治疗混合谱系白血病的小分子抑制剂研究进展

罗孟兰[1,2], 陈玉婷[2], 刘同超[2], 崔永梅[1*], 陈丹琦[2*], 熊 兵[2*]

(1. 上海大学理学院, 上海 200444; 2. 中国科学院大学, 中国科学院上海药物研究所, 上海 201203)

摘要: 急性白血病 (acute leukemia, AL) 是一类造血干细胞恶性克隆性疾病。在约5%～10%的AL患者体内都能观察到混合谱系白血病 (mixed-lineage leukemia, MLL) 基因易位重排现象。患有MLL易位重排 (MLL-rearranged, MLL-r) 的白血病患者目前缺乏治疗手段, 且预后差。大量研究表明, 许多表观遗传调节因子直接或间接参与了MLL的发生发展过程, 这为采用干预表观遗传的策略以治疗MLL提供了理论依据。本文从MLL的表观遗传学相关调控机制出发, 选择代表性的药物靶点, 分析各靶点与MLL的联系, 并综述相关抑制剂的研发进展, 希望为后续研发用于治疗MLL的药物提供参考。

关键词: 混合谱系白血病; 混合谱系白血病融合蛋白; 表观遗传调节因子; 抑制剂

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Research advances of small molecule inhibitors in the treatment of mixed lineage leukemia

LUO Meng-lan[1,2], CHEN Yu-ting[2], LIU Tong-chao[2], CUI Yong-mei[1*], CHEN Dan-qi[2*], XIONG Bing[2*]

(*1. College of Sciences, Shanghai University, Shanghai 200444, China; 2. University of Chinese Academy of Sciences, Chinese Academy of Sciences Shanghai Institute of Materia Medica, Shanghai 201203, China*)

Abstract: Acute leukemia (AL) is a kind of malignant clonal disease of hematopoietic stem cells. Rearrangement of mixed lineage leukemia (MLL) gene can be observed in about 5%−10% of AL patients. Currently, AL patients with MLL-rearrangements (MLL-r) lack effective treatment and are usually associated with poor prognoses. Recent studies have shown that many epigenetic regulators are directly or indirectly involved in the occurrence and development of AL carrying MLL-r (MLL), which provides a biological basis for the use of epigenetic regulation strategies to treat MLL. In this review, we start from the epigenetic regulation mechanism of MLL, and select representative drug targets to briefly analyze the relationship between each target and MLL and summarize the development progress of their inhibitors, hoping to provide reference for the subsequent research and development of drugs for the treatment of MLL.

Key words: mixed lineage leukemia; mixed lineage leukemia fusion protein; epigenetic regulator; inhibitor

急性白血病 (acute leukemia, AL) 是一类造血干细胞的恶性克隆性疾病, 可分为急性髓细胞白血病 (acute myeloid leukemia, AML) 和急性淋巴细胞白血病 (acute lymphoid leukemia, ALL)。混合谱系白血病 (mixed-lineage leukemia, MLL) 基因位于11号染色体长臂2区3带 (11q23), 该基因重排是AL的主要致病因素之一。研究表明, 超过70%的婴儿AL和近10%的成人AML的发生均与MLL易位重排 (MLL-rearranged, MLL-r) 有关[1,2]。已知有120多种易位伴侣基因 (translocation partner genes, TPGs) 可与MLL发生融合编码出致癌的MLL融合蛋白 (MLL fusion proteins, MLL-FPs), 从而导致MLL。其中, 6种TPGs是MLL发生的主要基

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*通讯作者 Tel: 13661970096, E-mail: bxiong@simm.ac.cn; dqchen@sibs.ac.cn; ymcui@shu.edu.cn
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因, 分别是AF4、AF6、AF9、AF10、ENL和ELL[3]。MLL患者一般预后较差[4], 患有MLL的婴儿5年不复发存活率 (34%) 约为非MLL的婴儿存活率 (60%) 的一半[5]; 与一般的儿童AML相比, 患有伴随MLL-r的AML的儿童5年不复发存活率 (55% *vs* 44%) 也相对较低[6]。

MLL患者采用传统手段治疗存在着诸多缺陷。当前MLL的治疗主要是通过运用大剂量的阿糖胞苷进行化疗。这种治疗手段虽然对70%～80%的年轻患者有效, 但对于60岁以上的老年人效果甚微, 导致患者5年不复发总生存率在40%左右[7-14]。此外, 由于传统治疗手段无法靶向癌变的造血干细胞使得MLL具有高复发率和低生存率的特点。基于当前临床治疗情况, MLL患者迫切需要新的有效治疗药物。

MLL, 又称赖氨酸特异性甲基转移酶2A (lysine specific methyltransferase 2A, KMT2A)。该基因可编码出含多个功能结构域、大小约为500 kDa的核蛋白, 即野生型MLL蛋白 (wild-type MLL protein, WT-MLL) (图1)。WT-MLL的氮端 (WT-MLL-N) 具有转录抑制活性, 由LMI、AT-hooks、CXXC、BRD、PHD1-4和FYRN组成[15]。其中, LMI结构域是MLL与Menin蛋白结合的区域[16]。MLL与Menin之间的相互作用对MLL-FPs致白血病活性至关重要。WT-MLL的碳端 (WT-MLL-C) 显示出转录激活特性, 由TAD、FYRC、WIN和SET构成[15]。其中, SET是具有甲基转移酶活性的功能结构域。该结构域[17]通过与蛋白复合物WDR5-RbBP5-ASH2L-DPY30 (WRAD) 作用催化组蛋白H3K4三甲基化, 进而调控一系列与造血相关的基因的表达; 这些基因主要包括HOXA基因 (如Hoxa7、Hoxa9、Hoxa10)[18]、Meis1[19]和PBX1[20]等。

重排后的MLL与TPGs发生融合继而编码得到MLL-FPs, 该蛋白在结构上发生了明显变化; 其结构中仅保留了WT-MLL结构中从LMI到CXXC的部分, 其余部分由TPGs蛋白代替 (图1)。由于部分结构的缺失, 导致MLL-FPs不能行使正确的转录调节功能, 进而引起相关靶基因的异常表达, 最终影响造血细胞的正常生化过程, 诱发白血病[21,22]。

表观遗传学是指在不改变DNA序列的前提下, 通过特定机制引起可遗传的基因表达或细胞表现型的改变。肿瘤细胞, 从某种角度可认为是一类具高度增殖能力的细胞。众所周知, 细胞的分化主要通过表观遗传相关机制进行调节, 因此细胞表观遗传的异常调节是促进恶性肿瘤发生的主要因素, 已成为肿瘤的标志性特征。因而从表观遗传角度对肿瘤进行治疗, 有望通过多种基因表达调控机制进行深层次干预。大量研究表明, 许多表观遗传调节因子直接或间接参与了MLL的发生发展过程[23-26]。这为运用表观遗传学的调控策略以治疗MLL提供了理论依据。许多表观遗传的改变是可逆的, 这为人类多种疾病的治疗提供了乐观的

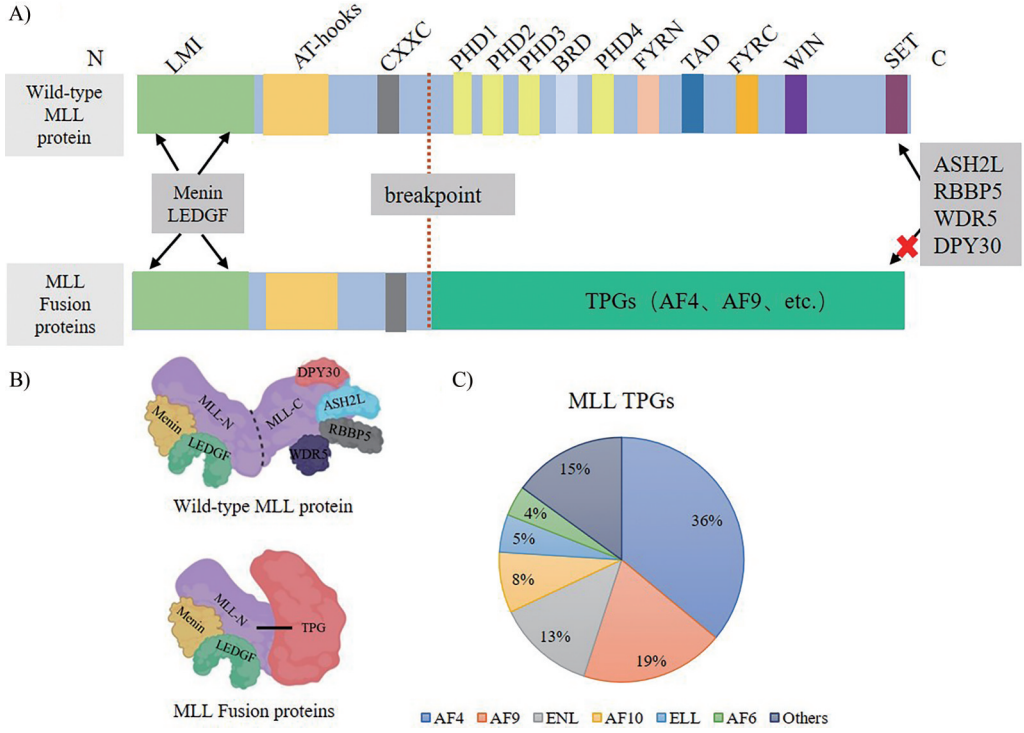


Figure 1 A) and B) The structures of mixed-lineage leukemia (MLL) proteins: wild-type and fusion proteins; C) Pie chart of the prevalence of each MLL TPG in MLL cases

前景。近年来大量可用于治疗MLL的表观遗传学的潜力靶点被相继报道。根据靶点是否直接参与MLL蛋白对MLL治疗的调控过程, 可将当前报道的用于治疗MLL的表观遗传学潜力靶点分为两大类, 即MLL蛋白参与类 (Menin、WDR5、DOT1L、PRMT1等) 和无MLL蛋白参与类 (LSD1、PRMT5、G9A等)。本文选择以上两类中研究较为丰富、具有较好发展前景的药物靶点, 对其作用机制进行分析 (图2), 并综述靶点相关抑制剂的研究进展, 以期为后续研究提供参考。

1 Menin-MLL PPI抑制剂

Menin蛋白是由多发性内分泌肿瘤1型 (multiple endocrine neoplasia type 1, MEN1) 基因所编码的一种核蛋白, 全长为610个氨基酸, 大小为67 kDa[27,28]。该蛋白是MLL-FPs致白血病的一种重要致癌辅助因子[29-31]。Menin与MLL蛋白 (MLL-FPs和WT-MLL) 的N端结合, 在染色质结合蛋白晶状体上皮衍生生长因子 (lens epithelium-derived growth factor, LEDGF) 的作用下, 形成一个巨大的蛋白复合物 (图3)。该复合物通过催化H3K4甲基化以上调Hox、Meis1和EZH2等基因的表达, 从而导致造血干细胞的恶性增殖与分化障碍, 最终引发白血病[19,32]。在MLL-FPs诱导的白血病细胞模型中, Menin蛋白的缺失或MLL-FPs N端突变均可下调Hox基因的表达[32-34], 而正常造血细胞中Menin蛋白的缺失并不会影响WT-MLL的造血功能[35-37]。因此, 靶向Menin-MLL PPI是治疗MLL的潜在靶点[38-40]。

