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Design, synthesis, and inhibition of platelet aggregation for some 1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline derivatives☆

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Abstract—Based on ticlopidine active as an ADP receptor antagonist for inhibiting platelet aggregation in clinical test, and upon finding (±)-1,2-substituted-7-sulfonylamide/amide-1,2,3,4-tetrahydroisoquinoline (**11**–**31**) inhibited of platelet aggregation, a series of (±)-1-*o*-chlorophenyl-2-substituted-tetrahydroisoquinoline derivatives was designed and synthesized. Four analogs proved to be potential antiplatelet aggregation agents, and compound **9** (TQP-3, applying for patent) which inhibits ADP-induced human platelet aggregation with IC_{50} values of approximately 0.206 nM was the most active. Compound **2** is more active than compound **1**, which (Type I) is similar to ticlopidine. This is because there is a spacial hindrance in compound **1**, and the *o*-chloro group of compound **2** may play the same a role as *o*-chloro group of ticlopidine. On the other hand, with the different substitutions at different positions on the 2-substituted phenylacyl group, their inhibition of platelet aggregation differs. These compounds with *m*-substituted group (**5**, **7**, **9**) showed a higher IC_{50} value for inhibiting ADP-induced human platelet aggregation than those with *o*-substituted group (**4**, **6**) or *p*-substituted group (**3**, **8**). It was observed that their inhibition is bromine-substituted derivative (**9**), chlorine-substituted derivative (**7**), and nitro-substituted derivative (**5**) in turn. Moreover, these compounds (Type II) may be more similar to clopidogrel than to ticlopidine due to the acyl group at 2 position of the nucleus playing a role as the ester group of clopidogrel. It was conjectured that these analogs function as a potential antiplatelet aggregation role by acting as ADP receptor antagonists.

1. Introduction

Cardiovascular and cerebrovascular diseases are still the main cause of morbidity and mortality in the world. The formation of platelet aggregates is an important pathogenetic factor in widespread cardiovascular disease. The sudden occlusion of an arterial vessel by formation of a thrombotic plug is the crucial event leading to deficient oxygen supply of important target organs like the heart or the brain. Human blood platelets play a significant role not only in normal hemostasis but also in arterial thrombosis, particularly under conditions of high shear stress.[1] Currently used antiplatelet drugs, including aspirin, ticlopidine, and others, are effective against certain but not all of the many endogenous platelet activators. Because of their limited efficacy, a significant number of serious thromboembolic complications still occur, highlighting the need for a more effective therapy. Fibrinogen interactions with vascular endothelial cells are implicated in various physiological and pathophysiological events, including angiogenesis and wound healing.[2] Fibrinogen has been identified in many prospective clinical studies as an independent risk factor for arterial, peripheral, and cerebrovascular events, which include stroke, myocardial infarction, peripheral circulatory deficiencies, angina, intermittent claudication, and deep vein thrombosis.[3] The binding of the glycoprotein IIb–IIIa complex (GP IIb–IIIa or integrin $\alpha_{IIb}\beta_3$) to the symmetrical molecules fibrinogen and/or vWF in solution was shown to be essential for platelet aggregation and thrombus growth. In addition, a variety of other platelet activators, including adenosine diphosphate (ADP), collagen, epinephrine, thromboxane A_2, platelet-activating factor (PAF), serotonin or thrombin, might be involved in the physiological process of platelet stimulation leading finally to the functionalization of GP IIb–IIIa and to platelet aggregation. Therefore, the blockade of GP IIb–IIIa receptor might constitute a superior approach in

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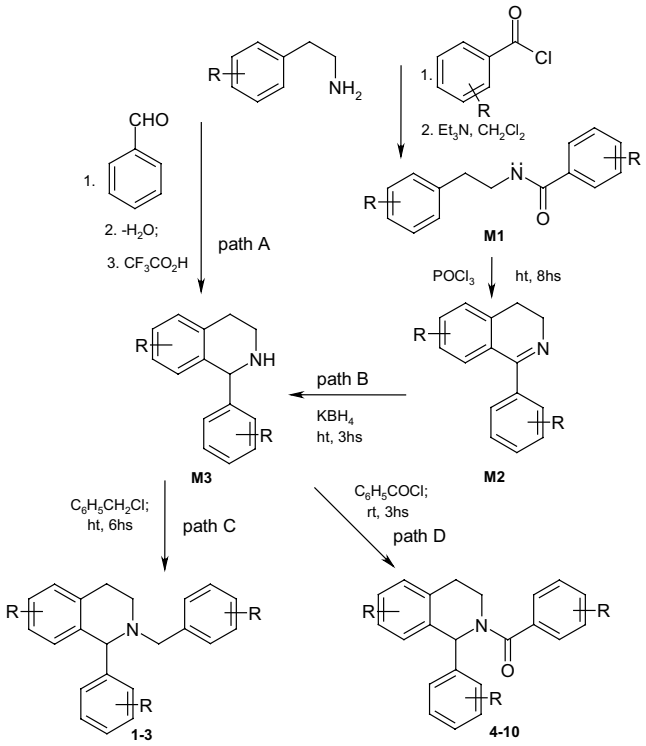
effectively preventing arterial thrombus formation.[1,3] There are some GP IIb–IIIa receptor antagonists, such as a systematically active peptide analog (DMP 728) of RGD motif,[4] decorsin of 39-residue RGD–protein,[5–8] ornatin,[6,7,9] a nonpeptide antagonist XR300, an ethyl ester prodrug of XR299.[10]

Ticlopidine, an antiplatelet drug with a broad scope of clinical applications, is claimed to be an antagonist of adenosine diphosphate on platelet receptors. Thienopyridine compounds, including ticlopidine and clopidogrel, have been found to selectively inhibit adenosine 5′ diphosphate (ADP)-induced platelet aggregation and adenylyl cyclase ex vivo, but the mechanism of their antiplatelet action remains to be determined.[11] The therapeutic efficacy of ticlopidine might be associated not only with its delayed antiplatelet effects but also with its immediate thrombolytic action, which is likely to be mediated by endothelial prostacyclin and tissue plasminogen activator rather than by platelet mechanisms.[12,13] Like ticlopidine, the ADP receptor antagonist clopidogrel is inactive in vitro and must be administered i.v. or orally to exhibit antiaggregatory and antithrombotic activities. The irreversible modification of the ADP receptor site, which is responsible for the biological activity could be explained by the formation of a disulfide bridge between the reactive thiol group of the active metabolite and a cysteine residue of the platelet ADP receptor.[14,15] Platelet-dependent occlusive thrombosis at sites of deep vessel wall injury elicited by electrical stimulation of rat carotid arteries was significantly reduced by thromboxane A2 (TXA2) synthetase inhibition and/or TXA2/prostaglandin endoperoxide receptor antagonism (ridogrel, dazoxiben, sulotroban), by inhibition of ADP-dependent platelet responses (ticlopidine) and by anticoagulation (heparin, warfarin). This points to an involvement of arachidonic acid metabolites, ADP, and thrombin as modulators of the thrombotic process. The antithrombotic effect of ridogrel was abolished by cyclooxygenase inhibition but enhanced by *c*AMP phosphodiesterase inhibition, demonstrating the importance of platelet inhibitory prostanoids such as PGD2, and prostacyclin formed after TXA2 synthetase inhibition. The antithrombotic effect of ridogrel, when combined with ticlopidine or heparin, exceeded that of the single compounds, pointing to interactions between arachidonic acid metabolites, ADP, and thrombin in the formation of occlusive thrombosis at sites of arterial injury (Fig. 1).[16–19]

Here, on the basis of the principles of isosterism and rational drug design, and in view of the structural features of ticlopidine, clopidogrel, *R*-(+)-trimetoquinol and some tetrahydroisoquinoline alkaloids possessing antiplatelet aggregation activities, as well as some synthesized antiplatelet aggregating agents (±)-1-phenyl-2-substituted-7-sulfonylamide/amide-1,2,3,4-tetrahydroisoquinoline (**11**–**31**, Table 2), a series of 1-*o*-chlorophenyl-2-substituted-1,2,3,4-tetrahydroisoquinoline derivatives were designed and synthesized in order to search for new type of platelet antiaggregants.



