An andrographolide derivative AGP-26b exhibiting anti-angiogenic activity in HUVECs and zebrafish via blocking VEGFA/VEGFR2 signaling pathway

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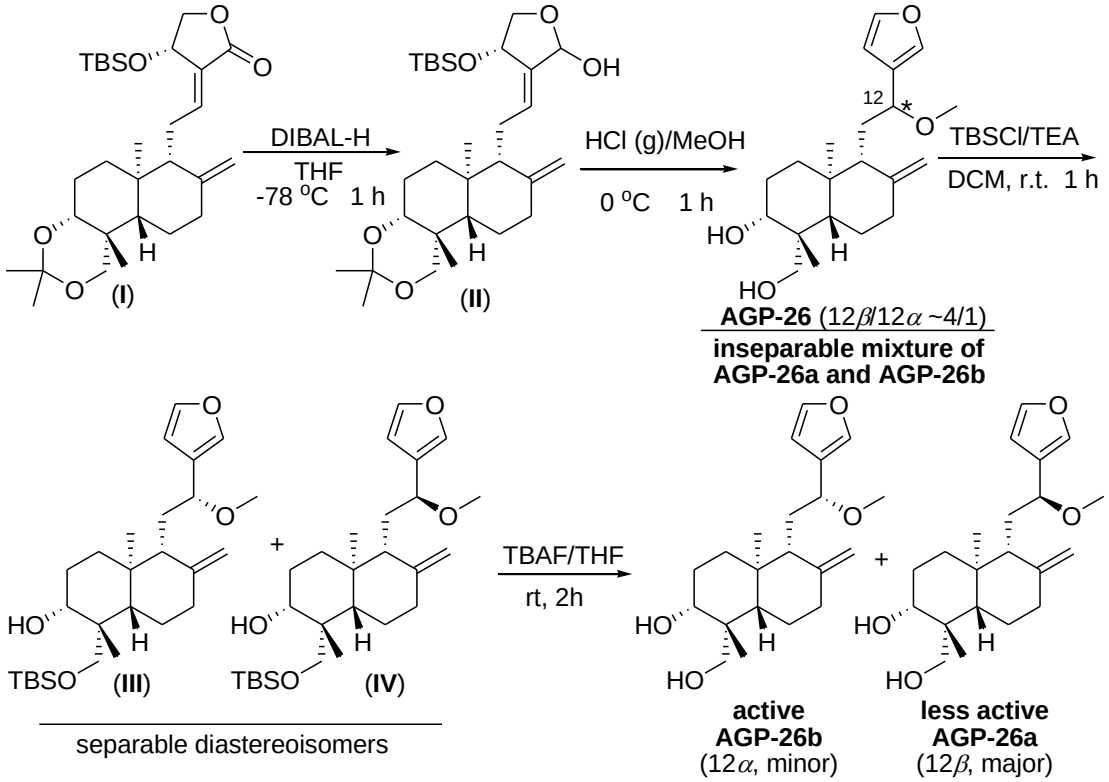
Contents:

Synthesis of AGP-26a and AGP-26b



(1) Preparation of 16-aldolactol-3,19-acetonylidene-andrographolide (**II**)

To -78 °C cooled solution of 10.0 mmol of compound (**I**) (prepared from reference 25) dissolved in 50 ml dry dichloromethane under N_2, 8.0 ml (1.5 N) DIBAL-H solution in hexane was added dropwise. The reaction was completed in about 1 hour and then 5 ml ethyl acetate was added carefully at -78 °C before 5 ml saturated potassium sodium tartrate solution was added. After stirred at -78 °C for 5 min, the mixture was extracted with 150 ml ethyl acetate and washed with 150 ml saturated potassium sodium tartrate solution. Aqueous phase was extracted with ethyl acetate (2 x 100 ml) again and combined organic phase was washed with saturated potassium sodium tartrate solution. Organic phase was dried over anhydrous Na2SO4 and filtered, evaporated, the residue was silica gel chromatographed (ethyl acetate/dichloromethane 1/130) to give 68% yield of compound **II**: white solid; m.p. 115.2 - 116.1 °C; ^{1}H NMR (400 MHz, C_6D_6) δ 9.20 (s, 1H), 6.17 (dd, J = 6.6, 5.5 Hz, 1H), 5.15 (dd, J = 6.8, 4.1 Hz, 1H), 4.91 (s, 1H), 4.60 (s, 1H), 3.89 (d, J = 11.6 Hz, 1H), 3.73 – 3.65 (m, 1H), 3.57 (ddd, J = 11.2, 7.2, 4.2 Hz, 1H), 3.51 (dd, J = 7.8, 3.5

Hz, 1H), 3.13 (d, *J* = 11.6 Hz, 1H), 2.80 (ddd, *J* = 18.4, 5.1, 3.3 Hz, 1H), 2.70 (ddd, *J* = 18.2, 11.1, 6.7 Hz, 1H), 2.27 – 2.20 (m, 1H), 1.96 (ddt, *J* = 13.1, 7.9, 4.3 Hz, 1H), 1.86 – 1.61 (m, 3H), 1.60 – 1.53 (m, 1H), 1.49 (t, *J* = 6.4 Hz, 1H), 1.45 (s, 3H), 1.40 (s, 3H), 1.38 (d, *J* = 2.8 Hz, 1H), 1.23 – 1.16 (m, 1H), 1.15 (s, 3H), 1.13 – 1.01 (m, 2H), 0.99 (s, 3H), 0.92 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (101 MHz, C_6D_6) *δ* 193.1, 159.3, 147.9, 142.2, 109.4, 99.5, 75.7, 70.0, 65.9, 64.2, 57.0, 51.9, 38.5, 38.3, 38.0, 34.4, 26.9, 26.2^4, 26.1^6, 25.7, 25.4, 25.1, 23.4, 18.5, 16.9, -4.6, -4.8; HRMS (ESI) *m/z*: 529.3321 $[M+Na]^+$, calculated for $C_{29}H_{50}O_5SiNa$, 529.3325.

(2) Preparation of TLC and silica gel chromatography inseparable mixture of (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan-3-yl)-2-methoxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylene-decahydronaphthalen-2-ol (**AGP-26a**, major) and (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan-3-yl)-2-methoxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylene-decahydronaphthalen-2-ol (**AGP-26b**, minor)

At 0 ^{o}C, 2.0 mmol of compound **II** was dissolved in 10 ml methanol and then 10 ml saturated hydrogen chloride methanol solution was added. The reaction was stirred at 0 ^{o}C for 1 hour before carefully treated with saturated $NaHCO_3$ solution at 0 ^{o}C. Extracted with ethyl acetate and washed with brine, organic phase was dried over anhydrous Na_2SO_4, filtered, evaporated to dryness. The residue was purified by silica gel chromatography (dichloromethane/methanol 90/1) to afforded (85% yield) the inseparable mixture of **AGP-26a** and **AGP-26b**.

