·论 文·

丹皮酚肟衍生物的设计、合成及抗血小板聚集活性

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摘 要 以丹皮酚为起始原料,经结构修饰得到25个新的丹皮酚肟类衍生物**4a**~**4y**,其结构经过高分辨质谱、核磁共振氢谱确证。所合成的目标化合物对二磷酸腺苷(ADP)和胶原诱导的血小板聚集均具有一定的抑制活性,其中化合物**4h**、**4j**对两种诱导剂诱导的血小板聚集优于阳性对照药阿司匹林,且化合物**4h**具有较好的水溶性和类药性,可作为新的抗血小板活性化合物进一步研究。

关键词 丹皮酚肟衍生物;合成;水溶性;抗血小板聚集

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Design, synthesis and anti-platelet aggregation activity of paeonol oxime derivatives

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Abstract In order to afford new antiplatelet agents with higher potency, a series of paeonol oxime derivatives (**4a**-**4y**) were designed and synthesized from paeonol. Their structures were confirmed by HRMS, ^{1}H NMR spectra. The anti-platelet aggregation activity of the target compounds was evaluated. The results revealed that most of them had moderate to good anti-platelet aggregation activity. Among them, compound **4h** and **4j** were the most potent on adenosine diphosphate (ADP)-induced platelet aggregation and collagen-induced platelet aggregation. Furthermore, the target compound **4h** not only showed strong antiplatelet aggregation activity, but also exhibited good water-solubility and drug-like properties, which can be used as a new antiplatelet active compound for further research.

Key words peaonol oxime derivatives; synthesis; aqueous solubility; anti-platelet aggregation

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#戴卫国与谭鸿舟为共同第一作者

血栓栓塞引发的心脑血管疾病具有较高的发病率和病死率,是一类严重危害人类健康和生命的疾病。血小板聚集对血栓的形成起着关键的作用,抑制血小板聚集对血栓栓塞性疾病的防治具有重要意义。目前,临床使用的抗血小板药物仍存在抗血小板聚集疗效不佳或增加出血风险等问题,进一步寻找高效、不良反应小的新型抗血小板药物依然任重道远。

丹皮酚是传统中药牡丹根皮的主要有效成分之一,具有广泛的药理活性,如抗炎、抗血小板聚集、抗菌、抗氧化、抗肿瘤等[1-5]。近年来,丹皮酚在降血脂、抗血小板聚集等方面的研究备受关注[6-9]。研究发现,丹皮酚具有易挥发、水溶性差及易代谢等方面的不足,因此其临床应用受到一定的限制。对药用活性成分进行结构修饰和改造,是创制新药的有效途径之一。本课题组前期研究表明,对丹皮酚2-位羟基和1-位酮羰基进行结构修饰和优化能够得到具有较高稳定性、较好水溶性及较强抗血小板聚集活性的衍生物[10-12]。文献报道,肟或肟醚类衍生物具有广泛的生物活性,部分肟醚类衍生物具有良好的抗血小板聚集作用[13-14],如临床药物利多格雷等[15]。本课题组也曾以丹皮酚结构中的酮羰基为修饰位点,与盐酸羟胺反应得到丹皮酚肟类化合物,结果发现丹皮酚酮羰基肟化有助于提高化合物的抗血小板聚集作用,且其抗血小板聚集活性强于丹皮酚肟醚类化合物[16-17]。

为获得更多结构新颖且具有良好抗血小板聚集作用的丹皮酚肟衍生物,本研究以丹皮酚为先导化合物,将其2-位酚羟基通过不同碳数的连接臂与有机胺片段偶联,再对其1-位羰基进行肟化得到25个目标化合物。采用Bron比浊法分别测试目标化合物对二磷酸腺苷(ADP)和胶原诱导的血小板聚集的抑制作用。在此基础上,对具有高抗血小板聚集活性的化合物进行水溶性测试和类药性分析,期望得到具有更强的抗血小板聚集活性和成药性的候选药物。

