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Design, synthesis and in vitro activities on anti-platelet aggregation of 4-methoxybenzene-1,3-isophthalamides

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ABSTRACT

On the purpose of searching for the structure–activity relationship (SAR) and obtaining novel anti-platelet drugs, 41 4-methoxybenzene-1,3-isophthalamides have been described the synthesis process and in vitro activities on anti-platelet aggregation. The target compounds have been classified into four series: series 1 (*ortho*-substituted phenyl: **1a**–**1j**), series 2 (*meta*-substituted phenyl: **2a**–**2k**), series 3 (*para*-substituted phenyl: **3a**–**3l**) and series 4 (aromatic of no substituted group and aromatic heterocyclic substituted groups: **4a**–**4h**). The chemical structures of the target compounds were confirmed by MS, IR, ^{1}H NMR, and their in vitro activities on anti-platelet aggregation were tested and assessed by using Born test. The result showed that thirteen compounds **1c**, **1d**, **1i**, **1j**, **2g**, **3a**, **3c**, **3d**, **3f**, **3h**, **3l**, **4b** and **4c** have superior anti-platelet aggregation activities than the reference drug Picotamide.

The use of anti-platelet therapy has become an integral part of the treatment for type-2 diabetes since platelet plays a dual role in thrombosis and atherosclerosis, the major causes of cardiovascular complications.[1] Picotamide (Scheme 1-1), *N*,*N*′-bis(3-picolyl)-4-methoxyl-isophthalamide, is a derivative of 4-methoxyl-isophtalic acid and a new anti-platelet aggregating drug that inhibits both thromboxane A_2 (TxA_2) receptors and TxA_2 synthesis, [2] and does not interfere with endothelial prostacyclin (PGI_2) production compared to Aspirin.[3,4] Since 2000 we have reported some arylamides and arylsulfonamides of the analogues of Picotamide, and some obtained compounds exhibited higher anti-platelet aggregation activities than Picotamide.[5] These compounds have the chemical structures of *N*,*N*′-disubstituted-phenyl-4-methoxyl-isophthalamide or *N*,*N*′-disubstituted-phenyl-4-methoxybenzene-1,3-disulfonamide, which are essential in anti-platelet aggregating activity. According to the anti-platelet aggregating mechanism and the principle of bioisosterism of Picotamide and in order to search for the structure–activity relationship (SAR) and obtain novel anti-platelet drugs, more and more active compounds were systematically designed and synthesized continuously with two *meta*-pyridinemethyl groups of Picotamide replaced by two different substituted phenyl groups.[6] And especially, of the *N*,*N*′-di(*ortho*-substituted-phenyl)-4-methoxyl-isophthalamides (series 1), compound **1i** was at least 10 times more active than Picotamide.[7]

Our program has designed and synthesized *meta*- and *para*-monosubstituted phenyl & other substituted aromatic Picotamide derivatives to compare the anti-platelet aggregation activities with the series 1 (Scheme 1-2 and Table 1). All 41 compounds reported in this Letter have been classified into four series based on their structures: series 1 (*ortho*-substituted phenyl: **1a**–**1j**), series 2 (*meta*-substituted phenyl: **2a**–**2k**), series 3 (*para*-substituted phenyl: **3a**–**3l**) and series 4 (aromatic of no substituted group and heterocyclic groups: **4a**–**4h**). First of all, 4-methoxy group was still maintained in 4-position of the parent benzene ring. Second, with two *ortho*-, *meta*- or *para*-monosubstituted phenyl groups replacing the two *meta*-pyridinemethyl groups, 21 compounds (**a**–**g**) were prepared to study the impact on the activities of the same substituents (F, Cl, Br, I, NO_2, CH_3, OCH_3) in different substituted positions (*ortho*-, *meta*- and *para*-). Third, in the same way, another 12 compounds, including compounds **1h**–**1j** (series 1), compounds **2h**–**2k** (series 2) and compounds **3h**–**3l** (series 3) were prepared to study different substituents and compare with the compounds (**a**–**g**) in the same series on the respective activities, especially the impact of different alkoxy substituted groups (OCH_3, OCH_2CH_3, $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH_2CH_2CH_2CH_3$) of series 3 on the activities. Fourth, by replacing the *meta*-pyridylmethyl with phenyl, benzyl, 2-phenylethyl, 1-phenylethyl, 1-naphthyl, 2-pyridyl, 2-furylmethyl, (2-benzo[3,4-*d*]1,3-dioxolan-5-yl)ethyl, eight compounds **4a**–**4h** were also prepared to compare the activities between Phenyl and other aromatic groups.