Scheme 1.

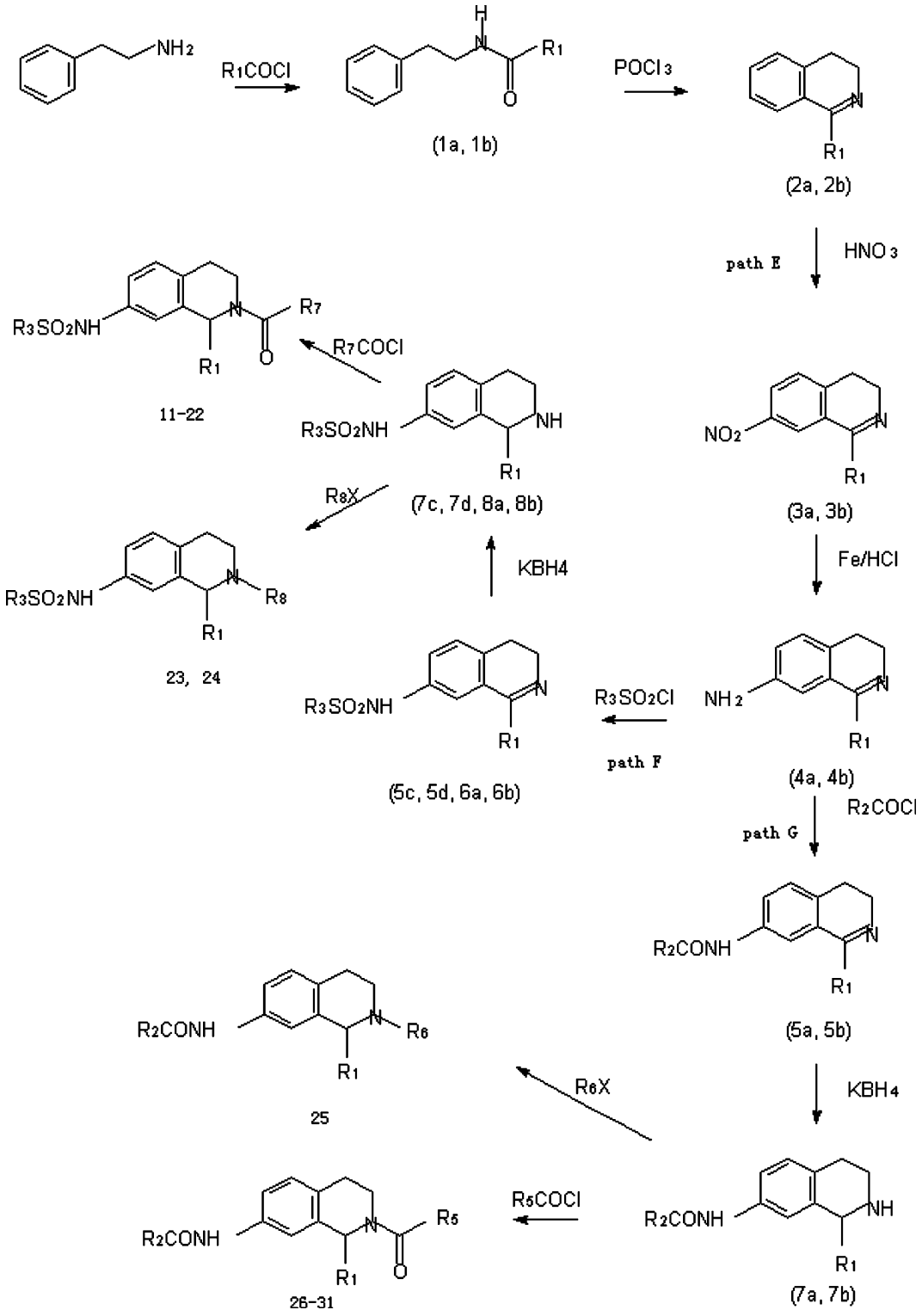
2. Chemistry

Two synthetic schemes were used to make the 2-unsubstituted tetrahydroisoquinolines. If the phenethylamine had a substituent, which activated the position *ortho* to the ethylamine side chain, such as methoxy group, the Pictet–Spengler reaction (Scheme 1, path A) was used. Trifluoroacetic acid proved to be a much better cyclization catalyst than the usual acids used in this reaction. When there was no activation *ortho* to the ethylamine, the Bischler–Napieralski reaction[20,21] (Scheme 1, path B) was used. The representative synthesis of 10 tetrahydroisoquinoline derivatives (**1**–**10**) were prepared by Scheme 1, path C or path D. The 21 tetrahydroisoquinoline derivatives (**11**–**31**) were prepared by Scheme 2, path F or path G. Table 1 lists 10 analogs of 1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline (**M3**) while Table 2 lists 21 tetrahydroisoquinoline derivatives with different substitutes at position 1, 2, and 7 of the tetrahydroisoquinoline nucleus used to make the various analogs. Table 3 lists the structural and spectrum parameters of 21 tetrahydroisoquinoline derivatives (**11**–**31**).

3. Results and discussion

3.1. Antiplatelet aggregation activity in vitro

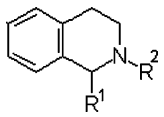
The effects of 10 tetrahydroisoquinoline derivatives upon ADP-induced (121.0 μmol L^{-1}, 20 μL) platelet aggregation in human platelet-rich plasma (PRP) were evaluated by a turbidimetric method. Inhibitory



Scheme 2.

concentrations (IC_{50}) of these compounds for antiplatelet aggregation measured with human platelet-rich plasma (PRP) are listed in Table 1. It seemed to reveal that four derivatives (**2**, **5**, **7**, **9**) showed platelet aggregation inhibitory activities with a range of potencies. Concentrations of these compounds as low as 1.0 μM inhibited human platelet aggregation induced by 121.0 μM ADP. The inhibitory concentration (IC_{50}) values for 2, 5, 7, and 9 were 60.60 nM, 2.080 μM, 54.21, and 0.206 nM, respectively. Furthermore, human platelet aggregation induced by ADP was inhibited by TQP-3 (**9**) with an IC_{50} of approximately 0.206 nM; complete inhibition of aggregation was observed at a concentration of 1 μM. Further evaluation is under investigation.

