

非核苷类逆转录酶抑制剂DB02氨基酸衍生物的合成及抗HIV-1活性研究

杨金轩^{1,3#}, 余乐^{2#}, 杨玉卓², 罗荣华¹, 何严萍^{2*}, 郑永唐^{1*}

(1. 中国科学院昆明动物研究所, 中国科学院动物模型与人类疾病机理重点实验室, 云南 昆明 650223; 2. 云南大学化学科学与工程学院, 自然资源药物化学教育部重点实验室, 云南 昆明 650091; 3. 云南中医药大学中药学院, 云南 昆明 650500)

摘要: 为提高非核苷类HIV-1逆转录酶抑制剂DB02氨基酸酯衍生物的稳定性和生物电子等排原理, 以具有更高化学稳定性的酰胺替代酯键, 设计合成了24个DB02氨基酸酰胺衍生物 $2a\sim 2x$ 。采用MTT法及合胞体计数评估了其体外抗HIV-1活性。研究发现大部分目标化合物具有良好的抗HIV-1活性, 其中活性最佳的5个化合物 $2d$ 、 $2i$ 、 $2l$ 、 $2s$ 、 $2w$ 的抗病毒效果均优于先导化合物DB02, 且具有优良的治疗指数(TI > 1 000.00)。这类化合物的构效关系研究为DB02衍生物的进一步开发提供了新的思路。

关键词: DB02; 非核苷类逆转录酶抑制剂; 氨基酸酰胺衍生物; 抗HIV-1活性; 构效关系

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Design, synthesis and biological activity of DB02 amino acid derivatives as HIV-1 non-nucleoside reverse transcriptase inhibitors

YANG Jin-xuan^{1,3#}, YU Le^{2#}, YANG Yu-zhuo², LUO Rong-hua¹, HE Yan-ping^{2*}, ZHENG Yong-tang^{1*}

(1. Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China; 2. Key Laboratory of Medicinal Chemistry for Natural Resource Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China; 3. College of Traditional Chinese Medicine, Yunnan University of Chinese Medicine, Kunming 650500, China)

Abstract: To improve the stability of amino acid ester derivatives of DB02, a series of 24 amide derivatives of DB02 amino acids as non-nucleoside HIV-1 reverse transcriptase inhibitor were designed and synthesized based on bioisosterism by replacing amino acid ester scaffold with more stable amide bond. The anti-HIV-1 activity of these compounds was evaluated by MTT assay and counting the number of syncytia. Most of the target compounds showed a potential anti-HIV-1 activity, among which compounds $2d$, $2i$, $2l$, $2s$, and $2w$ had better antiviral effect than lead compound DB02, with a therapeutic index > 1 000.00. Finally, the structure-activity relationship of these compounds was discussed, which provided new ideas for the further development of DB02 derivatives.

Key words: DB02; non-nucleoside reverse transcriptase inhibitor; amino acid amide derivative; anti-HIV-1 activity; structure-activity relationship

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#共同第一作者.

*通讯作者 Tel / Fax: 86-871-65031119, E-mail: yphe@ynu.edu.cn;

Tel / Fax: 86-871-65195684, E-mail: zhengyt@mail.kiz.ac.cn

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获得性免疫缺陷综合征 (acquired immune deficiency syndrome, AIDS) 是由人类免疫缺陷病毒 (human immunodeficiency virus, HIV) 引起的一种致命性传染病。截至2020年全球大约有3 770万感染人群, 新增150万感染人群, 68万人死于HIV相关的疾病^[1]。目前HIV/AIDS治疗最为有效的方法是联合抗逆转录病毒疗法 (combined antiretroviral therapy, cART), 一般

是2种核苷类逆转录酶抑制剂+1种非核苷类逆转录酶抑制剂 (non-nucleoside reverse transcriptase inhibitors, NNRTIs) 或整合酶链转移抑制剂或蛋白酶抑制剂^[2]。NNRTIs因其强有力的抗病毒活性、高选择性、适度的毒性和良好的药代动力学特性,在cART治疗中独具优势,也是药物研发的热点^[3]。尽管当前已有奈韦拉平、地拉韦啉、依法韦仑、依曲韦林、利匹韦林、多拉韦林、依磷韦林和艾诺维林等8种NNRTIs获批应用于临床,但由于临床治疗方案的选择、药物间相互作用、药代性质不理想、毒副作用及耐药株的产生等问题^[4-9],迫切需要不断开发新的抗HIV药物及先导化合物。

本课题组前期报道了系列二氢芳基/烷基硫-环己甲基嘧啶酮类 (dihydro-aryl/alkylsulfanyl-cyclohexylmethyl-oxypyrimidines, S-DACOs) NNRTIs^[10],其中优选化合物DB02对不同亚型不同嗜性的HIV-1病毒株均有较好的抑制作用 (EC_{50} 为2.40~41.80 nmol·L⁻¹),且细胞毒性低 (CC_{50} >1.00 mmol·L⁻¹)^[11]。但由于分子结构的疏水性,DB02水溶性极差,导致生物利用度较

低^[12],阻碍了其进一步开发。为提高该类化合物的生物利用度,在DB02的C-2侧链末端苯环通过酯键引入各种氨基酸,进一步合成高效低毒的DB02-氨基酸酯衍生物**1**,并利用其末端氨基制备相应的盐酸盐,从而显著提高了其水溶性^[13]。遗憾的是,在进一步的研究中发现化合物**1**由于氨基的碱性,容易导致酯键断裂、分解生成化合物**1b**(图1),影响了其化学稳定性。

