

## · 论 文 ·

# 氨基甲酸酯-异山梨醇-丁苯酞开环物三联体的合成及抗血小板聚集活性

杨春昱<sup>1</sup>, 黄张建<sup>1</sup>, 凌菁菁<sup>2</sup>, 季晖<sup>2</sup>, 赖宜生<sup>1</sup>, 徐进宜<sup>3\*</sup>, 彭司勋<sup>1</sup>, 张奕华<sup>1\*\*</sup>

(中国药科大学<sup>1</sup>新药研究中心; <sup>2</sup>药理学教研室; <sup>3</sup>药物化学教研室, 南京 210009)

**摘要** 合成一系列氨基甲酸酯-异山梨醇-丁苯酞开环物三联体(**8a~8i**), 其结构经波谱确证; 采用 Born 氏比浊法检测目标化合物对二磷酸腺苷(ADP)诱导的血小板聚集的抑制活性。结果表明, 化合物**8i**对ADP诱导的血小板聚集抑制活性及水溶性均显著优于丁苯酞, 具有深入研究价值。

**关键词** 氨基甲酸酯; 丁苯酞; 异山梨醇; 合成; 抗血小板聚集活性

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## Synthesis and evaluation of carbamate-isosorbide-3-n-butylphthalide ring opening derivative trihybrids as novel platelet aggregation inhibitors

YANG Chunyu<sup>1</sup>, HUANG Zhangjian<sup>1</sup>, LING Jingjing<sup>2</sup>, JI Hui<sup>2</sup>, LAI Yisheng<sup>1</sup>, XU Jinyi<sup>3\*</sup>, PENG Sixun<sup>1</sup>, ZHANG Yihua<sup>1\*\*</sup>

<sup>1</sup>Center of Drug Discovery; <sup>2</sup>Department of Pharmacology; <sup>3</sup>Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

**Abstract** A series of carbamate-isosorbide-3-n-butylphthalide ring opening derivative trihybrids (**8a-8i**) were synthesized, and their structures were confirmed by <sup>1</sup>H NMR and MS. The inhibitory activity of the target compounds against adenosine diphosphate (ADP)-induced platelet aggregation was evaluated *in vitro* by Born's turbidimetric assay. In comparison with 3-n-butylphthalide (NBP), compound **8i** possessed better antiplatelet aggregation activity and aqueous solubility. Therefore, compound **8i** may be a potential platelet aggregation inhibitor for further investigation.

**Key words** carbamate; 3-n-butylphthalide; isosorbide; synthesis; antiplatelet aggregation activity

血小板具有激活、黏附、聚集等多种功能。其表面受体种类繁多, 如二磷酸腺苷(ADP)受体、胶原受体、血小板活化因子(PAF)受体等, 能在多种刺激下导致血小板的活化。在动脉粥样硬化早期, 由于机械性和化学性损伤, 血管内膜破坏, 血管壁胶原暴露于血小板的接触面增加, 内源性凝血系统与纤溶平衡破坏, 触发一系列凝血反应, 形成富含血小板的血栓<sup>[1]</sup>。大量研究表明, 缺血性脑卒中是动脉粥样硬化后血栓形成的结果, 其中血小板与动脉粥样硬化病灶的相互作用是血栓形成的关

键<sup>[2]</sup>。目前治疗缺血性脑卒中的主要手段之一是使用抗血小板药物<sup>[3]</sup>, 但现有的药物尚不能取得令人满意的疗效, 故寻找新型抗血小板聚集药物已成为新药研究的热点之一。

3-正丁基-1(3H)-异苯并呋喃酮, 简称丁苯酞(NBP), 商品名恩必普, 是我国脑血管病治疗领域第一个拥有自主知识产权的国家一类新药, 于2004年11月被批准上市, 用于治疗脑血管疾病。大量临床实践表明, 丁苯酞可阻断缺血性脑卒中所致脑损伤的多个病理环节, 具有较强的抗脑缺血、缩小

\* 收稿日期 2013-02-06 通信作者 \* Tel: 025-83271445 E-mail: jinyixu@china.com

\*\* Tel: 025-83271015 E-mail: zyhtgl@163.com

脑梗死面积、减轻脑水肿、改善脑能量代谢和缺血区的微循环和血流量、抑制神经细胞凋亡等功效<sup>[4]</sup>。丁苯酞虽用药安全,但水溶性差,生物利用度不高,需要与其他抗血小板聚集药物联合应用才能发挥更好的疗效<sup>[5]</sup>。另据报道,丁苯酞的开环产物,2-( $\alpha$ -羟基正戊基)苯甲酸(HPBA),在体内能环合成丁苯酞,发挥抗脑缺血和抗血栓等作用<sup>[6]</sup>。

