 mL). Organic phase was washed with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel (EtOAc/petroleum ether) to provide **I-4** (0.18 g, 86%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.22 (br, 1H), 7.37 (d, 2H), 6.80 (d, 2H), 4.06 (s, 2H), 3.55 (t, 2H), 1.70 (m, 2H), 0.99 (t, 3H).

(E)-N-(4-hydroxy-3-(pent-1-en-1-yl)phenyl)acetamide **(I-5).** To a stirred solution of **7** (0.46 g, 2.0 mmol), K_2CO_3 (1.6 g, 11 mmol) and $Pd(PPh_3)_4$ (0.072 g, 0.062 mmol) in 12 mL of toluene/H_2O (3:2), the solution of **3** (0.25 g, 2.2 mmol) in EtOH (6 mL) was added. The mixture was degassed and purged with Argon and then stirred and refluxed for 20 h. After cooling to room temperature, water (20 mL) was added to the reaction solution and the resulting mixture was extracted with

EtOAc (20 mL× 3). The combined organic phases were washed with saturated NaClaq and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residual was purified by chromatography on silica gel (EtOAc/petroleum ether) to provide **8** (0.41 g, 93%).

To a solution of **8** (0.40 g, 1.8 mmol) in EtOH (20 mL), $Na_2S·9H_2O$ (0.72 g, 3.0 mmol) was added in one portion and the resulting solution was stirred and refluxed for 6 h. The solvent was eliminated in vacuo and the resulting residual was extracted with brine/EtOAc. The combined organic phases were dried over anhydrous Na_2SO_4 and purified by chromatography on silica gel (EtOAc/petroleum ether) to obtain **9** (0.18 g, 52%).

To a solution of **9** (0.18 g, 0.94 mmol) in THF (10 mL), acetyl chloride (71 μL, 1.0 mmol) and Et_3N (0.22 mL, 1.6 mmol) were added and the resulting mixture was stirred at room temperature for 6 h. The solvent was eliminated in vacuo and the resulting residual was extracted with brine/EtOAc. The combined organic layers were washed with 1 M HCl and brine, and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to obtain **10** (0.21 g, 96%). It was used in the next step without further purification.

To a degassed solution of **10** (0.21 g, 0.90 mmol) in DCM (10 mL) at -78 °C was added BBr_3 (2.5 mL, 1.0 M solution in DCM, 2.5 mmol) dropwise under an inert atmosphere. The solution was stirred at the same temperature for 0.5 h and then allowed to warm to room temperature gradually for 5h. The reaction was quenched

with MeOH (5 mL). EtOAc (40 mL) was added and the resulting organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residual was purified by chromatography on silica gel (EtOAc/petroleum ether) to provide **I-5** (0.08 g, 41%).

Scheme S-2 summarizes the general preparation route of second round designed and **II-1** derivatives. The reaction of benzoic acids and phenylpropanols under the condition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) gave the series compounds of benzoic esters. For amino analogues, a further reduction of nitro group with hydrogen in the presence of palladium/carbon catalyst was needed.

3-phenylpropyl 5-amino-2-hydroxybenzoate **(II-1).** To a stirred solution of 5-nitro salicylic acid (0.37 g, 2.0 mmol) in THF (10 mL), 3-phenylpropan-1-ol (0.27 g, 2.0 mmol), EDCI (0.46 g, 2.4 mmol) and DMAP (cat.) were added. The mixture was stirred at room temperature overnight. The solvent was eliminated in vacuo and the resulting residual was extracted with brine/EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and purified by chromatography on silica gel (EtOAc/petroleum ether) to provide **11** (0.43 g, 72%).

The solution of **11** (0.10 g, 0.33 mmol) and Pd/C (0.02 g) in 20 mL of EtOAc was put into a hydrogenation device. Setting the hydrogen pressure at 0.4 MPa, the solution was stirred at room temperature for 5 h. The mixture was then filter through celite and the solvent was removed under reduced pressure to obtain a brown oil **II-1**

(0.086 g, 95%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.26 (s, 1H), 7.30 (m, 2H), 7.21 (m, 3H), 7.07 (d, 1H), 6.87 (dd, 1H), 6.82 (d, 1H), 4.34 (t, 2H), 3.44 (br, 2H), 2.79 (t, 2H), 2.11 (m, 2H); **^{13}C NMR** (100 MHz, CDCl3): δ 170.0, 154.9, 141.0, 138.2, 128.5 (2), 128.4 (2), 126.1, 124.3, 118.2, 114.7, 112.3, 64.6, 32.3, 30.0; **HRMS (ESI)**: calcd for $C_{16}H_{18}NO_3$ [$(M+H)^+$] 272.1281, found 272.1280.

Using the procedure for **II-1** synthesis, **II-1** derivatives were synthesized.

