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# Antiplatelet Activity of Synthetic Pyrrolo-Benzylisoquinolines

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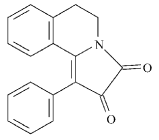
**Abstract**—Pyrrolo-benzylisoquinolines were prepared as target compounds and their antiplatelet aggregation activity, adrenoreceptor affinity, and cytotoxicity were screened. Compounds **1d**–**9d** showed specific antiplatelet aggregation activity induced by arachidonic acid and collagen. Among them, **8d** and **9d** exhibited better activity than the reference drug, aspirin and **9d** also showed inhibition of platelet aggregation by all four inducers.


## Introduction

Benzylisoquinolines, a class of the most commonly isolated alkaloids in higher plants, have been studied for their rich structural and pharmacological diversity.[1–5] They also play an important role in the biosynthetic pathway of morphines and papaverines.[6] Benzylisoquinoline derivatives have been extensively studied for their interaction with various biological functions, such as analgesic,[7,8] muscle relaxation,[9] anticancer activity,[10,11] and cardiovascular activities.[12–15] Among the benzylisoquinoline derivatives, pyrrolo-benzylisoquinolines were prepared as intermediates in the total synthesis of aporphines and protoberberines.[16,17] However, the biological activities of these compounds have never been discussed. In our ongoing research of Annonaceous alkaloids, pyrrolo-benzylisoquinolines, possessing an interesting skeleton, were prepared and their biological activities were studied.



Pyrrolobenzylisoquinoline

## Chemistry

Target compounds **1d**–**9d**[18] were synthesized as illustrated in Scheme 1. Substituted phenylacetic acids **1a**–**9a** were treated with thionyl chloride in dry dichloromethane to form the active acyl chloride and then compound 2-(3′,4′-dimethoxyphenyl)ethylamines were added to yield the amide derivatives **1b**–**9b**. The amides were purified and poured into a mixture of dry acetonitrile and excess phosphoryl chloride. The resulting mixture was refluxed for 3 h to give the products, 3,4-dihydrobenzylisoquinolines **1c**–**9c** via Bischler–Napieralski reaction. Compounds **1c**–**9c** were not purified because of their instability. Diluted oxalyl chloride in $CH_2Cl_2$ was added dropwise to the mixture of crude **1c**–**9c** and $CH_2Cl_2$ under $N_2$ at $-20\,^{\circ}C$. The deep red final products, **1d**–**9d**, were purified and identified by spectral data to confirm the structures.

## Pharmacological Evaluation and Discussion

As we mentioned above, benzylisoquinolines are associated with various pharmacological functions. Based on these surveys, we studied the antiplatelet aggregation activity of compounds **1d**–**9d**. In the platelet aggregation assays, four inducers were employed, including AA (arachidonic acid), Col (collagen), PAF (platelet activating factor), and Thr (thrombin). Table 1 shows the results of the experiments.

All compounds generally showed inhibitory effects on platelet aggregation induced by AA and Col in a concentration-dependent manner. This specific inhibitory effect shows similarity to the reference drugs, aspirin. In comparison of the reference drug with these tested compounds, **8d** and **9d** showed better antiaggregatory

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**Table 1.** Antiplatelet aggregation activity of compounds **1d–9d**[19]

| | $IC_{50}$ (μM)[a,b] | | | |
|---|---|---|---|---|
| | AA (100μM) | COL (10μg/mL) | PAF (2ng/mL) | Thr (0.1U) |
| Aspirin | 44.6±9.8 | 22.4±4.1 | >100 | >100 |
| **1d** | 78.3±3.9 | 44.9±10.8 | >100 | >100 |
| **2d** | 53.8±10.7 | 37.5±5.6 | >100 | >100 |
| **3d** | 52.8±8.6 | 30.0±1.7 | 84.8±8.7 | >100 |
| **4d** | >100 | 39.8±6.1 | >100 | >100 |
| **5d** | 45.5±10.5 | 23.3±3.9 | >100 | >100 |
| **6d** | 72.7±4.3 | 29.8±0.8 | >100 | >100 |
| **7d** | 65.5±4.1 | 35.8±12.7 | >100 | >100 |
| **8d** | 21.0±2.6 | 6.1±1.2 | >100 | >100 |
| **9d** | 15.2±3.8 | 9.15±2.4 | 33.2±1.3 | 30.7±1.1 |