(3) Preparation and separation of (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan-3-yl)-2-methoxyethyl)-1-((*t*-butyldimethylsilyloxy)methyl)-1,4a-dimethyl-6-methylene-decahydronaphthalen-2-ol (**IV**, major) and (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan-3-yl)-2-methoxyethyl)-1-((*t*-butyldimethylsilyloxy)methyl)-1,4a-dimethyl-6-methylene-decahydronaphthalen-2-ol (**III**, minor)

To the solution of 10.0 mml of the mixture of **AGP-26a** and **AGP-26b** in 30 ml dry chloromethane and 8.3 ml (60.0 mmol) of triethylamine, the solution of 7.5 g (50.0 mmol) of *t*-butyldimethylsilyl chloride in 10 ml dry dichloromethane were

added dropwise below 25 °C. The reaction was monitored by thin-layer chromatography and completed in 1 hour. The reaction mixture was treated with ethyl acetate and saturated $NaHCO_3$ solution, organic phase was washed with brine, dried over anhydrous Na_2SO_4. After filtered and evaporated, the residue was separated by silica gel chromatography to provide less polar compound **IV** (petroleum/ethyl acetate 35/1) and more polar compound **III** (petroleum/ethyl acetate 33/1).

More polar compound **III** as minor derivative**:** 26% yield; white solid; m.p. 87.1 - 88.4 °C; ^{1}H NMR (400 MHz, C_6D_6) δ 7.15 (t, J = 1.5 Hz, 1H), 7.10 (s, 1H), 6.35 – 6.30 (m, 1H), 4.95 (d, J = 1.2 Hz, 1H), 4.86 (s, 1H), 4.23 (dd, J = 10.6, 3.6 Hz, 1H), 4.19 (d, J = 10.0 Hz, 1H), 3.83 (d, J = 7.1 Hz, 1H), 3.39 (d, J = 9.7 Hz, 1H), 3.20 (ddd, J = 11.6, 7.0, 4.3 Hz, 1H), 3.11 (s, 3H), 2.33 – 2.24 (m, 1H), 2.16 (ddd, J = 13.6, 11.5, 3.7 Hz, 1H), 2.03 – 1.85 (m, 2H), 1.84 – 1.63 (m, 2H), 1.62 – 1.50 (m, 2H), 1.43 (d, J = 11.2 Hz, 1H), 1.23 – 1.08 (m, 4H), 0.92 (s, 9H), 0.91 – 0.82 (m, 2H), 0.65 (s, 3H), 0.00 (d, J = 5.5 Hz, 6H); ^{13}C NMR (101 MHz, C_6D_6) δ 148.4, 144.0, 141.4, 126.3, 108.8, 107.3, 79.7, 74.7, 65.5, 55.8, 55.4, 52.5, 43.0, 39.3, 38.7, 37.1, 31.4, 29.3, 25.9, 24.6, 23.2, 18.3, 15.7, -5.7, -5.8; HRMS (ESI) *m/z*: 485.3058 $[M+Na]^+$, calculated for $C_{27}H_{46}O_4SiNa$, 485.3063.

Less polar compound **IV** as major derivative**:** 67% yield; white solid; m.p. 107.5 - 109.7 °C; ^{1}H NMR (400 MHz, C_6D_6) δ 7.14 (d, J = 1.9 Hz, 2H), 6.38 – 6.29 (m, 1H), 4.92 (d, J = 1.2 Hz, 1H), 4.52 (s, 1H), 4.23 (d, J = 10.0 Hz, 1H), 4.18 (dd, J = 10.3, 1.2 Hz, 1H), 3.89 (d, J = 7.2 Hz, 1H), 3.48 – 3.32 (m, 2H), 3.06 (s, 3H), 2.39 – 2.28 (m, 1H), 2.24 (d, J = 11.0 Hz, 1H), 2.08 – 1.86 (m, 3H), 1.84 – 1.52 (m, 4H), 1.40 – 1.28 (m, 1H), 1.26 (s, 3H), 1.19 (td, J = 12.7, 4.1 Hz, 1H), 1.09 (dd, J = 12.9, 2.2 Hz, 1H), 0.91 (s, 9H), 0.60 (s, 3H), -0.00 (d, J = 4.1 Hz, 6H); ^{13}C NMR (101 MHz, C_6D_6) δ 148.8, 143.8, 139.9, 127.6, 109.0, 107.0, 79.9, 74.0, 65.6, 56.2, 55.3, 52.6, 43.1, 39.1, 38.7, 37.2, 32.8, 29.4, 26.0, 24.6, 23.4, 18.3, 15.7, -5.6^8, -5.7^3; HRMS (ESI) *m/z*: 485.3059 $[M+Na]^+$, calculated for $C_{27}H_{46}O_4SiNa$, 485.3063.

(4) Preparation of (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan- 3-yl)-2-methoxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylene-decahydronaphthalen-2-ol

(**AGP-26a**, major) from **IV** and preparation of (1R,2R,4aS,5R,8aS)-5-((S)-2-(furan-3-yl)-2-methoxyethyl)-1-(hydroxymethyl)-1,4a-dimethyl-6-methylene-deca hydronaphthalen-2-ol (**AGP-26b**, minor) from **III**

