倍半萜类化合物通过抑制乙酰胆碱酯酶治疗阿尔茨海默病的潜力

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摘要: 阿尔茨海默病 (Alzheimer's disease, AD) 是一种神经退行性疾病, 其发病机制假说多样, 其中胆碱能假说是其主要的发病机制之一, 乙酰胆碱酯酶 (acetylcholinesterase, AChE) 的天然抑制剂如加兰他敏和卡巴拉汀在临床上用于治疗AD。生物分子如倍半萜具有丰富的结构多样性, 是一类重要的萜类化合物, 并具有广泛的生物活性。过去的研究证明了各种倍半萜通过抑制AChE改善胆碱能传递的潜力。本文综述了近5年来各种倍半萜类化合物对乙酰胆碱酯酶抑制活性的研究进展, 通过分析发现多种倍半萜具有一定的AChE抑制活性, 显示出作为治疗AD潜在药物的良好前景。

关键词: 阿尔茨海默病; 倍半萜; 天然产物; 乙酰胆碱酯酶抑制; 构效关系

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The potential of sesquiterpenoids in the treatment of Alzheimer's disease *via* acetylcholinesterase inhibition

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder with a variety of etiological hypotheses, among which the cholinergic hypothesis is one of the primary theories. Natural inhibitors of acetylcholinesterase (AChE), such as galantamine and rivastigmine, are clinically approved for the treatment of AD. Biologically active molecules like sesquiterpenoids exhibit significant structural diversity and are an important class of terpenoids with a wide range of biological activities. Previous studies have demonstrated the potential of various sesquiterpenoids to improve cholinergic transmission through inhibiting AChE. This review summarizes the research progress on the activity of various sesquiterpenoids against AChE over the past five years. Through analysis, it was found that several sesquiterpenoids possess AChE inhibitory activity to varying degrees, demonstrating their potential as candidate medicines for the treatment of AD.

Key words: Alzheimer's disease; sesquiterpenoid; natural product; anti-acetylcholinesterase; structure-activity relationship

阿尔茨海默病 (Alzheimer's disease, AD) 是一种神经退行性类的痴呆疾病, 以65岁以上人群最为常见, 占痴呆症病例的60%～70%[1]。全世界现有5 500万人患有各种类型的痴呆症, 每年约有1 000万新病例被诊断出来, 预计到2050年痴呆症患者将达到1.52亿, 给世界各地的卫生系统带来了巨大的经济和社会成本[2]。

AD起病隐匿且病情发展不可逆, 轻度的AD患者的临床显著特征表现为记忆力下降、判断力下降; 后期

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AD患者则主要表现为严重的认知障碍, 如无法对环境做出反应、难以控制运动, 最终导致死亡[3,4]。AD的病理特征主要为以大脑皮质和海马区形成的β-淀粉样蛋白 (β-amyloid, Aβ) 为核心的细胞外神经炎症斑块和由Tau蛋白过度磷酸化组成的神经原纤维缠结 (neurofibrillary tangles, NFTs)[5-7]。AD的病理机制复杂, 国内外学者提出了许多关于其发病机制的假说, 包括胆碱能假说、Aβ级联假说、tau蛋白异常磷酸化假说、神经免疫炎症假说和氧化应激假说等[8,9]。

在AD患者脑内常发现神经递质异常现象, 如去甲肾上腺素浓度变化、5-羟色胺浓度下降等, 相比之下, 胆碱能系统障碍的程度最高。在中枢神经系统中, 胆碱能神经元广泛分布, 其传递与许多疾病有关。研究表明, 内源性乙酰胆碱 (acetylcholine, ACh) 是所有胆碱能神经元共用的神经递质, 通过与胆碱能受体结合来调节学习、认知及运动相关信息的传递, 对获取、编码、巩固、再巩固、消退和记忆恢复的调节极其重要, 由于ACh在认知过程中的重要性, 胆碱能假说被认为是AD发病机制中的一个重要理论, 其核心观点为AD患者的认知能力下降是皮层和海马基底前脑的胆碱乙酰转移酶活性降低而导致的ACh合成和释放的降低[10,11]。研究表明, 随着病情发展, 90%的胆碱能细胞受损, 通过上调ACh可显著提高患者的记忆力和认知功能。正常的生理状态下, 当ACh在突触间隙完成信号传递后, 乙酰胆碱酯酶 (acetylcholinesterase, AChE) 会将其快速水解, 因此可以通过抑制剂降低AChE的催化活性, 从而减少ACh的水解, 提高AD患者脑部ACh的含量, 改善胆碱能神经传递, 抵消症状的发展[12,13]。临床应用最广泛的治疗AD药物之一的卡巴拉汀 (rivastigmine), 其延缓胆碱能神经元对释放的ACh的降解速度, 加快胆碱能神经传导, 从而提高了ACh的含量, 减慢淀粉样前体蛋白片段的形成, 从而缓解AD[11]。

AChE作为治疗AD的关键靶点, 主要由运动神经元和肌管共同产生[14,15]。AChE的结合口袋是一个深而窄的凹陷区域, 由位于该区域入口和底部的外周阴离子位点 (PAS) 和催化位点 (CAS) 组成[16]。PAS是ACh的“过渡结合位点”, 其变构调节可影响酶活性中心与不同配基或底物的亲和性, 从而提高AChE的催化效率。此外, Aβ与PAS的结合会诱导Aβ的构象从无规则卷曲或α-螺旋转变为β折叠, 加速Aβ聚集, 表明抑制PAS位点结合有望减少脑内淀粉样斑块, 缓解AD症状[17]。CAS上包含胆碱结合位点、氧阴离子洞和催化三联体等3个主要结合位点。PAS和CAS二者协同工作, 促使ACh进入凹陷区域, 再通过催化ACh的酯键水解反应, 将ACh水解为乙酸和胆碱, 降低ACh的浓度[18]。