[a]Platelet were preincubated with DMSO (0.5%, control), aspirin or tested compounds at 37 °C for 3 min, then four inducers were added.
[b]The $IC_{50}$ values were presented as means±S.E. ($n=3$).

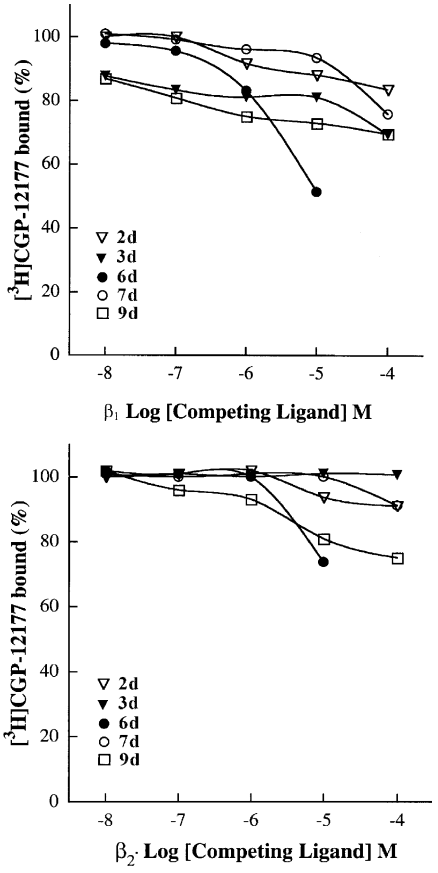
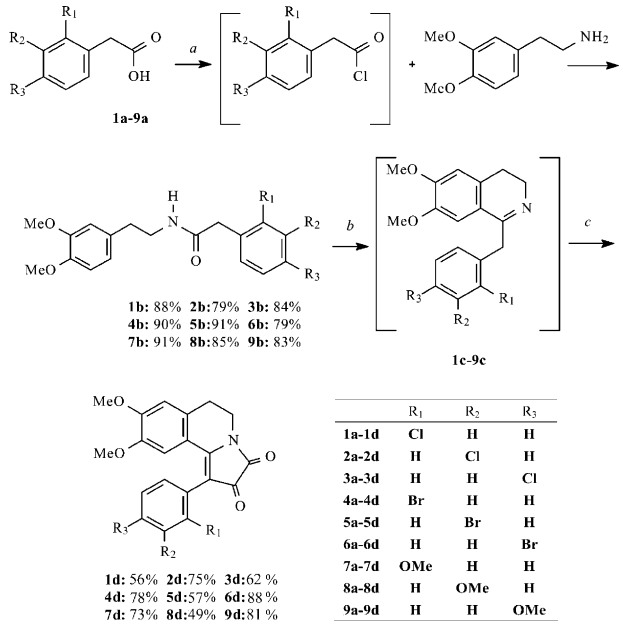



**Figure 1.** Inhibition of [$^3$H]CGP-12177 specific binding to guinea pig $\beta_1$ (ventricular membrane) and $\beta_2$ (lung membrane) adrenoreceptor by **2d**, **3d**, **6d**, **7d**, and **9d**.[20]

activity with $IC_{50}$ 15–21 μM and 6∼9 μM induced by AA and Col, respectively, than aspirin and **5d** demonstrated almost equal potency as aspirin. Compound **9d**, the most potent derivative, showed additional inhibition on platelet aggregation induced by the other two inducers, PAF and Thr. This result revealed that **9d** may act by a different mechanism from other derivatives.