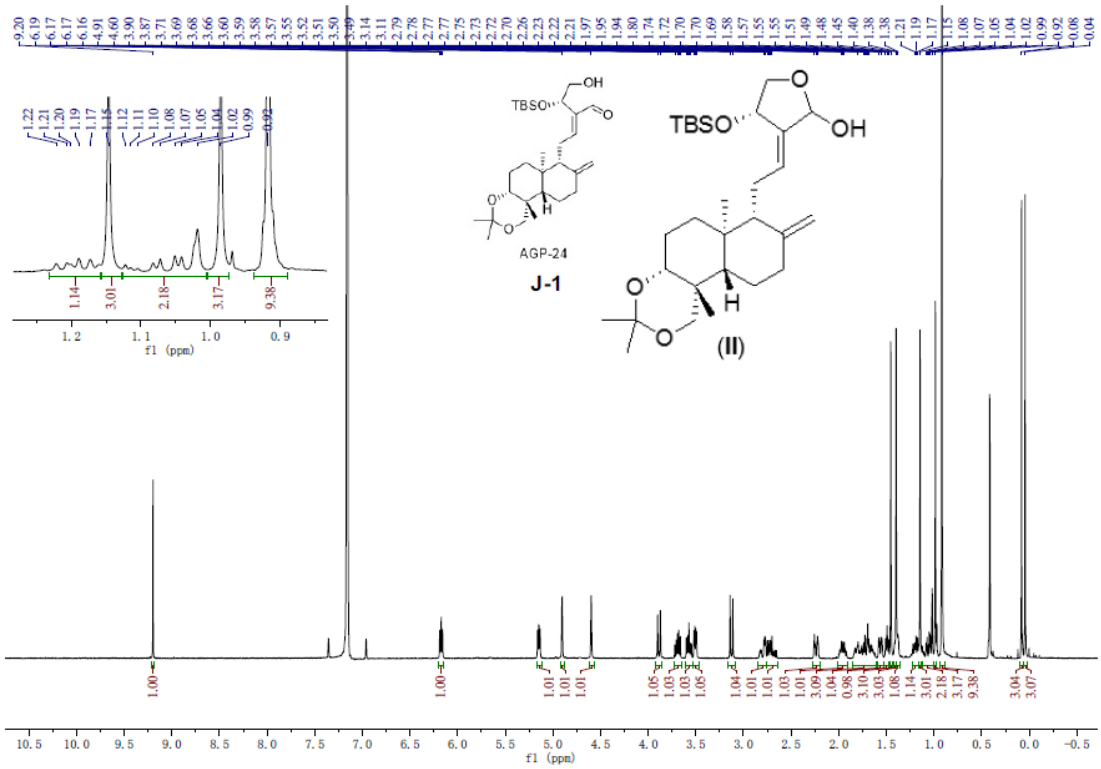
2.0 mmol of Compound **III** or **IV** was dissolved in 10 ml THF and treated with 2.0 mmol of tetrabutylammonium fluoride (TBAF) for 2 hour at ambient temperature. After the reaction was completed, ethyl acetate and saturated $NaHCO_3$ solution were added and organic phase was washed with saturated $NaHCO_3$ solution, and dried over anhydrous Na2SO4. After filtered and evaporated under depressed pressure, the residue was purified by silica gel chromatography (dichloromethane/methanol 90/1) to give **AGP-26a** or **AGP-26b**.

AGP-26a (major) from **IV**：92% yield; white solid; m.p. 159.2 - 161.1 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 – 7.57 (m, 2H), 6.46 (d, J = 1.4 Hz, 1H), 5.05 (d, J = 4.8 Hz, 1H), 4.83 (s, 1H), 4.42 (s, 1H), 4.13 (dd, J = 7.5, 2.7 Hz, 1H), 4.03 (d, J = 8.9 Hz, 1H), 3.83 (dd, J = 11.0, 2.6 Hz, 1H), 3.29 – 3.18 (m, 2H), 3.02 (s, 3H), 2.40 – 2.31 (m, 1H), 2.02 – 1.80 (m, 3H), 1.79 – 1.68 (m, 2H), 1.63 (td, J = 12.0, 11.0, 3.2 Hz, 2H), 1.54 – 1.42 (m, 1H), 1.32 (qd, J = 12.9, 4.0 Hz, 1H), 1.25 – 1.11 (m, 2H), 1.09 (s, 3H), 0.57 (s, 3H)；^{13}C NMR (101 MHz, DMSO-d_6) δ 148.1, 143.7, 140.0, 126.4, 108.8, 106.7, 78.5, 73.1, 62.7, 55.6, 54.7, 51.8, 42.3, 38.4, 38.0, 36.6, 31.3, 27.9, 24.1, 23.0, 14.9; HRMS (ESI) *m/z*: 371.2192 $[M+Na]^+$, calculated for $C_{21}H_{32}O_4Na$, 371.2198.

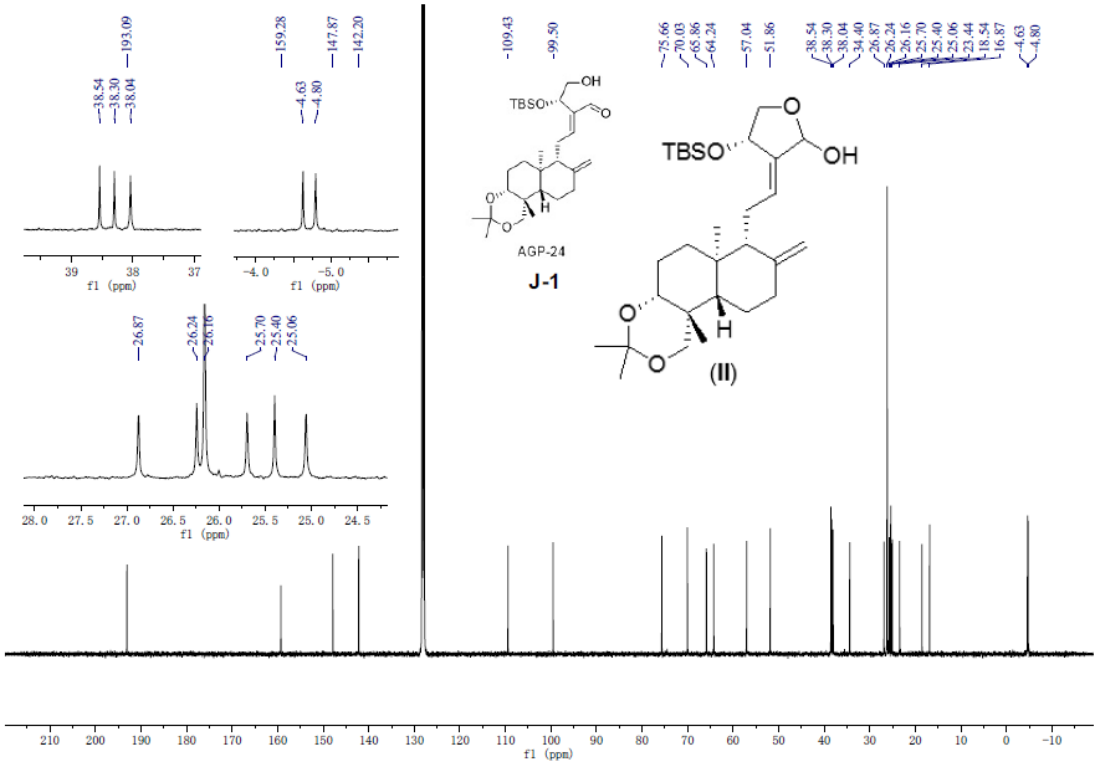
AGP-26b (minor) from **III**: 92% yield; white solid; m.p. 113.4 - 116.0 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (t, J = 1.5 Hz, 1H), 7.58 (s, 1H), 6.46 – 6.38 (m, 1H), 5.01 (d, J = 4.8 Hz, 1H), 4.84 (s, 1H), 4.64 (s, 1H), 4.08 (dd, J = 7.6, 2.8 Hz, 1H), 4.03 (dd, J = 10.3, 3.7 Hz, 1H), 3.79 (dd, J = 11.0, 2.8 Hz, 1H), 3.20 (dd, J = 10.8, 7.8 Hz, 1H), 3.09 (dt, J = 9.9, 5.3 Hz, 1H), 3.02 (s, 3H), 2.36 – 2.24 (m, 1H), 1.89 – 1.50 (m, 7H), 1.38 – 1.19 (m, 2H), 1.01 (s, 3H), 0.94 (dd, J = 12.6, 2.2 Hz, 1H), 0.74 (td, J = 12.3, 5.7 Hz, 1H), 0.60 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 147.9, 143.9, 141.5, 125.1, 108.4, 106.5, 78.4, 73.8, 62.6, 55.2, 54.6, 51.6, 42.2, 38.5, 37.9, 36.3, 30.1, 27.8, 24.0, 22.9, 15.0; HRMS (ESI) *m/z*: 371.2194 $[M+Na]^+$, calculated for $C_{21}H_{32}O_4Na$, 371.2198.

