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(*Z*)-/(*E*)-马鞭草烯酮肟醚的合成及抑菌活性

王晓宇[1], 段文贵[1*], 林桂汕[1], 康国强[1], 商明昊[1], 雷福厚[2]

(1.广西大学 化学化工学院,广西 南宁 530004; 2.广西林产化学与工程重点实验室,广西 南宁 530008)

WANG Xiaoyu

摘 要: 将 α-蒎烯选择性氧化制备马鞭草烯酮,对羰基进行肟化和分离,再发生亲核取代反应,合成得到 40 个新型(*Z*)-/(*E*)-马鞭草烯酮肟醚(**4a** ~ **4t**,包括 20 对 *Z*/*E* 异构体),采用 ^{1}H NMR、^{13}C NMR、FT-IR、UV-vis 和 ESI-MS 对目标化合物进行了结构表征,并测试其抑菌活性。研究结果表明:在质量浓度 50 mg/L 下,目标化合物对 8 种植物病原菌均显示出不同程度的抑菌活性,其中化合物(*E*)-**4r**(R = 2,6-Cl)对苹果轮纹病菌的抑制率为 77.8%,化合物(*E*)-**4s**(R = 2,6-F)对水稻纹枯病菌的抑制率为 72.7%,化合物(*E*)-**4n**(R = *p*-CN)对玉米小斑病菌的抑制率为 70.8%,(*Z*)-/(*E*)-异构体对一些植物病原菌的抑制活性显示一定差异。建立了(*E*)-马鞭草烯酮肟醚化合物对水稻纹枯病菌抑制活性的 CoMFA 模型(r^2 = 0.992, q^2 = 0.507),进行 3D-QSAR 研究,结果表明建立的模型可用于设计具有潜在高活性的先导化合物。

关键词: α-蒎烯;马鞭草烯酮;肟醚;*Z*-*E* 异构体;抑菌活性;3D-QSAR

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Synthesis and Antifungal Activity of (*Z*)-/(*E*)-Verbenone Oxime Ether Compounds

WANG Xiaoyu[1], DUAN Wengui[1], LIN Guishan[1], KANG Guoqiang[1], SHANG Minghao[1], LEI Fuhou[2]

(1.College of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, China; 2.Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, Nanning 530008, China)

Abstract: Verbenone was prepared by selective oxidation of α-pinene at first. Then, by oximation of the carbonyl group (*Z*)- and (*E*)-verbenone oxime compounds were synthesized and separaed, followed by the nucleophilic substitution reaction to afford forty novel (*Z*)- and (*E*)-verbenone oxime ether compounds **4a** – **4t**, including twenty couples of *Z*/*E* isomers. The structures of the target compounds were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, UV-vis, and ESI-MS. The antifungal activities of the target compounds were evaluated. The results showed that, at the mass concentration of 50 mg/L, all the target products exhibited certain inhibition activities against the eight kinds of plant pathogens. Compound(*E*)-**4r**(R = 2,6-Cl) had inhibition rates of 77.8% against *Physalospora piricola*. Compound(*E*)-**4s**(R = 2,6-F) had inhibition rates of 72.7% against *Rhizoctonia solani*. Compound (*E*)-**4n**(R = *p*-CN) had inhibition rates of 70.8% against *Helmithosporium maydis*. Certain difference were found in inhibition activity of *Z*-*E* isomers against some plant pathogens. The CoMFA model(r^2 = 0.992, q^2 = 0.507) of (*E*)-verbenone oxime ether compounds for inhibiting *Rhizoctonia solani* was established for the study of 3D-QSAR, and the results could provide a basis for the design of potential lead compounds with higher activity.

Key word: α-pinene; verbenone; oxime ether; *Z*-*E* isomer; antifungal activity; 3D-QSAR

马鞭草烯酮[1]是具有一定生物活性的双环单萜烯酮类化合物,有类似于樟脑、薄荷脑以及芹菜的

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作者简介:王晓宇(1992—),女,河北石家庄人,硕士生,从事天然产物改性及有机合成研究

* **通讯作者**:段文贵,教授,博士生导师,主要从事天然资源化学和有机合成研究;**E-mail**: wgduan@gxu.edu.cn。

香气,常被用作香料及医药中间体。马鞭草烯酮存在于天然产物中,如蓝桉和西班牙马鞭草精油,以及长期存放的被氧化的松节油中,也可由松节油主成分 α-蒎烯的烯丙位亚甲基氧化得到[2-3]。马鞭草烯酮自身具有驱虫[4-5]和抗惊厥[6]活性,在农药和医药领域有广泛应用,其衍生物可用作不对称合成[7]、氨基酸类药物合成的起始物[8-9]和医药中间体[10]等。肟醚类化合物具有抗菌[11-12]、抗肿瘤[13-14]、杀螨[15]、杀虫[16]和酶抑制[17]等生物活性。基于大量文献报道及课题组多年来在松香松节油基生物活性化合物的研究成果[18-20],作者将肟醚活性基团引入到马鞭草烯酮骨架中,合成系列具有潜在生物活性的(*Z*)-/(*E*)-马鞭草烯酮肟醚化合物,以期为我国天然优势生物质资源松节油的深度开发和利用提供新的途径。