1 材 料

1.1 试 剂

丹皮酚、1,2-二溴乙烷、1,3-二溴丙烷、1,4-二溴丁烷、1,5-二溴戊烷、1,6-二溴己烷、吗啉、哌啶、甲基哌嗪、乙基哌嗪、羟乙基哌嗪(上海麦克林生化科技有限公司);乙腈(江苏强盛功能化学股份有限公司);盐酸羟胺(上海化学试剂有限公司);氢氧化钠(NaOH)、*N*,*N*-二甲基甲酰胺(DMF)、吡啶,无水硫酸钠、乙醇、甲醇、二氯甲烷(DCM)、乙酸乙酯、石油醚(国药集团化学试剂有限公司)。所用试剂均为分析纯。

1.2 仪 器

WRS-1B数字熔点仪(上海索光光电技术有限公司);LCQ Advantage Max液质联用质谱仪(美国Finnigan公司);Nicolet Avatar 370 DTGS型红外光谱仪(美国ThermElectron公司);AV400和AV600型核磁共振仪(德国Bruker公司);薄层硅胶G板(100 mm × 100 mm,合肥森瑞有限公司);ZF-7型暗箱线分析三用紫外仪(上海嘉鹏科技有限公司);ML104电子天平(上海梅特勒-托利多仪器有限公司)。

1.3 动 物

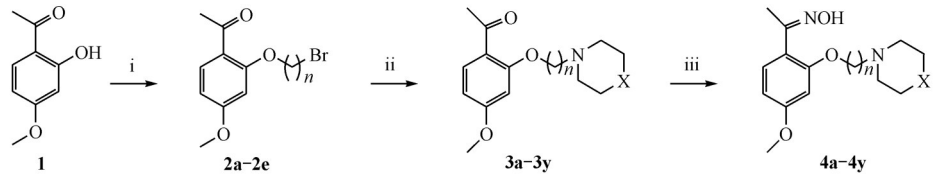
家兔2只,雄性,体重分别为2.5和2.2 kg,由安徽医科大学动物实验中心提供,合格证号:SCXK(苏)2019-0005,所有动物实验符合动物伦理委员会标准。

2 方法与结果

2.1 合成部分

以丹皮酚(**1**)为原料,通过Williamson合成法在碱性条件下利用2-位羟基与二溴烷烃Br-$(CH_2)_n$-Br进行反应,得到中间体丹皮酚溴代烷基醚(**2**);化合物**2**与不同的有机胺反应得到丹皮酚氨基醇醚衍生物(**3**);再利用化合物**3**中的羰基与盐酸羟胺进行加成消去反应,得到目标化合物(**4**)。化合物的结构及合成路线见路线1。

2.1.1 丹皮酚溴代烷基醚(**2**)的合成通法[11] 将丹皮酚(1.66 g,0.01 mol)、NaOH(2.4 g,0.06 mol),DMF 10 mL置于50 mL圆底烧瓶中,室温搅拌20 min。再加入相应的二溴烷烃(0.03 mol),室温搅拌反应,TLC跟踪反应进程。反应完毕,将反应液缓慢倒入冰水200 mL中,二氯甲烷(DCM)萃取(100 mL × 3)。有机相用饱和NaCl溶液洗涤(100 mL × 1),无水硫酸钠(Na_2SO_4)干燥。过滤,浓缩,柱色谱分离(乙酸乙酯-石油醚,1∶15),得白色



2a: n = 2; **2b**: n = 3; **2c**: n = 4; **2d**: n = 5; **2e**: n = 6
3a, **4a**: n = 2, X = O; **3b**, **4b**: n = 3, X = O; **3c**, **4c** n: = 4, X = O; **3d**, **4d**: n = 5, X = O; **3e**, **4e**: n = 6, X = O
3f, **4f**: n = 2, X = CH_2; **3g**, **4g**: n = 3, X = CH_2; **3h**, **4h**: n = 4, X = CH_2; **3i**, **4i**: n = 5, X = CH_2; **3j**, **4j**: n = 6, X = CH_2
3k, **4k**: n = 2, X = NCH_3; **3l**, **4l**: n = 3, X = NCH_3; **3m**, **4m**: n = 4, X = NCH_3; **3n**, **4n**: n = 5, X = NCH_3; **3o**, **4o**: n = 6, X = NCH_3
3p, **4p**: n = 2, X = NC_2H_5; **3q**, **4q**: n = 3, X = NC_2H_5; **3r**, **4r**: n = 4, X = NC_2H_5; **3s**, **4s**: n = 5, X = NC_2H_5; **3t**, **4t**: n = 6, X = NC_2H_5
3u, **4u**: n = 2, X = NC_2H_4OH; **3v**, **4v**: n = 3, X = NC_2H_4OH; **3w**, **4w**: n = 4, X = NC_2H_4OH; **3x**, **4x**: n = 5, X = NC_2H_4OH;
3y, **4y**: n = 6, X = NC_2H_4OH

