

半夏乙酸乙酯部位化学成分及抗炎活性研究

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摘要: 研究半夏 (*Pinelliae Rhizoma*) 的化学成分及其抗炎活性。采用硅胶、MCI、ODS、Toyopearl HW-40C 柱色谱及半制备液相等方法对半夏乙酸乙酯萃取部位进行分离纯化, 根据理化性质结合现代波谱技术鉴定化合物结构。从中共分离鉴定了 26 个化合物, 分别为环-(*L*-脯-8-羟基-*D*-异亮) 二肽(1)、环-(*L*-脯-*L*-亮) 二肽(2)、环-(*L*-脯-*L*-苯丙) 二肽(3)、环-(*L*-脯-*L*-缬) 二肽(4)、腺苷(5)、5'-硫甲基-5'-硫代腺(6)、2'-*O*-甲基腺苷(7)、腺嘌呤(8)、1,8,15-三氮杂环二十一烷-2,9,16-三酮(9)、1,8,15,22-四氮杂环二十八烷-2,9,16,23-四酮(10)、1,8,15,22,29-五氮杂环三十五烷-2,9,16,23,30-五酮(11)、壬二酸二甘油酯(12)、棕榈酸单甘油酯(13)、亚油酸甘油酯(14)、9,12-十八烷二烯基 *N*-羟乙基(15)、亚麻酸 2-丁氧基乙酯(16)、(2*S*)-1-*O*-(9*Z*,12*Z*-十八烷二烯基)-3-*O*- β -半乳糖基甘油(17)、胡萝卜苷-6'-*O*-棕榈酸酯(18)、邻苯二甲酸二-(2-乙基)-己酯(19)、落叶松脂素(20)、落叶松脂醇-9-*O*- β -吡喃葡萄糖苷(21)、(+)-表松脂素-4'-*O*- β -吡喃葡萄糖苷(22)、(+)-异落叶松脂素-9'-*O*- β -吡喃葡萄糖苷(23)、(+)-异落叶松脂素-9-*O*- β -吡喃葡萄糖苷(24)、香草酸(25)、对羟基肉桂醇(26)。其中, 化合物 1,3,4,8~12,15,16,19,22 和 23 为首次从半夏中分离得到。采用脂多糖 (LPS) 诱导小鼠巨噬细胞 (RAW 264.7) 模型对部分单体化合物进行抗炎活性筛选。结果显示, 化合物 2,3,11,16 和 21 具有抑制 NO 释放作用, 其中化合物 16 的 NO 抑制作用较强, 其 IC₅₀ 值为 10.47 ± 0.89 μ mol/L。

关键词: 半夏; 乙酸乙酯部位; 化学成分; 抗炎活性

中图分类号: R284.2

文献标识码: A

文章编号: 1001-6880(2024)8-1339-11

DOI: 10.16333/j.1001-6880.2024.8.007

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Abstract: This study aims to investigate the chemical constituents from *Pinelliae Rhizoma* and their anti-inflammatory activity. Twenty-six compounds were isolated and purified from the ethyl acetate extract of *Pinelliae Rhizoma* silica gel, MCI, ODS and Toyopearl HW-40C column chromatography and semi-preparative HPLC methods. Their chemical structures were elucidated by physicochemical properties and spectral data as cyclo-(*L*-Pro-8-hydroxy-*D*-Ile) (1), cyclo-(*L*-Pro-*L*-Leu) (2), cyclo-(*L*-Pro-*L*-Phe) (3), cyclo-(*L*-Pro-*L*-Val) (4), adenosine (5), 5'-*S*-methyl-5'-thioadenosine (6), 2'-methoxyadenosine (7), adenine (8), 1,8,15-triazacycloterpene-2,9,16-trione (9), 1,8,15,22-tetraazacyclooctacosane-2,9,16,23-tetraketone (10), 1,8,15,22,29-pentaaza-pentadecane-2,9,16,23,30-pentanone (11), diethylene glycol azelate (12), glycerol monopalmitate (13), glycerin monolinoleate (14), 9,12-octadecadienoic acid *N*-(2-hydroxyethyl) (15), 2-butoxyethyl linolenate (16), (2*S*)-1-*O*-(9*Z*,12*Z*-octadecandienyl)-3-*O*- β -galactosyl glycerol (17), β -sitosteryl-3 β -glucopyranoside-6'-*O*-palmitate

收稿日期: 2024-01-19 接受日期: 2024-07-01

基金项目: 2023 年度河南省高校科技创新团队项目 (23IRTSTHN028); 河南省科技研发计划联合基金重点项目 (222301420023); 河南省省级重大科技专项 (221100310400)

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(18), bis (2-ethylhexyl) phthalate (19), lariciresinol (20), lariciresinol-9-*O*- β -glucopyranoside (21), (+)-epipinosinol-4'-*O*- β -glucopyranoside (22), (+)-isolariciresinol-9'-*O*- β -glucopyranoside (23), (+)-isolariciresinol-9-*O*- β -glucopyranoside (24), vanillic acid (25), and (*E*)-*p*-coumaroyl alcohol (26). Compounds 1, 3, 4, 8-12, 15, 16, 19 and 23 were isolated from *Pinelliae Rhizoma* for the first time. Some of isolated compounds were further evaluated for their anti-inflammatory activity in lipopolysaccharide (LPS)-induced mouse macrophage (RAW 264.7) cells. The results showed that compounds 2, 3, 11, 16 and 21 had inhibitory effects on NO release, and compound 16 had stronger NO inhibition with IC₅₀ values of 10.47 ± 0.89 μ mol/L.

Key words: *Pinelliae Rhizoma*; ethyl acetate fraction; chemical compositions; anti-inflammatory activity

半夏 (*Pinelliae Rhizoma*) 为天南星科植物半夏 *Pinellia ternata* (Thunb.) Breit. 的干燥块茎^[1]。味辛, 性温, 有毒, 归脾、胃、肺经, 始载于《神农本草经》, 具有燥湿化痰, 降逆止呕, 消痞散结的功效^[2]。半夏为临床常用中药, 《伤寒杂论》使用半夏的方剂共计 42 首, 其中 20 首为生半夏^[3]。半夏在消化道疾病方面疗效显著, 以半夏为君药的经典名方半夏泻心汤、半夏厚朴汤是治疗慢性萎缩性胃炎、反流性食管炎的首选方剂。现代药理研究表明^[4], 半夏具有抗溃疡、抗肿瘤、降血脂、降压等作用。但迄今, 对半夏的研究多集中在经典名方的药效验证、有效部位药理研究方面, 而对于半夏单味药的化学成分及其作用机制研究较少, 仅报道了生物碱、挥发油、有机酸、甾醇类、氨基酸等少量成分。其传统功效物质基础尚不清晰, 临床疗效证据不充分。因此, 本实验以半夏乙酸乙酯萃取部位作为研究对象, 对其进行细致的化学成分分离和结构鉴定, 并对部分单体化合物采用脂多糖 (LPS) 诱导小鼠巨噬细胞 (RAW 264.7) 模型进行抗炎活性筛选, 以期阐明半夏药效物质奠定基础。

1 材料与方法

1.1 材料、仪器与试剂

半夏药材于 2021 年 9 月采自山东省菏泽市鄄城县, 经河南中医药大学药学院代丽萍教授鉴定为天南星科植物半夏 *Pinellia ternata* (Thunb.) Breit. 的干燥块茎, 样品 (NO. 2021-0901) 存放于豫药全产业链研发河南省协同创新中心。

Bruker AV-500 核磁共振仪 (德国 Bruker 公司); UPLC-Oribtrap-Exploris-120-MS 液相色谱-质谱系统 (赛默飞世尔科技公司); U3000 型高效液相色谱仪 (PDA 检测器, 赛默飞世尔科技公司); LC 52 型半制备液相 (北京清华博华公司)。

ODS (40 ~ 60 μ m, 日本 YMC 公司); Toyopearl 凝胶树脂 HW-40C (北京英莱克科技发展有限公司); 硅胶板 (GF₂₅₄ 型)、柱色谱硅胶 (100 ~ 200 目、