1 实 验

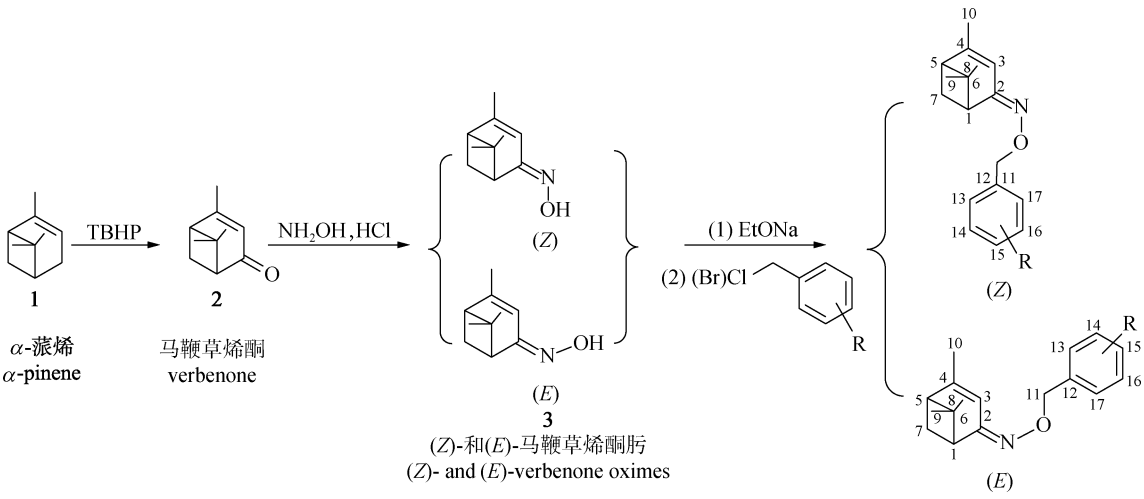
1.1 试剂与仪器

α-蒎烯(GC 纯度 98.0%),广西梧州松脂股份有限公司;盐酸羟胺、乙醇钠以及系列苄氯(苄溴)均为市售分析纯试剂,上海阿拉丁试剂公司。

AVANCE III HD 600 MHz 超导核磁共振波谱仪,瑞士 BRUKER 公司; NICOLET IS 50 FT-IR 红外光谱仪,美国 THERMO SCIENTIFIC 公司;TSQ QUANTUM ACCESS MAX 液相色谱-质谱联用仪,美国 THERMO SCIENTIFIC 公司;SHIMASZU UV-1800 紫外光谱仪,日本 SHIMASZU 公司;WATERS 1525 高效液相色谱仪,美国 WATERS 公司;AGILENT 6890 气相色谱仪,美国 AGILENT TECHNOLOGIES 公司;海能 MP420 全自动熔点仪,济南海能仪器股份有限公司。

1.2 (*Z*)-/(*E*)-马鞭草烯酮肟醚的合成

1.2.1 合成路线 (*Z*)-/(*E*)-马鞭草烯酮肟醚(**4a**~**4t**)的合成路线如下所示:



4a: R = H; **4b**: R = *o*-CH_3; **4c**: R = *m*-CH_3; **4d**: R = *p*-CH_3; **4e**: R = *o*-OCH_3; **4f**: R = *m*-OCH_3; **4g**: R = *o*-Cl;
4h: R = *m*-Cl; **4i**: R = *p*-Cl; **4j**: R = *o*-NO_2; **4k**: R = *m*-NO_2; **4l**: R = *p*-NO_2; **4m**: R = *m*-F; **4n**: R = *p*-CN;
4o: R = *p*-SCH_3; **4p**: R = *p*-vinyl; **4q**: R = 2,6-CH_3; **4r**: R = 2,6-Cl; **4s**: R = 2,6-F; **4t**: R = 3-Cl-4-F

1.2.2 中间产物的制备 参考文献[1-2]制备马鞭草烯酮(**2**),其为淡黄色油状液体。参考前期工作[18-19]制备得到(*Z*)-/(*E*)-马鞭草烯酮肟(**3**)(白色固体),并用 NOESY 鉴定(*Z*)-/(*E*)-异构体。

