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Synthesis, resolution, and antiplatelet activity of 3-substituted 1(3H)-isobenzofuranone

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Abstract—A series of 3-substituted-1(3H)-isobenzofuranone **6a**–**g** and **7a**–**g** were synthesized from phthalic anhydride. The compound **6a**–**g** was resolved. The antiplatelet activities of these compounds were evaluated using in vitro experiment of platelet aggregation. The levels of antiplatelet activity were displayed as following sequence: l-isomer > dl-isomer > d-isomer, respectively. The alkylphthalide is more active than the corresponding alkenephthalide. All these compounds were less active than *n*-butylphthalide (NBP, **6c**) and Aspirin (Asp).

Cardiovascular and cerebrovascular disorders are the main cause of deformity and death in recent years. As a result, seeking ideal medicine for the treatment of cerebralapoplexy with good effectiveness and low toxicity has become the interest of many investigators. It was found out that 1(3H)-isobenzofuranone had the antiplatelet activity. *n*-Butylphthalide (NBP) is a representive compound from such group, represent which has already been in the market as antiplatelet drug for ischemia-cerebralapoplexy. There was significant difference in the antiplatelet effect of NBP stereo isomers, which is known as l NBP > dl-NBP > d-NBP.[1–4] Structure modification of NBP has not been investigated yet. In addition, butylidenephthalide also has the activity of antiplatelet.[5] In this study, 3-alkyl-1(3H)-isobenzofuranone derivatives **6a**–**g** and 3-alkene-1(3H)-isobenzofuranone derivatives **7a**–**g** were designed and synthesized with the different substituent on the 3-position. Furthermore, the stereo isomers of 3-alkyl-1(3H)-isobenzofuranone were resolved using chemical method.

The antiplatelet effect of the target-compounds was screened by Borns' method[6] using Adenosine 5′-diphos-phate (ADP) and Arachidonic Acid (AA) as the inducers.

The compounds **6a**–**g** and **7a**–**g** were synthesized by five-step reaction as outlined in Scheme 1.[7] The 3-substituted-1(3H)-isobenzofuranone derivatives **6a**–**g** and **7a**–**h** were synthesized (Scheme 1) from RBr. The key intermediates **5a**–**g** were synthesized by the formation of RMgBr, followed by transforming to **3a**–**g** with the treatment of $CdCl_2$. 3-alkyl-1(3H)-isobenzo-furanone **6a**–**g** were prepared by the reduction of **5a**–**g** with $NaBH_4$, eluted by column chromatography (light petroleum/acetone = 100:1),[11] *Z*-3-alkene-1(3H)-isobenzofuranone **7a**–**g** were obtained by dehydration of **5a**–**g** with p-TsOH (Scheme 1 and Table 1).[13]

The resolution route of the compounds **6a**–**g** is outlined in Scheme 2.

The compounds **8a**–**g** was synthesized by hydrolysis in the presence of NaOH, followed by acidification with HCl to pH 3–4 at −20–0 °C. The compounds **9a**–**g** were obtained from the compounds **8a**–**g** and (−) α-phenylethylamine.[12] Similarly, the compounds **10a**–**g** were prepared using (+) α-phenylethylamine. Cyclization of the compounds **9a**–**g**/**10a**–**g** gave the corresponding compounds **11a**–**g**/**12a**–**g** under acid condition in good yield (Scheme 2 and Table 2).[13]

All compounds reported here were fully characterized on the basis of their ^1H NMR, ^{13}C NMR, IR and MS spectroscopic and analytical data. The purity of all compounds were examined by High Performance Liquid Chromatography.