Table 1. Information concerning seven new compounds



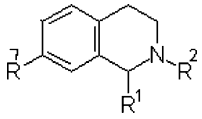
No.	Type	Substituent at 1	Substituent at 2	Procedure	Yield (%)	Mp (°C), solvent	Formula, MW	IC_{50} (μM)* (nmol/L)
1	I	*o*-C_6H_5Cl	*o*-Cl–$C_6H_5CH_2$	C	12.28	168–169, Ethanol	$C_{22}H_{19}NCl_2$, 368.30	[a]
2, TQP-1	I	*o*-C_6H_5Cl	$C_6H_5CH_2$	C	15.90	118–119, Ethanol	$C_{22}H_{20}NCl$, 333.90	60.60 ± 25.23
3	I	*o*-C_6H_5Cl	*p*-NO_2–$C_6H_5CH_2$	C	51.77	104–106, Ethanol	$C_{21}H_{19}N_2O_2Cl$, 378.857	>10 μM
4	II	*o*-C_6H_5Cl	*o*-NO_2–C_6H_5CO	D	47.02	143–145, Ethanol	$C_{22}H_{17}N_2O_3Cl$, 392.841	>10 μM
5	II	*o*-C_6H_5Cl	*m*-NO_2–C_6H_5CO	D	41.94	115–117, Ethanol	$C_{22}H_{17}N_2O_3Cl$, 392.841	2.080 μM
6	II	*o*-C_6H_5Cl	*o*-Cl–C_6H_5CO	D	15.58	178–180, Ethanol	$C_{22}H_{17}NOCl_2$, 382.30	[b]
7, TQP-2	II	*o*-C_6H_5Cl	*m*-Cl–C_6H_5CO	D	11.23	160–162, Ethanol	$C_{22}H_{17}NOCl_2$, 382.30	54.21 ± 18.72
8	II	*o*-C_6H_5Cl	*p*-Cl–C_6H_5CO	D	14.68	174–176, Ethanol	$C_{22}H_{17}NOCl_2$, 382.30	[c]
9, TQP-3	II	*o*-C_6H_5Cl	*m*-Br–C_6H_5CO	D	11.06	120–122, Ethanol	$C_{22}H_{17}NOBrCl$, 426.70	0.206 ± 0.033
10	II	*o*-C_6H_5Cl	$C_6H_5SO_2$	D	12.44	98–100, Ethanol	$C_{21}H_{18}NO_2SCl$, 383.90	[c]
Aspirin								9.673 ± 3.285

Note: The inhibitory concentrations (IC_{50}) of these compounds for antiplatelet aggregation were evaluated by a turbidimetric method based on ADP-induced (2.0 μM, 5 μL) platelet aggregation in rabbit platelet-rich plasma (PRP).

[a] The compound at 0.882 μmol/L possesses antiplatelet aggregation activity with aggregation inhibition rate of 26.02% while at 0.0882 μmol/L shows accelerating platelet aggregation.

[b–d] The compound at approximately 10^{-6} mol/L shows accelerating platelet aggregation.

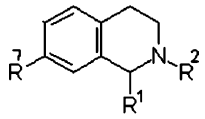
Table 2. Information concerning 21 new compounds



No.	R^7	R^1	R^2	Path	Yield (%)	Mp (°C), solvent	Formula, MW	IC_{50} (μM)*
11	CH_3SO_2NH	C_6H_5	*o*-NO_2–C_6H_5CO	F	42.39	208–210, Acetone	$C_{23}H_{21}N_3O_5S$, 451.427	0.0229
12	CH_3SO_2NH	C_6H_5	*p*-NO_2–C_6H_5CO	F	38.29	135–137, Acetone	$C_{23}H_{21}N_3O_5S$, 451.427	0.0763
13	CH_3SO_2NH	C_6H_5	CH_3CH_2OCO	F	38.63	145–146, Acetone	$C_{19}H_{22}N_2O_4S$, 374.454	0.543
14	CH_3SO_2NH	*p*-C_6H_5Cl	*o*-NO_2–C_6H_5CO	F	43.53	130–132, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	0.428
15	CH_3SO_2NH	*p*-C_6H_5Cl	*p*-NO_2–C_6H_5CO	F	61.34	147–149, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	0.171
16	CH_3SO_2NH	*p*-C_6H_5Cl	*m*-NO_2–C_6H_5CO	F	55.44	121–123, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	0.199
17	CH_3SO_2NH	*p*-C_6H_5Cl	CH_3CH_2OCO	F	46.19	148–150, Acetone	$C_{19}H_{21}N_2O_4SCl$, 408.954	2.040
18	$C_6H_5SO_2NH$	C_6H_5	*m*-NO_2–C_6H_5CO	F	33.18	149–151, Acetone	$C_{28}H_{23}N_3O_5S$, 513.544	0.156
19	$C_6H_5SO_2NH$	C_6H_5	*p*-NO_2–C_6H_5CO	F	43.34	225–227, Acetone	$C_{28}H_{23}N_3O_5S$, 513.544	>10
20	$C_6H_5SO_2NH$	C_6H_5	3,5-$(NO_2)_2$–C_6H_5CO	F	40.51	>250, Acetone	$C_{28}H_{22}N_4O_7S$, 558.554	>10
21	$C_6H_5SO_2NH$	*p*-C_6H_5Cl	*o*-NO_2–C_6H_5CO	F	49.34	185–187, Acetone	$C_{28}H_{22}N_3O_5SCl$, 548.012	>10
22	$C_6H_5SO_2NH$	C_6H_5	CH_3CH_2OCO	F	26.76	125–127, Acetone	$C_{24}H_{24}N_2O_4S$, 436.514	>10
23	CH_3SO_2NH	C_6H_5	$CH_3CH_2OCOCH_2$	F	26.53	153–155, Acetone	$C_{20}H_{24}N_2O_4S$, 388.474	1.710
24	$C_6H_5SO_2NH$	C_6H_5	$CH_3CH_2OCOCH_2$	F	20.49	122–124, Acetone	$C_{25}H_{26}N_2O_4S$, 450.544	2.287
25	*p*-NO_2–C_6H_5CONH	C_6H_5	CH_3CH_2OCO	G	20.10	107–109, Ethanol	$C_{25}H_{23}N_3O_5$, 455.45	1.500
26	*o*-NO_2–C_6H_5CONH	C_6H_5	*o*-NO_2–C_6H_5CO	G	35.56	257–259, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	0.730
27	*o*-NO_2–C_6H_5CONH	C_6H_5	*p*-NO_2–C_6H_5CO	G	48.29	111–113, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	1.720
28	*o*-NO_2–C_6H_5CONH	C_6H_5	*m*-NO_2–C_6H_5CO	G	29.53	145–147, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	2.420
29	*p*-NO_2–C_6H_5CONH	C_6H_5	*p*-NO_2–C_6H_5CO	G	58.56	224–226, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	>10
30	*p*-NO_2–C_6H_5CONH	C_6H_5	*o*-NO_2–C_6H_5CO	G	39.99	113–115, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	>10
31	*p*-NO_2–C_6H_5CONH	C_6H_5	3,5-$(NO_2)_2$–C_6H_5CO	G	50.15	223–225, Ethanol	$C_{29}H_{21}N_5O_8$, 567.50	0.869

Note: *The inhibitory concentrations (IC_{50}) of these compounds for antiplatelet aggregation were evaluated by a turbidimetric method based on ADP-induced (2.0 μM, 5 μL) platelet aggregation in rabbit platelet-rich plasma (PRP). The anticoagulant blood was taken from the auricular artery of rabbits, and platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared with centrifugation. Platelet aggregation was studied at 37 °C using Born's method[21] in a platelet aggregation module (DAM-1, Danyang Institute of Electronic Research, Suzhou, China). A final concentration of ADP 2.0 μmol L^{-1} was used in a volume of 5 μL. The new compound 0.05 mL at different concentrations and normal saline were added to 0.45 mL PRP, respectively. After 5 min, ADP (2.0 μmol L^{-1}, 5 μL) was given. Maximal change in light transmission was assumed to represent maximal platelet aggregation. Platelet aggregation was measured and the maximal deflection was obtained after 5 min of curve registration computed as a percentage of maximal aggregation.