In the structure–activity relationship of these synthesized compounds, the substitution variation at the benzyl group slightly changed the activity: 2′-substituted




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1a-1d | Cl | H | H |
| 2a-2d | H | Cl | H |
| 3a-3d | H | H | Cl |
| 4a-4d | Br | H | H |
| 5a-5d | H | Br | H |
| 6a-6d | H | H | Br |
| 7a-7d | OMe | H | H |
| 8a-8d | H | OMe | H |
| 9a-9d | H | H | OMe |

**Scheme 1.** Synthesis of pyrrolo-benzylisoquinoline derivatives **1d–9d**.

pyrrolobenzylisoquinolines possessed lower antiplatelet activity than 3′ or 4′-substituted ones. The presence of the methoxy group on the benzyl group exhibited better activity than halogenated ones. Compound **9d**, the derivative that fits in with the two conditions showed the best activity among these compounds.

Besides the antiaggregation activity, compounds **2d**, **3d**, **6d**, **7d**, and **9d** were submitted to screen the $\beta_1$ and $\beta_2$ adrenoreceptor binding affinities. However, all of them demonstrate mild affinity to both $\beta_1$ and $\beta_2$ receptors (Fig. 1).

Cytotoxic activity was also evaluated for compounds **1d–9d**.[21] Preliminary results of cytotoxicity toward HONE-1 (human nasopharyngeal carcinoma) and NUGC (human gastric cancer) cell lines revealed that all compounds showed no activity against two cell lines in a concentration of 10 μM-with survival percentage 86–105% and 93–104%, respectively, in comparison with the DMSO vehicle control.

In conclusion, we synthesized nine pyrrolo-benzylisoquinolines that displayed a specific activity toward platelet aggregation and have provided a new active skeleton in the development of antiplatelet aggregation drugs. Compound **9d**, the most potent alkaloid, could be further investigated as the lead compound and the relationship between 3-dimensional structure and activity of these derivatives should be the focus in a continuing study.