Scheme 1　Synthetic routes of target compounds **4a−4y**

Reagents and conditions: i: NaOH, DMF, 25 ℃; ii: Morpholine, Piperidine, *N*-methyl piperazine, *N*-ethyl piperazine, *N*-(2-hydroxyethyl) piperazine, MeCN, 50 ℃; iii: $NH_2OH\cdot HCl$, EtOH, pyridine, 60 ℃

固体**2a** ~ **2e**。

2.1.2　中间体丹皮酚氨基醇醚**3a** ~ **3y**的合成通法[11]　称取丹皮酚溴代烷基醚（3 mmol）于 50 mL 圆底烧瓶中，加入乙腈 10 mL溶解，滴加相应的有机胺（9 mmol），50 ℃搅拌反应，TLC 监测反应进程。反应完全，停止反应。冷却至室温后将其倒入水 50 mL中，用乙酸乙酯萃取（50 mL × 3），再将乙酸乙酯层用饱和氯化钠溶液 150 mL洗涤 1 次，无水硫酸钠（Na_2SO_4）干燥。过滤，浓缩，柱色谱分离（甲醇-二氯甲烷，1∶80），得淡黄色油状物**3a** ~ **3y**，收率、高分辨质谱数据列于表1。

2.1.3　目标化合物**4a** ~ **4y**的合成通法[16]　称取中间体 1 mmol 置于 50 mL圆底烧瓶中，加入无水乙醇 3 mL，60 ℃下搅拌使之充分溶解。滴加含 2 mmol盐酸羟胺的吡啶液 2.5 mL，60 ℃搅拌反应，TLC 监测反应进程。反应完毕，减压蒸馏除去溶剂，柱色谱分离（甲醇-二氯甲烷，1∶80），得白色固体化合物**4**。目标化合物**4a** ~ **4y**的收率、熔点、高分辨质谱及核磁共振氢谱数据列于表2。

Table 1　Yield and HRMS of compounds **3a−3y**

Compd.	Yield/%	HRMS m/z[M+H]$^+$	Compd.	Yield/%	HRMS m/z[M+H]$^+$
3a	78.6	280.154 0	**3n**	74.5	335.105 9
3b	79.8	294.169 6	**3o**	69.8	349.103 4
3c	80.9	308.184 9	**3p**	65.8	307.202 1
3d	76.1	322.200 9	**3q**	72.2	321.217 7
3e	74.2	336.216 1	**3r**	67.0	335.233 5
3f	78.5	278.172 5	**3s**	69.0	349.249 3
3g	76.7	292.192 4	**3t**	78.1	363.265 3
3h	74.9	306.208 3	**3u**	69.6	323.197 4
3i	71.3	320.223 9	**3v**	71.1	337.213 0
3j	69.5	334.239 9	**3w**	72.6	351.228 3
3k	69.4	293.145 2	**3x**	73.9	365.243 9
3l	67.5	307.224 5	**3y**	73.9	379.259 9
3m	73.4	321.162 3			