200 ~ 300 目) (青岛海洋化工厂); YMC-Triart C₁₈ 半制备柱 (10 mm × 250 mm, 5 μ m; 北京绿百草科技发展有限公司); 噻唑蓝 (methylthiazolyldiphenyl-tetrazolium bromide, MTT) 和脂多糖 (lipopolysaccharide, LPS) (北京索莱宝科技有限公司); 生化级 DM-SO、高糖培养基 (dulbecco's modified eagle medium, DMEM) 和胎牛血清 (fetal bovine serum, FBS) (武汉普诺赛生命科技有限公司)。氘代试剂 (上海麦克林生化科技公司); 甲醇、乙腈 (色谱纯, 美国天地公司); 其他试剂 (分析纯, 天津恒兴公司)。

1.2 提取与分离

取半夏 35 kg, 用 75% 乙醇浸泡 40 min, 回流提取 3 次, 每次 2 h。合并提取液并在 60 °C 下减压浓缩至浸膏无醇味, 得总浸膏约 2 kg。总浸膏加入 1 倍量超纯水并使其充分混合均匀后, 依次萃取, 得到乙酸乙酯部位 117.0 g 和正丁醇部位 280.0 g。采用硅胶柱色谱对乙酸乙酯部位进行分离, 以二氯甲烷-甲醇 (100% → 0%) 梯度洗脱, 得到 7 个组分 Fr. 1 ~ Fr. 7。

Fr. 3 (9.1 g) 经 Toyo 柱色谱, 用二氯甲烷-甲醇 (1:1) 洗脱, 得 Fr. 3-1 ~ Fr. 3-4。Fr. 3-2 (6.0 g) 经 Sephadex LH 20 凝胶 (二氯甲烷-甲醇 1:1) 柱色谱, 得 Fr. 3-2-1 ~ Fr. 3-2-3。Fr. 3-2-1 (1.0 g) 用半制备液相色谱 (70% 乙腈-水) 纯化得到化合物 12 (4.0 mg, t_R = 37.2 min)。Fr. 3-2-2 (1.9 g) 用半制备液相色谱 (70% 乙腈) 分离得到化合物 14 (8.3 mg, t_R = 43.1 min)。Fr. 3-3 (0.9 g) 经 ODS 柱色谱, 乙腈-水 (5% → 25%) 梯度洗脱, 得 Fr. 3-3-1 ~ Fr. 3-3-12。Fr. 3-3-1 (84.7 mg) 用半制备液相纯化 (8% 乙腈-水) 得到化合物 25 (5.2 mg, t_R = 46.3 min)。Fr. 3-3-2 (44.2 mg) 用半制备液相纯化 (5% 乙腈-水) 得到化合物 4 (7.4 mg, t_R = 53.2 min)。Fr. 3-3-6 (149.2 mg) 用半制备液相纯化 (10% 乙腈-水) 得到化合物 1 (20.6 mg, t_R = 41.6 min)、26 (5.5 mg, t_R = 48.0 min) 和 2 (7.2 mg, t_R = 55.8 min)。Fr. 3-3-8 (98.5

mg)用半制备液相纯化(15%乙腈-水)得到化合物**3**(8.7 mg, $t_R = 38.2$ min)。Fr. 3-3-12(83.9 mg)用半制备液相纯化(21%乙腈-水)得到化合物**20**(6.0 mg, $t_R = 40.1$ min)。

Fr. 4(15.2 g)经 ODS 柱色谱,甲醇-水(30% → 90%)梯度洗脱,得 Fr. 4-1 ~ Fr. 4-2。Fr. 4-2(1.5 g)经 ODS 柱色谱,乙腈-水(30% → 65%)梯度洗脱,得 Fr. 4-2-1 ~ Fr. 4-2-18。Fr. 4-2-13(126.0 mg)经半制备液相纯化(50%乙腈-水)得到化合物**17**(18.5 mg, $t_R = 95.4$ min)。Fr. 4-2-15(81.4 mg)经半制备液相纯化(55%乙腈-水)得到化合物**15**(18.4 mg, $t_R = 116.3$ min)和化合物**16**(4.4 mg, $t_R = 126.0$ min)。

Fr. 6(20.0 g)经 ODS 柱色谱,甲醇-水(30% → 90%)梯度洗脱,得 Fr. 6-1 ~ Fr. 6-2。Fr. 6-1 经重结晶得到化合物**18**(10.0 mg)。Fr. 6-1(5.7 g)经 ODS 中压柱,甲醇-水(5% → 50%)梯度洗脱,得 Fr. 6-1-1 ~ Fr. 6-1-24。Fr. 6-1-1(2.8 g)经半制备液相纯化(8%甲醇-水)得到化合物**8**(6.6 mg, $t_R = 34.2$ min)。Fr. 6-1-2(239.7 mg)经重结晶得到化合物**5**(8.0 mg)。Fr. 6-1-5(30.1 mg)经半制备液相纯化(13%甲醇-水)得到化合物**7**(7.2 mg, $t_R = 29.0$ min)。Fr. 6-1-13(59.5 mg)经半制备液相纯化(8%乙腈-水)得到化合物**9**(5.8 mg, $t_R = 45.8$ min)。Fr. 6-1-15(69.2 mg)经半制备液相纯化(10%乙腈-水)得到化合物**6**(13.2 mg, $t_R = 50.2$ min)。Fr. 6-1-17(24.2 mg)经半制备液相纯化(12%乙腈-水)得到化合物**23**(2.5 mg, $t_R = 72.1$ min)。Fr. 6-1-20(91.4 mg)经半制备液相纯化(15%乙腈-水)得到化合物**21**(8.8 mg, $t_R = 58.3$ min)。Fr. 6-1-22(63.7 mg)经半制备液相纯化(15%乙腈-水)得到化合物**11**(19.1 mg, $t_R = 40.6$ min)和化合物**22**(4.2 mg, $t_R = 91.1$ min)。Fr. 6-2(6.6 g)经 ODS 中压柱,乙腈-水(10% → 50%)梯度洗脱,得 Fr. 6-2-1 ~ Fr. 6-2-19。Fr. 6-2-6(25.0 mg)经半制备液相纯化(13%乙腈-水)得到化合物**10**(5.5 mg, $t_R = 27.3$ min)。Fr. 6-2-9(30.2 mg)经半制备液相纯化(13%乙腈-水)得到化合物**24**(2.0 mg, $t_R = 45.3$ min)。Fr. 6-2-18(1.5 g)经 ODS 中压柱,乙腈-水(65% → 85%)梯度洗脱,得 Fr. 6-2-18-1 ~ Fr. 6-2-18-13。Fr. 6-2-18-4(191.9 mg)经半制备液相纯化(76%乙腈-水)得到化合物**13**(13.7 mg, $t_R = 47.0$ min)。Fr. 6-2-18-12(90.3 mg)经半制备液相纯化(86%乙腈-

水)得到化合物**19**(4.7 mg, $t_R = 61.5$ min)。

1.3 抗炎活性测定

1.3.1 细胞活力实验

采用 MTT 比色法测定细胞存活率^[5]。取对数生长期状况良好的 RAW 264.7 细胞,以 1×10^5 个/mL 浓度稀释,将每孔 100 μ L 细胞稀释液均匀加至 96 孔板中,孵育 24 h,分为空白组、对照组、样品组加入不同浓度的化合物,其给药浓度分别为(6.25、12.5、25、50、100 μ mol/L),每组 3 个复孔,孵育 24 h 后,每孔加入 20 μ L 的 MTT 溶液,避光放置 4 h。用排枪吸出上清液,每孔加入 150 μ L DMSO,用摇床震荡 10 min,在酶标仪 490 nm 处的吸光度下测定。计算细胞存活率。

