

噻唑类衍生物二氢乳清酸脱氢酶抑制剂的三维定量构效关系研究

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摘要: **目的** 应用三维定量构效关系(3D-QSAR)研究噻唑类衍生物结构的二氢乳清酸脱氢酶抑制活性, 为该类药物设计和筛选提供可靠的理论依据。**方法** 针对38个以噻唑为基本骨架的二氢乳清酸脱氢酶抑制剂, 分别应用分子力场分析(CoMFA)和比较分子相似性指数分析(CoMSIA)2种经典的方法进行了三维定量构效关系(3D-QSAR)研究, 建立相关模型, 验证模型的预测能力, 三维等势图分析噻唑类衍生物结构与活性的关系。**结果** CoMFA模型的交叉验证系数 q^2 为0.796, 相关系数 r^2 为0.978; CoMSIA模型的 q^2 以及 r^2 分别为0.721和0.976; 2种模型对化合物的活性预测与实际值接近; 三维等势图可以全面直观的分析化合物结构对其活性的影响。**结论** 该3D-QSAR模型三维等势图揭示了结构特征与抑制活性的关系, 模型具有较好的预测能力和较强的稳定性, 为进一步开发研究打下了较好的基础。

关键词: 二氢乳清酸脱氢酶抑制剂; 比较分子力场分析; 比较分子相似性指数分析; 三维定量构效关系

中图分类号: R965 **文献标志码:** A **文章编号:** 1674-6376(2017)01-0020-08

DOI: 10.7501/j.issn.1674-6376.2017.01.004

3D-QSAR studies on thiazole derivatives as potent inhibitors of dihydroorotate dehydrogenase

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Abstract: Objective The three-dimensional quantitative structure activity relationship (3D-QSAR) method was applied to study thiazole derivatives as potent inhibitors of dihydroorotate dehydrogenase, which provided useful guidance for more discovery of potent inhibitors of dihydroorotate dehydrogenase. **Methods** Molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were applied to systematically investigate 3D-QSAR of 38 thiazole derivatives as potent inhibitors of dihydroorotate dehydrogenase. Established models of CoMFA and CoMSIA and the predictive ability of models were validated. Three dimensional map was applied to analyzing the relationship between structure and activity of thiazole derivatives. **Results** The coefficients of cross validation q^2 and non-cross validation r^2 for CoMFA model were 0.796 and 0.978, and for CoMSIA model were 0.721 and 0.976 respectively. The prediction of activity of compound was close to the actual value of the two models. Effect of compound structure on its activity could be analyzed comprehensively and intuitively by three dimensional map. **Conclusion** The model reveals the relationship between the structure characteristics and the inhibitory activity, and has good predictive capability and stability to lay a good foundation for further development and research.

Keywords: inhibitors of dihydroorotate dehydrogenase; CoMFA; CoMSIA; 3D-QSAR

二氢乳清酸脱氢酶(dihydroorotate dehydrogenase, DHODH)是一种含铁的黄素依赖的线粒体酶,是核酸中嘧啶合成的关键酶,催化从头嘧啶生物合成途径中的第四步反应。抑制二氢乳清酸脱氢酶,可以阻断新生嘧啶合成,致使DNA合成障碍,从而

抑制活化淋巴细胞的增殖和B淋巴细胞中的T细胞依赖性自体抗体的形成^[1-2]。目前二氢乳清酸脱氢酶已被证实为治疗类风湿关节炎以及其他免疫性疾病的有效靶点^[3-4],特立氟胺^[5-6]以及阿托伐醌^[7]已作为二氢乳清酸脱氢酶抑制剂进行研究,已经上市的来

收稿日期: 2016-07-12

基金项目: 国家自然科学基金资助项目(81171508); 重庆市自然科学基金重点项目(CSTC 2013 JJB10004)

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氟米特 (leflunomide, LEF) 是临床常用的 DHODH 有效抑制剂, 通过阻断嘧啶的从头合成途径和抑制酪氨酸激酶的活性, 阻断细胞信号传导过程, 是具有抗炎及免疫抑制作用的异恶唑类衍生物^[8]。但由于长期服用来氟米特会导致秃头^[9]、肺动脉高压^[10]、肾损伤、皮疹^[11]、腹泻、肝酶异常以及肺结节形成等不良反应^[12-13], 寻求新型的、不良反应少的免疫抑制剂成为一项长期工作。