Table 2　Yield, melting, HRMS and ^{1}H NMR of compounds **4a−4y**

Compd.	Yield/%	mp/℃	HRMS m/z [M+H]$^+$	^{1}H NMR(400 Hz), δ
4a	83.3	121.6−123.2	295.168 1	7.10(d, J = 8.4 Hz, 1H), 6.59(d, J = 8.2 Hz, 1H), 6.47(dd, J = 8.4, 2.2 Hz, 1H), 4.08(t, J = 5.6 Hz, 2H), 3.74(s, 3H), 3.58−3.53(m, 4H), 3.33−3.26(m, 6H), 2.03(s, 3H)
4b	80.2	105.8−107.6	309.177 0	7.11(d, J = 8.4 Hz, 1H), 6.57(d, J = 8.3 Hz, 1H), 6.47(dd, J = 8.4, 2.3 Hz, 1H), 4.05(t, J = 5.8 Hz, 2H), 3.74(s, 3H), 3.58−3.53(m, 4H), 3.33−3.26(m, 6H), 2.03(s, 3H), 1.85−1.80(m, 2H)
4c	79.5	91.3−93.1	323.192 6	7.12(d, J = 8.3 Hz, 1H), 6.56(d, J = 8.2 Hz, 1H), 6.48(dd, J = 8.4, 2.3 Hz, 1H), 3.99(t, J = 6.3 Hz, 2H), 3.76(s, 3H), 3.58−3.53(m, 4H), 3.35−3.28(m, 6H), 2.05(s, 3H), 1.75−1.70(m, 2H), 1.58−1.54(m, 2H)
4d	80.1	83.5−85.1	337.208 3	7.08(d, J = 8.4 Hz, 1H), 6.57(d, J = 8.2 Hz, 1H), 6.45(dd, J = 8.4, 2.2 Hz, 1H), 4.05(t, J = 5.6 Hz, 2H), 3.74(s, 3H), 3.55−3.49(m, 4H), 3.29−3.25(m, 6H), 2.02(s, 3H).1.77−1.65(m, 4H), 1.50−1.43(m, 2H)
4e	83.1	69.5−71.2	351.227 4	7.12(d, J = 8.4 Hz, 1H), 6.57(d, J = 8.2 Hz, 1H), 6.49(dd, J = 8.4, 2.3 Hz, 1H), 3.99(t, J = 5.3 Hz, 2H), 3.76(s, 3H), 3.42−3.35(m, 4H), 3.34(s, 3H), 3.06−2.95(m, 6H), 2.06−2.01(m, 2H), 1.76−1.66(m, 2H), 1.47−1.40(m, 2H), 1.38−1.31(m, 2H)

(Continued)

