Effect of Isoquinoline Alkaloids of Different Structural Types on Antiplatelet Aggregation *in Vitro*

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Abstract

Forty-one isoquinoline alkaloids were tested for antiplatelet aggregation effects. Among them, (–)-discretamine (**6**), protopine (**7**), ochotensimine (**18**), *O*-methylarmepavinemethine (**23**), lindoldhamine (**25**), isotetrandrine (**26**), thalicarpine (**27**), papaverine (**28**), and D-(+)-*N*-norarmepavine (**32**) exhibited significant inhibitory activity towards adenosine 5′-diphosphate (ADP)-, arachidonic acid (AA)-, collagen-, and/or platelet-activating factor (PAF)-induced platelet aggregation. The results are discussed on the basis of structure-activity relationships.

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Platelets play an important role in the haemostatic process, and their aggregation can cause arterial thrombosis. Accordingly, compounds with antiplatelet aggregation activity can be useful therapeutic agents. Isoquinoline alkaloids were well known to display numerous biological activities [1], [2], [3]. Therefore, we evaluated various isoquinoline alkaloids in antiplatelet aggregation assays and, in our past report, described the target activity of thirty-seven aporphine alkaloids [4], [5]. In our continuing investigation on the activities of different types of isoquinoline alkaloids, we chose and tested forty-one compounds of different structural types, including six protoberberines: berberine chloride (**1**) [6], berberine iodine (**2**) [7], jatrorrhizine picrate (**3**) [8], (±)-tetrahydroberberine (**4**) [8], (–)-tetrahydropalmatine (**5**) [9], and (–)-discretamine (**6**) [10]; three protopines: protopine (**7**) [11], protopine *N*-oxide (**8**) [1], and α-allocryptopine (**9**) [12]; eight pavines: pavine (**10**) [13], *N*-methylpavine (**11**) [13], L-caryachine (**12**) [14], dl-caryachine (**13**) [14], d-*O*-methylcaryachine HCl (**14**) [14], (+)-*O*-methylcaryachine *N*-oxide (**15**) [1], crychine

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Table **1** Effects of test compounds on the platelet aggregation induced by ADP, AA, collagen and PAF in washed rabbit platelets [a]