为分析化合物**1**与HIV-1逆转录酶 (reverse transcriptase, RT)的作用机制及为进一步的结构修饰提供指导,采用AutoDock 4.2程序进行分子模拟^[14,15]建立了优选化合物**1a**与RT (PDB: 1RT)复合物模型。如图2A所示,化合物**1a**结合位点与DB02相似^[11],其嘧啶环的N₃-NH与Lys101通过氢键作用将抑制剂分子锚定在RT内,C-6位的环己基以椅式柔性构象嵌入Trp229、Tyr181、Tyr188所组成的疏水口袋;C-2位β-羰基氧与Tyr318通过形成氢键对C-2位侧链起定向作用,使其末端朝向溶剂开口处。与DB02结合位点不同的是,化合物**1a**的C-2侧链酯羰基及末端氨基与

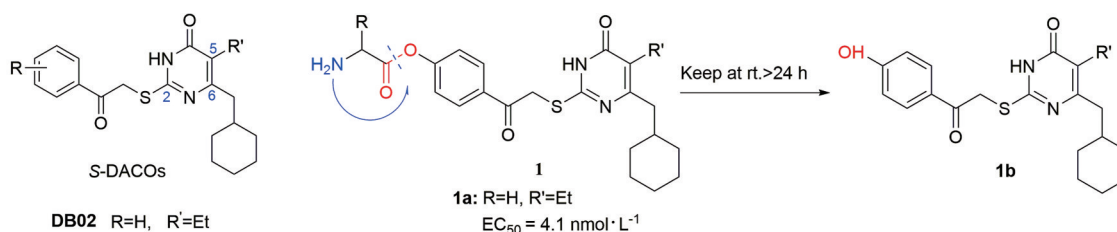


Figure 1 Structures of S-DACOs-like NNRTIs

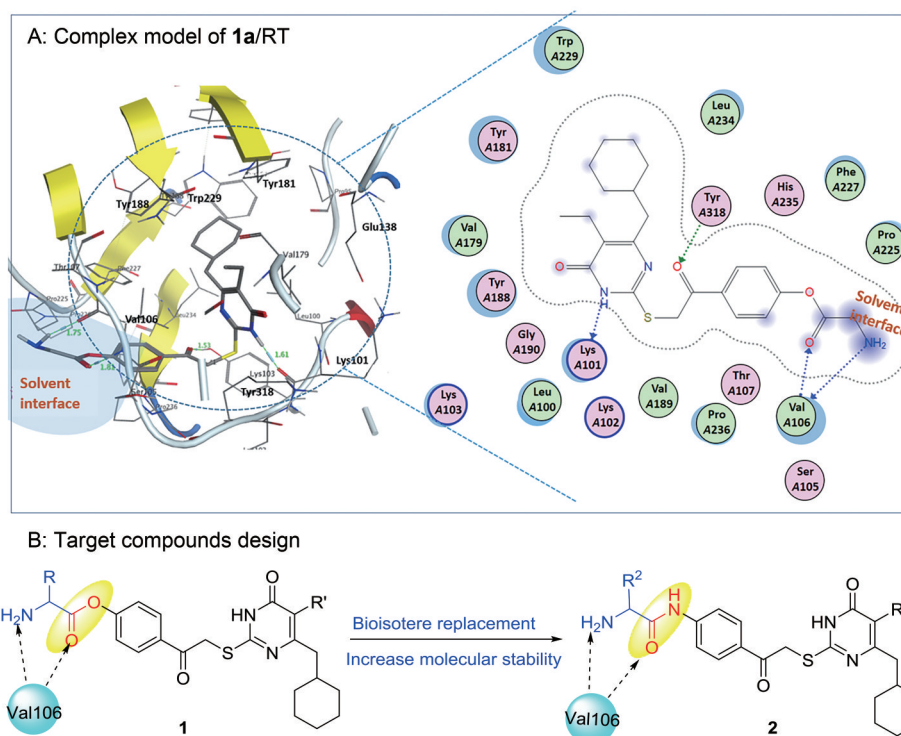


Figure 2 (A) Predicted binding mode of compound **1a** in the allosteric site of HIV-1 wt RT (PDB: 1RT). (B) Target compounds design

Val106 形成了双重氢键, 从而加强了小分子抑制剂与 RT 的结合, 这是其活性较 DB02 增强的重要原因。基于该复合物模型, 本研究用化学稳定性更好的酰胺键替代酯键设计了目标分子 **2** (图 2B), 酰胺键作为酯键的生物电子等排体, 同样可与 Val106 形成双重氢键, 因而有利于活性。另外, 由于 C-2 侧链末端处于溶剂开口处, 周围有较大空间可容纳不同取代基, 因此, 在该位置引入不同体积和构型的取代基 (R^2) 以寻找更优活性基团。期望化合物 **2** 在增加分子稳定性的同时保持其良好的抗 HIV 活性。