Keywords: 1(3H)-Isobenzofuranone; Synthesize; Specific rotation; Antiplatelet.
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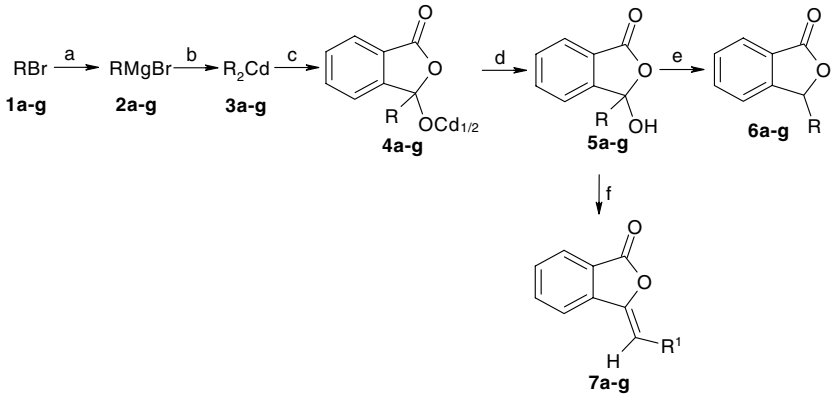


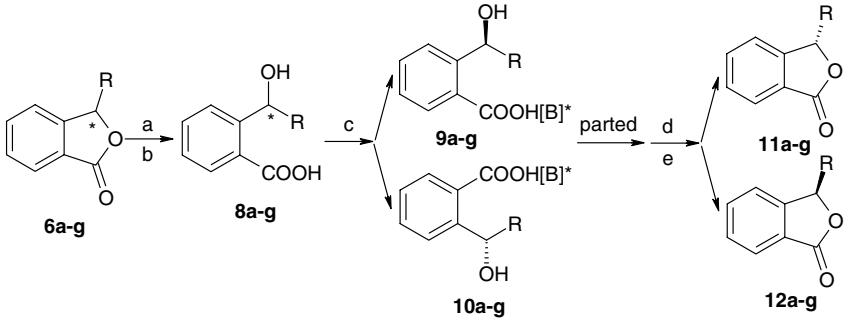
Scheme 1. Reagents and conditions: (a) Mg, ether, 40 °C, reflux, 3 h; (b) $CdCl_2$, ether, 40 °C, reflux, 1 h; (c) phthalic anhydride, ether, 40 °C, 3 h; (d) 3 M HCl; (e) $NaBH_4$, 2–3 h; (f) p-TsOH, benzene, rt, 1.5 h.

Table 1. IE_{50} (μmol L^{-1}) of compounds **6a**–**g** and **7a**–**g** against platelet aggregation

Compound	R	R^1	IE_{50} (μmol L^{-1})	
			Induced by ADP	Induced by AA
6a	C_2H_5		164.09	15.16
6b	C_3H_7		133.26	14.94
6c	C_4H_9		169.96	14.49
6d	C_5H_{11}		180.4	16.37
6e	*i*-C_5H_{11}		201.34	15.39
6f	C_6H_{13}		220.34	15.85
6g	C_7H_{15}		153.13	16.06
7a		CH_3	170.12	15.98
7b		C_2H_5	171.21	16.05
7c		C_3H_7	204.22	16.11
7d		C_4H_9	201.21	17.61
7e		*i*-C_4H_9	204.34	17.88
7f		C_5H_{11}	242.99	17.13
7g		C_6H_{13}	289.47	18.04
Asp			117.94	12.3

Table 2. $[\alpha]_D$ and IE_{50} (μmol L^{-1}) against platelet aggregation of compounds **11a**–**g** and **12a**–**g**

Compound	$[\alpha]_D$/°C		IE_{50} (μmol L^{-1})	
	Measured	Reported	Induced by ADP	Induced by AA
11a	+73.3	+77.3[8]	209.17	16.95
11b	+69.7		142.43	16.92
11c	+69.7		193.06	16.12
11d	+59.7		203.24	17.91
11e	+63.7		207.01	17.22
11f	+62.5		217.34	18.54
11g	+68.4		175.86	18.66
12a	−64.5	−76.0[9]	113.85	14.13
12b	−63.7		111.51	13.81
12c	−64.6	−61.3[10]	94.72	13.25
12d	−59.7		178.3	15.14
12e	−61.4		115.65	14.32
12f	−60.3		138.72	14.36
12g	−65.1		142.86	14.69
Asp			117.94	12.3



Scheme 2. Reagents and conditions: (a) 10 M NaOH, reflux, 1 h; (b) 3 M HCl, −20–0 °C; (c) (−)/(+) α-phenylethylamine, −20–0 °C; filtering after 10 h; (d) 2 M NaOH; (e) 3 M HCl.