Spectra of ^{1}H NMR and ^{13}C NMR

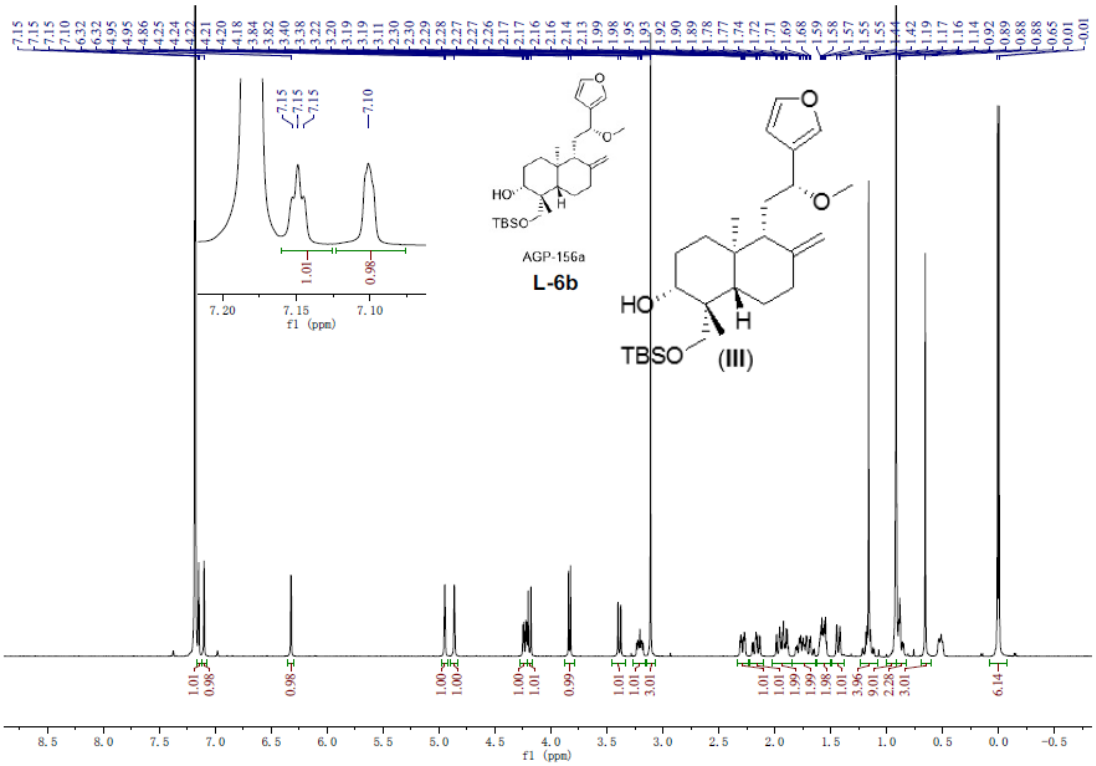
^{1}H NMR of Compound **II**



^{13}C NMR of Compound **II**



^{1}H NMR of Compound **III**