异山梨醇(IS)作为利尿药用于治疗脑积水和青光眼等疾病。文献报道,IS衍生物具有显著的血管舒张和抗感染等活性<sup>[7]</sup>。有趣的是,Gilmer等<sup>[8]</sup>发现异山梨醇-2-阿司匹林-5-水杨酸酯(**1**)的抗血小板活性优于异山梨醇-2-阿司匹林-5-硝酸酯(**2**),提示化合物**2**分子中的硝酸酯并非是必须的药效基团,抑或硝酸酯并非处于最佳位置。

本课题组曾合成了一系列结构类似于化合物**2**的单硝酸异山梨醇/丁苯酞开环物偶联体(**3**)。药理结果显示,大多数化合物的血小板聚集抑制活性均弱于丁苯酞<sup>[9]</sup>。考虑到氨基甲酸酯结构有助于异山梨醇与体内丁酰胆碱酯酶结合,加快前药酯键断裂水解成原药的速度,同时考虑到氨基甲酸酯具有较好的水溶性<sup>[10]</sup>,本研究设想用氨基甲酸酯取代杂合物**3**结构中的硝酸酯片段,有可能改善目标化合物的生物活性和水溶性,故合成了氨基甲酸酯-异山梨醇-丁苯酞开环物三联体;另考虑到NO可抑制血小板聚集,NO供体型化合物(化合物**2**除外)一般都具有这一活性,本研究在部分目标化合物末端引入硝酸酯基团,以考察其对血小板聚集抑制活性的影响。化合物**1-3**结构式见图1。

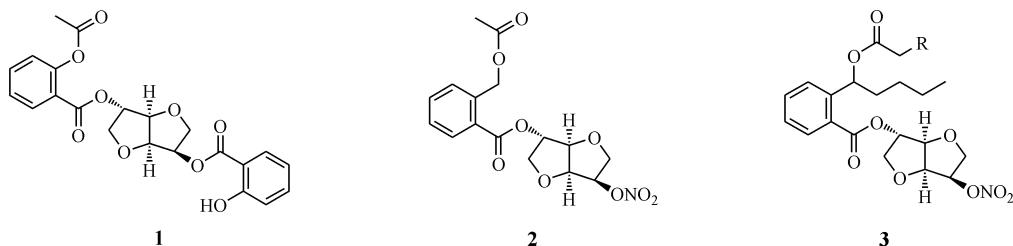
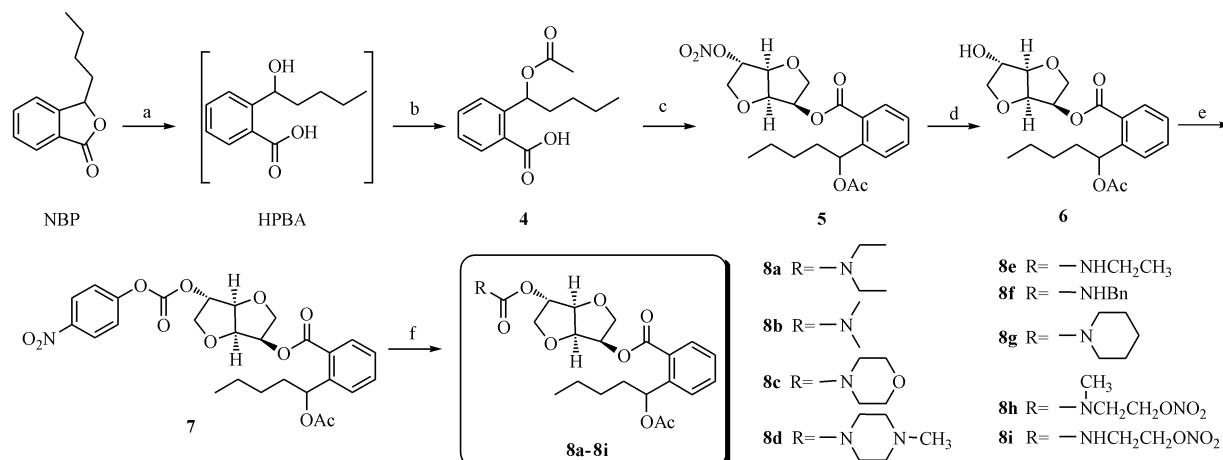