1.3.2 抗炎活性筛选

将分离得到的单体化合物通过 LPS 诱导的 RAW 264.7 细胞炎症模型进行活性筛选^[5]。将 RAW 264.7 细胞加到 96 孔板中,按 1×10^5 个/mL 浓度稀释,孵育 24 h,将不同浓度的化合物加入给药组,再用 1 μ g/mL 的 LPS 刺激细胞,增设空白对照组、模型组和阳性对照组(地塞米松),每组 3 个复孔,孵育 24 h。采用 Griess 试剂系统的比色测定法测量 NO 含量,将 100 μ L 的细胞培养基与等体积的 Griess 试剂混合,并在室温下孵育 10 min。采用酶标仪测量 540 nm 处的吸光度测定 OD 值,根据公式(1)计算 NO 抑制率(R),并通过 Graphpad 软件进行拟合计算 IC₅₀。

$$R = (OD_2 - OD_1) / (OD_2 - OD_0) \times 100\% \quad (1)$$

式中, OD_2 、 OD_1 和 OD_0 分别为模型组、给药组和空白对照组的 OD 值。

2 实验结果

2.1 结构鉴定

化合物**1** 白色粉末; $[\alpha]_D^{25} -27.6$ (c 1.0, MeOH); HR-ESI-MS: m/z 227.1389 $[M + H]^+$ (计算值 $C_{11}H_{19}N_2O_3$, 227.1396), 分子式为 $C_{11}H_{18}N_2O_3$ 。¹H NMR(500 MHz, CD₃OD) δ : 4.25 ~ 4.18(1H, m, H-9), 4.09(1H, s, H-6), 3.63 ~ 3.47(2H, m, H-3), 2.40 ~ 2.28(1H, m, H-5), 2.24 ~ 2.12(1H, m, H-10), 2.08 ~ 2.01(1H, m, H-4), 2.01 ~ 1.96(1H, m, H-5), 1.95 ~ 1.90(1H, m, H-4), 1.55 ~ 1.40(1H, m, H-11), 1.40 ~ 1.27(m, 1H, H-11), 1.09(3H, d, $J = 7.2$ Hz, H-13), 0.95(3H, t, $J = 7.5$ Hz, H-12); ¹³C NMR(125 MHz, CD₃OD) δ : 172.6(C-1), 46.3(C-3), 23.4(C-4), 29.7(C-5), 61.5(C-6),

167.8(C-7), 60.2(C-9), 25.6(C-10), 37.3(C-11), 12.7(C-12), 15.7(C-13)。以上数据与文献^[6]报道的一致,故鉴定该化合物为环-(*L*-脯-8-羟基-*D*-异亮)二肽。

化合物 2 白色固体; $[\alpha]_D^{25}$ -76.8 (*c* 1.1, MeOH); HR-ESI-MS: m/z 211.144 0 $[M + H]^+$ (计算值 $C_{11}H_{19}N_2O_2$, 211.144 7), 分子式为 $C_{11}H_{18}N_2O_2$ 。 1H NMR (500 MHz, CD_3OD) δ : 4.26 (1H, t, $J = 7.1$ Hz, H-9), 4.13 (1H, dd, $J = 6.7, 3.9$ Hz, H-6), 3.54 ~ 3.48 (2H, m, H-3), 2.35 ~ 2.25 (1H, m, H-5), 2.07 ~ 2.02 (1H, m, H-4), 2.01 ~ 1.97 (1H, m, H-4), 1.96 ~ 1.93 (1H, m, H-10), 1.93 ~ 1.90 (1H, m, H-5), 1.89 ~ 1.84 (1H, m, H-11), 1.57 ~ 1.48 (1H, m, H-10), 0.97 (3H, d, $J = 3.2$ Hz, H-13), 0.95 (3H, d, $J = 3.2$ Hz, H-12); ^{13}C NMR (125 MHz, CD_3OD) δ : 172.8 (C-1), 46.4 (C-3), 23.7 (C-4), 29.1 (C-5), 60.3 (C-6), 168.9 (C-7), 54.6 (C-9), 39.4 (C-10), 25.8 (C-11), 23.3 (C-12), 22.2 (C-13)。以上数据与文献^[6]报道的一致,故鉴定该化合物为环-(*L*-脯-*L*-亮)二肽。

化合物 3 白色固体; $[\alpha]_D^{25}$ -35.8 (*c* 0.9, MeOH); HR-ESI-MS: m/z 245.128 5 $[M + H]^+$ (计算值 $C_{14}H_{17}N_2O_2$, 245.129 0), 分子式为 $C_{14}H_{16}N_2O_2$ 。 1H NMR (500 MHz, CD_3OD) δ : 7.33 ~ 7.22 (5H, m, H-2', 3', 4', 5', 6'), 4.46 (1H, dt, $J = 5.1, 2.8$ Hz, H-9), 4.14 ~ 4.04 (1H, m, H-6), 3.56 (1H, dt, $J = 12.0, 8.3$ Hz, H-3a), 3.39 (1H, dt, $J = 12.4, 6.5$ Hz, H-3b), 3.18 (2H, dd, $J = 5.1, 1.5$ Hz, H-10), 2.19 ~ 2.05 (1H, m, H-5b), 1.92 ~ 1.75 (2H, m, H-4), 1.35 ~ 1.15 (1H, m, H-5a); ^{13}C NMR (125 MHz, CD_3OD) δ : 166.9 (C-1), 46.0 (C-3), 22.8 (C-4), 29.4 (C-5), 60.1 (C-6), 170.9 (C-7), 57.7 (C-9), 38.2 (C-10), 137.3 (C-1'), 131.0 (C-2', 6'), 129.5 (C-3', 5'), 128.1 (C-4')。以上数据与文献^[7]报道的一致,故鉴定该化合物为环-(*L*-脯-*L*-苯丙)二肽。

化合物 4 白色粉末; $[\alpha]_D^{25}$ -4.5 (*c* 1.2, MeOH); HR-ESI-MS: m/z 197.129 1 $[M + H]^+$ (计算值 $C_{10}H_{17}N_2O_2$, 197.129 0), 分子式为 $C_{10}H_{16}N_2O_2$ 。 1H NMR (500 MHz, CD_3OD) δ : 4.26 ~ 4.17 (1H, m, H-6), 4.07 ~ 4.02 (1H, m, H-9), 3.62 ~ 3.55 (1H, m, H-3), 3.56 ~ 3.46 (1H, m, H-3), 2.57 ~ 2.44 (1H, m, H-10), 2.38 ~ 2.27 (1H, m, H-5), 2.08

~ 1.99 (1H, m, H-5), 2.01 ~ 1.88 (2H, m, H-4), 1.10 (3H, d, $J = 7.2$ Hz, H-12), 0.94 (3H, d, $J = 7.0$ Hz, H-11); ^{13}C NMR (125 MHz, CD_3OD) δ : 167.6 (C-1), 46.2 (C-3), 23.3 (C-4), 29.9 (C-5), 60.0 (C-6), 172.6 (C-7), 61.5 (C-9), 29.5 (C-10), 16.6 (C-11), 18.9 (C-12)。以上数据与文献^[8]报道的一致,故鉴定该化合物为环-(*L*-脯-*L*-缬)二肽。

化合物 5 白色粉末; HR-ESI-MS: m/z 268.103 1 $[M + H]^+$ (计算值 $C_{10}H_{14}N_5O_4$, 268.104 6), 分子式为 $C_{10}H_{13}N_5O_4$ 。 1H NMR (500 MHz, $DMSO-d_6$) δ : 8.35 (1H, s, H-8), 8.14 (1H, s, H-2), 7.35 (2H, s, 6-NH₂), 5.88 (1H, d, $J = 6.2$ Hz, H-1'), 4.61 (1H, q, $J = 5.5$ Hz, H-2'), 4.15 (1H, dd, $J = 3.7$ Hz, H-3'), 3.97 (1H, q, $J = 3.4$ Hz, H-4'), 3.60 ~ 3.50 (2H, m, H-5'); ^{13}C NMR (125 MHz, $DMSO-d_6$) δ : 152.4 (C-2), 149.1 (C-4), 119.4 (C-5), 156.9 (C-6), 140.0 (C-8), 88.0 (C-1'), 73.3 (C-2'), 70.5 (C-3'), 86.0 (C-4'), 61.7 (C-5')。以上数据与文献^[9]报道的一致,故鉴定该化合物为腺苷。