1.2.3 目标产物的合成 参考文献[11]方法,在 50.0 mL 两口瓶中分别加入(*Z*)-或(*E*)-马鞭草烯酮肟(0.5 g, 3.0 mmol)、乙醇钠(0.3 g, 3.6 mmol),无水乙醇(15 mL),室温搅拌 1 h,缓慢滴加苄氯或苄溴(3.6 mmol)的乙醇溶液到反应混合物中。采用 TLC 监测反应过程,待反应完全后,旋转蒸发除去乙醇。再加入适量去离子水,用乙酸乙酯萃取反应液 3 次,将有机层合并,再旋转蒸发除去溶剂。经柱层析纯化,得到目标化合物(*Z*)-或(*E*)-马鞭草烯酮肟醚(**4a**~**4t**),均为黄色液体。

1.3　结构表征

对目标产物进行结构表征。以 CH_2Cl_2 为溶剂,采用紫外可见分光光度计分析紫外吸收;采用 KBr 压片法测定 FT-IR 光谱;以 $CDCl_3$ 为溶剂,TMS 为内标,用 600 MHz 核磁共振仪进行 ^{1}H NMR 和 ^{13}C NMR 分析;采用电喷雾电离源(ESI)在液相色谱-质谱联用仪上进行 ESI-MS 分析。

1.4　抑菌活性测试

参照文献[21],在质量浓度 50 mg/L 下,采用离体法,测试目标产物的抑菌活性,计算相对抑菌率。活性分级指标为 A 级:≥90%,B 级:≥70% ~90%,C 级:≥50% ~70%,D 级:<50%。

1.5　3D-QSAR 研究

参照文献[22-23],采用 SYBYL-X 2.1.1 软件对(*E*)-马鞭草烯酮肟醚(**4a ~ 4t**)进行建模。所有建模目标化合物均采用 Tripos 力场、Gasteiger-Huckel 电荷、Conjugate Gradient 法,在最大循环次数为 10 000 次、终止收敛能量为 0.05 kJ/mol,分子力场计算以 +1 价 sp^3 杂化的碳原子作为探针、步长为 0.2 nm,其余参数均为缺省值条件下进行结构优化。对水稻纹枯病菌的抑制活性建立 CoMFA 模型,进行 3D-QSAR 研究。

2　结果与讨论

2.1　(*Z*)-/(*E*)-马鞭草烯酮肟醚的表征

本研究合成了一系列(*Z*)-/(*E*)-马鞭草烯酮肟醚(**4a ~ 4t**),选择化合物 **4f**、**4n**、**4r** 和 **4s** 进行表征。(其余化合物的表征数据见辅助材料)

(*Z*)-**4f**:(*Z*)-马鞭草烯酮肟间-甲氧基苄基醚,淡黄色液体,产率 76.5%。UV-vis(CH_2Cl_2) λ_{max}: 257.31 nm; IR(KBr, cm^{-1}) ν: 3045(═CH), 2954, 2929, 2870, 2836(C—H), 1628, 1604, 1492, 1468, 1436(C═N, Ar—C═C, C═C), 1264, 1045(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ: 7.24(dd, J = 12.7, 4.9 Hz, 1H, H-16), 6.91(d, J = 7.5 Hz, 1H, H-17), 6.89(s, 1H, H-13), 6.81(dd, J = 8.2, 2.5 Hz, 1H, H-15), 5.79(dd, J = 2.9, 1.4 Hz, 1H, H-3), 5.07(s, 2H, H-11), 3.79(s, 3H, H-18), 3.61(td, J = 5.9, 1.5 Hz, 1H, H-1), 2.58(dt, J = 8.9, 5.5 Hz, 1H, H-5), 2.25 ~ 2.19(m, 1H, H-7), 1.86(d, J = 1.5 Hz, 3H, H-10), 1.61(d, J = 8.9 Hz, 1H, H-7), 1.43(s, 3H, H-9), 0.88(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ: 162.30(C-2), 159.61(C-4), 154.44(C-14), 140.05(C-12), 129.28(C-16), 119.97(C-17), 115.70(C-13), 113.12(C-15), 113.09(C-3), 75.29(C-11), 55.19(C-18), 49.10(C-1), 47.36(C-6), 42.40(C-5), 36.35(C-7), 26.10(C-8), 23.14(C-10), 22.25(C-9); ESI-MS m/z: 285.96$[M+H]^+$。