天然产物的化学结构多样, 为发现新的药物提供了丰富的资源, 例如从蛇足石杉中提取分离的石杉碱甲, 是一种具有AChE抑制活性的生物碱, 能穿过血脑屏障 (blood-brain barrier, BBB), 改善啮齿目动物、哺乳动物和人的认知障碍[19-21]。以前从天然产物中寻找AChE抑制剂的研究多集中在生物碱、黄酮及香豆素等化合物类型上, 近年来发现萜类尤其一些倍半萜类化合物表现出神经保护方面的活性, 具有防治神经退行性病变的潜力[22]。

萜类化合物又称类异戊二烯化合物, 是自然界种类最丰富, 化学结构最多样的植物次级代谢产物, 具有抗癌、抗过敏、抗病毒、抗溃疡等生物活性。萜类化合物普遍存在于高等植物, 已鉴定结构的化合物超过23 000种, 主要存在于植物的叶片、茎、花、果实和根中[23,24]。其中倍半萜是萜类化合物中最大的一类, 已报道的化合物有7 000多种, 主要以挥发性植物油的形式存在, 它由3个异戊二烯单元组成, 含有15个碳原子[25,26]。大多数倍半萜为手性分子, 包括单环倍半萜 (榄香烯型、吉马烷型)、双环倍半萜 (杜松烷型、桉烷型)、三环倍半萜、倍半萜二聚体 (棉酚)、倍半萜内酯等[27]。由于倍半萜化合物具有复杂的分子结构、固有的生物活性和芳香气味, 常被用作香水、药物和生物能源的前体物质, 在化妆品、食品、药物、能源和化学生产行业有较高的应用价值[28,29]。倍半萜如青蒿素在抗疟疾中发挥重要作用[30]。此外, 一些具有各种药理活性的精油和芳香成分也是由倍半萜组成的[31]。本文主要综述了近5年从自然界中发现的具有AChE抑制活性的倍半萜类化合物。

1 具有AChE抑制活性的倍半萜类化合物的当前进展

1.1 具有AChE抑制活性的倍半萜 倍半萜是一类重要的萜类化合物, 是法尼基焦磷酸 (FPP) 的15-C前体的衍生物, 主要存在于植物的分泌细胞、乳管中, 倍半萜的结构多样性使得其具有抗肿瘤、抗炎、抗病毒、保护神经系统等广泛的生物活性[32,33]。研究表明, 一些倍半萜, 如红没药烷型、桉烷型、原伊鲁烷型、愈创木烷型倍半萜均有明显的AChE抑制活性, 具体化合物如图1所示, 为进一步研发治疗AD药物提供新的方向。

Alimi等[34]研究了两种倍半萜α-红没药醇 (**1**) 和红没药醇氧化物A (**2**) 的AChE抑制活性, 其半数抑制浓度 (IC_{50}) 分别为37.09和28.14 $\mu g \cdot mL^{-1}$, 并发现化合物**1**和**2**在分子对接中表现出良好的结合活性, 通过氢键和疏水相互作用和AChE的活性位点进行结合, 结合能分别为−7.289和−7.139 $kcal \cdot mol^{-1}$。Li等[35]从红树

林来源的胶孢炭疽菌属 (*Colletotrichum* sp.) 真菌的丙酮提取物中分离出一种具有AChE抑制活性的多手性倍半萜红没药酸A (**3**), 具有中等抑制活性, 其IC_{50}为2.2 μmol·L^{-1}。Yusuf等[36]研究了姜黄 (*Curcuma longa*) 中的姜黄酮 (**4**), 其与AChE的对接活性较好, 具有强氢键的相互作用, 结合能为−7.9 kcal·mol^{-1}, 且其ADMET性能良好, 成为治疗AD的候选药物。

Li等[37]在翼齿六棱菊 (*Laggera pterodonta*) 的乙酸乙酯和石油醚提取物中分离出laggeranines A (**5**) 和laggeranines B (**6**) 及6种已知的桉烷型倍半萜**7**～**12**, 化合物**5**～**12**对AChE均有抑制作用, 且呈剂量依赖性, 其中化合物**12**的IC_{50}为437.33 ± 8.33 μmol·L^{-1}, 并具有良好的ADMET性能。Evelyn等[38]评价了具有桉烷骨架的化合物**13**、**14**的生物学活性, 二者均具有显著的AChE抑制活性, IC_{50}分别为0.26 ± 0.016和0.77 ± 0.016 μmol·L^{-1}, 酶动力学研究及分子对接表明, 化合物**13**和**14**均为非竞争性酶抑制剂。Xu等[39]从一种来自烟草 (*Nicotiana tabacum*) 的内生拟青霉属真菌 *Purpureocillium* TE-540的乙酸乙酯提取物中分离出杜松烷型倍半萜, 其中化合物**15**、**16**抑制AChE的IC_{50}值分别为43.02 ± 6.01和35.97 ± 2.12 μmol·L^{-1}, 通过对接分析预测, 化合物**15**与Trp84、Gly117、Ser122和Tyr121残基通过疏水相互作用结合在AChE的催化口袋中, 而化合物**16**与Asp72和Ser122残基结合。Li等[40]从沉香 (*Aquilaria sinensis*) 的乙醚提取物中分离出化合物**17**, 其在50 μg·mL^{-1}浓度下表现出对AChE的抑制活性, 抑制率为48.33% ± 0.17%。硝基是一种独特的官能团, 其可以产生与生物靶标相互作用的电子密度缺陷位点, 故可在倍半萜类化合物中引入硝基[41,42]。Jiménez-Estrada等[43]在印第安菊 (*Psacalium sinuatum*) 根提取物分离得到cacalol acetate, 其在10 μg·mL^{-1}浓度时对AChE抑制率为39.3%, 使用硝酸铈 (IV) 铵和乙酸酐将cacalol acetate硝化, 获得2-nitrocacalol acetate (**18**), 其对AChE的抑制活性显著提高, 在10 μg·mL^{-1}浓度时的抑制率为69.9%, 且该化合物与AChE具有良好的结合能 (−8.68 kcal·mol^{-1})。此研究证明通过引入硝基对倍半萜提供新的修饰, 提高了化合物抑制AChE的能力, 提供了对倍半萜的研究方向。