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18. **General Experimental Procedure for the synthesis of compounds 1d–9d:** A mixture of **1b–9b** (each 1 mmol) and $POCl_3$ (1.2 mL) in dry MeCN (6mL) was heated at reflux for 4 h. After the reaction was finished, the resulting mixture was diluted with $H_2O$ (40 mL), made basic, and then extracted with $CH_2Cl_2$ (3×50 mL). The $CH_2Cl_2$ was evaporated in vacuo to give viscous yellowish oil, the crude **1c–9c**. The oil was dissolved in a mixture of dry $CH_2Cl_2$ (3 mL) and pyridine (0.2 mL) cooled at −20 °C. Excess oxalyl chloride (0.5 mL) in dry $CH_2Cl_2$ (3 mL) was added dropwise to the mixture. After 5 min, the temperature was raised to 40 °C, and the mixture was stirred for 1 h. The deep red precipitate was collected by filtration and purified by column chromatography. The $^1H$ and $^{13}C$ NMR spectra were recorded at 200 and 50 MHz, respectively, using $CDCl_3$ as solvent. **1d**: $^1H$ NMR: δ 7.42 (1H, m, Ar-H), 7.26 (3H, m, Ar-H), 6.76 and 6.57 (each 1H, s, Ar-H), 3.85 and 3.20 (each 3H, s, 2×OMe), 3.77 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–) and 3.02 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 181.3, 158.3, 158.2, 153.5, 147.7, 134.8, 133.0, 132.4, 129.9, 129.5, 129.4, 127.0, 116.2, 111.0, 110.8, 105.4, 55.9, 54.8, 36.0, 27.9; EI–MS $m/z$: 371 $[M]^+$, 369 $[M-2]^+$; UV: 233, 280(sh), 318 nm; IR (KBr): 1744, 1700 $cm^{-1}$. **2d**: $^1H$ NMR: δ 7.31 (4H, m, Ar-H), 6.96 and 6.77 (each 1H, s, Ar-H), 3.95 and 3.40 (each 3H, s, 2×OMe), 3.83 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–), 3.08 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 181.4, 158.3, 158.2, 153.6, 148.0, 133.0, 132.9, 132.7, 132.3, 129.8, 127.9, 125.7, 116.5, 111.1 (2 signals), 107.7, 56.1, 55.0, 36.2, 28.2; EI–MS $m/z$: 371$[M]^+$, 369 $[M-2]^+$; UV: 260, 288 (sh), 317, 379 nm; IR (KBr): 1740, 1695 $cm^{-1}$. **3d**: $^1H$ NMR: δ 7.39 (2H, d, $J$=8.4 Hz, Ar–H), 7.30 (2H, d, $J$=8.4 Hz, Ar–H), 6.94 and 6.77 (each 1H, s, Ar–H), 3.95 and 3.40 (each 3H, s, 2×OMe), 3.83 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–) and 3.07 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.6, 158.1, 157.5, 153.8, 147.9, 133.7, 133.3, 131.4 (2 signals), 129.0 (2 signals), 128.9, 116.3, 111.8, 111.2, 107.0, 56.2, 55.4, 36.2, 28.5; EI–MS $m/z$: 371$[M]^+$, 369 $[M-2]^+$; UV: 232, 267 (sh), 318 nm; IR (KBr): 1741, 1696 $cm^{-1}$. **4d**: $^1H$ NMR: δ 7.68 (1H, d, $J$=7.8 Hz, Ar-H), 7.26 (3H, m, Ar–H), 6.76 and 6.60 (each 1H, s, Ar–H), 3.90 and 3.25 (each 3H, s, 2×OMe), 3.83 (2H, t, $J$=6.2 Hz, $N$-$CH_2$–), 3.06 (2H, t, $J$=6.2 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.3, 158.5, 157.7, 153.9, 148.0, 134.5, 133.4, 132.3, 130.1, 130.0, 128.4, 127.9, 116.2, 111.9, 111.3, 106.6, 56.3, 55.4, 36.3, 28.6; EI–MS $m/z$: 415 $[M]^+$, 413 $[M-2]^+$; UV: 260, 288(sh), 317, 379 nm; IR (KBr): 1743, 1701 $cm^{-1}$. **5d**: $^1H$ NMR: δ 7.52 (1H, d, $J$=1.2 Hz, Ar–H), 7.46 (1H, m, Ar–H), 7.30 (2H, m, Ar–H), 6.94 and 6.77 (each 1H, s, Ar–H), 3.93 and 3.39 (each 3H, s, 2×OMe), 3.80 (2H, t, $J$=6.2 Hz, $N$-$CH_2$–), 3.06 (2H, t, $J$=6.2 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.2, 