9-取代巴马汀衍生物抗幽门螺杆菌活性研究

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摘要: 本研究共设计合成了15个9-取代巴马汀 (palmatine, **1**, 异喹啉生物碱类) 衍生物, 首次评价了其体外抗幽门螺杆菌 (*Helicobacter pylori*, Hp) 活性。初步构效关系表明9-位引入适当的二级胺取代基利于抗菌活性提高。其中, 代表性化合物**5a**对部分甲硝唑耐药菌株显示较好活性, 最低抑菌浓度 (MICs) 值为4 μg·mL^{-1}, 优于先导物**1**。另外, **5a**显示良好安全性, 口服LD_{50}>1 000 mg·kg^{-1}。分子对接实验结果提示, 化合物**5a**可能通过作用于Hp脲酶而发挥抑菌作用。本研究结果为巴马汀类衍生物发展成一类新颖抗Hp联合用药组分提供了重要科学数据。

关键词: 9-取代巴马汀; 幽门螺杆菌; 构效关系; 分子对接

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Anti-*Helicobacter pylori* activities of 9-substituted palmatine derivatives

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Abstract: Fifteen 9-substituted palmatine (**1**) derivatives were synthesized and evaluated for their anti-*Helicobacter pylori* (Hp) activities *in vitro*. Structure-activity relationship studies revealed that introducing appropriate substituted secondary amino group at position 9 of lead **1** might be beneficial for potency. Among them, compound **5a** showed the most potential activity against metronidazole (Met) resistant Hp isolates with minimal inhibitory concentrations (MICs) of 4 μg·mL^{-1}, much better than that of lead **1**. Compound **5a** displayed satisfactory safety profile in acute toxicity assay. Molecular docking suggested that **5a** might act on Hp urease. The results provided key scientific evidence for the development of **1** derivatives into a new class of anti-Hp component.

Key words: 9-substitute palmatine; *Helicobacter pylori*; structure-activity relationship; molecular docking

幽门螺旋杆菌 (*Helicobacter pylori*, Hp) 是一类具有严重致病性和传染性的革兰阴性菌, 与黄曲霉素、砒霜等被世界卫生组织共列为I类致癌物[1], 不仅可诱发胃癌和胃淋巴瘤等肿瘤, 还与心脑血管疾病、肝胆疾病、慢性支气管炎和缺铁性贫血等其他系统疾病发生有关[2-5]。1982年澳大利亚学者Marshall和Warren首次报道Hp, 并为此共享2005年诺贝尔生理学与医学奖。流行病学研究显示Hp感染形式严峻, 在家庭内有明显的聚集现象, 自愈率几乎为零; 全球感染率超过50%[6], 而我国Hp感染率约为58%～64%, 明显高于发达国家水平。因单一用药不能有效清除Hp, 目前临床多采用联合用药治疗方案[7-9], 以三联疗法质子泵抑制剂 (PPI) +克拉霉素+阿莫西林/甲硝唑 (Met) 最为常见。但由于抗生素的大量使用, Hp对抗生素耐药率不

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断提高, 如克拉霉素为20%～50%, Met为40%～70%。因此, 寻找新的结构骨架和作用机制的Hp治疗药物尤为迫切。

本课题组长期致力于从生物碱天然产物中寻找化学结构新颖、作用机制独特的创新药物, 如在小檗碱、苦参碱等结构修饰和生物活性挖掘方面进行了深入研究[10-14]。Hp脲酶在人类和动物Hp感染的发病机制中起着重要作用[15,16], 是研究和寻找抗Hp先导物的热门靶点之一[17]。来源于黄连的原小檗碱类生物碱巴马汀 (palmatine, **1**) 是一类新颖的Hp脲酶抑制剂, 虽然其对Hp感染引起的胃炎和消化性溃疡以及其他与脲酶相关的疾病有潜在的治疗作用, 然而其最低抑Hp浓度 (MIC) 介于100～200 μg·mL^{-1}之间[18,19], 抗菌活性不理想, 并且其衍生物抗Hp活性和构效关系未见文献报道。综上, 为获得抗Hp活性更好的候选物, 本文以**1**为先导物, 保留A环二甲氧基侧链, 针对其9-位衍生物易合成特点, 设计合成了一系列**1**的9-位取代衍生物 (图1), 探讨了9-位取代衍生物的抗Hp构效关系 (structure-activity relationship, SAR), 并对活性较好的化合物开展了急性毒性与初步作用机制等研究。