自2012年第一个Menin-MLL PPI小分子抑制剂

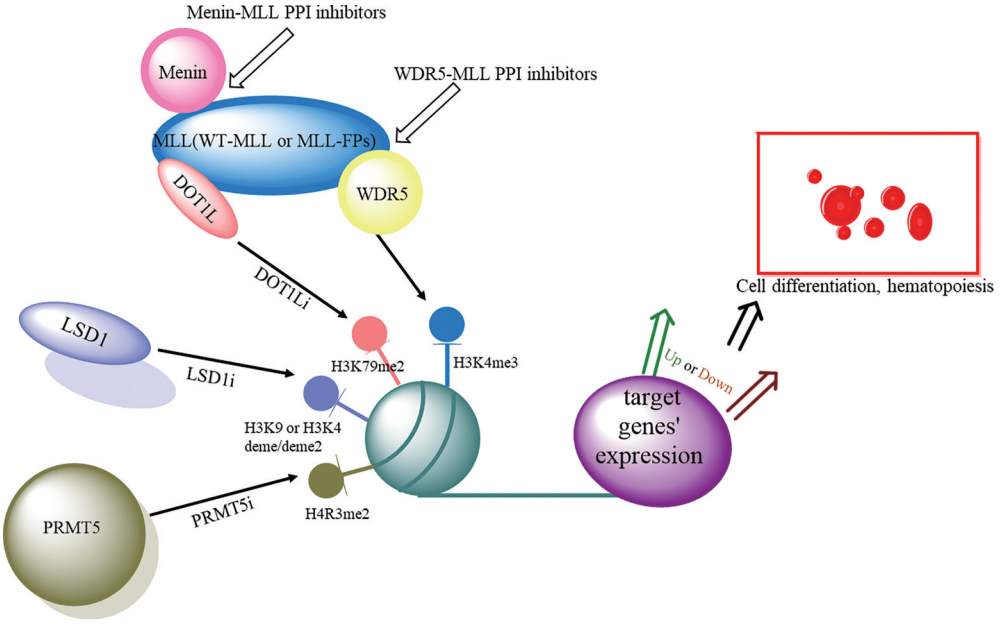


Figure 2 The diagram of progresses of representative epigenetic regulators (Menin, WDR5, DOT1L, LSD1 and PRMT5)*via* histone modifications to regulate genes' expression in MLL

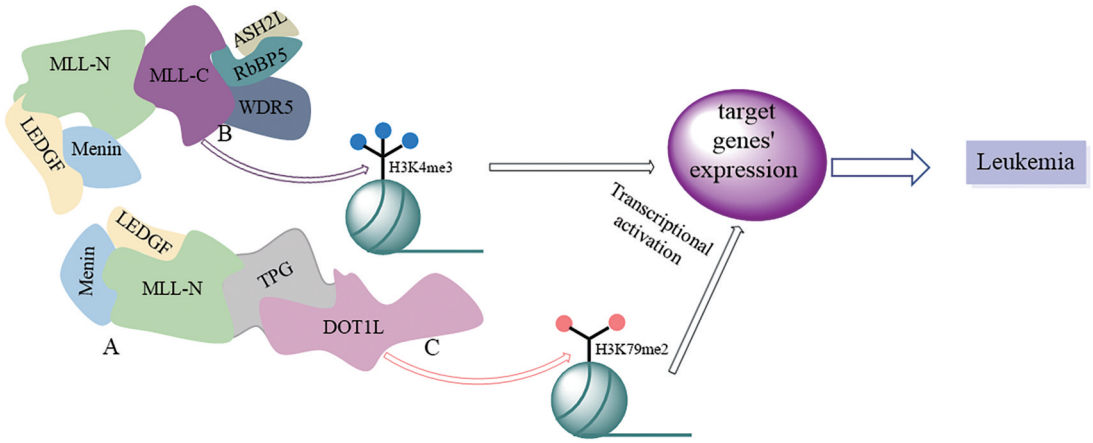


Figure 3 Epigenetic regulator *via* MLL fusion proteins and/or wild-type MLL to activate MLL target genes, leading to leukemogenesis. A, B and C represent three regulatory pathways (Menin, WDR5 and DOT1L), respectively

被发现以来, 越来越多的小分子抑制剂被报道, 按结构母核的差异主要分为噻吩嘧啶类、嘧啶类、哌啶类、环肽类和其他。

噻吩嘧啶类抑制剂 (图 4) 是研究较多的一类 Menin-MLL PPI 抑制剂[41-46]。2012 年, Grembecka 等[41] 通过高通量筛选, 从 49 000 个小分子中发现了具有较高亲和力的 **1-1**, 经化学改造得到了首个 Menin-MLL 小分子抑制剂 **1-2**。同年, 该课题组[47] 成功解析了 Menin-MLL 蛋白复合物和 **1-2**/Menin 复合物的晶体结构。通过对晶体结构进行分析, 作者发现 **1-2** 与 Menin 竞争性的占据了 MLL 与 Menin 的结合口袋, 并模拟了 MLL 与 Menin 之间的相互作用。**1-3** 中的一个氟原子可与 Menin 中的组氨酸 181 (His181) 发生偶极作用从而使其活性对比 **1-2** 提高约 10 倍。**1-3** 可有效下调 Hoxa9 和 Meis1 基因的表达, 对白血病细胞具有明显的抑制活性[42,47]。但此类抑制剂药代性质不佳, 易代谢失活, 未见体内药效学评价的研究报道。Borkin 等[43] 通过氨基哌啶将母核噻吩嘧啶与氰基吲哚环进行连接, 得到了先导化合物 **1-4**。基于 **1-4** 的结构, 对氰基吲哚环上的取代基不断优化以改善其成药性, 得到了化合物 **1-5** 至 **1-10**。2020 年, Brzezinka 等[48] 用螺环胺代替氨基哌啶得到了高选择性且成药性更佳的 **1-11**。**1-11** 在 MV4-11 异种移植模型中表现出显著的下调靶基因和减小肿瘤体积的生物活性。此外, **1-11** 对乳腺、前列腺和滑膜肉瘤细胞株没有抗增殖作用, 具有较高的肿瘤细胞特异性。

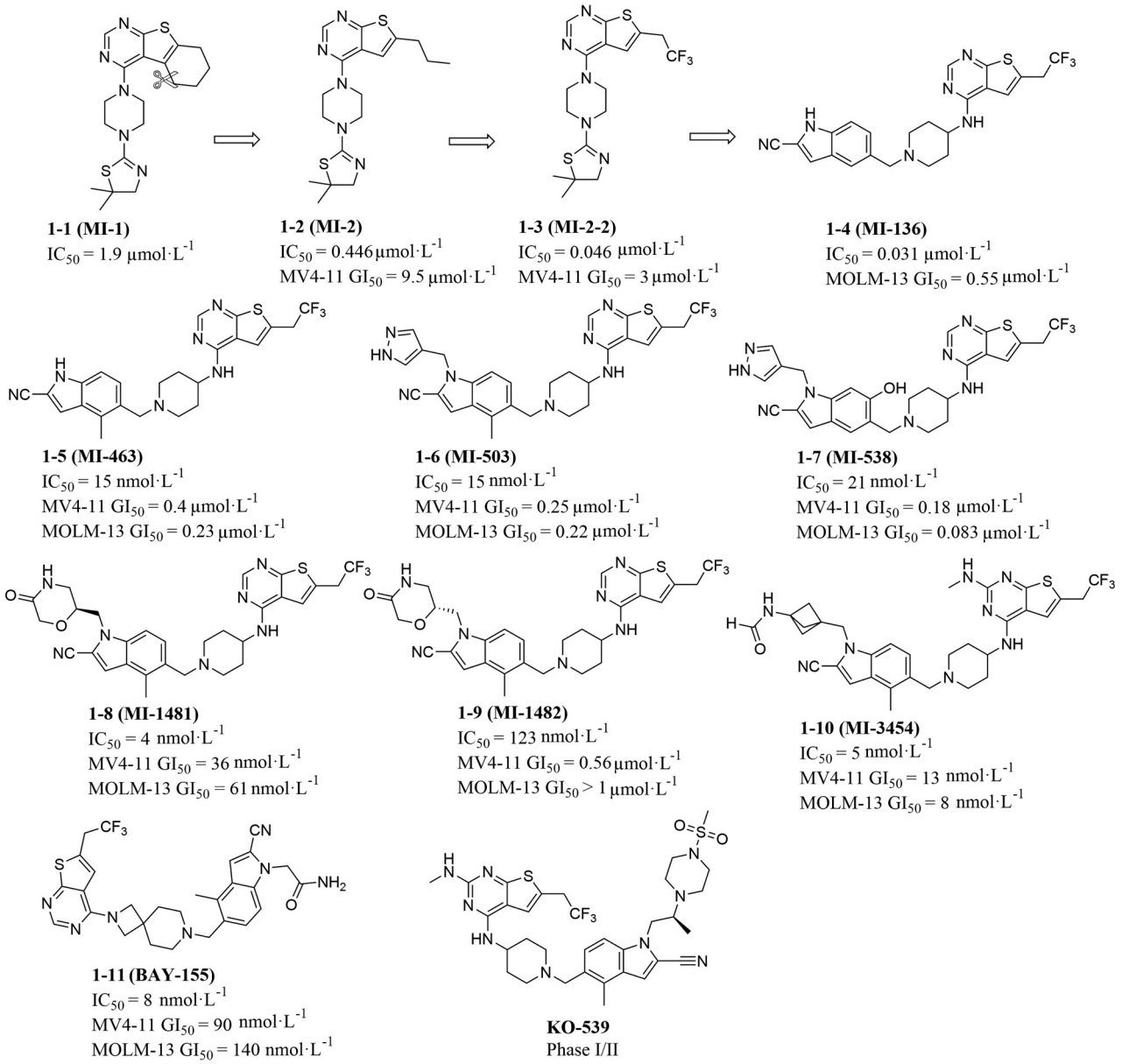


Figure 4 Chemical structures of thienopyrimidines' Menin-MLL PPI inhibitors

由 Kura Oncology 公司开发的 **KO-539** 是一个高活性、口服有效、生物利用度和安全性较高的噻吩嘧啶类小分子抑制剂。2019 年, **KO-539** 获得了美国食品和药品管理局的罕见药物认证, 被指定为治疗 AML 的孤儿药, 可用于治疗包括由 NPM1 基因突变和 MLL-r 所引发的复发性/难治性 AML, 目前处于 I/II 期临床试验。

根据对噻吩嘧啶类抑制剂 **1-2** 晶体结构的分析, 通过进一步的结构简化, 获得了嘧啶类 Menin 抑制剂 (图 5)。Krivtsov 团队[49, 50]以 **1-12** 为基础, 在 **1-12** 上先后引入 4-氟苯胺基和 2-异丙基苯基, 并以螺环胺代替哌嗪, 最终得到了高效、高选择性且可口服的抑制剂 **1-15**。2021 年, **1-15** 的类似物 **SNDX-5613** 通过了美国 FDA 的快速审评通道, 用于治疗复发性/难治性 AL, 其中包含由 MLL-r 和 NPM1 突变引发的白血病。

He 等[51]基于高通量筛选, 发现了首个哌啶类 Menin-MLL 小分子抑制剂 **1-16**。经过一系列衍生设计后, 哌啶类抑制剂 (图 6) 根据头部所连基团的不同, 可分为羟甲基哌啶类抑制剂 **1-17**[52]和氨甲基哌啶类抑制剂 **1-19**[51], 其中氨甲基哌啶类抑制剂又可进一步细分为共价抑制剂[53-55]和非共价抑制剂两类[56]。

2015 年, Senter 等[52]通过对 **1-17** 的头部进行优化, 发现了具有良好体外药物代谢及动力学性质的 **1-18**。但该系列小分子抑制剂的选择性、药代性质、有效性及安全性还未见报道。

