浅析抗病毒药物化学的底层逻辑、创新思维与研究范式

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摘要: 抗病毒药物研发是当前及未来生物医药领域的重要研究方向。抗病毒药物研发不仅需要新策略与新技术的应用, 更需要项目团队优势互补、密切配合。本文根据该领域的最新进展, 结合笔者的药物研究实践, 总结了抗病毒药物化学的底层逻辑、创新思维与研究范式。

关键词: 药物化学; 病毒感染; 药物设计; 研究范式; 小分子药物

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The underlying logic, innovative thinking and research paradigm of antiviral medicinal chemistry

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Abstract: Antiviral drug research and development is an important research direction in the current and future biomedical field. The research and development of antiviral drugs not only requires the application of new strategies and new technologies, but also requires the complementary advantages and close cooperation of project teams. Based on the latest progress in this field and the author's drug research practice, this paper summarizes the underlying logic, innovative thinking and research paradigm of antiviral medicinal chemistry.

Key words: medicinal chemistry; viral infection; drug design; research paradigm; small molecule drug

1 抗病毒药物的临床需求与关键科学问题

目前, 病毒感染性疾病是世界上发病率最高的疾病, 此外新的病毒不断被发现并造成广泛传播。不断变化的自然环境以及病毒具有的人际传播的能力, 使病毒性传染病往往具有较高的致病率和致死率。特异性的疫苗与药物被认为是抗击突发病毒疫情最重要的“武器”之一, 然而, 对大多数新发病毒而言, 依然缺乏有效疫苗和特效药物[1,2]。因此, 抗病毒药物的研发仍具有重要意义。

小分子药物是指一类能够靶向作用于蛋白、降低蛋白活性或者阻碍生化反应、相对分子质量小于1 000的化合物。小分子抑制剂在口服吸收性、透细胞以及透屏障 (如血脑屏障) 给药、成药性能、药物代谢动力学性质、工业化生产等方面具有显著优势。因此, 小分子化合物成为抗病毒药物研究的重点领域。

尽管如此, 目前小分子抗病毒药物依然存在未满足的临床需求。例如, 针对高价值靶点的药物研究无实质性的突破; 上市药物涉及的靶标过于集中, 同质化严重; 药物分子的骨架及结构新颖性不足; 首创药物的数量非常有限等。总之, 无论对于慢性传染病还是突发传染病, 高效、低毒、抗耐药性的抗病毒药物依然是未满足的重大临床需求和迫切需要解决攻克的“卡脖子”难题。

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药物的发现有其自身的规律性, 从靶标结构确证、先导化合物的发现和优化到临床试验, 整个流程周期长、投入大且成功率低。无论临床需求多么迫切, 突发疫情多么严重, 药物的发现与临床试验都要遵循基本原理与科学方法, 并不以人的意志为转移。因此, 总结抗病毒药物化学的底层逻辑、创新思维与研究范式, 对于当前及未来的抗病毒药物研发将具有指导意义。

2　抗病毒药物化学的底层逻辑

古语"为学须有本原, 须从本原上用力"底层逻辑就是本原, 即遵从事物的底层与本质, 寻找解决问题路径的思维方法。万变不离其宗, 以不变应万变。病毒学与感染免疫、耐药机制的基础知识以及基本的生物和化学原理, 就是抗病毒药物研究的底层逻辑, 基础越坚固, 学术创新和新药创制的能力就越强。例如, 蛋白降解和核酸降解等多特异性结合策略以及相分离、共价结合与动态组合化学成为抗病毒药物领域的前沿方向[3-8], 其背后的底层逻辑就是化学邻近效应, 明白了其基本原理, 也就清楚了该原理在抗病毒药物研究领域的适用性。

药物与靶标的结合模式[9]、药物设计中的多价效应[10-12]以及超分子药物设计等的底层原理是化学热力学与动力学、熵焓效应 (图1)[13], 掌握这些基本原理, 不仅有助于提高精准药物设计的成功率, 而且有助于技术创新。例如, 自从1992年DNA编码化合物库 (DNA encoded libraries, DELs) 概念提出以来, 由于其库容量大、化学空间广阔以及筛选通量高等优势, 近年来DELs的设计与筛选已成为当前药物研发领域的前沿技术之一[14]。该技术通过模拟自然进化过程中的优胜劣汰原则, 用特异性的DNA序列标记分子的合成过程及结构信息, 将可扩增的"基因型"与无法自我复制的"化学表型"相联系。通过多步的化学合成, 探索数量庞大和结构多样的化学空间。

总之, 底层逻辑是学术创新的基石, 是为学的本原, 正因为如此, 笔者认为, 在研究生科研训练中, 应加强学术前沿与化学基本原理之间的联系, 强化底层逻辑。

3　抗病毒药物研究中的创新思维

众所周知, 归纳与演绎是两种重要的思维方式。此外, 逆向思维, 也称求异思维, 它是对似乎已成定论以及司空见惯的观点或事物反向思考的一种思维方式。根据这几种思维方式, 结合抗病毒药物研究的代表成果, 笔者将该领域的创新思维方式可分为求同思维、求异思维和仿生思维 (图2), 下文结合具体实例进行阐释。

3.1　抗病毒药物研究中的求同思维

在过去的二十年里, 暴发过三次高致病性冠状病毒感染疫情, 包括2002年的非典肺炎疫情、2012年的中东呼吸综合征以及2019年新型冠状病毒 (SARS-CoV-2) 引起的新型冠状病毒感染 (COVID-19) 疫情。由于缺乏安全有效的药物, 每一次疫情的暴发对人类

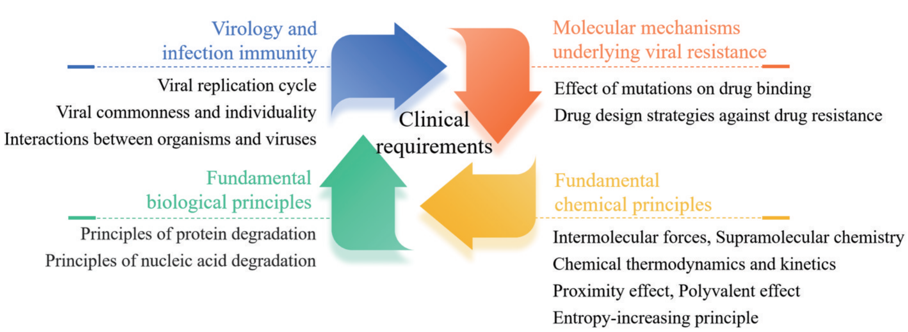

Figure 1　Underlying logic of antiviral medicinal chemistry

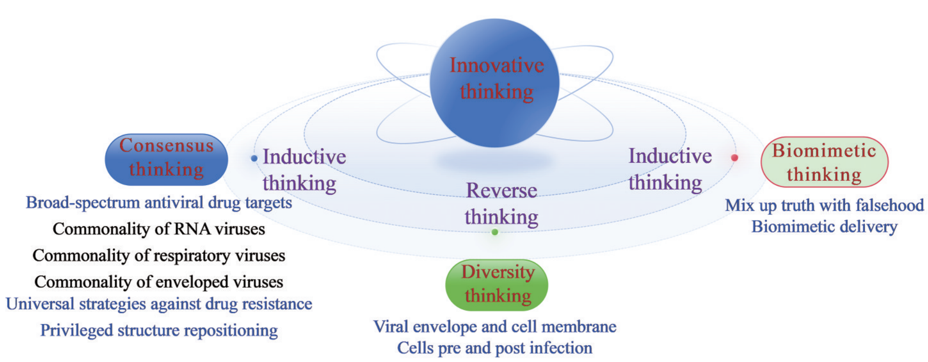

Figure 2　Innovative thinking in antiviral medicinal chemistry

都造成了巨大威胁, 这凸显了研发广谱抗病毒药物的极端重要性。在抗SARS-CoV-2药物应急攻关中, 研究者不约而同地从具有抗SARS冠状病毒 (SARS-CoV) 活性的分子以及具有共性特征的抗病毒药物靶标入手, 采取老药新用策略[15,16], 致使瑞德西韦 (remdesivir) 及多种蛋白酶抑制剂上市, 这就是求同思维的最鲜明体现。

在后疫情时代, 研究广谱抗病毒药物的着力点依旧是重点关注病毒复制周期中的共性环节或蛋白, 包括聚合酶、蛋白酶、甲基转移酶以及螺旋酶等共性靶标[17-27]。例如, 近期研究者报道了一类猴痘病毒甲基转移酶VP39抑制剂, 并揭示了与SARS-CoV-2 nsp14甲基转移酶的相似性。此外, 膜融合等病毒感染共性环节和宿主蛋白也是广谱抗病毒药物研究备受瞩目的方向[28-30]。

高效抗耐药性是优秀的抗病毒药物的基本特征。针对新发病毒以及新颖靶标的药物设计, 可以从具有普适性的抗耐药性药物设计策略获得启迪, 例如靶向保守区域、形成主链氢键、保持构象的柔性和底物包膜假说等[31-33]。

此外, 在抗病毒药物策略方面, 靶向蛋白二聚体(分子胶)、蛋白降解、核酸降解、抗体募集、纳米酶以及共价药物设计等均是普适性的策略[34-36]。例如, "侦察片段"作为化学蛋白质组学分析的小分子探针, 可以与蛋白质的催化位点或变构位点形成共价键。近期, 研究者基于"侦查片段"策略, 开发了选择性地靶向构象动态的"机械酶"SARS-CoV-2解旋酶nsp13变构位点的共价抑制剂。"侦察片段"有望成为开发其他"机械酶"的共价抑制剂的普适方法[37]。

优势结构是可以与多个靶点高亲和力结合的"共性"片段。在对靶标共性特征认识的基础上, 将优势结构的灵活运用, 如采用"优势结构再定位"策略, 可以大大提高药物发现的成功率[38-40]。最典型的例子是抗流感病毒原创药物巴洛沙韦 (baloxavir, **2**) 的发现, 源自研究者对流感内切酶与HIV整合酶共同特征的认识——二者均属于二价镁离子依赖性蛋白, 其活性的发挥需要镁离子的催化。参考多替拉韦 (dolutegravir, **1**) 中螯合镁离子的三环结构, 引入与流感病毒内切酶催化位点契合的取代基便得到**2** (图3)[41,42]。

王正强课题组及笔者课题组运用"优势结构再定位"策略, 发现了一系列的HIV RNA核糖核酸酶H (HIV ribonuclease H, HIV RNase H) 抑制剂[43]、人巨细胞病毒 (human cytomegalovirus, HCMV) pUL89-C核酸内切酶抑制剂[44,45]及HIV整合酶抑制剂[46]。

早在2007年, 香港大学孙红哲团队发现铋类化合物具有抗SARS-CoV活性[47,48]。2020年, 该团队发现临床常用含有金属铋的抗溃疡药物——枸橼酸铋雷尼替丁 (ranitidine bismuth citrate), 在细胞水平及动物水平均可以有效抑制SARS-CoV-2感染, 这是基于求同思维的"老树新花"式学术创新的经典案例 (详见4.1部分)[49]。