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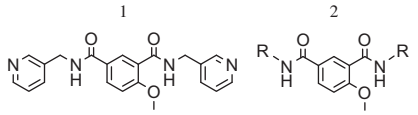
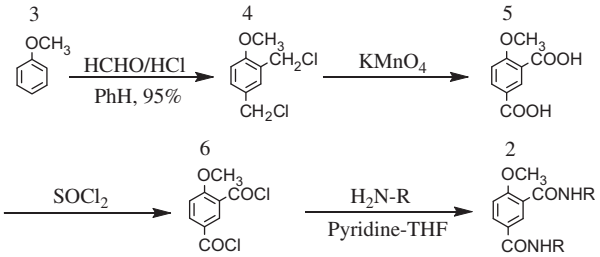


Scheme 1. Structures of Picotamide (1) and target compounds (2).



Scheme 2. Synthesis routes of the target compounds.

The synthesis of the target compounds 4-methoxybenzene 1,3-isophthalamides (**2**) were achieved following a described procedure in Scheme 2. Reaction of anisole **3** with formaldehyde and hydrochloric acid in benzene gave **4**, then compound **4** was oxidized with potassium permanganate and key intermediate 4-methoxyisophthalic acid **5** was obtained. Subsequent treatment of acid **5** with thionyl chloride, 4-methoxylisophthaloyl dichloride **6** was prepared. Finally, the target compounds **2** were easily obtained by reacting of compound **6** in anhydrous tetrahydrofuran and dry pyridine with different aromatic amines.

N,*N*′-di(2-fluorophenyl)-4-methoxyl-isophthalamide (**1a**): Equipped a stirrer in a three-necked flask (100 ml), to a mixture of 2-fluoroaniline (1.11 g, 10.0 mmol), anhydrous tetrahydrofuran (15 ml) and dry pyridine (1 ml), dropwise added 4-methoxyl-isophthaloyl dichloride (1.17 g, 5.0 mmol) which had been dissolved in dry tetrahydrofuran (10 ml). After the mixture reaction at room temperature for 8 h under continuous stirring, the excess tetrahydrofuran was distilled off in vacuum. The residue was recrystallized by acetone. Its melting point is 204–206 °C, and yield is 67%. The other compounds **1b**–**1j**, **2a**–**2k**, **3a**–**3l** and **4a**–**4h** were prepared in the same manner.

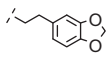
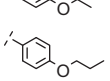
1a. Yield = 67%; mp 204–206 °C; MS (*m*/*z*) = 405 (M+Na); IR (KBr σ/cm^{-1}): 3431, 3325, 1656, 1601, 1553, 1501, 1459, 1310, 1259, 1017, 755; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 4.18 (s, 3H, OCH_3), 7.12–7.26, 8.21–8.25 (m, 8H, 2 × C_6H_4), 8.42 (t, 1H, H-5), 8.59 (t, 1H, H-6), 8.78 (d, 1H, H-2), 8.15 (s, 1H, CONH), 10.28 (s, 1H, CONH).

1b. Yield = 39%; mp 198–200 °C; MS (*m*/*z*) = 437 (M+Na-1); IR (KBr σ/cm^{-1}): 3283, 1671, 1591, 1491, 1285, 851, 825; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.30 (s, 3H, OCH_3), 7.16–8.73 (m, 8H, 2 × C_6H_4), 7.51 (d, H, H-5), 8.30 (dd, H, H-6), 8.89 (d, H, H-2), 9.35 (s, H, CONH), 10.65 (s, H, CONH).

1c. Yield = 36%; mp 199–200 °C; MS (*m*/*z*) = 505 (M+1); IR (KBr σ/cm^{-1}): 3430, 1667, 1522, 1493, 1431, 1255, 1147, 1076, 751; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 4.22 (s, 3H, –OCH_3), 7.05 (q, 2H, 2 × H-4), 7.24 (d, 1H, H-5), 7.40 (q, 2H, 2 × H-5), 7.62 (d, 2H, 2 × H-3), 8.27, 8.69 (d, 2H, 2 × H-6), 8.53 (d, 1H, H-6), 8.57 (s, 1H, CONH), 8.90 (s, 1H, H-2), 10.45 (s, 1H, –CONH).