Figure 1 Chemical structures of compounds 1-3

## 1 目标化合物的合成路线

以消旋丁苯酞为起始原料,经皂化、酸化反应制得内酯开环物,然后与乙酰氯反应成酯得到2-( $\alpha$ -乙酰氧基正戊基)苯甲酸(中间体**4**),再将**4**与草酰氯反应制得相应的酰氯,继续与2-单硝酸异

山梨酯(2-ISMN)成酯,得含有硝酸酯类的中间体**5,5**通过H<sub>2</sub>/Pd还原,脱去2位硝基,得到中间体**6,6**再与对硝基苯酚氯甲酸酯反应,生成活泼中间体**7**,化合物**7**再与不同仲胺进行氨解反应,得到目标物**8a~8i**(路线1)。所有目标物均未见文献报道,结构均经MS及<sup>1</sup>H NMR确证。



Reagents and conditions: (a) (i) 2 mol/L NaOH, CH<sub>3</sub>OH/H<sub>2</sub>O, 50 °C, 0.5 h; (ii) 5% HCl, -10~0 °C; (b) AcCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, -10~0 °C, 5 h; (c) (i) (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, DMF, rt, 6 h; (ii) 2-ISMN, CH<sub>2</sub>Cl<sub>2</sub>, NaH, 0 °C, 2 h; (d) H<sub>2</sub>, Pd/C, EtOAc/MeOH, rt, 12 h; (e) 4-nitrophenyl chloroformate, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 12 h; (f) RH, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 5 h

Scheme 1 Synthetic route of the target compounds

## 2 合成实验

质谱采用 Hewlett-Packard 1100 LC/MSD 质谱仪测定。核磁共振氢谱采用 Bruker ACF-300Q 型核磁共振仪测定, CDCl<sub>3</sub> 为溶剂, TMS 为内标。起始原料丁苯酞、2-单硝酸异山梨醇酯(2-ISMN)按照文献[10-11]合成。实验所用试剂均为市售化学纯或分析纯,除特别说明外,不经处理直接使用。

### 2.1 2-(1-乙酰氧基正戊基)苯甲酸(4)的合成

将丁苯酞 1.24 g(6.5 mmol)溶于甲醇 10 mL 中,加入 2 mol/L NaOH 溶液 10 mL,回流 0.5 h,减压蒸除除去甲醇,加入蒸馏水 10 mL 稀释,冷却至 -5℃,剧烈搅拌下用 5% 稀盐酸酸化至 pH 2~3,水层用乙醚(50 mL × 3)萃取,未经纯化直接投入下一步反应。将上述乙醚溶液用二氯甲烷 200 mL 稀释,分别加入三乙胺 2.7 mL(19.6 mmol)和 4-二甲氨基吡啶(DMAP)0.5 g(4.1 mmol),-10℃下滴加乙酰氯 1.5 mL(19.6 mmol),滴毕于 -10℃下搅拌 5 h,加入水 10 mL,室温搅拌 0.5 h,分出有机层,无水 Na<sub>2</sub>SO<sub>4</sub> 干燥,过滤,浓缩得腊状固体,正己烷重结晶,得白色针状晶体 0.98 g。mp 65~66℃,收率 60%。ESI-MS *m/z*: 251 [M + H]<sup>+</sup>。<sup>1</sup>H NMR(CDCl<sub>3</sub>) δ: 0.93(t, 3H, *J* = 8.5 Hz, CH<sub>3</sub>), 1.37~1.42(m, 4H, 2 × CH<sub>2</sub>), 1.88(m, 2H, CH<sub>2</sub>), 2.13(s, 3H, CH<sub>3</sub>), 6.61(m, 1H, CH), 7.29(m, 1H, ArH), 7.37~7.58(m, 2H, ArH), 8.05 d,(m, 1H, *J* = 7.1 Hz, ArH), 10.98(br. s, 1H, COOH)。