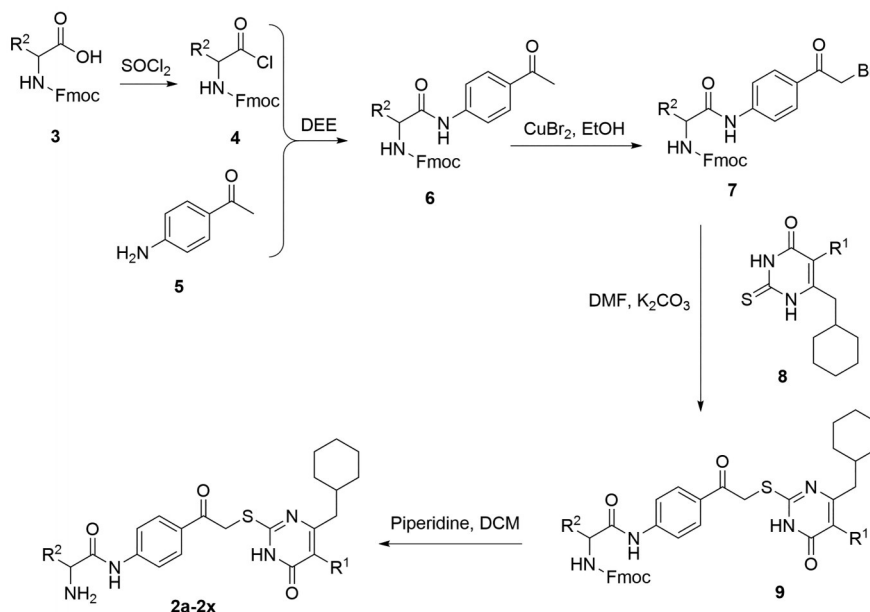
结果与讨论

1 化学实验

以 9-芴基甲氧基羰基 (Fmoc) 保护的氨基酸 **3** 为起始原料, 与二氯亚砷反应生成 *N*-Fmoc-氨基酸酰氯 **4**, 再与对氨基苯乙酮 **5** 反应生成中间体 **6**, 然后经溴化铜溴代得到中间体 **7**。中间体 **7** 与 5-烷基-2-噻嘧啶酮 **8** 在 K_2CO_3 催化下经烷基化反应生成氨基酸酰胺衍生物 **9**。最后, **9** 在哌啶和二氯甲烷作用下脱保护得到目标化合物 **2**。化合物的合成见合成路线 1。

通过上述方法合成了 24 个目标新化合物, 并采用 1H NMR、 ^{13}C NMR 和 HRMS-ESI 进行了结构表征, 其理化性质和谱学数据见表 1 和表 2。

DB02-氨基酸酯衍生物 **1** 在冰箱内 $-2\text{ }^\circ\text{C}$ 密封储存, TLC 检测 24 h 后即有杂点产生, 表明化合物开始分解。本研究合成的目标化合物 **2** 同条件储存, 经 TLC 跟踪检测 60 天以上仍无杂点生成, 说明以酰胺键替代酯键后的化合物具有良好的稳定性。



Scheme 1 Synthetic route of target compounds

Table 1 Physical data of compounds **2a-2x**

Compd.	Yield/%	mp/ $^\circ\text{C}$	Compd.	Yield/%	mp/ $^\circ\text{C}$
2a	55	160.8-161.8	2m	62	148.1-149.1
2b	45	157.3-158.3	2n	58	111.7-112.7
2c	41	172.1-172.1	2o	55	106.1-107.1
2d	53	174.2-175.2	2p	44	138.9-139.9
2e	40	174.7-175.7	2q	39	141.3-142.3
2f	49	176.3-177.3	2r	43	124.8-125.8
2g	58	199.6-200.6	2s	56	141.6-142.6
2h	53	142.2-143.2	2t	62	115.0-116.0
2i	49	159.8-160.8	2u	54	117.7-118.7
2j	40	197.1-198.1	2v	64	136.2-137.2
2k	48	203.7-204.7	2w	61	139.3-149.3
2l	52	162.5-163.5	2x	61	131.5-132.5

2 抗 HIV-1 活性评价

以临床使用的 NNRTI 拉米夫定 (lamivudine, 3TC) 为阳性对照, 采用 MTT 法及合胞体计数评估了目标化合物 **2a-2x** 的体外抗病毒活性 (表 3)。由表 3 可知, 目标化合物普遍具有良好的抗 HIV-1 活性, 其 EC_{50} 介于 $0.01\sim 2.59\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 之间。除化合物 **2a**、**2e**、**2g**、**2j** 和 **2p** 外, 其余化合物的 EC_{50} 值均低于阳性对照药物 3TC ($EC_{50} = 0.61\text{ }\mu\text{mol}\cdot\text{L}^{-1}$), 其中 **2d**、**2i**、**2l**、**2s**、**2w** 这 5 个化合物的 EC_{50} 值均优于 DB02 ($EC_{50} = 0.03\text{ }\mu\text{mol}\cdot\text{L}^{-1}$)。此外, 大部分目标化合物的细胞毒性较 DB02 明显增加, 除化合物 **2a**、**2b** 和 **2f** 的细胞毒性较小 ($CC_{50} > 200.00\text{ }\mu\text{mol}\cdot\text{L}^{-1}$), 其余化合物的 CC_{50} 均介于 $15.00\sim 150.60\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 。体外抗 HIV-1 活性研究显示化合物 **2b**、**2d**、**2i**、**2o**、**2s** 和 **2w** 的抗病毒效果最好, TI 值均大于 1 000.00, 其中活性最好的化合物 **2w** 的 TI 值高达 4 881.85, 较阳性对照药物 3TC 提高了 15 倍。