Table 3. Structural Information concerning 21 new compounds



No.	Yield %	Mp (°C) solvent	Formula MW	IR (KBr) (cm^{-1})	^1H NMR ($CDCl_3$, δ)	MS, *m/z*
11	42.39	208–210, Acetone	$C_{23}H_{21}N_3O_5S$, 451.427	3267 (NH), 1616 (C=O), 1523, 1341 (NO_2), 1335, 1146 (SO_2–N)	2.90 (s, 3H, CH_3SO_2), 3.30 (m, 4H, $ArCH_2CH_2N$), 6.80 (s, 1H, C_1–H), δ 7.00–8.30 (m, 12H, aromatic), 9.40 (s, 1H, NH)	452 (M^{+1})
12	38.29	135–137, Acetone	$C_{23}H_{21}N_3O_5S$, 451.427	3398 (NH), 3094 (ArH), 3206 (C=O double), 1610 (C=O), 1516, 1340 (NO_2), 1340, 1146 (SO_2–N), 1275 (CN)	2.90 (s, 3H, CH_3SO_2), 3.35 (m, 4H, $ArCH_2CH_2N$), 6.90 (s, 1H, C_1–H), 7.10–8.00 (m, 12H, aromatic), 9.45 (s, 1H, NH)	452 (M^{+1})
13	38.63	145–146, Acetone	$C_{19}H_{22}N_2O_4S$, 374.454	3306 (NH), 1724, 1668 (C=O), 1371, 1227 (SO_2–N), 1101 (C–O)	1.25 (t, 3H, CH_3), 2.90 (s, 3H, CH_3SO_2), 2.70–3.50 (m, 4H, $ArCH_2CH_2N$), 4.10 (q, 2H, CH_2O), 6.40 (s, 1H, C_1–H), δ 7.30 (m, 8H, aromatic)	375 (M^{+1})
14	43.53	130–132, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	3022 (ArH), 2878 (CH); 1678 (C=O), 1528, 1360 (NO_2), 1483, 1412 (aromatic), 1360, 1133 (SO_2–N), 1290 (CN)	3.00 (s, 3H, CH_3), 3.30 (m, 4H, $ArCH_2CH_2N$), 6.8 (s, 1H, C_1–H), 7.30–7.90 (m, 11H, aromatic)	487 (M^{+1})
15	61.34	147–149, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	3246 (NH), 1624 (C=O), 1518, 1347 (NO_2), 1316, 1142 (SO_2–N)	2.90 (s, 3H, CH_3), 3.40 (m, 4H, $ArCH_2CH_2N$), 7.0 (s, 1H, C_1–H), 7.20–8.30 (m, 11H, aromatic), 9.50 (s, 1H, NH)	487 (M^{+1})
16	55.44	121–123, Acetone	$C_{23}H_{20}N_3O_5SCl$, 485.927	3246 (NH), 3084 (ArH), 2934 (CH); 1616 (C=O), 1529, 1348 (NO_2), 1481, 1442 (aromatic), 1317, 1148 (SO_2–N)	3.00 (s, 3H, CH_3), 3.40 (m, 4H, $ArCH_2CH_2N$), 7.0 (s, 1H, C_1–H), 7.20–8.30 (m, 11H, aromatic)	487 (M^{+1})
17	46.19	148–150, Acetone	$C_{19}H_{21}N_2O_4SCl$, 408.954	2918 (CH); 1634 (C=O), 1346, 1163 (SO_2–N), 1285 (CN)	1.30 (t, 3H, CH_3), 2.90 (s, 3H, CH_3SO_2), 2.70–3.40 (m, 4H, $ArCH_2CH_2N$), 4.10 (m, H, CH_2O), 6.30 (s, 1H, C_1–H), 7.20 (m, 7H, aromatic), 7.80 (s, 1H, NH)	373 (M−Cl, base peak)
18	33.18	149–151, Acetone	$C_{28}H_{23}N_3O_5S$, 513.544	3437 (NH), 1662 (C=O), 1341, 1164 (SO_2–N), 1079 (C–O)	2.70–3.40 (m, 4H, $ArCH_2CH_2N$), 6.90 (s, 1H, C_1–H), 7.20–8.30 (m, 17H, aromatic), 10.00 (s, 1H, NH)	513 (M)
19	43.34	225–227, Acetone	$C_{28}H_{23}N_3O_5S$, 513.544	3435 (NH), 3099, 3049 (ArH), 2920 (CH), 1666 (C=O), 1529, 1427 (aromatic), 1339, 1159 (SO_2–N), 1097 (C–O), 1003, 932, 864 (CH)	2.70–4.60 (m, 4H, $ArCH_2CH_2N$), 6.90 (s, 1H, C_1–H), 7.10–8.30 (m, 17H, aromatic), 9.90 (s, 1H, NH)	513 (M)
20	40.51	>250, Acetone	$C_{28}H_{22}N_4O_7S$, 558.554	3084 (ArH), 2920 (CH), 1639 (C=O), 1539, 1371 (NO_2), 1344, 1173 (SO_2–N)	2.80–3.70 (m, 4H, $ArCH_2CH_2N$), 6.70 (s, 1H, C_1–H), 6.90–9.00 (m, 16H, aromatic)	557 (M^{-1})
21	49.34	185–187, Acetone	$C_{28}H_{22}N_3O_5SCl$, 548.012		2.60–3.60 (m, 4H, $ArCH_2CH_2N$), 6.85 (s, 1H, C_1–H), 7.20–8.10 (m, 16H, aromatic)	547 (M^{-1})
22	26.76	125–127, Acetone	$C_{24}H_{24}N_2O_4S$, 436.514	1686 (C=O), 1445, 1414 (aromatic), 1375, 1167 (SO_2–N), 1232 (CN), 1084 (C–O), 933 (CH)	1.25 (t, 3H, CH_3), 2.90 (m, 4H, $ArCH_2CH_2N$), 4.20 (m, 2H, CH_2O), 6.35 (s, 1H, C_1–H), 6.80–7.90 (m, 13H, aromatic)	435 (M^{-1})

(*continued on next page*)

Table 3 (*continued*)