1d. Yield = 30%; mp 228–229 °C; MS (*m*/*z*) = 600 (M+2); IR (KBr σ/cm^{-1}): 3349, 1677, 1657, 1556, 1504, 1331, 1249, 1224, 1127, 1055, 833; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 4.25 (s, 3H, –OCH_3), 7.25 (d, 1H, H-5), 6.92, 7.43, 7.87 (q, 6H, 2 × H-4, 2 × H-5, 2 × H-3), 7.2 (d, 1H, H-5), 8.29, 8.51 (d, 2 × 1H, 2 × H-6), 8.42 (d, 1H, H-6), 8.96 (s, 1H, H-2), 10.15 (s, 2H, −CONH).

Table 1
Structures of the target compounds

Compound	R	Compound	R	Compound	R	Compound	R
1a		**2a**		**3a**		**4a**	
1b		**2b**		**3b**		**4b**	
1c		**2c**		**3c**		**4c**	
1d		**2d**		**3d**		**4d**	
1e		**2e**		**3e**		**4e**	
1f		**2f**		**3f**		**4f**	
1g		**2g**		**3g**		**4g**	
1h		**2h**		**3h**		**4h**	
1i		**2i**		**3i**			
1j		**2j**		**3j**			
		2k		**3k**			
				3l			

1e. Yield = 37%; mp 234–236 °C; MS (*m*/*z*) = 459 (M+Na); IR (KBr σ/cm^{-1}): 3359, 3284, 1679, 1606, 1583, 1498, 1339, 1273, 823, 737; ^{1}H NMR δ (*J*/Hz): (DMSO) 11.65 (s, 1H, NH), 10.88 (s, 1H, NH), 8.66 (d, 1H, benzene-H), 8.58 (d, 1H, benzene-H), 8.21 (m, 2H, NHC_6H_4), 8.02 (d, 1H, benzene-H), 7.82 (m, 3H, NHC_6H_4), 7.43 (m, 3H, NHC_6H_4), 4.17 (s, 3H, OCH_3).

1f. Yield = 41%; mp 218–220 °C; MS (*m*/*z*) = 375 (M+1); IR (KBr σ/cm^{-1}): 3372, 3273, 1667, 1642, 1604, 1588, 1523, 1497, 1266, 1047, 754; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 9.77 (s, 1H, –NH–), 8.80 (d, 1H, Ar-H), 8.24 (q, 2H, Ar-H), 7.99 (s, 1H, NH), 7.82 (d, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.19 (m, 6H, Ar-H), 4.16 (S, 3H, OCH_3), 2.37 (d, 6H, 2 × CH_3).

1g. Yield = 58%; mp 238–240 °C; MS (*m*/*z*) = 407 (M+1); IR (KBr σ/cm^{-1}): 3437, 1669, 1602, 1530, 1496, 1460, 1252, 1029, 855; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 3.97 (d, 6H, 2 × OCH_3), 4.16 (s, 3H, OCH_3), 6.94–8.63 (m, 8H, 2 × C_6H_4), 7.19 (d, 1H, 5-H), 8.23 (s, 1H, CONH), 8.51 (m, 1H, 6-H), 8.77 (d, 1H, 2-H), 10.57 (s, 1H, CONH).

1h. Yield = 39%; mp 272–273 °C;MS (*m*/*z*) = 433 (M-1); IR (KBr σ/cm^{-1}): 3447, 3284, 1669, 1605, 1537, 1499, 1449, 1300, 1261, 1018, 832, 818; ^{1}H NMR δ (*J*/Hz): (DMSO-d_6) 4.09 (s, 3H, OCH_3), 7.20–8.75 (m, 8H, 2 × C_6H_4), 7.45 (d, 1H, H-5), 8.17 (dd, 1H, H-6), 882 (s, 1H, H-2), 12.30 (d, 2H, 2 × CONH), 13.64 (br s, 2H, 2 × COOH).

1i. Yield = 59%; mp 197–198 °C; MS (*m*/*z*) = 527 (M^+); IR (KBr σ/cm^{-1}): 3301, 3025, 2922, 1653, 1606, 1587, 1493, 1451, 1300, 1262, 1027, 825; ^{1}H NMR δ (*J*/Hz): (DMSO) 3.85 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2), 4.07 (s, 2H, CH_2), 7.11–7.36 (m, 18H, 2 × C_6H_4, 2 × C_6H_5), 7.75 (d, 1H, H-5), 8.04 (d, 1H, H-6), 8.40 (d, 1H, H-2), 9.79 (s, 1H, CONH), 9.96 (s, 1H, CONH).