三维定量构效关系 (three-dimensional quantitative structure-activity relationship, 3D-QSAR) 研究被广泛应用于各类药物的研究与开发中^[14-15]。本研究采用 3D-QSAR 中常用的比较分子力场分析 (CoMFA) 和比较分子相似性指数分析 (CoMSIA) 方法建立模型^[16]。通过验证, 建立的 CoMFA 和 CoMSIA 模型是稳定且具有良好预测能力的。根据三维等势图分析, 全面直观了解噻唑类衍生物结构的二氢乳清酸脱氢酶抑制活性。

1 材料与方法

1.1 数据优化与叠合

噻唑类二氢乳清酸脱氢酶抑制剂的结构与活性来自于参考文献^[17]。为了使建立的 CoMFA 和 CoMSIA 模型具有可靠性, 建模所需的化合物分子必须经过筛选, 共筛选出 38 个化合物分子, 其基本骨架见图 1 和表 1, 根据随机和活性梯度分布原则, 化合物分为 2 组, 其中 32 个化合物作为训练集, 6 个化合物作为测试集。所有化合物对二氢乳清酸脱氢酶抑制作用均以 IC_{50} 值表示, IC_{50} 值均转换成 pIC_{50} 值。

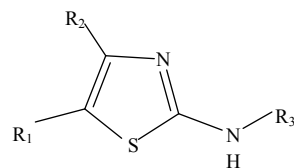


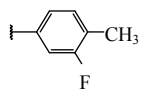
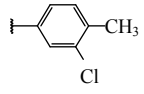
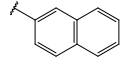
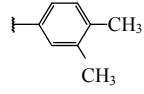
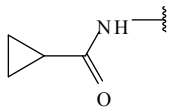
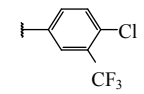
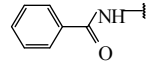
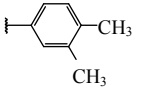
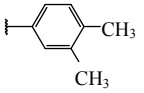
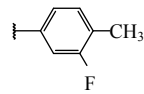
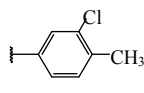
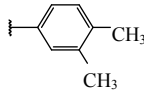
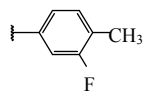
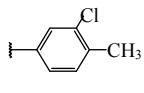
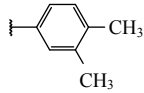
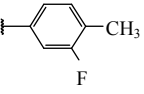
图 1 噻唑类衍生物基本骨架图

Fig. 1 Structure of thiazole derivatives

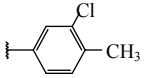
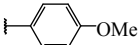
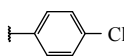
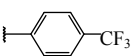
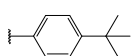
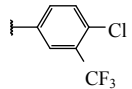
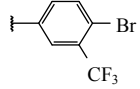
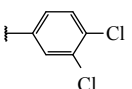
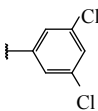
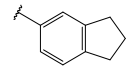
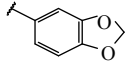
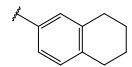
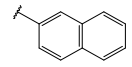
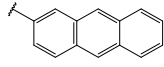
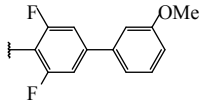
表 1 分子结构与抑制二氢乳清酸脱氢酶活性
Table 1 Structure and activity inhibition of DHODH

编号	R ₁	R ₂	R ₃	活性值 pIC_{50}
1	-COCH ₃	-CH ₃		5.4048
2*	-COCH ₃	-CH ₃		5.4378
3	-COCH ₃	-CH ₃		5.4148
4	-COCH ₃	-CH ₃		5.6297
5	-COCH ₃	-CH ₃		5.7153
6	-COCH ₃	-CH ₃		6.2503
7	-COOEt	-CH ₃		5.0422
8	-COOEt	-CH ₃		5.8671