3.2　抗病毒药物研究中的求异思维

病毒膜与宿主细胞膜在组成与化学成分上具有很大的类似性, 但是病毒大小远远小于细胞, 因此二者的曲率存在明显差别。鉴于此, 研究者设计合成了一系列特异性靶向病毒膜的分子钳, 通过与病毒膜结合, 从而极大增强病毒膜的表面张力, 包膜破裂会导致寨卡病毒、埃博拉病毒和流感病毒等感染性完全丧失。例如化合物CP025 (**3**, 图3) 抑制SARS-CoV-2 (EC_{50} = 6.9 μmol·L^{-1}), 且对Caco-2细胞无明显细胞毒性 (CC_{50} = 106.1 μmol·L^{-1})。**3**对不同包膜病毒的EC_{50}分别为6.1 μmol·L^{-1} (呼吸道合胞病毒, respiratory syncytial virus, RSV)、3.2 μmol·L^{-1} (甲型流感病毒, influenza A virus, IAV)、7.0 μmol·L^{-1} (麻疹病毒, measles virus, MeV) 和1.1 μmol·L^{-1} (单纯疱疹病毒, herpes simplex virus, HSV-1)[50-53]。

细胞被病毒感染前后膜表面成分存在差别, 例如流感病毒感染的细胞表面会有病毒自身的糖蛋白[54]。为了利用这个差别, 研究者设计并合成了一种双功能小分子, 通过将神经氨酸酶抑制剂扎那米韦 (zanamivir) 与具有高度免疫原性的半抗原二硝基苯偶联, 该半抗原能特异性靶向游离病毒和病毒感染细胞的表面, 这不仅抑制了释放出来的病毒, 而且清除了免疫介导产生的游离病毒和感染细胞。在给予100倍半数致死量

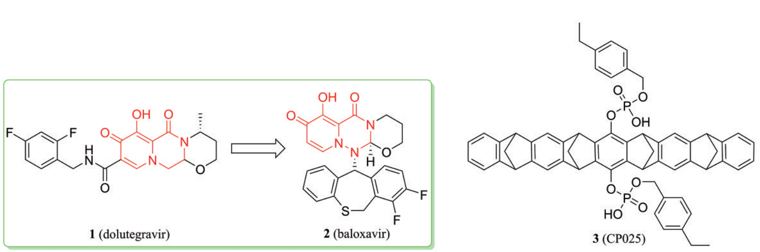

Figure 3　Chemical structures of compounds **1**−**3**

浓度的流感病毒进行晚期感染时, 在小鼠体内单次给药可以根除甲型和乙型流感毒株。重症感染的治疗在接种致死疫苗后3天内仍有效, 该方法显示出很好的应用前景[55]。

在病毒感染宿主的过程中, 病毒离子通道蛋白会诱导宿主膜表面的pH发生改变。鉴于此, 研究人员成功建立了对pH敏感的聚合物探针, 并精确测定了被病毒感染的宿主细胞膜的酸化水平。并从中筛选出一种可以特异性结合并裂解病毒包膜的聚合物, 最终证明这一新型聚合物具有广谱高效的抗病毒效果[56]。

3.3　抗病毒药物研究中的仿生思维

疫苗是预防病毒感染的有效手段。当前临床使用的疫苗, 或因病毒灭活致免疫原性和安全性差, 或因制备工艺复杂而不通用, 或因病毒突变致免疫逃逸失效。

通常来说, 疫苗对病毒的信息还原程度越高其效应潜力越大, 病毒本身就是最仿真的疫苗。中国科学家[57]以流感病毒为模型, 发明了人工控制病毒复制技术, 通过突变病毒基因组三联码将病毒直接转化为疫苗。该技术简化了疫苗的开发过程, 颠覆了传统的病毒疫苗开发理念, 且具有通用性。

冠状病毒作为RNA病毒, 极易突变。其最关键的抗原信息存在于病毒表面冠状突起顶端的受体结合域 (receptor binding domain, RBD) 蛋白上, 这种空间离散的结构难以被免疫系统有效识别。针对冠状病毒的特点, 研究者用聚合物材料重塑病毒“真身”, 发现了增强免疫识别的仿病毒结构纳米颗粒疫苗 (viromimetic polymer nanoparticle vaccine, VPNVax)[58]。VPNVax通过马来酰亚胺–巯基的点击化学反应将冠状病毒的RBD蛋白重新排列并密集分布修饰到聚合物纳米颗粒表面。这种模块化策略相较基于融合蛋白技术的VLP (virus-like particle) 疫苗具有显著优势, 例如可替换抗原蛋白以快速响应不同病毒变种的暴发、可灵活调控纳米颗粒疫苗表面抗原密度 (价数) 并能实现从亚单位蛋白向颗粒疫苗的直接转化, 有利于快速大规模制备。

生物中和是利用治疗剂与有害分子或传染性病原体结合, 阻断其生物活性, 从而防止其引起疾病的一种策略。与传统的中和策略相比, 细胞纳米海绵创造仿生诱饵与病毒中和, 从而抑制病毒进入真正的宿主细胞。研究表明, SARS-CoV-2在与受体蛋白细胞血管紧张素转换酶2 (angiotensin converting enzyme 2, ACE2) 结合之前, RBD首先与肝素或硫酸乙酰肝素等膜成分相互作用, 导致增强了开放构象S蛋白与ACE2的结合。因此, 当SARS-CoV-2进入人体后, 就会被细胞纳米海绵上的受体所欺骗“吸走”病毒, 从而抑制病毒感染细胞[59-61]。

已知几何匹配有利于在自然系统中形成稳定的生物相互作用。鉴于此, 研究者报道了一种以DNA正四面体作为载体连接SARS-CoV-2适配体的DNA纳米结构, 该结构与Spike蛋白三聚体的等边三角形构象互补, 实现了空间结构的多价相互作用, 可高效阻断Spike三聚体与宿主ACE2的相互作用, 抑制SARS-CoV-2感染[62]。类似地, 研究者构建了一种与IAV病毒相匹配的针状纳米结构, 可高效抑制病毒复制[63]。

蛋白质作为病毒正常生命活动和结构组成所必需的共性物质, 为人们操控病毒进而利用病毒、开发蛋白质调控的病毒减毒策略提供了契机。例如, 抑制或阻断蛋白质合成可以减少子代病毒组装所需的“原料”生产; 还可以加速蛋白质降解以及时将子代病毒组装所需的“原料”清除。研究者基于合成生物学理念, 以流感病毒为模式病毒, 建立了蛋白降解靶向病毒作为减毒疫苗的技术-PROTAC疫苗 (proteolysis-targeting chimeric virus vaccine), 通过操控病毒蛋白质的降解降低病毒的复制能力, 将野生型病毒减毒成为疫苗, 为疫苗开发提供了新思路[64,65]。此外, 还可以将PROTAC技术与表型筛选相结合, 探明抗病毒药物的作用靶标[66,67]。

4　抗病毒药物化学的研究范式

抗病毒药物化学的创新思维包括演绎、归纳等思维方式, 具体研究中需要多方协作, 有效的合作需要差异化与互补性。笔者将具体研究范式分为老树新花式拓展、优势互补型合作、化学赋能型融通和领域开辟型创新 (图4)。

4.1　老树新花式拓展

Arnold E擅长晶体学片段筛选 (crystallographic fragment screening) 技术, 将之成功用于HIV逆转录酶及流感病毒内切酶等靶标[68-70]; Jorgensen WL多年深耕自由能微扰技术, 陆续将之用于HIV逆转录酶、SARS-CoV-2蛋白酶等靶标抑制剂的发现与优化[71-78]。Hannon MJ[79-82]多年来一直在基于配位超分子的病毒RNA配体领域深耕, 取得一系列创新成果。Gendelman HE近几年的工作通过聚焦基于抗HIV前药的研发, 发现了一系列长效核苷前药 [M1TFA (**4**)、M2TFA (**5**)] 及HIV整合酶抑制剂前药 [M2CAB (**6**)、MDTG (**7**)] (图5)[83-86]。

在抗SARS-CoV-2药物研发中, 多个团队通过“老树新花”式创新, 将积淀多年的技术或资源用于抗SARS-CoV-2药物研发, 做出了创新性的成果, 例如杨朝勇团队的核酸适配体[87-90]、曲晓刚团队的G四连体[91-93]、王磊团队的蛋白共价[7,94,95]以及孙红哲团队的

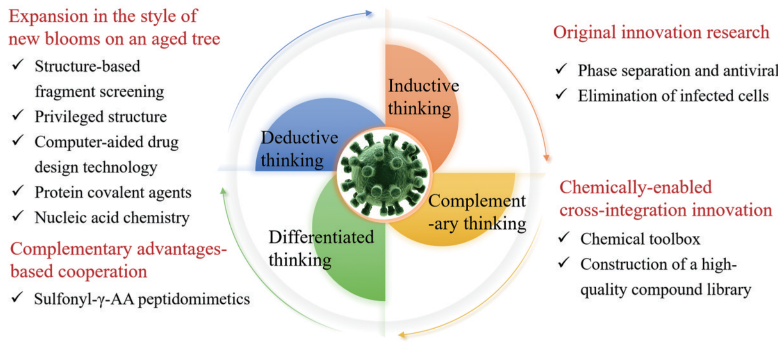

Figure 4　Research paradigm of antiviral drug research and development.

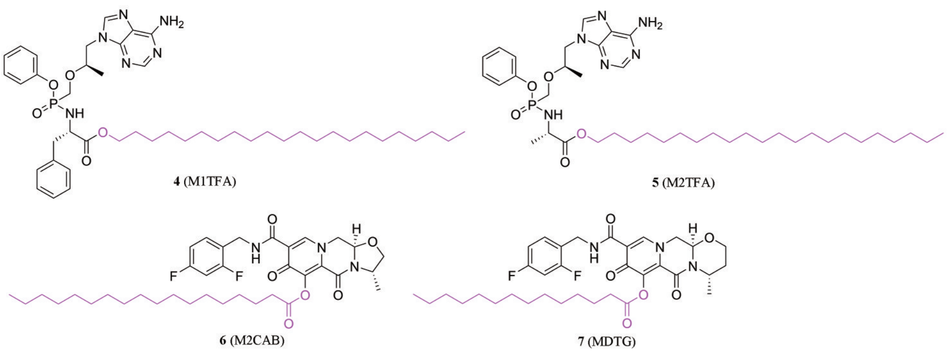

Figure 5　Anti-HIV agents based on long chain fatty acid prodrug

铋类配合物[47-49]等。

4.2　优势互补型合作

美国南佛罗里达大学蔡健峰教授团队一直致力于基于磺基-γ-AA的螺旋拟肽的研究和应用。复旦大学姜世勃/陆路团队长期从事基于病毒融合过程的肽类抑制剂。针对肽类药物具有较低的生物稳定性和生物利用度、半衰期较短的缺点，鉴于拟肽折叠体具有稳定的和可预测的结构、结构修饰多样化等优点，可以用于提高肽类药物的半衰期和口服利用度。

鉴于此，蔡健峰和姜世勃/陆路团队强强联合、优势互补，共同研发了基于γ-AA拟肽的HIV及冠状病毒融合抑制剂[96-98]，例如发现一种模拟七肽重复序列2 (heptad repeat 2, HR2) 的磺酰基-γ-AA螺旋拟肽是一种有效的泛冠状病毒融合抑制剂，具有很强的血脑屏障通透性、长半衰期和良好的口服生物利用度。此外，蔡健峰团队开发的AApeptides (γ-取代-*N*-酰化-*N*-氨基乙基拟肽) 拟肽结构可以抵抗蛋白水解，具有易于官能团化的巨大潜力和广阔的应用潜力。鉴于此，与王俊课题组合作发现了基于AApeptides结构的广谱冠状病毒蛋白酶抑制剂[99]。

笔者课题组发挥抗病毒药物化学特色优势，与国际知名的结构生物学家Müller CE、Arnold E等合作，发现了一系列抗SARS-CoV-2及HIV候选药物[100-102]。