Wu等[44,45]通过蜜环菌 (*Armillaria mellea*) 与内生真菌附球菌 (*Epicoccum nigrum* YUD17002) 共培养, 从其发酵提取物中分离出两种原伊鲁烷型倍半萜**19**、**20**, 二者均显示对AChE的抑制活性, IC_{50}分别为23.85和9.03 μmol·L^{-1}。Dong等[46]将藏东瑞香 (*Daphne bholua*) 全草以80%乙醇提取, 从中分离得到愈创木烷型倍半萜类化合物**21**、**22**, 二者对AChE具有显著的抑制活性, IC_{50}分别为0.332 ± 0.034和0.184 ± 0.015 μmol·L^{-1}。

Al-Nagar等[47]研究了姜科植物生姜 (*Zingiber officinale*) 金合欢类倍半萜 (*Z*,*E*)-橙花叔醇 (**23**) 的药理活性, 发现其对AChE产生抑制作用, IC_{50}为0.02 mmol·L^{-1}。Formagio等[48]对茜草科植物 *Palicourea tomentosa* 的叶子加氢蒸馏提取出精油, 精油的主要成分吉马烷型倍半萜烯香叶醇D (29.38%) (**24**) 和橄榄烷型倍半萜双环大牻牛儿烯 (25.21%) (**25**), 二者对大鼠海马、大脑皮层、下丘脑AChE均产生抑制活性, 且酶−配体分子模型显示其具有作为AChE抑制剂的潜力。Aggarwal等[49]对紫茎泽兰 (*Ageratina adenophora*) 的花进行加氢蒸馏提取并分离出一种新的杜松烷型倍半萜**26**, 其对AChE显示出抑制作用, IC_{50}为49.020 ± 0.005 μg·mL^{-1}。

Bendjedou等[50]从茄科植物欧洲枸杞 (*Lycium europaeum*) 的根和叶子的乙醇提取物中分离出两种螺环倍半萜**27**、**28**及两种倍半萜醇**29**、**30**, 均显示AChE抑制活性。Liu等[51]从小花八角 (*Illicium micranthum*) 中分离出一种倍半萜醇illifunone B (**31**), 在50 μmol·L^{-1}浓度下对AChE的抑制率为46.0%。Kovács等[52]从菊科植物秘鲁豚草 (*Ambrosia peruviana*) 和银香菊 (*Santolina chamaecyparissus*) 的二氯甲烷提取物中分离出4个倍半萜类化合物, 分别为吉马烷型倍半萜germacrene B (**32**)、愈创木烷型倍半萜dehydroaromadendrene (**33**) 及倍半萜醇 *α*-bisabolol (**34**) 和manool (**35**), 4个化合物及两种二氯甲烷提取物均显示出AChE抑制活性, 其中从银香菊分离得到的化合物**34**对AChE抑制活性的IC_{50}为6.04 ± 0.23 μmol·L^{-1}。

应用海洋天然产物数据库的化学基因组学技术分析表明红藻 (*Porphyra yezoensis*) 中存在的卤代倍半萜是潜在的AChE抑制剂, 因此, Gonçalves等[53]使用分子对接和酶抑制实验研究了里约热内卢海岸红藻的代谢物, 结果表明 (−)-elatol (**36**)、(−)-dendroidiol (**37**) 和 (−)-cartilagineol (**38**) 3种卤代倍半萜在300 μmol·L^{-1}的浓度下均对AChE产生抑制活性, 抑制率分别为78.5%、72.9%和61.3%, 同时化合物**36**的分子对接结果也显示了卤素Br和Cl与Try86和Try286之间的相互作用有助于其AChE抑制活性的产生。

综上所述, 一些愈创木烷型倍半萜与桉烷型倍半萜对AChE的抑制活性最为显著, 如愈创木烷型倍半萜化合物**21**及**22**对AChE的抑制活性的IC_{50}值分别为0.332 ± 0.034和0.184 ± 0.015 μmol·L^{-1}, 两种桉烷型倍半萜化合物**13**及**14**对AChE的抑制活性的IC_{50}值分别为0.26 ± 0.016和0.77 ± 0.016 μmol·L^{-1}。进一步分析发现, 这4种化合物均含有 *α*,*β*-不饱和羰基结构单元, 这可能是其表现出显著抑制活性的关键因素之一。此