Compd.	Yield/%	mp/°C	HRMS *m/z* [M+H]$^+$	^{1}H NMR(400 Hz),δ
4f	68.6	175.1−176.8	293.182 0	7.15(d, *J* = 8.4 Hz, 1H), 6.61(d, *J* = 8.4 Hz, 1H), 6.52(dd, *J* = 8.4, 2.3 Hz, 1H), 4.08(t, *J* = 6.1 Hz, 2H), 3.77(s, 3H), 3.09(s, 3H), 2.25−2.13(m, 2H), 2.08−1.97(m, 4H), 1.85−1.66(m, 6H)
4g	68.0	163.8−165.2	307.203 5	7.17(d, *J* = 8.4 Hz, 1H), 6.50(d, *J* = 8.4 Hz, 1H), 6.41(s, 1H), 4.05(t, *J* = 6.0 Hz, 2H), 3.80(s, 3H), 3.11(s, 3H), 2.05−1.94(m, 2H), 1.86−1.78(m, 4H), 1.70−1.66(m, 2H), 1.46−1.38(m, 6H)
4h	75.9	173.7−175.2	321.219 6	7.13(d, *J* = 8.4 Hz, 1H), 6.59(d, *J* = 8.4 Hz, 1H), 6.50(dd, *J* = 8.4, 2.3 Hz, 1H), 4.03(t, *J* = 6.0 Hz , 2H), 3.78(s, 3H), 3.25−3.17(m, 2H), 3.11(s, 3H), 2.16(m, 2H), 2.02−1.96(m, 4H), 1.69−1.60(m, 2H), 1.44−1.35(m, 6H)
4i	70.1	149.5−151.3	335.235 3	7.19(d, *J* = 8.3 Hz, 1H), 6.46(d, *J* = 8.4 Hz, 2H), 3.99(t, *J* = 5.8 Hz, 2H), 3.81(s, 3H), 3.10(s, 3H), 2.25-2.19(m, 6H), 1.61−1.55(m, 2H), 1.47−1.39(m, 4H), 1.27−1.24(m, 6H)
4j	73.8	144.7−146.2	359.250 9	7.15(d, *J* = 8.4 Hz, 1H), 6.60(d, *J* = 8.4 Hz, 1H), 6.52(dd, *J* = 8.4, 2.3 Hz, 1H), 4.01(t, *J* = 5.8 Hz, 2H), 3.78(s, 3H), 3.12(s, 3H), 2.28−2.10(m, 6H), 1.84−1.81(m, 2H), 1.75−1.71(m, 4H), 1.43−1.41(m, 2H), 1.31−1.26(m, 6H)
4k	52.9	197.1−198.8	308.200 6	7.21(d, *J* = 8.3 Hz, 1H), 6.47(d, *J* = 8.0 Hz, 2H), 4.09(t, *J* = 5.7 Hz, 2H), 3.79(s, 3H), 2.82(t, *J* = 5.7 Hz, 2H), 2.61(m, 8H), 2.33(s, 3H), 2.20(s, 3H)
4l	48.6	189.7−191.3	322.216 1	7.23(d, *J* = 8.3 Hz, 1H), 6.49(d, *J* = 8.0 Hz, 2H), 4.11(t, *J* = 5.7 Hz, 2H), 3.80(s, 3H), 2.79(t, *J* = 5.7 Hz, 2H), 2.59(m, 8H), 2.31(s, 3H), 2.16(s, 3H), 1.85−1.80(m, 2H)
4m	46.5	185.1−186.4	336.232 7	7.20(d, *J* = 7.9 Hz, 1H), 6.47(d, *J* = 8.0 Hz, 2H), 3.96(t, *J* = 5.0 Hz, 2H), 3.79(s, 3H), 2.48−2.43(m, 2H), 2.32(s, 3H), 2.21−2.12(m, 8H), 1.67−1.59(m, 4H), 1.23(s, 3H)
4n	48.6	167.8−169.5	350.247 3	7.18(d, *J* = 8.3 Hz, 1H), 6.44(d, *J* = 8.0 Hz, 2H), 4.06(t, *J* = 5.7 Hz, 2H), 3.78(s, 3H), 2.75(t, *J* = 5.7 Hz, 2H), 2.54(m, 8H), 2.28(s, 3H), 2.13(s, 3H), 1.80−1.76(m, 2H), 1.64−1.58(m, 4H)
4o	54.7	165.3−166.9	364.263 6	7.21(d, *J* = 8.3 Hz, 1H), 6.47(d, *J* = 8.0 Hz, 2H), 4.08(t, *J* = 5.7 Hz, 2H), 3.79(s, 3H), 2.77(t, *J* = 5.7 Hz, 2H), 2.56(m, 8H), 2.27(s, 3H), 2.14(s, 3H), 1.89−1.84(m, 4H), 1.71−1.63(m, 4H)