1j. Yield = 82%; mp 193–194 °C; MS (*m*/*z*) = 425 (M+1); IR (KBr σ/cm^{-1}): 3426, 2926, 2251, 1650, 1605, 1494, 1455, 1414, 1267, 1015, 830, 758; ^{1}H NMR δ (*J*/Hz): (Acetone) 4.24 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2), 4.06 (s, 2H, CH_2), 7.31–7.57 (m, 8H, 2 × C_6H_4), 7.81 (d, 1H, H-5), 8.25 (d, 1H, H-6), 8.75 (d, 1H, H-2), 9.72 (s, 1H, CONH–), 9.81 (s, 1H, CONH).

2a. Yield = 67%; mp 210–212 °C; MS (*m*/*z*) = 405 (M+Na); IR (KBr σ/cm^{-1}): 3349, 1679, 1602, 1546, 1494, 1442, 1264, 850, 827; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.17 (s, 3H, OCH_3), 6.88–7.91 (m, 8H, 2 × C_6H_4), 7.53 (d, H, H-5), 8.23 (dd, H, H-6), 8.58 (d, H, H-2), 9.96 (s, H, CONH), 10.06 (s, H, CONH).

2b. Yield = 62%; mp 216–218 °C; MS (*m*/*z*) = 437 (M+Na-1); IR (KBr σ/cm^{-1}): 3334, 1681, 1592, 1546, 1481, 1273, 828, 783; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.17 (s, 3H, OCH_3), 7.15–8.11 (m, 8H, 2 × C_6H_4), 8.05 (d, H, H-5), 8.23 (dd, H, H-6), 8.59 (d, H, H-2), 9.88 (s, H, CONH), 9.99 (s, H, CONH).

2c. Yield = 51%; mp 214–216 °C; MS (*m*/*z*) = 505 (M+1); IR (KBr σ/cm^{-1}): 3333, 2922, 1682, 1589, 1541, 1493, 1478, 1273, 854; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.17 (s, 3H, –OCH_3), 7.29–8.24 (m, 8H, 2 × C_6H_4), 7.16 (m, H, H-5), 8.10 (dd, H, H-6), 8.59 (d, H, H-2), 9.86 (d, H, CONH), 9.98 (d, H, CONH).

2d. Yield = 52%; mp 202–204 °C; MS (*m*/*z*) = 600 (M+2); IR (KBr σ/cm^{-1}): 3334, 1681, 1591, 1540, 1493, 1273, 827, 778; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.17 (s, 3H, OCH_3), 7.30–8.24 (m, 8H, 2 × C_6H_4), 7.15 (m, H, H-5), 8.04 (dd, H, H-6), 8.58 (d, H, H-2), 9.84 (d, H, CONH), 9.97 (d, H, CONH).

2e. Yield = 40%; mp 298–300 °C; MS (*m*/*z*) = 459 (M+Na); IR (KBr σ/cm^{-1}): 3347, 1678, 1605, 1529, 1496, 1349, 820; ^{1}H NMR δ (*J*/Hz): (DMSO) 10.68 (d, 2H, 2 × NH), 8.79 (s, 2H, NHC_6H_4), 8.32 (d, 1H, benzene-H), 8.21 (m, 2H, NHC_6H_4), 8.08 (d, 1H, benzene-H), 7.96 (m, 2H, NHC_6H_4), 7.65 (m, 2H, NHC_6H_4), 7.37 (d, 1H, benzene-H), 3.98 (s, 3H, OCH_3).

2f. Yield = 51%; mp 179–180 °C; MS (*m*/*z*) = 375 (M+1); IR (KBr σ/cm^{-1}): 3264, 1618, 1593, 1521, 1487, 1236, 1070, 732; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$)9.72 (s, 1H, NH), 8.71 (d, 1H, benzene-H), 8.24 (q, 1H, benzene-H), 7.99 (s, 1H, NH), 7.53 (s, 2H, C_6H_4), 7.45 (t, 2H, C_6H_4), 7.30 (d, 1H, C_6H_4), 7.27 (m, 1H, C_6H_4), 7.18 (d, 1H, benzene -H), 6.99 (m, 2H, C_6H_4), 4.16 (s, 3H, OCH_3), 2.39 (s, 6H, 2 × CH_3).