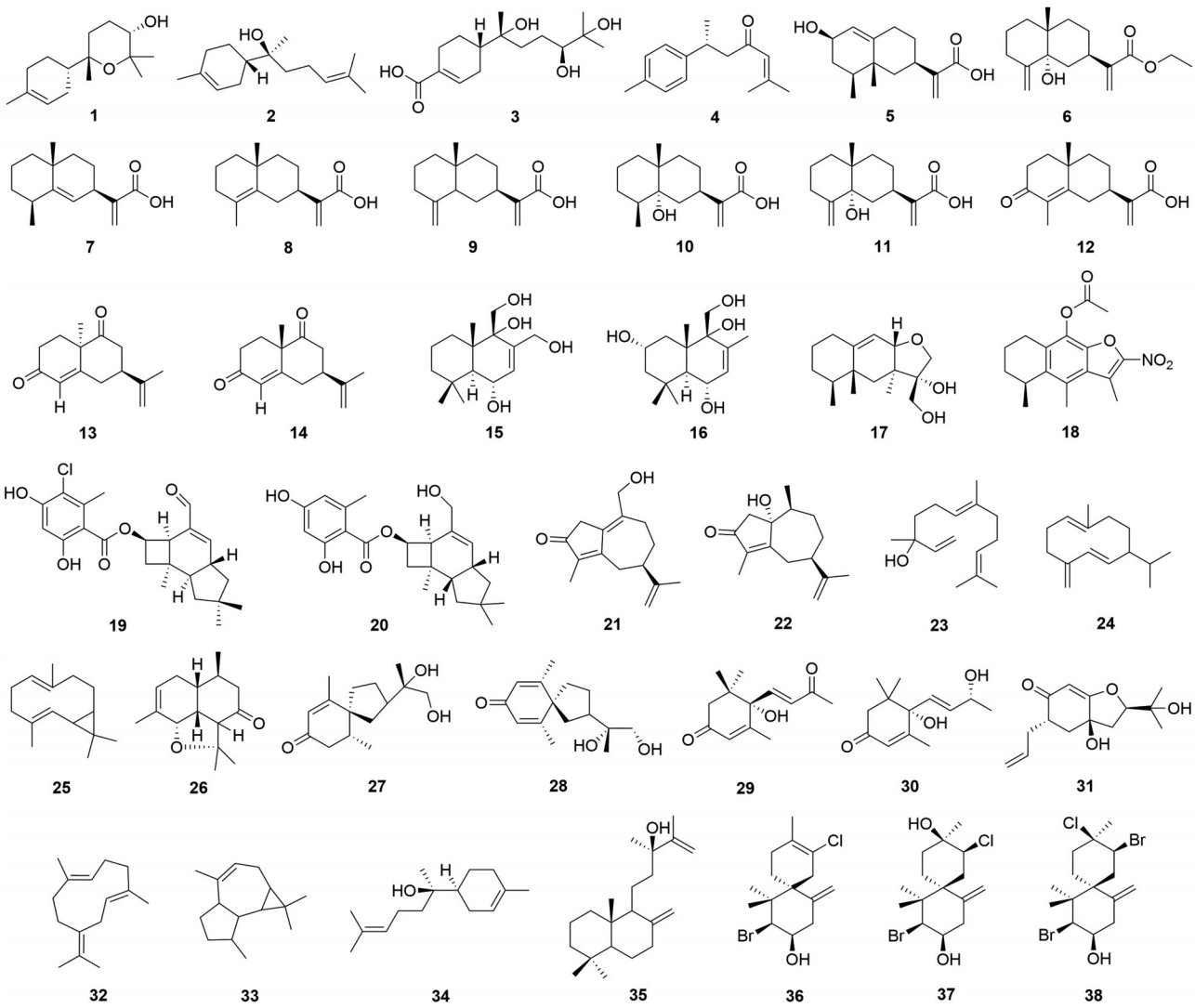


Figure 1 Structures of compounds **1**–**38**

外, 在分子对接中, 结合能 ≤ −5.0 kJ·mol^{-1}时, 认为两者有一定的结合活性; 结合能 ≤ −7.0 kJ·mol^{-1}时, 认为两者有较好的结合活性。化合物**4**与AChE的对接结合能为−7.9 kcal·mol^{-1}; 化合物**18**与AChE的结合能为−8.68 kcal·mol^{-1}, 且其在10 μg·mL^{-1}浓度时对AChE抑制率为69.9%, 显示出二者均具有较好AChE结合活性。这些研究均为AD的治疗提供了有效的先导化合物。

1.2 具有AChE抑制活性的含倍半萜的精油 精油(essential oil, EO)是一种由芳香植物生物合成的、天然的、具有挥发性的化合物所构成的混合物, 其大多数呈油滴状存在[54-56]。它是植物体内次生代谢产物, 主要由脂肪族、芳香族、萜类(主要为单萜和倍半萜)、含氮或含硫化合物等易挥发的成分组成, 是天然产物资源多样化和独特性的代表。在植物中含精油较为丰富的科有: 松科、柏科、木兰科、樟科、芸香科、伞形科、唇形科、姜科、菊科、桃金娘科、龙脑香科等。精油的提取主要是利用其挥发性及能溶于有机溶剂的性质, 既有传统技术也有衍生出来的新技术[57,58], 如水蒸汽蒸馏法、溶剂萃取、冷榨法、超临界流体萃取、膜辅助溶剂萃取及固相微萃取等。

含萜类的精油生物活性多样, 研究表明一些芳香植物的精油具有明显的AChE抑制活性, 如罗勒精油、胡椒精油等, 并发现与含有单萜类的精油相比, 含有倍半萜类的精油对AChE的抑制活性更为有效[59-61]。由于这些精油成分不仅具有AChE抑制活性, 而且易于吸入, 故为疾病的治疗提供了新方法[62]。表1[60, 63-76]总结了植物中主要成分为倍半萜和含氧倍半萜的精油及其在抑制AChE中的重要作用。

由表1可知, 从毛番樱(*Myrciaria floribunda*)的果实中提取精油的AChE抑制活性最好, 其IC_{50}为0.08 μg·mL^{-1}。这些精油为缓解AD症状带来了新的希望。

Table 1 Essential oils containing sesquiterpenoids with acetylcholinesterase (AChE) inhibitory activity. BuChE: Butyrocholinesterase