通过分析 **1-19**/Menin 复合物的晶体结构, 王少萌课题组[56]设计并开发了非共价抑制剂 **1-20**。**1-20** 以 K_d = 1.4 nmol·L^{-1} 的亲和力与 Menin 蛋白结合, 可有效抑制 MV4-11 (IC_{50} = 25 nmol·L^{-1}) 和 MOLM-13 (IC_{50} = 55 nmol·L^{-1}) 细胞株的生长。此外, 该团队[53]发现 Menin 蛋白上的半胱氨酸 329 (Cys329) 是开发共价抑制剂的关键, 初步设计得到了 **1-21** 和 **1-22**。在细胞活性上, 两者显示出约 43 倍的差距。**1-21** 的成功验证了开发共价抑制剂的可行性, 经进一步优化得到了水溶性较好的 Menin-MLL PPI 共价抑制剂 **1-23**。随后基于 **1-23**, 得到了具有更好生物活性的 **1-24**[54]。虽然 **1-24** 活性相对 **1-23** 有所提高, 且在 MLL 白血病细胞模型中表现出良好的肿瘤抑制活性, 但其口服生物利用度低, 无法开展进一步的生物活性测试。对此, 该课题组[55]基于 **1-23** 和 **1-24**, 通过在砜基和迈克尔受体之间连接不同的基团对分子构象进行限制, 得到了活性略有下降但口服生物利用度提高 (F = 49%) 的化合物 **1-25**。

2012 年, 王少萌课题组[57]为探测 Menin 与 MLL 的结合口袋, 通过模拟 WT-MLL-N 的 MBM1 区, 获得了一类环肽结构的 Menin-MLL 小分子抑制剂 (图 7)。**1-26** 是一类具有较高亲和力和低分子量的环肽类小分子抑制剂。2020 年, Fortuna 等[58]基于 Menin-MLL 复合物的晶体结构, 通过复分解反应得到了一系列环肽类小分子, 随后对环的大小、N 端、C 端等方面进行改造得到了含一个 17 元环的环肽类抑制剂 **1-27**。近年来, 基于老药新用策略, 通过运用虚拟筛选和分子对接等手段, 人们发现了大量靶向 Menin-MLL PPI 的小分子抑制剂 **1-28** 至 **1-33**[37,59-61]。这为靶向 Menin-MLL 抑制剂的设计与开发提供了新思路。

2 WDR5-MLL PPI 抑制剂

WDR5 (WD repeat-containing protein 5) 属于 WD40 蛋白家族的一员, 是一种重要的表观遗传调节因子。WDR5 可识别组蛋白 H3K4 和组蛋白 H3R2 上的甲基化修饰进而参与相关基因的表达[62,63]。值得关注的是, WDR5 是通过招募蛋白并形成蛋白复合物对基因表达进行调节的。在 MLL-AF9 白血病细胞模型中, 由 MLL 蛋白引起的 H3K4 超甲基化可使具有致白血病活性的靶基因过度表达从而引发白血病 (图 3)[64]。研究表明, 蛋白复合物 MLL-WDR5-RbBP5-ASH2L 的稳定

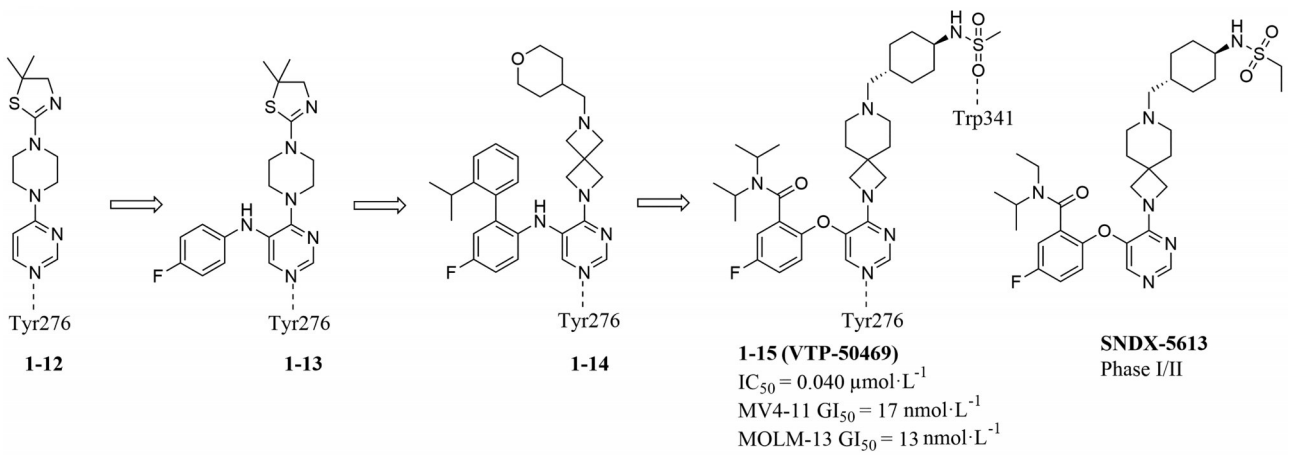


Figure 5 Chemical structures of pyrimidines' Menin-MLL PPI inhibitors

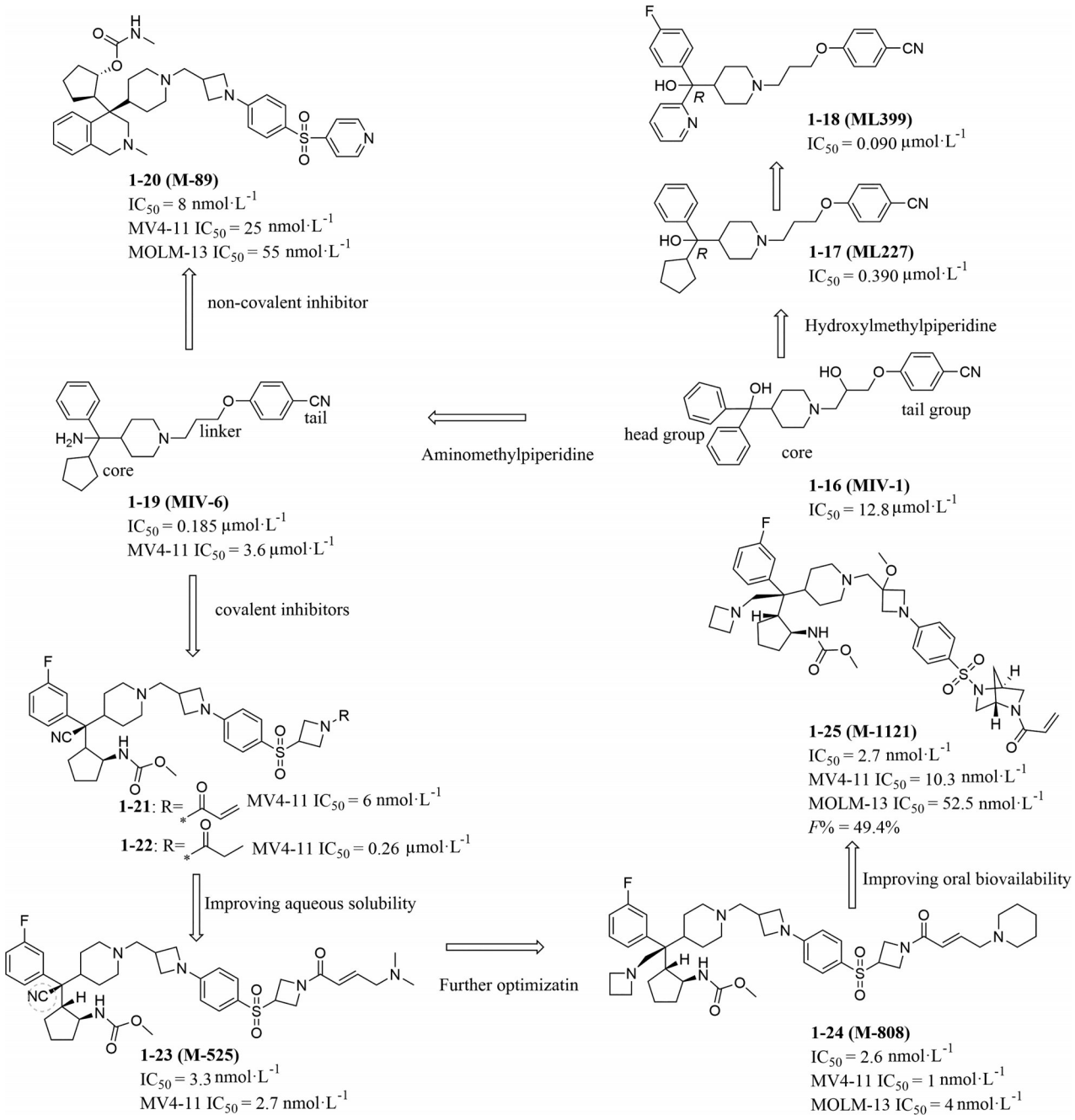


Figure 6 Chemical structures of piperidines' Menin-MLL PPI inhibitors

存在决定了细胞内 H3K4 甲基化水平[17], 而维持整个蛋白复合物稳定存在的核心取决于 WDR5 与 MLL 之间的相互作用[65]。因此, 靶向 WDR5-MLL PPI 是治疗 MLL 的有效策略。

根据设计思路及结构特征, 可将 WDR5-MLL 抑制剂分为多肽类[66-68]和非多肽类抑制剂[69-72], 其主要通过占据 WDR5 的 WIN 结构域发挥作用 (图 8)。

通过模拟 MLL 的一段氨基酸序列, 王少萌团队[66]得到了靶向 WDR5-MLL PPI 的多肽类小分子抑制剂 **2-1** 至 **2-3**。**2-2** 可以在一定程度上抑制 Hoxa9 和 Meis1 基因的表达, 但 **2-2** 对 H3K4 甲基化的抑制能力较差。为进一步提高多肽类抑制剂在体内的活性, 该团队[67]设计并合成了环肽类的抑制剂 **2-4**。随后为进一步改善 **2-4** 在代谢稳定性方面的缺陷, 该课题组[68]通过封闭 **2-4** 的代谢位点并缩小整个环的大小得到了 **2-5**。**2-5** 是目前得到的最有潜力的多肽类小分子抑制剂。但与非多肽类抑制剂相比, 该分子在体内易代谢、分子极性较大且分子含有碱性较大的胍基。这些因素均会限制 **2-5** 在临床上的运用。为此, 研究者们又把目标转向了非多肽类抑制剂的开发上。