4p	43.9	157.5−159.1	322.213 4	7.13(d, *J* = 8.2 Hz, 1H), 6.59(s, 1H), 6.50(d, *J* = 7.6 Hz, 1H), 4.03(t, 2H), 3.79(s, 3H), 3.44−3.32(m, 10H), 3.16(s, 3H), 2.14−2.06(m, 2H), 1.15(t, 3H)
4q	48.4	119.5−121.0	336.228 8	7.15(d, *J* = 8.2 Hz, 1H), 6.61(d, 1H), 6.52(d, *J* = 7.6 Hz, 1H), 4.10(t, 2H), 3.77(s, 3H), 3.48−3.34(m, 12H), 3.17(s, 3H), 2.10−2.03(m, 2H), 1.26(t, 3H)
4r	40.9	112.8−114.5	350.244 8	7.12(d, *J* = 8.4 Hz, 1H), 6.56(d, *J* = 2.4 Hz, 1H), 6.48(dd, *J* = 8.4, 2.4 Hz, 1H), 3.99(t, *J* = 6.3 Hz, 2H), 3.75(s, 3H), 3.45−3.38(m, 10H), 3.17(s, 3H), 1.74−1.68(m, 2H), 1.60−1.52(m, 2H), 1.33−1.22(m, 2H), 1.00(t, *J* = 7.2 Hz, 3H)
4s	43.4	103.2−104.9	364.260 6	7.13(d, *J* = 8.4 Hz, 1H), 6.54(d, *J* = 2.4 Hz, 1H), 6.46(dd, *J* = 8.4, 2.4 Hz, 1H), 3.96(t, *J* = 6.3 Hz, 2H), 3.72(s, 3H), 3.43−3.35(m, 12H), 3.16(s, 3H), 1.72−1.66(m, 2H), 1.59−1.56(m, 2H), 1.31−1.26(m, 2H), 1.12(t, *J* = 7.2 Hz, 3H)
4t	40.2	99.5−101.2	378.276 0	7.12(d, *J* = 8.3 Hz, 1H), 6.57(d, *J* = 2.4 Hz, 1H), 6.48(dd, *J* = 8.4, 2.3 Hz, 1H), 3.98(t, *J* = 5.3 Hz, 2H), 3.76(s, 3H), 3.43−3.35(m, 12H), 3.15(s, 3H), 1.79−1.65(m, 4H), 1.50−1.32(m, 4H), 1.25(t, *J* = 7.2 Hz, 3H)
4u	62.3	109.1−110.8	338.208 3	7.15(d, *J* = 8.4 Hz, 1H), 6.64(d, *J* = 2.3 Hz, 1H), 6.56(dd, *J* = 8.4, 2.3 Hz, 1H), 4.42(t, *J* = 4.6 Hz, 2H), 3.78(t, t, *J* = 4.6 Hz, 2H), 3.76(s, 3H), 3.57−3.44(m, 8H), 3.25(s, 3H), 2.48(t, *J* = 7.2 Hz, 2H), 2.04(t, *J* = 7.2 Hz, H)
4v	60.1	103.8−105.5	352.224 0	7.19(d, *J* = 8.4 Hz, 1H), 6.67(d, *J* = 2.3 Hz, 1H), 6.59(dd, *J* = 8.4, 2.3 Hz, 1H), 4.23(t, *J* = 5.2 Hz, 2H), 3.88(t, *J* = 5.2 Hz, 2H), 3.79(s, 3H), 3.62−3.57(m, 8H), 3.20(s, 3H), 2.44(t, *J* = 7.2 Hz, 2H), 2.08(t, *J* = 7.2 Hz, 2H), 1.87−1.80(m, 2H)
4w	63.8	97.8−99.6	366.234 8	7.11(d, *J* = 8.1 Hz, 1H), 6.56(d, 1H), 6.48(d, *J* = 8.3 Hz, 1H), 4.38(t, *J* = 5.2 Hz, 2H), 3.99(t, *J* = 5.2 Hz, 2H), 3.75(s, 3H), 3.18(s, 3H), 2.46−2.25(m, 12H), 2.04−2.01(m, 2H), 1.58−1.49(m, 2H)
4x	65.3	78.9−80.5	380.255 3	7.11(d, *J* = 8.1 Hz, 1H), 6.56(d, 1H), 6.48(d, *J* = 8.3 Hz, 1H), 4.38(t, *J* = 5.2 Hz, 2H), 3.99(t, *J* = 5.2 Hz, 2H), 3.75(s, 3H), 3.17(s, 3H), 2.46−2.25(m, 12H), 2.04−2.01(m, 4H), 1.58−1.49(m, 2H)
4y	64.7	67.8−69.5	394.270 9	7.11(d, *J* = 8.4 Hz, 1H), 6.56(d, *J* = 2.4 Hz, 1H), 6.48(dd, *J* = 8.4, 2.4 Hz, 1H), 3.98(t, *J* = 5.3 Hz, 2H), 3.79(t, *J* = 5.3 Hz, 2H), 3.75(s, 3H), 3.18(s, 3H), 2.42−2.20(m, 12H), 1.77−1.65(m, 4H), 1.50−1.38(m, 4H)