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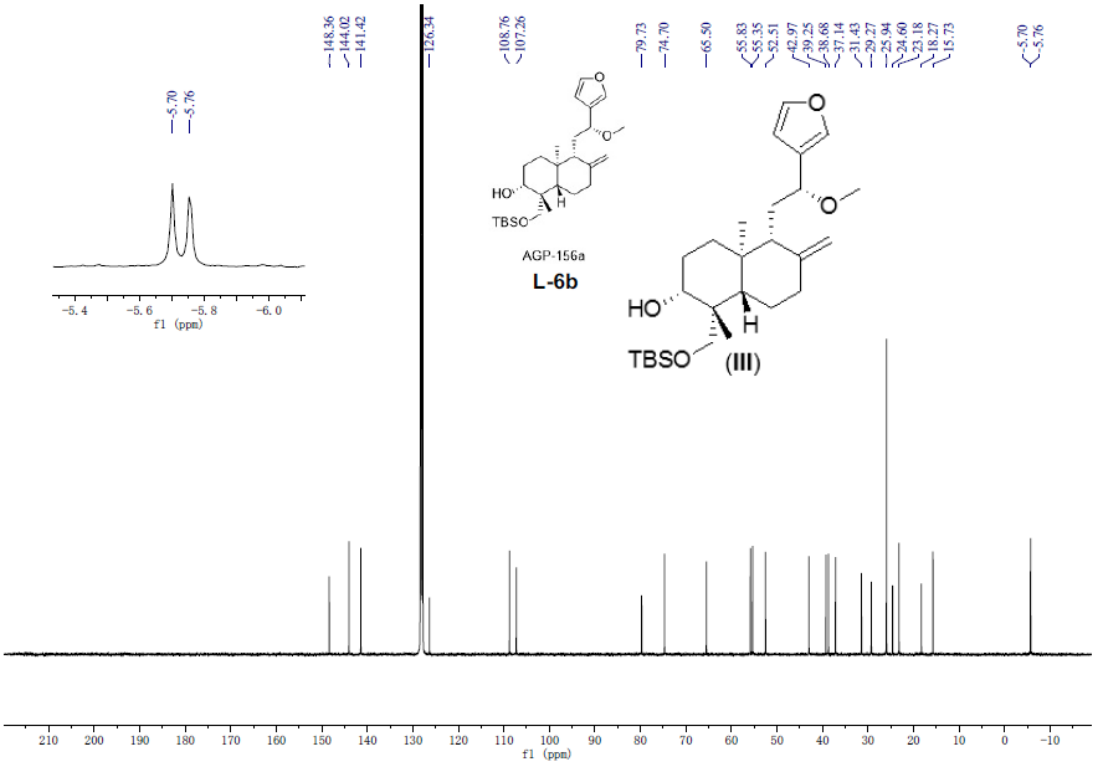
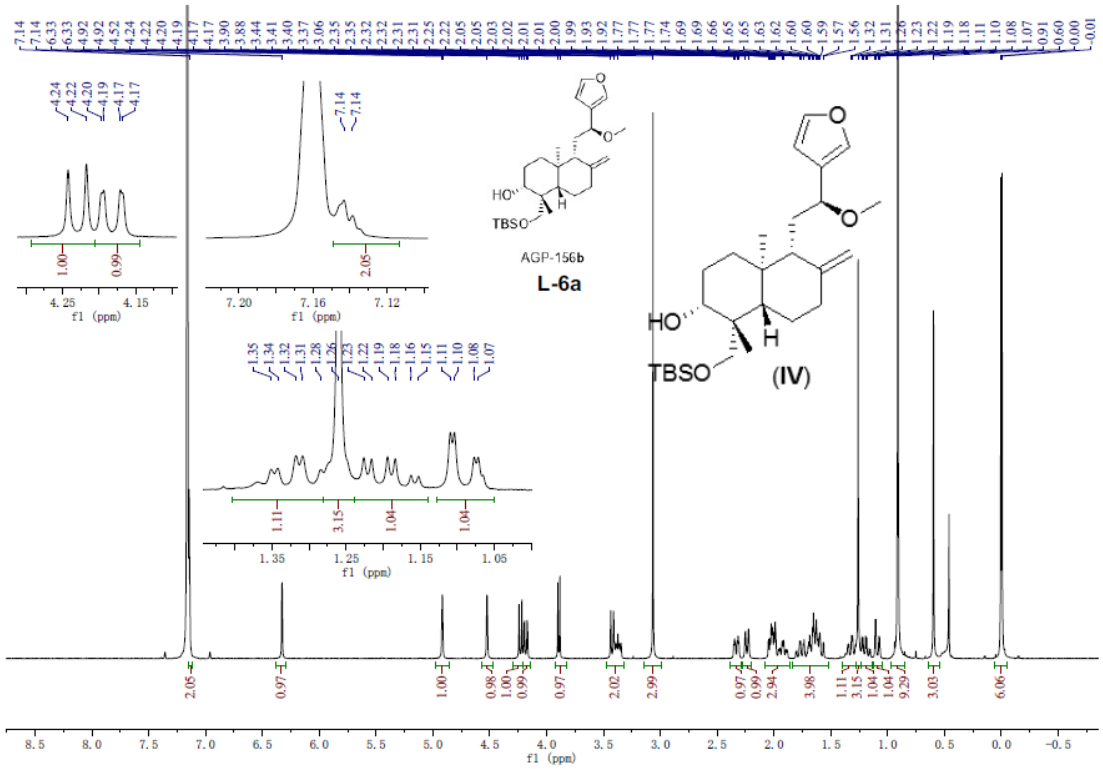
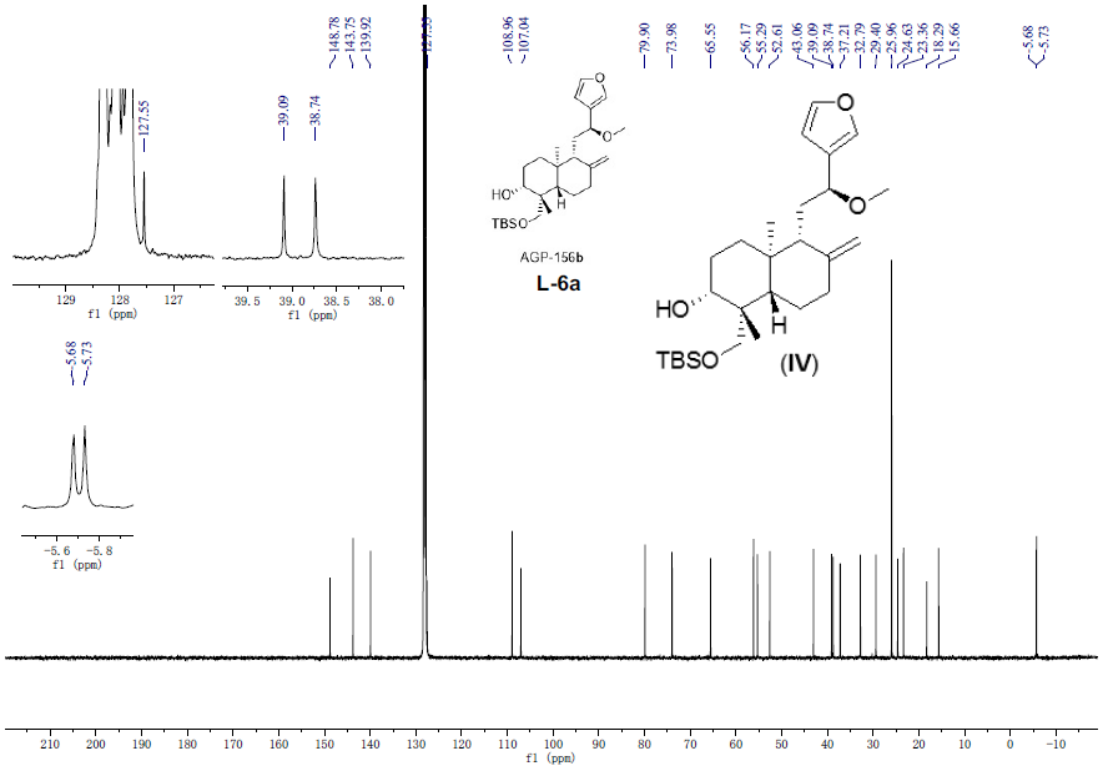