Table 2 Spectral data of compounds **2a–2x**

Compd.	¹ H NMR, ¹³ C NMR, HRMS-ESI
2a	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 7.86 (d, <i>J</i> = 9.2 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.61 (d, <i>J</i> = 2.9 Hz, 2H, NH ₂ CH ₂), 2.09 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.79 (s, 3H, Me), 1.66 (m, 7H, Cyclohexyl), 1.54 (m, 4H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 190.3, 174.3, 161.5 (2C), 155.9, 142.8, 131.1, 130.0 (2C), 118.7 (2C), 115.3, 43.9, 37.6 (2C), 36.8 (2C), 32.9, 32.6 (3C), 10.9; HRMS-ESI: <i>m/z</i> calcd. for C ₂₂ H ₂₈ N ₄ O ₃ S [M+H] ⁺ 429.194 6, found 429.194 8.
2b	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.03 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 7.83 (d, <i>J</i> = 9.9 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.60 (d, <i>J</i> = 2.9 Hz, 2H, NH ₂ CH ₂), 2.29–2.26 (m, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 6.9 Hz, 2H, CH ₂ -cyclohexyl), 1.65 (m, 7H, cyclohexyl), 1.53 (dd, <i>J</i> = 6.1, 2.1 Hz, 4H, cyclohexyl), 0.91 (s, 3H, CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.3, 174.4, 161.7 (2C), 156.4, 143.2, 131.0, 130.0 (2C), 118.7 (2C), 117.2, 43.9, 37.6, 36.6 (2C), 32.9 (2C), 32.6 (3C), 22.5, 13.7; HRMS-ESI: <i>m/z</i> calcd. for C ₂₃ H ₃₀ N ₄ O ₃ S [M+H] ⁺ 443.210 3, found 443.210 5.
2c	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (s, 2H, ArH), 7.88–7.79 (m, 2H, ArH), 4.63–4.55 (m, 2H, CH ₂ -S), 3.62–3.55 (m, 1H, CH-NH ₂), 2.15–2.04 (m, 2H, CH ₂ -cyclohexyl), 1.83–1.77 (m, 3H, Me), 1.68–1.60 (m, 1H, cyclohexyl), 1.54–1.48 (m, 3H, CHCH ₃), 1.35–1.21 (m, 7H, cyclohexyl), 1.02–0.90 (m, 3H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.9, 174.7, 164.1, 160.9, 156.4, 143.4, 131.0, 129.5 (2C), 118.3 (2C), 114.7, 50.87, 36.6, 36.3, 32.4 (2C), 25.8, 25.5 (3C), 20.7, 10.4; HRMS-ESI: <i>m/z</i> calcd. for C ₂₃ H ₃₀ N ₄ O ₃ S [M+H] ⁺ 443.210 3, found 443.210 6.
2d	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.82 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.54 (q, <i>J</i> = 7.1 Hz, 1H, CH-NH ₂), 2.28 (q, <i>J</i> = 7.4 Hz, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.50–1.40 (m, 3H, CHCH ₃), 1.36–1.28 (m, 2H, cyclohexyl), 1.25 (t, <i>J</i> = 6.1 Hz, 5H, cyclohexyl), 0.91 (t, <i>J</i> = 7.2 Hz, 7H, cyclohexyl and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.9, 174.9, 163.2, 160.44, 156.32, 143.51, 130.96, 129.55 (2C), 118.33 (2C), 114.63, 50.95, 36.76, 36.14, 32.50 (2C), 25.83, 25.55 (3C), 20.87, 17.97, 13.27; HRMS-ESI: <i>m/z</i> calcd. for C ₂₄ H ₃₂ N ₄ O ₃ S [M+H] ⁺ 457.225 9, found 457.226 1.
2e	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.07 (s, 2H, ArH), 7.86–7.72 (m, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.72 (m, 1H, CH-NH ₂), 2.15–2.01 (m, 2H, CH ₂ -cyclohexyl), 1.80–1.74 (s, 3H, Me), 1.71–1.62 (m, 1H, cyclohexyl), 1.52–1.44 (m, 3H, CHCH ₃), 1.36–1.09 (m, 7H, cyclohexyl), 1.03–0.90 (m, 3H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.6, 174.7, 163.2, 160.5, 154.1, 144.0, 131.8, 129.0 (2C), 117.8 (2C), 114.0, 51.0, 36.8, 36.3, 32.8 (2C), 25.9, 25.3 (3C), 18.8, 10.3; HRMS-ESI: <i>m/z</i> calcd. for C ₂₃ H ₃₀ N ₄ O ₃ S [M+H] ⁺ 443.210 3, found 443.210 7.
2f	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.04 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.80 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 4.61 (s, 2H, CH ₂ -S), 3.60 (q, <i>J</i> = 7.8 Hz, 1H, CH-NH ₂), 2.18 (q, <i>J</i> = 7.2 Hz, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 7.0 Hz, 2H, CH ₂ -cyclohexyl), 1.56–1.40 (m, 3H, CHCH ₃), 1.35–1.26 (m, 2H, cyclohexyl), 1.21 (t, <i>J</i> = 6.1 Hz, 5H, cyclohexyl), 0.91 (t, <i>J</i> = 7.2 Hz, 7H, cyclohexyl and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.2, 174.6, 163.8, 161.1, 156.3, 144.7, 131.0, 129.5 (2C), 118.1 (2C), 114.3, 51.8, 36.9 (2C), 35.4 (2C), 31.6, 26.6 (2C), 25.1, 20.2, 18.3, 14.1; HRMS-ESI: <i>m/z</i> calcd. for C ₂₄ H ₃₂ N ₄ O ₃ S [M+H] ⁺ 457.225 9, found 457.226 3.