续表 1

编号	R ₁	R ₂	R ₃	活性值 pIC ₅₀
9	-COOEt	-CH ₃		5.7018
10	-COOEt	-CH ₃		6.0137
11	-COOEt	-CH ₃		6.0521
12	-COOEt	-NH ₂		5.6755
13	-COOEt			5.2190
14*	-COOEt			5.0444
15*	-COOEt			6.4881
16	-COOEt			6.1904
17	-COOEt			6.4295
18	-COOEt	-t-Bu		6.2882
19	-COOEt	-t-Bu		6.1858
20	-COOEt	-t-Bu		6.2581
21	-COOEt	-Ph		7.2366
22	-COOEt	-Ph		7.3098

续表 1

编号	R ₁	R ₂	R ₃	活性值 pIC ₅₀
23	-COOEt	-Ph		7.4559
24	-COOEt	-Ph		6.1057
25*	-COOEt	-Ph		5.8416
26*	-COOEt	-Ph		6.6216
27	COOEt	-Ph		6.3893
28	-COOEt	-Ph		6.5467
29	-COOEt	-Ph		6.8827
30	-COOEt	-Ph		6.8928
31	-COOEt	-Ph		6.7595
32	-COOEt	-Ph		6.4056
33	-COOEt	-Ph		7.5850
34	-COOEt	-Ph		5.9555
35	-COOEt	-Ph		7.7447
36	-COOEt	-Ph		7.5376
37*	-COOEt	-Ph		6.3439
38	-COOEt	-Ph		6.5072

采用 Sybyl 2.1 搭建, 通过在 Tripos 立场下加 Gasteiger-MMFF94 电荷对所有分子进行能量优化, 其余设置参数均使用默认值。选取最高活性分子 35 号作为模板, 应用 Align Database 模块将所有优化后的最低能量构象进行叠合, 结果见图 2。

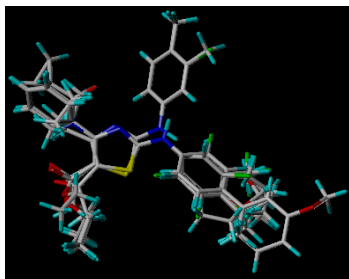


图 2 分子叠合图

Fig. 2 Superposition of compounds

1.2 3D-QSAR 建模

CoMFA 是构建 3D-QSAR 模型中的一种, 特点是配体分子用立体场和静电场来描述并用三维交叉格点取样建模^[18-19]。CoMSIA 是配体分子用 5 个场,

即立体场、静电场、疏水性场、氢键供体场、氢键受体场, 来进行描述^[20-21]。在 CoMFA 建模中, 使用偏最小二乘 (PLS) 进行相互验证方法得到交叉验证系数 q^2 以及最佳主成分数 n 。最终用非交叉验证方法得到相关系数 r^2 , 标准偏差 SEE、方差分析 F 值、立体场 S%、静电场 E%、疏水场 H%、氢键供体场 D%、氢键受体场 A% 等值。CoMSIA 模型的建立与 CoMFA 相似。

2 结果

2.1 3D-QSAR 模型

CoMFA 和 CoMSIA 模型总结在表 2 中, CoMFA 模型包括立体场与静电场, 相互验证系数 $q^2=0.796$, 最佳主成分数 $n=8$, 回归系数 $r^2=0.978$, 标准偏差 SEE=0.124。CoMSIA 模型包括 5 个场, 相互验证系数 $q^2=0.721$, 最佳主成分数 $n=9$, 回归系数 $r^2=0.976$, 标准偏差 SEE=0.132。这些数值预示了 CoMFA 和 CoMSIA 是可信的。表 3 中显示了预测值与活性值 pIC_{50} 。

表 2 训练集 CoMFA 与 CoMSIA 模型的统计参数

Table 2 Statistical parameters of CoMFA and CoMSIA models for training set model

模型	q^2	n	r^2	SEE	F	S/%	E/%	H/%	D/%	A/%
CoMFA	0.796	8	0.978	0.124	125.509	84.4	15.6	—	—	—
CoMSIA	0.721	9	0.976	0.132	99.552	16.5	16.9	45.8	10.8	10.0

表 3 化合物实验的活性值 (pIC_{50}) 与预测值 (pIC_{50})

Table 3 Experimental activities (pIC_{50} value) and predicted activities (pIC_{50} value)