3-(4-chlorophenyl)propyl5-amino-2-hydroxybenzoate **(III-1).** Pale brown oil (0.07 g, 57 % two steps): **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.25 (s, 1H), 7.30 (m, 2H), 7.18 (m, 3H), 7.06 (dd, 1H), 6.87 (dd, 1H), 6.82 (d, 1H), 4.33 (t, 2H), 3.45 (br, 2H), 2.79 (t, 2H), 2.11 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.0, 155.0, 141.0, 138.2, 128.5 (d, 4), 126.1, 124.3, 118.2, 114.7, 112.3, 64.6, 32.3, 30.0; **HRMS (ESI)**: calcd for $C_{16}H_{17}ClNO_3$ [$M + H^+$] 306.0891, found 306.0893.

3-(4-fluorophenyl)propyl5-amino-2-hydroxybenzoate **(III-2).** Yellow solid (0.06 g, 61% two steps): **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.23 (s, 1H), 7.16 (q, 2H), 7.07 (d, 1H), 6.99 (t, 2H), 6.88 (dd, 1H), 6.83 (d, 1H), 4.33 (t, 2H), 3.43 (br, 2H), 2.76 (t, 2H), 2.08 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 168.9, 160.4 (d), 154.0, 137.2, 135.5 (d), 128.8, 128.7, 123.4, 117.2, 114.4, 114.1, 113.6, 111.3, 63.4, 30.5, 29.2; **HRMS (ESI)**: calcd for $C_{16}H_{17}FNO_3$ [$M + H^+$] 290.1187, found 290.1185; m. p. 63~64°C.

3-(4-methoxyphenyl)propyl5-amino-2-hydroxybenzoate **(III-3).** Pale brown oil(0.04 g, 43% two steps): **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.26 (s, 1H), 7.13 (d, 2H), 7.04 (d, 1H), 6.85 (m, 4H), 4.33 (t, 2H), 3.79 (s, 3H), 3.37 (br, 2H), 2.73 (t, 2H), 2.06 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.0, 158.0, 155.0, 138.3, 133.1, 129.4 (2), 124.3, 118.2, 114.7, 114.0 (2), 112.4, 64.6, 55.3, 31.4, 30.2; **HRMS (ESI)**: calcd for $C_{17}H_{20}NO_4$ [M + H^+] 302.1387, found 302.1389.

3-(4-chloro-3-fluorophenyl)propyl5-amino-2-hydroxybenzoate **(III-4).** Faint yellow oil(0.06g, 50% two steps): **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.24 (s, 1H), 7.26 (m, 1H), 7.07 (dd, 1H), 6.99 (dd, 1H), 6.89 (m, 2H), 6.82 (d, 1H), 4.33 (t, 2H), 3.45 (t, 2H), 2.78 (t, 2H), 2.10 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.9, 163.0 (d), 154.9, 143.5 (d), 138.3, 129.9 (d), 124.4, 124.1 (d), 118.2, 115.3 (d), 114.6, 113.0 (d), 112.2, 64.4, 32.1, 29.8; **HRMS (ESI)**: calcd for $C_{16}H_{16}ClFNO_3$ [M + H^+] 324.0797, found 324.0801.

Biological Methods

All the target compounds were tested for their inhibition activities against purified COX-2 and LTA_4H.[3, 4] The LTA_4H hydrolyse activity was measured using an ELISA assay kit to quantify the amount of LTB_4 generated. The known LTA_4H inhibitor, 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine **Ref-1** was used as a positive control. The enzyme activity of the purified COX-2 was measured by a chromogenic assay based on monitoring the absorption of oxidized

N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2 at 610 nm.[5] The known COX-2 inhibitor, flurbiprofen **Flur**, was used as a positive control.

The activity of lead compounds **II-1** and **III-1** were also evaluated using human whole blood (HWB) assay. In this study, *E. coli* liopolysaccharide (LPS) was used to induce the COX-2/PGES pathway in human whole blood, and A23187 was used to induce the 5-LOX/LTA_4H pathway.[6, 7] PGE_2 and LTB_4 ELISA kits were used respectively to monitor the production of PGE_2 and LTB_4 of HWB assay.