化合物 6 白色粉末; HR-ESI-MS: m/z 298.096 0 $[M + H]^+$ (计算值 $C_{11}H_{16}N_5O_3S$, 298.097 4), 分子式为 $C_{11}H_{15}N_5O_3S$ 。 1H NMR (500 MHz, $DMSO-d_6$) δ : 8.36 (1H, s, H-2), 8.16 (1H, s, H-8), 7.29 (2H, s, 6-NH₂), 5.89 (1H, d, $J = 5.7$ Hz, H-1'), 5.50 (1H, d, $J = 5.9$ Hz, 3'-OH), 5.32 (1H, d, $J = 4.9$ Hz, 2'-OH), 4.75 (1H, q, $J = 5.0$ Hz, H-2'), 4.15 (1H, q, $J = 4.1$ Hz, H-3'), 4.05 ~ 4.01 (1H, m, H-4'), 2.88 (1H, dd, $J = 13.9, 5.8$ Hz, H-5'a), 2.78 (1H, dd, $J = 13.9, 5.8$ Hz, H-5'b), 2.05 (3H, s, S-CH₃); ^{13}C NMR (125 MHz, $DMSO-d_6$) δ : 152.7 (C-2), 149.5 (C-4), 119.2 (C-5), 156.1 (C-6), 139.9 (C-8), 87.3 (C-1'), 72.6 (C-2'), 72.5 (C-3'), 83.7 (C-4'), 36.1 (C-5'), 15.6 (S-CH₃)。以上数据与文献^[10]报道的一致,故鉴定该化合物为 5'-硫甲基-5'-硫代腺。

化合物 7 白色粉末; HR-ESI-MS: m/z 282.121 2 $[M + H]^+$ (计算值 $C_{11}H_{16}N_5O_4$, 282.120 2), 分子式为 $C_{11}H_{15}N_5O_4$ 。 1H NMR (500 MHz, CD_3OD) δ : 8.33 (1H, s, H-2), 8.19 (1H, s, H-8), 6.06 (1H, d, $J = 6.1$ Hz, H-1'), 4.50 (1H, dd, $J = 4.9, 2.9$ Hz, H-3'), 4.43 (1H, t, $J = 5.5$ Hz, H-2'), 4.17 (1H, q, $J = 2.8$ Hz, H-4'), 3.89 (1H, dd, $J = 12.6, 2.8$ Hz, H-5'a), 3.76 (1H, dd, $J = 12.6, 2.8$ Hz, H-5'b), 3.42 (3H, s, 2'-OCH₃); ^{13}C NMR (125 MHz, CD_3OD)

δ : 153.6 (C-2), 150.0 (C-4), 120.9 (C-5), 157.6 (C-6), 141.9 (C-8), 89.2 (C-1'), 84.6 (C-2'), 70.8 (C-3'), 88.4 (C-4'), 63.2 (C-5'), 58.8 (2'-OCH₃)。以上数据与文献^[11]报道的一致,故鉴定该化合物为2'-*O*-甲基腺苷。

化合物 8 白色固体; HR-ESI-MS: m/z 136.0614 [M + H]⁺ (计算值 C₅H₆N₅, 136.0623), 分子式为 C₅H₅N₅。¹H NMR (500 MHz, DMSO-*d*₆) δ : 12.82 (1H, s, H-7), 8.07 (2H, d, J = 14.6 Hz, H-2, 8), 7.07 (2H, s, 6-NH₂); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 152.4 (C-2), 150.2 (C-4), 118.5 (C-5), 155.9 (C-6), 138.9 (C-8)。以上数据与文献^[12]报道的一致,故鉴定该化合物为腺嘌呤。

化合物 9 白色固体; HR-ESI-MS: m/z 340.2599 [M + H]⁺ (计算值 C₁₈H₃₄O₃N₃, 340.2600), 分子式为 C₁₈H₃₃O₃N₃。¹H NMR (500 MHz, CD₃OD) δ : 3.20 (6H, t, J = 6.4 Hz, H-7, 14, 21), 2.21 (6H, t, J = 6.9 Hz, H-3, 10, 17), 1.64 (6H, p, J = 7.1 Hz, H-4, 11, 18), 1.57 ~ 1.48 (6H, m, H-6, 13, 20), 1.40 ~ 1.29 (6H, m, H-5, 12, 19); ¹³C NMR (125 MHz, CD₃OD) δ : 175.4 (C-2, 9, 16), 36.7 (C-3, 10, 17), 25.0 (C-4, 11, 18), 28.5 (C-5, 12, 19), 26.4 (C-6, 13, 20), 39.0 (C-7, 14, 21)。根据 NMR 数据,推测为一分子的己内酰胺 (C₆H₁₁NO), 结合其分子式 C₁₈H₃₃O₃N₃, 提示该化合物由 3 分子的氨基己酸首尾酰胺化成环而成。以上数据与文献^[13]报道的一致,故鉴定该化合物为 1,8,15-三氮杂环二十一烷-2,9,16-三酮。

化合物 10 白色固体; HR-ESI-MS: m/z 453.3424 [M + H]⁺ (计算值 C₂₄H₄₅O₄N₄, 453.3441), 分子式为 C₂₄H₄₄O₄N₄。¹H NMR (500 MHz, CD₃OD) δ : 3.19 (8H, t, J = 6.6 Hz, H-7, 14, 21, 28), 2.21 (8H, t, J = 7.0 Hz, H-3, 10, 17, 24), 1.64 (8H, p, J = 7.2 Hz, H-4, 11, 18, 25), 1.52 (8H, p, J = 6.8 Hz, H-6, 13, 20, 27), 1.39 ~ 1.29 (8H, m, H-5, 12, 19, 26); ¹³C NMR (125 MHz, CD₃OD) δ : 174.6 (C-2, 9, 16, 23), 36.0 (C-3, 10, 17, 24), 25.2 (C-4, 11, 18, 25), 28.7 (C-5, 12, 19, 26), 26.0 (C-6, 13, 20, 27), 39.0 (C-7, 14, 21, 28)。对比化合物 10 与化合物 9 的¹H NMR 谱,数据基本一致,结合其分子式 C₂₄H₄₄O₄N₄, 提示该化合物由 4 分子的氨基己酸首尾酰胺化成环而成。以上数据与文献^[13]报道的一

致,故鉴定该化合物为 1,8,15,22-四氮杂环二十八烷-2,9,16,23-四酮。

化合物 11 白色固体; HR-ESI-MS: m/z 588.4099 [M + Na]⁺ (计算值 C₃₀H₅₅O₅N₅Na, 588.4101), 分子式为 C₃₀H₅₅O₅N₅。¹H NMR (500 MHz, CD₃OD) δ : 3.23 (10H, t, J = 6.6 Hz, H-7, 14, 21, 28, 35), 2.24 (10H, t, J = 7.0 Hz, H-3, 10, 17, 24, 31), 1.69 (10H, p, J = 7.2 Hz, H-4, 11, 18, 25, 32), 1.58 (10H, p, J = 6.8 Hz, H-6, 15, 20, 27, 34), 1.45 ~ 1.34 (10H, m, H-5, 12, 19, 26, 33); ¹³C NMR (125 MHz, CD₃OD) δ : 175.7 (C-2, 9, 16, 23, 30), 36.8 (C-3, 10, 17, 24, 31), 26.4 (C-4, 11, 18, 25, 32), 29.8 (C-5, 12, 19, 26, 33), 27.1 (C-6, 13, 20, 27, 34), 39.0 (C-7, 14, 21, 28, 35)。对比化合物 11 与化合物 9 的¹H NMR 谱图,数据基本一致,结合其分子式 C₃₀H₅₅O₅N₅, 提示该化合物由 5 分子的氨基己酸首尾酰胺化成环而成。以上数据与文献^[13]报道的一致,故鉴定该化合物为 1,8,15,22,29-五氮杂环三十五烷-2,9,16,23,30-五酮。

化合物 12 无色油状物; HR-ESI-MS: m/z 359.1674 [M + Na]⁺ (计算值 C₁₅H₂₈O₈Na, 359.1682), 分子式为 C₁₅H₂₈O₈。¹H NMR (500 MHz, CD₃OD) δ : 4.16 (2H, dd, J = 11.3, 4.4 Hz, H-1'), 4.07 (2H, dd, J = 11.3, 4.4 Hz, H-1''), 3.87 ~ 3.80 (2H, m, H-2', 2''), 3.63 ~ 3.50 (4H, m, H-3', 3''), 2.37 (4H, t, J = 7.3 Hz, H-2, 8), 1.67 ~ 1.59 (4H, m, H-3, 7), 1.37 ~ 1.35 (6H, m, H-4, 5, 6); ¹³C NMR (125 MHz, CD₃OD) δ : 175.3 (C-1, 9), 34.7 (C-2), 25.7 (C-3, 7), 29.9 (C-4, 6), 25.9 (C-5), 34.7 (C-8), 66.3 (C-1', 1''), 71.0 (C-2', 2''), 63.9 (C-3', 3'')。以上数据与文献^[14]报道的一致,故鉴定该化合物为壬二酸二甘油酯。