### 2.2 5-[2-[1-(2-乙酰氧基)戊基]苯甲酸-2-硝基异山梨醇酯(5)的合成

将化合物 4(1.20 g, 4.8 mmol)溶于无水二氯甲烷(20 mL)中,加入草酰氯(0.61 mL, 6.24 mmol),室温搅拌 8 h,减压蒸除溶剂得相应酰氯。将 2-ISMN(0.86 g, 4.5 mmol)溶于无水二氯甲烷(20 mL)中,0℃下加入氢化钠(1.12 g, 45.0 mmol)搅拌 10 min。将上述得到的酰氯溶于无水二氯甲烷(20 mL)中,滴加到此反应混合液中,0℃搅拌反应 4 h,反应液倾入冰水中,乙酸乙酯萃取 3 次,有机相用饱和食盐水洗涤,无水 Na<sub>2</sub>SO<sub>4</sub> 干燥,过滤,浓缩,快速柱色谱[乙酸乙酯-石油醚(1:4)],得无色油状物 1.05 g,收率 52%。ESI-MS *m/z*: 446 [M + Na]<sup>+</sup>。<sup>1</sup>H NMR(CDCl<sub>3</sub>) δ: 0.89(t, 3H, *J* = 6.8 Hz, CH<sub>3</sub>), 1.34~1.46(m, 4H, 2 × CH<sub>2</sub>), 1.74~1.84(m, 2H, CH<sub>2</sub>), 2.06(s, 3H, COCH<sub>3</sub>), 3.84~4.12(m, 4H, IsH-6, IsH-6', IsH-1, IsH-1'), 4.63(d, 1H, *J* = 4.9 Hz, IsH-3), 4.98(t, 1H, *J* = 5.1 Hz, IsH-4), 5.40(m, 2H, IsH-5, IsH-2), 6.46~6.50(m, 1H, OCH), 7.29~7.35(m, 1H, ArH), 7.53(m, 2H, ArH), 7.88(d, 1H, *J* = 8.6 Hz, ArH)。

### 2.3 5-[2-[1-(2-乙酰氧基)戊基]苯甲酸-2-羟基异山梨醇酯(6)的合成

将化合物 5(1.35 g, 3.2 mmol)溶于甲醇-乙酸乙酯(1:1)200 mL 溶液中,加入 10% Pd/C,常压通入氢气,室温搅拌 8 h,过滤,浓缩,快速柱色谱[乙酸乙酯-石油醚(1:2)],得无色油状物 0.88 g,收率 73%。ESI-MS *m/z*: 401 [M + Na]<sup>+</sup>。<sup>1</sup>H NMR(CDCl<sub>3</sub>) δ: 0.90(t, 3H, *J* = 6.9 Hz, CH<sub>3</sub>), 1.23~1.45(m, 4H, 2 × CH<sub>2</sub>), 1.78~1.84(m, 2H, CH<sub>2</sub>), 2.06(s, 3H, COCH<sub>3</sub>), 3.64~4.15(m, 4H, IsH-6, IsH-6', IsH-1, IsH-1'), 4.32(s, 1H, IsH-3), 4.45(d, 1H, *J* = 4.5 Hz, IsH-4), 4.96(t, 1H, *J* = 4.8 Hz, IsH-5), 5.33~5.39(m, 1H, IsH-2), 6.52~6.56(m, 1H, OCH), 7.26~7.34(m, 1H, ArH), 7.52(m, 2H, ArH), 7.90(d, 1H, *J* = 7.8 Hz, ArH)。

### 2.4 5-[2-[1-(2-乙酰氧基)戊基]苯甲酸-2-(对硝基苯酚碳酸酯)异山梨醇酯(7)的合成

将化合物 6(0.83 g, 2.2 mmol)溶于无水二氯甲烷 200 mL,加入对硝基苯酚氯甲酸酯(0.50 g, 2.5 mmol),室温搅拌 3 h,反应液用饱和 NaHCO<sub>3</sub> 洗涤,无水 Na<sub>2</sub>SO<sub>4</sub> 干燥,过滤,浓缩,快速柱色谱[乙酸乙酯-石油醚(1:6)],得无色油状物 0.99 g,收率 83%。ESI-MS *m/z*: 566 [M + Na]<sup>+</sup>。<sup>1</sup>H NMR(CDCl<sub>3</sub>) δ: 0.91(t, 3H, *J* = 6.8 Hz, CH<sub>3</sub>), 1.23~1.47(m, 4H, 2 × CH<sub>2</sub>), 1.79~1.85(m, 2H, CH<sub>2</sub>), 2.06(s, 3H, COCH<sub>3</sub>), 3.99~4.19(m, 4H, IsH-6, IsH-6', IsH-1, IsH-1'), 4.70(d, 1H, *J* = 4.71 Hz, IsH-3), 5.06(t, 1H, *J* = 5.0 Hz, IsH-4), 5.22(d, 1H, *J* = 3 Hz, IsH-5), 5.39~5.44(m, 1H, IsH-2), 6.49~6.53(m, 1H, OCH), 7.30~7.40(m, 3H, ArH), 7.52(m, 2H, ArH), 7.90(d, 1H, *J* = 7.8 Hz, ArH), 8.29(d, 2H, *J* = 6.9 Hz, ArH)。