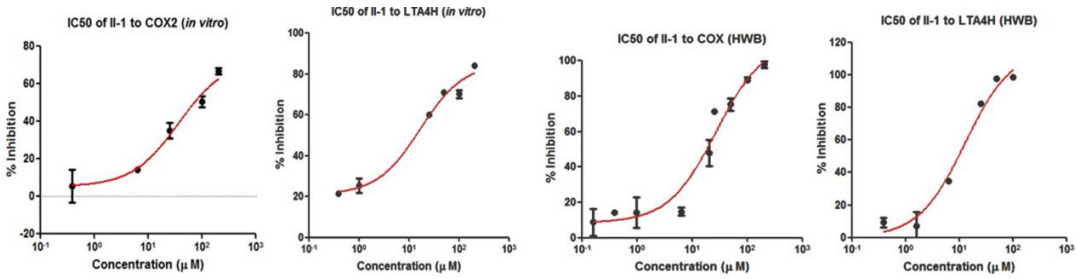


Figure S-1. IC_{50} curves of **II-1.**

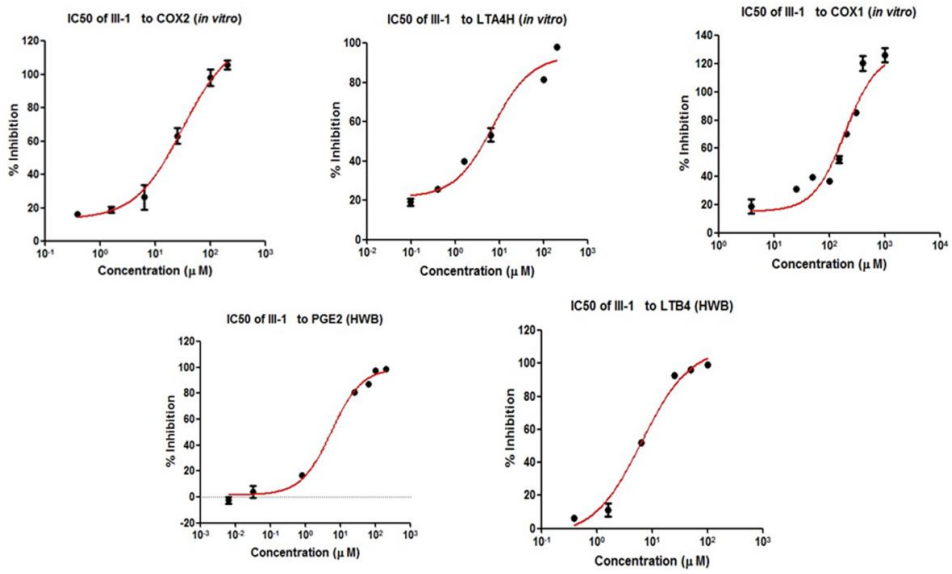


Figure S-2. IC_{50} curves of **III-1.**

References

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5. M. Ouellet, J. Falgueyret and M. Percival, *Biochem. J.