化合物 13 黄色油状物; HR-ESI-MS: m/z 353.2676 [M + Na]⁺ (计算值 C₁₉H₃₈O₄Na, 353.2668), 分子式为 C₁₉H₃₈O₄。¹H NMR (500 MHz, CDCl₃) δ : 4.19 (1H, dd, J = 11.7, 4.7 Hz, H-1a), 4.14 (1H, dd, J = 11.6, 5.9 Hz, H-3a), 3.99 ~ 3.88 (1H, m, H-2), 3.70 (1H, dd, J = 11.7, 4.7 Hz, H-1b), 3.60 (1H, dd, J = 11.6, 5.9 Hz, H-3b), 2.34 (2H, t, J = 5.3 Hz, H-2'), 0.87 (3H, t, J = 7.0 Hz, H-16'); ¹³C NMR (125 MHz, CDCl₃) δ : 65.0 (C-1), 70.2 (C-2), 63.2 (C-3), 174.3 (C-1'), 34.0 (C-2'), 31.8 (C-3'), 29.6 (C-4'), 29.6 (C-5'), 29.5 (C-6'),

29.5 (C-7'), 29.5 (C-8'), 29.3 (C-9'), 29.2 (C-10'), 29.1 (C-11'), 29.0 (C-12'), 29.0 (C-13'), 24.8 (C-14'), 22.6 (C-15'), 14.0 (C-16')。以上数据与文献^[15]报道的一致,故鉴定该化合物为棕榈酸单甘油酯。

化合物 14 黄色油状物; HR-ESI-MS: m/z 355.2817 [M + H]⁺ (计算值 C₂₁H₃₉O₄, 355.2848), 分子式为 C₂₁H₃₈O₄。¹H NMR (500 MHz, CD₃OD) δ : 5.36 (4H, m, H-9', 10', 12', 13'), 4.17 (1H, dd, J = 11.4, 4.4 Hz, H-1b), 4.08 (1H, dd, J = 11.4, 4.4 Hz, H-1a), 3.87 ~ 3.80 (1H, m, H-2), 3.60 ~ 3.53 (2H, m, H-3), 2.80 (2H, t, J = 6.6 Hz, H-11'), 2.37 (2H, t, J = 7.5 Hz, H-2'), 2.15 ~ 2.03 (4H, m, H-8', 14'), 1.63 (2H, t, J = 7.5 Hz, H-3'), 1.43 ~ 1.36 (6H, m, H-4', 7', 15'), 1.36 ~ 1.30 (8H, m, H-5', 6', 16', 17'), 0.93 (3H, t, J = 6.8 Hz, H-18'); ¹³C NMR (125 MHz, CD₃OD) δ : 64.1 (C-1), 71.2 (C-2), 66.5 (C-3), 175.5 (C-1'), 34.9 (C-2'), 30.2 (C-3'), 30.2 (C-4'), 30.3 (C-5'), 30.5 (C-6'), 32.7 (C-7'), 28.2 (C-8'), 130.9 (C-9'), 129.1 (C-10'), 26.5 (C-11'), 129.1 (C-12'), 130.9 (C-13'), 28.2 (C-14'), 30.7 (C-15'), 26.0 (C-16'), 23.7 (C-17'), 14.4 (C-18')。以上数据与文献^[16]报道的一致,故鉴定该化合物为亚油酸甘油酯。

化合物 15 黄色油状物; HR-ESI-MS: m/z 324.2891 [M + H]⁺ (计算值 C₂₀H₃₈O₂N, 324.2903), 分子式为 C₂₀H₃₇O₂N。¹H NMR (500 MHz, CD₃OD) δ : 5.41 ~ 5.29 (4H, m, H-9, 10, 12, 13), 3.59 (2H, t, J = 5.9 Hz, H-1'), 3.30 (2H, t, J = 5.9 Hz, H-2'), 2.79 (2H, t, J = 6.6 Hz, H-11), 2.21 (2H, t, J = 7.6 Hz, H-2), 2.08 (4H, q, J = 6.9 Hz, H-8, 14), 1.62 (2H, q, J = 7.3 Hz, H-3), 1.43 ~ 1.26 (14H, m, H-4 ~ 7, 15 ~ 17), 0.92 (3H, t, J = 6.9 Hz, H-18); ¹³C NMR (125 MHz, CD₃OD) δ : 176.6 (C-1), 37.1 (C-2), 26.5 (C-3), 30.3 (C-4), 30.3 (C-5), 30.4 (C-6), 30.5 (C-7), 28.2 (C-8, 14), 130.9 (C-9), 130.9 (C-10), 27.0 (C-11), 129.1 (C-12), 129.1 (C-13), 30.7 (C-15), 32.7 (C-16), 23.6 (C-17), 14.4 (C-18), 42.9 (C-1'), 61.6 (C-2')。以上数据与文献^[17]报道的一致,故鉴定该化合物为 9,12-十八烷二烯基 *N*-羟乙基。

化合物 16 黄色油状物; HR-ESI-MS: m/z 379.3198 [M + H]⁺ (计算值 C₂₄H₄₃O₃, 379.321

2), 分子式为 C₂₄H₄₂O₃。¹H NMR (500 MHz, CD₃OD) δ : 5.44 ~ 5.27 (6H, m, H-9, 10, 12, 13, 15, 16), 4.16 (2H, dd, J = 11.1, 4.6 Hz, H-1'), 4.07 (2H, dd, J = 11.3, 6.3 Hz, H-2'), 3.56 (2H, dd, J = 5.6, 2.3 Hz, H-3'), 2.82 (2H, t, J = 6.1 Hz, H-14), 2.36 (2H, t, J = 7.5 Hz, H-11), 2.13 ~ 2.06 (2H, m, H-2), 1.62 (4H, m, H-8, 17), 1.36 ~ 1.29 (14H, m, H-3 ~ 7, 4', 5'), 0.99 (3H, t, J = 7.2 Hz, H-18), 0.91 (3H, t, J = 6.8 Hz, H-6'); ¹³C NMR (125 MHz, CD₃OD) δ : 175.5 (C-1), 34.9 (C-2), 23.8 (C-3), 28.2 (C-4), 30.2 (C-5), 30.2 (C-6), 30.3 (C-7), 26.4 (C-8), 131.1 (C-9), 128.2 (C-10), 26.5 (C-11), 129.2 (C-12), 129.2 (C-13), 26.0 (C-14), 128.9 (C-15), 132.7 (C-16), 21.5 (C-17), 14.7 (C-18), 71.1 (C-1'), 66.5 (C-2'), 64.1 (C-3'), 30.7 (C-4'), 19.5 (C-5'), 14.5 (C-6')。以上数据与文献^[18]报道的一致,故鉴定该化合物为亚麻酸 2-丁氧基乙酯。

化合物 17 黄色油状物; $[\alpha]_D^{25}$ -27 (c 0.1, MeOH); HR-ESI-MS: m/z 540.3293 [M + Na]⁺ (计算值 C₂₇H₄₉O₉Na, 540.3274), 分子式为 C₂₇H₄₉O₉。¹H NMR (500 MHz, CD₃OD) δ : 5.35 (4H, m, H-9'', 10'', 12'', 13''), 4.25 (1H, d, J = 7.5 Hz, H-1'), 4.21 ~ 4.13 (2H, m, H-1a, 1b), 4.00 (1H, p, J = 5.0 Hz, H-2), 3.93 (1H, dd, J = 10.5, 5.1 Hz, H-3b), 3.84 (1H, d, J = 3.3 Hz, H-4'), 3.75 (2H, dd, J = 11.3, 6.2 Hz, H-6'a, 6'b), 3.67 (1H, dd, J = 10.5, 5.1 Hz, H-3a), 3.59 ~ 3.52 (1H, m, H-2'), 3.54 ~ 3.51 (1H, m, H-5'), 3.49 (1H, dd, J = 9.7, 3.5 Hz, H-3'), 2.79 (2H, t, J = 6.6 Hz, H-11''), 2.37 (2H, t, J = 7.5 Hz, H-2''), 2.15 ~ 2.03 (4H, m, H-8'', 14''), 1.63 (2H, t, J = 7.3 Hz, H-3''), 1.44 ~ 1.27 (14H, m, H-4'' ~ 7'', H-15'' ~ 17''), 0.92 (3H, t, J = 6.8 Hz, H-18''); ¹³C NMR (125 MHz, CD₃OD) δ : 66.6 (C-1), 69.6 (C-2), 71.9 (C-3), 105.3 (C-1'), 72.6 (C-2'), 74.8 (C-3'), 70.3 (C-4'), 76.8 (C-5'), 62.5 (C-6'), 175.5 (C-1''), 34.9 (C-2''), 26.0 (C-3''), 30.2 (C-4''), 30.2 (C-5''), 30.3 (C-6''), 30.5 (C-7''), 28.2 (C-8'', 14''), 129.1 (C-9''), 129.1 (C-10''), 26.5 (C-11''), 130.9 (C-12''), 131.0 (C-13''), 30.7 (C-15''), 32.7 (C-16''), 23.6 (C-17''), 14.4 (C-18'')。以上数据与文献^[19]报道的一致,故鉴定该化合物为 (2*S*)-1-*O*-(9*Z*,12*Z*-十八烷二烯基)-3-*O*- β -半乳糖基甘油。