2g	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.09 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.82 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 4.68 (s, 2H, CH ₂ -S), 3.11 (m, 2H, NH ₂ CH ₂ CH ₂), 2.60 (m, 2H, NH ₂ CH ₂ CH ₂), 2.28 (m, 2H, CH ₂ CH ₂), 2.08 (d, <i>J</i> = 7.8 Hz, 2H, CH ₂ -cyclohexyl), 1.56–1.29 (m, 11H, cyclohexyl), 0.91 (t, <i>J</i> = 7.2 Hz, 3H, CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.6, 173.6, 162.1, 160.8, 157.9, 143.6, 131.0, 129.6 (2C), 119.2 (2C), 114.3, 39.8, 36.9, 36.0, 34.5, 33.8 (2C), 32.7, 26.4, 25.3 (2C), 20.2, 14.2; HRMS-ESI: <i>m/z</i> calcd. for C ₂₄ H ₃₂ N ₄ O ₃ S [M+H] ⁺ 457.225 9, found 457.226 0.
2h	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 7.82 (d, <i>J</i> = 8.8 Hz, 2H, ArH), 4.58 (s, 2H, CH ₂ -S), 3.40 (t, <i>J</i> = 6.6 Hz, 1H, CH-NH ₂), 2.07 (s, 3H, Me), 1.79 (m, 4H, CH ₂ -cyclohexyl and CH ₂ CH ₂ CH ₂ CH ₃), 1.66–0.91 (m, 15H, cyclohexyl and CH ₂ CH ₂ CH ₂ CH ₃), 0.84 (t, <i>J</i> = 6.9 Hz, 3H, CH ₂ CH ₂ CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.0, 174.7, 163.7, 161.0, 156.2, 143.5, 131.0, 129.5 (2C), 118.3 (2C), 114.9, 55.4, 36.6, 36.2, 34.4, 32.4 (2C), 27.3, 25.8 (3C), 25.5, 22.1, 13.9, 10.4; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.256 8.
2i	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.06 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.84 (d, <i>J</i> = 8.7 Hz, 2H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.37 (t, <i>J</i> = 6.5 Hz, 1H, CH-NH ₂), 2.28 (q, <i>J</i> = 7.3 Hz, 2H, CH ₂ CH ₃), 2.06 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.69–1.17 (m, 13H, cyclohexyl and CH ₂ CH ₂ CH ₂ CH ₃), 0.88 (dt, <i>J</i> = 25.0, 7.2 Hz, 10H, CH ₂ CH ₃ and CH ₂ CH ₂ CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.9, 174.8, 163.1 (2C), 156.2, 143.5, 130.9, 129.5 (2C), 120.9 (2C), 118.3, 55.5, 36.7, 36.1, 34.5, 32.4 (2C), 27.4, 25.8, 25.5 (2C), 22.1, 17.9, 13.8, 13.2; HRMS-ESI: <i>m/z</i> calcd. for C ₂₇ H ₃₈ N ₄ O ₃ S [M+H] ⁺ 499.272 9, found 499.272 6.
2j	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.82 (d, <i>J</i> = 8.8 Hz, 2H, ArH), 4.63 (s, 1H, CH ₂ -S), 2.78 (t, <i>J</i> = 7.6 Hz, 2H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 2.40 (t, <i>J</i> = 7.3 Hz, 2H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 2.28 (d, <i>J</i> = 7.6 Hz, 2H, CH ₂ -cyclohexyl), 2.08 (s, 3H, Me), 1.66 (q, <i>J</i> = 5.8 Hz, 4H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 1.61–1.57 (m, 4H, cyclohexyl and NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 1.57–1.50 (m, 4H, cyclohexyl), 1.37–1.25 (m, 5H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.1, 175.5, 164.1 (2C), 155.3, 144.2, 131.0, 129.8 (2C), 118.0 (2C), 117.0, 43.0, 37.1, 32.9 (2C), 27.2, 26.4 (2C), 25.7 (3C), 24.6, 13.7; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.257 7.
2k	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.00 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 7.82 (d, <i>J</i> = 8.6 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 2.76 (t, <i>J</i> = 7.6 Hz, 2H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 2.40 (t, <i>J</i> = 7.3 Hz, 2H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 2.30 (m, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 6.8 Hz, 2H, CH ₂ -cyclohexyl), 1.66 (q, <i>J</i> = 5.8 Hz, 4H, NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 1.61–1.57 (m, 4H, cyclohexyl), 1.57–1.50 (m, 4H, cyclohexyl and NH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂), 1.37–1.25 (m, 5H, cyclohexyl), 0.92 (m, 3H, CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.3, 175.8, 164.3 (2C), 155.2, 144.5, 131.1, 129.9 (2C), 118.6 (2C), 117.2, 42.8, 37.2 (2C), 32.9 (2C), 27.2, 26.2 (2C), 25.9 (2C), 24.9 (2C), 18.4, 13.7; HRMS-ESI: <i>m/z</i> calcd. for C ₂₇ H ₃₈ N ₄ O ₃ S [M+H] ⁺ 499.272 9, found 499.273 0.
2l	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.03 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.84 (d, <i>J</i> = 8.6 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.21 (d, <i>J</i> = 5.5 Hz, 1H, CH-NH ₂), 2.28 (q, <i>J</i> = 7.4 Hz, 2H, CH ₂ CH ₃), 2.07 (d, <i>J</i> = 6.8 Hz, 2H, CH ₂ -cyclohexyl), 1.94 (dq, <i>J</i> = 13.4, 6.8 Hz, 1H, CH(CH ₃) ₂), 1.49–1.39 (m, 6H, CH(CH ₃) ₂), 1.30–1.20 (m, 3H, CH ₂ CH ₃), 0.89 (m, 11H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.8, 174.3, 163.0, 160.8, 156.1, 143.3, 130.8, 129.4 (2C), 120.8 (2C), 118.2, 60.6, 36.6, 35.9, 32.3 (2C), 31.7, 30.1, 25.7, 25.4 (2C), 19.3, 17.8, 17.2, 13.2; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.257 5.