2g Yield = 59%; mp 153–155 °C; MS (*m*/*z*) = 407 (M+1); IR (KBr σ/cm^{-1}): 3277, 3246, 2957, 2822, 1657, 1636, 1610, 1494, 1453, 1249, 1160, 860; ^{1}H NMR δ (*J*/Hz): (DMSO) 3.75 (s, 6H, OCH_3), 3.96 (s, 3H, OCH_3), 8.26 (d, 1H, H-2), 8.15 (dd, 1H, H-6), 7.39 (d, 1H, H-5), 7.73 (d, 2H, 2 × C_6H_4), 7.29 (m, 6H, 2 × C_6H_4), 10.2 (d, 2H, 2 × CONH).

2h. Yield = 24%; mp 165–167 °C; MS (*m*/*z*) = 431 (M+1); IR (KBr σ/cm^{-1}): 3351, 1669, 1591, 1546, 1491, 1431, 1358, 1258, 793; ^{1}H NMR δ (*J*/Hz): (Acetone) 10.03 (s, 1H, –NH–), 9.92 (s, 1H, –NH–), 8.66 (d, 1H, benzene-H), 8.46 (d, 2H, –NHC_6H_4–), 8.26 (q, 1H, benzene-H), 8.17 (d, 1H, –NHC_6H_4–), 8.08 (d, 1H, –NHC_6H_4–), 7.76 (t, 2H, –NHC_6H_4–), 7.52 (q, 2H, –NHC_6H_4–), 7.40 (d, 1H, benzene-H), 4.19 (s, 3H, –OCH_3), 2.60 (d, 6H, 2 × –$COCH_3$).

2i. Yield = 51%; mp 175–177 °C; MS (*m*/*z*) = 435 (M+1); IR (KBr σ/cm^{-1}): 3432, 2981, 1692, 1513, 1512, 1365, 1218, 913, 862; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 4.1 (s, 3H, OCH_3), 8.66 (d, 1H, H-2), 8.17 (dd, 1H, H-6), 7.11 (d, 1H, H-5), 6.88 (m, 5H, 2 × C_6H_4), 7.32 (m, 3H, 2 × C_6H_4), 3.93 (m, 4H, 2 × OCH_2), 0.98 (m, 6H, 2 × OCH_2CH_3), 9.62 (s, 1H, 2 × CONH).

2j. Yield = 42%; mp 110–112 °C; MS (*m*/*z*) = 444 (M+1); IR (KBr σ/cm^{-1}): 3366, 1681, 1603, 1530, 1492, 1267, 829, 766; ^{1}H NMR δ (*J*/Hz): (CD_3OCD_3) 4.05 (s, 3H, OCH_3), 4.65 (m, 4H, 2 × $NHCH_2$), 7.25–7.43 (m, 8H, 2 × C_6H_4), 7.28 (d, H, H-5), 8.41 (dd, H, H-6), 8.57 (d, H, H-2), 8.49 (s, H, CONH), 8.69 (s, H, CONH).

2k. Yield = 27%; mp 136–137 °C; MS (*m*/*z*) = 533 (M+1); IR (KBr σ/cm^{-1}): 3364, 1644, 1602, 1570, 1535, 1491, 1271, 1069, 764; ^{1}H NMR δ (*J*/Hz): (Acetone) 8.68 (s, 1H, NH), 8.57 (d, 1H, benzene-H), 8.49 (d, 1H, NH), 8.10 (dd, 1H, benzene-H), 7.58 (d, 2H, NHC_6H_4), 7.26–7.43 (m, 7H, benzene-H, NHC_6H_4), 4.62 (t, 4H, 2 × CH_2), 4.05 (m, 3H, OCH_3).

3a. Yield = 49%; mp 283–284 °C; MS (*m*/*z*) = 405 (M+Na); IR (KBr σ/cm^{-1}): 3339, 3272, 1678, 1650, 1614, 1562, 1508, 1222, 829; ^{1}H NMR δ (*J*/Hz): (Acetone) 9.91 (s, 1H, NH), 9.80 (s, 1H, NH), 8.60 (d, 1H, benzene-H), 8.21 (dd, 1H, benzene-H), 7.36 (d, 1H, benzene-H), 7.89 (m, 4H, NHC_6H_4), 7.15 (m, 4H, NHC_6H_4), 4.16 (s, 3H, OCH_3).

3b. Yield = 84%; mp 267–268 °C; MS (*m*/*z*) = 437 (M+Na-1); IR (KBr σ/cm^{-1}): 3291, 1681, 1595, 1550, 1493, 1261, 823; ^{1}H NMR δ (*J*/Hz): (DMSO)3.95 (s, 3H, OCH_3), 7.38–7.82 (m, 8H, 2 × C_6H_4), 7.31 (d, H, H-5), 8.13 (dd, H, H-6), 8.24 (d, H, H-2), 10.33 (d, 2H, 2 × CONH).