编号	pIC_{50}			编号	pIC_{50}		
	活性值	CoMFA 预测值	CoMSIA 预测值		活性值	CoMFA 预测值	CoMSIA 预测值
1	5.4048	5.417	5.414	20	6.2581	6.220	6.189
2*	5.4378	5.390	5.099	21	7.2366	7.325	7.244
3	5.4148	5.409	5.411	22	7.3098	7.105	7.034
4	5.6297	5.533	5.623	23	7.4559	7.433	7.468
5	5.7153	5.758	5.790	24	6.1057	5.993	6.128
6	6.2503	6.162	6.200	25*	5.8416	6.312	6.262
7	5.0422	5.115	4.992	26*	6.6216	6.363	6.285
8	5.8671	5.859	5.813	27	6.3893	6.802	6.793
9	5.7018	5.681	5.600	28	6.5467	6.473	6.516
10	6.0137	5.957	6.030	29	6.8827	6.730	6.751
11	6.0521	6.097	6.091	30	6.8928	6.908	6.915
12	5.6755	5.665	5.641	31	6.7595	6.839	6.983
13	5.2190	5.254	5.266	32	6.4056	6.596	6.335
14*	5.0444	5.871	5.960	33	7.5850	7.571	7.527
15*	6.4881	6.627	6.636	34	5.9555	5.893	5.958
16	6.1904	6.236	6.269	35	7.7447	7.672	7.719
17	6.4295	6.421	6.424	36	7.5376	7.504	7.438
18	6.2882	6.344	6.401	37*	6.3439	7.258	7.354
19	6.1858	6.214	6.183	38	6.5072	6.472	6.513

“*”: 测试集

“*”: test set

2.2 模型验证

通过线性回归,图3中显示的CoMFA和CoMSIA模型具有很强的预测能力,几乎所有化合物都位于或接近趋势线,说明这两种模型具有较强说服力。

2.3 CoMFA 和 CoMSIA 模型三维等势图分析

以化合物35为参照样本的CoMFA模型三维等势分析如图4。绿色表示增大基团会增加化合物活性,黄色表示增大基团会降低化合物活性。图中,可明显看见氨基上的R₃取代基有黄色的等势域,也

有绿色的等势域。预示着与氨基直接相连的取代基若增大,会减少化合物的活性,如,38号分子R₃苯环上有3个取代基团,相较于35号分子,38号R₃苯环上多取代2个F,使得黄色区域的空间结构增大,故38号分子活性降低。相反,在R₁、R₂取代基相同的化合物分子中比较,绿色区域取代基结构较大的4、5、6号分子活性较1、2、3号分子活性大;10、11号分子活性较7、8、9号分子活性大。这与化合物本身的pIC₅₀值一致,表现出很好的分子模型预测。

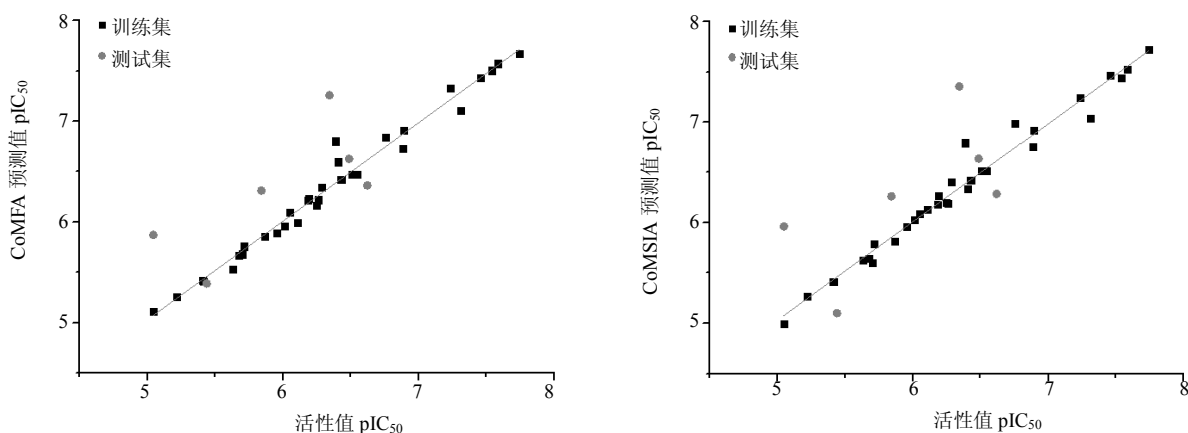


图3 3D-QSAR模型中训练集和测试集预测值数据与活性值数据之间的相关性

Fig. 3 Correlation between experimental and predicted values of 3D-QSAR model for training and test sets