化合物 18 白色固体; HR-ESI-MS: m/z 815.676 6 $[M + H]^+$ (计算值 $C_{51}H_{91}O_7$, 815.676 5), 分子式为 $C_{51}H_{90}O_7$ 。 1H NMR (500 MHz, $CDCl_3$) δ : 5.40 ~ 5.31 (1H, m, H-6), 4.44 (1H, dd, $J = 12.3, 2.2$ Hz, H-6'b), 4.38 (1H, d, $J = 7.7$ Hz, H-1'), 4.28 (1H, dd, $J = 12.3, 2.2$ Hz, H-6'a), 3.35 ~ 3.60 (4H, m, H-2' ~ 5'), 2.34 (2H, t, $J = 7.6$ Hz, H-2''), 1.25 (2H, s, nCH₂), 1.00 (3H, s, H-19), 0.92 (3H, d, $J = 6.2$ Hz, H-21), 0.88 (3H, t, $J = 6.8$ Hz, H-16''), 0.84 (3H, d, $J = 1.3$ Hz, H-27), 0.68 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 39.0 (C-1), 29.9 (C-2), 79.8 (C-3), 39.1 (C-4), 140.4 (C-5), 122.3 (C-6), 32.1 (C-7), 34.1 (C-8), 50.3 (C-9), 37.4 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.3 (C-17), 12.0 (C-18), 19.5 (C-19), 36.9 (C-20), 18.9 (C-21), 36.3 (C-22), 26.3 (C-23), 46.0 (C-24), 29.3 (C-25), 19.2 (C-26), 20.0 (C-27), 23.2 (C-28), 12.1 (C-29), 101.4 (C-1'), 73.7 (C-2'), 76.2 (C-3'), 70.3 (C-4'), 74.0 (C-5'), 63.4 (C-6'), 174.8 (C-1''), 34.4 (C-2''), 25.1 (C-3''), 29.4 (C-4''), 29.7 (C-5''), 29.8 (C-6''), 29.9 (C-7'' ~ 12''), 29.5 (C-13''), 32.0 (C-14''), 22.8 (C-15''), 14.3 (C-16'')。以上数据与文献^[20]报道的一致,故鉴定该化合物为胡萝卜苷-6'-*O*-棕榈酸酯。

化合物 19 无色油状物; HR-ESI-MS: m/z 413.266 2 $[M + Na]^+$ (计算值 $C_{24}H_{38}O_4Na$, 413.266 8), 分子式为 $C_{24}H_{38}O_4$ 。 1H NMR (500 MHz, CD_3OD) δ : 7.70 (2H, dd, $J = 5.8, 3.3$ Hz, H-3, 6), 7.61 (2H, dd, $J = 5.8, 3.3$ Hz, H-4, 5), 4.20 (4H, dd, $J = 5.7, 2.1$ Hz, H-1', 1''), 1.71 ~ 1.63 (2H, m, H-2', 2''), 1.45 ~ 1.39 (4H, m, H-a', a''), 1.37 (4H, d, $J = 6.6$ Hz, H-3', 3''), 1.34 ~ 1.30 (8H, m, H-4', 4'', 5', 5''), 0.98 ~ 0.86 (12H, m, H-6', 6'', b', b''); ^{13}C NMR (125 MHz, CD_3OD) δ : 169.3 (2 \times CO), 132.4 (C-1, 2), 129.9 (C-3, 6), 133.6 (C-4, 5), 69.1 (C-1', 1''), 40.2 (C-2', 2''), 31.6 (C-3', 3''), 30.1 (C-4', 4''), 24.0 (C-5', 5''), 14.4 (C-6', 6''), 25.0 (C-a', a''), 11.4 (C-b', b'')。以上数据与文献^[21]报道的一致,故鉴定该化合物为邻苯二甲酸二-(2-乙基)-己酯。

化合物 20 白色粉末; HR-ESI-MS: m/z 361.164 6 $[M + H]^+$ (计算值 $C_{20}H_{25}O_6$, 361.165

1), 分子式为 $C_{20}H_{24}O_6$ 。 1H NMR (500 MHz, CD_3OD) δ : 6.92 (1H, d, $J = 1.9$ Hz, H-2), 6.81 (1H, d, $J = 1.9$ Hz, H-2'), 6.79 ~ 6.76 (2H, m, H-5, H-6), 6.73 (1H, d, $J = 8.0$ Hz, H-5'), 6.66 (1H, dd, $J = 8.0, 1.9$ Hz, H-6'), 4.76 (1H, d, $J = 6.9$ Hz, H-7), 4.00 (1H, dd, $J = 8.4, 6.3$ Hz, Hb-9'), 3.86 (3H, s, 3'-OCH₃), 3.85 (3H, s, 3-OCH₃), 3.85 (1H, overlapped, Hb-9), 3.74 (1H, dd, $J = 8.4, 5.8$ Hz, Ha-9'), 3.65 (1H, dd, $J = 11.0, 6.4$ Hz, Ha-9), 2.95 (1H, dd, $J = 13.5, 4.9$ Hz, Hb-7'), 2.75 (1H, m, H-8'), 2.51 (1H, dd, $J = 13.5, 11.2$ Hz, Ha-7'), 2.39 (1H, m, H-8); ^{13}C NMR (125 MHz, CD_3OD) δ : 135.7 (C-1), 110.6 (C-2), 149.0 (C-3, C-3'), 147.1 (C-4), 116.0 (C-5), 119.8 (C-6), 84.1 (C-7), 54.1 (C-8), 60.5 (C-9), 56.4 (3-OCH₃), 133.5 (C-1'), 113.4 (C-2'), 145.8 (C-4'), 116.2 (C-5'), 122.2 (C-6'), 33.7 (C-7'), 43.9 (C-8'), 73.5 (C-9'), 56.4 (3'-OCH₃)。以上数据与文献^[22]报道的一致,故鉴定该化合物为落叶松脂素。

化合物 21 白色固体; HR-ESI-MS: m/z 567.208 3 $[M + HCOO]^-$ (计算值 $C_{27}H_{35}O_{13}$, 567.207 8), 分子式为 $C_{26}H_{34}O_{11}$ 。 1H NMR (500 MHz, CD_3OD) δ : 6.94 (1H, d, $J = 1.9$ Hz, H-2), 6.81 (1H, d, $J = 1.9$ Hz, H-2'), 6.80 (1H, d, $J = 1.9$ Hz, H-6), 6.76 (1H, d, $J = 8.1$ Hz, H-5), 6.72 (1H, d, $J = 8.0$ Hz, H-5'), 6.67 (1H, dd, $J = 8.0, 1.9$ Hz, H-6'), 4.59 (1H, s, H-7), 4.31 (1H, d, $J = 7.7$ Hz, H-1'), 4.06 (1H, dd, $J = 9.8, 6.3$ Hz, H-9), 4.00 (1H, t, $J = 7.5$ Hz, H-9'a), 3.88 (1H, d, $J = 2.1$ Hz, H-6''a), 3.85 (3H, s, 3-OCH₃), 3.84 (3H, s, 3'-OCH₃), 3.76 (1H, m, H-9'b), 3.71 ~ 3.65 (1H, m, H-6''b), 3.40 ~ 3.23 (2H, m, H-2'', 4''), 2.96 (1H, dd, $J = 10.3, 2.9$ Hz, H-7'a), 2.82 ~ 2.72 (1H, m, H-8'), 2.53 (1H, dd, $J = 10.3, 2.9$ Hz, H-7'b), 2.50 (1H, t, $J = 7.0$ Hz, H-8); ^{13}C NMR (125 MHz, CD_3OD) δ : 135.6 (C-1), 110.8 (C-2), 149.0 (C-3), 145.8 (C-4), 116.2 (C-5), 122.3 (C-6), 84.2 (C-7), 51.7 (C-8), 68.5 (C-9), 56.4 (3-OCH₃), 133.7 (C-1'), 113.5 (C-2'), 149.0 (C-3'), 147.0 (C-4'), 116.0 (C-5'), 119.8 (C-6'), 33.9 (C-7'), 43.9 (C-8'), 73.6 (C-9'), 56.5 (3'-OCH₃), 104.8 (C-1''), 75.2 (C-2''), 78.1 (C-3''), 71.7 (C-4''), 78.0 (C-5''), 62.7 (C-6'')。以上数据与文献^[23]报道的