Continued

Compd.	¹ H NMR, ¹³ C NMR, HRMS-ESI
2m	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.03 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.83 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.25 (d, <i>J</i> = 5.6 Hz, 1H, CH-NH ₂), 2.07 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.96 (m, 1H, CH(CH ₃) ₂), 1.80 (s, 3H, Me), 1.61 (d, <i>J</i> = 21.3 Hz, 1H, cyclohexyl), 1.45 (d, <i>J</i> = 10.2 Hz, 4H, cyclohexyl), 1.32-1.19 (m, 4H, cyclohexyl), 1.05 (t, <i>J</i> = 7.0 Hz, 2H, cyclohexyl), 0.93 (d, <i>J</i> = 6.7 Hz, 3H, CH(CH ₃) ₂), 0.86 (d, <i>J</i> = 6.7 Hz, 3H, CH(CH ₃) ₂); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.0, 174.0, 163.6, 161.0, 156.0, 143.4, 131.0, 129.5 (2C), 120.5 (2C), 118.3, 60.5, 36.6, 36.2 (2C), 32.4 (2C), 31.7, 25.8, 25.5 (2C), 18.5 (2C), 10.42; HRMS-ESI: <i>m/z</i> calcd. for C ₂₅ H ₃₄ N ₄ O ₃ S [M+H] ⁺ 471.241 6, found 471.242 0.
2n	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.03 (d, <i>J</i> = 8.9 Hz, 2H, ArH), 7.89-7.79 (m, 2H, ArH), 4.58 (s, 2H, CH ₂ -S), 3.27-3.20 (m, 1H, CH-NH ₂), 2.06 (q, <i>J</i> = 5.7 Hz, 2H, CH ₂ -cyclohexyl), 1.95 (m, 1H, CH(CH ₃) ₂), 1.83-1.76 (m, 3H, Me), 1.52-1.18 (m, 6H, CH(CH ₃) ₂), 1.04-0.79 (m, 11H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.0, 174.1, 163.6 (2C), 156.1, 143.4, 130.9, 129.5 (2C), 118.3 (2C), 114.8, 60.6, 36.6, 36.2, 32.4 (2C), 31.7 (2C), 25.8, 25.5 (2C), 19.4 (2C), 10.4; HRMS-ESI: <i>m/z</i> calcd. for C ₂₅ H ₃₄ N ₄ O ₃ S [M+H] ⁺ 471.241 6, found 471.241 8.
2o	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.83 (d, <i>J</i> = 8.8 Hz, 2H, ArH), 4.58 (s, 2H, CH ₂ -S), 3.19 (d, <i>J</i> = 5.5 Hz, 1H, CH-NH ₂), 2.28 (q, <i>J</i> = 7.3 Hz, 2H, CH ₂ CH ₃), 2.07 (d, <i>J</i> = 6.8 Hz, 2H, CH ₂ -cyclohexyl), 1.98-1.88 (m, 1H, CH(CH ₃) ₂), 1.65-1.19 (m, 9H, CH(CH ₃) ₂ and CH ₂ CH ₃), 0.88 (m, 11H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.0, 174.5, 163.6 (2C), 156.1, 143.5, 130.9, 129.5 (2C), 118.3 (2C), 114.6, 60.8, 36.6, 36.1, 32.5 (2C), 31.8 (2C), 25.8, 25.5 (2C), 19.4, 17.9, 17.2, 13.3; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.257 0.
2p	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.01 (d, <i>J</i> = 8.7 Hz, 2H, ArH), 7.83 (d, <i>J</i> = 8.2 Hz, 2H, ArH), 4.57 (s, 2H, CH ₂ -S), 3.43-3.40 (m, 1H, CH-NH ₂), 2.08 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.77-1.70 (m, 1H, CH ₂ CH(CH ₃) ₂), 1.61 (m, 8H, Me and cyclohexyl), 1.54-1.51 (m, 5H, cyclohexyl and CH ₂ CH(CH ₃) ₂), 1.17 (m, 3H, cyclohexyl), 0.91 (m, 6H, CH ₂ CH(CH ₃) ₂); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.2, 175.8, 160.9, 144.0, 131.4, 131.2, 129.9 (2C), 118.8 (2C), 112.9, 54.5, 44.4, 36.7 (2C), 33.1 (2C), 33.0, 26.3, 26.1 (2C), 23.2, 22.6 (2C), 13.8; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.257 5.
2q	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.06 (d, <i>J</i> = 8.7 Hz, 2H, ArH), 7.85 (t, <i>J</i> = 8.2 Hz, 2H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.44-3.41 (m, 1H, CH-NH ₂), 2.29 (m, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 6.7 Hz, 2H, CH ₂ -cyclohexyl), 1.77-1.70 (m, 1H, CH ₂ CH(CH ₃) ₂), 1.64-1.60 (m, 8H, cyclohexyl), 1.54-1.50 (m, 5H, cyclohexyl and CH ₂ CH(CH ₃) ₂), 1.17 (t, <i>J</i> = 7.1 Hz, 3H, CH ₂ CH ₃), 0.92 (m, 6H, CH ₂ CH(CH ₃) ₂); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.2, 175.8, 160.9, 144.0, 131.4, 131.2 (2C), 129.9 (2C), 118.8, 112.9, 54.5, 44.4, 36.7 (2C), 33.1 (2C), 33.0, 26.3, 26.1 (2C), 23.2, 22.6 (2C), 18.5, 13.8; HRMS-ESI: <i>m/z</i> calcd. for C ₂₇ H ₃₈ N ₄ O ₃ S [M+H] ⁺ 499.272 9, found 499.273 3.
2r	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.83 (d, <i>J</i> = 8.7 Hz, 2H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.44 (dd, <i>J</i> = 8.5, 5.8 Hz, 1H, CH-NH ₂), 2.08 (d, <i>J</i> = 6.6 Hz, 2H, CH ₂ -cyclohexyl), 1.80 (s, 3H, Me), 1.77-1.69 (m, 1H, CH ₂ CH(CH ₃) ₂), 1.54-1.20 (m, 10H, cyclohexyl and CH ₂ CH(CH ₃) ₂), 0.89 (m, 9H, cyclohexyl and CH ₂ CH(CH ₃) ₂); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.5, 175.5, 163.3, 161.4, 156.1, 144.0, 130.9, 129.9 (2C), 120.5 (2C), 118.4, 54.2, 44.2, 37.1, 36.7, 32.8 (2C), 30.1, 25.9 (2C), 25.8, 23.5, 22.4 (2C), 13.8; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 485.257 2, found 485.257 6.