Name of the plant (Species)	Part used	Solvent/Method used for extraction	Extract/Volatile oil/ Phytoconstituents	Acetylcholinesterase inhibitory activities	Ref.
Valeriana officinalis/ *V. jatamansi*/ *Nardostachys jatamansi* (Caprifoliaceae)	Roots and rhizomes	Steam distillation	Contains essential oils of monoterpenes and sesquiterpenes	Possessing the potential to inhibit AChE, Vo IC_{50} = 127.30 μg·mL^{-1}; Vj IC_{50} = 246.84 μg·mL^{-1}; Nj IC_{50} = 67.15 μg·mL^{-1}	[63]
Syzygiella rubricaulis (Adelanthaceae)	Aerial parts	Steam distillation	Contains hydrocarbon sesquiterpenes (48.35%) and essential oil containing oxygen-sesquiterpenes (46.89%)	Potential inhibitory activity against AChE, IC_{50} = 26.75 ± 1.03 μg·mL^{-1}	[64]
Hedychium spicatum (Zingiberaceae)	Rhizomes	Chloroform extraction	Essential oils containing oxygen-containing sesquiterpenes (43.1%)	Molecular docking studies were performed using AChE, which is considered a potential target for organophosphorus and carbamate insecticides	[65]
Myrcianthes discolor (Myrtaceae)	Leaf	Steam distillation	Sesquiterpenes make up more than 75% of the composition. The main compounds detected were: (*E*)-caryophyllene (29.40% ± 0.21%); bicyclogermacrene (7.45% ± 0.16%); *β*-elemene (6.93% ± 0.499%); *α*-cubebene (6.06% ± 0.053%); *α*-humulene (3.96% ± 0.023%); *δ*-cadinene (3.02% ± 0.002%)	Moderate inhibitory effect on AChE with IC_{50} = 6.68 ± 1.07 μg·mL^{-1}	[66]
Salvia leucantha (Lamiaceae)	Aerial parts	Steam distillation	Essential oils containing 6 major sesquiterpenes: 6,9-guaiadiene (19.14%); (*E*)-caryophyllene (16.80%); germacrene D (10.22%); (*E*)-*β*-farnesene (10.00%); bicyclogermacrene (7.52%); monoterpenoid bornyl acetate (14.74%)	Possessing the potential to inhibit BuChE with IC_{50} = 32.60 μg·mL^{-1} and weak inhibitory activity against AChE with IC_{50} > 250 μg·mL^{-1}	[67]
Elaeosilenum thapsioides (Apiaceae)	Aerial parts	Hydrodistillation	Monoterpene hydrocarbons (72.78%−99.13%) were the main essential oils, and the content of sesquiterpene hydrocarbons collected in different regions and different parts was 0.69%−13.44%	The IC_{50} of essential oils of leaves, stems, flowers and seeds of plants in Mahouane area were 67.66, 52.71, 154.52 and 158.35 μg·mL^{-1}. The IC_{50} levels of essential oils of leaves, stems, flowers and seeds in Megres were 93.86, 67.15, 62.65 and 78.15 μg·mL^{-1}, respectively	[60]
Artemisia nakaii (Asteraceae)	Aerial parts	Hydrodistillation	Contains monoterpenes (49.01%) and sesquiterpenes (50.76%), the highest concentrations of terpenes were feropodin (200.46 ± 1.42 μg·mL^{-1}), (+)-camphor (154.93 ± 9.72 μg·mL^{-1}), *β*-selinene (57.73 ± 2.48 μg·mL^{-1}) and 1,8-cineole (17.99 ± 1.06 μg·mL^{-1})	Potential inhibitory activity against AChE, the IC_{50} of EOs is 37.75 ± 3.59 μg mL^{-1}; the IC_{50} of *β*-selinene is 6.88 ± 0.48 μg·mL^{-1}	[68]
Eugenia valvata (Myrtaceae)	Leaf	Steam distillation	Contains hydrocarbon sesquiterpenes (37.43%), essential oil containing oxygen-sesquiterpenes (31.08%)	Possessing the potential to inhibit AChE, IC_{50} = 53.08 ± 1.13 μg·mL^{-1}	[69]
(1) *Chamaecyparis obtusa* (Cupressaceae) (2) *Cymbopogon nardus* (Poaceae) (3) *Lavandula coronopifolia* (Lamiaceae)	(1) Fruits (2) Roots (3) Aerial parts	Hydrodistillation	The content of oxidized sesquiterpenes was the highest in (2), and cedr-8-en-15-ol (28.69%) was the main content. Monoterpenes, sesquiterpenes and monoterpene oxide lavandulol (26.56%) were the most abundant in (3)	(1) exhibited AChE inhibitory activity and BuChE inhibitory activity; (2) exhibited BuChE inhibitory activity	[70]

Continued

Name of the plant (Species)	Part used	Solvent/Method used for extraction	Extract/Volatile oil/ Phytoconstituents	Acetylcholinesterase inhibitory activities	Ref.
Clinopodium taxifolium (Govaerts)	Aerial parts	Hydrodistillation	Contains sesquiterpenes and sesquiterpenoids (>70%), mainly (*E*)-*β*-caryophyllene (17.8%), *α*-copaene (10.5%), *β*-bourbonene (9.9%), *δ*-cadinene (6.6%), *cis*-cadina-1 (6%),4-diene (6.4%) and germacrene D (4.9%)	Possessing the potential to inhibit AChE, IC_{50} = 31.3 ± 3.0 μg·mL^{-1}	[71]
Jungia rugosa (Asteraceae)	Leaf	Steam distillation	Contains *γ*-curcumene (47.1% and 49.7%); *β*-sesquiphellandrene (17.0% and 17.9%), and two unknown oxygenated sesquiterpenes (6.7%−7.2% and 4.7%−3.3%)	Weak inhibitory activity against AChE, the inhibition rate was 25.9% ± 13.9% (38 μg·mL^{-1})	[72]
Anisophyllea disticha (Anisophylleaceae)	Leaf	Hydrodistillation	Contains sesquiterpene hydrocarbons (67.5%) and oxygen-fold, the main components of semiterpene (30.8%) essential oil are germacrene D (25.3%), *δ*-cadinene (15.2%), caryophyllene oxide (7.0%), and spathulenol (6.4%)	Possessing the potential to inhibit AChE, the inhibition rate was 62.5% ± 0.3% (1.00 μg·mL^{-1})	[73]
Mitrephora poilanei (Annonaceae)	Leaf	Hydrodistillation	Contains sesquiterpene hydrocarbons (63.1%) and oxidized sesquiterpenes (22.9%)	Possessing the potential to inhibit AChE, IC_{50} = 31.16 ± 3.06 μg·mL^{-1}	[74]
Tithonia diversifolia (Asteraceae)		Methylene chloride extraction	Crude extract containing sesquiterpenes	IC_{50} = 73.9 ± 11.06 μg·mL^{-1}	[75]
Myrciaria floribunda (Myrtaceae)	Fruit	Hydrodistillation	The main ingredients are: *δ*-cadinene (26.8%), *γ*-cadinene (15.69%), *γ*-muurolene (6.21%), essential oils of *α*-muurolene (6.11%) and (*E*)-caryophyllene (5.54%)	High inhibitory effect on AChE with IC_{50} = 0.08 μg·mL^{-1}	[76]