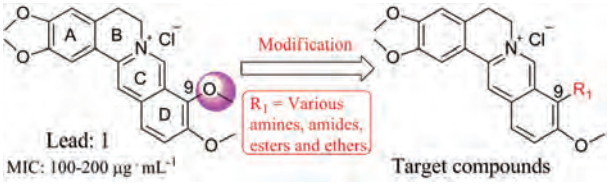


Figure 1 Chemical structure of **1**, and its structure modification strategy

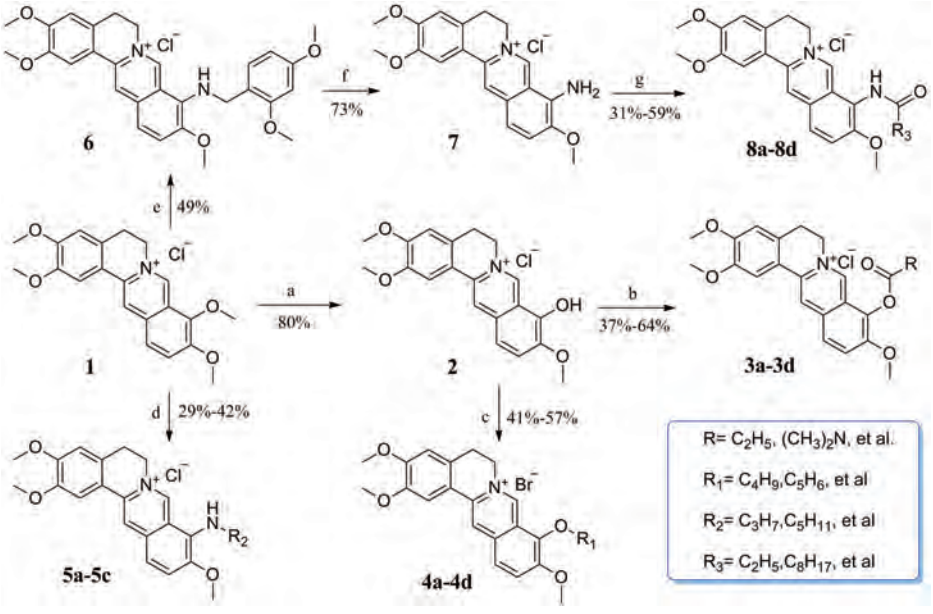
结果和讨论

1 化合物的合成

目标化合物的合成见合成路线1。首先, 以市售**1**为原料, 选择性脱除9位甲基后, 经盐酸乙醇酸化得到关键中间体9-羟基巴马汀**2**[20], 随后在碱性条件下, 与相应的酰氯或卤代烃经过酯化或者醚化反应得到目标物**3a**～**3d**和**4a**～**4d**[13,20-22]。其次, 以**1**为原料, 在95～120 ℃条件下, 与相应的胺 (既是反应溶剂, 也是反应试剂) 反应, 得到胺类衍生物**5a**～**5c**[22,23]。经上述方法, **1**与2,4-二甲氧基苄胺反应得到关键产物**6**, 在酸性条件下脱除保护基后得到中间体**7**[22], 随后以吡啶为缚酸剂, **7**与相应的酰氯反应得到酰胺类衍生物**8a**～**8d**[22]。所有目标化合物结构经^{1}H NMR、^{13}C NMR以及HR-MS分析确证。目标化合物的收率、理化参数和波谱数据见表1。

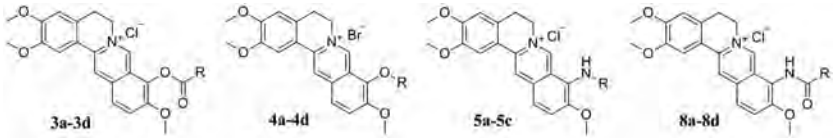
2 抗菌活性测定

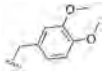
2.1 目标化合物抗Hp活性SAR研究 以Met和**1**为阳性对照, 以纸片法药敏试验评价了所有目标化合物对5株 (ATCC43504、CCPMAP160010、CCPMAP160008、CCPMAP160007和CCPMAP160017) Met耐药的Hp的体外抑菌活性 (表2)。9-位烷基、芳香基和含氮杂原子基等酯类衍生物**3a**～**3d**抗菌活性部分或完全丧失, 均弱于先导物, 说明9-位被酯基取代可能不利于抗菌活性提高。随后将9-位酯连接臂转化为醚, 由此获得



Scheme 1 Reagents and conditions: (a) 195−210 ℃, 30−40 mmHg, 60 min; (b) RCOCl, triethylamine, CH_3CN, 65 ℃, 3−8 h; (c) Halogenated hydrocarbons, K_2CO_3 for **4a**,**4b** and **4d**, K_2CO_3 and NaI for **4c**, CH_3CN, 71 ℃, 2−24 h; (d) RNH_2, 95−120 ℃, 4−72 h; (e) 2,4-Dimethoxybenzylamine, 116 ℃, 7 h; (f) 1∶1 HCl/CH_3OH, rt, 24 h; (g) R_3COCl, pyridine, CH_3CN, 71 ℃, 3−24 h

Table 1 Structures, physical properties and spectra data of all synthesized compounds