### 2.5 氨基甲酸酯-异山梨醇-丁苯酞开环物三联体(8a~8i)的合成通法

将中间体 7(1.0 mmol)溶于无水二氯甲烷(20 mL)中,加入相应胺类化合物(2.0 mmol)和三乙胺(2.0 mmol),室温反应 8 h,反应液分别用饱和 NaHCO<sub>3</sub>、水及饱和食盐水洗涤,无水 Na<sub>2</sub>SO<sub>4</sub> 干燥,过滤,浓缩,快速柱色谱[乙酸乙酯-石油醚(1:2)],分离得到产物。合成的 9 个目标化合物的收率、质谱及核磁共振氢谱数据见表 1。

## 3 活性评价

### 3.1 血小板聚集抑制活性

采用 Born 氏比浊法,以丁苯酞为阳性对照药,测试目标化合物 8a~8i 对 ADP 诱导的血小板聚集抑制活性。结果如表 2 所示,当浓度为 0.1 mmol/L 时,化合物 8i(18.9%) 的抑制率高于丁苯酞(10.7%)。

**Table 1** Yield, MS and  $^1\text{H}$  NMR data of the target compounds **8a-8i**

Compd.	Yield /%	MS $m/z$	$^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) $\delta$
<b>8a</b>	69.4	500 [ $\text{M} + \text{Na}$ ] $^+$	0.88 (t, 3H, $J = 6.8$ Hz, $\text{CH}_3$ ), 1.10 (t, 6H, $J = 6.3$ Hz, $2 \times \text{CH}_2$ ), 1.32-1.42 (m, 4H, $2 \times \text{CH}_2$ ), 1.77-1.83 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.27 (s, 4H, $2 \times \text{CH}_2$ ), 3.92-4.19 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.56 (d, 1H, $J = 4.4$ Hz, $\text{IsH-3}$ ), 4.93 (t, 1H, $J = 4.7$ Hz, $\text{IsH-4}$ ), 5.18 (s, 1H, $\text{IsH-5}$ ), 5.34-5.39 (m, 1H, $\text{IsH-2}$ ), 6.54-6.59 (m, 1H, $\text{OCH}$ ), 7.26-7.34 (m, 1H, $\text{ArH}$ ), 7.52 (m, 2H, $\text{ArH}$ ), 7.93 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8b</b>	70.1	472 [ $\text{M} + \text{Na}$ ] $^+$	0.88 (t, 3H, $J = 6.3$ Hz, $\text{CH}_3$ ), 1.21-1.49 (m, 4H, $2 \times \text{CH}_2$ ), 1.82 (m, 2H, $\text{CH}_2$ ), 2.06, (s, 3H, $\text{COCH}_3$ ), 2.90 (d, 6H, $J = 5.5$ Hz, $2 \times \text{CH}_3$ ), 3.87-4.19 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.57 (d, 1H, $J = 4.3$ Hz, $\text{IsH-3}$ ), 4.94 (t, 1H, $J = 4.6$ Hz, $\text{IsH-4}$ ), 5.14 (s, 1H, $\text{IsH-5}$ ), 5.33-5.45 (m, 1H, $\text{IsH-2}$ ), 6.53-6.57 (m, 1H, $\text{OCH}$ ), 7.26-7.34 (m, 1H, $\text{ArH}$ ), 7.52 (m, 2H, $\text{ArH}$ ), 7.92 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8c</b>	78.2	514 [ $\text{M} + \text{Na}$ ] $^+$	0.89 (t, 3H, $J = 6.8$ Hz, $\text{CH}_3$ ), 1.23-1.42 (m, 4H, $2 \times \text{CH}_2$ ), 1.78-1.83 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.45 (s, 4H, $2 \times \text{NCH}_2$ ), 3.65 (s, 4H, $2 \times \text{OCH}_2$ ), 3.94-4.15 