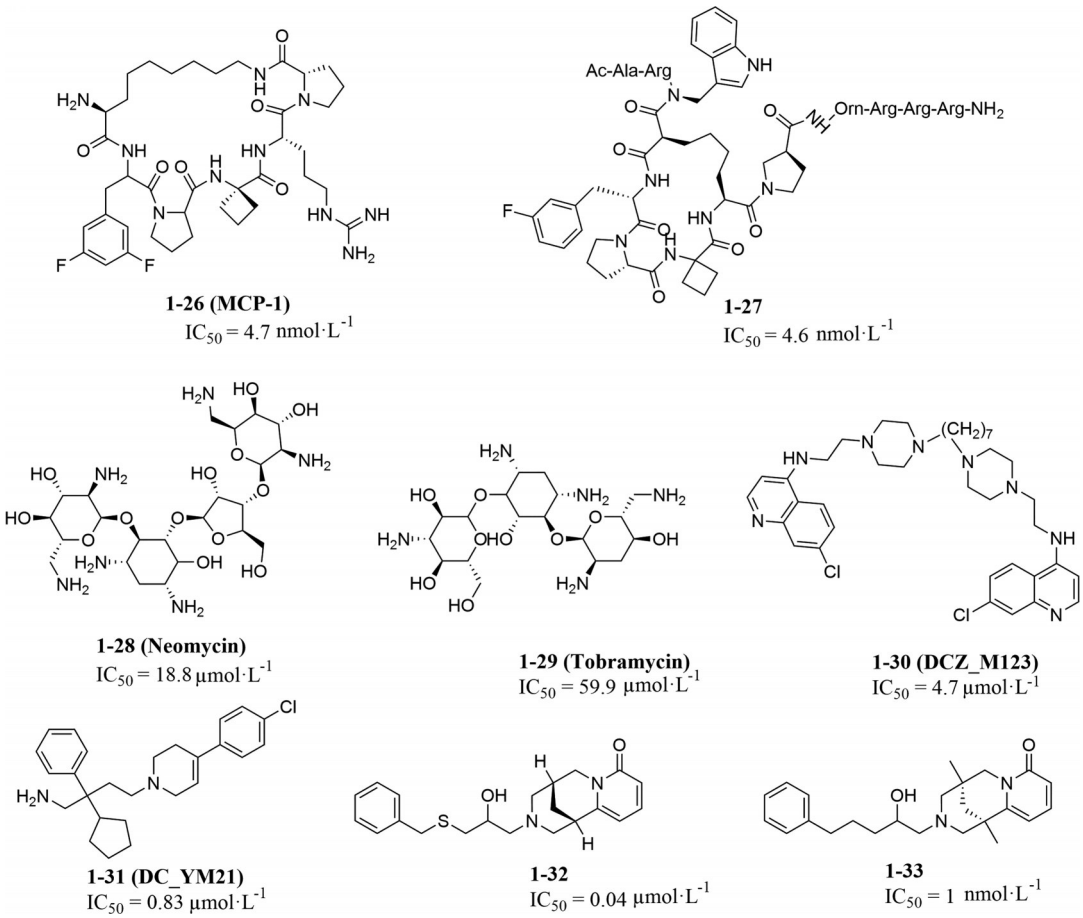


Figure 7 Chemical structures of peptides' Menin-MLL PPI inhibitors and others

在安大略癌症研究所科研人员[69]的努力下, 得到了具有较高亲和力和选择性的非多肽类抑制剂**2-6**。随后, 通过进一步的合理优化, 得到了首个具有皮摩尔亲和力的化合物**2-7**。Chen等[72]基于安大略癌症研究所的报道, 通过SAR分析, 设计并合成了化合物**2-8**。**2-8**对MV4-11细胞显示出良好的抑制能力, GI_{50} = 7.6 μmol·L^{-1}。通过进一步探索, 相继得到了**2-9**、**2-10**和**2-11**。这些化合物均可下调Hox和Meis1基因的表达, 从而诱导白血病细胞凋亡并抑制白血病细胞的增殖。2018年, Fesik团队[70,71]基于结构片段的虚拟筛选和优化改造, 相继得到了**2-12**、**2-13**和**2-14**。其中, **2-14**对MV4-11和MOLM-13均显示出优异的抑制活性, 具有较好的抗增殖能力和成药性。

3 DOT1L抑制剂

染色质结构在内部扩散并抑制附近基因转录的现象称为端粒沉默。1988年, Singer等[73]在对啤酒中的酵母进行筛查时, 发现了端粒沉默干扰因子 (disruptor of telomeric silencing-1, DOT1)。DOT1L是DOT1在脊椎动物中的同源物。人类全长的DOT1L由1 537个氨基酸组成, N端的360个氨基酸与酵母菌具有高度同源性[74]。DOT1L是人体唯一的H3K79甲基化的赖氨酸甲基转移酶[75,76], 参与了多种表观修饰过程。在AML发展过程中 (图3), MLL-FPs通过蛋白之间的协同作用将DOT1L招募到MLL靶基因的启动子上[77], 使启动子的H3K79甲基化水平增加, 导致靶基因 (Hoxa9、Meis1等) 持续激活, 从而诱导白血病的发生[78-83]。因此, 开发DOT1L抑制剂也是治疗MLL的一种有效手段。

自发现DOT1L抑制剂EPZ004777对MLL具有显著抑制作用以来, 已有多个DOT1L抑制剂被报道。根据抑制剂的结构特点和作用模式的不同, 可将已报道的DOT1L抑制剂分为两大类: 核苷类抑制剂 (图9) 和非核苷类抑制剂 (图10)。SAH (K_i = 160 nmol·L^{-1}) 是一种天然的SAM竞争性DOT1L核苷类抑制剂。但SAH易被体内水解酶代谢, 且对多种甲基转移酶均有作用, 不具选择性。目前文献报道的核苷类DOT1L抑制剂多为SAH的衍生物。2013年Anglin等[84]将核苷类DOT1L抑制剂进一步细分为SAH类似物抑制剂、基于机制类抑制剂、氨基甲酸酯类抑制剂和脲类/苯并咪唑类抑制剂四类。具体说来, SAH类似物抑制剂是基于SAH的结构, 通过合理设计获得的一类代谢较为稳定的选择性

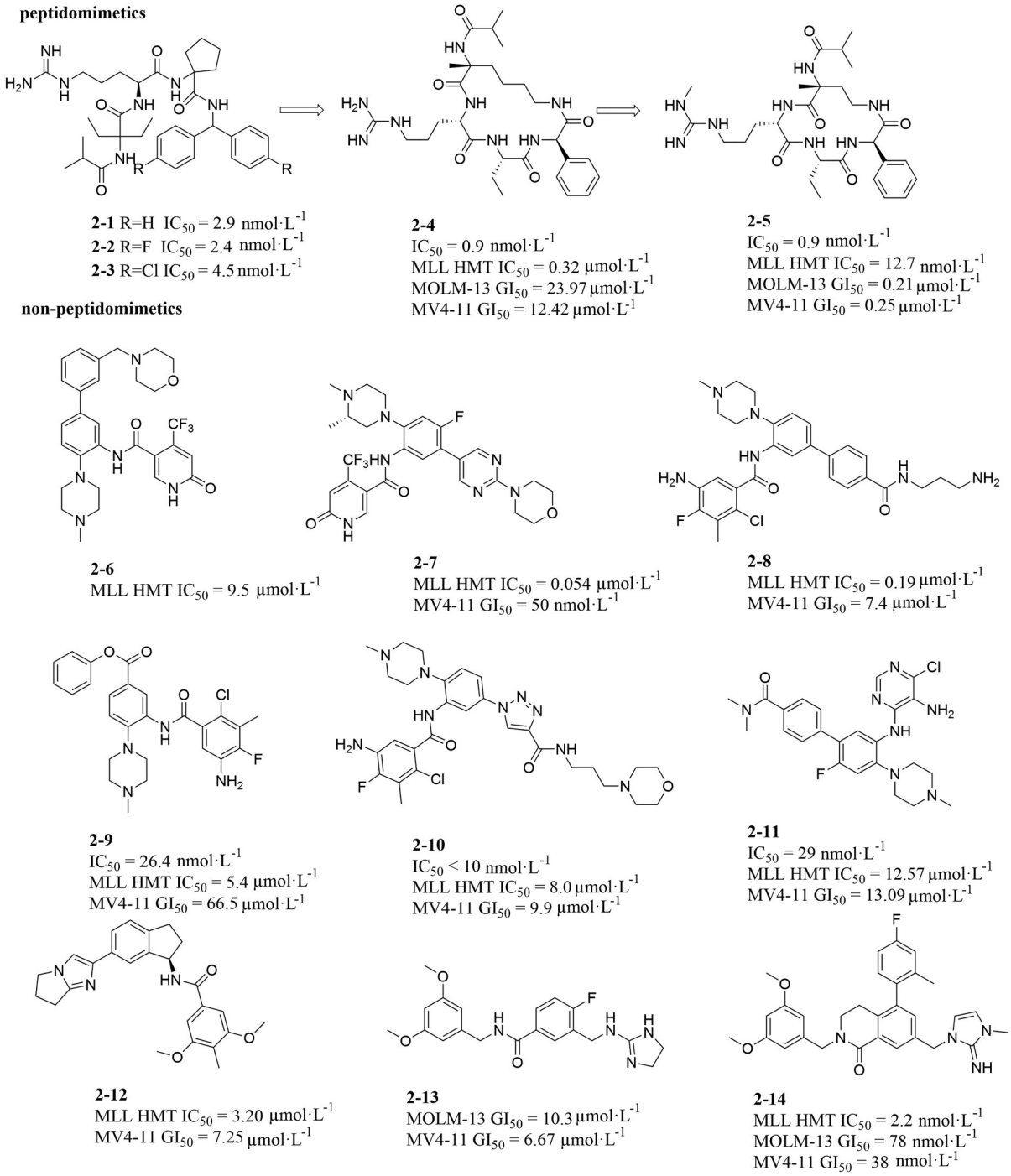


Figure 8 Chemical structures of WDR5-MLL PPI inhibitors

DOT1L 抑制剂 (如**3-1**)。基于机制类抑制剂是一类可抑制 SAM 甲基转移过程的新型核苷类抑制剂 (如**3-2**)。氨基甲酸酯类是通过优化 SAH 的氨基酸片段以改善其类药性而获得的抑制剂 (如**3-3**)。值得注意的是, 脲类/苯并咪唑类抑制剂是基于前者的基础上, 获得的一类高活性的 DOT1L 抑制剂 (如**3-4** 和**3-5**)。

非核苷类 DOT1L 抑制剂是利用计算机虚拟筛选和基于片段的药物设计等手段发现的具有多种新型骨架的 DOT1L 抑制剂。2016 年, 罗成团队[85]利用药效团筛选、分子对接和生物活性测试等方法从约 20 万个小分子中发现了对 DOT1L 分子活性为 1.5 μmol·L^{-1} 的先导化合物**3-6**。该化合物对 MV4-11 细胞株具有一定的增殖抑制活性 (IC_{50} = 37.1 μmol·L^{-1}), 经进一步修饰优化得到了对 DOT1L 和 MV4-11 细胞抑制活性更好的化合物**3-7** 至**3-9**[86], IC_{50} 分别为 30.54、1.62 和 1.29 μmol·L^{-1}。Wang 等[87]和 Luo [88]通过运用相同的手