(*E*)-**4f**:(*E*)-马鞭草烯酮肟间-甲氧基苄基醚,淡黄色液体,产率 75.4%。UV-vis(CH_2Cl_2) λ_{max}: 267.62 nm; IR(KBr, cm^{-1}) ν: 3054(═CH), 2998, 2955, 2932, 2869, 2838(C—H), 1621, 1602, 1588, 1492, 1436(C═N, Ar—C═C, C═C), 1266, 1041(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ: 7.25(dd, J = 10.4, 5.3 Hz, 1H, H-16), 6.94(dd, J = 13.0, 4.8 Hz, 2H, H-15, H-17), 6.82(dd, J = 8.2, 2.3 Hz, 1H, H-13), 6.45(dd, J = 3.1, 1.5 Hz, 1H, H-3), 5.07(s, 2H, H-11), 3.80(s, 3H, H-18), 2.69(td, J = 6.0, 1.5 Hz, 1H, H-1), 2.64(dt, J = 8.9, 5.5 Hz, 1H, H-5), 2.25 ~ 2.21(m, 1H, H-7), 1.90(d, J = 1.6 Hz, 3H, H-10), 1.71(d, J = 8.9 Hz, 1H, H-7), 1.41(s, 3H, H-9), 0.90(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ: 159.94(C-2), 159.63(C-4), 158.89(C-14), 140.07(C-12), 129.30(C-16), 120.01(C-17), 113.17(C-13), 113.07(C-15), 110.30(C-3), 75.22(C-11), 55.20(C-18), 49.36(C-1), 48.32(C-6), 48.09(C-5), 37.60(C-7), 26.13(C-8), 23.56(C-10), 21.83(C-9); ESI-MS m/z: 286.10$[M+H]^+$。

(*Z*)-**4n**:(*Z*)-马鞭草烯酮肟对-氰基苄基醚,淡黄色液体,产率 76.8%。UV-vis(CH_2Cl_2) λ_{max}: 235.53 nm; IR(KBr, cm^{-1}) ν: 3045(═CH), 2977, 2955, 2931, 2870(C—H), 2229(C≡N), 1634, 1610, 1506, 1442(C═N, Ar—C═C, C═C), 1242, 1017(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ:

7.65~7.59(m, 2H, H-14, H-16), 7.41(dd, J=8.0, 0.5 Hz, 2H, H-13,H-17), 5.75(dd, J=3.2, 1.6 Hz, 1H, H-3), 5.12(s, 2H, H-11), 3.60(td, J=5.9, 1.6 Hz, 1H, H-1), 2.61(dt, J=9.0, 5.5 Hz, 1H, H-5), 2.29~2.19(m, 1H, H-7), 1.87(d, J=1.6 Hz, 3H, H-10), 1.66~1.60(m, 1H, H-7), 1.45(s, 3H, H-9), 0.88(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:162.91(C-2), 155.13(C-4), 144.34(C-12), 132.10(C-14,C-16), 127.85(C-13,C-17), 118.94(C-18), 115.40(C-15), 111.13(C-3), 74.23(C-11), 49.08(C-1), 47.60(C-6), 42.42(C-5), 36.42(C-7), 26.12(C-8),23.15(C-10), 22.26(C-9); ESI-MS m/z: 281.08$[M+H]^+$。

(*E*)-**4n**:(*E*)-马鞭草烯酮肟对-氰基苄基醚,淡黄色液体,产率75.4%。UV-vis(CH_2Cl_2) λ_{max}: 267.33 nm; IR(KBr,cm^{-1}) ν: 3069(═CH), 2953, 2931, 2868(C—H), 2230(C≡N), 1626, 1468, 1438(C═N, Ar—C═C, C═C), 1244, 1017(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ:7.64~7.60(m, 2H, H-14, H-16), 7.45(d, J=8.4 Hz, 2H, H-13,H-17), 6.43(dd, J=2.9, 1.4 Hz, 1H, H-3), 5.13(s, 2H, H-11), 2.68~2.62(m, 2H, H-1, H-5), 2.26(td, J=5.9, 1.3 Hz, 1H, H-7), 1.93(d,J=1.6 Hz, 3H, H-10), 1.78~1.69(m, 1H, H-7), 1.41(s, 3H, H-9), 0.89(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:160.65(C-2), 159.86(C-4), 144.42(C-12), 132.12(C-14,C-16), 127.83(C-13,C-17), 118.98(C-18), 111.08(C-15), 109.97(C-3), 74.11(C-11), 49.38(C-1), 48.33(C-6), 48.24(C-5), 37.69(C-7), 26.08(C-8), 23.65(C-10), 21.77(C-9); ESI-MS m/z: 281.11$[M+H]^+$。