The antiplatelet activities of the compounds **6a**–**g**, **7a**–**g**, **11a**–**g**, **12a**–**g** were evaluated in vitro by antiplatelet aggregation induced by ADP and AA. At first, platelet-rich plasma (PRP) was prepared from the whole blood of healthy volunteers. The target-compounds **6a**–**g**, **7a**–**g**, **11a**–**g**, **12a**–**g** and Asp were added to PRP (The concentrations of the target-compounds were 10, 20, 50 and 100 μmol L^{-1}. The concentration of Asp was 5 μmol L^{-1}). PEG-400 was also added to PRP as the control. Then, the mixtures were pre-heated at 37 °C for 5 min. ADP (5μmol L^{-1}) and AA (0.5 μmol L^{-1}) were added subsequently. The most

platelet aggregation rate was determined after 5 min. The compounds **6c** (NBP) and Asp were used for comparison. All the compounds' inhibit-rates of platelet aggregation were calculated. IE_{50} is shown in Tables 1 and 2.

All these compounds showed activity against platelet aggregation when tested at the range between 10 and 100 μmol L^{-1}. The compound **12c** (1-NBP) exhibited the highest activity. The inhibition of platelet aggregation by all the compounds exhibited dose dependence. The magnitude of antiplatelet activity was displayed as following sequence, l-isomer > dl-isomer > d-isomer, respectively. The alkylphthalide is more active than the corresponding alkenephthalide. It appears that, with the increase of substituting-group magnitude, the effects became weaker. All these compounds were less active than NBP and Asp.

In conclusion, a series of chiral 3-alkyl-1(3H)-isobenzofuranone and *Z*-3-alkene-1(3H)-isobenzofuranone were designed and synthesized systematically. The antiplatelet activities of all compounds were screened. These results suggest that the antiplatelet activities of 1(3H)-isobenzofuranone maybe associated with the substituent and configuration of the group on the 3-position.