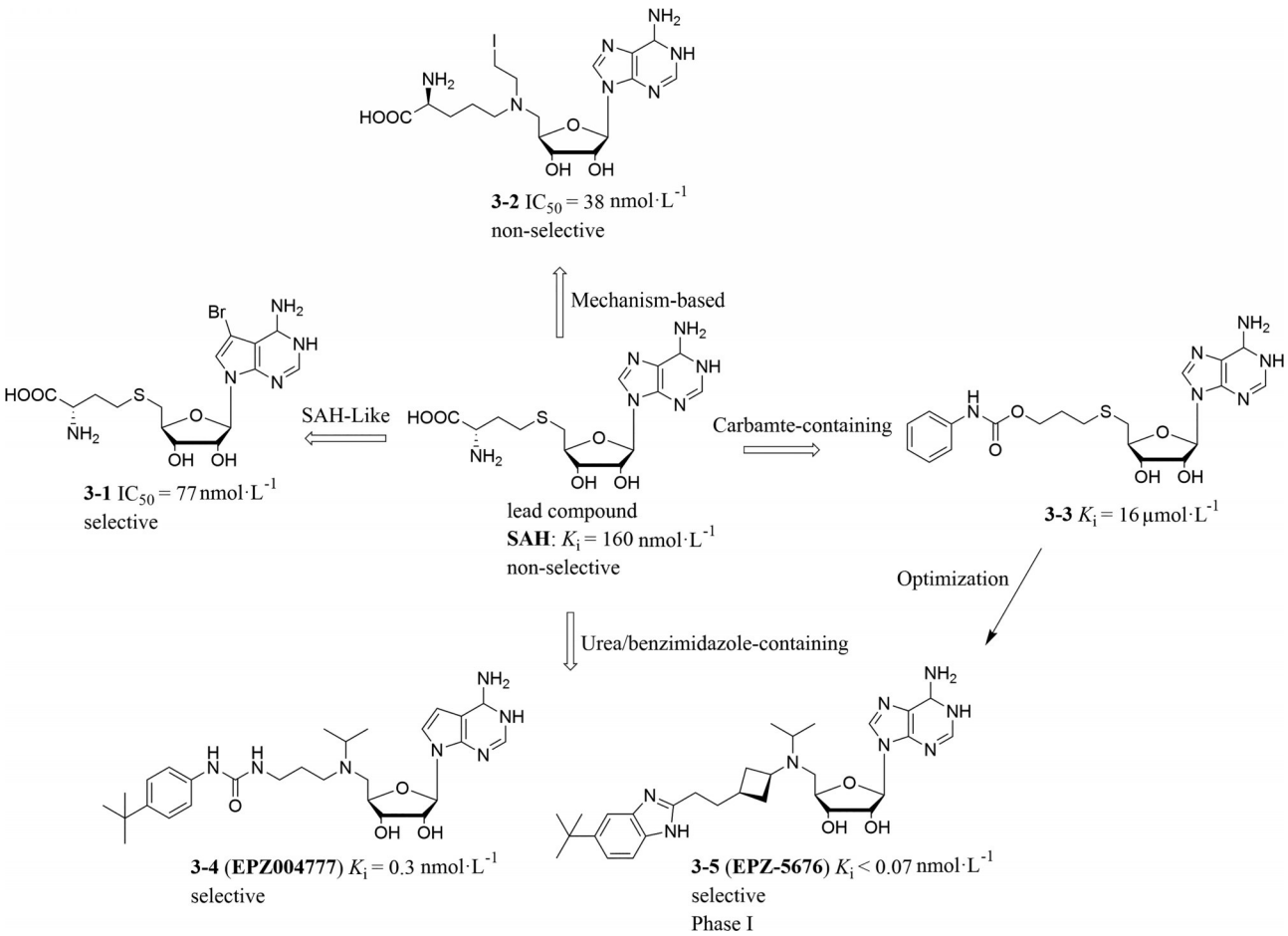


Figure 9 Chemical structures of representative nucleoside DOT1L inhibitors

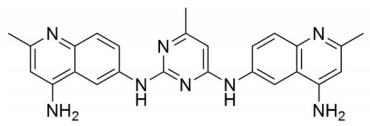
段发现了两类新型母核的DOT1L抑制剂**3-10**和**3-11**, **3-10**对MLL-r细胞株表现出一定的选择性抑制能力(IC_{50} = 21.91 μmol·L^{-1})。

诺华制药公司[89]通过高通量筛选发现了具有一定DOT1L抑制活性的苗头化合物**3-12**。DOT1L/**3-12**共晶结构 (PDB: 5DRT) 显示, **3-12**与DOT1L结合于SAM结合口袋旁边形成的新口袋。初步改造后得到了具有明显选择性活性的**3-13** (IC_{50} = 20 nmol·L^{-1}), 但由于**3-13**药代动力学性质差, 无法开展进一步的活性评价。为改善其成药性, 用*N*-甲氨基嘧啶代替**3-13**结构中整个苯环四唑脲得到**3-14** (IC_{50} = 280 nmol·L^{-1})、**3-15**至**3-18**。其中**3-17**和**3-18**可有效抑制MV4-11细胞株的增殖, **3-17**表现出良好的药代动力学性质, 口服生物利用度为40%。通过运用相同的改造手段, 诺华制药公司[90]对基于虚拟筛选得到的先导化合物**3-19**进行优化改造, 得到了**3-20** (IC_{50} = 14 nmol·L^{-1})。此外, 诺华公司[91]基于拼接策略, 将筛选得到的片段和SAM模拟片段通过碳链进行连接得到了高活性的DOT1L抑制剂**3-21** (IC_{50} < 0.1 nmol·L^{-1})。诺华公司的研究为开发高活性、高选择性的新型DOT1L抑制剂提供了新思路。2018年, Song等[92]通过高通量筛选从2万个小分子中确定了具有DOT1L抑制活性的小分子**3-22**至**3-25**, 其中**3-22** (IC_{50} = 51.47 μmol·L^{-1})和**3-25** (IC_{50} = 1.07 μmol·L^{-1})可通过抑制MLL细胞MV4-11的增殖, 从而诱导细胞凋亡。

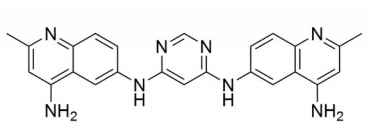
最近文献报道的新型DOT1L抑制剂**3-27**是属于糖苷类的天然产物[93]。在分子水平上, **3-27**对DOT1L表现出优越的选择性。在细胞水平上, **3-27**可有效抑制MV4-11细胞中H3K79的单甲基化和双甲基化水平(IC_{50} = 12.5 μmol·L^{-1}), 进而抑制MLL-FPs相关靶基因的表达。Du等[94]发现了DOT1L的一段多肽片段**3-28**。通过对此段多肽的氨基酸残基进行优化得到了**3-29**和**3-30**, 可通过破坏MLL-FPs (MLL-AF9和MLL-ENL)与DOT1L之间的相互作用界面从而抑制H3K79甲基化水平。

4 LSD1抑制剂

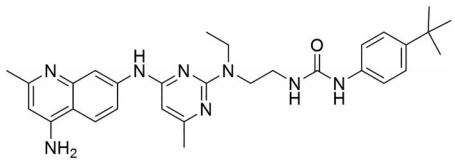
赖氨酸特异性去甲基化酶1A (lysine-specific demethylase 1A, KDM1A或LSD1)全长为852个氨基酸, 是催化组蛋白H3K9和H3K4单双甲基化主要的去甲基化酶[95]。LSD1在基因表达和细胞内生化过程的调控方面具有重要作用。对LSD1进行抑制或基因敲除可以下调MLL靶基因 (Hoxa9和Meis1)的表达, 从



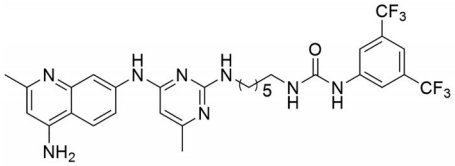
3-6
IC_{50} = 1.5 μmol·L^{-1}



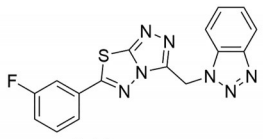
3-7
IC_{50} = 1.06 μmol·L^{-1}
MV4-11 IC_{50} = 30.54 μmol·L^{-1}



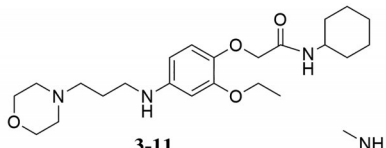
3-8
IC_{50} = 5.72 μmol·L^{-1}
MV4-11 IC_{50} = 1.62 μmol·L^{-1}



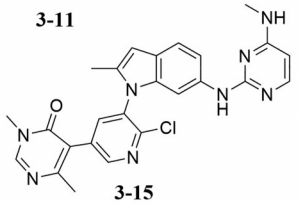
3-9
IC_{50} = 3.55 μmol·L^{-1}
MV4-11 IC_{50} = 1.29 μmol·L^{-1}



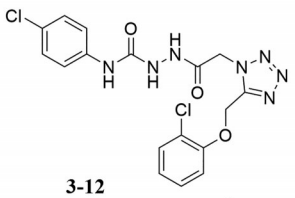
3-10
IC_{50} = 8.29 μmol·L^{-1}
MV4-11 IC_{50} = 21.91 μmol·L^{-1}



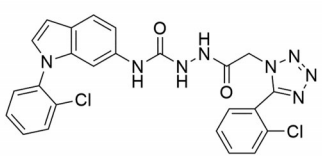
3-11



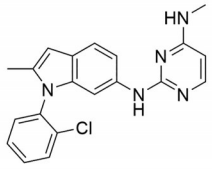
3-15
IC_{50} = 10 nmol·L^{-1}



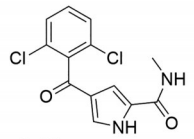
3-12
IC_{50} = 4.4 μmol·L^{-1}



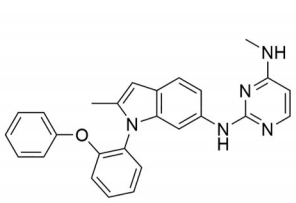
3-13
IC_{50} = 20 nmol·L^{-1}



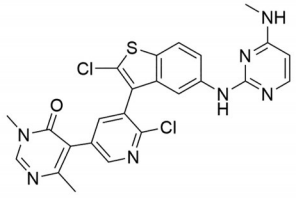
3-14
IC_{50} = 280 nmol·L^{-1}



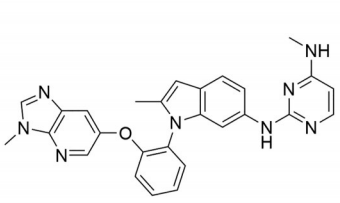
3-19
IC_{50} = 320 μmol·L^{-1}



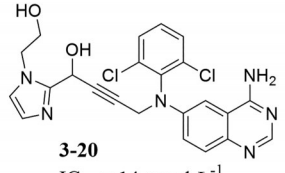
3-16
IC_{50} = 150 nmol·L^{-1}



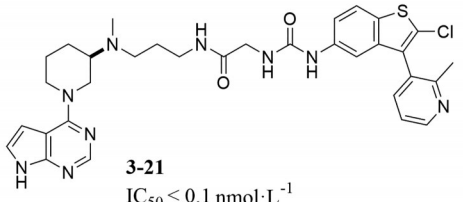
3-17
IC_{50} = 1.4 nmol·L^{-1}



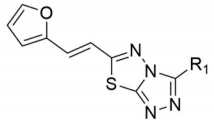
3-18
IC_{50} = 0.4 nmol·L^{-1}



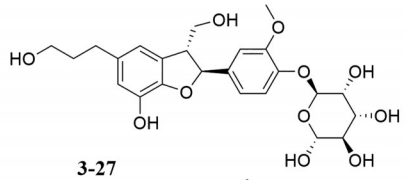
3-20
IC_{50} = 14 nmol·L^{-1}



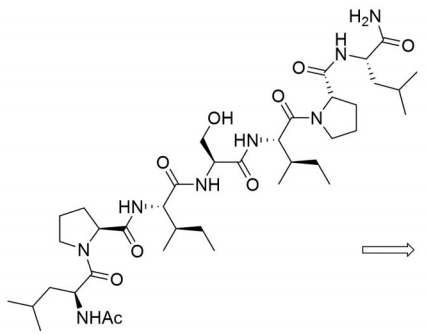
3-21
IC_{50} < 0.1 nmol·L^{-1}
MV4-11 IC_{50} = 5 nmol·L^{-1}
MOLM-13 IC_{50} = 17 nmol·L^{-1}