(*Z*)-**4r**:(*Z*)-马鞭草烯酮肟2′,6′-二氯苄基醚,淡黄色液体,产率76.8%。UV-vis(CH_2Cl_2) λ_{max}: 256.16 nm; IR(KBr,cm^{-1}) ν: 3045(═CH), 2980, 2955, 2927, 2868(C—H), 1634, 1584, 1468, 1438(C═N, Ar—C═C, C═C), 1244, 1034(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ:7.32~7.28(m, 2H, H-14, H-16), 7.17(dd, J=8.4, 7.7 Hz, 1H, H-15), 5.78(dd, J=3.1, 1.5 Hz, 1H, H-3), 5.32(dd, J=29.0, 10.7 Hz, 2H, H-11), 3.53(td, J=5.9, 1.6 Hz, 1H, H-1), 2.53(dt, J=8.9, 5.5 Hz, 1H, H-5), 2.24~2.13(m, 1H, H-7), 1.85(d, J=1.6 Hz, 3H, H-10),1.57(d, J=8.9 Hz, 1H, H-7), 1.38(s, 3H, H-9), 0.80(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:163.04(C-2), 154.73(C-4), 137.13(C-12), 133.02(C-13,C-17), 129.78(C-15), 128.24(C-14,C-16), 115.64(C-3), 69.83(C-11), 49.05(C-1), 47.43(C-6), 42.20(C-5), 36.25(C-7), 26.04(C-8), 23.12(C-10), 22.06(C-9); ESI-MS m/z: 324.00$[M+H]^+$。

(*E*)-**4r**:(*E*)-马鞭草烯酮肟2′,6′-二氯苄基醚,淡黄色液体,产率76.2%。UV-vis(CH_2Cl_2) λ_{max}: 266.48 nm; IR(KBr,cm^{-1}) ν: 3076(═CH), 2955, 2929, 2870(C—H), 1625, 1594, 1471, 1437(C═N, Ar—C═C, C═C), 1236, 1029(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ 7.31(d, J=8.0 Hz, 2H, H-14, H-16), 7.20~7.13(m, 1H, H-15), 6.35(dd, J=2.9, 1.4 Hz, 1H, H-3), 5.37~5.30(m, 2H, H-11), 2.70(td, J=6.1, 1.5 Hz, 1H, H-1), 2.63(dt, J=8.9, 5.5 Hz, 1H, H-5), 2.27~2.18(m, 1H, H-7), 1.86(d, J=1.6 Hz, 3H, H-10), 1.69(t, J=10.8 Hz, 1H, H-7), 1.40(s, 3H, H-9), 0.87(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:160.55(C-2), 158.70(C-4), 137.20(C-12), 133.03(C-13,C-17), 129.80(C-15), 128.27(C-14,C-16), 110.26(C-3), 69.88(C-11), 49.32(C-1), 48.29(C-6), 48.15(C-5), 37.53(C-7), 26.13(C-8), 23.52(C-10), 21.71(C-9); ESI-MS m/z:324.02$[M+H]^+$。

(*Z*)-**4s**:(*Z*)-马鞭草烯酮肟2′,6′-二氟苄基醚,淡黄色液体,产率75.8%。UV-vis(CH_2Cl_2) λ_{max}: 259.60 nm; IR(KBr,cm^{-1}) ν: 3069(═CH), 2952, 2929, 2871(C—H), 1628, 1595, 1471(C═N, Ar—C═C, C═C), 1237, 1026(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ:7.25(ddd, J=8.4, 7.2, 4.2 Hz, 1H, H-15), 7.01~6.73(m, 2H, H-14, H-16), 5.77(dd, J=3.1, 1.6 Hz, 1H, H-3), 5.14(s, 2H, H-11), 3.50(td, J=5.9, 1.6 Hz, 1H, H-1), 2.53(dt, J=9.0, 5.5 Hz, 1H, H-5), 2.20~2.15(m, 1H, H-7), 1.84(d, J=1.6 Hz, 3H, H-10), 1.56(d, J=9.0 Hz, 1H, H-7), 1.39(s, 3H, H-9), 0.80

(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:162.99(C-13,C-17), 162.63(C-2), 154.56(C-4), 129.98(C-15), 115.67(C-3), 113.75(C-12), 111.18(C-14,C-17), 62.87(C-11), 49.03(C-1), 47.36(C-6), 42.19(C-5), 36.27(C-7), 26.02(C-8), 23.09(C-10), 22.04(C-9); ESI-MS *m/z*: 292.09 $[M+H]^+$。

(*E*)-**4s**:(*E*)-马鞭草烯酮肟 2′,6′-二氟苄基醚,淡黄色液体,产率 76.9%。UV-vis(CH_2Cl_2) λ_{max}: 257.31 nm; IR(KBr, cm^{-1}) ν: 3076(═CH), 2953, 2901, 2873(C—H), 1626, 1595, 1471(C═N, Ar—C═C, C═C), 1237, 1026(C—O); ^{1}H NMR(600 MHz, $CDCl_3$) δ:7.26(ddd, J = 8.4, 5.5, 2.0 Hz, 1H, H-15), 6.91 ~ 6.86(m, 2H, H-14, H-16), 6.33(dd, J = 3.1, 1.5 Hz, 1H, H-3), 5.14(d, J = 0.9 Hz, 2H, H-11), 2.68(td, J = 6.0, 1.5 Hz, 1H, H-1), 2.64 ~ 2.61(m, 1H, H-5), 2.21 ~ 2.19(m, 1H, H-7), 1.86(d, J = 1.6 Hz, 3H, H-10), 1.68(d, J = 8.9 Hz, 1H, H-7), 1.40(s, 3H, H-9), 0.85(s, 3H, H-8); ^{13}C NMR(151 MHz, $CDCl_3$) δ:161.35(C-13,C-17), 160.21(C-2), 158.74(C-4), 129.99(C-15), 113.69(C-12), 111.25(C-14,C-17), 110.17(C-3), 62.81(C-11), 49.30(C-1), 48.29(C-6), 48.06(C-5), 37.54(C-7), 26.10(C-8), 23.48(C-10), 21.70(C-9); ESI-MS *m/z*: 292.08$[M+H]^+$。