1.3 具有AChE抑制活性的倍半萜内酯 倍半萜内酯是一大类次生代谢产物, 其化学结构以15个碳原子的骨架为基础, 具有*γ*内酯环结构片段, 通常由细胞色素P450单加氧酶 (cytochrome P450 monooxygenases, CYP450) 和其他氧化酶修饰倍半萜烯骨架派生而来, 具有丰富多样的结构类型[32,77]。根据结构骨架、取代基的类型和位置, 倍半萜内酯类化合物被分为吉马烷型、桉烷型、苍耳烷型、愈创木烷型倍半萜内酯等[56,78]。国内外很多研究团队推测吉马烷型倍半萜内酯是以上各种倍半萜内酯类型的生物起源中间物质, 其他类型倍半萜内酯可能是由吉马烷型碳骨架在形成内酯环前或者成环后经过各种各样的修饰而成, 例如环化、裂环和甲基迁移等[55,79]。倍半萜内酯类化合物具有抗肿瘤、抗炎、抗菌等多种生物活性, *γ*内酯环结构被认为是倍半萜内酯化合物的功能基团, 与生物活性密切相关, 其他基团如过氧桥、羟基、氯乙醇、不饱和酮基或氧酰基与其功能基团的偶联进一步增强倍半萜内酯化合物的生物亲核能力从而影响生物活性[56,80]。研究表明, 一些倍半萜内酯, 如具有桉烷型、吉马烷型、苍耳烷型等骨架的倍半萜内酯具有显著的AChE抑制活性, 具体化合物如图2所示, 成为治疗AD的候选物。

Zhang等[81]基于MS/MS的分子网络指导策略, 从菊科植物天名精 (*Carpesium abrotanoides*) 的全株75%乙醇提取物中分离出一种桉烷型倍半萜内酯**39**, 与多奈哌齐 (donepezil) 相比, 其表现出中等的AChE抑制作用, 在10 μmol·L^{-1}的浓度下抑制率为77.86%。分子对接结果表明, 化合物**39**与Glu199和Hid440残基形成两个氢键, 同时也被Gly117、Gly118、Tyr121、Ser122等疏水性氨基酸包围, 通过分子动力学模拟可知, 在整个模拟过程中, 图形的波动保持在RMSD的标准范围0.20～2.80 Å内, 表明了对接中的稳定性。Ma等[82]从大叶土木香花 (*Inula grandis*) 的90%乙醇提取物中分离出一种桉烷型倍半萜内酯5*α*-epoxyalantolactone (5*α*-EAL) (**40**), 研究其对认知障碍的改善作用, 5*α*-EAL抑制脂多糖 (lipopplysaccharides, LPS) 刺激的小鼠小胶质细胞 (BV2) 中NO的产生, 半数效应浓度 (EC_{50}) 为6.2 μmol·L^{-1}, 并通过平行人工膜渗透测定了5*α*-EAL穿透BBB的能力, 采用东莨菪碱 (scopolamine, SCOP)

诱导的AD小鼠模型评估5α-EAL在体内对认知损伤的改善情况，结果表明，在用5α-EAL（每天10和30 mg·kg^{-1}，腹腔注射）预处理小鼠21天后可以显著减轻记忆损伤并显著抑制SCOP诱导的AD小鼠脑内AChE活性，这些发现突出了天然产物5α-EAL减轻AD小鼠认知缺陷的作用。

筛选能够与AChE的PAS和CAS相互作用的先导化合物是寻找治疗AD创新药物的重要趋势。为筛选潜在的AChE双位点抑制剂，Zhu等[83]评估了菊科植物白术（*Atractylodes macrocephala*）中分离的倍半萜内酯化合物的活性，双倍半萜内酯biatractylenolide II（**41**）和桉烷型倍半萜内酯atractylenolide III（**42**）具有AChE抑制活性，IC_{50}分别为19.61 ± 1.11和61.26 ± 3.01 μg·mL^{-1}。通过分子对接研究了化合物**41**与AChE的相互作用，其结合能为−8.2 kcal·mol^{-1}，单体之一位于PAS位点，被残基Leu76、Phe297和Trp286包围，形成强疏水结合，另一单体位于由Trp86、Phe295和Phe338组成的CAS位点，形成稳定的疏水结合，并在**41**与残基Tyr72、Tyr124和Ser125之间观察到一个氢键，键长为2.6 Å。表明化合物**41**可与AChE的双重位点CAS和PAS相互作用。log*P*值是亲脂性的量度，亲脂性是预测治疗AD药物通过BBB能力的重要理化性质，通常具有最佳中枢神经系统穿透的log*P*值约为2 ± 0.7，使用ChemDraw Ultra2010计算化合物**41**的log*P*值为3.47，表明其亲脂性较强，可透过BBB，可作为治疗AD的先导化合物进一步研究。