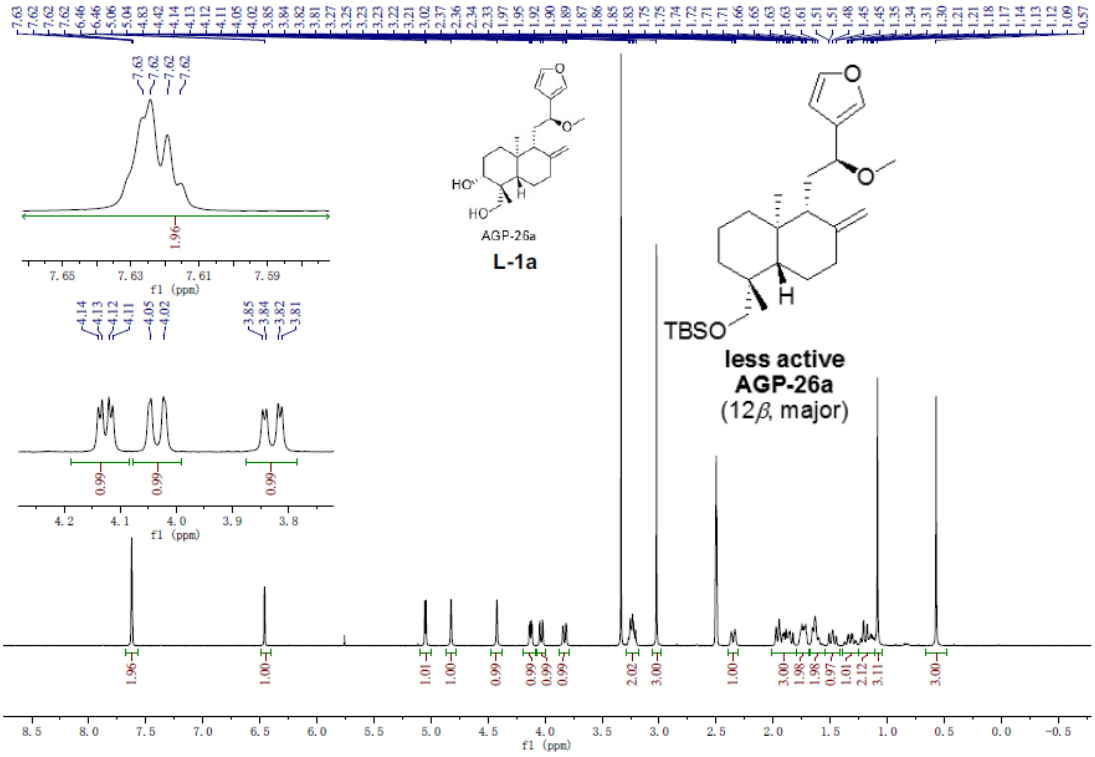
^{1}H NMR of Compound **IV**



^{13}C NMR of Compound **IV**



^{1}H NMR of Compound **AGP-26a**



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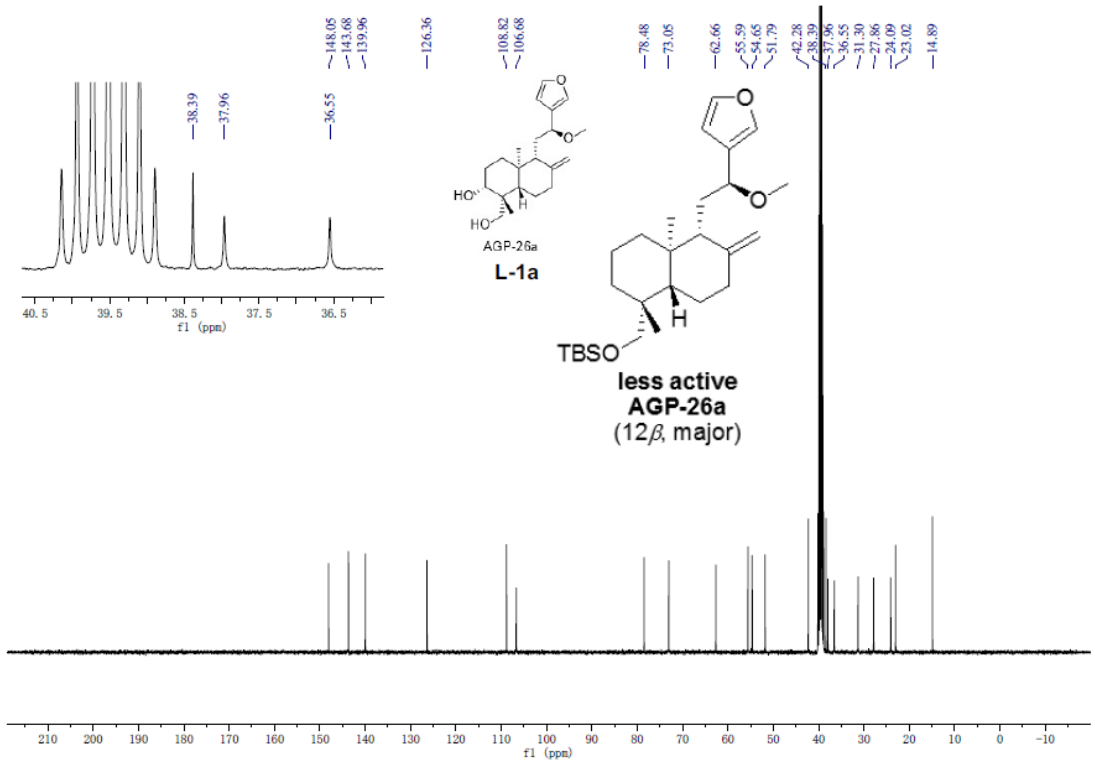
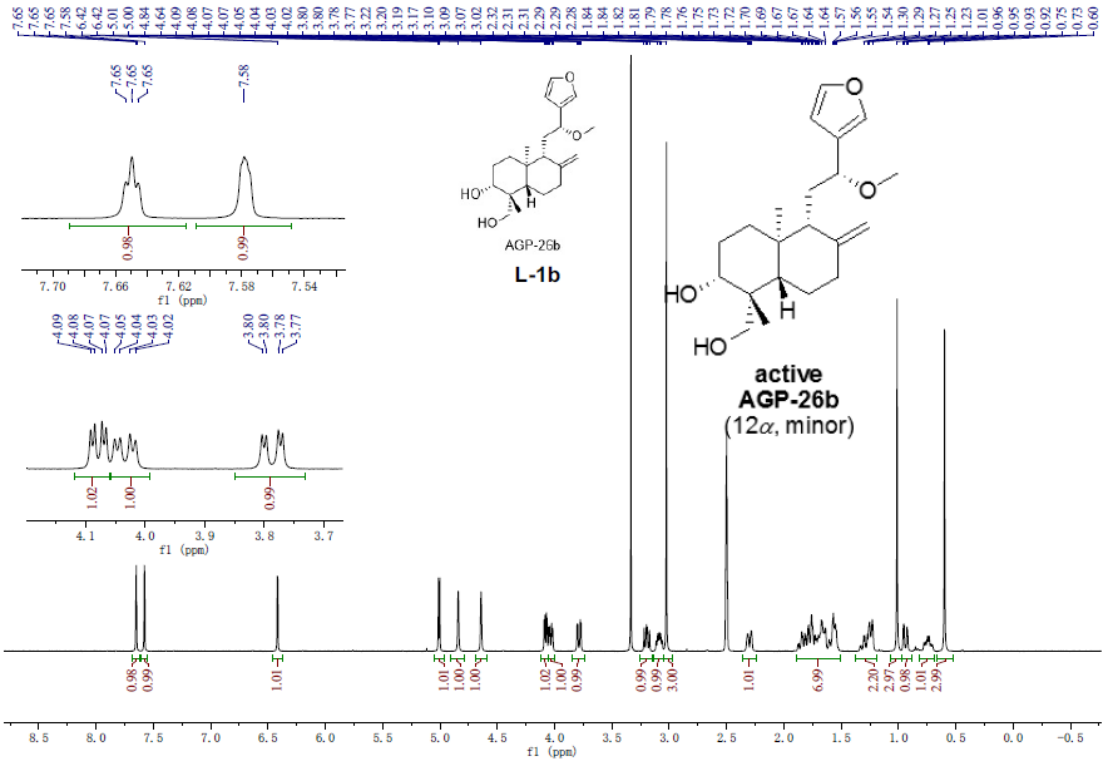