No.	R	Yield /%	mp/°C (Dec.)	^{1}H NMR (DMSO-d_6)	^{13}C NMR (DMSO-d_6)	HR-ESI-MS or ESI (m/z)
3a	C_2H_5	53	186−188	^{1}H NMR (600 MHz) δ 9.94 (s, 1H), 9.15 (s, 1H), 8.31−8.27 (m, 1H), 8.24 (d, J = 9.6 Hz, 1H), 7.74 (s, 1H), 7.10 (s, 1H), 4.97 (t, J = 6.6 Hz, 2H), 4.04 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.24 (t, J = 6.6 Hz, 2H), 2.91 (q, J = 7.8 Hz, 2H), 1.24 (t, J = 7.8 Hz, 3H).	^{13}C NMR (151 MHz) δ 171.5, 151.6, 150.3, 148.7, 144.4, 138.3, 133.5, 133.0, 128.8, 126.5, 125.9, 121.1, 120.3, 118.8, 111.3, 108.9, 57.2, 56.2, 55.9, 55.5, 26.6, 25.8, 8.8.	$C_{23}H_{24}NO_5Cl$ $[M-Cl]^+$: 394.164 9, found: 394.164 9.
3b	$(CH_3)_2N$	44	195−197	^{1}H NMR (400 MHz) δ 9.89 (s, 1H), 9.16 (s, 1H), 8.27 (d, J = 9.0 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 7.75 (s, 1H), 7.10 (s, 1H), 5.00 (t, J = 6.6 Hz, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.28−3.21 (m, 5H), 3.01 (s, 3H).	^{13}C NMR (101 MHz) δ 153.1, 152.0, 151.2, 149.2, 144.9, 138.5, 135.0, 133.4, 129.2, 126.5, 126.4, 122.1, 120.7, 119.3, 111.7, 109.3, 57.6, 56.6, 56.3, 55.9, 37.2, 37.0, 26.3.	$C_{23}H_{25}N_2O_5Cl$ $[M-Cl]^+$: 409.175 8, found: 409.175 8.
3c		37	180−182	^{1}H NMR (500 MHz) δ 9.97 (s, 1H), 9.15 (s, 1H), 8.34 (d, J = 9.0 Hz, 1H), 8.28 (d, J = 9.0 Hz, 1H), 7.74 (s, 1H), 7.11 (s, 1H), 4.95 (t, J = 6.6 Hz, 2H), 4.16 (s, 2H), 4.08 (s, 3H), 3.99 (t, J = 7.8 Hz, 2H), 3.95 (s, 3H), 3.88 (s, 3H), 3.44 (s, 3H), 3.26 (t, J = 6.6 Hz, 2H).	^{13}C NMR (126 MHz) δ 151.7, 150.5, 149.6, 148.8, 144.3, 138.6, 133.1, 132.2, 129.6, 128.8, 127.2, 126.0, 121.0, 120.3, 118.8, 111.3, 108.9, 57.3, 56.2, 55.9, 55.7, 41.1, 40.6, 31.3, 25.8.	$C_{25}H_{26}N_3O_8SCl$ $[M-Cl]^+$: 528.143 5, found: 528.143 5.
3d	C_5H_6	64	199−202	^{1}H NMR (600 MHz) δ 10.00 (s, 1H), 9.23 (s, 1H), 8.35 (d, J = 9.6 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.29−8.26 (m, 2H), 7.88−7.82 (m, 1H), 7.77 (s, 1H), 7.73−7.69 (m, 2H), 7.09 (s, 1H), 4.93 (t, J = 6.6 Hz, 2H), 4.03 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.22 (t, J = 6.6 Hz, 2H).	^{13}C NMR (151 MHz) δ163.9, 152.1, 150.7, 149.2, 144.9, 138.8, 135.0, 134.0, 133.5, 130.9 (2), 129.6 (2), 129.2, 128.4, 127.3, 126.3, 121.6, 120.8, 119.3, 111.7, 109.3, 57.7, 56.6, 56.3, 55.9, 26.2.	$C_{27}H_{24}NO_5Cl$ $[M-Cl]^+$: 442.164 9, found: 442.164 8.