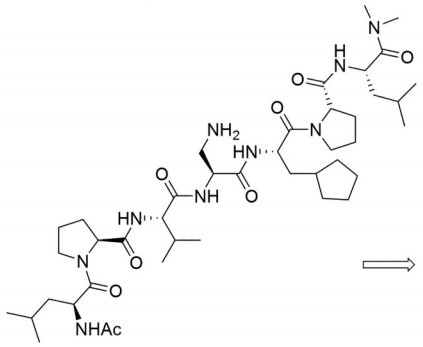
3-22 R_1=4-*N*,*N*-dimethylaminophenyl
3-23 R_1=phenyl
3-24 R_1=3-methylphenyl
3-25 R_1=2-methoxyphenyl
3-26 R_1=4-pyridyl



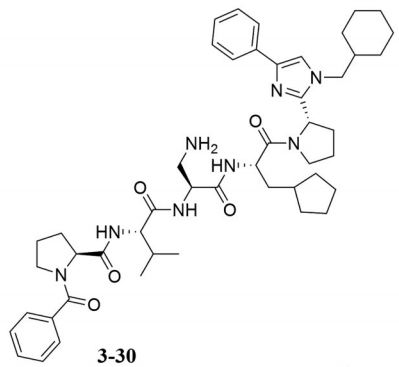
3-27
IC_{50} = 339 nmol·L^{-1}
MV4-11 IC_{50} = 12.5 μmol·L^{-1}



3-28
DOT1L-AF9 K_i = 160 nmol·L^{-1}
DOT1L-ENL K_i = 3.37 μmol·L^{-1}



3-29
DOT1L-AF9 K_i = 20 nmol·L^{-1}
DOT1L-ENL K_i = 90 nmol·L^{-1}



3-30
DOT1L-AF9 K_i = 9 nmol·L^{-1}
DOT1L-ENL K_i = 119 nmol·L^{-1}

Figure 10 Chemical structures of non-nucleoside DOT1L inhibitors

而诱导细胞分化并抑制MLL的发展[96,97]。LSD1也可以与转录因子REST (RE1 silencing transcription factor)的辅助抑制因子RCOR1 (又称CoREST)和组蛋白去乙酰化酶 (HDAC)[98]形成蛋白复合物, 进而招募转录抑制因子GFI1并与GFI1氮端的SNAG结构域结合, GFI1进而结合在相关基因的增强子上以调控髓样分化基因 (IRF8、KLF4和MEF2C)的表达 (图11)[99]。

基于小分子与LSD1作用机制的差异, 可将LSD1抑制剂分为共价类抑制剂和非共价类抑制剂。LSD1共价类抑制剂主要包括苯环丙胺 (TCP)及其衍生物、多胺类和多肽类。其中, TCP类是研究较早的一类LSD1抑制剂[100-102], 在目前进入临床试验阶段并公开结构的9个LSD1抑制剂中大部分为TCP的衍生物 (图12) (数据来源于Cortellis Drug Discovery Intelligence)。其中, **GSK2879552**和**IMG-7289**等LSD1抑制剂还开展了用于治疗AML、骨髓异常增殖综合征 (MDS)等白血病的临床试验[103,104]。

由于共价型LSD1抑制剂会与FAD发生加成反应, 而细胞内有多种氧化还原酶以FAD作为辅酶, 可能存在选择性不高的问题。近年来, 随着对LSD1研究的不断深入, 非共价型LSD1抑制剂被不断发现。目前, 已有两个小分子进入临床试验阶段, 分别为Celgene公司的**CC-90011**和Salarius公司的**SP-2577**。2020年, Dai等[105]对LSD1抑制剂进行了详细的综述, 本文不再赘述, 仅补充2021年新报道的LSD1抑制剂的结构及其研究情况 (图13)[106-119]。

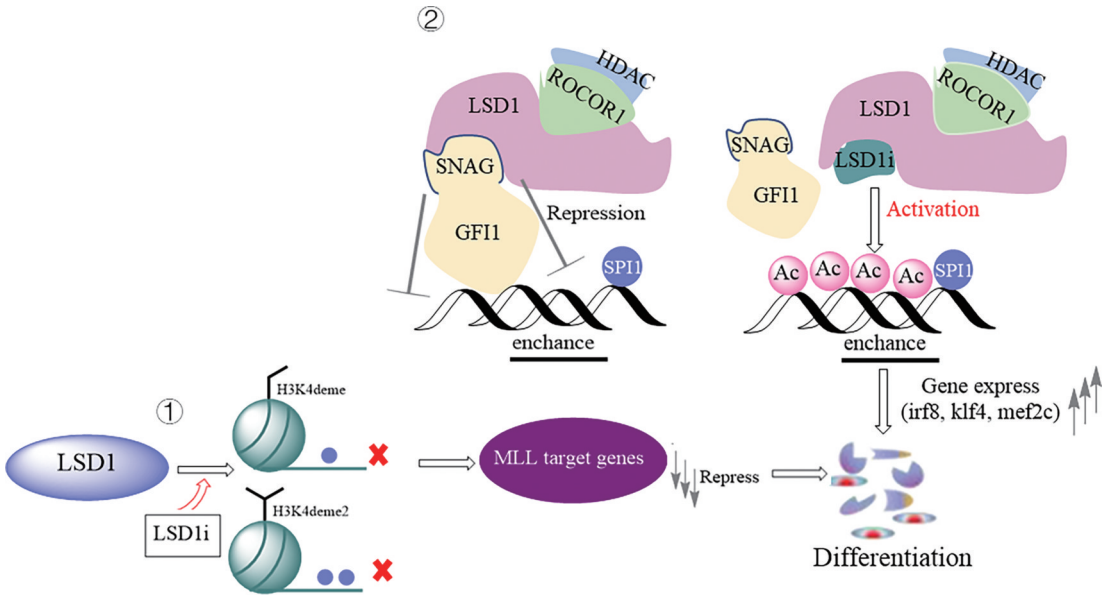


Figure 11 Two potential mechanism of lysine-specific demethylase 1A (LSD1) inhibitors regulating hematopoietic stem cell differentiation

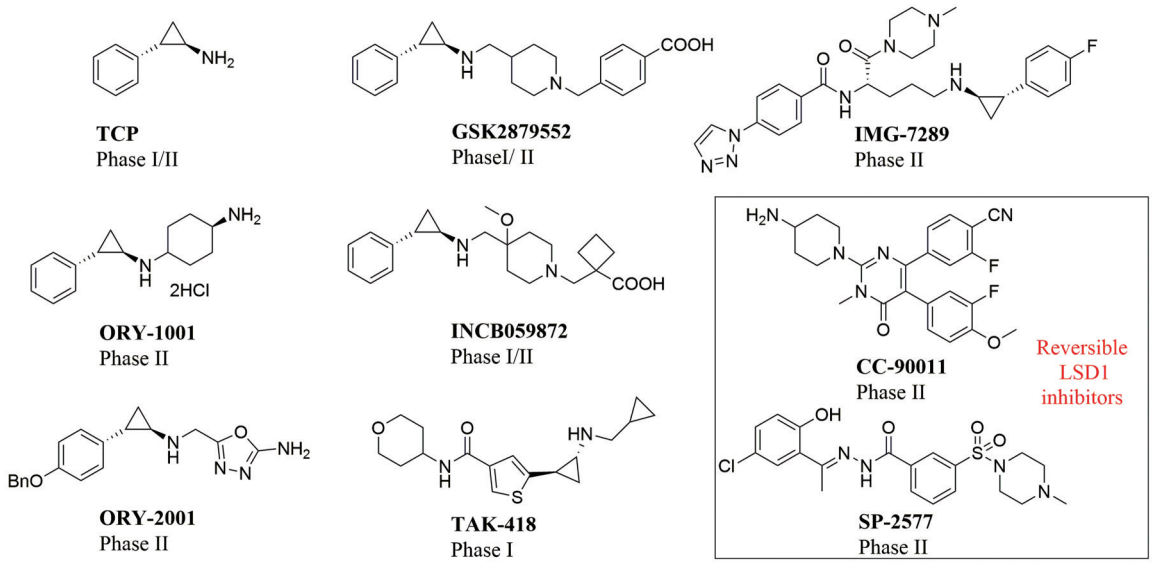
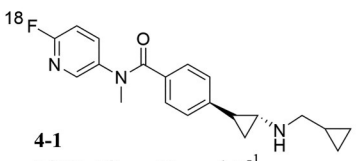
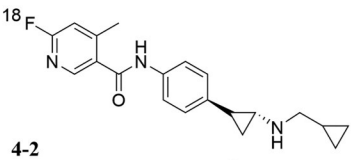


Figure 12 Chemical structures of LSD1 inhibitors in clinical trials

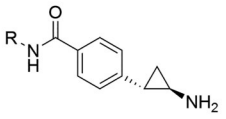
TCP derivatives



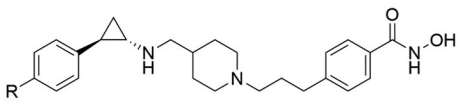
4-1
LSD1: IC_{50} = 11 nmol·L^{-1}



4-2
LSD1: IC_{50} = 11 nmol·L^{-1}

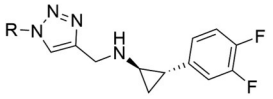


4-3 R=Bn
LSD1: IC_{50} = 0.3 μmol·L^{-1}
4-4 R=Phenylethyl
LSD1: IC_{50} = 0.4 μmol·L^{-1}

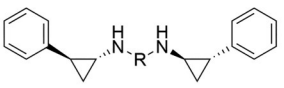


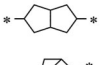
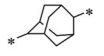
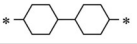
4-5 R=H
LSD1: IC_{50} = 5 nmol·L^{-1}
HDAC6: IC_{50} = 48 nmol·L^{-1}

4-6 R=F
LSD1: IC_{50} = 6 nmol·L^{-1}
HADC6: IC_{50} = 51 nmol·L^{-1}

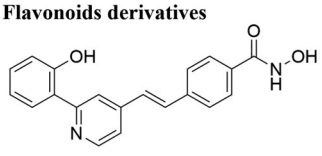


4-7 R=4-methoxyphenyl
IC_{50} = 17 nmol·L^{-1}
4-8 R=2,5-dimethoxyphenyl
IC_{50} = 11 nmol·L^{-1}

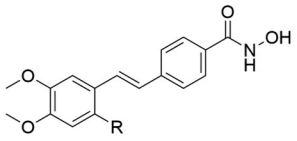


No.	R	LSD1 (IC_{50}, nmol·L^{-1})	MV4-11 (IC_{50}, μmol·L^{-1})
4-9		7.29	0.14
4-10		22.90	0.01
4-11		8.25	0.03
4-12		36.22	0.18

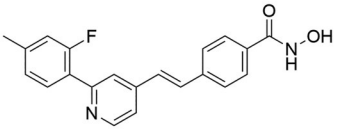
Flavonoids derivatives



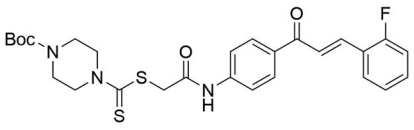
4-13
LSD1: IC_{50} = 0.93 μmol·L^{-1}
HDAC6: IC_{50} = 47 nmol·L^{-1}



4-15 R=F
LSD1: IC_{50} = 0.197 μmol·L^{-1}
4-16 R=Br
LSD1: IC_{50} = 0.123 μmol·L^{-1}