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11. The reaction is high stereoselectivity, *Z*-isom is the most production.
12. When (−) α-phenylethylamine was added, compounds **9a**–**g** were separated as solid, while compounds **10a**–**g** were dissolved in the solution; when (+) α-phenylethylamine was added, compounds **10a**–**g** were separated as solid, while compounds **9a**–**g** were dissolved in the solution.
13. Analytical data for compounds **6a**–**g** and **7a**–**g** ^{1}H NMR spectra were recorded on a Varian INOVA 400 MHz instrument as solutions ($CDCl_3$) using TMS as internal reference, and chemical shift values are express in δ units. Mass spectra were run on an Applied Biosystems QStar instrument using a direct inlet system under positive ion electrospray ionization source. Compound **6a**: yellow oil (99.8%), yield: 46.7%. ^{1}H NMR: δ 7.90 (1H, d, *J* = 8.0 Hz), 7.68 (1H, t, *J* = 7.2, 8.0 Hz), 7.53 (1H, t, *J* = 7.2, 8.0 Hz), 7.45 (1H, d, *J* = 8.0 Hz), 5.46 (1H, q, J=4.8 Hz), 2.13 (1H, m), 1.83 (1H, m), 1.01 (3H, t). ^{13}C NMR: δ 170.62, 149.70, 133.88, 129.00, 126.34, 125.62, 121.69, 82.25, 27.61, 8.75. MS: *m*/*z* 162 (M^+).
Compound **6b**: yellow oil (99.2%), yield: 43.9%. ^{1}H NMR: δ 7.89 (1H, d, *J* = 7.6 Hz), 7.67 (1H, t, *J* = 7.6, 7.6 Hz), 7.52 (1H, t, *J* = 7.6, 7.6 Hz), 7.44 (1H, d, *J* = 7.6 Hz), 5.48 (1H, q, *J* = 4.0 Hz), 2.02 (1H, m), 1.76 (1H, m), 1.53 (2H, m), 0.98 (3H, t). ^{13}C NMR: δ 170.55, 150.10, 133.85, 128.94, 126.13, 125.62, 121.67, 81.18, 36.78, 18.17, 13.72. MS: *m*/*z* 176 (M^+).
Compound **6c**: yellow oil (99.5%), yield: 42.7%. ^{1}H NMR: δ 7.89 (1H, d, *J* = 7.6 Hz), 7.68 (1H, t, *J* = 7.6, 7.6 Hz), 7.51 (1H, t, *J* = 7.6, 7.6 Hz), 7.44 (1H, d, *J* = 7.6 Hz), 5.48 (1 H, q, *J* = 4.0 Hz), 2.04 (1H, m), 1.75 (1H, m), 1.42 (4H, m), 0.91 (3H, t). ^{13}C NMR: δ 170.62, 150.10, 133.86, 128.96, 126.21, 125.64, 121.68, 81.38, 34.41, 26.83, 22.38, 13.78. MS: *m*/*z* 190 (M^+).
Compound **6d**: yellow oil (98.1%), yield: 42.5%. ^{1}H NMR: δ 7.89 (1H, d, *J* = 7.6 Hz), 7.68 (1H, t, *J* = 7.6, 7.6 Hz), 7.52 (1H, t, *J* = 7.6, 7.6 Hz), 7.44 (1H, d, *J* = 7.6 Hz), 5.48 (1H, q, *J* = 4.0 Hz), 2.04 (1H, m), 1.76 (1H, m), 1.50 (2H, m), 1.34 (4H, m), 0.89 (3H, t). ^{13}C NMR: δ 170.59, 150.13, 133.87, 128.98, 126.21, 125.67, 121.68, 81.41, 34.72, 31.47, 24.45, 22.39, 13.90. MS: *m*/*z* 204(M^+).
Compound **6e**: yellow oil (92.3%), yield: 38.9%. ^{1}H NMR: δ 7.90 (1H, d, *J* = 7.6 Hz), 7.68 (1H, m, *J* = 0.8, 7.6, 7.6 Hz), 7.53 (1H, t, *J* = 7.6, 7.6 Hz), 7.44 (1H, m, *J* = 0.8, 7.6 Hz), 5.47 (1H, q, *J* = 4.0 Hz), 2.06 (1H, m), 1.76 (1H, m), 1.60 (1H, m), 1.35 (2H, m), 0.89 (6H, m). ^{13}C NMR: δ 170.68, 150.06, 133.92, 129.00, 126.18, 125.68, 121.68, 81.62, 33.56, 32.61,27.84, 22.45, 22.29. MS: *m*/*z* 204 (M^+).