4.3　化学赋能型融通

4.3.1　基于高效反应的抗病毒药物化学　大环是重要的抗病毒化合物类型。发展新颖成环反应以快速且高效地构建结构多样性的大环库，是发现抗病毒活性分子的有效途径。近期，杨伟波课题组运用Rh (III) 催化双分子双碳氢键活化大环化反应和Rh (III) 催化C-H/O_2双活化大环化发现了新颖的流感病毒H1N1抑制剂 (**8**、**9**)[103,104]。柳红课题组运用Pd (II) 催化的C-H烯化反应发现了新颖的SARS-CoV-2核蛋白抑制剂**10** (图6)[105]。

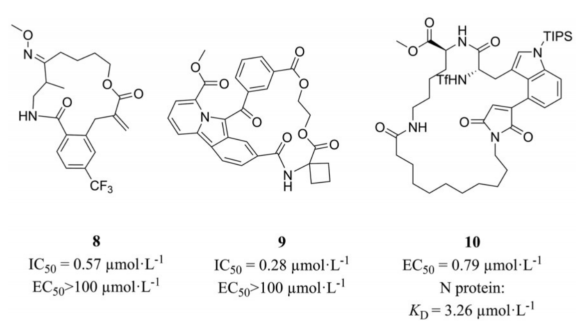

Figure 6　Antiviral agents based on macrocyclic reactions

点击化学是用于构筑活性分子的有效工具[106,107]。本课题组运用该反应发现了痘病毒和腺病毒抑制剂 (**11**、**12**)[108]、HIV整合酶抑制剂**13**[46]、HIV衣壳调控剂

14[109]及HIV非核苷类逆转录酶抑制剂 (**15**、**16**)[110,111]; 此外, 还结合微量合成与直接筛选技术[112,113], 发现了新颖的HIV非核苷类逆转录酶抑制剂 (**17**、**18**)[114,115]及SARS-CoV-2主蛋白酶抑制剂 (**19**、**20**)[116], 见图7, 成为候选药物研发的优质“种子”。

六价硫氟交换反应作为新一代点击化学[117], 正逐渐在抗病毒药物研究领域得以应用。例如, 针对靶向蛋白–蛋白相互作用靶标的共价抑制剂存在的选择性和反应性等问题, 2023年, 研究者用六价硫氟交换反应建立了一个高通量的共价抑制剂筛选的通用平台, 从数万亿个磺酰氟修饰的寡核苷酸中发现共价抑制剂, 靶向SARS-CoV-2 S蛋白与ACE2之间互作界面[118]。

蛋白质药物往往具有高度的特异性, 但它们的非共价相互作用常常导致药物解离和不完全抑制。针对该问题, 2022年, 研究者[7]报道了能够通过近端激活的反应性治疗 (proxime-enabled reactive therapeutic, PERx) 机制与SARS-CoV-2不可逆结合的共价纳米抗体。研究者设计了一种潜在的生物反应性氨基酸, 并将其基因编码到纳米抗体中以加快PERx反应速率。与非共价野生型纳米抗体相比, 加入生物反应性氨基酸的共价纳米抗体以显著更高的效力中和了野生型SARS-CoV-2及其Alpha、Delta、Epsilon、Lambda和Omicron变异株。这种PERx激活的共价纳米抗体策略有望用于开发针对各种病毒感染的普适疗法。

多组分反应是构筑多样性和立体性分子库的常用工具[119], 目前Ugi四组分反应已用于SARS-CoV/SARS-CoV-2抑制剂 (**21**～**24**)[120-124]及HIV衣壳调控剂**25**的发现[125](图8)。

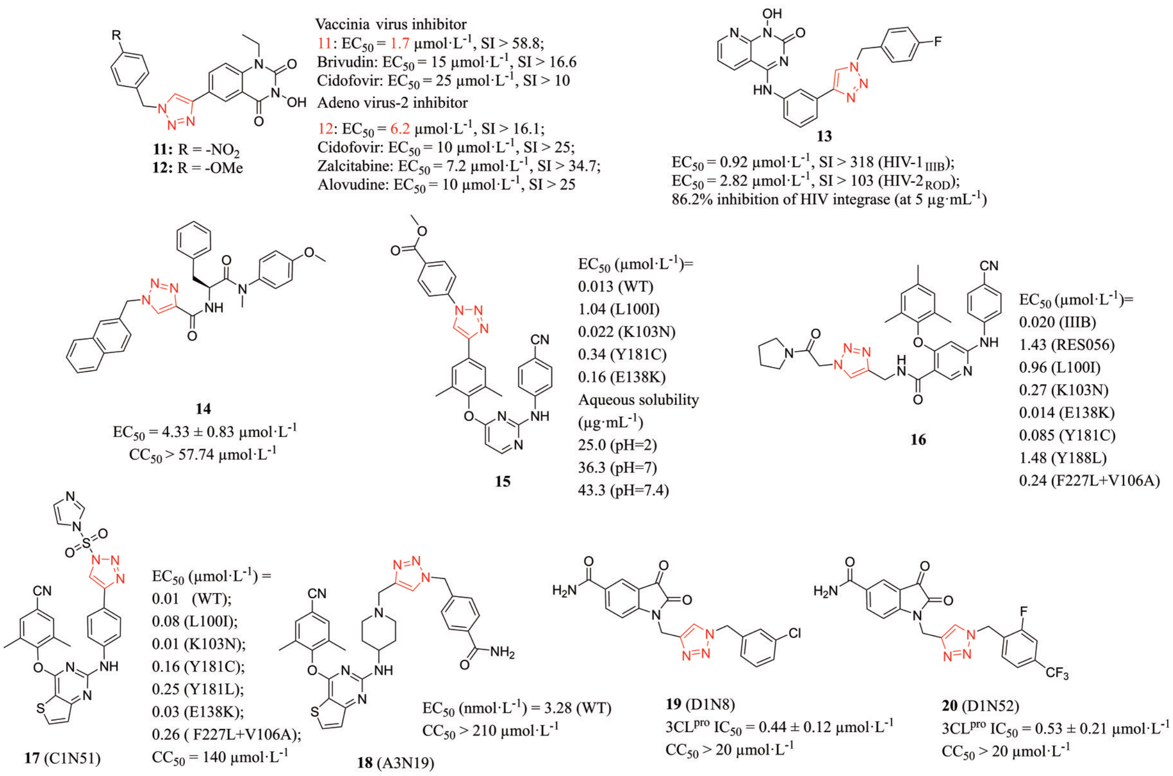

Figure 7　Antiviral agents based on CuAAC click chemistry

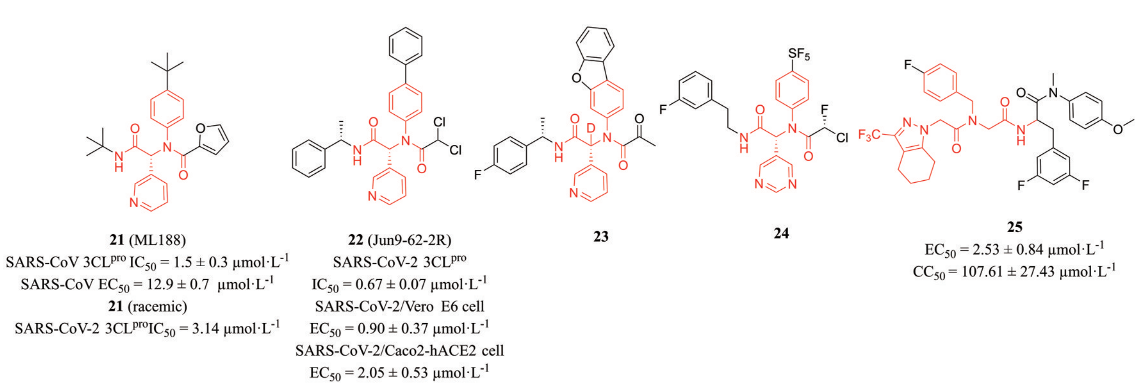

Figure 8　Antiviral agents based on multi-component reactions

生物催化是抗病毒药物合成工艺优化的重要方法。近期, 改造的胞苷脱氨酶及核糖基-1激酶分别用于核苷类抗SARS-CoV-2药物莫诺拉韦 (molnupiravir) 的合成[126,127], 这些优化的方法大幅提高了反应收率, 缩短了反应步骤, 该类生物催化方法有望在简化核苷类合成方面有广泛的应用。

4.3.2　基于优势结构的抗病毒药物化学　优势结构可以与多个靶点高亲和力结合, 从而有助于新型生物活性药物的发现[40,128]。类肽类化合物是一类重要的抗病毒活性分子[129]。本课题组基于类肽结构的HIV衣壳调控剂PF74 (**26**), 经过基于靶标的合理设计发现了磺酰胺哌嗪类化合物**27**和**28** (图9)[130,131]。

在化学基团的功能性模块化方面, Protide技术是最常见的抗病毒药物前药技术之一[132]。近年来, 长链脂肪酸成为另一类重要的前药类型[133], 逐渐应用于抗HIV药物研发中[83-86]。苯硼酸和磺酰胺是抗病毒活性化合物中频繁出现的"高效药效团元素" (或称"优势药效基团")[134,135]。例如, 含有苯硼酸的HIV蛋白酶抑制剂**29** (图10)[136]、HIV非核苷类逆转录酶抑制剂**30**和**31**[137,138]、流感病毒神经氨酸酶抑制剂**32**[139]及HBV衣壳抑制剂**33**和**34**等[140,141]。笔者课题组发现了一系列含有苯磺酰胺的HIV非核苷类逆转录酶抑制剂**35**～**54** (图11)[142-153]。此外, 值得关注的"高效药效团元素"还包括硒元素[154]和磺酸基团[155]。

4.3.3　基于新颖技术的抗病毒药物化学　DNA编码库技术多次用于SARS-CoV-2主蛋白酶抑制剂**55**～**57**的发现 (图12A)[156-158]。特别是WU-04 (**57**) 有效阻断了人细胞中的SARS-CoV-2复制, EC_{50}值在10 nmol·L^{-1}内。**57**也能高效抑制SARS-CoV和MERS-CoV的主蛋白酶 (3-chymotrypsin-like protease, 3CL^{pro}), 表明它是冠状病毒3CL^{pro}的泛抑制剂。**57**在K18-hACE2小鼠体内表现出与奈玛特韦相似的抗SARS-CoV活性。因此, **57**是一种很有前景的冠状病毒治疗候选药物[158]。

高通量微量合成和高通量蛋白质晶体学的联合运用加快了SARS-CoV-2主蛋白酶抑制剂的发现[159]。动态组合化学技术广泛用于病毒蛋白酶抑制剂的发现, 包括HIV蛋白酶抑制剂**58**、EV-A71蛋白酶抑制剂**59**、SARS-CoV蛋白酶抑制剂**60**及肠病毒D68蛋白酶抑制剂**61**等 (图12B)[160-163]。

后期官能团化 (late-stage functionalization, LSF) 是优化药物特性和研究构效关系的便捷途径。药物化学中的LSF方法包括氟化、胺化、芳基化、甲基化、三氟甲基化、硼化、酰化和氧化。其中光催化分子内氢胺化反应、铃木反应等后期官能团化用于抗病毒活性分子的结构优化, 得到了抗冠状病毒分子**62**、流感病毒抑制剂**63**和**64** (图12C)[164-166]。不过, 药物分子的化学复