一致,故鉴定该化合物为落叶松脂醇-9-*O*- β -吡喃葡萄糖苷。

化合物 22 白色粉末; HR-ESI-MS: m/z 543.183 0 [M + Na]⁺ (计算值 C₂₆H₃₂O₁₁Na, 543.183 7), 分子式为 C₂₆H₃₂O₁₁。¹H NMR (500 MHz, CD₃OD) δ : 7.16 (1H, d, J = 8.3 Hz, H-5), 7.04 (1H, d, J = 2.0 Hz, H-2), 6.98 (1H, d, J = 1.7 Hz, H-2'), 6.93 (1H, dd, J = 8.3, 2.0 Hz, H-6), 6.83 ~ 6.80 (1H, m, H-6'), 6.79 (1H, d, J = 8.0 Hz, H-5'), 4.49 (1H, d, J = 6.8 Hz, H-7'), 4.14 (1H, d, J = 9.3 Hz, H-7), 3.88 (3H, s, 3-OCH₃), 3.87 (3H, s, 3'-OCH₃), 2.94 (1H, q, J = 7.1 Hz, H-8); ¹³C NMR (125 MHz, CD₃OD) δ : 137.6 (C-1), 111.6 (C-2), 147.6 (C-3), 151.0 (C-4), 118.0 (C-5), 119.4 (C-6), 83.5 (C-7), 55.8 (C-8), 72.0 (C-9), 56.4 (3-OCH₃), 131.3 (C-1'), 110.6 (C-2'), 148.9 (C-3'), 146.7 (C-4'), 116.0 (C-5'), 119.9 (C-6'), 89.1 (C-7'), 51.3 (C-8'), 70.8 (C-9'), 56.7 (3'-OCH₃), 102.9 (C-1''), 74.9 (C-2''), 77.9 (C-3''), 71.3 (C-4''), 78.2 (C-5''), 62.5 (C-6'')。以上数据与文献^[24]报道的一致,故鉴定该化合物为(+)-表松脂素-4'-*O*- β -吡喃葡萄糖苷。

化合物 23 白色固体; HR-ESI-MS: m/z 545.200 4 [M + Na]⁺ (计算值 C₂₆H₃₄O₁₁Na, 545.199 3), 分子式为 C₂₆H₃₄O₁₁。¹H NMR (500 MHz, CD₃OD) δ : 6.80 (1H, d, J = 1.9 Hz, H-2'), 6.75 (1H, d, J = 8.0 Hz, H-5'), 6.66 (1H, s, H-2), 6.19 (1H, s, H-5), 6.64 (1H, d, J = 1.9 Hz, H-6'), 4.13 (1H, d, J = 7.8 Hz, H-1''), 4.09 (1H, d, J = 10.8 Hz, H-7'), 4.06 (1H, d, J = 2.7 Hz, H-9'b), 3.84 (1H, dd, J = 12.1, 2.5 Hz, H-6''b), 3.82 (3H, s, 3-OCH₃), 3.81 (3H, s, 3'-OCH₃), 3.78 (1H, dd, J = 11.1, 3.7 Hz, H-9b), 3.73 (1H, dd, J = 11.0, 6.0 Hz, H-9a), 3.66 (1H, dd, J = 11.9, 5.7 Hz, H-6''a), 3.36 (1H, d, J = 2.7 Hz, H-3''), 3.29 (1H, d, J = 9.5 Hz, H-4''), 3.26 (1H, d, J = 4.0 Hz, H-9'a), 3.24 ~ 3.19 (2H, m, H-2'', 5''), 2.84 (1H, d, J = 8.0 Hz, H-7b), 2.81 (1H, d, J = 10.2 Hz, H-7a), 2.10 (1H, dd, J = 10.3, 4.7 Hz, H-8), 1.87 (1H, dd, J = 10.5, 5.1 Hz, H-8'); ¹³C NMR (125 MHz, CD₃OD) δ : 129.2 (C-1), 112.4 (C-2), 147.2 (C-3), 145.9 (C-4), 117.4 (C-5), 134.4 (C-6), 33.9 (C-7), 39.5 (C-8), 69.5 (C-9), 56.4 (3-OCH₃),

138.7 (C-1'), 114.3 (C-2'), 148.9 (C-3'), 145.2 (C-4'), 116.1 (C-5'), 123.1 (C-6'), 47.9 (C-7'), 45.9 (C-8'), 65.2 (C-9'), 56.5 (3'-OCH₃), 105.2 (C-1''), 75.2 (C-2''), 78.1 (C-3''), 71.7 (C-4''), 77.9 (C-5''), 62.8 (C-6'')。以上数据与文献^[25]报道的一致,故鉴定该化合物为(+)-异落叶松脂素-9'-*O*- β -吡喃葡萄糖苷。

化合物 24 白色结晶(甲醇); HR-ESI-MS: m/z 545.199 3 [M + Na]⁺ (计算值 C₂₆H₃₄O₁₁Na, 545.199 3), 分子式为 C₂₆H₃₄O₁₁。¹H NMR (500 MHz, DMSO-*d*₆) δ : 8.72 (s, 0H), 8.41 (s, 0H), 6.80 (1H, d, J = 1.9 Hz, H-2), 6.68 (1H, d, J = 8.0 Hz, H-5), 6.60 (1H, s, H-2'), 6.48 (1H, dd, J = 8.0, 1.9 Hz, H-6), 6.07 (1H, s, H-5'), 4.03 (1H, d, J = 10.6 Hz, H-7), 3.94 (1H, d, J = 7.7 Hz, H-1''), 3.90 (1H, dd, J = 10.0, 2.6 Hz, H-9'a), 3.72 (3H, s, 3-OCH₃), 3.70 (3H, s, 3'-OCH₃), 3.67 ~ 3.60 (1H, m, H-9a), 3.61 ~ 3.55 (1H, m, H-9'b), 3.48 ~ 3.39 (2H, m, H-6''), 3.12 (1H, t, J = 8.4 Hz, H-9b), 3.05 ~ 2.94 (4H, m, H-2'' ~ 5''), 2.72 (2H, d, J = 7.9 Hz, H-7'), 1.96 ~ 1.84 (1H, m, H-8'), 1.76 ~ 1.66 (1H, m, H-8); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 136.9 (C-1), 113.9 (C-2), 147.2 (C-3), 144.5 (C-4), 115.5 (C-5), 121.1 (C-6), 45.5 (C-7), 44.2 (C-8), 67.6 (C-9), 55.6 (3-OCH₃), 127.0 (C-1'), 111.8 (C-2'), 145.5 (C-3'), 144.1 (C-4'), 116.3 (C-5'), 132.7 (C-6'), 32.6 (C-7'), 37.5 (C-8'), 62.8 (C-9'), 55.5 (3'-OCH₃), 104.2 (C-1''), 73.6 (C-2''), 76.9 (C-3''), 70.1 (C-4''), 76.7 (C-5''), 61.1 (C-6'')。以上数据与文献^[26]报道的一致,故鉴定该化合物为(+)-异落叶松脂素-9-*O*- β -吡喃葡萄糖苷。