* **2004**, *377*, 675-684.
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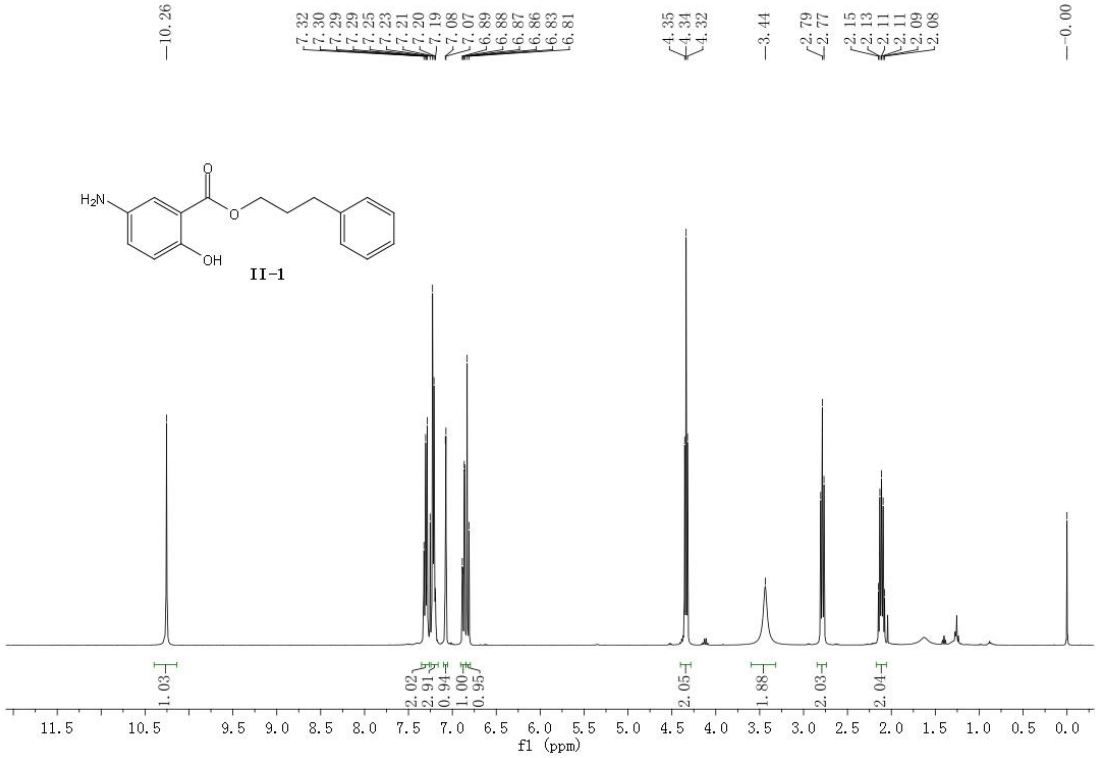
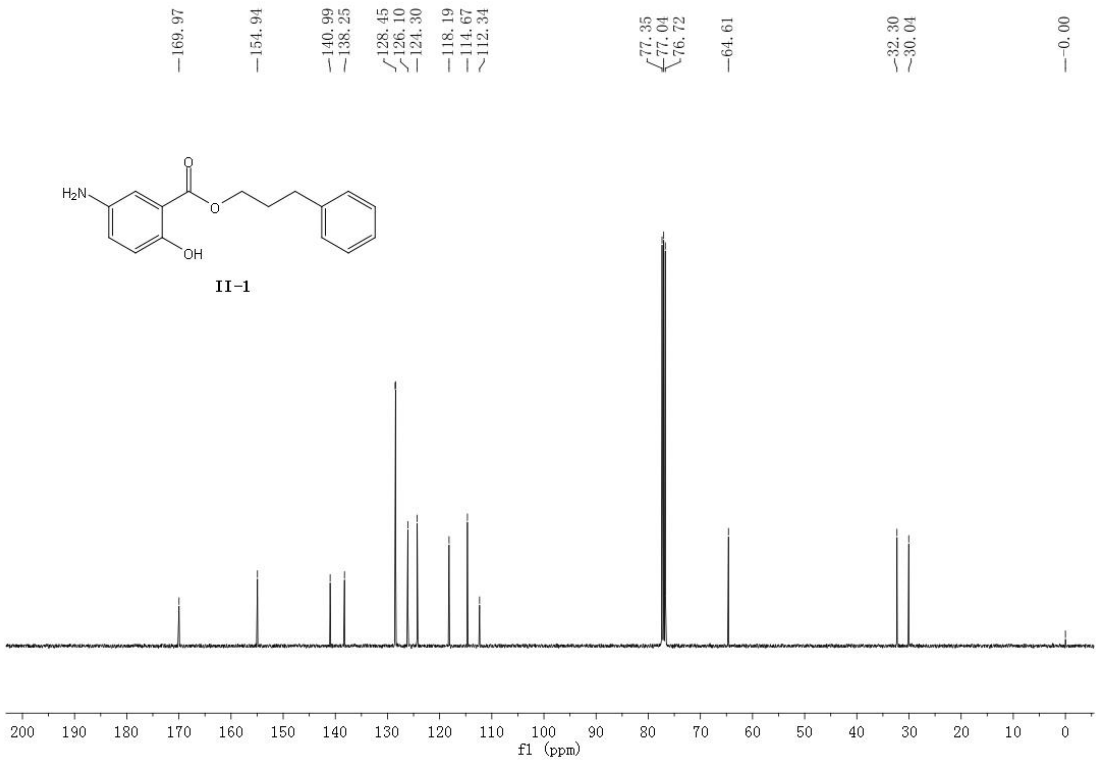
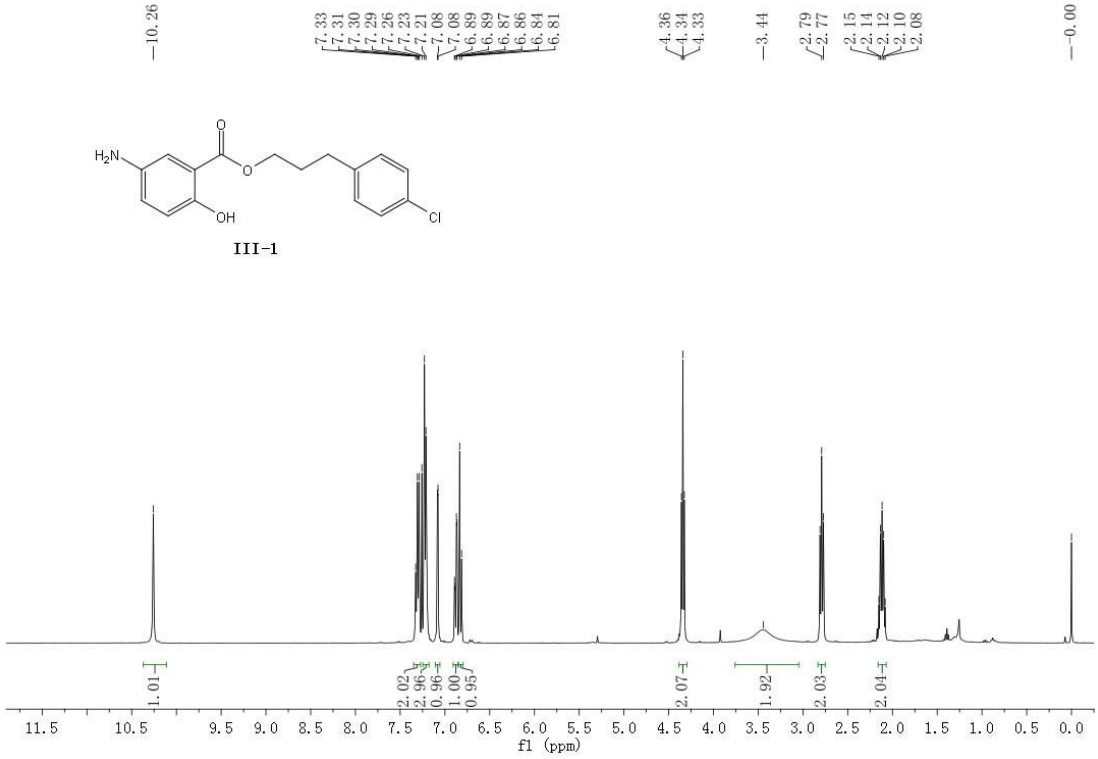
NMR Spectra of Representative Compounds