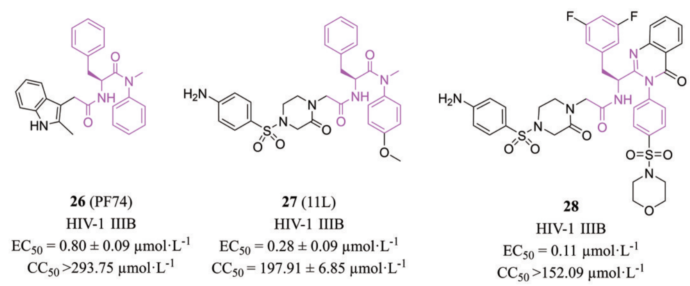

Figure 9　Peptidomimetic HIV capsid modulators

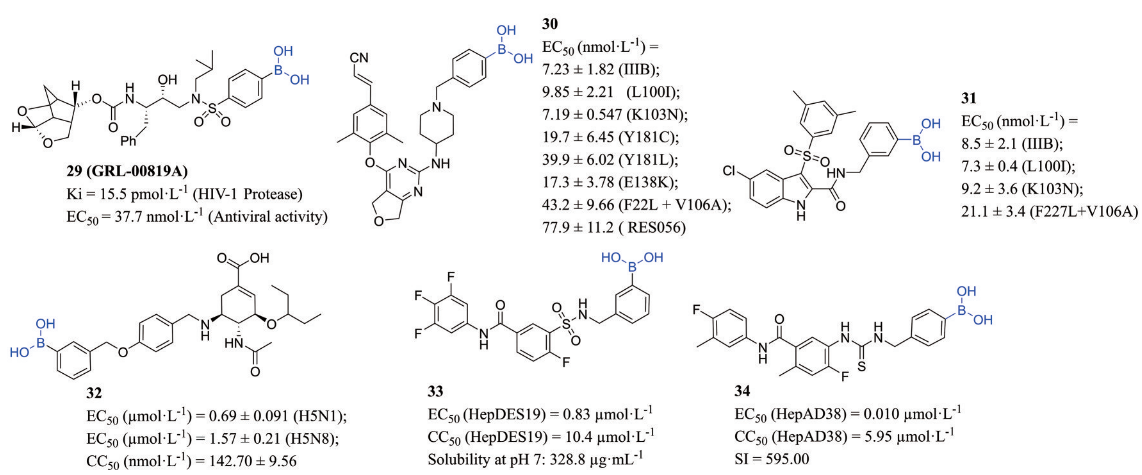

Figure 10　Antiviral agents containing phenylboronic acid motif

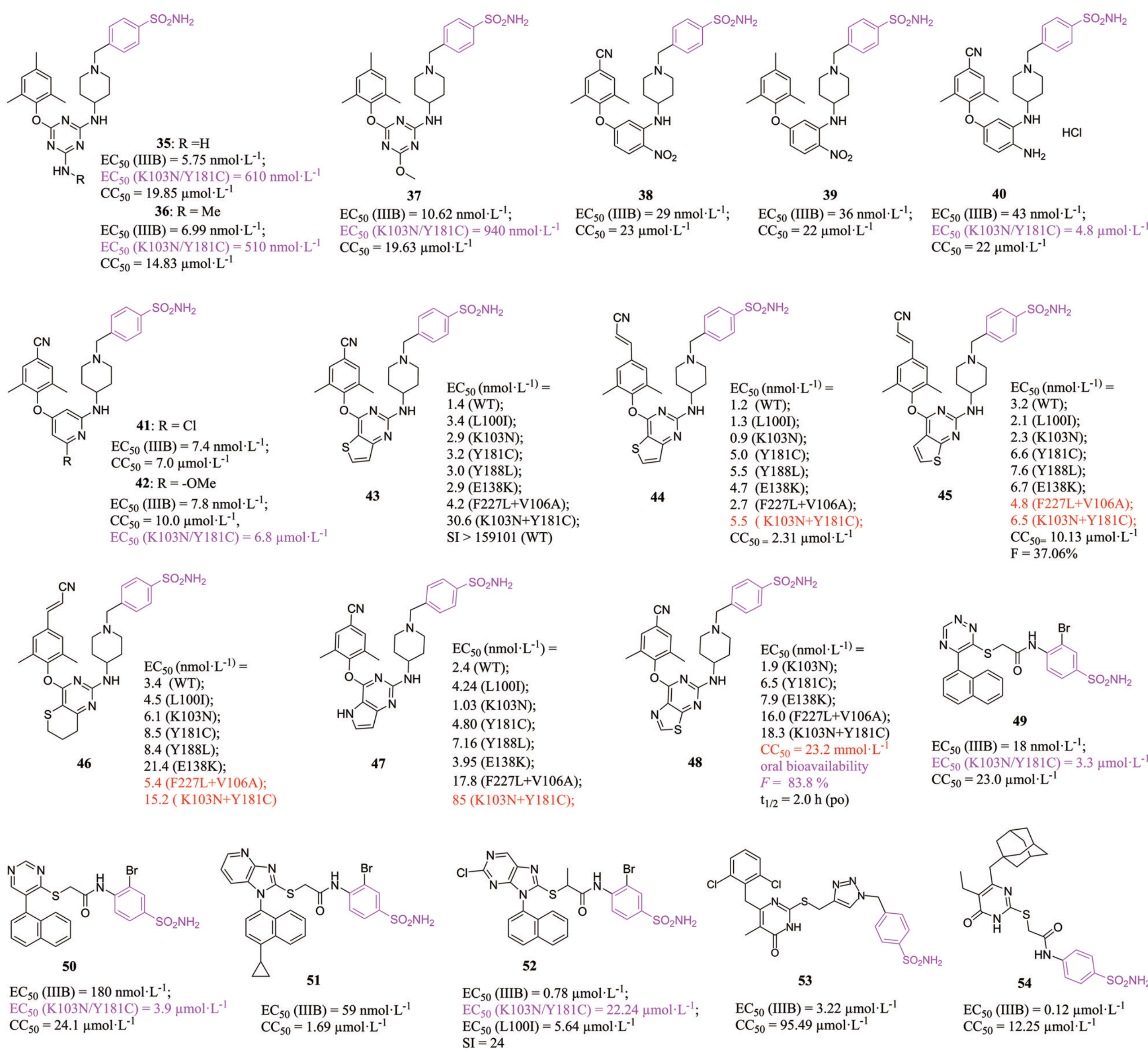

Figure 11　HIV non-nucleoside reverse transcriptase inhibitors containing benzene sulfonamide motif

杂性往往会让LSF面临诸多挑战。已报道的大多也都集中在单一的LSF反应类型上。与传统实验形式相比, 高通量实验能实现半自动化的快速高效率筛选, 结合数据分析和机器学习, 能极大拓展LSF在药物发现领域的应用[167]。

增加sp³杂化碳原子的比例 (fraction of sp^3 carbon atoms, Fsp^3) 有可能覆盖更广泛的生物学相关化学空间。已经表明, 增加Fsp^3特性可以改善几种化合物特性并有助于临床成功[168]。特别是, 在3D结构中掺入面外官能团可以潜在地实现更强的受体/配体相互作用, 从而提高对给定靶标的效力和选择性。本课题组成功将Fsp^3类药性参数用于HIV非核苷类逆转录酶抑制剂的结构优化, 得到了活性及成药性均较好的分子**65**～**67** (图12D)[169-171]。

4.4　领域开辟型创新

生物大分子凝聚体已经变成多种亚细胞结构组装的重要机制之一。许多病毒的复制发生在病毒诱导产生的内含体或者病毒发生基质之中。负链RNA病毒的内含体是由液–液相分离介导形成的凝聚体。最近, 研究者们发现液–液相分离介导形成的凝聚体可以通过类固醇生物碱类环丙胺药物处理逐渐硬化, 从而造成RSV感染后RNA复制过程的抑制, 为RSV感染的治疗提供了新见解[172]。这项开辟性研究表明通过靶向凝聚物形成以及病毒复制至关重要的蛋白, 对开发广谱活性药物具有重要参考价值[173]。

抗逆转录病毒治疗可抑制HIV-1复制, 但由于病毒已经整合入基因组, 此方法无法达到治愈的效果, 因此减少病毒的蓄积十分重要。2023年研究者报道了

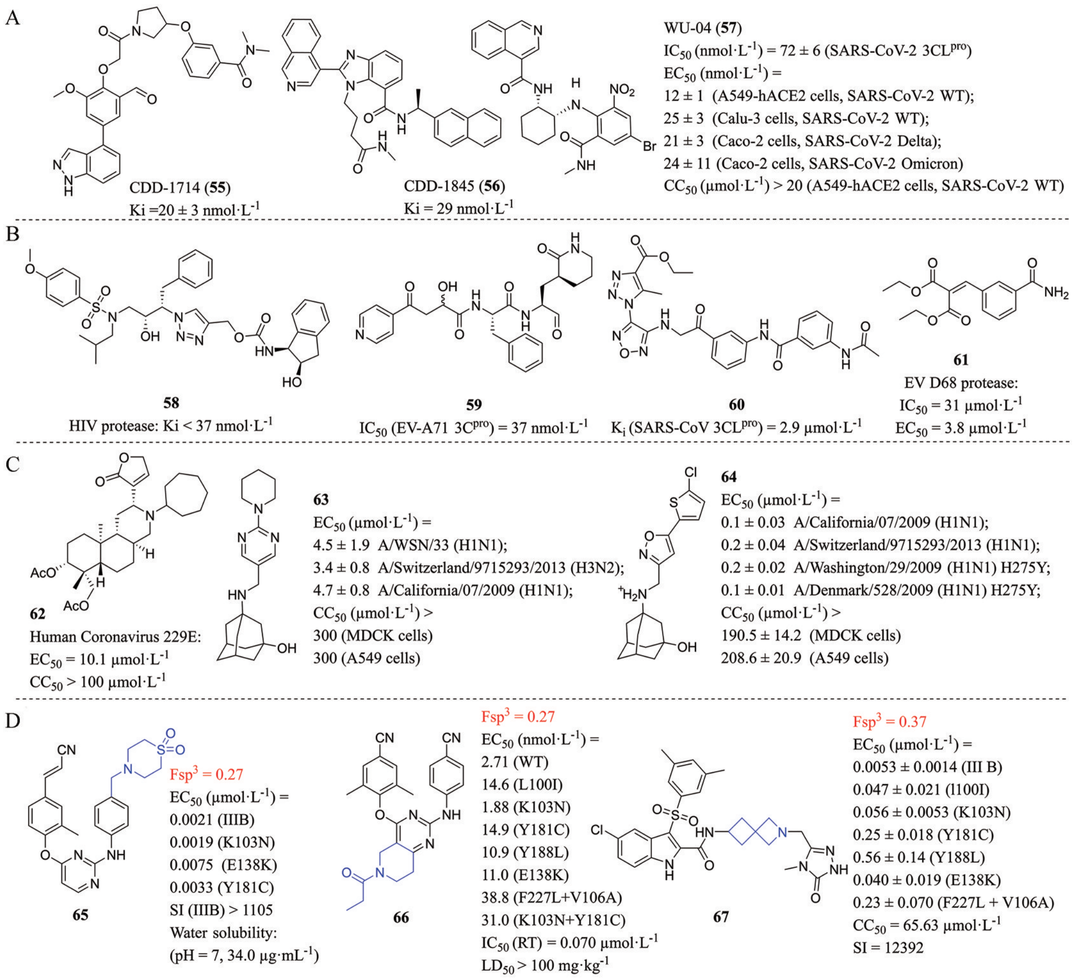

Figure 12 Chemical structures of compounds **55−67**. A: Discovery of SARS-CoV-2 main protease inhibitors based on DNA-encoded chemistry technology; B: Discovery of viral protease inhibitors based on dynamic combinatorial chemistry; C: Antiviral agents discovered by late-stage functionalization; D: Discovery of HIV non-nucleoside reverse transcriptase inhibitors based on Fsp^3 parameter