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.56 (d, 1H, $J = 4.5$ Hz, $\text{IsH-3}$ ), 4.95 (t, 1H, $J = 4.8$ Hz, $\text{IsH-4}$ ), 5.18 (s, 1H, $\text{IsH-5}$ ), 5.33-5.39 (m, 1H, $\text{IsH-2}$ ), 6.52-6.61 (m, 1H, $\text{OCH}$ ), 7.29-7.34 (m, 1H, $\text{ArH}$ ), 7.52 (m, 2H, $\text{ArH}$ ), 7.90 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8d</b>	67.2	505 [ $\text{M} + \text{H}$ ] $^+$	0.90 (t, 3H, $\text{CH}_3$ , $J = 6.9$ Hz), 1.35-1.44 (m, 4H, $2 \times \text{CH}_2$ ), 1.63-1.89 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 2.32 (s, 3H, $\text{NCH}_3$ ), 2.38 (s, 4H, $2 \times \text{NCH}_2$ ), 3.49 (s, 4H, $2 \times \text{NCH}_2$ ), 3.94-4.08 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.57 (d, 1H, $J = 4.3$ Hz, $\text{IsH-3}$ ), 4.97 (t, 1H, $J = 4.7$ Hz, $\text{IsH-4}$ ), 5.20 (s, 1H, $\text{IsH-5}$ ), 5.34-5.46 (m, 1H, $\text{IsH-2}$ ), 6.49-6.53 (m, 1H, $\text{OCH}$ ), 7.29-7.34 (m, 1H, $\text{ArH}$ ), 7.52 (d, 2H, $J = 4.1$ Hz, $\text{ArH}$ ), 7.89 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8e</b>	57.0	450 [ $\text{M} + \text{H}$ ] $^+$	0.90 (t, 3H, $J = 6.9$ Hz, $\text{CH}_3$ ), 1.14 (t, 3H, $J = 7.2$ Hz, $\text{CH}_3$ ), 1.26-1.36 (m, 4H, $2 \times \text{CH}_2$ ), 1.82-1.89 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.17-3.26 (m, 2H, $\text{NCH}_2$ ), 3.93-4.01 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.54 (d, 1H, $J = 4.3$ Hz, $\text{IsH-3}$ ), 4.70 (s, 1H, $\text{NH}$ ), 4.95 (t, 1H, $J = 4.7$ Hz, $\text{IsH-4}$ ), 5.20 (s, 1H, $\text{IsH-5}$ ), 5.35-5.40 (m, 1H, $\text{IsH-2}$ ), 6.50-6.54 (m, 1H, $\text{OCH}$ ), 7.29-7.34 (m, 1H, $\text{ArH}$ ), 7.52 (d, 2H, $J = 3.9$ Hz, $\text{ArH}$ ), 7.91 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8f</b>	55.5	529 [ $\text{M} + \text{NH}_4$ ] $^+$	0.89 (t, 3H, $J = 6.8$ Hz, $\text{CH}_3$ ), 1.11-1.42 (m, 4H, $2 \times \text{CH}_2$ ), 1.73-1.83 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.92-4.09 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.36 (d, 2H, $J = 5.7$ Hz, $\text{PhCH}_2\text{N}$ ), 4.55 (d, 1H, $J = 3.8$ Hz, $\text{IsH-3}$ ), 4.92 (t, 1H, $J = 4.4$ Hz, $\text{IsH-4}$ ), 5.04 (s, 1H, $\text{NH}$ ), 5.20 (d, 1H, $J = 