2s	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.06-7.99 (m, 2H, ArH), 7.82 (t, <i>J</i> = 6.2 Hz, 2H, ArH), 4.60 (d, <i>J</i> = 4.0 Hz, 2H, CH ₂ -S), 3.43-3.39 (m, 1H, CH-NH ₂), 2.28 (m, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 5.5 Hz, 2H, CH ₂ -cyclohexyl), 1.73 (dp, <i>J</i> = 13.4, 6.6 Hz, 1H, CH ₂ CH(CH ₃) ₂), 1.51-1.23 (m, 9H, cyclohexyl and CH ₂ CH(CH ₃) ₂), 0.97-0.83 (m, 13H, cyclohexyl and CH ₂ CH(CH ₃) ₂ and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.9, 175.3, 163.2, 160.5, 156.3, 143.6, 130.9, 129.6 (2C), 120.9 (2C), 118.4, 54.1, 43.9, 36.7, 36.1, 32.5 (2C), 25.8, 25.6 (2C), 24.2, 23.1 (2C), 21.9, 17.9, 13.3; HRMS-ESI: <i>m/z</i> calcd. for C ₂₇ H ₃₈ N ₄ O ₃ S [M+H] ⁺ 499.272 9, found 499.272 7.
2t	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.80 (d, <i>J</i> = 8.6 Hz, 2H, ArH), 7.27-7.16 (m, 5H, ArH), 4.61 (s, 2H, CH ₂ -S), 3.66 (m, 1H, CH-NH ₂), 3.01 (dd, <i>J</i> = 13.4, 5.7 Hz, 1H, CH ₂ -Ph), 2.77 (dd, <i>J</i> = 13.4, 7.7 Hz, 1H, CH ₂ -Ph), 2.09 (d, <i>J</i> = 6.6 Hz, 2H, CH ₂ -cyclohexyl), 1.80 (s, 3H, Me), 1.26 (t, <i>J</i> = 14.8 Hz, 7H, cyclohexyl), 0.92 (h, <i>J</i> = 13.4, 12.3 Hz, 4H, cyclohexyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.3, 174.4, 163.9 (2C), 156.4, 143.9, 138.7, 131.4, 130.0, 129.7 (2C), 128.5 (2C), 126.6 (2C), 118.8 (2C), 115.4, 57.5, 37.1, 36.7 (2C), 32.9 (2C), 26.3, 26.0 (3C), 10.8; HRMS-ESI: <i>m/z</i> calcd. for C ₂₉ H ₃₄ N ₄ O ₃ S [M+H] ⁺ 519.241 6, found 519.242 0.
2u	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.81 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.26-7.16 (m, 5H, ArH), 4.61 (s, 2H, CH ₂ -S), 3.69 (m, 1H, CH-NH ₂), 3.02 (dd, <i>J</i> = 13.4, 5.7 Hz, 1H, CH ₂ -Ph), 2.77 (dd, <i>J</i> = 13.4, 7.7 Hz, 1H, CH ₂ -Ph), 2.29 (q, <i>J</i> = 7.3 Hz, 2H, CH ₂ CH ₃), 2.08 (d, <i>J</i> = 6.8 Hz, 2H, CH ₂ -cyclohexyl), 1.52-1.18 (m, 7H, cyclohexyl), 0.91 (t, <i>J</i> = 7.6 Hz, 7H, cyclohexyl and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.3, 174.4, 163.6, 160.9, 156.7, 143.9, 138.7, 131.4, 129.9 (2C), 129.8 (2C), 128.5 (2C), 126.6, 121.4 (2C), 118.9, 57.5, 37.2, 36.6 (2C), 32.9 (2C), 26.3, 26.0 (3C), 18.4, 13.7; HRMS-ESI: <i>m/z</i> calcd. for C ₃₀ H ₃₆ N ₄ O ₃ S [M+H] ⁺ 533.257 2, found 533.257 0.
2v	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 10.44 (s, 1H, C=O-NH), 8.02 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.85 (d, <i>J</i> = 8.5 Hz, 2H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.80 (m, 1H, pyrrolidinyl), 2.92 (t, <i>J</i> = 6.6 Hz, 2H, pyrrolidinyl), 2.08 (d, <i>J</i> = 7.0 Hz, 2H, CH ₂ -cyclohexyl), 1.79 (s, 3H, Me), 1.73-1.18 (m, 11H, cyclohexyl), 0.91 (m, 4H, pyrrolidinyl); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.5, 174.0, 164.1, 161.4, 156.5, 143.5, 131.6, 130.0 (2C), 118.8 (2C), 115.4, 61.2, 47.1, 37.1, 36.7, 32.8 (2C), 30.9 (2C), 26.3, 26.1 (2C), 25.9, 10.9; HRMS-ESI: <i>m/z</i> calcd. for C ₂₅ H ₃₂ N ₄ O ₃ S [M+H] ⁺ 469.225 9, found 469.226 3.
2w	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 10.69 (s, 1H, C=O-NH), 8.20-7.70 (m, 4H, ArH), 4.59 (s, 2H, CH ₂ -S), 3.90 (s, 1H, pyrrolidinyl), 2.95 (m, 2H, pyrrolidinyl), 2.16 (m, 4H, CH ₂ -cyclohexyl and CH ₂ CH ₃), 1.72-1.10 (m, 12H, cyclohexyl and pyrrolidinyl), 1.01-0.75 (m, 6H, pyrrolidinyl and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 192.5, 173.2, 163.8, 160.8, 156.8, 143.5, 131.6, 129.9 (2C), 121.3 (2C), 118.8, 61.0, 46.9, 43.9, 37.2, 36.5, 32.9, 30.8, 26.3, 25.9, 25.8, 22.5, 22.2, 18.4, 13.7; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₄ N ₄ O ₃ S [M+H] ⁺ 483.241 6, found 483.241 8.
2x	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 10.69 (s, 1H, C=O-NH), 8.20 (d, <i>J</i> = 8.4 Hz, 2H, ArH), 7.80 (d, <i>J</i> = 8.6 Hz, 2H, ArH), 4.60 (s, 2H, CH ₂ -S), 3.90 (t, 1H, pyrrolidinyl), 2.95 (m, 2H, pyrrolidinyl), 2.16 (q, <i>J</i> = 8.5 Hz, 2H, CH ₂ CH ₃), 2.06 (d, <i>J</i> = 7.2, 2H, CH ₂ -cyclohexyl), 1.72-1.10 (m, 12H, cyclohexyl and pyrrolidinyl), 1.01-0.75 (m, 6H, pyrrolidinyl and CH ₂ CH ₃); ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) δ: 191.4, 172.2, 163.8, 159.8, 156.8, 146.2, 131.8, 129.9 (2C), 121.3 (2C), 118.8, 62.4, 46.9, 43.9, 37.2, 36.5, 32.9, 30.8, 26.3, 26.0, 25.8, 22.6, 22.3, 18.4, 14.0; HRMS-ESI: <i>m/z</i> calcd. for C ₂₆ H ₃₄ N ₄ O ₃ S [M+H] ⁺ 483.241 6, found 483.242 0.