在 IR 谱图中,C═N、Ar—C═C 和 C═C 伸缩振动的吸收峰出现在 1650 ~ 1400 cm^{-1}之间;C—O 伸缩振动吸收峰出现在 1250 和 1070 cm^{-1}左右。在^{1}H NMR 谱图中,目标化合物(*Z*)-**4a** ~ (*Z*)-**4t** 和(*E*)-**4a** ~ (*E*)-**4t** 的马鞭草烯酮骨架中═C—H 的 δ 分别在 5.7 和 6.4 附近,马鞭草烯酮骨架上的饱和氢在 δ3.6 ~ 0.5 和 δ3.0 ~ 0.5;在^{13}C NMR 谱图中,目标产物(*Z*)-**4a** ~ (*Z*)-**4t** 和(*E*)-**4a** ~ (*Z*)-**4t** 的 C═N双键化学位移分别在 δ163 和 δ159 左右,C═C 双键分别在 δ162 ~ 113 和 δ159 ~ 110。质谱数据与目标化合物相对分子质量相符合。

2.2　抑菌活性测试结果

在质量浓度 50 mg/L 下,目标化合物(*Z*)-/(*E*)-**4a** ~ (*Z*)-/(*E*)-**4t** 对所测 8 种植物病原菌均有一定的抑制活性,结果见表 1(文中仅列出 **4f**、**4n**、**4r** 和 **4s** 的抑菌活性,其余见辅助材料)。其中,(*E*)-**4r**(R = 2,6-Cl)对苹果轮纹病菌的抑制率为 77.8%,与阳性对照百菌清相当;(*E*)-**4s**(R = 2,6-F)对水稻纹枯病菌的抑制率为 72.7%;(*E*)-**4n**(R = *p*-CN)对玉米小斑病菌的抑制率为 70.8%。此外,(*E*)-、(*Z*)-异构体对一些植物病原菌的抑制作用有一定的差异。例如,(*E*)-**4f**(R = *m*-OCH_3)对苹果轮纹病菌的抑制率是(*Z*)-**4f**(R = *m*-OCH_3)的 5.5 倍,抑制率分别为 61.1% 和 11.1%。总体上,中间体马鞭草烯酮肟经过醚化作用后抑菌活性有所提高。

表 1　目标产物对不同菌种的抑菌率(50 mg/L)

Table 1　Antibacterial rate of target products against different strains(50 mg/L)　　%

化合物 compounds	黄瓜枯萎病菌 *Fusarium oxysporum*	花生褐斑病菌 *Cercospora arachidicola*	苹果轮纹病菌 *Physalos-pora piricola*	番茄早疫病菌 *Alternaria solani*	小麦赤霉病菌 *Gibberella zeae*	水稻纹枯病菌 *Rhizoctonia solani*	玉米小斑病菌 *Helmithos-porium maydis*	西瓜炭疽病菌 *Colletot-richum lagenarium*
(*Z*)-**4f**	22.7	35.7	11.1	36.4	37.5	59.1	30.4	34.6
(*E*)-**4f**	18.2	14.3	61.1	36.4	25.0	57.6	26.1	19.2
(*Z*)-**4n**	23.8	35.7	66.7	63.2	45.0	57.5	29.2	29.4
(*E*)-**4n**	28.6	50.0	33.3	63.2	40.0	61.3	70.8	41.2
(*Z*)-**4r**	9.5	42.9	44.4	36.8	25.0	45.0	29.2	11.8
(*E*)-**4r**	18.2	28.6	77.8	9.1	41.7	68.2	39.1	42.3
(*Z*)-**4s**	19.0	50.0	50.0	52.6	45.0	55.0	41.7	35.3
(*E*)-**4s**	31.3	42.9	40.0	40.0	46.2	72.7	44.4	11.1
百菌清 chlorothalonil	100.0	73.3	75.0	73.9	73.1	96.1	90.4	91.3