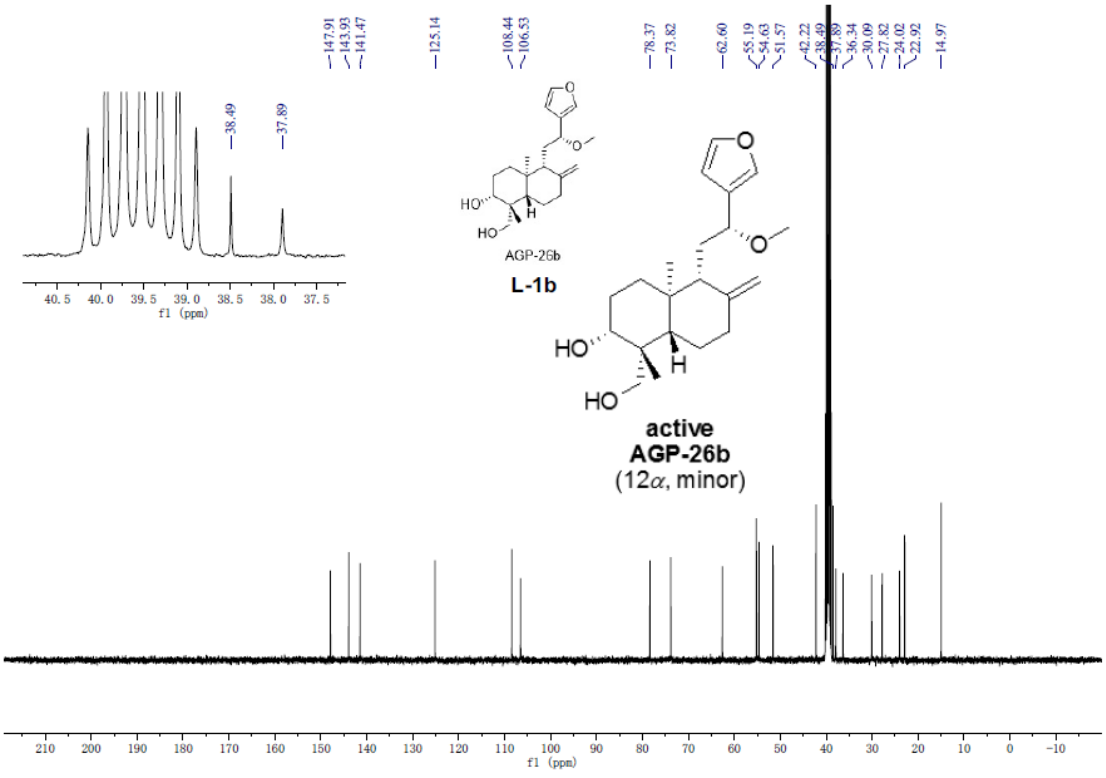


^{1}H NMR of Compound **AGP-26b**



^{13}C NMR of Compound **AGP-26b**



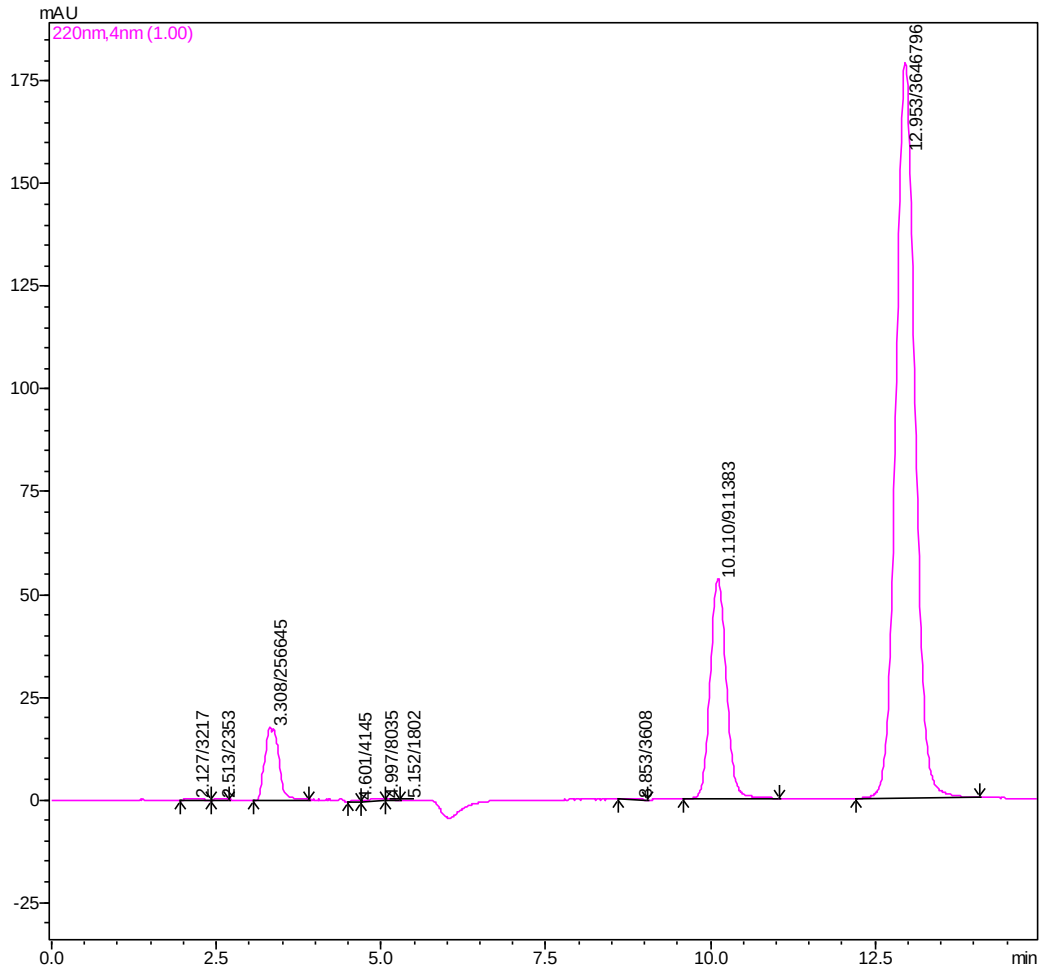
HPLC data for AGP-26 (TLC and silica gel chromatography inseparable mixture of AGP-26a and AGP-26b)