2.2　体外抗血小板聚集活性实验[11]

取家兔2只，利多卡因局部麻醉，手术分离颈总动脉取血，加9倍体积3.8%枸橼酸钠抗凝，以500 r/min离心10 min，制备富血小板血浆（PRP），剩余部分再以3 000 r/min离心，制备贫血小板血浆（PPP），按比浊法进行血小板聚集实验. 测定管中加入PRP 240 μL、不同浓度受试药物30 μL，37 ℃温孵5 min，分别以ADP或胶原30 μL（ADP终浓度为25 μmol/L、胶原终浓度为10 μg/mL）为诱导剂，观察记录5 min内最大聚集率。以DMSO作空白对照，丹皮酚和阿司匹林作阳性对照，计算目标化合物对ADP和胶原诱导的血小板聚集的抑制率，并根据线性回归方程计算出化合物的半数抑制浓度IC_{50}。首先测试了试药质量浓度在100 μg/mL时对ADP或胶原诱导的血小板聚集的抑制率，选择抗血小板聚集活性高于阿司匹林的目标化合物进行IC_{50}测算，结果如表3～表4所示。

从表3～表4可以看出，所合成丹皮酚肟类衍

Table 3　Inhibitory activity of target compounds **4a**–**4y** (100 μg/mL) on adenosine diphosphate (ADP)/collagen-induced platelet aggregation *in vitro*

Compd.	Aggregation inhibition/%		Compd.	Aggregation inhibition/%	
	ADP-induced	Collagen-induced		ADP-induced	Collagen-induced
4a	57.34	14.08	**4n**	36.42	36.42
4b	17.24	32.68	**4o**	31.40	59.70
4c	12.92	41.83	**4p**	50.83	44.78
4d	38.45	62.90	**4q**	43.39	47.26
4e	30.69	75.54	**4r**	40.50	68.16
4f	23.79	39.92	**4s**	62.80	59.20
4g	40.17	37.10	**4t**	39.52	40.30
4h	75.86	59.68	**4u**	58.62	32.62
4i	28.10	45.97	**4v**	63.10	40.32
4j	84.48	80.65	**4w**	68.45	48.39
4k	37.19	59.68	**4x**	71.72	53.23
4l	36.78	40.80	**4y**	65.49	45.49
4m	39.47	32.34	Blank	0	0
Paeonol	11.11	21.77	Aspirin	35.08	43.66
Heparin sodium		59.20			

Table 4　IC_{50} of some target compounds against platelet aggregation *in vitro*

Compd.	Antiplatelet aggregation IC_{50}/(μmol/L)		Compd.	Antiplatelet aggregation IC_{50}/(μmol/L)	
	ADP-induced	Collagen-induced		ADP-induced	Collagen-induced
4a	1.04×10^3	–	**4n**	4.70×10^2	–
4b	–	–	**4o**	–	41.4
4c	–	–	**4p**	1.30×10^3	3.1×10^2
4d	–	28.06	**4q**	1.23×10^3	–
4e	–	15.44	**4r**	2.79×10^3	5.18
4f	–	–	**4s**	4.78	–
4g	1.27×10^3	–	**4t**	–	–
4h	9.52	21.25	**4u**	7.34	–
4i	–	–	**4v**	40.0	–
4j	2.87	15.84	**4w**	20.15	3.01×10^2
4k	1.80	1.30×10^2	**4x**	3.50	2.82×10^2
4l	4.70×10^2	–	**4y**	65.5	2.41×10^2
4m	4.30×10^2	–	Aspirin	8.90×10^2	90.4
			Paeonol	1.34×10^3	3.60×10^2

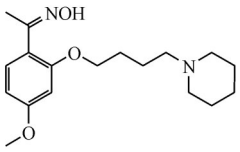
生物均可不同程度地抑制ADP、胶原诱导的血小板聚集,但其作用强度各不相同。化合物对ADP诱导的血小板聚集具有更强的抑制作用,部分化合物如**4h**,**4j**,**4k**,**4s**,**4u**和**4x**的IC_{50}达到较低微摩尔浓度,其抑制活性远强于阳性对照药阿司匹林;化合物抑制胶原诱导的血小板聚集作用相对较弱,抑制作用最强的化合物为**4r**,其IC_{50}为5.18 μmol/L,其次为化合物**4e**,**4h**和**4j**,IC_{50}分别为15.44,21.25和15.84 μmol/L。其中,化合物**4h**,**4j**对ADP诱导的血小板聚集抑制作用(IC_{50}分别为9.52,2.87 μmol/L)是阿司匹林(IC_{50} = 890 μmol/L)的93.5和310倍,是丹皮酚(IC_{50} =1.34 mmol/L)的141和467倍;对胶原诱导的血小板聚集抑制作用(IC_{50}分别为21.3,15.8 μmol/L)是阿司匹林(IC_{50}为90.4 μmol/L)的4.2和5.7倍,是丹皮酚(IC_{50} = 360 μmol/L)的17.0和22.7倍。

2.3 活性化合物**4h**,**4j**水溶性测试[18]