2.6$ Hz, $\text{IsH-5}$ ), 5.34-5.48 (m, 1H, $\text{IsH-2}$ ), 6.52-6.62 (m, 1H, $\text{OCH}$ ), 7.25-7.52 (m, 6H, $\text{ArH}$ ), 7.66 (m, 2H, $\text{ArH}$ ), 7.89 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8g</b>	75.3	490 [ $\text{M} + \text{H}$ ] $^+$	0.89 (t, 3H, $J = 6.8$ Hz, $\text{CH}_3$ ), 1.25-1.36 (m, 4H, $2 \times \text{CH}_2$ ), 1.53 (m, 6H, $3 \times \text{CH}_2$ ), 1.78-1.87 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.39 (s, 4H, $2 \times \text{CH}_2$ ), 3.93-4.08 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.57 (d, 1H, $J = 4.4$ Hz, $\text{IsH-3}$ ), 4.97 (t, 1H, $J = 4.4$ Hz, $\text{IsH-4}$ ), 5.20 (s, 1H, $\text{IsH-5}$ ), 5.34-5.40 (m, 1H, $\text{IsH-2}$ ), 6.49-6.53 (m, 1H, $\text{OCH}$ ), 7.29-7.34 (m, 1H, $\text{ArH}$ ), 7.64 (d, 2H, $J = 3.9$ Hz, $\text{ArH}$ ), 7.90 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )
<b>8h</b>	45.2	542 [ $\text{M} + \text{NH}_4$ ] $^+$	0.89 (t, 3H, $J = 6.6$ Hz, $\text{CH}_3$ ), 1.34-1.44 (m, 4H, $2 \times \text{CH}_2$ ), 1.78-1.87 (m, 2H, $\text{CH}_2$ ), 2.06 (3H, s, $\text{COCH}_3$ ), 2.97 (d, 3H, $J = 9.0$ Hz, $\text{NCH}_3$ ), 3.48-3.61 (m, 2H, $\text{NCH}_2$ ), 3.94-4.21 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.53-4.62 (m, 3H, $\text{IsH-3, CH}_2\text{ONO}_2$ ), 4.98 (d, 1H, $J = 4.4$ Hz, $\text{IsH-4}$ ), 5.20 (d, 1H, $J = 7.2$ Hz, $\text{IsH-5}$ ), 5.37-5.46 (m, 1H, $\text{IsH-2}$ ), 6.49-6.53 (m, 1H, $\text{OCH}$ ), 7.29-7.34 (m, 1H, $\text{ArH}$ ), 7.60 (d, 2H, $J = 3.9$ Hz, $\text{ArH}$ ), 7.89 (d, 1H, $\text{ArH}$ )
<b>8i</b>	45.4	528 [ $\text{M} + \text{NH}_4$ ] $^+$	0.89 (t, 3H, $J = 6.9$ Hz, $\text{CH}_3$ ), 1.23-1.46 (m, 4H, $2 \times \text{CH}_2$ ), 1.80-1.86 (m, 2H, $\text{CH}_2$ ), 2.06 (s, 3H, $\text{COCH}_3$ ), 3.51-3.56 (m, 2H, $\text{NCH}_2$ ), 3.94-4.10 (m, 4H, $\text{IsH-6, IsH-6', IsH-1, IsH-1'}$ ), 4.53 (m, 3H, $\text{IsH-3, CH}_2\text{ONO}_2$ ), 4.95 (t, 1H, $J = 5.0$ Hz, $\text{IsH-4}$ ), 5.07 (m, 1H, $\text{NH}$ ), 5.19 (s, 1H, $\text{IsH-5}$ ), 5.35-5.41 (m, 1H, $\text{IsH-2}$ ), 6.50-6.65 (m, 1H, $\text{OCH}$ ), 7.29-7.41 (m, 1H, $\text{ArH}$ ), 7.61 (m, 2H, $\text{ArH}$ ), 7.90 (d, 1H, $J = 7.8$ Hz, $\text{ArH}$ )