Compound **6f**: yellow oil (99.6%), yield: 42.8%. ^{1}H NMR: δ 7.89 (1H, d, *J* = 7.2 Hz), 7.68 (1H, t, *J* = 0.8, 7.2, 7.2 Hz), 7.52 (1H, t, *J* = 7.2, 7.6 Hz), 7.44 (1H, d, *J* = 0.8, 7.6 Hz), 5.48 (1H, q, *J* = 4.0 Hz), 2.04 (1H, m), 1.76 (1H, m), 1.48(2H, m), 1.33 (6H, m), 0.88 (3H, t). ^{13}C NMR: δ 170.56, 150.11, 133.85, 128.94, 126.16, 125.62, 121.67, 81.37, 34.72, 31.52, 28.92, 24.71, 22.45, 13.94. MS: *m*/*z* 218 (M^+).
Compound **6g**: yellow oil (99.8%), yield: 41.6%. ^{1}H NMR: δ 7.90 (1H, d, *J* = 7.6 Hz), 7.68 (1H, t, *J* = 0.8, 7.6, 7.6 Hz), 7.53 (1H, t, *J* = 7.6, 8.0 Hz), 7.43 (1H, d, *J* = 0.8, 8.0 Hz), 5.48 (1H, q, *J* = 4.0 Hz), 2.04 (1H, m), 1.77 (1H, m), 1.37 (10H, m), 0.88 (3H, t). ^{13}C NMR: δ 170.68, 150.12, 133.90, 128.98, 126.13, 125.67, 121.68, 81.44, 34.73, 31.68, 29.27, 29.03, 24.79, 22.58, 14.04. MS: *m*/*z* 232 (M^+).
Compound **7a**: yellow oil (93.3%), yield: 39.8%. ^{1}H NMR: δ 7.48–7.90 (4H, m), 5.68 (1H, q, *J* = 7.6 Hz), 2.03 (3H, m).
Compound **7b**: yellow oil (74.8%), yield: 40.8%. ^{1}H NMR: δ 7.48–7.90 (4H, m), 5.68 (1H, q, *J* = 8.0 Hz), 2.03 (3H, m).
Compound **7c**: yellow oil (90.8%), yield: 39.5%. ^{1}H NMR: δ 7.49–7.90 (4H, m), δ 5.65 (1H, t, *J* = 8.0 Hz), 2.46 (2 H, dt), 1.56 (2H, m), 0.99 (3H, t).
Compound **7d**: yellow oil (81.2%), yield: 36.1%. ^{1}H NMR: δ 7.49–7.90 (4H, m), 5.64 (1H, t, *J* = 8.0 Hz), 2.48 (2H, dt), 1.52 (2H, m), 1.42 (2H, m), 0.94 (3H, t).
Compound **7e**: yellow oil (81.5%), yield: 38.7%. ^{1}H NMR: δ 7.49–7.90 (4H, m), 5.66 (1H, t, *J* = 8.0 Hz), 2.38 (2H, q), 1.82 (1H, m), 0.83–1.00 (2H, m).
Compound **7f**: yellow oil (88.3%), yield: 38.4%. ^{1}H NMR: δ 7.49–7.90 (4H, m), 5.65 (1H, t, *J* = 8.0 Hz), 2.47 (2H, q), 1.38 (2H, m), 1.34–1.38 (4H, m), 0.90 (3H, t).
Compound **7g**: yellow oil (92.8%), yield: 36.9%. ^{1}H NMR: δ 7.50–7.90 (4H, m), 5.65 (1H, t, *J* = 8.0 Hz), 2.47 (2H, q), 1.52 (2H, m), 1.26–1.41 (6H, m), 0.88 (3H, m).

Compound **11a**: yellow oil (99.8%), yield: 32.5%.
Compound **11b**: yellow oil (96.7%), yield: 33.5%.
Compound **11c**: yellow oil (99.8%), yield: 48.9%.
Compound **11d**: yellow oil (99.3%), yield:47.9%.
Compound **11e**: yellow oil (90.5%), yield: 48.7%.
Compound **11f**: yellow oil (99.7%), yield: 49.6%.
Compound **11g**: yellow oil (99.8%), yield: 48.2%.

Compound **12a**: yellow oil (99.8%), yield: 31.9%.
Compound **12b**: yellow oil (99.1%), yield: 32.8%.
Compound **12c**: yellow oil (99.8%), yield: 49.4%.
Compound **12d**: yellow oil (99.8%), yield: 49.1%.
Compound **12e**: yellow oil (92.1%), yield: 48.3%.
Compound **12f**: yellow oil (99.3%), yield: 48.7%.
Compound **12g**: yellow oil (99.7%), yield: 48.5%.