4a	C_4H_9	41	201−203	^{1}H NMR (600 MHz) δ 9.74 (s, 1H), 9.03 (s, 1H), 8.21 (dd, J = 9.6, 2.4 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.72 (d, J = 2.4 Hz, 1H), 7.10 (s, 1H), 4.97 (t, J = 6.6 Hz, 2H), 4.30 (t, J = 6.6 Hz, 2H), 4.06 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H), 3.23 (t, J = 6.6 Hz, 2H), 1.92−1.82 (m, 2H), 1.53 (qd, J = 7.8, 2.4 Hz, 2H), 0.99 (td, J = 7.8, 2.4 Hz, 3H).	^{13}C NMR (151 MHz) δ 151.5, 150.2, 148.7, 145.3, 142.8, 137.7, 133.1, 128.6, 126.7, 123.1, 121.6, 119.9, 118.9, 111.3, 108.7, 73.9, 57.0, 56.2, 55.9, 55.5, 31.6, 26.0, 18.6, 13.8.	$C_{24}H_{28}NO_4Cl$ $[M-Br]^+$: 394.201 3, found: 394.201 4.
4b	C_5H_6	48	233−235	^{1}H NMR (500 MHz) δ 9.73 (s, 1H), 9.03 (s, 1H), 8.23 (dd, J = 9.0, 1.8 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.71 (s, 1H), 7.59 (d, J = 7.8 Hz, 2H), 7.44−7.31 (m, 3H), 7.10 (s, 1H), 5.36 (s, 2H), 4.93 (t, J = 6.6 Hz, 2H), 4.09 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.21 (t, J = 6.6 Hz, 2H).	^{13}C NMR (126 MHz) δ 151.9, 151.0, 149.2, 145.8, 142.4, 138.0, 136.9, 133.5, 129.2 (2), 129.0, 128.9, 128.8 (2), 127.0, 124.1, 122.2, 120.3, 119.3, 111.7, 109.2, 75.8, 57.5, 56.6, 56.3, 56.0, 26.4.	$C_{27}H_{26}NO_4Cl$ $[M-Br]^+$: 428.
4c		43	114−116	^{1}H NMR (600 MHz) δ 9.76 (s, 1H), 9.02 (s, 1H), 8.27−8.17 (m, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.70 (s, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.10 (s, 1H), 7.02 (dd, J = 7.8, 1.8 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 5.32 (s, 2H), 4.91 (t, J = 6.6 Hz, 2H), 4.12 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.76 (s, 3H), 3.71 (s, 3H), 3.20 (t, J = 6.6 Hz, 2H).	^{13}C NMR (151 MHz) δ 151.9, 151.1, 149.4, 149.2, 149.0, 145.9, 142.3, 138.0, 133.4, 129.0, 128.9, 126.9, 123.9, 122.5, 122.3, 120.3, 119.3, 113.3, 111.7, 111.6, 109.2, 75.7, 57.4, 56.6, 56.3, 56.0, 55.9, 55.9, 26.4.	$C_{29}H_{30}NO_6Cl$ $[M-Br]^+$: 488.206 8, found: 488.206 8.
4d	4-$NO_2C_6H_4$	57	176−178	^{1}H NMR (600 MHz) δ 9.86 (s, 1H), 9.07 (s, 1H), 8.30 (dd, J = 8.4, 2.4 Hz, 2H), 8.24 (dd, J = 9.0, 1.9 Hz, 1H), 8.08 (d, J = 9.0 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.73 (s, 1H), 7.11 (d, J = 1.8 Hz, 1H), 5.51 (s, 2H), 4.96 (t, J = 6.6 Hz, 2H), 4.07 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H), 3.23 (t, J = 6.6 Hz, 2H).	^{13}C NMR (151 MHz) δ 151.5, 150.3, 148.7, 147.2, 145.3, 144.4, 141.7, 137.8, 133.1, 129.1 (2), 128.7, 126.6, 123.9, 123.5 (2), 121.5, 119.9, 118.9, 111.3, 108.8, 74.0, 57.1, 56.2, 55.9, 55.5, 26.0	$C_{27}H_{25}N_2O_6Cl$ $[M-Br]^+$: 473.170 7, found: 473.170 7.