**Table 2** Effect of the target compounds **8a-8i** (100  $\mu\text{mol/L}$ ) on the ADP-induced platelet aggregation *in vitro*

Compd.	Max aggregation at 5 min/%	Inhibition/%	Compd.	Max aggregation at 5 min/%	Inhibition/%
Control	46.30 $\pm$ 3.14	-	<b>8e</b>	45.90 $\pm$ 3.92	0.9
NBP	41.36 $\pm$ 3.17	10.7	<b>8f</b>	44.40 $\pm$ 4.94	4.1
<b>8a</b>	44.88 $\pm$ 5.51	3.0	<b>8g</b>	43.97 $\pm$ 3.57	5.0
<b>8b</b>	43.89 $\pm$ 4.87	5.2	<b>8h</b>	45.05 $\pm$ 2.35	6.7
<b>8c</b>	46.01 $\pm$ 3.56	0.6	<b>8i</b>	37.53 $\pm$ 3.88	18.9 *
<b>8d</b>	43.89 $\pm$ 3.60	6.2			

NBP:3-n-butylphthalide; \*  $P < 0.05$  vs control group

根据表2,选取初筛中具有较高抑制活性的化合物 $\mathbf{8i}$ ,按照上述方法,在不同浓度下测定该化合物对血小板聚集的抑制率。同样以丁苯酞为阳性对照药,计算 $IC_{50}$ 。结果显示, $\mathbf{8i}$ 的 $IC_{50}$ (0.44 mmol/L)低于阳性对照药丁苯酞(1.52 mmol/L),进一步表明, $\mathbf{8i}$ 对ADP诱导的血小板聚集抑制活性高于丁苯酞。

### 3.2 水溶性

选取活性较好的化合物 $\mathbf{8i}$ ,以NBP为阳性对照药,采用紫外分光光度法测定溶解度。结果表明,在生理盐水5 mL中,化合物 $\mathbf{8i}$ 的溶解度(2.13 mmol/L)约为NBP(0.53 mmol/L)的4倍,提示氨基甲酸酯的引入增加了化合物的水溶性。

### 3.3 体外NO释放

采用Griess法测试化合物 $\mathbf{8h}$ 和 $\mathbf{8i}$ 的体外NO释放,按试剂盒说明书进行检测,如图2所示,化合物 $\mathbf{8h}$ 和 $\mathbf{8i}$ 的NO释放量分别为1.36  $\mu\text{mol}/\text{L}$ 和3.54  $\mu\text{mol}/\text{L}$ 。结果表明,相对于化合物 $\mathbf{8h}$ ,活性较好的化合物 $\mathbf{8i}$ 释放较高浓度的NO。

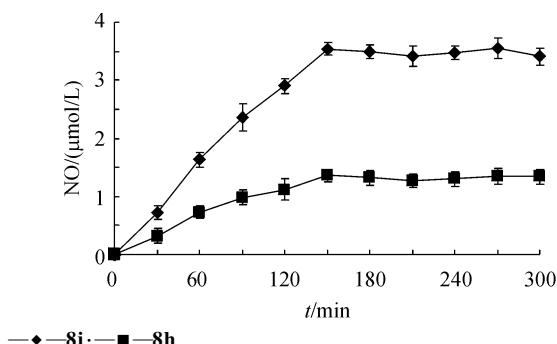


Figure 2 Levels of NO produced by compounds  $\mathbf{8h}$  and  $\mathbf{8i}$  in vitro ( $\bar{x} \pm s, n = 4$ )

## 4 结果与讨论

抑制血小板聚集实验结果表明:①氨基甲酸酯片段中的氨基对血小板聚集抑制活性有一定的影响,其活性大小依次为N-甲基哌嗪、二甲胺、哌啶、苄胺、二乙胺、乙胺、吗啡啉;②氨基甲酸酯片段中含NO供体的化合物( $\mathbf{8h}$ 和 $\mathbf{8i}$ )的活性优于不含NO供体的化合物( $\mathbf{8a} \sim \mathbf{8g}$ ),其中化合物 $\mathbf{8i}$ 活性最强。化合物 $\mathbf{8h}$ 和 $\mathbf{8i}$ 结构相似,但血小板聚集抑制活性和NO释放量却相差较大,究其原因可能是两个化合物所连氨基不同(分别为叔胺基和仲胺基)所致。前者空间位阻较大,不利于氨基甲酸酯的酯键断裂及NO释放,导致活性相对较低。

与阳性对照药丁苯酞( $IC_{50} = 1.52 \text{ mmol/L}$ )相比,化合物 $\mathbf{8i}$ ( $IC_{50} = 0.44 \text{ mmol/L}$ )对ADP诱导的血小板聚集抑制活性有显著提高;其水溶性是丁苯酞的4倍。此外, $\mathbf{8i}$ 能在体外释放适量浓度的NO,有可能与异山梨醇、丁苯酞开环物协同发挥作用,从而增强活性。该结果也提示,除引入氨基甲酸酯外, $\mathbf{8i}$ 与化合物 $\mathbf{2}$ 和 $\mathbf{3}$ 结构中硝酸酯位置的差异可能是导致它们具有不同生物活性的另一个重要因素。

鉴于化合物 $\mathbf{8i}$ 具有较好的血小板聚集抑制活性和水溶性,故可作为先导化合物进行深入研究。

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