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6.81
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4.34
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2.14
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2.10
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O
OH
Cl
III-1
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2.02
2.96
0.96
1.00
0.95
2.07
1.92
2.03
2.04
11.5
10.5
9.5
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7.5
7.0
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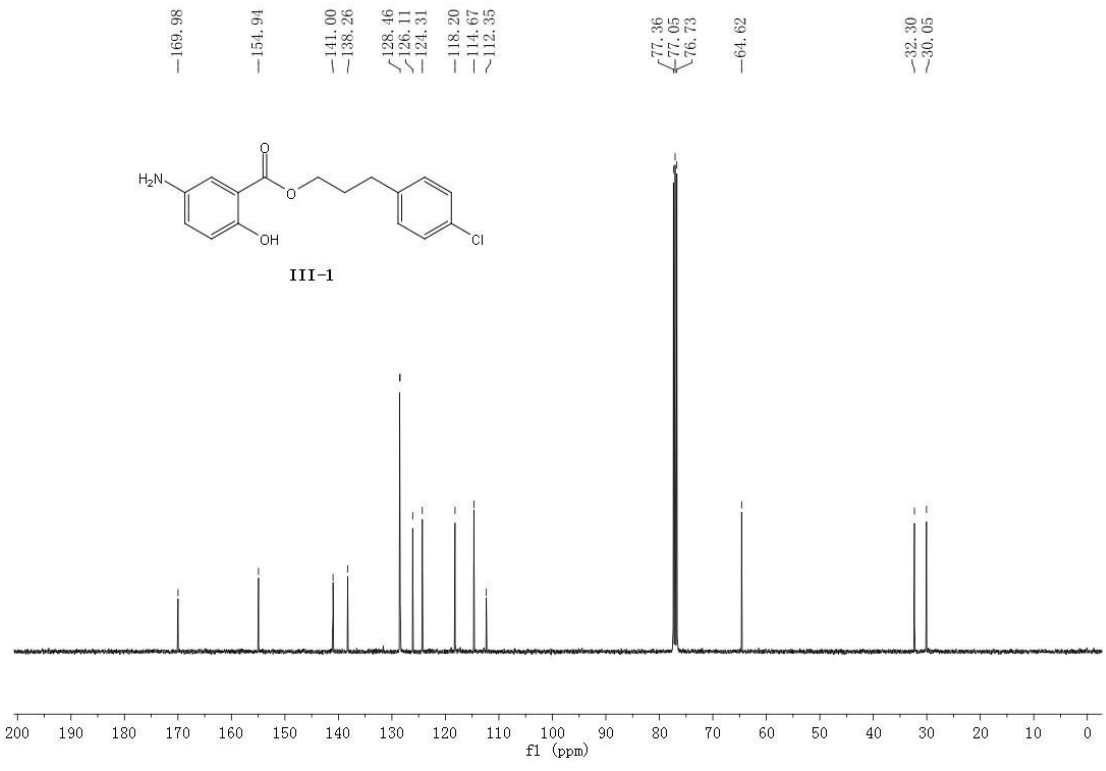
f1 (ppm)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O
OH
Cl
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180
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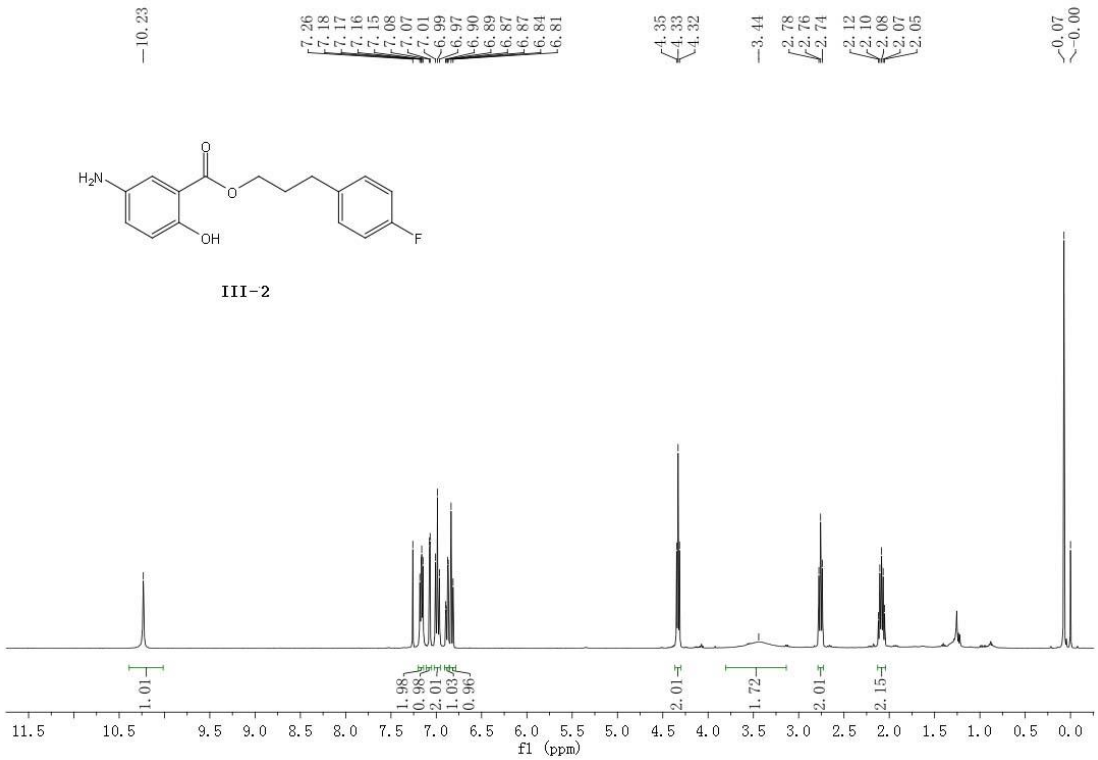
f1 (ppm)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4.33
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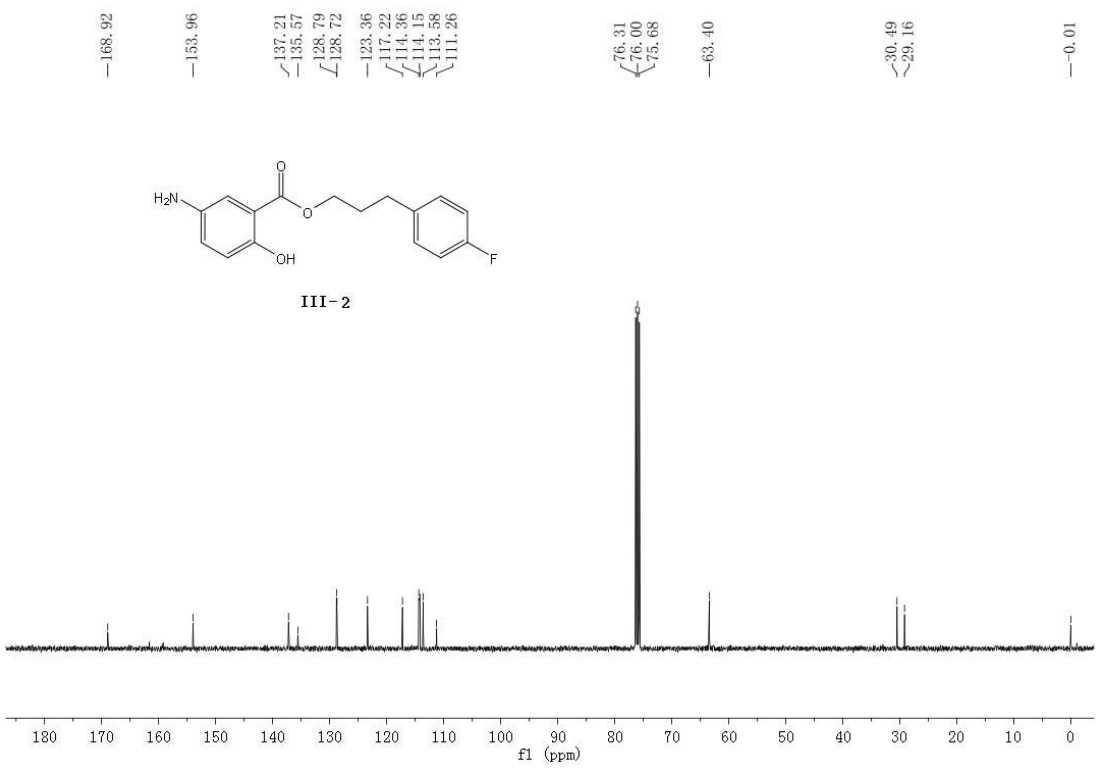
f1 (ppm)