Continued

No.	R	Yield /%	mp/℃ (Dec.)	^{1}H NMR (DMSO-d_6)	^{13}C NMR (DMSO-d_6)	HR-ESI-MS or ESI (*m*/*z*)
5a	C_3H_7	34	239−241	^{1}H NMR (600 MHz) δ 10.13 (s, 1H), 8.79 (s, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.68 (s, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 7.08 (s, 1H), 6.44 (t, *J* = 6.0 Hz, 1H), 4.82 (t, *J* = 6.6 Hz, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.86 (s, 3H), 3.55 (q, *J* = 7.2 Hz, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 1.63 (q, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).	^{13}C NMR (151 MHz) δ 151.1, 148.7, 146.5, 146.4, 137.2, 135.8, 133.2, 128.2, 124.6, 119.3, 119.1, 116.9, 115.8, 111.3, 108.5, 57.0, 56.1, 55.8, 55.0, 49.0, 26.3, 23.7, 11.4.	$C_{23}H_{27}N_2O_3Cl$ $[M-Cl]^+$: 379.201 6, found: 379.201 6.
5b	C_4H_9	29	197−199	^{1}H NMR (600 MHz) δ 10.22−10.09 (m, 1H), 8.79 (s, 1H), 7.90 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.68 (s, 1H), 7.50 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.07 (d, *J* = 1.8 Hz, 1H), 6.54−6.33 (m, 1H), 4.82 (t, *J* = 6.6 Hz, 2H), 3.96 (d, *J* = 1.8 Hz, 3H), 3.93 (d, *J* = 1.2 Hz, 3H), 3.86 (d, *J* = 1.8 Hz, 3H), 3.59 (q, *J* = 6.6 Hz, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 1.66−1.54 (m, 2H), 1.43−1.32 (m, 2H), 0.90 (td, *J* = 7.2, 1.8 Hz, 3H).	^{13}C NMR (151 MHz) δ 151.1, 148.7, 146.4 (2), 137.2, 135.8, 133.2, 128.2, 124.6, 119.3, 119.1, 116.9, 115.8, 111.3, 108.5, 56.9, 56.1, 55.8, 54.9, 46.9, 32.7, 26.3, 19.6, 13.9.	$C_{24}H_{29}N_2O_3Cl$ $[M-Cl]^+$: 393.217 3, found: 393.217 1.
5c	C_5H_{11}	42	205−207	^{1}H NMR (600 MHz) δ 10.09 (s, 1H), 8.79 (s, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.68 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.08 (s, 1H), 6.39 (t, *J* = 6.0 Hz, 1H), 4.82 (t, *J* = 6.6 Hz, 2H), 3.96 (s, 3H), 3.93 (s, 3H), 3.86 (s, 3H), 3.58 (q, *J* = 6.6 Hz, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.33 (dq, *J* = 7.8, 4.2, 3.0 Hz, 4H), 0.87 (t, *J* = 6.6 Hz, 3H).	^{13}C NMR (151 MHz) δ 151.1, 148.7, 146.5, 146.4, 137.1, 135.8, 133.1, 128.2, 124.5, 119.3, 119.1, 116.9, 115.9, 111.3, 108.5, 56.9, 56.1, 55.8, 55.0, 47.3, 30.2, 28.6, 26.3, 21.9, 14.0.	$C_{25}H_{31}N_2O_3Cl$ $[M-Cl]^+$: 407.232 9, found: 407.232 6.
8a	C_2H_5	41	245−247	^{1}H NMR (600 MHz) δ 10.06 (s, 1H), 9.65 (s, 1H), 9.13 (s, 1H), 8.23 (d, *J* = 4.8 Hz, 2H), 7.75 (s, 1H), 7.11 (s, 1H), 4.98 (t, *J* = 6.6 Hz, 2H), 4.04 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.23 (t, *J* = 6.6 Hz, 2H), 2.57 (q, *J* = 7.8 Hz, 2H), 1.16 (t, *J* = 7.8 Hz, 3H).	^{13}C NMR (151 MHz) δ 173.3, 154.7, 154.1, 151.5, 148.7, 146.3, 137.5, 133.3, 128.6, 125.2, 123.9, 121.9, 120.4, 118.9, 111.3, 108.8, 56.9, 56.2, 55.9, 55.4, 28.5, 26.0, 9.5.	$C_{23}H_{25}N_2O_4Cl$ $[M-Cl]^+$: 393.180 9, found: 393.181 1.
8b	C_5H_{11}	59	216−218	^{1}H NMR (600 MHz) δ 10.03 (s, 1H), 9.59 (s, 1H), 9.10 (s, 1H), 8.22 (d, *J* = 1.8 Hz, 2H), 7.74 (s, 1H), 7.10 (s, 1H), 4.97 (t, *J* = 6.6 Hz, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.23 (t, *J* = 6.6 Hz, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 1.68 (t, *J* = 7.2 Hz, 2H), 1.49−1.30 (m, 4H), 0.92 (t, *J* = 7.2 Hz, 3H).	^{13}C NMR (151 MHz) δ 172.7, 154.1, 151.5, 148.7, 146.2, 137.6, 133.2, 128.6, 127.2, 125.2, 123.9, 121.9, 120.4, 118.9, 111.3, 108.8, 56.9, 56.2, 55.9, 55.5, 35.5, 31.0, 26.0, 24.7, 22.0, 14.0.	$C_{26}H_{31}N_2O_4Cl$ $[M-Cl]^+$: 435.227 8, found: 435.227 9.
8c	C_6H_{13}	31	238−240	^{1}H NMR (600 MHz) δ 9.99 (s, 1H), 9.56 (s, 1H), 9.07 (s, 1H), 8.19 (s, 2H), 7.71 (s, 1H), 7.08 (s, 1H), 4.94 (t, *J* = 6.6 Hz, 2H), 4.00 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.21 (t, *J* = 6.6 Hz, 2H), 2.51 (t, *J* = 7.8 Hz, 2H), 1.64 (p, *J* = 7.8 Hz, 2H), 1.44−1.15 (m, 6H), 0.97−0.78 (m, 3H).	^{13}C NMR (151 MHz) δ 172.7, 154.1, 151.5, 148.7, 146.2, 137.6, 133.2, 128.6, 127.2, 125.2, 123.9, 121.9, 120.4, 118.9, 111.3, 108.8, 56.9, 56.2, 55.9, 55.5, 35.5, 31.1, 28.4, 26.0, 25.0, 22.1, 14.0.	$C_{27}H_{33}N_2O_4Cl$ $[M-Cl]^+$: 449.243 5, found: 449.243 4.
8d		52	209−211	^{1}H NMR (600 MHz) δ 10.26 (s, 1H), 9.72 (s, 1H), 9.12 (s, 1H), 8.28 (d, *J* = 3.0 Hz, 2H), 8.18−8.07 (m, 2H), 7.75 (s, 1H), 7.15−7.11 (m, 2H), 7.10 (s, 1H), 4.98 (t, *J* = 6.6 Hz, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.21 (t, *J* = 6.6 Hz, 2H).	^{13}C NMR (151 MHz) δ 165.7, 162.3, 154.7, 151.5, 148.7, 146.1, 137.7, 133.3, 130.2, 130.2, 128.7, 127.5, 125.8, 125.3, 124.5, 122.2, 120.5, 119.0, 113.6, 113.6, 111.3, 108.8, 56.9, 56.2, 55.9, 55.6, 55.3, 25.9.	$C_{28}H_{27}N_2O_5Cl$ $[M-Cl]^+$: 471.191 5, found: 471.191 4.