No.	Yield %	Mp (°C) solvent	Formula MW	IR (KBr) (cm^{-1})	^1H NMR ($CDCl_3$, δ)	MS, *m/z*
23	26.53	153–155, Acetone	$C_{20}H_{24}N_2O_4S$, 388.474	3491 (NH), 2991, 2924 (CH), 1730 (C=O), 1493, 1416 (aromatic), 1366, 1271 (SO_2–N), 1161, 1109 (C–O), 974, 930, 872 (CH)	1.25 (t, 3H, CH_3), 2.90 (s, 3H, CH_3SO_2), 3.00–3.50 (m, 6H, $ArCH_2CH_2N$, CH_2N), 4.15 (q, 2H, CH_2O), 6.70 (s, 1H, C_1–H), 7.10–7.65 (m, 8H, aromatic)	388 (M)
24	20.49	122–124, Acetone	$C_{25}H_{26}N_2O_4S$, 450.544	3464 (C=O double), 1732 (C=O), 1491, 1445 (aromatic), 1375, 1167 (SO_2–N)	1.20 (t, 3H, CH_3), 2.70–3.60 (m, 8H, $ArCH_2CH_2N$, CH_2N, CH_2O), 6.70 (s, 1H, C_1–H), 7.10–8.00 (m, 13H, aromatic)	449 (M^{-1})
25	20.10	107–109, Ethanol	$C_{25}H_{23}N_3O_5$, 455.45	3294 (NH), 1690, 1661 (C=O), 1535, 1339 (NO_2), 1501, 1431 (aromatic), 1231 (C–N)	1.30 (t, 3H, CH_3), 2.70–3.30 (m, 4H, $ArCH_2CH_2N$), 4.20 (m, 2H, CH_2O), 6.30 (s, 1H, C_1–H), 7.20–8.40 (m, 12H, aromatic)	445 (M)
26	35.56	257–259, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	3170 (NH), 1676 (C=O), 1595, 1437 (aromatic), 1523, 1342 (NO_2), 1244 (C–N), 1197 (C–O), 1021, 886 (CH)	2.70–3.50 (m, 4H, $ArCH_2CH_2N$), 7.00 (s, 1H, C_1–H), 7.30–8.30 (m, 16H, aromatic), 10.05 (s, 1H, NH)	523 (M^{+1})
27	48.29	111–113, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	2854 (CH), 1649 (C=O), 1529, 1346 (NO_2), 1411 (aromatic), 1259 (C–N), 1164, 1077 (C–O), 1020, 853 (CH)	2.70–3.60 (m, 4H, $ArCH_2CH_2N$), 6.90 (s, 1H, C_1–H), 7.10–8.30 (m, 16H, aromatic), 10.30 (s, 1H, NH)	523 (M^{+1})
28	29.53	145–147, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	3101 (NH), 2950 (CH), 1681 (C=O), 1512, 1345 (NO_2), 1477, 1406 (aromatic), 1258 (C–N), 1062 (C–O), 893 (CH)	2.70–3.60 (m, 4H, $ArCH_2CH_2N$), 6.90 (s, 1H, C_1–H), 7.10–8.30 (m, 16H, aromatic), 10.15 (s, 1H, NH)	523 (M^{+1})
29	58.56	224–226, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	3284 (NH), 1665 (C=O), 1591, 1423 (aromatic), 1516, 1340 (NO_2), 1300 (C–N), 932, 848 (CH)	2.70–3.60 (m, 4H, $ArCH_2CH_2N$), 7.00 (s, 1H, C_1–H), 7.20–8.30 (m, 16H, aromatic), 10.19 (s, 1H, NH)	523 (M^{+1})
30	39.99	113–115, Ethanol	$C_{29}H_{22}N_4O_6$, 522.50	2867 (CH), 1686 (C=O), 1526, 1348 (NO_2), 1479, 1411 (aromatic), 1263 (C–N), 1067 (C–O), 895 (CH)	2.70–3.60 (m, 4H, $ArCH_2CH_2N$), 7.00 (s, 1H, C_1–H), 7.30–8.30 (m, 16H, aromatic), 10.20 (s, 1H, NH)	523 (M^{+1})
31	50.15	223–225, Ethanol	$C_{29}H_{21}N_5O_8$, 567.50	3291 (NH), 2985 (CH), 1624 (C=O), 1587, 1408 (aromatic), 1513, 1393 (NO_2), 1240 (C–N), 1153 (C–O), 900, 863 (CH)	2.70–3.60 (m, 4H, $ArCH_2CH_2N$), 6.80 (s, 1H, C_1–H), 7.30–8.90 (m, 15H, aromatic), 10.35 (s, 1H, NH)	568 (M^{+1})

3.2. Design of 1-*o*-chlorophenyl-2-substituted-1,2,3,4-tetrahydroisoquinoline derivatives

A series of 1-*o*-chlorophenyl-2-substituted-1,2,3,4-tetrahydroisoquinoline derivatives were designed and synthesized in order to search for a new type of platelet antiaggregants on the basis of the principles of isosterism and rational drug design, and in view of the structural features of ticlopidine, clopidogrel, *R*-(+)-trimetoquinol, and some tetrahydroisoquinoline alkaloids possessing antiplatelet aggregation activities, as well as some synthesized antiplatelet aggregating agents (±)-1-phenyl-2-substituted-7-sulfonylamide/amide-1,2,3,4-tetrahydroisoquinoline (**11**–**31**, Table 2).

Thienopyridine compounds, including ticlopidine and clopidogrel, have been found to selectively inhibit adenosine 5′ diphosphate (ADP)-induced platelet aggregation and adenylyl cyclase ex vivo.[11] The (±)-1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline nucleus (**M3**) is similar to ticlopidine, and tetrahydroisoquinoline nucleus and thienopyridine are biological isosteres. So, we designed and synthesized some analogs of (±)-1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline nucleus (**M3**) with several substituent groups at position 2 of the nucleus.

Fifteen synthesized (±)-1-phenyl-2-substituted-7-sulfonylamide/amide-1,2,3,4-tetrahydroisoquinoline compounds (**11**–**31**, Table 2) inhibited of platelet aggregation, PLS (partial least-square method) analysis result shows that the CoMFA model based upon superposition of the 15 compounds with compound **11** as the template produced a valid model. CoMFA analysis of these compounds was performed on a Silicon Graphics

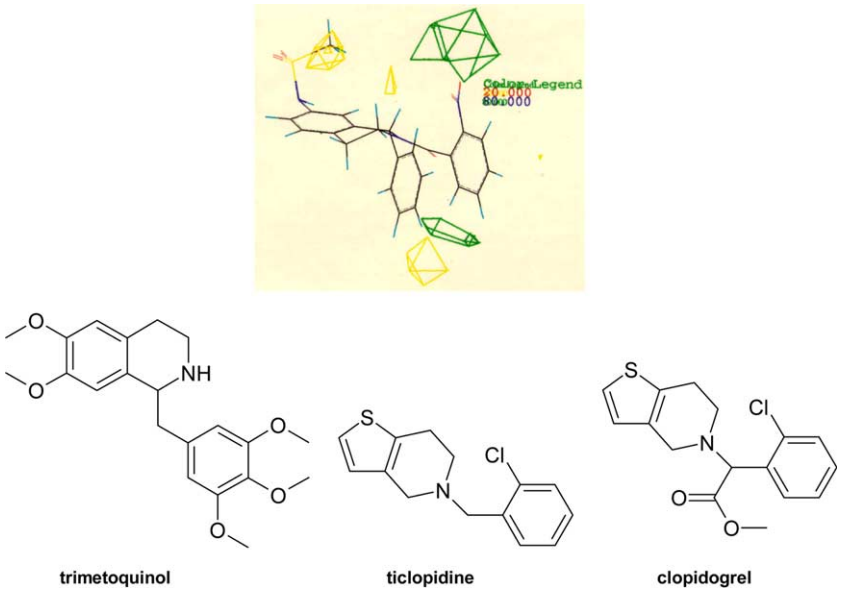


Figure 1. Steric field contribution contour map based on the superposition of the 15 compounds using compound **11** as the template. CoMFA results reveal that the steric interactive energy is a major contribution to antiplatelet aggregatory activity, and the sulfonylamide group is necessary to the activity. Here a small group favorable area exists near the 7 position of the tetrahydroisoquinoline nucleus, which means that the smaller substituent at the 7 position of the tetrahydroisoquinoline nucleus, the more inhibition of platelet aggregation.

Iris Indigo XZ4000 (SGI Inc., Silicon, CA, USA) workstation using the QSAR/CoMFA module within SYBYL V 6.3 software (Tripos Inc., St. Louis, MI, USA). The CoMFA study reveals that the steric interactive energy is a major contribution to antiplatelet aggregatory activity, and the sulfonylamide group is necessary for activity (Fig. 1). An area accommodating a small group exists near the 7 position of the tetrahydroisoquinoline nucleus. Smaller substituent at the 7 position of the tetrahydroisoquinoline nucleus, favor platelet aggregation inhibitory activity. It seems to show that removing a substituent from the 7 position of the tetrahydroisoquinoline nucleus would improve antiplatelet aggregation activity. So, we designed a type of (±)-1-phenyl-2-substituted-1,2,3,4-tetrahydroisoquinoline derivatives, which is similar to ticlopidine.