购自大连美仑生物技术有限公司。人T淋巴细胞C8166细胞系、实验株HIV-1_{111B}由NIH AIDS Reagent Progro惠赠。

Bruker DRX 400 (瑞士Bruker公司), 内标TMS, 溶剂DMSO-*d*₆; 数字熔点仪(WRS-1, 中国卓光科技优先公司); Agilent LC/MSD TOF 质谱仪(美国Agilent公司); 离心机(Allegra X-30, 美国Beckman公司); 二级生物安全柜(1287)、CO₂细胞培养箱(3111)、-80 °C冰箱(386L -86 °C)(美国Thermo公司); 2~8 °C冰箱(HYC-360)、-20 °C冰箱(BCD-196E/D)(中国Haier公司); 酶标仪(800TS, 美国Bioteck公司); 倒置显微镜(TS-100F, 日本Nikon公司)。

1 化合物的合成

1.1 *N*-Fmoc-(2-(4-乙酰苯氨基)-2-氧代乙基)氨基甲酸酯的合成 取250 mL的干燥圆底烧瓶, 将*N*-Fmoc-氨基酸**3** (10 mmol) 溶于150 mL二氯甲烷溶液中, 滴加0.75 mL二氯亚砷(10 mmol), 加热回流反应5 h后, 减压蒸馏除去溶剂, 真空干燥2 h, 得中间体*N*-Fmoc-氨基酸酰氯**4**, 用150 mL无水乙醚溶解中间体**4**, 快速加入氨基苯乙酮**5** (10 mmol), 剧烈震荡5 min, 有固体生成, 抽滤, 滤饼用50 mL的水洗涤, 真空干燥48 h得化合物**6**, 收率为62%~89%。可不经纯化直接用于下一步反应。

1.2 (9*H*-苻-9-基)甲基-(((4-(2-溴乙酰基)苯基)氨基))氨基甲酸酯的合成 在三口烧瓶中加入上一步的固体产物**6**, 再加入4.467 g (约20 mmol) 溴化铜粉末、120 mL (乙酸乙酯-氯仿=1:1, *V/V*) 的混合溶剂, 加热回流20 min, 加入20 mL无水乙醇, 反应2 h后停止加热, 趁热抽滤, 滤饼用冷氯仿(30 mL)洗涤。合并滤液, 用无水硫酸镁干燥, 减压蒸馏得淡黄色固体; 无水乙醇重结晶得化合物**7**, 该粗产物可直接投下一步。

1.3 目标化合物的合成 在50 mL干燥圆底烧瓶中加入2 mmol的5-烷基-2-噻嘧啶酮**8**和10 mL DMF, 待完全溶解后加入2 mmol无水碳酸钾, 常温搅拌0.5 h, 加入上一步所得中间体**7** (2 mmol), TLC跟踪检测反应至原料点消失, 将反应液倒入冰水中, 剧烈搅拌, 产生黄色浑浊物, 用乙酸乙酯萃取3次, 合并有机层并用饱和食盐水洗涤3次, 无水硫酸钠干燥, 减压蒸馏除去溶剂, 得到黄色固体粗产品**(9)**, 用6 mL二氯甲烷溶解**(9)**, 再加入3 mL哌啶, 常温下反应30 min, 减压蒸馏除去溶剂。即获得油状物质, 用无水乙醚少量多次洗去哌啶, 干燥后经过重结晶纯化得到目标化合物**(2)**。收率: 30%~69%。

2 目标化合物的抗HIV-1活性测试

2.1 化合物对C8166细胞的毒性实验 96孔板内将

药物进行5倍梯度稀释, 共设置6个药物浓度, 每个药物浓度设3个复孔(每孔100 μL), 同时设置无药物的阴性对照孔(NC)和空白对照孔(BC)。除BC孔外, 每孔加入100 μL C8166细胞悬液(细胞密度为每毫升4×10⁵个)。在37 °C、5% CO₂培养箱培养3天, 采用MTT法测定化合物对C8166细胞系的毒性。800TS酶标仪在570/630 nm波长下测定OD值, 计算半数细胞毒性浓度(the 50% cytotoxicity concentration, CC₅₀)^[16,17]。

2.2 化合物对HIV-1_{111B}致C8166细胞病变的抑制作用 96孔板内将药物进行5倍梯度稀释, 共设置6个药物浓度, 每个药物浓度设3个复孔(每孔100 μL)。每孔加50 μL C8166细胞悬液(细胞密度为每毫升8×10⁵个)与50 μL HIV-1_{111B}病毒稀释液(1300 TCID₅₀), 同时设置含C8166细胞与病毒的阳性对照孔(PC), 置于37 °C、5% CO₂培养箱内培养。第3天在倒置显微镜下(100×)观察合胞体的形成并计数, 计算半数有效浓度(the 50% effective concentration, EC₅₀)^[18]。

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利益冲突: 无利益冲突。

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