9-位烷基和芳香基醚衍生物**4a**～**4d**。除化合物**4d**仅对临床菌株CCPMAP160008和CCPMAP160007的抗菌活性稍强于先导物外, 其他衍生物的抗菌活性均弱于先导物, 其中**4a**～**4d**对菌株CCPMAP160017均未显示活性。基于含氨基等可离子化氮原子的小分子更有利于在阴性菌内聚集进而杀灭细菌[24], 设计合成了3个9-位链状氨基取代的胺类衍生物**5a**～**5c**, 其对测试菌株的抗菌活性均优于**1**, 说明9-位氨基取代时可能有利于衍生物抗Hp活性的提高。同时, 为进一步证实氨基取代对抗菌活性的影响, 相继合成并测定了离子化能力相对较弱的9-位烷基、芳香基巴马汀酰胺类衍生物**8a**～**8d**的抗Hp活性, 活性结果符合推断: 仅**8b**

Table 2 Antibacterial activities of the target compounds against *H. pylori* strains. [a]The American Type Culture Collection (ATCC); [b]Clinical isolated strains from patients in Chinese hospitals provided by CAMS-CCPM; [c]NA: Inhibition zone diameters less than 12 mm

Compd.	Inhibition zone diameter/mm				
	ATCC43504[a]	CCPMAP160010[b]	CCPMAP160008[b]	CCPMAP160007[b]	CCPMAP160017[b]
1	17.5 ± 1.4	13.4 ± 0.5	13.9 ± 0.5	15.3 ± 1.8	12.0 ± 0.4
3a	NA[c]	NA	NA	NA	NA
3b	NA	NA	NA	NA	NA
3c	NA	NA	NA	NA	NA
3d	NA	NA	13.5 ± 0.45	14.2 ± 0.71	NA
4a	NA	NA	NA	14.5 ± 1.41	NA
4b	14.4 ± 1.1	12.4 ± 0.3	14.5 ± 0.5	16.8 ± 1.3	NA
4c	12.2 ± 0.3	NA	12.6 ± 0.3	15.1 ± 1.4	NA
4d	16.3 ± 0.6	NA	14.5 ± 0.7	17.8 ± 1.8	NA
5a	19.3 ± 1.8	14.6 ± 0.9	17.2 ± 1.8	21.5 ± 1.4	18.7 ± 1.8
5b	18.3 ± 1.6	15.1 ± 1.4	15.5 ± 0.7	21.5 ± 1.7	15.2 ± 1.6
5c	17.5 ± 1.2	19.6 ± 1.9	17.7 ± 1.3	23.3 ± 1.6	20.6 ± 1.7
8a	NA	NA	NA	NA	NA
8b	17.7 ± 1.5	NA	14.2 ± 0.7	17.1 ± 0.7	12.2 ± 0.9
8c	NA	NA	13.4 ± 0.5	14.6 ± 0.5	NA
8d	NA	NA	NA	NA	NA
Met	NA	NA	NA	NA	NA

对菌株CCPMAP160007抗菌活性强于先导物外, 其他衍生物抗菌活性均降低甚至丧失。

2.2 二倍稀释法测定衍生物抗Hp活性 选择对Met耐药的致病菌具有较好的抑制作用的化合物**5a**～**5c**, 采用平皿二倍稀释法测定对5株致病菌的MIC, 结果见表3。化合物**5a**～**5c**的抗Hp活性均优于先导物**1**和Met, 其中化合物**5a**抗菌活性最好, 对菌株CCPMAP160008的抗菌活性强于先导物32倍, 为此选取化合物**5a**进行下一步初步安全性和初步机制研究。

3 初步安全性评价

3.1 化合物5a毒性预测结果 首先, 使用ADMET Predictor 9.5软件预测了化合物**5a**的致癌性、生殖毒性和肝毒性, 并对整体毒性性质进行打分 (表4)。结果显示, 化合物**5a**对上述毒性都在合理范围内, 提示化合物**5a**可能具有较好的安全性。

3.2 化合物5a急性毒性实验结果 昆明小鼠单次灌胃 (i.g.) 给药, 化合物**5a**剂量分别为0、250、500和1 000 mg·kg^{-1}, 密切观察7天。小鼠灌胃急性毒性结果显示半数致死量 (LD_{50}) 大于1 000 mg·kg^{-1}, 说明化合物**5a**具有较好的安全性。

4 化合物5a分子对接研究

鉴于**1**是较好的Hp脲酶抑制剂[19], 本研究将化合物**5a**和先导物**1**分别与Hp脲酶进行了分子对接研究。使用Discovery Studio 4.5软件, 以分子对接方法模拟代表性化合物**5a**和**1**分别与Hp脲酶 (1E9Y) 活性腔的相互作用[25,26]。化合物**5a**和Hp脲酶对接打分 (LibDock Score = 101.74) 明显大于先导物**1** (LibDock Score = 92.14)。另外, 如图2所示, 化合物**5a**与**1**相比, 与Hp脲酶作用模式除主要有氢键、分子间范德华力、碳氢键等外, 还有其9-位氨基氢与Hp脲酶残基

Table 3 The MIC values of compounds **5a**–**5c**

Compd.	MIC/μg·mL^{-1}				
	ATCC43504	CCPMAP160010	CCPMAP160008	CCPMAP160007	CCPMAP160017
1	64	64	128	64	128
5a	16	4	4	4	64
5b	32	8	16	8	32
5c	32	4	8	4	64
Met	128	16	32	64	256