From 10 new compounds, (±)-1-phenyl-2-substituted-1,2,3,4-tetrahydroisoquinoline (**1**–**10**), it is clear that four analogs, of which (±)-1-*o*-chlorophenyl-2-*m*-bromicophenylacyl-1,2,3,4-tetrahydroisoquinoline (**9**, TQP-3) was the most active, proved to be potential antiplatelet aggregation agents. It seemed to reveal that four derivatives (**2**, **5**, **7**, **9**) showed, which inhibited human platelet aggregation induced by 121.0 μM ADP with IC_{50} values ranging over 0.206 nM–2.080 μM. The compound TQP-3 (**9**) inhibits ADP-induced human platelet aggregation with IC_{50} values of approximately 0.206 nM. Here, (±)-1-phenyl-2-*o*-chlorobenzyl/benzyl-1,2,3,4-tetrahydroisoquinoline (Type I) compounds **1** and **2** are similar to ticlopidine because there are *o*-chlorobenzyl or benzyl at the 2 position of the nucleus. However, compound **2** with benzyl at the 2 position of the tetrahydroisoquinoline nucleus is more active than compound **1** with *o*-chlorobenzyl. This is different from ticlopidine, which has a *o*-chlorobenzyl group at the 2 position of the thienopyridine nucleus. This is because there are steric hindrance to both *o*-chloro groups of *o*-chlorophenyl at the 1 position and of *o*-chlorobenzyl at the 2 position of the nucleus of compound **1**. The *o*-chloro group of *o*-chlorophenyl at the 1 position of the tetrahydroisoquinoline nucleus in compound **2** may play the same role as *o*-chloro group of *o*-chlorobenzyl at the 2 position of the thienopyridine nucleus in ticlopidine. This may be the reason that compound **2** is superior to compound **1**.

On the other hand, compound with the different substitutions at position 2 of the nucleus (such as 2-substituted phenylacyl group), have different effects on activity. These compounds of 2-substituted phenylacyl group with *m*-substituted group (**5**, **7**, **9**) showed a higher IC_{50} values for inhibiting ADP-induced human platelet aggregation than those with *o*-substituted group (**4**, **6**) or *p*-substituted group (**3**, **8**), where these substituents were chlorine, bromine, and nitro group. Of these compounds, bromine-substituted derivative (TQP-3, **9**) exhibited the highest IC_{50} value. The bromine-substituted derivative (TQP-3, **9**) was more potent than that of two other derivatives, namely the chlorine-substituted derivative (**7**) and the nitro-substituted derivative (**5**). Moreover, compound **7** was more active than compound **5**, (±)-1-*o*-chlorophenyl-2-substituted phenylacyl-1,2,3,4-tetrahydroisoquinoline analogs (Type II **4**–**10**) is more similar to clopidogrel than to ticlopidine. This is due to the acyl group at 2 position of the nucleus, which plays the role of the ester group of clopidogrel. It was conjectured that these analogs could take on a potential antiplatelet aggregation role acting as ADP receptor antagonists.

4. Conclusion

Ten tetrahydroisoquinoline derivatives were designed and synthesized by the aid of computer drug design, basis on the principles of isosterism and the reported SAR (structure–activity relationship) of synthesized tetrahydroisoquinoline derivatives[22]. It was found that compound TQP-3 possessed more potent antiplatelet aggregatory activity with an IC_{50} values of approximately 0.206 nM. 2-Benzyl-1,2,3,4-tetrahydroisoquinoline compounds **1** and **2** are similar to ticlopidine, and compound **2** with benzyl at the 2 position of the tetrahydroisoquinoline nucleus is more active than compound **1** with *o*-chlorobenzyl. This is because there is a steric interaction between *o*-chlorophenyl and *o*-chlorobenzyl of compound **1**, and the *o*-chloro group of *o*-chlorophenyl of compound **2** may play a role as *o*-chloro group of *o*-chlorobenzyl of ticlopidine. The 2-substituted phenylacyl group, their inhibition of platelet with *m*-substituted group (**5**, **7**, **9**) showed a higher IC_{50} value of inhibiting ADP-induced human platelet aggregation than those with *o*-substituted group (**4**, **6**) or *p*-substituted group (**3**, **8**). Of these compounds, bromine-substituted derivative (TQP-3, **9**) exhibited the highest IC_{50} value. It is observed that their inhibition is bromine-substituted derivative, chlorine-substituted derivative (**7**), and nitro-substituted derivative (**5**) in turn. (±)-1-*o*-Chlorophenyl-2-substituted phenylacyl-1,2,3,4-tetrahydroisoquinoline analogs is more similar to clopidogrel than to ticlopidine due to the acyl group at 2 position of the nucleus playing a role as the ester group of clopidogrel.

It was conjectured that these analogs could take on a potential antiplatelet aggregation role acting as ADP receptor antagonists. The results may be useful for further investigation on antiplatelet aggregatory activity and mechanism of tetrahydroisoquinoline compounds.

5. Experimental

5.1. General

Ten 1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline derivatives (**1**–**10**) with different substitutes at position 2 of the nucleus were designed and synthesized. The structures of the new compounds synthesized were identified by means of elemental analysis, MS, H NMR, and IR. Reactions using anhydrous solvents that had been dried by sodium thread were carried out in a nitrogen atmosphere. Melting points were determined on a DX-5 double-eyes micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were taken with a Bruker IFS-48 FT-IR or Nexus 870 FT-IR spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a FX-90Q or FX-900 spectrometer using deuteriochloroform unless otherwise stated with tetramethylsilane as an internal or external (D_2O) reference. Mass spectra (MS) were obtained on a Finnigan FTMS-2000 mass spectrometer or ZAB-HS (VG) inversion double-focusing high-definition mass spectrometer. To dry the organic extraction solutions, anhydrous magnesium sulfate was used. For column chromatography, silica gel (Merck silica gel 60). Elemental analysis was performed with PK2400CHN instrument, and C, H, and N results were within 0.4% of theoretical values.

5.2. Synthesis of *N*-phenylethyl *o*-chlorophenylamide (M1)

o-Chlorophenylformyl acid (16.0 g, 0.11 mol) was dissolved in 50 mL dry CH_2Cl_2, and 10.0 mL $SOCl_2$ was added to the solution at 0 °C. The mixture was stirred at room temperature for 1 h and concentrated (<30 °C) to give the unstable chloride, which was used in the next reaction without purification. The fresh chloride (17.5 mL, 14.6 g, 0.10 mol) dissolved in 20 mL CH_2Cl_2 was added dropwise to a solution containing a mixture of β-phenylethylamine (12.0 g, 0.10 mol) and triethylamine (14.0 mL, 0.10 mol) in 100 mL dry CH_2Cl_2 at ice bath. The resultant mixture was stirred at room temperature for 3 h. After being concentrated under reduced pressure, the reaction mixture was cooling and 150 mL ether was added. The colorless powders were separated out and were filtered off. The filtrate was washed with water and ether, dried, and 23.8 g (92.90%) of *N*-phenylethyl *o*-chlorophenylamide (**M1**) was given as white needles: mp 99–101 °C (acetone).