2.3 3D-QSAR 研究

2.3.1 CoMFA 模型 以图 1 中 * 标记的原子作为公共叠合点,对优化后的目标化合物进行叠合和计算,叠合结果如图 2 所示,计算 CoMFA 值见表 2。活性因子(E_D)通过 $E_D = \lg\{I/[(100-I)M_w]\}$ 计算得到(其中 I 为化合物对水稻纹枯病菌的抑制率,M_w为相对分子质量)。以 CoMFA 值作为自变量,活性因子 E_D作为因变量建立 CoMFA 模型。

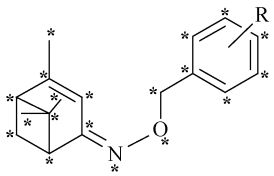


图 1 目标产物的公共骨架

Fig. 1 Asterisk skeleton of the target compounds

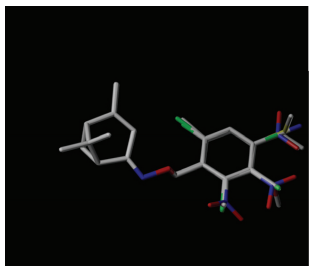


图 2 目标产物的叠合示意图

Fig. 2 Superposition models of the target compounds

2.3.2 CoMFA 的主成分分析 采用的留一法(Leave-one-out)交叉验证和非交叉验证对模型进行验证,相关验证参数如下:交叉验证相关系数(q^2)0.507,非交叉验证相关系数(r^2)0.992,估计标准误差(S)0.033,Fisher 验证值(F)170,表明该模型具有可靠性和预测能力。主成分(PLS)分析显示,立体场和静电场的贡献分别为 55.4% 和 44.6%,表明对活性的贡献主要是立体场。利用建立的 CoMFA 模型对建模的分子进行预测,其预测 E_D'值及残差值(E_D值与 E_D'值之差)列于表 2。

表 2 化合物(*E*)-4a ~ (*E*)-4t 的实验活性因子(E_D)和预测活性因子(E_D')

Table 2 The experimental factor(E_D) and predicted factor (E_D') of compounds(*E*)-4a – (*E*)-4t

化合物 compounds	CoMFA 值 CoMFA value	E_D	E_D'	残差 residue
(*E*)-**4a**(R = H)	120	−2.77	−2.72	−0.05
(*E*)-**4b**(R = *o*-CH_3)	130	−2.38	−2.39	0.01
(*E*)-**4c**(R = *m*-CH_3)	128	−2.96	−2.96	0.00
(*E*)-**4d**(R = *p*-CH_3)	132	−2.19	−2.20	0.01
(*E*)-**4e**(R = *o*-OCH_3)	132	−2.53	−2.52	−0.01
(*E*)-**4f**(R = *m*-OCH_3)	132	−2.32	−2.32	0.00
(*E*)-**4g**(R = *o*-Cl)	128	−2.54	−2.50	−0.04
(*E*)-**4h**(R = *m*-Cl)	122	−2.44	−2.46	0.02
(*E*)-**4i**(R = *p*-Cl)	126	−2.49	−2.51	0.02
(*E*)-**4j**(R = *o*-NO_2)	132	−3.25	−3.27	0.02
(*E*)-**4k**(R = *m*-NO_2)	126	−2.43	−2.43	0.00
(*E*)-**4l**(R = *p*-NO_2)	132	−2.41	−2.42	0.01
(*E*)-**4m**(R = *m*-F)	120	−2.25	−2.31	0.06
(*E*)-**4n**(R = *p*-CN)	126	−2.25	−2.25	0.00
(*E*)-**4o**(R = *p*-SCH_3)	140	−2.35	−2.35	0.00
(*E*)-**4p**(R = *p*-vinyl)	132	−2.32	−2.32	0.00
(*E*)-**4q**(R = 2,6-CH_3)	138	−2.53	−2.55	0.02
(*E*)-**4r**(R = 2,6-Cl)	132	−2.18	−2.17	−0.01
(*E*)-**4s**(R = 2,6-F)	122	−2.04	−2.04	0.00
(*E*)-**4t**(R = 3-Cl-4-F)	124	−2.40	−2.36	−0.04

以实验 E_D值为横坐标、预测 E_D'值为纵坐标作图,结果见图 3。图中实验值和预测值的数据都集中在 45°对角线附近,进一步说明该模型的可靠性且具有较好的预测能力。

2.3.3　CoMFA 等势图分析　图 4 所示即为(*E*)-**4s** 模板分子的 CoMFA 三维等势图,其中(a)和(b)分别表示立体场三维等势图和静电场三维等势图。在图 4(a)中,绿色表示引入大体积基团会增强活性,黄色区域定义相反。在图(a)中,红色区域表示引入负电荷基团会增强活性,而蓝色区域定义相反。