C 18 Sunfire 4.6x250mm 5µm

Eluents: 80% methanol + 20% purified water

rate = 0.8mL/min

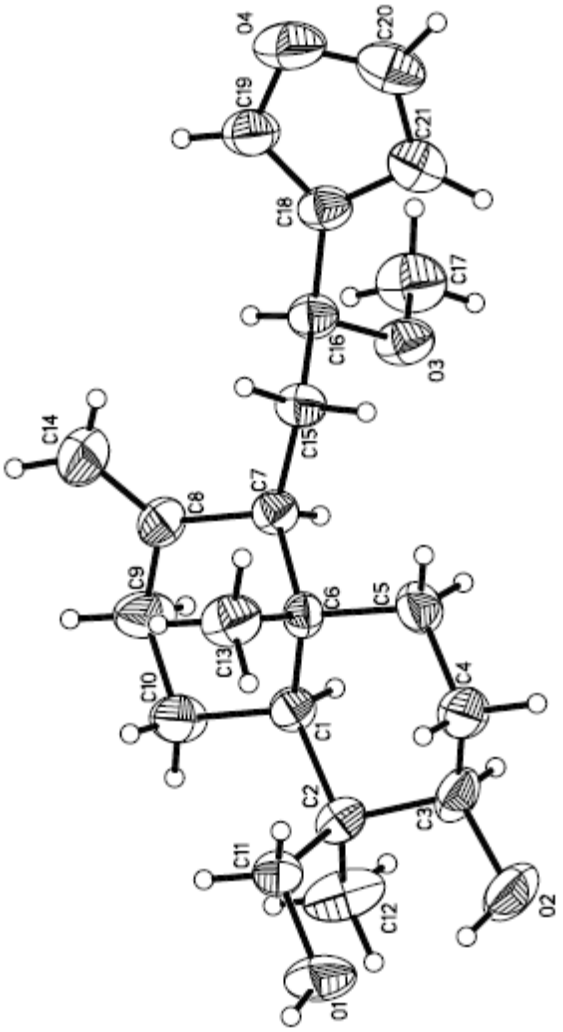
detection wavelength: 220 nm

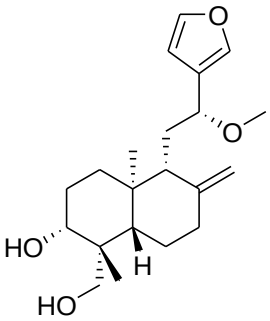


10.110 min AGP-26b, 20%

12.953 min AGP-26a, 80%

Crystal structure of AGP-26a (CCDC 14156500)





active
AGP-26b
(12α, minor)

Morphology observation of Andro, AGP-26, AGP-26a and AGP-26b on Zebrafish

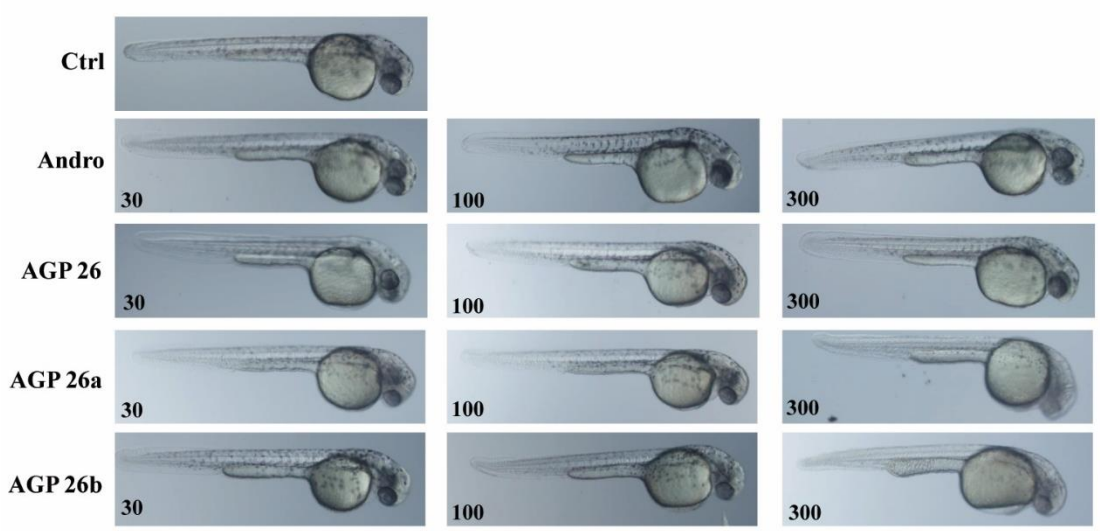


Figure S14 Morphology observation of Andro, AGP-26, AGP-26a and AGP-26b on zebrafish at 8 hpt. 24 hpf embryos were treated with 30-300μM Andro, AGP-26, AGP-26a and AGP-26b for 8 h. Then take photos of zebrafish embryos at 32hpf (24hpf+8hpt). Embryos receiving DMSO served as a vehicle control.