一类非核苷逆转录酶抑制剂, 可作为细胞杀伤分子靶向激活剂, 通过与HIV逆转录酶结合, 产生别构效应, 加速细胞杀伤, 其具有强大的抗病毒活性, 能够有选择性地消除HIV-1病人的感染CD4$^+$ T细胞, 可作为免疫非依赖型的清除策略[174]。近期研究发现, 衣壳蛋白调控剂通过诱导细胞凋亡来根除具有高核心蛋白水平的HBV感染的肝细胞, 这可能是一种有希望实现功能性治愈的方案[175]。

5 总结与展望

尽管结构生物学和计算化学的发展大大促进了小分子抗病毒药物的研发, 但是要做到"指哪打哪"的真正精准设计, 依然存在一定的难点。例如病毒蛋白靶标的极易突变、蛋白互作界面的诱导契合效应、构象的柔性等, 导致配体结合位点难以预测; 一些金属离子依赖型病毒蛋白, 配体与之结合所需要的配位作用是动态的多方参与 (蛋白、核酸、金属离子) 的过程; 当前前沿的共价结合策略, 其共价键的形成也往往是多方协作的过程。AI技术在药物研发领域的驱动作用尚处于起步阶段[176]。

以上因素导致目前的抗病毒药物化学主流依然是合理设计与通量筛选联合运用, 这不仅体现在新冠疫情期间的攻关研发[177-179], 而且在抗登革病毒药物研发中也有广泛体现[180-182]。

抗病毒药物化学研究需要"致广大而尽精微", 优秀的研究者应"无所不用其极", 不仅要不遗余力地学习并运用最先进的药物设计策略与技术, 要善于将经典策略用于新场景中抗病毒药物的研发, 例如多靶标策略在发现广谱抗耐药抗病毒药物方面常用常新[183]; 更要注重整合与集成式创新, 例如DNA编码库与共价结合的整合[184]、PROTAC与分子胶的结合[185]、DNA编码库与动态组合化学的集成[186]、自组装与蛋白降解的融合[187]等。可以坚信整合创新也必将是抗病毒药物领域的极具前景的研究范式。作为信息化、智能化时代的药物化学研究者, 除了做到以上几点, 更要实时总

结复盘实践经验, 用于指导新一轮实践, 形成知行合一的闭环, 是药物研发的精进之道。例如抗病毒药物化学“三易”原则, 即不易 (保守位点、优势结构、成药参数)、变易 (构象灵活、新颖骨架、全新机制)、简易 (最小修饰、热点残基、模块反应), 毕竟抗病毒药物研发是策略、技术与经验紧密融合的系统性工程。

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利益冲突: 所有作者均声明不存在利益冲突。

References

[1] Meganck RM, Baric RS. Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases [J]. Nat Med, 2021, 27: 401-410.

[2] Edwards AM, Baric RS, Saphire EO, et al. Stopping pandemics before they start: lessons learned from SARS-CoV-2 [J]. Science, 2022, 375: 1133-1139.

[3] Stanton BZ, Chory EJ, Crabtree GR. Chemically induced proximity in biology and medicine [J]. Science, 2018, 359: eaao5902.

[4] Liu X, Ciulli A. Proximity-based modalities for biology and medicine [J]. ACS Cent Sci, 2023, 9: 1269-1284.

[5] Zhou Y, Xu SJ, Ding D, et al. Recent advances of antiviral drugs based on multispecific binding strategy [J]. Acta Pharm Sin (药学学报), 2023, 58: 2203-2217.

[6] Sang X, Wang J, Zhou J, et al. A chemical strategy for the degradation of the main protease of SARS-CoV-2 in cells [J]. J Am Chem Soc, 2023, 145: 27248-27253.

[7] Yu B, Li S, Tabata T, et al. Accelerating PERx reaction enables covalent nanobodies for potent neutralization of SARS-CoV-2 and variants [J]. Chem, 2022, 8: 2766-2783.

[8] Yu B, Cao L, Li S, et al. The proximity-enabled sulfur fluoride exchange reaction in the protein context [J]. Chem Sci, 2023, 14: 7913-7921.

[9] Zhan P, Wang X, Liu X. Contemporary molecular targeted drug in the context of "precision medicine": an attempting discussion of "precision drug design" [J]. Prog Chem, 2016, 28: 1363-1386.

[10] Ramos-Soriano J, Reina JJ, Illescas BM, et al. Synthesis of highly efficient multivalent disaccharide/[60]fullerene nanoballs for emergent viruses [J]. J Am Chem Soc, 2019, 141: 15403-15412.

[11] Muñoz A, Sigwalt D, Illescas BM, et al. Synthesis of giant globular multivalent glycofullerenes as potent inhibitors in a model of Ebola virus infection [J]. Nat Chem, 2016, 8: 50-57.

[12] Patino-Alonso J, Cabrera-González J, Merino J, et al. C60-based multivalent glycoporphyrins inhibit SARS-CoV-2 specific interaction with the DC-SIGN transmembrane receptor [J]. Small, 2024, 20: e2307045.

[13] Kawasaki Y, Freire E. Finding a better path to drug selectivity [J]. Drug Discov Today, 2011, 16: 985-990.

[14] Ma P, Zhang S, Huang Q, et al. Evolution of chemistry and selection technology for DNA-encoded library [J]. Acta Pharm Sin B, 2024, 14: 492-516.

[15] Huang T, Sun L, Zhan P, et al. Recent advances in the research of broad-spectrum antiviral agents [J]. Acta Pharm Sin (药学学报), 2020, 55: 679-693.

[16] Li J, Jiang X, Xu S, et al. Medicinal chemistry strategies in seeking coronavirus inhibitors [J]. Acta Pharm Sin (药学学报), 2020, 55: 537-553.

[17] Lu L, Su S, Yang H, et al. Antivirals with common targets against highly pathogenic viruses [J]. Cell, 2021, 184: 1604-1620.

[18] Dsouza L, Pant A, Offei S, et al. Antiviral activities of two nucleos(t)ide analogs against vaccinia, mpox, and cowpox viruses in primary human fibroblasts [J]. Antiviral Res, 2023, 216: 105651.

[19] Guo S, Zhang Y, Liu Z, et al. Brincidofovir is a robust replication inhibitor against African swine fever virus *in vivo* and *in vitro* [J]. Emerg Microbes Infect, 2023, 12: 2220572.

[20] Wang J, Shahed-Ai-Mahmud M, Chen A, et al. An overview of antivirals against monkeypox virus and other orthopoxviruses [J]. J Med Chem, 2023, 66: 4468-4490.

[21] Noble CG, Li SH, Dong H, et al. Crystal structure of dengue virus methyltransferase without *S*-adenosyl-*L*-methionine [J]. Antiviral Res, 2014, 111: 78-81.

[22] Dostalik P, Krafcikova P, Silhan J, et al. Structural analysis of the OC43 coronavirus 2'-*O*-RNA methyltransferase [J]. J Virol, 2021, 95: e0046321.

[23] Rosas-Lemus M, Minasov G, Shuvalova L, et al. High-resolution structures of the SARS-CoV-2 2'-*O*-methyltransferase reveal strategies for structure-based inhibitor design [J]. Sci Signal, 2020, 13: eabe1202.

[24] Kottur J, Rechkoblit O, Quintana-Feliciano R, et al. High-resolution structures of the SARS-CoV-2 *N*7-methyltransferase inform therapeutic development [J]. Nat Struct Mol Biol, 2022, 29: 850-853.

[25] Chen H, Lin S, Yang F, et al. Structural and functional basis of low-affinity SAM/SAH-binding in the conserved MTase of the multi-segmented Alongshan virus distantly related to canonical unsegmented flaviviruses [J]. PLoS Pathog, 2023, 19: e1011694.

[26] Silhan J, Klima M, Otava T, et al. Discovery and structural characterization of monkeypox virus methyltransferase VP39 inhibitors reveal similarities to SARS-CoV-2 nsp14 methyltransferase [J]. Nat Commun, 2023, 14: 2259.

[27] Ferrero DS, Albentosa-González L, Mas A, et al. Structure and function of the NS5 methyltransferase domain from Usutu virus [J]. Antiviral Res, 2022, 208: 105460.

[28] Zhu X, Yi Y, Fan Z, et al. Novel mono- and multivalent N-acetylneuraminic acid glycoclusters as potential broad-spectrum entry inhibitors for influenza and coronavirus infection [J]. Eur J Med Chem, 2023, 260: 115723.

[29] Kazakova O, Ma X, Tretyakova E, et al. Evaluation of A-ring hydroxymethylene-amino-triterpenoids as inhibitors of SARS-CoV-2 spike pseudovirus and influenza H1N1 [J]. J Antibiot (Tokyo), 2024, 77: 39-49.

[30] He Y, Zhou J, Gao H, et al. Broad-spectrum antiviral strategy: host-targeting antivirals against emerging and re-emerging viruses [J]. Eur J Med Chem, 2024, 265: 116069.

[31] Ma Y, Frutos-Beltrán E, Kang D, et al. Medicinal chemistry strategies for discovering antivirals effective against drug-resistant viruses [J]. Chem Soc Rev, 2021, 50: 4514-4540.

[32] Du S, Hu X, Menéndez-Arias L, et al. Target-based drug design strategies to overcome resistance to antiviral agents: opportunities and challenges [J]. Drug Resist Updat, 2024, 73: 101053.

[33] Shaqra AM, Zvornicanin SN, Huang QYJ, et al. Defining the substrate envelope of SARS-CoV-2 main protease to predict and avoid drug resistance [J]. Nat Commun, 2022, 13: 3556.

[34] Bonnard D, Le Rouzic E, Singer MR, et al. Biological and structural analyses of new potent allosteric inhibitors of HIV-1 integrase [J]. Antimicrob Agents Chemother, 2023, 67: e0046223.

[35] Singer MR, Dinh T, Levintov L, et al. The drug-induced interface that drives HIV-1 integrase hypermultimerization and loss of function [J]. MBio, 2023, 14: e0356022.

[36] Giri-Rachman EA, Effendy VV, Azmi MHS, et al. The SARS-CoV-2 M^{pro} dimer-based screening system: a synthetic biology tool for identifying compounds with dimerization inhibitory potential [J]. ACS Synth Biol, 2024, 13: 509-520.

[37] Ramsey JR, Shelton PMM, Heiss TK, et al. Using a function-first "scout fragment"-based approach to develop allosteric covalent inhibitors of conformationally dynamic helicase mechanoenzymes [J]. J Am Chem Soc, 2024, 146: 62-67.

[38] Urvashi, Senthil Kumar JB, Das P, et al. Development of azaindole-based frameworks as potential antiviral agents and their future perspectives [J]. J Med Chem, 2022, 65: 6454-6495.

[39] Song Y, Zhan P, Liu X. Heterocycle-thioacetic acid motif: a privileged molecular scaffold with potent, broad-ranging pharmacological activities [J]. Curr Pharm Des, 2013, 19: 7141-7154.

[40] Song Y, Zhan P, Zhang Q, et al. Privileged scaffolds or promiscuous binders: a glance of pyrrolo[2,1-f][1,2,4]triazines and related bridgehead nitrogen heterocycles in medicinal chemistry [J]. Curr Pharm Des, 2013, 19: 1528-1548.