5.3. 1-*o*-Chlorophenyl-3,4-dihydroisoquinoline intermediates (M2)

According to Bischler–Napieralski reaction,[20,21] a mixture of compound **M1** (20 g, 0.077 mol) with anhydrous methylbenzene (50 mL) and fresh $POCl_3$ (30 mL) was refluxed for 8 h in a nitrogen atmosphere. The solvent was evaporated under reduced pressure. The concentrated solution was added to a 10 mL acetone solution, poured into ice water, neutralized with strong aqua ammonia to pH 9–10, and extracted with 250 mL ether three times. The extractant was washed with water, dried with anhydrous $MgSO_4$, filtered, and concentrated. The crude product was to give 11.2 g (46.38%) of 1-*o*-chlorophenyl-3,4-dihydroisoquinoline (**M2**) as light yellow needles, which was used in the next reaction without purification. ^{1}H NMR ($CDCl_3$): δ 2.75 (m, 2H, CH_2N), δ 3.70 (m, 2H, $ArCH_2$), δ 7.45 (m, 8H, aromatic).

5.4. 1-*o*-Chlorophenyl-1,2,3,4-tetrahydroisoquinoline (M3)

The above intermediate (3.5 g, 0.014 mol) was dissolved in 40 mL anhydrous methanol, adjusted to pH 8.5 with diethylamine, and KBH_4 (1.5 g, 0.027 mol) was slowly added under 50 °C. The mixture was stirred at 50–70 °C for 4 h. After evaporating solvent, addition of water can decompose KBH_4. The extraction with ether was washed with brine and water, dried with $MgSO_4$, filtering, and concentration, to produce next intermediate, namely 1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinolines (**M3**) as light yellow oleaginous dope: 1.7 g (48.57%), which was used in next reaction without purification.

5.5. Synthesis of 1-*o*-chlorophenyl-2-*o*-chlorobenzyl-1,2,3,4-tetrahydroisoquinoline (1)

The above intermediate (**M3**, 3.50 g, 0.014 mol) was dissolved in 50 mL anhydrous ethanol, added a little triethylamine and fresh *o*-chlorobenzyl chloride (2.20 g, 0.014 mol), and stirred at reflux for 6 h. After evaporating solvent, 5 mL acetone was added to the concentrated solution, poured into water, and extracted with 250 mL $CHCl_3$ three times. The extraction was washed with brine and water, dried with $MgSO_4$, colored with active carbon, filtering, and concentration. The crude product was crystallized with 40 mL anhydrous alcohol to give 0.64 g (12.28%) 1-*o*-chlorophenyl-2-*o*-chlorobenzyl-1,2,3,4-tetrahydroisoquinolines (**1**) as white powders: mp 168–169 °C (EtOH); IR (KBr) 2912, 2863, 1616 (CH_2), 1489, 1471, 1451 (aromatic), 1244 (C–N), 1021, 886 (CH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.619 (m, 2H, Ar–CH_2–$C(H_2)$–), δ 3.066 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 3.621 (m, 2H, –N–CH_2–Ar), δ 5.361 (s, 1H, C_1–H), δ 6.744 (d, 1H, C_7–H), δ 7.134 (m, 1H, C_5–H of 1-*o*-chlorophenyl), δ 7.206 (m, 6H, C_5–H, C_8–H, C_4–H, and C_6–H of 1-*o*-chlorophenyl, C_4–H and C_6–H of 2-*o*-chlorobenzyl), δ 7.281 (m, 1H, C_5–H of 2-*o*-chlorobenzyl), δ 7.397 (m, 2H, C_3–H of 1-*o*-chlorophenyl, C_3–H of 2-*o*-chlorobenzyl), δ 7.523 (d, 1H, C_6–H); MS, *m*/*z* 369 (M^{+1}), 368 (M), 367 (M^{-1}). Analyses ($C_{22}H_{19}NCl_2$) C, H, N.

5.6. 1-*o*-Chlorophenyl-2-benzyl-1,2,3,4-tetrahydroisoquinoline (2)

A white powder: 0.78 g (15.90%); mp 118–119 °C (EtOH); IR (KBr) 2938 (–CH_2–), 2861 (–CH_2–), 1603, 1495, 1456 (aromatic), 757, 707 (CH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.501 (m, 2H, Ar–CH_2–$C(H_2)$–), δ 3.093 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 3.282 (d, 2H, –N–CH_2–Ar), δ 5.311 (s, 1H, C_1–H), δ 6.767 (d, 1H, C_7–H), δ 7.134 (m, 1H, C_5–H of 1-*o*-chlorophenyl), δ 7.194 (m, 2H, C_5–H, C_8–H), δ 7.226 (m, 2H, C_4–H and C_6–H of 1-*o*-chlorophenyl), δ 7.258 (d, 1H, C_4–H of 2-benzyl), δ 7.306 (d, 4H, C_2–H, C_3–H, C_5–H, and C_6–H of 2-benzyl), δ 7.417 (d, 1H, C_3–H of 1-*o*-chlorophenyl), δ 7.486 (m, 1H, C_6–H); MS, *m*/*z* 333 (M^{-1}), 334 (M), 335 (M^{+1}). Analyses ($C_{22}H_{20}NCl$) C, H, N.

5.7. 1-*o*-Chlorophenyl-2-*p*-nitrobenzyl-1,2,3,4-tetrahydroisoquinoline (3)

A light yellow powder: 0.42 g (51.77%); mp 104–106 °C (EtOH); IR (KBr) 1661 (C=O), 1512, 1340 (NO_2), 1086 (C–O), 1013, 851 (CH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.40–3.90 (m, 6H, $ArCH_2CH_2N$, $ArCH_2$), 6.70 (s, 1H, C_1–H), 6.90–8.10 (m, 12H, aromatic); MS, *m*/*z* 379 (M). Analyses ($C_{22}H_{19}N_2O_2Cl$) C, H, N.

5.8. 1-*o*-Chlorophenyl-2-*o*-nitrophenylacyl-1,2,3,4-tetrahydroisoquinoline (4)

A light yellow powder: 0.78 g (47.02%); mp 143–145 °C (EtOH); IR (KBr) 3059 (ArH), 2912 (CH), 1634 (C=O), 1522, 1340 (NO_2), 1481, 1431 (aromatic), 1228 (C–N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.70–3.40 (m, 4H, $ArCH_2CH_2N$), 7.0 (s, 1H, C_1–H), 7.20–8.20 (m, 12H, aromatic); MS, *m*/*z* 393 (M). Analyses ($C_{22}H_{17}N_2O_3Cl$) C, H, N.

5.9. 1-*o*-Chlorophenyl-2-*m*-nitrophenylacyl-1,2,3,4-tetrahydroisoquinoline (5)

A light yellow powder: 0.59 g (41.94%); mp 115–117 °C (EtOH); IR (KBr) 1673, 1622 (C=O), 1528, 1344 (NO_2), 1479, 1429 (aromatic), 1234 (C–N), 1084 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.70–3.70 (m, 4H, $ArCH_2CH_2N$), 7.0 (s, H, C_1–H), 7.20–8.30 (m, 12H, aromatic); MS, *m*/*z* 393 (M). Analyses ($C_{22}H_{17}N_2O_3Cl$) C, H, N.