III-2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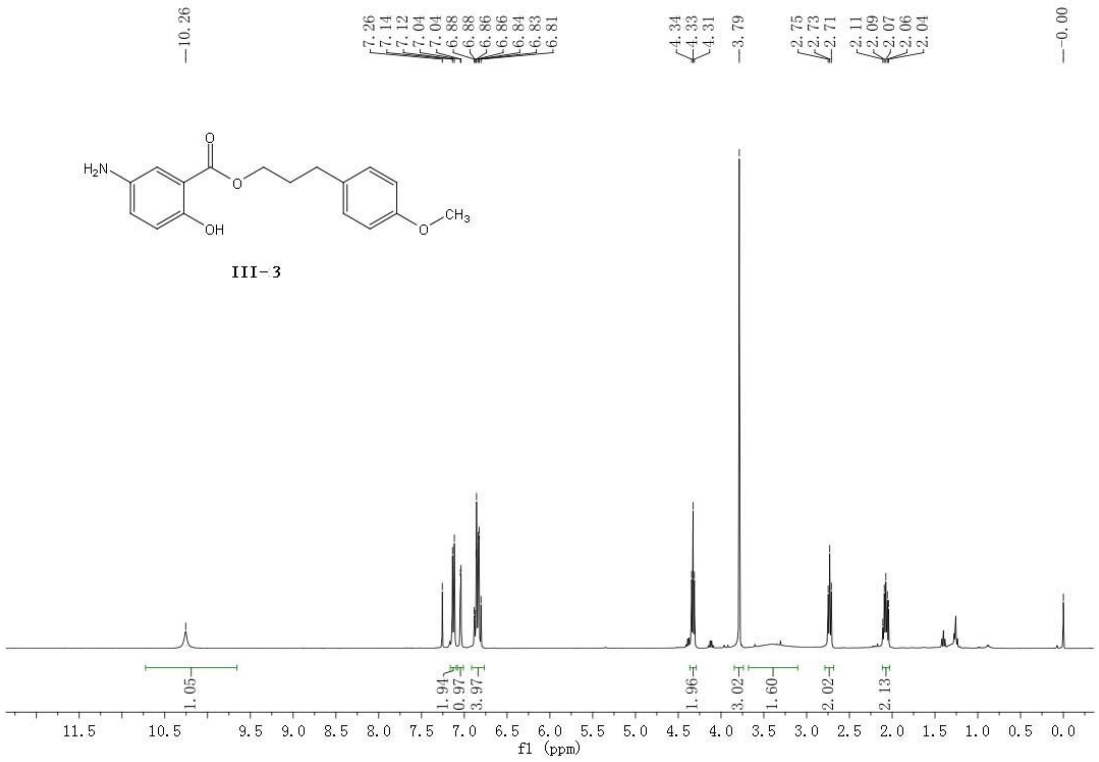
f1 (ppm)