化合物 25 白色固体; HR-ESI-MS: m/z 169.049 4 [M + H]⁺ (计算值 C₈H₉O₄, 169.050 1), 分子式为 C₈H₉O₄。¹H NMR (500 MHz, CD₃OD) δ : 7.51 (1H, s, H-2), 6.78 (1H, d, J = 8.2 Hz, H-5), 7.49 (1H, d, J = 1.9 Hz, H-6), 3.85 (3H, s, 3-OCH₃); ¹³C NMR (125 MHz, CD₃OD) δ : 113.8 (C-2), 152.4 (C-3), 148.6 (C-4), 115.8 (C-5), 125.3 (C-6), 56.4 (3-OCH₃)。以上数据与文献^[27]报道的一致,故鉴定该化合物为香草酸。

化合物 26 白色粉末; HR-ESI-MS: m/z 151.075 2 [M + H]⁺ (计算值 C₉H₁₁O₂, 151.075 9), 分子式为 C₉H₁₁O₂。¹H NMR (500 MHz, CD₃OD)

δ : 7.25 (2H, d, $J = 8.6$ Hz, H-6, H-2), 6.73 (2H, d, $J = 8.6$ Hz, H-5), 6.51 (1H, d, $J = 15.8$ Hz, H-7), 6.17 (1H, dt, $J = 15.8, 6.0$ Hz, H-8), 4.19 (2H, dd, $J = 6.0, 1.5$ Hz, H-9); ^{13}C NMR (125 MHz, CD_3OD) δ : 130.3 (C-1), 128.7 (C-2), 116.3 (C-3), 158.3

(C-4), 116.1 (C-5), 131.9 (C-7), 126.7 (C-8), 64.0 (C-9)。以上数据与文献^[28]报道的一致,故鉴定该化合物为对羟基肉桂醇。

化合物 1~26 的结构见图 1。

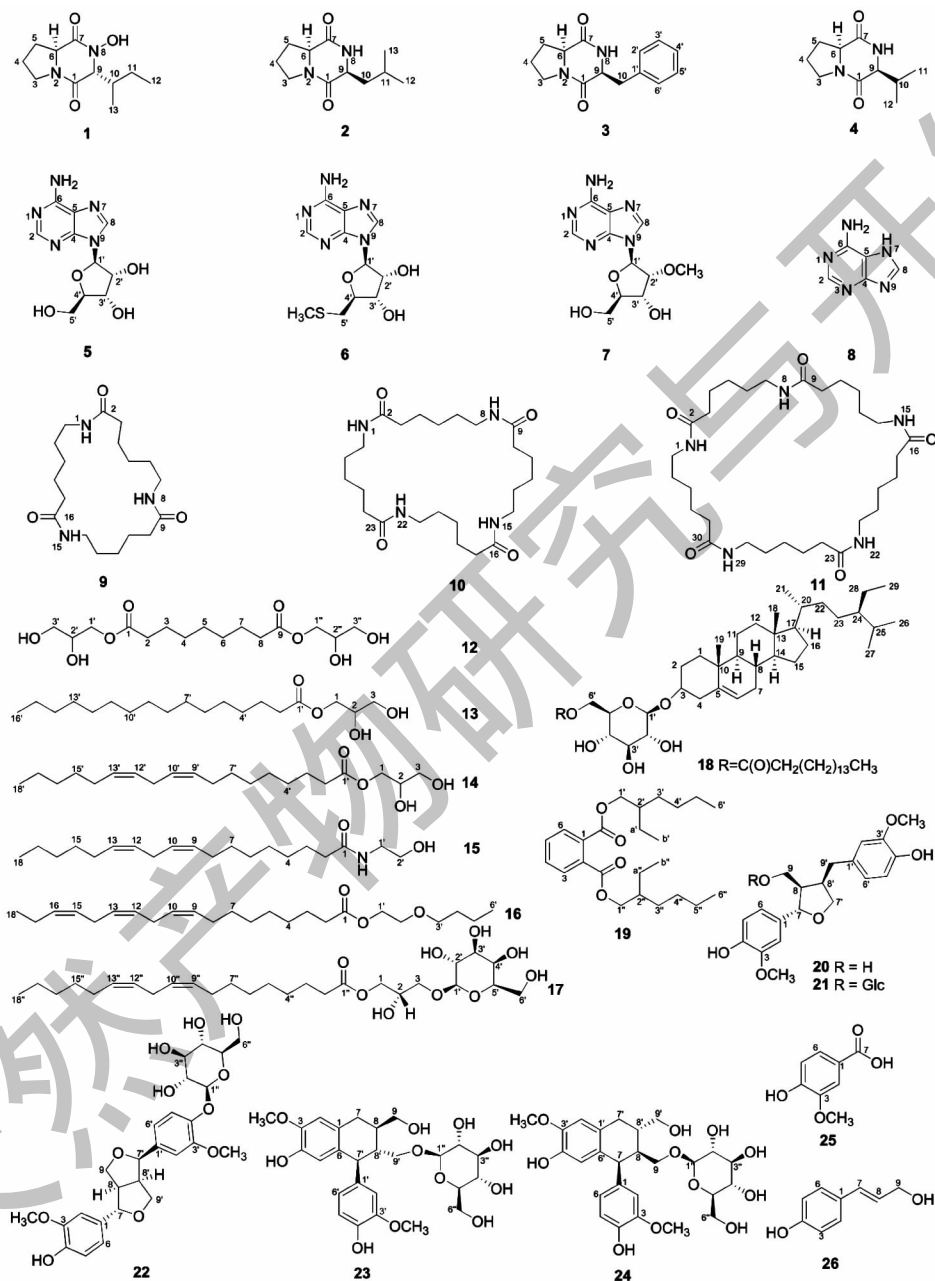


图 1 化合物 1~26 的化学结构

Fig. 1 The chemical structures of compounds 1-26

2.2 抗炎活性结果

2.2.1 RAW 264.7 细胞活力

采用 MTT 实验检测单体化合物对 RAW 264.7 细胞活力的影响。与空白对照组相比,所测单体化

合物在不高于 $50 \mu\text{mol/L}$ 浓度下的细胞活力均在 90% 以上,无明显差异,未出现细胞毒性。因此进一步选择在低于 $50 \mu\text{mol/L}$ 浓度下评价不同单体化合物的抑制 NO 释放活性。

2.2.2 抑制 NO 释放活性

评价不同单体化合物抑制 LPS 诱导 RAW 264.7 细胞释放 NO 作用,结果见表 1。化合物 **2**、**3**、**11**、**16** 和 **21** 具有一定的抑制 NO 释放作用;其中,化合物 **16** 的 NO 抑制作用最为显著,其 IC_{50} 为 $(10.47 \pm 0.89) \mu\text{mol/L}$ 。

表 1 化合物对 LPS 诱导 RAW 264.7 细胞释放 NO 水平影响 ($\bar{x} \pm s, n = 3$)

Table 1 Effect of compounds on NO levels in LPS-induced RAW 264.7 cells ($\bar{x} \pm s, n = 3$)

化合物 Compound	IC_{50} ($\mu\text{mol/L}$)
1	>50
2	46.64 ± 1.92
3	47.50 ± 2.59
4	>50
9	>50
10	>50
11	37.25 ± 0.64
15	>50
16	10.47 ± 0.89
21	47.73 ± 1.80
24	>50
地塞米松 Dexamethasone	7.46 ± 0.92

3 结论

半夏为一味在临床经典名方中广泛应用的中药,在治疗胃肠道疾病治疗方面疗效显著。为阐释其药效物质基础,从生半夏乙酸乙酯萃取部位中共分离鉴定了 26 个化合物,包括哌嗪二酮类(**1**~**4**)、腺苷类(**5**~**8**)、脂肪酸类(**12**~**19**)和木脂素类(**20**~**24**)等,其中 13 个化合物为首次从半夏中分离得到,丰富了半夏的化学成分库。化合物 **2**、**3**、**11**、**16** 和 **21** 具有抑制 LPS 诱导 RAW 264.7 细胞释放 NO 的作用,其中化合物 **16** 抗炎活性较为显著,提示哌嗪二酮类、脂肪酸类、木脂素类成分可能是半夏发挥抗炎作用的活性物质,尤其是大量成分脂肪酸类。以上研究结果为阐明半夏治疗慢性萎缩性胃炎的药效物质基础提供一定的实验依据。

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