5.10. 1-*o*-Chlorophenyl-2-*o*-chlorophenylacyl-1,2,3,4-tetrahydroisoquinoline (6)

o-Chlorophenyl formyl chloride (fresh) 1.5 g (0.014 mol) dissolved in 5 mL CH_2Cl_2 was added to a solution containing 1-*o*-chlorophenyl-1,2,3,4-tetrahydroisoquinoline (**M3**, 3.50 g, 0.014 mol) and 2.0 mL triethylamine in 80 mL CH_2Cl_2 at 0 °C. The mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated. Then, the concentrated solution was added 5 mL acetone solution, poured into water, and extracted with 250 mL CH_2Cl_2 three times. The combined extracts were washed with brine and water, dried with $MgSO_4$, colored with active carbon, filtering, and concentration. The crude product was crystallized with 40 mL anhydrous alcohol to give 0.86 g (15.58%) 1-*o*-chlorophenyl-2-*o*-chlorobenzoyl-1,2,3,4-tetrahydroisoquinolines (**6**) as white powder: mp 178–180 °C (EtOH); IR (KBr) 1644 (C=O), 1588, 1488, 1455 (aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.193 (s, 1H, C_1–H), δ 2.780 (m, 2H, Ar–CH_2–$C(H_2)$–), δ 3.076 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 6.157 (s, 1H, C_7–H), δ 6.390 (s, 1H, C_8–H), δ 6.557 (m, 2H, C_5–H, C_6–H), δ 6.947 (m, 2H, C_4–H and C_6–H of 1-*o*-chlorophenyl), δ 7.183 (m, 2H, C_3–H and C_5–H of 1-*o*-chlorophenyl), δ 7.313 (m, 2H, C_4–H and C_5–H of 2-*o*-chlorobenzoyl), δ 7.434 (m, 2H, C_3–H and C_6–H of 2-*o*-chlorobenzoyl); MS, *m*/*z* 381 (M^{-1}), 382 (M). Analyses ($C_{22}H_{17}NOCl_2$) C, H, N.

5.11. 1-*o*-Chlorophenyl-2-*m*-chlorophenylacyl-1,2,3,4-tetrahydroisoquinoline (7)

A white powder: 0.61 g (11.23%); mp 159–160 °C (EtOH); IR (KBr) 2945, 2836 (–CH_2–), 1637 (C=O), 1593, 1475, 1455 (aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.193 (s, 1H, C_1–H), δ 2.826 (m, 2H, Ar–CH_2–$C(H_2)$–N–), δ 3.446 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 6.890 (s, 1H, C_7–H), δ 7.067 (s, 1H, C_5–H), δ 7.138 (m, 5H, C_6–H, C_8–H, 3H at C_4–H, C_5–H, and C_6–H of 1-*o*-chlorophenyl), δ 7.341 (m, 5H, C_3–H of 1-*o*-chlorophenyl, C_2–H, C_4–H, C_5–H, and C_6–H of 2-*m*-chlorobenzoyl); MS, *m*/*z* 381 (M^{-1}), 382 (M), 383 (M^{+1}). Analyses ($C_{22}H_{17}NOCl_2$) C, H, N.

5.12. 1-*o*-Chlorophenyl-2-*p*-chlorophenylacyl-1,2,3,4-tetrahydroisoquinoline (8)

A white powder: 0.82 g (14.68%); mp 174–176 °C (EtOH); IR (KBr) 1640 (C=O), 1591, 1488, 1455

(aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.820 (s, 1H, C_1–H), δ 3.438 (s, 2H, Ar–CH_2–$C(H_2)$–), δ 3.843 (s, 2H, Ar–$C(H_2)$–CH_2–N–), δ 6.871 (m, 1H, C_7–H), δ 6.901 (s, 2H, C_5–H, C_8–H), δ 7.133 (m, 4H, C_6–H of tetrahydroisoquinoline, C_4–H, C_5–H, and C_6–H of 1-*o*-chlorophenyl), δ 7.280 (m, 4H, C_3–H of 1-*o*-chlorophenyl, C_3–H, C_4–H, and C_5–H of 2-*p*-chlorobenzoyl), δ 8.085 (d, 1H, C_6–H of 2-*p*-chlorobenzoyl); MS, *m*/*z* 381 (M^{-1}), 382 (M). Analyses ($C_{22}H_{17}NOCl_2$) C, H, N.

5.13. 1-*o*-Chlorophenyl-2-*m*-bromicophenylacyl-1,2,3,4-tetrahydroisoquinoline (9)

A white powder: 0.68 g (11.06%); mp 120–122 °C (EtOH); IR (KBr) 1673 (C═O), 1595, 1469, 1434 (aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.191 (s, 1H, C_1–H), δ 3.226 (m, 2H, Ar–CH_2–$C(H_2)$–), δ 3.843 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 7.254 (m, 3H, C_5–H, C_7–H, C_8–H), δ 7.315 (d, 1H, C_6–H of 1-*o*-chlorophenyl), δ 7.490 (m, 4H, C_6–H, C_3–H, C_4–H, and C_5–H of 1-*o*-chlorophenyl), δ 7.534 (m, 1H, C_5–H of 2-*m*-bromicobenzoyl), δ 7.763 (d, 2H, C_4–H and C_6–H of 2-*m*-bromicobenzoyl), δ 7.992 (s, 1H, C_2–H of 2-*m*-bromicobenzoyl); MS, *m*/*z* 426 (M^{-1}), 427 (M). Analyses ($C_{22}H_{17}NOBrCl$) C, H, N.

5.14. 1-*o*-Chlorophenyl-2-phenylsulfonyl-1,2,3,4-tetrahydroisoquinoline (10)

The residue was chromatographed on silica gel with oil ether/EtOAc (5/1) as effluent to give 0.68 g (12.44%) of 1-*o*-chlorophenyl-2-phenylsulfonyl-1,2,3,4-tetrahydroisoquinoline (**10**) as a light yellow powder: mp 98–100 °C (EtOH); IR (KBr) 1603, 1487, 1444 (aromatic), 1307 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.856 (m, 2H, Ar–CH_2–$C(H_2)$–), δ 3.603 (m, 2H, Ar–$C(H_2)$–CH_2–N–), δ 3.811 (m, 1H, C_1–H), δ 6.559 (s, 1H, C_7–H), δ 6.968 (m, 5H, C_5–H, C_6–H, C_8–H, C_4–H, and C_6–H of 1-*o*-chlorophenyl), δ 7.103 (m, 4H, C_3–H and C_5–H of 1-*o*-chlorophenyl, C_3–H and C_4–H of 2-phenylsulfonyl), δ 7.188 (d, 2H, C_2–H and C_6–H of 2-phenylsulfonyl); MS, *m*/*z* 382 (M^{-1}), 383 (M). Analyses ($C_{22}H_{17}NOBrCl$) C, H, N.

5.15. Pharmacology. Inhibitory effect on human PRP aggregation. Preparation of human PRP

Blood was withdrawn from the cubitus artery of human volunteers, who were healthy male, limosis and avoiding meat or fish, through a cannulation tube using a syringe containing sodium citrate (3.8%, 1/10 volume). The sample was left standing for 20 min at room temperature and then centrifuged at 210 g for 10 min at 37 °C to obtain platelet-rich plasma (PRP). The remaining blood was centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP).

5.16. Measurement of inhibition of platelet aggregation

Platelet aggregation was examined by the method of Born[19,20], with a LBY-NJ2 type four-channel aggregometer (Plysion Co., Ltd). Four samples of PRP (350 μL) placed in a cuvette were warmed at 37 °C for 1 min with stirring (1200 rpm), and then a saline solution of the test compound (30 μL) or saline was added. Exactly 2 min later, a solution of ADP (20 μL, 121 μM) was added to each of the samples, and the changes in light transmission were recorded, with the light transmission for PRP and PPP taken as 0% and 100%, respectively, and the maximum light transmission after addition of ADP as the maximum aggregations. Platelet aggregation was measured and the maximal deflection was obtained after 5 min of curve registration computed as a percentage of maximal aggregation. The percent inhibition of platelet aggregation (Pi) was expressed as the difference between 1 and the ratio of the maximum aggregation with the test compounds to that with the saline.

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