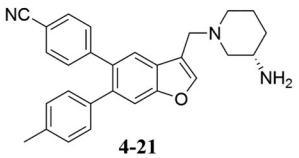


4-14
LSD1: IC_{50} = 1.64 μmol·L^{-1}
HDAC6: IC_{50} = 15 nmol·L^{-1}



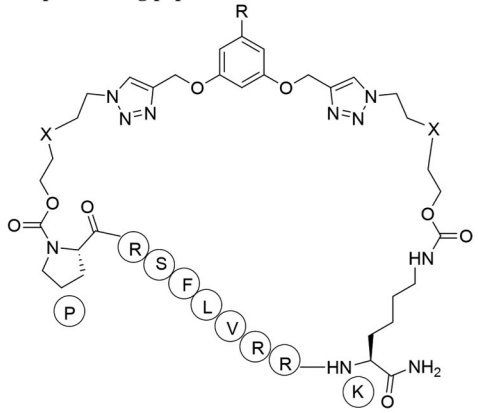
4-17
LSD1: IC_{50} = 0.14 μmol·L^{-1}

Others

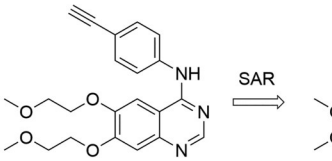


4-21
LSD1: IC_{50} = 65 nmol·L^{-1}

Cell-penetrating peptide

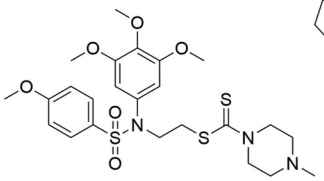


No.	X	R	LSD1/CoREST (K_i, μmol·L^{-1})
4-18	*—S-S—*	*–C(=O)-$(Arg)_{10}$-NH_2	0.06
4-19	*—O—*	*–C(=O)-$(Arg)_{10}$-NH_2	0.08
4-20	*—S-S—*	*–H	0.64

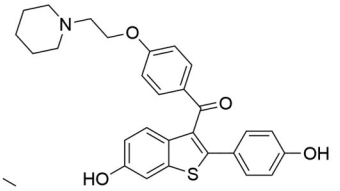


Erlotinib

4-22
LSD1: IC_{50} = 0.69 μmol·L^{-1}



4-23
LSD1: IC_{50} = 63 nmol·L^{-1}



4-24 (Raloxifene)
LSD1: IC_{50} = 2.08 μmol·L^{-1}

Figure 13 Chemical structures of LSD1 inhibitors reported in 2021

作为显像剂的**4-1**[115]和**4-2**[110]可用于检测脑内LSD1的表达水平, 这为因LSD1表达失调所引起的神经发育障碍类疾病提供了治疗的新思路。化合物**4-3**和**4-4**在AML细胞系中, 以剂量依赖的方式抑制了细胞中LSD1的生物活性, 具有一定的抗白血病能力[107]。**4-5**和**4-6**[109]是一类靶向LSD1和HDAC6的双靶抑制剂。其中, **4-5** (EC_{50} = 2 nmol·L^{-1}) 在多发性骨髓瘤细胞系中表现出优越的抑制活性。通过改造TCP的结构, Huang等[114]发现了可有效抑制LSD1的小分子抑制剂**4-7**和**4-8**。2021年, Sun等[108]基于TCP的结构, 以饱和的脂肪环作为连接子, 得到了一类新颖的二价LSD1抑制剂**4-9**至**4-12**。这些抑制剂对MV4-11细胞株显示了良好的抗增殖能力, 具有潜在的治疗白血病的活性。从结构上分析, 化合物**4-13**至**4-17**可视为黄酮类衍生物。其中, **4-17**[112]是一种新型查尔酮衍生物, 具有潜在的抗白血病活性。通过分析LSD1/CoREST的晶体结构, Kitagawa等[113]设计并合成了一系列环肽类LSD1抑制剂**4-18**至**4-20**。此类抑制剂在细胞内可被选择性激活, 转化为含有细胞穿透肽的氧化还原激活环肽并在低微摩尔浓度范围内抑制AML细胞的增殖。

4-21[106]代表了一类含苯并呋喃结构的新型的LSD1抑制剂, 可有效抑制LSD1的酶活性和肿瘤细胞的增殖。基于老药新用策略, Li等[111]发现经FDA批准的肺癌药物EGFR抑制剂erlotinib对LSD1具有一定的抑制能力, 经优化改造得到的**4-22**对LSD1抑制能力明显增强。**4-23**是一种靶向微观蛋白聚合和LSD1的双重抑制剂, 可用于治疗肝癌。Ma等[119]通过化合物库筛选, 发现**4-24** (raloxifene) 可与LSD1结合从而抑制肾癌细胞的增殖和迁移, 从而证明了LSD1可能也是治疗肾癌的潜在药物靶点。

5 PRMT5抑制剂

1999年, 学者从酵母双杂交系统中发现了具有一定甲基转移酶活性[120]、且能在组蛋白H2A和H4上添加甲基化标记的蛋白[121], 并将其命名为组蛋白精氨酸甲基转移酶5 (protein arginine methyltransferase 5, PRMT5)。PRMT5全长637个氨基酸, 其中包括TIM桶状结构、Rossmann折叠和β桶状蛋白[122], 后面两者是PRMT5主要的催化结构域。PRMT5参与的表观遗传和翻译后修饰对细胞生长与增殖是必需的, 具有重要的生物学功能。近来研究表明, PRMT5是治疗MLL的潜在药物靶点。首先, PRMT5的甲基转移酶活性的持续表达可促进体内白血病细胞的生长与发育, 而PRMT5抑制剂可通过抑制H4R3双甲基化的表达直接或间接的下调细胞中FLT3的表达, 以实现体内AML的有效抑制[123] (图14)。此外, PRMT5抑制剂也可通过介导细胞周期环素依赖性激酶抑制剂p21 (CDKN1a)的转录沉默阻断细胞分化最终实现对MLL的有效抑制[124]。

目前报道的PRMT5抑制剂大部分是根据PRMT5的结构以及甲基转移的生化过程来设计开发的, 主要分为底物竞争性抑制剂 (图15)[125-129]和SAM竞争性抑制剂 (图16)[130-137]两大类, 目前有4个小分子处于临床试验阶段 (图17)。

化合物**5-1**是首次报道的底物竞争性抑制剂, 具有良好的选择性和较好的药物代谢动力学性质[125]。而后, PRMT5的底物竞争性抑制剂**5-2**至**5-6**被先后报道[138]。其中值得注意的是, Epizyme公司研发的**GSK3326595**于2016年进入临床I期试验, 评估其在晚期或复发性实体瘤和非霍奇金淋巴瘤患者中的安全性、药代动力学和临床活性; 2018年, 该化合物进入临床II期试验, 以评估其用于治疗MDS和AML患者的安全性和临床活性。

2017年, Janssen公司对SAM进行结构改造, 得到了高选择性、高活性的SAM竞争性抑制剂**JNJ-64619178**, 目前正处于I期临床研究阶段, 用于评价治疗实体瘤和非霍奇金B细胞淋巴瘤的效果。学者们受到Janssen公司对PRMT5的SAM竞争性抑制剂研发成功的鼓舞, 相继展开了大量研究。

罗成课题组[131]基于药效团和分子对接的虚拟筛选, 相继发表了化合物**5-7**、**5-8**和**5-9**。**5-9**对PRMT5具有

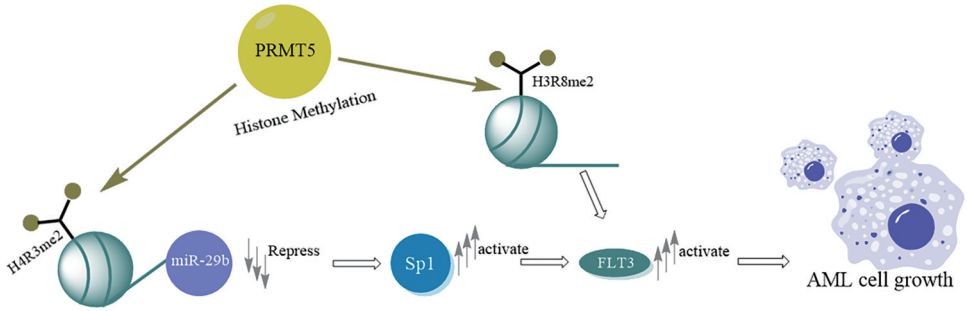


Figure 14 Mechanism of protein arginine methyltransferase 5 (PRMT5) regulating leukemia, PRMT5 inhibitors reverse this process by inhibiting PRMT5′s histone methylation activity