[41] Johns BA, Kawasuji T, Weatherhead JG, et al. Carbamoyl pyridone HIV-1 integrase inhibitors 3. A diastereomeric approach to chiral nonracemic tricyclic ring systems and the discovery of dolutegravir (S/GSK1349572) and (S/GSK1265744) [J]. J Med Chem, 2013, 56: 5901-5916.

[42] Takashita E, Morita H, Ogawa R, et al. Susceptibility of influenza viruses to the novel cap-dependent endonuclease inhibitor baloxavir marboxil [J]. Front Microbiol, 2018, 9: 3026.

[43] Wang L, Sarafianos SG, Wang Z. Cutting into the substrate dominance: pharmacophore and structure-based approaches toward inhibiting human immunodeficiency virus reverse transcriptase-associated ribonuclease H [J]. Acc Chem Res, 2020, 53: 218-230.

[44] Jagtap AD, Geraghty RJ, Wang Z. Inhibiting HCMV pUL89-C endonuclease with metal-binding compounds [J]. J Med Chem, 2023, 66: 13874-13887.

[45] He T, Edwards TC, Xie J, et al. 4,5-Dihydroxypyrimidine methyl carboxylates, carboxylic acids, and carboxamides as inhibitors of human cytomegalovirus pUL89 endonuclease [J]. J Med Chem, 2022, 65: 5830-5849.

[46] Gao P, Zhang L, Sun L, et al. 1-Hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-ones as novel selective HIV integrase inhibitors obtained *via* privileged substructure-based compound libraries [J]. Bioorg Med Chem, 2017, 25: 5779-5789.

[47] Yang N, Tanner JA, Zheng BJ, et al. Bismuth complexes inhibit the SARS coronavirus [J]. Angew Chem Int Ed Engl, 2007, 46: 6464-6468.

[48] Yang N, Tanner JA, Wang Z, et al. Inhibition of SARS coronavirus helicase by bismuth complexes [J]. Chem Commun (Camb), 2007, 42: 4413-4415.

[49] Yuan S, Wang R, Chan JF, et al. Metallodrug ranitidine bismuth citrate suppresses SARS-CoV-2 replication and relieves virus-associated pneumonia in Syrian hamsters [J]. Nat Microbiol, 2020, 5: 1439-1448.

[50] Weil T, Groß R, Röcker A, et al. Supramolecular mechanism of viral envelope disruption by molecular tweezers [J]. J Am Chem Soc, 2020, 142: 17024-17038.

[51] Le MH, Taghuo K ES, Schrader T. Molecular tweezers-a new class of potent broad-spectrum antivirals against enveloped viruses [J]. Chem Commun (Camb), 2022, 58: 2954-2966.

[52] Weil T, Kirupakaran A, Le MH, et al. Advanced molecular tweezers with lipid anchors against SARS-CoV-2 and other respiratory viruses [J]. JACS Au, 2022, 2: 2187-2202.

[53] Shahpasand-Kroner H, Siddique I, Malik R, et al. Molecular tweezers: supramolecular hosts with broad-spectrum biological applications [J]. Pharmacol Rev, 2023, 75: 263-308.

[54] Liu X, Luo W, Zhang B, et al. Design of neuraminidase-targeted imaging and therapeutic agents for the diagnosis and treatment of influenza virus infections [J]. Bioconjug Chem, 2021, 32: 1548-1553.

[55] Liu X, Zhang B, Wang Y, et al. A universal dual mechanism immunotherapy for the treatment of influenza virus infections [J]. Nat Commun, 2020, 11: 5597.

[56] Sun Y, Gong L, Yin Y, et al. A gradient pH-sensitive polymer-based antiviral strategy *via* viroporin-induced membrane acidification [J]. Adv Mater, 2022, 34: e2109580.

[57] Si L, Xu H, Zhou X, et al. Generation of influenza A viruses as

live but replication-incompetent virus vaccines [J]. Science, 2016, 354: 1170-1173.

[58] Huang Z, Zhuang X, Liu L, et al. Modularized viromimetic polymer nanoparticle vaccines (VPNVaxs) to elicit durable and effective humoral immune responses [J]. Natl Sci Rev, 2023, 11: nwad310.

[59] Mostafavi E, Iravani S, Varma RS. Nanosponges: an overlooked promising strategy to combat SARS-CoV-2 [J]. Drug Discov Today, 2022, 27: 103330.

[60] Zhang Q, Honko A, Zhou J, et al. Cellular nanosponges inhibit SARS-CoV-2 infectivity [J]. Nano Lett, 2020, 20: 5570-5574.

[61] Ai X, Wang D, Honko A, et al. Surface glycan modification of cellular nanosponges to promote SARS-CoV-2 inhibition [J]. J Am Chem Soc, 2021, 143: 17615-17621.

[62] Zhang J, Xu Y, Huang Y, et al. Spatially patterned neutralizing icosahedral DNA nanocage for efficient SARS-CoV-2 blocking [J]. J Am Chem Soc, 2022, 144: 13146-13153.

[63] Nie C, Stadtmüller M, Yang H, et al. Spiky nanostructures with geometry-matching topography for virus inhibition [J]. Nano Lett, 2020, 20: 5367-5375.

[64] Si L, Shen Q, Li J, et al. Generation of a live attenuated influenza A vaccine by proteolysis targeting [J]. Nat Biotechnol, 2022, 40: 1370-1377.

[65] Li Z, Bai H, Xi X, et al. PROTAC vaccine: a new way to live attenuated vaccines [J]. Clin Transl Med, 2022, 12: e1081.

[66] Li Y, Zeng ZW, Chen D, et al. Facilitated drug repurposing with artemisinin-derived PROTACS: unveiling PCLAF as a therapeutic target [J]. J Med Chem, 2023, 66: 11335-11350.

[67] Chen S, Bi K, Liang H, et al. PROTAC derivatization of natural products for target identification and drug discovery: design of evodiamine-based PROTACs as novel REXO4 degraders [J]. J Adv Res, 2023. DOI: 10.1016/j.jare.2023.10.014.

[68] Bauman JD, Patel D, Dharia C, et al. Detecting allosteric sites of HIV-1 reverse transcriptase by X-ray crystallographic fragment screening [J]. J Med Chem, 2013, 56: 2738-2746.

[69] Patel D, Antwi J, Koneru PC, et al. A new class of allosteric HIV-1 integrase inhibitors identified by crystallographic fragment screening of the catalytic core domain [J]. J Biol Chem, 2016, 291: 23569-23577.

[70] Sagong HY, Bauman JD, Nogales A, et al. Aryl and arylalkyl substituted 3-hydroxypyridin-2(1*H*)-ones: synthesis and evaluation as inhibitors of influenza A endonuclease [J]. Chem Med Chem, 2019, 14: 1204-1223.

[71] Jorgensen WL, Bollini M, Thakur VV, et al. Efficient discovery of potent anti-HIV agents targeting the Tyr181Cys variant of HIV reverse transcriptase [J]. J Am Chem Soc, 2011, 133: 15686-15696.

[72] Zeevaart JG, Wang L, Thakur VV, et al. Optimization of azoles as anti-human immunodeficiency virus agents guided by free-energy calculations [J]. J Am Chem Soc, 2008, 130: 9492-9499.

[73] Kim JT, Hamilton AD, Bailey CM, et al. FEP-guided selection of bicyclic heterocycles in lead optimization for non-nucleoside inhibitors of HIV-1 reverse transcriptase [J]. J Am Chem Soc, 2006, 128: 15372-15373.

[74] Jorgensen WL. Computer-aided discovery of anti-HIV agents [J]. Bioorg Med Chem, 2016, 24: 4768-4778.

[75] Ghahremanpour MM, Saar A, Tirado-Rives J, et al. Computation of absolute binding free energies for noncovalent inhibitors with SARS-CoV-2 main protease [J]. J Chem Inf Model, 2023, 63: 5309-5318.

[76] Saar A, Ghahremanpour MM, Tirado-Rives J, et al. Assessing metadynamics and docking for absolute binding free energy calculations using severe acute respiratory syndrome coronavirus 2 main protease inhibitors [J]. J Chem Inf Model, 2023, 63: 7210-7218.

[77] Zhang CH, Spasov KA, Reilly RA, et al. Optimization of triarylpyridinone inhibitors of the main protease of SARS-CoV-2 to low-nanomolar antiviral potency [J]. ACS Med Chem Lett, 2021, 12: 1325-1332.

[78] Zhang CH, Stone EA, Deshmukh M, et al. Potent noncovalent inhibitors of the main protease of SARS-CoV-2 from molecular sculpting of the drug perampanel guided by free energy perturbation calculations [J]. ACS Cent Sci, 2021, 7: 467-475.

[79] Melidis L, Hill HJ, Coltman NJ, et al. Supramolecular cylinders target bulge structures in the 5 UTR of the RNA genome of SARS-CoV-2 and inhibit viral replication [J]. Angew Chem Int Ed Engl, 2021, 60: 18144-18151.

[80] Melidis L, Styles IB, Hannon MJ. Targeting structural features of viral genomes with a nano-sized supramolecular drug [J]. Chem Sci, 2021, 12: 7174-7184.

[81] Cardo L, Nawroth I, Cail PJ, et al. Metallo supramolecular cylinders inhibit HIV-1 TAR-TAT complex formation and viral replication in cellulo [J]. Sci Rep, 2018, 8: 13342.

[82] Malina J, Hannon MJ, Brabec V. Iron(II) supramolecular helicates interfere with the HIV-1 Tat-TAR RNA interaction critical for viral replication [J]. Sci Rep, 2016, 6: 29674.

[83] Cobb DA, Smith N, Deodhar S, et al. Transformation of tenofovir into stable ProTide nanocrystals with long-acting pharmacokinetic profiles [J]. Nat Commun, 2021, 12: 5458.

[84] Kulkarni TA, Bade AN, Sillman B, et al. A year-long extended release nanoformulated cabotegravir prodrug [J]. Nat Mater, 2020, 19: 910-920.

[85] Gautam N, McMillan JM, Kumar D, et al. Lipophilic nanocrystal prodrug-release defines the extended pharmacokinetic profiles of a year-long cabotegravir [J]. Nat Commun, 2021, 12: 3453.

[86] Sillman B, Bade AN, Dash PK, et al. Creation of a long-acting nanoformulated dolutegravir [J]. Nat Commun, 2018, 9: 443.

[87] Huang M, Song J, Huang P, et al. Molecular crowding evolution for enabling discovery of enthalpy-driven aptamers for robust

biomedical applications [J]. Anal Chem, 2019, 91: 10879-10886.

[88] Lin B, Tian T, Lu Y, et al. Tracing tumor-derived exosomal PD-L1 by dual-aptamer activated proximity-induced droplet digital PCR [J]. Angew Chem Int Ed Engl, 2021, 60: 7582-7586.

[89] Sun M, Liu S, Wei X, et al. Aptamer blocking strategy inhibits SARS-CoV-2 virus infection [J]. Angew Chem Int Ed Engl, 2021, 60: 10266-10272.

[90] Song Y, Song J, Wei X, et al. Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein [J]. Anal Chem, 2020, 92: 9895-9900.

[91] Zhao A, Howson SE, Zhao C, et al. Chiral metallohelices enantioselectively target hybrid human telomeric G-quadruplex DNA [J]. Nucleic Acids Res, 2017, 45: 5026-5035.