Table 4 Toxicity prediction results of **5a**. [a]Rat-TD_{50}, suggested values: ⩾ 6.5; [b]Mouse-TD_{50}, suggested values: ⩾ 35; [c]Repro-Tox, reproduction toxicity; [d]Ser-AlkPhos, alkaline phosphatase level; [e]Ser-GGT, γ-glutamyl transpeptidase level; [f]Ser-LDH, lactic dehydrogenase level; [g]Ser-AST, serum glutamic oxalacetic transaminase; [h]Ser-ALT, serum glutamic pyruvic transaminase; [i]Tox-Risk, druglike risk about toxicity, suggested values: TOX Risk ⩽ 2.0

Code	Rat-TD_{50}[a] /mg·kg^{-1}·d^{-1}	Mouse-TD_{50}[b] /mg·kg^{-1}·d^{-1}	Repro-Tox[c]	Ser-AlkPhos[d]	Ser-GGT[e]	Ser-LDH[f]	Ser-AST[g]	Ser-ALT[h]	Tox-Risk[i]
5a	26.74	40.08	Non toxic	Normal	Normal	Normal	Normal	Normal	2

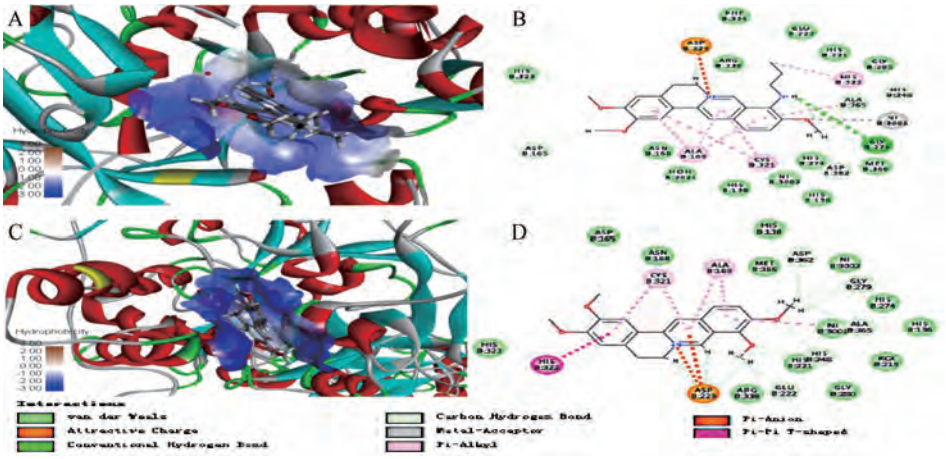


Figure 2 (A) Solid surface map of the interaction pocket with compound **5a**. (B) Binding modes within the receptor Hp urease active pocket with compound **5a**. (C) Solid surface map of the interaction pocket with compound **1**. (D) Binding modes within the receptor Hp urease active pocket with compound **1**. The receptor structure is shown in surface form. Key bonds are indicated by dashed lines between the atoms involved, and the colors of key bonds and residues are shown according to the interaction mode

GLYB:279产生强的氢键相互作用, 进而能更好的匹配Hp脲酶的活性腔。上述结果说明, 化合物**5a**可能同样是通过抑制Hp脲酶活性来起到较强的抗Hp活性。

结论

本研究以**1**为先导物, 以抗Hp活性为导向, 共设计合成了15个化合物9-位取代衍生物, 初步构效关系表明: 9-位引入适当的可离子化氮原子取代基利于抗菌活性的提高。代表性化合物**5a**对5株Met耐药的Hp活性明显优于阳性对照药Met和先导物**1**, 对于CCPMAP160010、CCPMAP160008和CCPMAP160007等菌株的MIC可达4 μg·mL^{-1}。另外, 化合物**5a**小鼠口服急毒LD_{50}大于1 000 mg·kg^{-1}, 表明具有较好的安全性特征。并且, 分子对接实验结果提示**5a**作用靶点可能是Hp脲酶。巴马汀类衍生物有希望发展成一类化学骨架新颖的抗Hp化合物, 值得进一步研究。

实验部分

熔点用CXM-300型精密熔点仪测定, 温度未校正; ^{1}H NMR和^{13}C NMR用Bruker Avance III 600核磁共振仪测定, 溶剂均为DMSO-d_6; HR-MS用Autospec Ultima-TOF质谱测定仪测定; Flash柱分离纯化用Combiflash Rf 200快速制备液相; 荧光检测用ZF-20D暗箱式紫外分析仪; 薄层色谱(TLC)采用E-Merck公司预铺硅胶铝箔卷; 试剂均为分析纯。

1 化学合成

1.1 化合物3a～3d和4a～4d的合成 在195～210 ℃负压(20～30 mmHg, 1 mmHg = 133 Pa)下, 对**1**加热30 min后, 将反应体系冷却, 将紫黑色固体产物用5%盐酸/乙醇酸化, 减压除去溶剂, 以二氯甲烷/甲醇为流动相, 经硅胶柱梯度洗脱, 分离纯化得棕黄色固体**2**。