Herrera-Acevedo等[84]从包含8 593个次级代谢物的SistematX数据集中选择潜在的AChE抑制剂，并使用3种类型的分子描述符、虚拟筛选等实验方法预测了两种吉马烷型倍半萜内酯**43**、**44**能够穿过BBB，并在VolSurf+定量模型中得到验证，揭示了这两种倍半萜内酯是8 593个分子中最有希望抑制AChE的次级代谢物。Lan等[85]从肿柄菊（*Tithonia diversifolia*）和小白菊（*Leucanthemella linearis*）的乙醇提取物中分别分离到两种吉马烷型倍半萜内酯tirotundin（**45**）和parthenolide（**46**），二者显示出对AChE的中等抑制活性，IC_{50}分别为6.89 ± 0.30和5.51 ± 0.23 μg·mL^{-1}，且都是AChE的可逆抑制剂，证明了两者可能是具有双结合位点的AChE抑制剂。

Jaśkiewicz等[86]研究了通过逆流分配色谱技术纯化从菊苣（*Cichorium intybus*）根的甲醇−水提取物中获得富含倍半萜内酯的提取物，使用两种模型等温滴定量热法（isothermal titration calorimetry, ITC）和对接模拟分析对AChE的抑制情况，酶和配体络合的ITC分析结果显示菊苣提取物及其中主要成分之一的愈创木烷型倍半萜内酯lactucopicrin（**47**）与AChE的强相互作用。在酶活性抑制测试观察到化合物**47**具有强抑制神经递质水解的能力，同时表现出其结合AChE活性位点的互补模式，该研究显示使用菊苣治疗AD的巨大潜力。Aydin等[87]从粘毛旋覆花（*Inula britannica*）地上部分的二氯甲烷提取物中分离得到的苍耳烷型倍半萜内酯tomentosin（**48**）对AChE和BuChE均显示中等抑制活性，IC_{50}分别为6.75 ± 0.208和6.67 ± 0.307 μmol·L^{-1}。Nguyen等[88]从大千年健（*Homalomena pendula*）根茎中分离得到teucmosin（**49**），其具有中等AChE抑制作用，IC_{50}为4.53 ± 0.39 μmol·L^{-1}，分子对接和分子动力学模拟表明该化合物可以作为AChE双结合位点（CAS和PAS）抑制剂，成为具有治疗AD潜力的候选化合物。

综上所述，在具有AChE抑制活性的倍半萜内酯中，苍耳烷型倍半萜内酯**48**与单环倍半萜内酯**49**的效果最好，二者的IC_{50}分别为6.75 ± 0.208和4.53 ± 0.39 μmol·L^{-1}；桉烷型倍半萜内酯**39**在10 μmol·L^{-1}的浓度下对AChE的抑制率为77.86%。此外，分子对接表明，双倍半萜内酯**41**可与AChE的双重位点CAS和PAS相互作用，具有较好的结合活性，其结合能为−8.2 kcal·mol^{-1}，可对其进行进一步的体内评估实验。

1.4 具有AChE抑制活性的杂倍半萜 不同类别的杂倍半萜如倍半萜香豆素、具有琼脂呋喃骨架的倍半萜类化合物，如图3所示，其AChE抑制作用如下：

Çiçek Kaya等[89]从伞形科三叶罗伞（*Heptaptera triquetra*）果实的二氯甲烷提取物中分离出4种倍半萜香豆素umbelliprenin（**50**）、colladin（**51**）、colladonin（**52**）和samarcandin（**53**），都表现出AChE抑制活性，其中化合物**52**活性显著，IC_{50}为8.25 nmol·L^{-1}。结果表明，从该植物中分离出的化合物成为治疗AD新的候选物。

二氢-β-琼脂呋喃倍半萜类化合物显示高度氧化，可形成聚酯和吡啶倍半萜生物碱，这些生物合成衍生物具有广谱的生物活性，如免疫抑制剂、抗昆虫剂、抗HIV等[31]。González-Chavarría等[90]发现二列美登木（*Maytenus disticha*）总提取物具有显著AChE抑制活性，其主要活性成分为二氢-β-琼脂呋喃倍半萜；Alarcón-Enos等[91]从二列美登木和麦哲伦美登木（*Maytenus magellanica*）的种子中分离出6种二氢-β-琼脂呋喃倍半萜，并通过Robinson环化反应合成了5种具有相同骨架的衍生物，天然产物**54**和其4个衍生物**55**～**58**均显示出对AChE的抑制活性，IC_{50}范围为17.0～43.0 μmol·L^{-1}。化合物**57**对AChE具有选择性且抑制活性最佳，IC_{50}为17.0 ± 0.016 μmol·L^{-1}。分子