157.9, 157.6, 153.8, 147.8, 133.6, 132.6, 130.7, 130.2, 129.1, 122.4, 116.1, 111.7, 111.2, 108.0, 106.6, 56.2, 55.4, 36.2, 27.1; EI–MS: $m/z$: 415 $[M]^+$, 413 $[M-2]^+$; UV: 234 (sh), 262, 284 (sh), 319, 381 nm; IR (KBr): 1742, 1697 $cm^{-1}$. **6d**: $^1H$ NMR: δ 7.53 (2H, d, $J$=8.4 Hz, Ar–H), 7.24 (2H, d, $J$=8.4 Hz, Ar–H), 6.92 and 6.77 (each 1H, s, Ar–H), 3.95 and 3.40 (each 3H, s, 2xOMe), 3.83 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–) and 3.07 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.6, 158.2, 157.6, 153.9, 148.0, 133.4, 132.0 (2 signals), 131.9 (2 signals), 129.5, 122.0, 116.4, 111.9, 111.4, 107.1, 56.4, 55.5, 36.4, 28.7; EI–MS $m/z$: 415 $[M]^+$, 413 $[M-2]^+$; UV: 240, 284 (sh), 322 nm; IR (KBr): 1739, 1697 $cm^{-1}$. **7d**: $^1H$ NMR: δ 7.33 (1H, td, $J$=7.8, 1.8 Hz, Ar-H), 7.22 (1H, dd, $J$=7.8, 1.8 Hz, Ar–H), 7.02 (1H, brt, $J$=7.6 Hz, Ar–H), 6.99 (1H, brd, $J$=7.6 Hz, Ar–H), 6.86 and 6.73 (each 1H, s, Ar–H), 3.93, 3.68, and 3.30 (each 3H, s, 3×OMe), 3.84 (2H, t, $J$=6.2 Hz, $N$-$CH_2$–), 3.07 (2H, t, $J$=6.2 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.9, 158.7, 157.7 (2 signals), 153.1, 147.8, 132.3, 132.1, 129.8, 121.1, 119.5, 117.4, 111.5, 111.3, 110.9, 105.0, 56.1, 55.6, 55.2, 36.3, 29.4; EI–MS $m/z$: 365 $[M]^+$; UV: 256, 284 (sh), 324 (sh), 336 nm; IR (KBr): 1742, 1697 $cm^{-1}$. **8d**: $^1H$ NMR: δ 7.27 (1H, m, Ar–H), 6.96 and 6.74 (each 1H, s, Ar–H), 6.84 (3H, m, Ar–H), 3.91, 3.73 and 3.21 (each 3H, s, 3×OMe), 3.77 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–), 3.04 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 182.6, 159.8, 158.1, 157.3, 153.4, 147.6, 133.0, 131.6, 129.7, 122.3, 116.4, 115.4, 113.5, 112.0, 111.1, 108.1, 56.1, 55.2 (2 signals), 36.1, 28.3; EI–MS $m/z$: 365 $[M]^+$; UV: 256, 330 (sh), 336 nm; IR (KBr): 1739, 1695 $cm^{-1}$. **9d**: $^1H$ NMR: δ 7.26 and 6.93 (each 2H, d, $J$=8.4 Hz, Ar–H), 7.03 and 6.75 (each 1H, s, Ar–H), 3.95, 3.80 and 3.38 (each 3H, s, 3×OMe), 3.80 (2H, t, $J$=5.8 Hz, $N$-$CH_2$–), 3.07 (2H, t, $J$=5.8 Hz, Ar-$CH_2$–); $^{13}C$ NMR: δ 183.1, 159.0, 158.0, 156.7, 153.1, 147.4, 132.8, 131.1 (2 signals), 122.1, 116.4, 114.0 (2 signals), 111.6, 111.0, 107.7, 56.0, 55.1 (2 signals), 36.0, 28.2; EI–MS $m/z$: 365$[M]^+$; UV: 229, 281, 325 (sh) nm; IR (KBr): 1744, 1696 $cm^{-1}$.
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21. (a) Cytotoxicity assay: Cancer cells were seeded in 96-well microtiter plates at a density of 6000/well in 100 μL culture medium. After an overnight adaptation period, 10 μM/mL (final concentration) of test compounds in serium-free medium were added to individual wells. Cells were treated with test compounds for three days. Cell viability was determined by the 5-(3-carbonylmethoxyphenyl)-2-(4,5-dimethylthazolyl)-3-(4-silfophenyl)tetrazolium salt (MTS) reduction. Actinomycin D 5μM (final concentration) and DMSO 0.1% (final concentration) were used as positive and vehicle controls. Results were expressed as percent of DMSO control. For details of the assay protocols, see: Gieni, R. S.; Li, Y.; HayGlass, K. T. *J. Immunol. Meth.* **1995**, *187*, 85. (b) Malich, G.; Markovic, B.; Winder, C. *Toxicology* **1997**, *124*, 179.