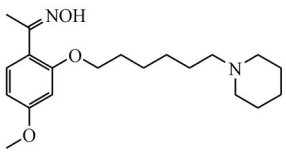
参照文献[18]方法,精密称取化合物**4h**和**4j**用甲醇定容至100 mL,分别移取0.5,1,1.5,2,2.5 mL,定容至10 mL中。平行测其吸收度3次,计算线性回归方程。取适量过量的化合物**4h**和**4j**加入一定量水中,37 ℃恒温摇床72 h,离心取上清液,紫外分光光度法检测其含量,计算出溶解度。测试结果表明,化合物**4j**在水中的溶解度为0.87 mg/mL,而化合物**4h**具有较高的水溶性,在水中的溶解度为2.08 mg/mL。

2.4 活性化合物**4h**,**4j**类药性分析

根据Lipinski规则,对活性化合物**4h**,**4j**进行了类药性分析,结果如表5所示。



4h



4j

Table 5 Drug-likeness correlation analysis of compound **4h** and **4j**

Compd.	M_r	HBD	HBA	*clgP*
4h	320	1	5	4.09
4j	358	1	5	5.15

HBD: hydrogen bond donor;HBA:hydrogen bond acceptor

3 讨 论

本研究以丹皮酚为先导化合物,先对其2-位羟基进行成醚修饰,再引入有机胺片段,最后对其1-位羰基进行肟化得到25个目标化合物,并对其合成工艺进行优化,最佳的反应条件为:采用Williamson合成法,以NaOH固体为缚酸剂,DMF为反应溶剂,n(丹皮酚):n(二溴烷烃):n(NaOH)=1∶3∶6,20～25 ℃下反应,所得中间体**2a**～**2e**的收率最高;以反应物有机胺自身作为缚酸剂,乙腈作为反应溶剂,50℃下反应中间体**3a**～**3y**的合成收率最高;以吡啶作为缚酸剂,无水乙醇为反应溶剂,n(**3a**～**3y**):n(盐酸羟胺)=1∶2,60 ℃下反应,目标化合物**4a**～**4y**的收率最佳。所合成的目标化合物均经HRMS、^{1}H NMR进行了结构确证。

抗血小板聚集活性研究表明,设计合成的目标化合物均具有一定的抗血小板聚集活性,部分化合物的活性优于阳性对照,其中目标化合物**4h**,**4j**,**4k**,**4s**,**4u**和**4x**对ADP诱导的血小板聚集显示出强的抑制作用,其IC_{50}远优于丹皮酚和阳性对照药阿司匹林;目标化合物**4d**,**4e**,**4h**,**4j**,**4o**和**4r**对胶原诱导的血小板聚集表现出较强的抑制活性,优于丹皮酚和阳性对照药阿司匹林。

初步的构效关系分析显示,在丹皮酚2-位羟基通过不同碳数的连接链引入不同的氨基片段,所得目标化合物对ADP和胶原诱导的血小板聚集的抑制作用影响各不相同。比如有机胺为吗啉时,所得目标化合物**4a**～**4e**对ADP诱导的血小板聚集抑制作用不强,连接臂长短对其抗血小板聚集活性影响不大;与之相反,连接臂碳数为5和6的目标化合物对胶原诱导的血小板聚集有着显著的抑制作用,如化合物**4d**(IC_{50}为28.0 μmol/L),化合物**4e**(IC_{50}为15.4 μmol/L)。有机胺为羟乙基哌嗪时,所得目标化合物**4u**～**4y**对ADP诱导的血小板聚集均具有较强的抑制作用,IC_{50}为3.50～65.5 μmol/L,但对胶原诱导的血小板聚集聚集影响不大;有机胺为甲基哌嗪时,连接臂碳数为2的化合物**4k**对ADP诱导的血小板聚集表现出最强的抑制作用,IC_{50}达1.8 μmol/L;而连接臂碳数为6的化合物**4o**对胶原诱导的血小板聚集有着较强的抑制作用。有机胺为乙基哌嗪时,连接臂碳数为4的化合物**4r**对胶原诱导的血小板聚集抑制作用最强,IC_{50}为5.18 μmol/L;连接臂碳数为5的化合物**4s**对ADP诱导的血小板聚集抑制作用最强,IC_{50}为4.78 μmol/L。有机胺为哌啶,连接臂碳数为4和6

的化合物**4h**和**4j**，对两种诱导剂诱导的血小板聚集均具有强的抑制作用。对活性化合物**4h**和**4j**的水溶性测试和类药性分析表明，化合物**4h**具有良好的水溶性，且为类药性化合物，值得进一步研究。

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