Compound[b]	*Aggregation (%)*			
	ADP (20 μM)	*AA (100 μM)*	*Collagen (10 μg/mL)*	*PAF (2 ng/mL)*
1	69.2 ± 3.0*	84.7 ± 2.5	85.6 ± 0.7	73.2 ± 4.3*
2	90.2 ± 4.5	80.7 ± 2.9**	83.3 ± 3.6	91.2 ± 0.5
3	89.6 ± 1.1	86.6 ± 0.8	92.4 ± 1.2	87.5 ± 2.4
4	85.6 ± 1.2*	88.8 ± 1.3	82.0 ± 0.7**	91.4 ± 1.4
5	91.7 ± 0.8	89.0 ± 0.9	78.0 ± 6.9	90.0 ± 1.9
6	65.0 ± 4.3**	0.0 ± 0.0***	76.4 ± 8.4	84.3 ± 2.8
7	83.6 ± 0.5***	48.5 ± 0.7*	0.0 ± 0.0***	0.0 ± 0.0***
8 (25 μM)	81.8 ± 1.1	88.0 ± 0.7	89.5 ± 2.4	77.6 ± 10.7
9	86.3 ± 0.5*	87.6 ± 1.5	80.5 ± 1.7**	77.6 ± 0.4***
10	90.2 ± 1.6	80.2 ± 1.8**	74.9 ± 5.8	80.6 ± 4.6
11	86.2 ± 2.2	87.6 ± 1.7	78.3 ± 3.5*	73.5 ± 3.3***
12	88.5 ± 1.0	70.8 ± 4.9**	53.8 ± 5.3***	85.3 ± 1.8**
13	88.4 ± 2.4	67.6 ± 2.3**	31.7 ± 16.9***	84.8 ± 2.3
14	78.2 ± 2.0	87.6 ±1.9	87.7 ± 1.1	87.5 ± 1.4
15	65.7 ± 4.1**	85.8 ± 1.1	89.7 ± 0.9	90.8 ± 0.9
16	76.5 ± 1.1	93.2 ± 0.5**	87.2 ± 2.0	83.7 ± 4.7
17	73.3 ± 1.7	86.0 ± 0.8	89.3 ± 2.4	87.9 ± 0.6
18	85.4 ± 2.9	81.9 ± 1.9	71.8 ± 2.8***	0.0 ± 0.0***
19	79.3 ± 3.4	89.3 ± 1.8	87.9 ± 1.3	87.9 ± 1.1
20	91.6 ± 1.9	84.4 ± 3.2	61.8 ± 7.0*	87.0 ± 0.8
21 (50 μM)	90.0 ± 0.5	63.2 ± 14.8	85.1 ± 4.2	80.3 ± 3.6*
22	51.0 ± 7.2***	59.9 ± 6.7*	9.5 ± 8.2***	13.8 ± 6.2***
23	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
24 (50 μM)	67.2 ± 6.5***	26.2 ± 3.3***	37.0 ± 7.9***	22.6 ± 6.1***
25	30.2 ± 10.9***	3.2 ± 1.7***	0.0 ± 0.0***	0.0 ± 0.0***
26	90.2 ± 1.3	0.0 ± 0.0***	0.0 ± 0.0***	79.8 ± 2.0***
27	78.5 ± 2.2***	35.2 ± 4.7***	2.8 ± 2.3***	34.9 ± 3.0***
28	10.1 ± 3.0***	0.0 ± 0.0***	7.6 ± 4.6***	0.0 ± 0.0***
29 (25 μM)	18.7 ± 7.1***	25.3 ± 12.4***	42.0 ± 8.6***	75.5 ± 13.8
30	90.2 ± 1.5	62.4 ± 8.3**	44.4 ± 17.9*	82.2 ± 2.9
31	91.2 ± 1.9	67.3 ± 8.1**	53.2 ± 16.2*	82.1 ± 2.6
32	91.6 ± 1.4	50.8 ± 15.7*	0.0 ± 0.0***	71.1 ± 4.4**
33	91.6 ± 0.7	84.4 ± 0.8	78.9 ± 3.6**	92.9 ± 1.0
34	89.2 ± 0.6	85.9 ± 0.8	79.8 ± 6.6	81.8 ± 2.7*
35	93.5 ± 0.7	86.8 ± 3.0	86.5 ± 1.4	74.6 ± 2.9**
36	90.3 ± 2.1	70.2 ± 4.1***	27.6 ± 10.8***	70.8 ± 8.0
37	92.4 ± 3.0	81.5 ± 3.3	86.3 ± 0.4	88.5 ± 1.8
38	90.0 ± 1.7	67.1 ± 7.8*	53.0 ± 14.6*	85.9 ± 2.6
39	93.4 ± 1.7	88.1 ± 1.1	89.5 ± 3.8	91.0 ± 1.3
40	88.1 ± 0.7**	56.0 ± 8.7***	84.4 ± 1.5*	84.9 ± 0.7***
41	53.5 ± 11.0*	79.8 ± 5.5	77.3 ± 9.8	85.5 ± 4.3
Aspirin	77.9 ± 1.9	0.0 ± 0.0***	87.8 ± 1.5	90.4 ± 1.1
Control	92.9 ±0.3	88.4 ±1.1	88.5 ± 0.4	90.5 ± 1.1

[a] Platelets were preincubated with DMSO (0.5%, control), aspirin or test compounds at 37 °C for 3 min, then ADP (20 μM), AA (100 μM), collagen (10 μg/mL) or PAF (3.6 nM) was added. Percentage of aggregation are presented as means ± S.E.(n = 3–5). * P < 0.05, ** P < 0.01, *** P < 0.001 as compared with the respective control.

[b] The concentration of each test compound was 100 μM, aspirin was 25 μM.

picrate (**16**) [14], and (–)-crychine *N*-oxide (**17**) [1]; four spirobenzylisoquinolines: ochotensimine (**18**) [15], dihydroochotensimine (**19**) [16], yenhusomidine (**20**) [15], and yenhusomine (**21**) [15]; three stilbene alkaloids: *O*-methylarmepavinemethine MeI (**22**) [17], *O*-methylarmepavinemethine (**23**) [3], and *O*,*O*-diethylcoclaurine methine (**24**) [18]; three bisbenzylisoquinolines: lindoldhamine (**25**) [19], isotetrandrine (**26**) [8], and thalicarpine (**27**) [20]; nine benzylisoquinolines: papaverine (**28**) [16], papaverine *N*-oxide HCl (**29**) [21], (±)-armepavine (**30**) [2], D-(–)-armepavine (**31**) [22], d-(+)-*N*-norarmepavine (**32**) [22], L-(–)-*N*-norarmepavine (**33**) [17], *N*,*O*-dimethyl-*N*-norarmepavine MeI (**34**) [22], *O*,*O*,*N*-trimethylcoclaurine MeI (**35**) [18], and (+)-laudanosine (**36**) [23]; three proaporphines: litsericine (**37**) [24], *N*-methyllitsericine (**38**) [24], and *N*-methyllitsericine HBr (**39**)

[24], as well as adlumidine (**40**) [15] and (+)-*O*-methylflavinandine (**41**) [25], which were isolated from Formosan plants or were semi-synthetic derivatives.