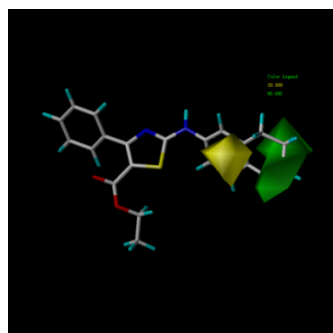


图4 CoMFA立体静电场三维等势图

Fig. 4 Contour map of CoMFA steric and electrostatic fields

图5为以35号化合物分子为参照样本的CoMSIA模型三维等势图。其中5a为立体静电场等势图,各个颜色代表的意义与CoMFA模型相同。通过观察比较,CoMSIA模型与CoMFA模型基本保持一致。图5b为疏水场等势域,黄色等势域代表疏水有利区域,白色等势域代表亲水有利区域。图5b中氨基所连接的苯环上有大块白色等势域,在此区域增加疏水性有利于活性的增加;而苯环上取代基区域显示大片黄色区域,增加亲水性基团可以提

高化合物活性。图5c为氢键供体与受体的等势分布图。蓝绿色表示增加氢键供体有利于化合物活性,紫色区域表示增加氢键供体不利于化合物活性,而红色区域表示增加氢键受体不利于化合物活性。如,23与26号分子相比较,23号分子R₃苯环上多一个甲基,增加了分子的疏水性,故活性大于26号分子;26与27号分子相比较,26号分子R₃苯环上连接氯原子,而27号分子连接的是三氟甲基,26号分子R₃苯环极性大于27号分子R₃苯环,亲水性增强,故26号分子活性较27号分子大。因此根据各个不同的区域,合理选择氢键供体与受体的改造方式,可以优化化合物结构,提高化合物的活性。

3 讨论

本研究依据38个噻唑类二氢乳清酸脱氢酶抑制剂的结构与活性,用CoMFA和CoMSIA得到稳定且具有较好预测能力的3D-QSAR模型,通过分子对接与计算打分,预测潜在活性分子,并且模拟出对接的三维立体图,显示良好的对接空间结构。通过三维等势图分析可了解其更多的化学结构与生物活性关系,为其提供结构改造的方向。该模型的建立

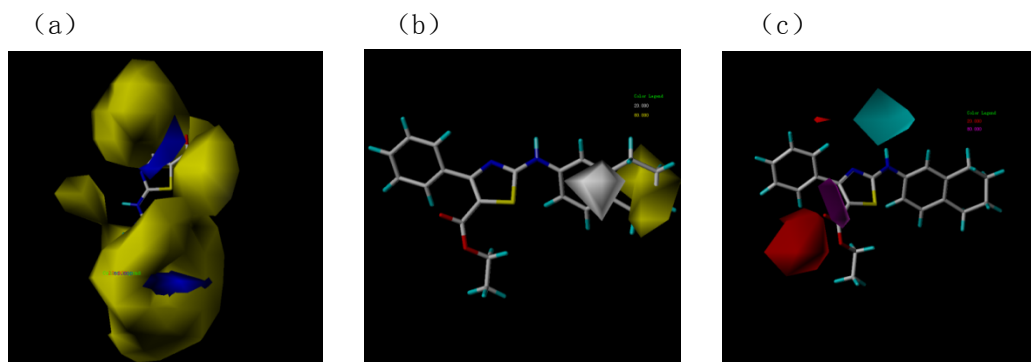


图5 CoMSIA 立体静电场 (a)、疏水场 (b)、氢键供体场与受体场 (c) 三维等势图

Fig. 5 Contour map of CoMSIA steric and electrostatic (a), hydrophobic (b), and H-bonding donor and acceptor (c) fields

对二氢乳清酸脱氢酶抑制剂活性和选择性的优化改造具有一定意义, 为今后该类化合物设计提供新思路。

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