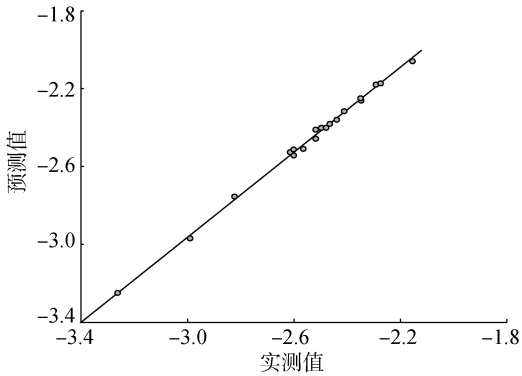


图 3　化合物(*E*)-4a ~ (*E*)-4t 实测值与预测值的线性回归图

Fig. 3　Linear regression of the experimental and predicted E_D values of Compounds(*E*)-4a – (*E*)-4t

在立体场图中,在苯环邻位和对位有大体积取代基有利于活性增加,例如化合物(*E*)-**4e**(R = *o*-OCH_3)比(*E*)-**4g**(R = *o*-Cl)活性好;化合物(*E*)-**4l**(R = *p*-NO_2)比(*E*)-**4i**(R = *p*-Cl)活性好。静电场图中,在苯环邻位上有吸电子取代基有利于活性增加,例如化合物(*E*)-**4s**(R = 2,6-F) > (*E*)-**4r**(R = 2,6-Cl) > (*E*)-**4q**(R = 2,6-CH_3)。在苯环间位上有吸电子取代基也有利于活性增加,例如化合物(*E*)-**4k**(R = *m*-NO_2) > (*E*)-**4h**(R = *m*-Cl) > (*E*)-**4c**(R = *m*-CH_3)。在苯环对位上有推电子基团可增强其活性,例如,化合物(*E*)-**4d**(R = *p*-CH_3)比(*E*)-**4i**(R = *p*-Cl)活性好。由此可知,建立的 CoMFA 模型可用于设计具有潜在高活性的先导化合物。

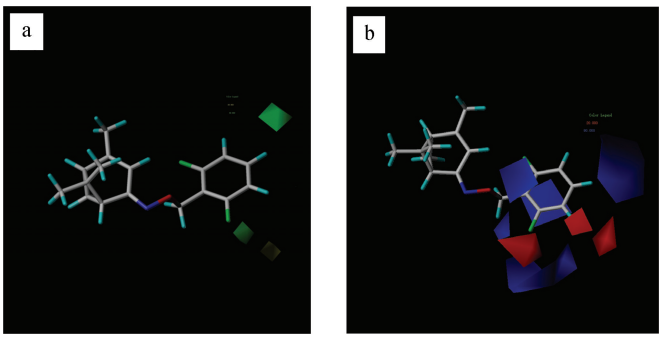


图 4　化合物(*E*)-4s 的 CoMFA 模型的立体场(a)和静电场(b)分布图

Fig. 4　3D contour plot of the CoMFA steric field(a) and electrostatic field(b) for compound(*E*)-4s

3　结 论

3.1　将 α-蒎烯的烯丙位亚甲基进行选择性氧化制备马鞭草烯酮,再与盐酸羟胺发生肟化反应并分离,得到(*Z*)-/(*E*)-马鞭草烯酮肟,继而与系列苄氯或苄溴发生亲核取代反应,合成得到 40 个新型(*Z*)-/(*E*)-马鞭草烯酮肟醚化合物 **4a** ~ **4t**(20 对 *Z*/*E* 异构体)。利用 ^{1}H NMR、^{13}C NMR、FT-IR、UV-Vis 和 ESI-MS 等多种波谱技术对目标产物做了结构表征。

3.2　抑菌活性测试表明,在质量浓度 50 mg/L 下,目标产物(*Z*)-/(*E*)-**4a** ~ (*Z*)-/(*E*)-**4t** 对所测 8 种植物病原菌均有一定的抑制活性。其中,(*E*)-**4r**(R = 2,6-Cl) 对苹果轮纹病菌的抑制率为 77.8%;(*E*)-**4s**(R = 2,6-F)对水稻纹枯病菌的抑制率为 72.7%;(*E*)-**4n**(R = *p*-CN)对玉米小斑病菌的抑制率为 70.8%。此外,(*Z*)-、(*E*)-异构体对一些植物病原菌的抑制作用有一定的差异。

3.3　建立了马鞭草烯酮肟醚化合物对抑制水稻纹枯病菌活性的 CoMFA 模型(r^2 = 0.992, q^2 = 0.507),进行 3D-QSAR 研究,其结果可为设计具有潜在高活性的先导化合物提供依据。

辅助材料(Supporting Information)　化合物 **4a** ~ **4t**(除 **4f**、**4n**、**4r** 和 **4s**)的表征及抑菌活性。这些材料可免费从本刊网站(http://www.cifp.ac.cn)下载。

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