[92] Zhao C, Qin G, Niu J, et al. Targeting RNA G-quadruplex in SARS-CoV-2: a promising therapeutic target for COVID-19? [J] Angew Chem Int Ed Engl, 2021, 60: 432-438.

[93] Qin G, Zhao C, Liu Y, et al. RNA G-quadruplex formed in SARS-CoV-2 used for COVID-19 treatment in animal models [J]. Cell Discov, 2022, 8: 86.

[94] Wang N, Yang B, Fu C, et al. Genetically encoding fluorosulfate-l-tyrosine to react with lysine, histidine, and tyrosine *via* SuFEx in proteins *in vivo* [J]. J Am Chem Soc, 2018, 140: 4995-4999.

[95] Li Q, Chen Q, Klauser PC, et al. Developing covalent protein drugs *via* proximity-enabled reactive therapeutics [J]. Cell, 2020, 182: 85-97.e16.

[96] Xue S, Xu W, Wang L, et al. Rational design of sulfonyl-γ-AApeptides as highly potent HIV-1 fusion inhibitors with broad-spectrum activity [J]. J Med Chem, 2023, 66: 13319-13331.

[97] Xue S, Xu W, Wang L, et al. An HR2-mimicking sulfonyl-γ-AApeptide is a potent pan-coronavirus fusion inhibitor with strong blood-brain barrier permeability, long half-life, and promising oral bioavailability [J]. ACS Cent Sci, 2023, 9: 1046-1058.

[98] Xue S, Wang X, Wang L, et al. A novel cyclic γ-AApeptide-based long-acting pan-coronavirus fusion inhibitor with potential oral bioavailability by targeting two sites in spike protein [J]. Cell Discov, 2022, 8: 88.

[99] Wang L, Ma C, Sacco MD, et al. Development of the safe and broad-spectrum aldehyde and ketoamide M^{pro} inhibitors derived from the constrained α, γ-AA peptide scaffold [J]. Chemistry, 2023, 29: e202300476.

[100] Gao S, Song L, Sylvester K, et al. Design, synthesis, and biological evaluation of trisubstituted piperazine derivatives as noncovalent severe acute respiratory syndrome coronavirus 2 main protease inhibitors with improved antiviral activity and favorable druggability [J]. J Med Chem, 2023, 66: 16426-16440.

[101] Gao S, Sylvester K, Song L, et al. Discovery and crystallographic studies of trisubstituted piperazine derivatives as non-covalent SARS-CoV-2 main protease inhibitors with high target specificity and low toxicity [J]. J Med Chem, 2022, 65: 13343-13364.

[102] Jiang X, Huang B, Rumrill S, et al. Discovery of diarylpyrimidine derivatives bearing piperazine sulfonyl as potent HIV-1 non-nucleoside reverse transcriptase inhibitors [J]. Commun Chem, 2023, 6: 83.

[103] Wang H, Li Z, Chen X, et al. Multicomponent coupling and macrocyclization enabled by Rh(III)-catalyzed dual C–H activation: macrocyclic oxime inhibitor of influenza H1N1 [J]. Chem, 2023, 9: 607–623.

[104] Song B, Guo X, Yang L, et al. Rhodium(III)-catalyzed C-H/O_2 dual activation and macrocyclization: synthesis and evaluation of pyrido[2,1-a]isoindole grafted macrocyclic inhibitors for influenza H1N1 [J]. Angew Chem Int Ed Engl, 2023, 62: e202218886.

[105] Li J, Sun J, Zhang X, et al. Synthesis of maleimide-braced peptide macrocycles and their potential anti-SARS-CoV-2 mechanisms [J]. Chem Commun (Camb), 2023, 59: 868-871.

[106] Meng G, Guo T, Ma T, et al. Modular click chemistry libraries for functional screens using a diazotizing reagent [J]. Nature, 2019, 574: 86-89.

[107] Xin Y, Liu S, Liu Y, et al. Affinity selection of double-click triazole libraries for rapid discovery of allosteric modulators for GLP-1 receptor [J]. Proc Natl Acad Sci U S A, 2023, 120: e2220767120.

[108] Kang D, Zhang H, Zhou Z, et al. First discovery of novel 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones as specific anti-vaccinia and adenovirus agents *via* 'privileged scaffold' refining approach [J]. Bioorg Med Chem Lett, 2016, 26: 5182-5186.

[109] Wu G, Zalloum WA, Meuser ME, et al. Discovery of phenylalanine derivatives as potent HIV-1 capsid inhibitors from click chemistry-based compound library [J]. Eur J Med Chem, 2018, 158: 478-492.

[110] Zhou Z, Liu T, Wu G, et al. Targeting the hydrophobic channel of NNIBP: discovery of novel 1,2,3-triazole-derived diarylpyrimidines as novel HIV-1 NNRTIs with high potency against wild-type and K103N mutant virus [J]. Org Biomol Chem, 2019, 17: 3202-3217.

[111] Tian Y, Liu Z, Liu J, et al. Targeting the entrance channel of NNIBP: discovery of diarylnicotinamide 1,4-disubstituted 1,2,3-triazoles as novel HIV-1 NNRTIs with high potency against wild-type and E138K mutant virus [J]. Eur J Med Chem, 2018, 151: 339-350.

[112] Wang X, Huang B, Liu X, et al. Discovery of bioactive molecules from CuAAC click-chemistry-based combinatorial libraries [J]. Drug Discov Today, 2016, 21: 118-132.

[113] Tao Y, Xu S, Zhang X, et al. Discovery of bioactive molecules *via* miniaturized parallel modular reactions and rapid screening (2016-2021 update) [J]. Mini-Rev Org Chem, 2022, 19: 546-548.

[114] Kang D, Feng D, Jing L, et al. In situ click chemistry-based

rapid discovery of novel HIV-1 NNRTIs by exploiting the hydrophobic channel and tolerant regions of NNIBP [J]. Eur J Med Chem, 2020, 193: 112237.

[115] Sun Y, Feng D, Zhou Z, et al. *In situ* click chemistry-based discovery of 1, 2, 3-triazole-derived diarylpyrimidines as novel HIV-1 NNRTIs by exploiting the tolerant region I in binding pocket [J]. Bioorg Med Chem, 2023, 96: 117484.

[116] Jiang X, Li J, Viayna A, et al. Identification of novel 1, 2, 3-triazole isatin derivatives as potent SARS-CoV-2 3CLpro inhibitors *via* click-chemistry-based rapid screening [J]. RSC Med Chem, 2023, 14: 2068-2078.

[117] Zeng D, Deng WP, Jiang X. Advances in the construction of diverse SuFEx linkers [J]. Natl Sci Rev, 2023, 10: nwad123.

[118] Qin Z, Zhang K, He P, et al. Discovering covalent inhibitors of protein-protein interactions from trillions of sulfur(VI) fluoride exchange-modified oligonucleotides [J]. Nat Chem, 2023, 15: 1705-1714.

[119] Xu S, Zhang X, Tao Y, et al. Application of multi-component reactions in the search of bioactive molecules [J]. Mini-Rev Org Chem, 2022, 19: 894-897.

[120] Jacobs J, Grum-Tokars V, Zhou Y, et al. Discovery, synthesis, and structure-based optimization of a series of N-(tert-butyl)-2-(N-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease [J]. J Med Chem, 2013, 56: 534-546.

[121] Lockbaum GJ, Reyes AC, Lee JM, et al. Crystal structure of SARS-CoV-2 main protease in complex with the non-covalent inhibitor Ml188 [J]. Viruses, 2021, 13: 174.

[122] Ma C, Xia Z, Sacco MD, et al. Discovery of di- and trihaloacetamides as covalent SARS-CoV-2 main protease inhibitors with high target specificity [J]. J Am Chem Soc, 2021, 143: 20697-20709.

[123] Quan BX, Shuai H, Xia AJ, et al. An orally available M^{pro} inhibitor is effective against wild-type SARS-CoV-2 and variants including Omicron [J]. Nat Microbiol, 2022, 7: 716-725.

[124] Yamane D, Onitsuka S, Re S, et al. Selective covalent targeting of SARS-CoV-2 main protease by enantiopure chlorofluoroacetamide [J]. Chem Sci, 2022, 13: 3027-3034.

[125] Ji X, Li J, Sharma PP, et al. Design, synthesis and structure-activity relationships of phenylalanine-containing peptidomimetics as novel HIV-1 capsid binders based on Ugi four-component reaction [J]. Molecules, 2022, 27: 5995.

[126] Burke AJ, Birmingham WR, Zhuo Y, et al. An engineered cytidine deaminase for biocatalytic production of a key intermediate of the COVID-19 antiviral molnupiravir [J]. J Am Chem Soc, 2022, 144: 3761-3765.

[127] McIntosh JA, Benkovics T, Silverman SM, et al. Engineered ribosyl-1-kinase enables concise synthesis of molnupiravir, an antiviral for COVID-19 [J]. ACS Cent Sci, 2021, 7: 1980-1985.

[128] Song Y, Chen W, Kang D, et al. "Old friends in new guise": exploiting privileged structures for scaffold re-evolution/refining [J]. Comb Chem High Throughput Screen, 2014, 17: 536-553.

[129] Ding D, Xu S, da Silva-Júnior EF, et al. Medicinal chemistry insights into antiviral peptidomimetics [J]. Drug Discov Today, 2023, 28: 103468.

[130] Sun L, Dick A, Meuser ME, et al. Design, synthesis, and mechanism study of benzenesulfonamide-containing phenylalanine derivatives as novel HIV-1 capsid inhibitors with improved antiviral activities [J]. J Med Chem, 2020, 63: 4790-4810.

[131] Xu S, Sun L, Barnett M, et al. Discovery, crystallographic studies, and mechanistic investigations of novel phenylalanine derivatives bearing a quinazolin-4-one scaffold as potent HIV capsid modulators [J]. J Med Chem, 2023, 66: 16303-16329.

[132] Mehellou Y, Rattan HS, Balzarini J. The ProTide prodrug technology: from the concept to the clinic [J]. J Med Chem, 2018, 61: 2211-2226.

[133] Shrivastava P, Gautam L, Jain A, et al. Lipid drug conjugates for improved therapeutic benefits [J]. Curr Pharm Des, 2020, 26: 3187-3202.

[134] Wang S, Ren Y, Wang Z, et al. The current progress in the use of boron as a platform for novel antiviral drug design [J]. Expert Opin Drug Discov, 2022, 17: 1329-1340.

[135] Song S, Gao P, Sun L, et al. Recent developments in the medicinal chemistry of single boron atom-containing compounds [J]. Acta Pharm Sin B, 2021, 11: 3035-3059.

[136] Ghosh AK, Xia Z, Kovela S, et al. Potent HIV-1 protease inhibitors containing carboxylic and boronic acids: effect on enzyme inhibition and antiviral activity and protein-ligand X-ray structural studies [J]. Chem Med Chem, 2019, 14: 1863-1872.

[137] Feng D, Wei F, Sun Y, et al. Boronic acid-containing diarylpyrimidine derivatives as novel HIV-1 NNRTIs: design, synthesis and biological evaluation [J]. Chin Chem Lett, 2021, 32: 4053-4057.

[138] Xu S, Song S, Sun L, et al. Indolylarylsulfones bearing phenylboronic acid and phenylboronate ester functionalities as potent HIV-1 non-nucleoside reverse transcriptase inhibitors [J]. Bioorg Med Chem, 2022, 53: 116531

[139] Jia R, Zhang J, Zhang J, et al. Discovery of novel boron-containing *n*-substituted oseltamivir derivatives as anti-influenza a virus agents for overcoming N1-H274Y oseltamivir-resistant [J]. Molecules, 2022, 27: 6426.