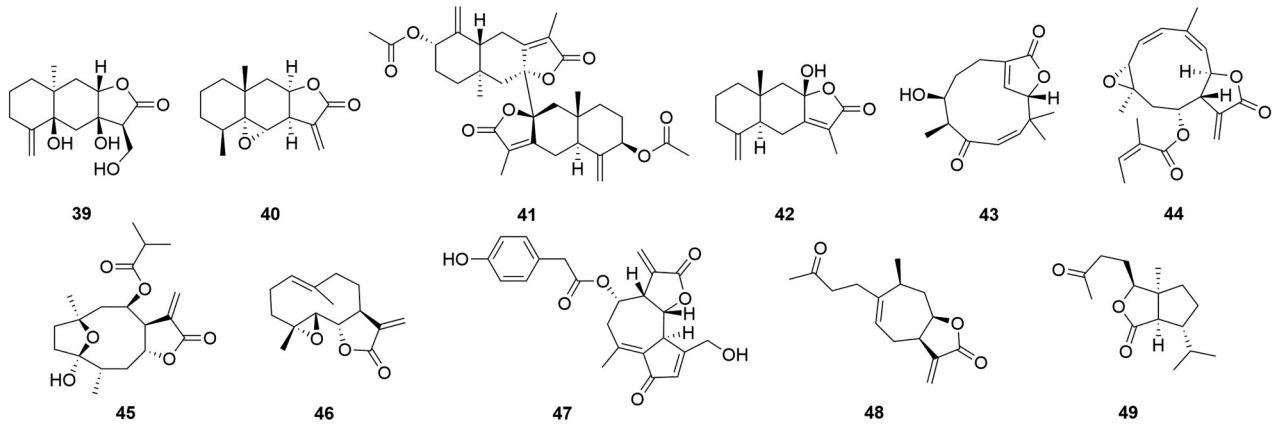


Figure 2 Structures of compounds **39**–**49**

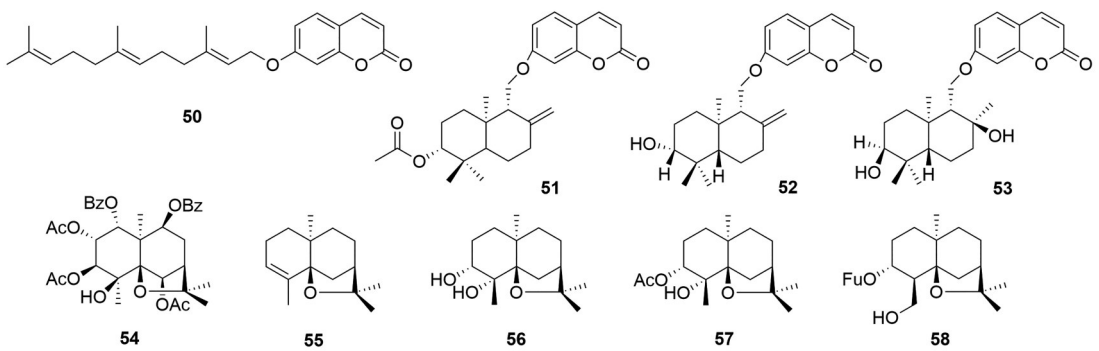


Figure 3 Structures of compounds **50**–**58**

对接和吡啶置换测定表明, 化合物**56**不结合AChE的CAS, 而是结合酶的PAS, 这些结果表明其具有阻断AChE诱导的β淀粉样蛋白聚集的潜力。基于药物相似性研究、ADMET研究, 也确定其具有良好的药代动力学性质, 这些小分子AChE抑制剂为治疗神经系统疾病如AD提供了新的可能。

如上所述, 在杂倍半萜中, 倍半萜香豆素类化合物**52**含有α,β-不饱和羰基, 为有效的AChE抑制剂, IC_{50}达到8.25 nmol·L^{-1}, 可以作为治疗AD的候选物。

2 总结与展望

目前, 治疗AD的药物非常有限, 以AChE为靶标开发的抑制剂是目前被批准用于治疗AD认知缺陷的主要临床药物[92]。应用最广泛的为第二代药物多奈哌齐、卡巴拉汀和加兰他敏 (galantamine)。加兰他敏是一种可逆的竞争性AChE抑制剂和ACh受体变构调节剂[93]; 卡巴拉汀具有双重抑制作用, 能同时抑制大脑中AChE和BuChE的活性[94]; 多奈哌齐通过抑制AChE水解ACh来增强大脑中胆碱能神经传递, 从而改善AD患者的认知功能[95]。临床研究表明, 当前应用的AD药物虽然能延缓疾病进程, 然而大多数情况下, 患者由于其不良反应而停止使用药物, 评估抑制AChE新候选物的毒理学特征至关重要, 研究人员未来可进行有潜力化合物的衍生化, 以获得临床有效且无毒性或毒性较小的AChE抑制剂[96-98]。

倍半萜是一类具有广泛生物活性的化合物, 本综述大多应用改良的Ellman法, 在特定条件下, 通过测定AChE水解底物产生的5-巯基-2-硝基苯甲酸的吸光值变化来计算抑制率, 以及通过计算模拟化合物与AChE的结合模式和亲和力, 预测其抑制活性。一方面, 有些活性物质, 如从三叶罗伞分离的倍半萜香豆素对AChE抑制作用显著, IC_{50}达到8.25 nmol·L^{-1}, 且不会产生细胞毒性, 从白术中分离的双倍半萜内酯与AChE的结合活性良好, 结合能小于−8 kcal·mol^{-1}, 进一步的结构分析表明, 这些具有显著AChE抑制活性的化合物中存在的α,β-不饱和羰基可能是其AChE抑制活性的关键结构基础。另一方面AD的病理生理学包括大脑海马和皮质区域等脆弱点的神经元变性, 故化合物必须通过BBB, 而倍半萜类化合物的亲脂性和低极性使其在大脑特定位点具有足够的生物利用度以满足治疗AD所需[99,100]。但是, 倍半萜类化合物结构复杂, 分子内通常存在多个手性中心, 绝对构型难以确定; 在分离过程中经常会遇到差向异构体和对映异构

体, 导致传统色谱分离方法难以高效分离, 这些异构体构型和构象的差异对其活性的影响尚未深入研究; 另外, 目前对倍半萜类化合物的研究主要集中在化合物体外 AChE 抑制活性评估上, 体内药效评价和构效关系研究较少, 目前的研究存在一定的局限性[101]。因此, 可以利用分子对接与分子动力学模拟进一步预测倍半萜类化合物的构型和构象对生物活性的影响, 并通过高通量筛选技术快速评估大量倍半萜类化合物的 AChE 抑制活性; 目前在体外活性实验中展现显著 AChE 抑制活性的化合物也应进一步在动物模型中进行评估。总体来说, 天然倍半萜及其衍生物种类繁多, 功能多样, 具有广阔的研究前景, 可以作为改善学习和记忆的候选药物[102]。

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