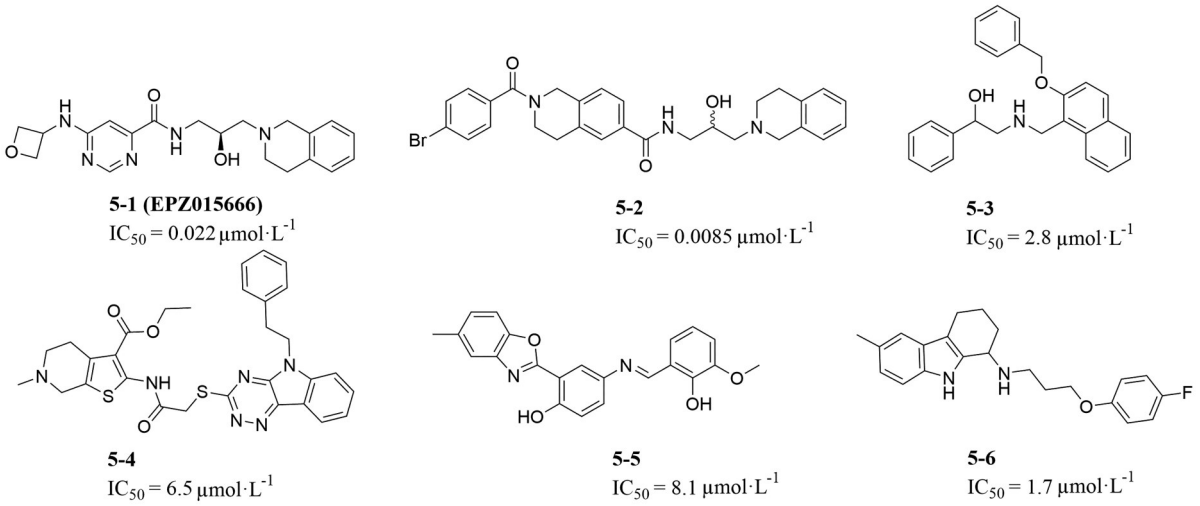


Figure 15 Chemical structures of substrate competitive PRMT5 inhibitors

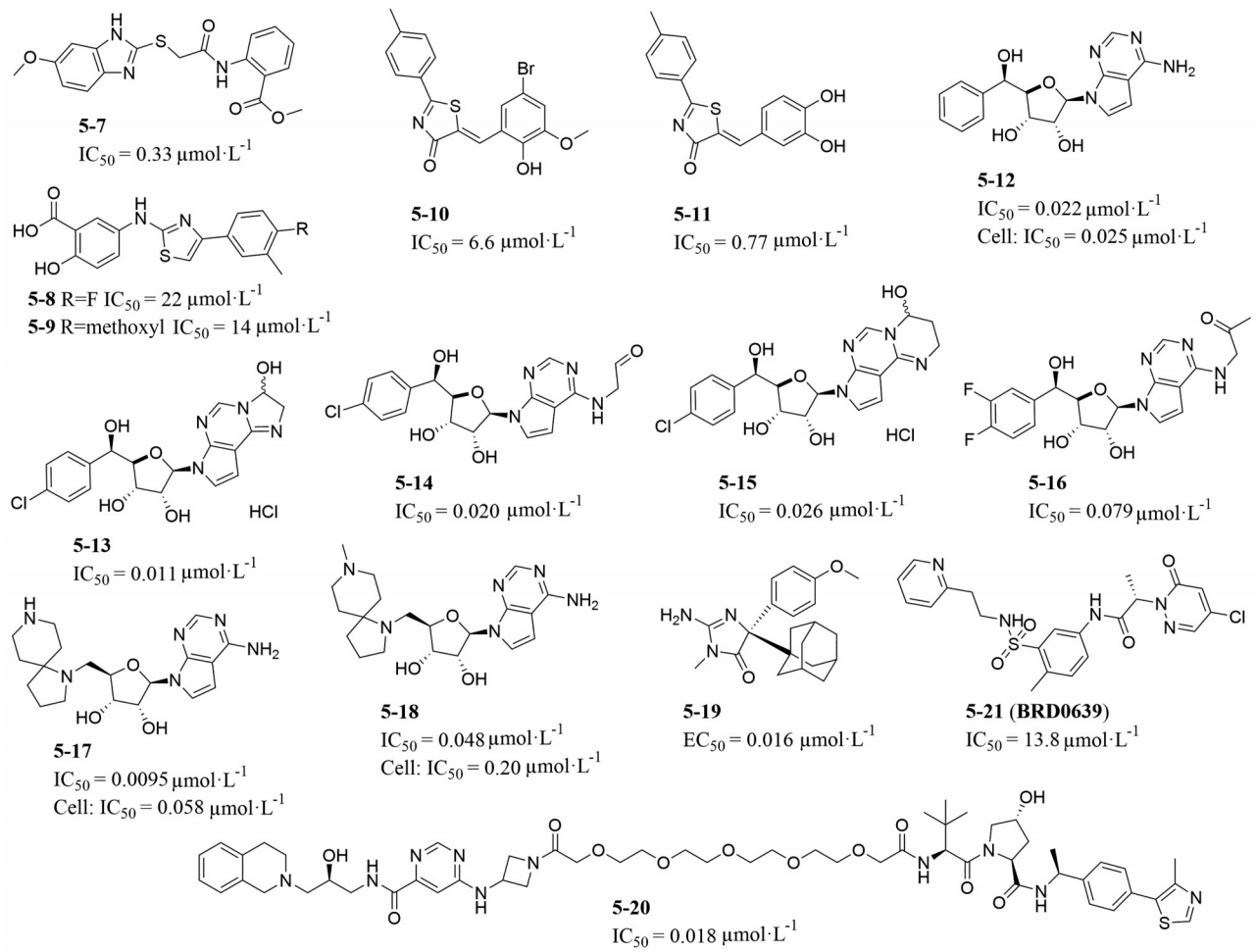


Figure 16 Chemical structures of SAM competitive PRMT5 inhibitors

良好的选择性。2018, Zhu等[133]报道了一类具有5-亚苄基-2-苯基噻唑酮 (5-benzylidene-2-phenylthiazolone) 结构的新型SAM竞争性抑制剂**5-10**和**5-11**。同年, 礼来公司[132]对SAM进行结构改造, 得到了在体内外活性都较好的选择性PRMT5抑制剂**5-12**。

在PRMT5蛋白的SAM结合位点附近存在的Cys449为开发共价PRMT5抑制剂提供了可能性。2019年, Prelude公司[134]报道了一类具有半缩醛胺结构的PRMT5共价抑制剂**5-13**, 该抑制剂在生理条件下转变为醛**5-14**, 然后与PRMT5蛋白的Cys449形成共价

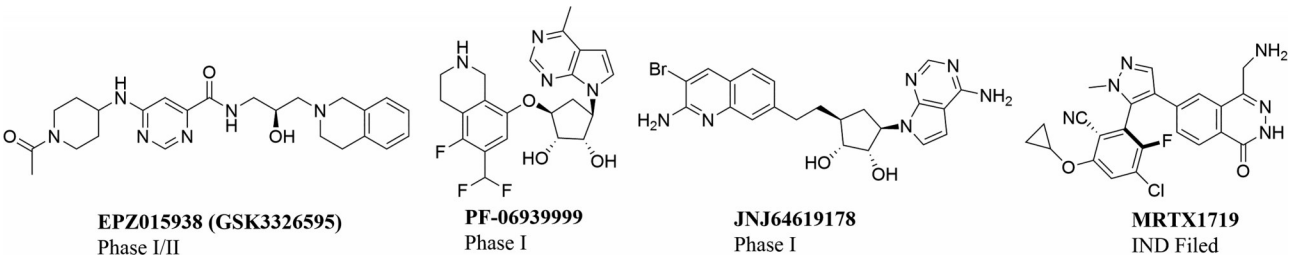


Figure 17 Chemical structures of PRMT5 inhibitors in clinical trials

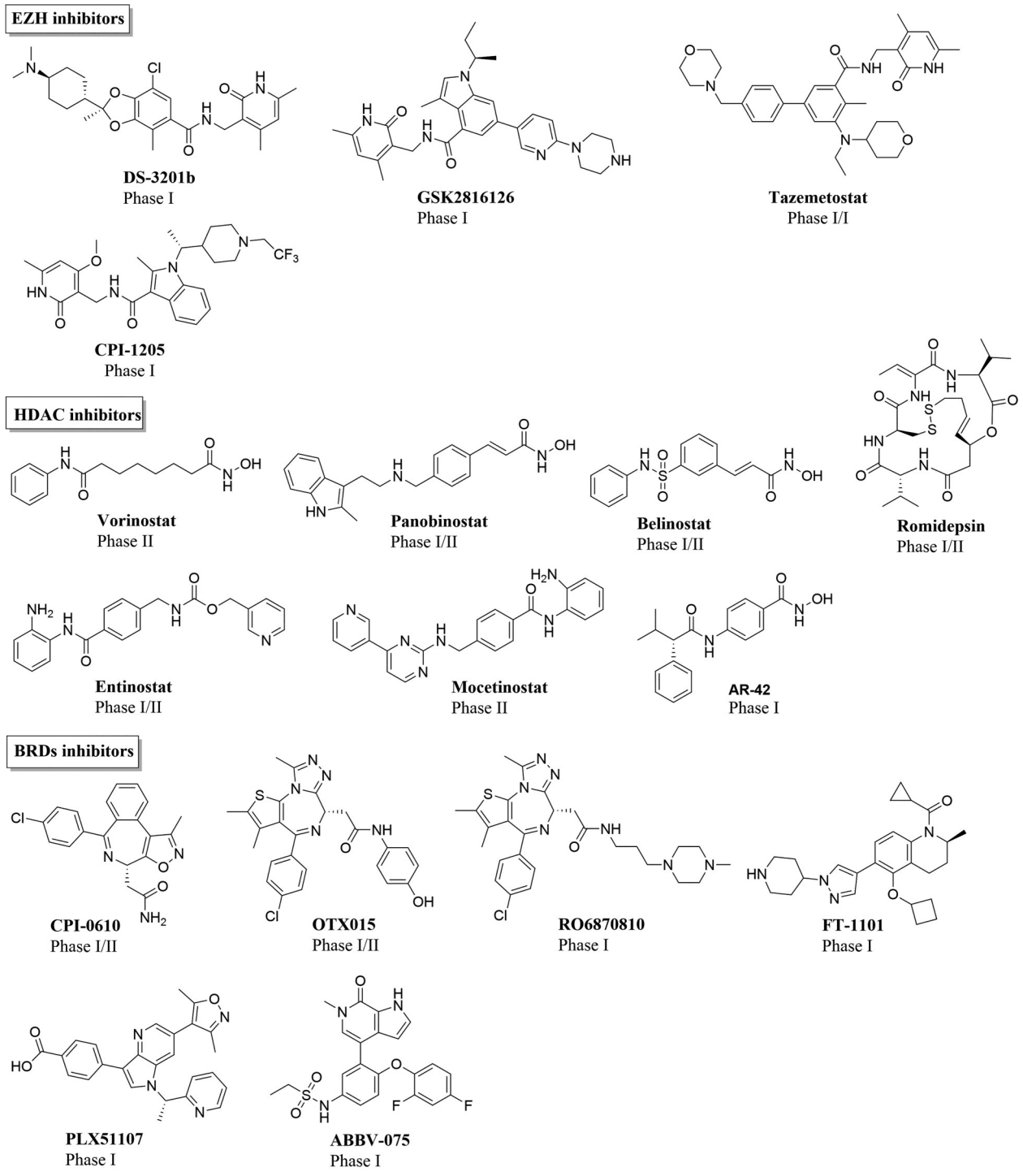


Figure 18 Chemical structures of EZH, HDAC and BRDs inhibitors for MLL in clinical trials

加合物, 进而抑制PRMT5的活性。

2020年, Janssen公司[135]设计了一类含有螺环胺的PRMT5抑制剂**5-17**和**5-18**, 可同时占据SAM结合口袋和部分的底物结合口袋, 从而显现出优良的细胞活性和较高的选择性。Merck公司[139]通过高通量筛选发现了一个之前作为BACE1和BACE2抑制剂的小分子**5-21**对PRMT5具有抑制活性。值得关注的是, 此化合物结合在PRMT5蛋白的变构位点, 是一类结构新颖的小分子抑制剂。同年, Jin课题组[137]基于**EPZ015666**, 通过对连接子 (linker) 和结合E3泛素连接酶的配体进行简单的构效关系 (structure activity relationship, SAR) 研究, 发现了第一个选择性靶向PRMT5的蛋白降解靶向嵌合体 (proteolysis targeting chimeric, PROTAC) 分子**5-20**。**5-20**在多种肿瘤细胞株中均能有效降低PRMT5蛋白水平, 抑制肿瘤细胞生长, 并在小鼠的药代动力学研究中表现出了良好的血浆暴露水平。PRMT5及其底物衔接蛋白 (substrate adaptor proteins, SAPs) pICln和Riok1是MTAP缺失癌细胞的合成致死的关键, SAPs共享一个保守的PRMT5结合基序 (PRMT5 binding motif, PBM)。对于一些PRMT5底物甲基化来说, 该基序与PRMT5之间的相互作用是需要的。**5-21**[136]是一种有效的PBM竞争性抑制剂, 它能使靶细胞与细胞结合, 并减少底物甲基化。

6 其他

此外, 还有大量的表观遗传学靶点 (如EZH、HDAC、BRDs等) 具有潜在的治疗MLL的能力[140-150]。目前针对这些靶点已有较多的小分子抑制剂进入临床试验阶段。Wong等[151]在2020发表的文章中对这些小分子用于治疗白血病的临床进展进行了总结, 本文在此仅对结构进行简要展示 (图18)。

7 总结与展望

随着近年来大量研究, 人们对表观遗传调节因子和蛋白复合物是如何通过与MLL-FPs协作以启动和维持基因过表达从而引发白血病的过程有了更为深入的了解。这种复杂的基因调控网络为MLL的治疗提供了一系列新的调控策略, 并涌现了大量可用于治疗MLL的潜在药物靶点。研究者们针对这些靶点也设计、开发了多种新颖结构的小分子抑制剂。部分抑制剂对MLL的治疗显示出较好的临床前或临床结果。但目前还未有针对MLL的表观遗传学相关靶点的小分子抑制剂获批上市。因此, 针对亟需有效治疗MLL的新药开发仍然是目前的研究热点, 希望本文的综述为该类药物提供参考。

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