III-2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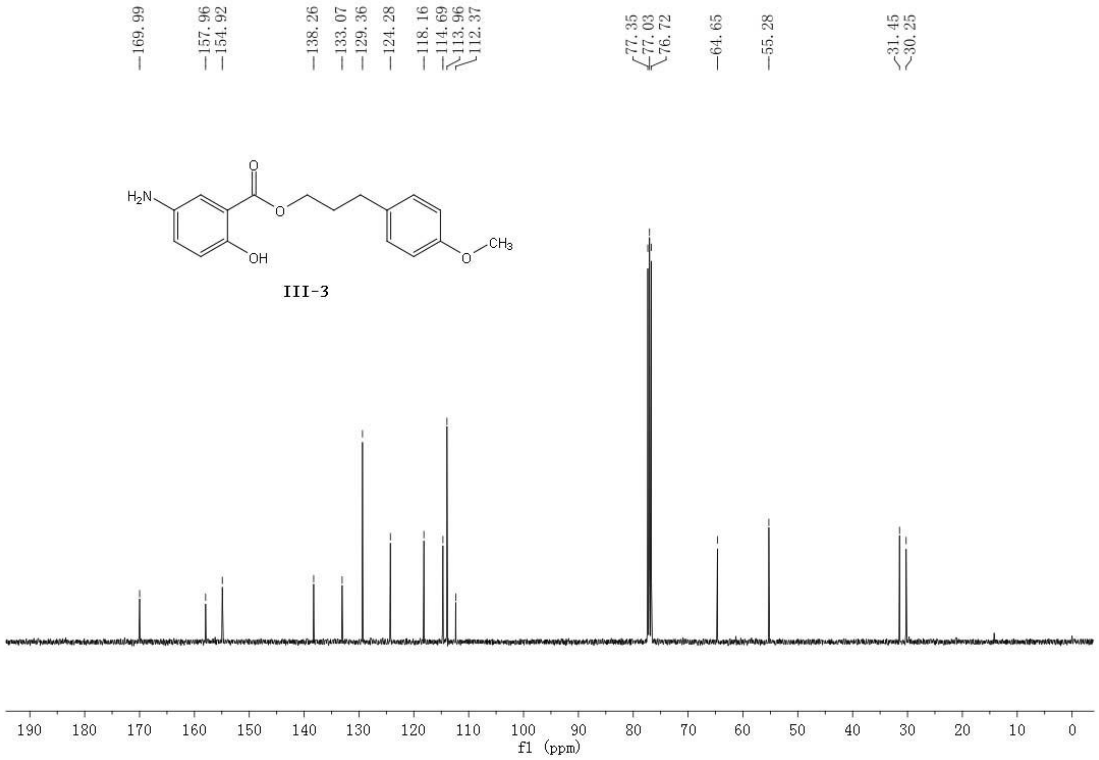
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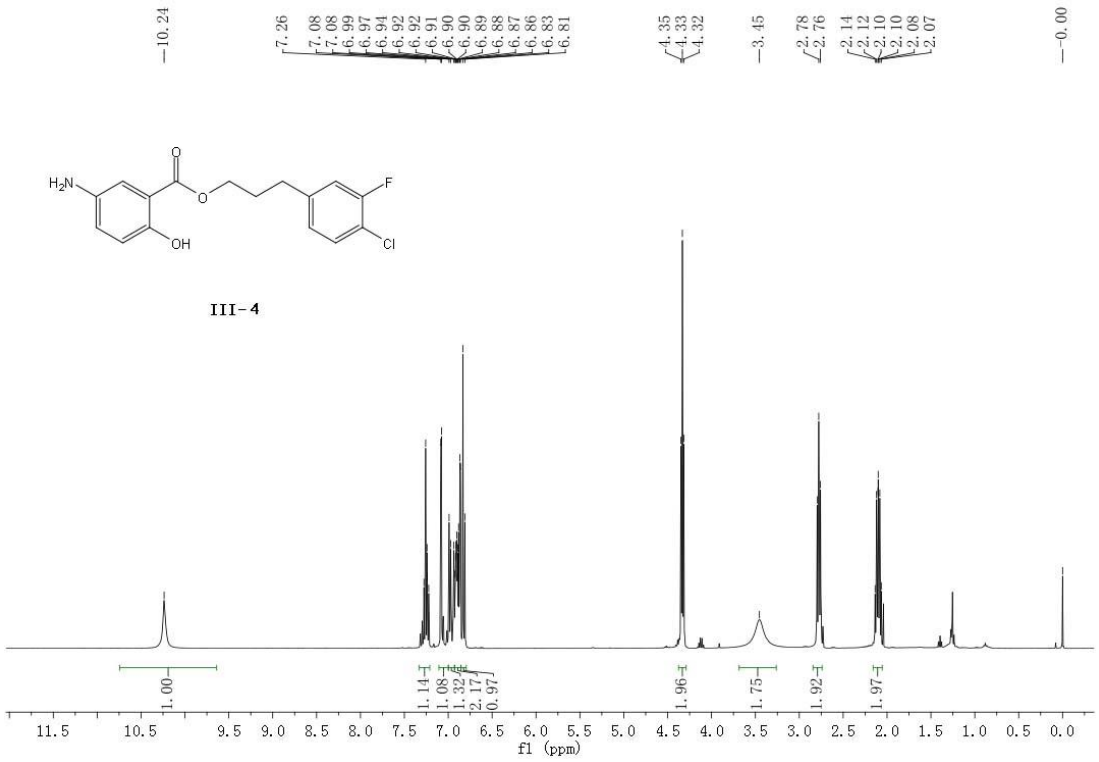
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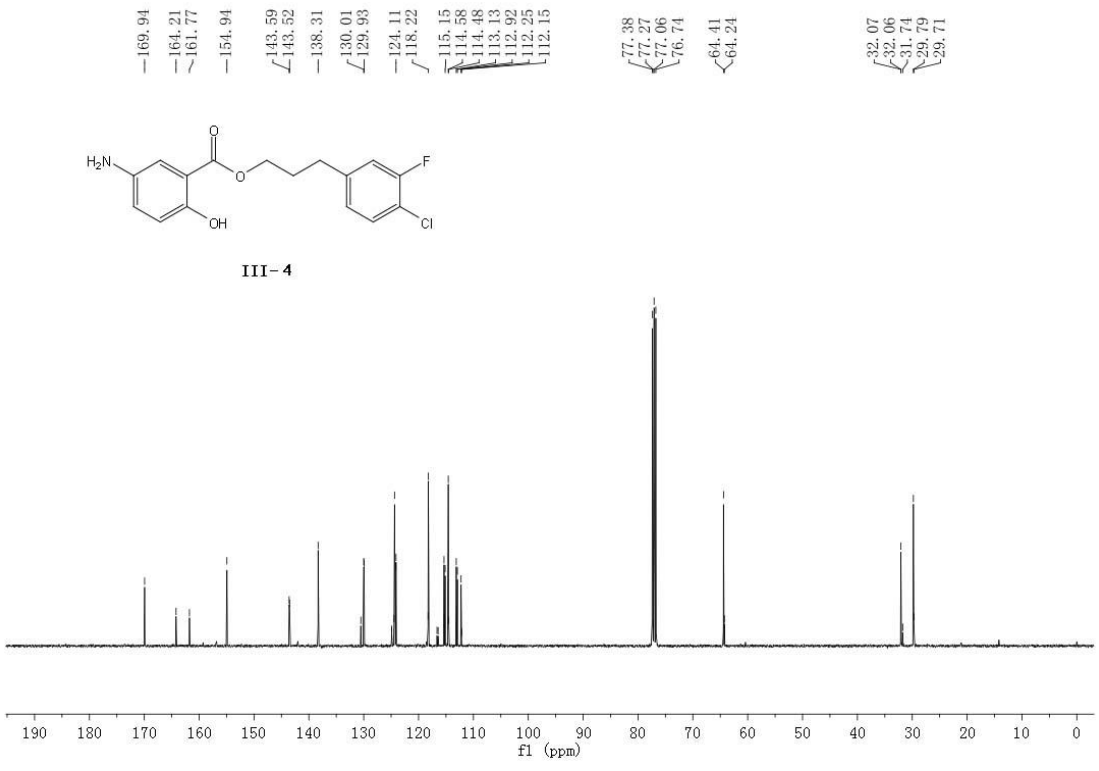
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