将**2** (100 mg, 0.27 mmol)溶于无水乙腈(5 mL)中, 65 ℃条件下, 加入三乙胺(1.22 mmol)和相应酰氯(0.81 mmol), 继续反应3～8 h。冷却反应体系, 减压抽滤, 滤渣以二氯甲烷/甲醇为流动相, 经硅胶柱梯度洗脱, 分离纯化得目标物**3a**～**3d**。

将**2** (100 mg, 0.27 mmol)溶于无水*N*,*N*-二甲基甲酰胺(5 mL)中, 71 ℃条件下, 加入K_2CO_3 (1.08 mmol)或者K_2CO_3 (1.08 mmol)和NaI (1.08 mmol), 10 min后加入相应的卤代烷烃, 继续反应2～24 h。冷却反应体系, 减压抽滤, 滤渣以二氯甲烷/甲醇为流动相, 经硅胶柱梯度洗脱, 分离纯化得目标物**4a**～**4d**。

1.2 化合物5a～5c 在95～120 ℃、N_2保护条件下, 将**1** (500 mg, 1.29 mmol)加入到相应的胺(5 mL)中, 继续反应4～72 h, 趁热将反应体系抽滤, 用乙酸乙酯洗涤滤渣, 再用二氯甲烷/甲醇为流动相, 经硅胶柱分离纯化得**5a**～**5c**。

1.3 化合物8a～8d 中间体化合物**6**的合成方法与化合物**5a**～**5c**的合成方法一致。

将**6** (522 mg, 1.0 mmol)加入到体积比1∶1 HCl/CH_3OH (10 mL)溶液中, 室温继续反应24 h, 真空减压旋干反应体系, 所得固体用二氯甲烷/甲醇为流动相, 经硅胶柱分离纯化得**7**。

将**7** (100 mg, 0.27 mmol)溶于无水乙腈(5 mL)中, 65 ℃条件下, 加入吡啶(1.22 mmol)和相应酰氯(0.81 mmol), 继续反应3～24 h。冷却反应体系, 减压抽滤, 滤渣以二氯甲烷/甲醇为流动相, 经硅胶柱梯度洗脱, 分离纯化得目标物**8a**～**8d**。

2 生物实验

2.1 药敏纸片法测定抑菌活性 菌株在含有5%脱纤维羊血和选择性抗生素的哥伦比亚琼脂培养基上培养72 h后收集, 调节至麦氏浓度2.0 (约1×10^8 CFU·mL^{-1})的细菌悬液。将100 μL菌液涂布于不含药物的MHA平板上, 贴上空白药敏纸片, 滴加10 μL含有浓度为10 mg·mL^{-1}的不同药液。将平皿放入培养罐中并放入产气包, 细菌在微需氧条件下 (10% CO_2, 5% O_2和85% N_2) 于37 ℃孵育72 h。使用游标卡尺测定抑菌圈直径, 判读不同药物对幽门螺杆菌抑菌活性。菌株 ATCC43504、CCPMAP160010、CCPMAP160008、CCPMAP160007和CCPMAP160017由中国医学科学院病原微生物菌 (毒) 种保藏中心药用微生物相关菌 (毒) 种保藏分中心 (Chinese Academy of Medical Sciences-Collection Center of Pathogen Microorganisms, CAMS-CCPM) 提供。

2.2 抗Hp活性MIC测定 参照CLSI标准, 采用平皿二倍稀释法进行抗菌药敏实验。菌株在含有5%脱纤维羊血和选择性抗生素的哥伦比亚琼脂培养基上培养72 h后收集, 调节至麦氏浓度2.0 (约1×10^8 CFU·mL^{-1})的细菌悬液。将2.5 μL/点的细菌悬液接种至含有不同浓度药液 (0.5～256 μg·mL^{-1}) 及5%脱纤维羊血的MHA琼脂培养基上, 每个接种点均设立3个重复。将平皿放入培养罐中并放入产气包, 细菌在微需氧条件下 (10% CO_2, 5% O_2和85% N_2) 于37 ℃孵育72 h。幽门螺杆菌ATCC43504菌株用作质控菌株。肉眼观察若无细菌生长或只有多个离散的菌落生长均记为该药物的MIC。Met的耐药折点定义为≥8 μg·mL^{-1}。

2.3 急性毒性实验 以昆明种小鼠 (18～20 g) 为动物模型, 称重后随机分组, 每组10只, 雌雄各半。动物实验遵循中国医学科学院药物研究所动物实验中心标准操作规程。将化合物**5a**在研钵中充分研磨, 分别配制成0、25、50和100 mg·mL^{-1}混悬液, 给药前充分震荡, 使药物混悬均匀, 每只小鼠给药0.2 mL, 按照0、250、500和1 000 mg·kg^{-1}的剂量灌胃给药, 密切观察动物7天内的死亡情况, 计算LD_{50}。

3 分子对接实验

本实验用以对接的活性腔选自Hp脲酶和化合物乙酰氧肟酸共结晶中配体所占的蛋白腔体 (PDB code 1E9Y, resolution: 3 Å)[26]。对接软件为Discovery Studio 4.5工作站中LibDock。对接之前先将小分子配体和目标蛋白进行预处理, 如小分子配体能量最低化、目标蛋白加氢、去水等[25]。最后选择打分最高相互作用模式作为最终对接结果。

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