These alkaloids were studied for their effects on the aggregation of washed rabbit platelets as induced by adenosine 5′-diphosphate (ADP, 20 μM), arachidonic acid (AA, 100 μM), collagen (10 μg/mL), and platelet-activating factor (PAF, 2 ng/mL).

As shown in Table **1**, (–)-discretamine (**6**) showed complete inhibition of AA-induced platelet aggregation, while the other protoberberines **1 – 5** were inactive. The protoberberine **6** possesses a completely different structural skeleton from acetylsalicylic acid (ASA), but it exhibits ASA-like activity to prevent AA-induced platelet aggregation [26]. Thus, the precise mechanism of action still needs to be explored. The phenolic moieties may play an important role in activity, because when the C-3 and C-10 hydroxy groups of **6** were changed to two methoxy groups in **5**, the antiplatelet effects were eliminated.

Protopine **7**, which contains a methylenedioxy group at C-9 and C-10, completely inhibited collagen- and PAF-induced platelet aggregation, and significantly prevented that by induced AA. Modification of the methylenedioxy functionality to two methoxy groups as in **9** totally abolished the antiplatelet activities. Thus, we suggest that the C-9,10 methylenedioxy group is critical to the action of protopines.

As shown in the assay results for pavine alkaloids **10 – 17**, only **13** exhibited significant inhibition of platelet aggregation induced by collagen. If the hydroxy group at C-8 of **13** was converted to a methoxy group as in **14**, no antiplatelet effects were seen.

We also assayed the unusual spirobenzylisoquinolines **18 – 21**. Interestingly, compound **18**, which possesses an *exo*-methylene group at C-13, demonstrated excellent activity against platelet aggregation induced by PAF. Converting the methylene group of **18** to the methyl group of **19** eliminated the antiplatelet activity.

The three stilbene alkaloids **22 – 24** were effective against platelet aggregation induced by ADP, AA, collagen, and PAF. Among them, the most potent compound **23** completely inhibited platelet aggregation induced by all four activators. By changing the stilbene-type base to the methyl iodide salt (**22**) or converting the C-5,4′-dimethoxy groups to C-5,4′-diethoxy groups (**24**), the antiplatelet effects were reduced.

Among the series of bisbenzylisoquinoline alkaloids **25 – 27**, compound **25** completely inhibited platelet aggregations induced by AA, collagen and PAF, and significantly inhibited that by ADP. On the other hand, **26** completely inhibited platelet aggregation induced by only AA and collagen, while **27** partially inhibited collagen-, AA- and PAF-induced platelet aggregation.

Furthermore, in the class of the benzylisoquinoline alkaloids **28 – 36**, compound **28** showed a wide range of antiplatelet aggregation effects. Another active member of this class is **29**, which showed strong inhibition of both ADP- and AA-induced aggregation, and significant inhibition of collagen-induced platelet aggregation. In addition, platelet aggregation induced by collagen was completely inhibited by **32,** strongly inhibited by **36**, and significantly inhibited by **30.** The other members of this class were inactive. Two interesting SAR observations were made. Benzylisoquinoline alkaloid **28**, which has four methoxy groups at C-6, 7, 3′, 4′, showed strong inhibition of platelet aggregation; however, modification to the *N*-methylbenzyltetrahydroisoquinoline **36** reduced the antiplatelet effects. Also saturation of the benzylisoquinoline B ring sharply reduced the antiplatelet activities.

The proaporphines **37 – 39** as well as the two miscellaneous alkaloids **40** and **41** did not exhibit any significant activity.

Among all structural types investigated, some compounds, e. g., **23** and **28**, exhibited potent antiplatelet activities against aggregation induced by all four activators, while others, such as **18** and **32**, demonstrated highly selective inhibition toward specific targets. The mechanism(s) of antiplatelet actions of those potent compounds is(are) not clear at this time. As also observed in the previous results [4], [5], in general, a tiny change in the structure of different sub-types of isoquinolines will cause significant changes in anti-platelet aggregation activity.

Materials and Methods

Compounds: Alkaloids **1 – 41** were isolated or semi-synthesized from various Formosan plants in our past investigation. All references were cited. The preparation of compounds **34** and **35** has been detailed in the Supporting Information.

Assay methods for platelet aggregation: The assay protocol is detailed in the Supporting Information.

Data analysis: The experimental results are expressed as means ± S.E. and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparisons, and if there was significant variation between treatment groups, then the mean values for inhibitors were compared with those for control by Student′s *t* test, and P values of less than 0.05 were considered to be statistically significant.

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