[140] Wang S, Ren Y, Li Q, et al. Design, synthesis, and biological evaluation of novel sulfamoylbenzamide derivatives as HBV capsid assembly modulators [J]. Bioorg Chem, 2022, 129: 106192.

[141] Wang M, Zhang J, Dou Y, et al. Design, synthesis, and biological evaluation of novel thioureidobenzamide (TBA) derivatives as HBV capsid assembly modulators [J]. J Med Chem, 2023, 66: 13968-13990.

[142] Chen X, Zhan P, Liu X, et al. Design, synthesis, anti-HIV evalua-

tion and molecular modeling of piperidine-linked amino-triazine derivatives as potent non-nucleoside reverse transcriptase inhibitors [J]. Bioorg Med Chem, 2012, 20: 3856-3864.

[143] Chen X, Zhan P, Pannecouque C, et al. Synthesis and biological evaluation of piperidine-substituted triazine derivatives as HIV-1 non-nucleoside reverse transcriptase inhibitors [J]. Eur J Med Chem, 2012, 51: 60-66.

[144] Zhang L, Zhan P, Chen X, et al. Design, synthesis and preliminary SAR studies of novel N-arylmethyl substituted piperidine-linked aniline derivatives as potent HIV-1 NNRTIs [J]. Bioorg Med Chem, 2014, 22: 633-642.

[145] Chen W, Zhan P, Daelemans D, et al. Structural optimization of pyridine-type DAPY derivatives to exploit the tolerant regions of the NNRTI binding pocket [J]. Eur J Med Chem, 2016, 121: 352-363.

[146] Huang B, Li C, Chen W, et al. Fused heterocycles bearing bridgehead nitrogen as potent HIV-1 NNRTIs. Part 3: Optimization of [1, 2, 4]triazolo[1, 5-*α*]pyrimidine core *via* structure-based and physicochemical property-driven approaches [J]. Eur J Med Chem, 2015, 92: 754-765.

[147] Kang D, Fang Z, Li Z, et al. Design, synthesis, and evaluation of thiophene[3,2-d]pyrimidine derivatives as HIV-1 non-nucleoside reverse transcriptase inhibitors with significantly improved drug resistance profiles [J]. J Med Chem, 2016, 59: 7991-8007.

[148] Kang D, Fang Z, Huang B, et al. Structure-based optimization of thiophene[3, 2-d]pyrimidine derivatives as potent HIV-1 non-nucleoside reverse transcriptase inhibitors with improved potency against resistance-associated variants [J]. J Med Chem, 2017, 60: 4424-4443.

[149] Kang D, Feng D, Sun Y, et al. Structure-based bioisosterism yields HIV-1 NNRTIs with improved drug-resistance profiles and favorable pharmacokinetic properties [J]. J Med Chem, 2020, 63: 4837-4848.

[150] Kang D, Zhang H, Wang Z, et al. Identification of dihydrofuro[3, 4-d]pyrimidine derivatives as novel HIV-1 non-nucleoside reverse transcriptase inhibitors with promising antiviral activities and desirable physicochemical properties [J]. J Med Chem, 2019, 62: 1484-1501.

[151] Kang D, Wang Z, Zhang H, et al. Further exploring solvent-exposed tolerant regions of allosteric binding pocket for novel HIV-1 NNRTIs discovery [J]. ACS Med Chem Lett, 2018, 9: 370-375.

[152] Kang D, Fang Z, Huang B, et al. Synthesis and preliminary antiviral activities of piperidine-substituted purines against HIV and influenza A/H1N1 infections [J]. Chem Biol Drug Des, 2015, 86: 568-577.

[153] Zhan P, Li X, Li Z, et al. Structure-based bioisosterism design, synthesis and biological evaluation of novel 1, 2, 4-triazin-6-ylthioacetamides as potent HIV-1 NNRTIs [J]. Bioorg Med Chem Lett, 2012, 22: 7155-7162.

[154] Shi D, Xu S, Ding D, et al. Advances in drug structure-activity-relationships for the development of selenium-based compounds against HIV [J]. Expert Opin Drug Discov, 2024, 19: 139-146.

[155] Hu L, Jia H, Zhang J, et al. Sulfonic acid: key drug design elements with potent, broad-ranging pharmacological activities [J]. Future Med Chem, 2023, 15: 2029-2032.

[156] Chamakuri S, Lu S, Ucisik MN, et al. DNA-encoded chemistry technology yields expedient access to SARS-CoV-2 M^{pro} inhibitors [J]. Proc Natl Acad Sci U S A, 2021, 118: e2111172118.

[157] Jimmidi R, Chamakuri S, Lu S, et al. DNA-encoded chemical libraries yield non-covalent and non-peptidic SARS-CoV-2 main protease inhibitors [J]. Commun Chem, 2023, 6: 164.

[158] Hou N, Shuai L, Zhang L, et al. Development of highly potent noncovalent inhibitors of SARS-CoV-2 $3CL^{pro}$ [J]. ACS Cent Sci, 2023, 9: 217-227.

[159] Sutanto F, Shaabani S, Oerlemans R, et al. Combining high-throughput synthesis and high-throughput protein crystallography for accelerated hit identification [J]. Angew Chem Int Ed Engl, 2021, 60: 18231-18239.

[160] Whiting M, Muldoon J, Lin YC, et al. Inhibitors of HIV-1 protease by using in situ click chemistry [J]. Angew Chem Int Ed Engl, 2006, 45: 1435-1439.

[161] Fu S, Nie Q, Ma Y, et al. Target-guided screening of fragments (TGSOF) in the discovery of inhibitors against EV-A71 3C protease [J]. Chem Commun (Camb), 2018, 54: 2890-2893.

[162] Schmidt MF, Isidro-Llobet A, Lisurek M, et al. Sensitized detection of inhibitory fragments and iterative development of non-peptidic protease inhibitors by dynamic ligation screening [J]. Angew Chem Int Ed Engl, 2008, 47: 3275-3278.

[163] Tauber C, Wamser R, Arkona C, et al. Chemical evolution of antivirals against enterovirus D68 through protein-templated knoevenagel reactions [J]. Angew Chem Int Ed Engl, 2021, 60: 13294-13301.

[164] Che C, Jiang D, Zhang J, et al. Tricyclic aza-andrographolide derivatives from late-stage hydroamination and their anti-human coronavirus (anti-HCoV) activity [J]. ACS Omega, 2022, 7: 24824-24837.

[165] Li F, Ma C, Hu Y, et al. Discovery of potent antivirals against amantadine-resistant influenza a viruses by targeting the M2-S31N proton channel [J]. ACS Infect Dis, 2016, 2: 726-733.

[166] Li F, Hu Y, Wang Y, et al. Expeditious lead optimization of isoxazole-containing influenza a virus M2-S31N inhibitors using the Suzuki-Miyaura cross-coupling reaction [J]. J Med Chem, 2017, 60: 1580-1590.

[167] Nippa DF, Atz K, Hohler R, et al. Enabling late-stage drug diversification by high-throughput experimentation with geometric deep learning [J]. Nat Chem, 2024, 16: 239-248.

[168] Wei W, Cherukupalli S, Jing L, et al. Fsp3: a new parameter for drug-likeness [J]. Drug Discov Today, 2020, 25: 1839-1845.

[169] Jiang X, Huang B, Olotu FA, et al. Exploiting the tolerant region

I of the non-nucleoside reverse transcriptase inhibitor (NNRTI) binding pocket. Part 2: Discovery of diarylpyrimidine derivatives as potent HIV-1 NNRTIs with high Fsp^3 values and favorable drug-like properties [J]. Eur J Med Chem, 2021, 213: 113051.

[170] Gao S, Cheng Y, Song S, et al. Chemical space exploration around indolylarylsulfone scaffold led to a novel class of highly active HIV-1 NNRTIs with spiro structural features [J]. Eur J Med Chem, 2022, 238: 114471.

[171] Wang Z, Sharma PP, Rathi B, et al. Escaping from flatland: multiparameter optimization leads to the discovery of novel tetrahydropyrido[4, 3-d]pyrimidine derivatives as human immunodeficiency virus-1 non-nucleoside reverse transcriptase inhibitors with superior antiviral activities against non-nucleoside reverse transcriptase inhibitor-resistant variants and favorable drug-like profiles [J]. J Med Chem, 2023, 66: 8643-8665.

[172] Risso-Ballester J, Galloux M, Cao J, et al. A condensate-hardening drug blocks RSV replication *in vivo* [J]. Nature, 2021, 595: 596-599.

[173] Du S, Hu X, Liu X, et al. Revolutionizing viral disease treatment: phase separation and lysosome/exosome targeting as new areas and new paradigms for antiviral drug research [J]. Drug Discov Today, 2024, 29: 103888.

[174] Balibar CJ, Klein DJ, Zamlynny B, et al. Potent targeted activator of cell kill molecules eliminate cells expressing HIV-1 [J]. Sci Transl Med, 2023, 15: eabn2038.

[175] Berke JM, Tan Y, Sauviller S, et al. Class A capsid assembly modulator apoptotic elimination of hepatocytes with high HBV core antigen level *in vivo* is dependent on *de novo* core protein translation [J]. J Virol, 2024, 98: e0150223.

[176] van Tilborg D, Alenicheva A, Grisoni F. Exposing the limitations of molecular machine learning with activity cliffs [J]. J Chem Inf Model, 2022, 62: 5938-5951.

[177] Riva L, Yuan S, Yin X, et al. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing [J]. Nature, 2020, 586: 113-119.

[178] Boby ML, Fearon D, Ferla M, et al. Open science discovery of potent noncovalent SARS-CoV-2 main protease inhibitors [J]. Science, 2023, 382: eabo7201.

[179] Unoh Y, Uehara S, Nakahara K, et al. Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19 [J]. J Med Chem, 2022, 65: 6499-6512.

[180] Bardiot D, Koukni M, Smets W, et al. A pan-serotype dengue virus inhibitor targeting the NS3-NS4B interaction [J]. Nature, 2021, 598: 504-509.

[181] Goethals O, Kaptein SJF, Kesteleyn B, et al. Blocking NS3-NS4B interaction inhibits dengue virus in non-human primates [J]. Nature, 2023, 615: 678-686.

[182] Kesteleyn B, Bardiot D, Bonfanti JF, et al. Discovery of acyl-indole derivatives as pan-serotype dengue virus NS4B inhibitors [J]. J Med Chem, 2023, 66: 8808-8821.

[183] Xie X, Lan Q, Zhao J, et al. Structure-based design of pan-coronavirus inhibitors targeting host cathepsin L and calpain-1 [J]. Signal Transduct Target Ther, 2024, 9: 54.

[184] Jiang L, Liu S, Jia X, et al. ABPP-CoDEL: activity-based proteome profiling-guided discovery of tyrosine-targeting covalent inhibitors from DNA-encoded libraries [J]. J Am Chem Soc, 2023, 145:25283-25292.

[185] Hsia O, Hinterndorfer M, Cowan AD, et al. Targeted protein degradation *via* intramolecular bivalent glues [J]. Nature, 2024, 627: 204-211.

[186] Zhou Y, Shen W, Gao Y, et al. Protein-templated ligand discovery *via* the selection of DNA-encoded dynamic libraries [J]. Nat Chem, 2024, 16: 543-555.

[187] Wang Q, Yang X, Yuan R, et al. A co-assembly platform engaging macrophage scavenger receptor A for lysosome-targeting protein degradation [J]. Nat Commun, 2024, 15: 1663.