3c. Yield = 10%; mp 259–260 °C; MS (*m*/*z*) = 505 (M+1); IR (KBr σ/cm^{-1}): 3328, 1655, 1604, 1546, 1491, 1261, 1072, 820; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$)9.75 (s, 1H, NH), 8.14 (s, 1H, NH), 8.70 (d, 1H, benzene-H), 8.26 (dd, 1H, benzene-H), 7.21 (d, 1H, benzene-H), 7.57 (m, 4H, NHC_6H_4), 7.49 (m, 4H, NHC_6H_4), 4.17 (s, 3H, OCH_3).

3d. Yield = 52%; mp 264–265 °C; MS (*m*/*z*) = 600 (M+2); IR (KBr σ/cm^{-1}): 3333, 1656, 1586, 1532, 1489, 1260, 1060, 817; ^{1}H NMR δ (*J*/Hz): (Acetone) 9.93 (s, 1H, NH), 9.82 (s, 1H, NH), 8.57 (d, 1H, benzene-H), 8.21 (dd, 1H, benzene-H), 7.39 (d, 1H, benzene-H), 7.72 (m, 8H, NHC_6H_4), 4.16 (s, 3H, OCH_3).

3e. Yield = 48%; mp >300 °C; MS (*m*/*z*) = 459 (M+Na); IR (KBr σ/cm^{-1}): 3409, 1684, 1559, 1507, 1335, 1092, 988; ^{1}H NMR δ (*J*/*Hz*): ($CDCl_3$) 10.82 (d, 2H, 2 × NH), 8.26 (m, 1H, benzene-H), 8.21 (dd, 1H, benzene-H), 7.38 (d, 1H, benzene-H), 8.28 (m, 4H, NHC_6H_4), 8.06 (m, 4H, NHC_6H_4), 3.98 (s, 3H, OCH_3).

3f. Yield = 30%; mp 246–247 °C; MS (*m*/*z*) = 375 (M+1); IR (KBr σ/cm^{-1}): 3347, 1677, 1605, 1551, 1515, 1319, 1091, 816; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$)9.68 (s, 1H, NH), 8.71 (s, 1H, NH), 8.25 (s, 1H, benzene-H), 7.99 (s, 1H, benzene-H), 7.26 (dd, 1H, benzene-H), 7.55 (t, 4H, NHC_6H_4), 7.20 (m, 4H, NHC_6H_4), 4.14 (s, 3H, OCH_3), 2.35 (d, 6H, 2 × CH_3).

3g Yield = 72%; mp 218–220 °C; MS (*m*/*z*) = 407 (M+1); IR (KBr σ/cm^{-1}): 3352, 1665, 1638, 1605, 1535, 1510, 828; ^{1}H NMR δ (*J*/

Hz): ($CDCl_3$) 9.65 (s, 1H, NH), 8.70 (d, 1H, benzene-H), 8.24 (m, 1H, benzene-H), 7.96 (s, 1H, NH), 7.57 (m, 4H, 22′, 66′2 × NHC_6H_4), 7.17 (d, 1H, benzene-H), 6.92 (m, 4H, 33′, 55′2 × NHC_6H_4), 4.14 (s, 3H, OCH_3), 3.82 (d, 6H, 2 × CH_3).

3h. Yield = 79%; mp 250–252 °C; MS (*m*/*z*) = 431 (M+1); IR (KBr σ/cm^{-1}): 3348, 1676, 1592, 1527, 1491, 1268, 838; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 9.90 (s, 1H, NH), 8.71 (d, 1H, benzene-H), 8.37 (s, 1H, NH), 8.23 (q, 1H, benzene-H), 7.96 (d, 4H, NHC_6H_4), 7.76 (q, 4H, NHC_6H_4), 7.17 (d, 1H, benzene-H), 4.16 (s, 3H, OCH_3), 2.58 (s, 6H, 2 × CH_3).

3i. Yield = 70%; mp 243–245 °C; MS (*m*/*z*) = 435 (M+1); IR (KBr σ/cm^{-1}): 3306, 2981, 1650, 1600, 1511, 1233, 1046, 823; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 9.60 (s, 1H, NH), 8.66 (s, 1H, benzene-H), 8.18 (d, 1H, benzene-H), 8.02 (s, 1H, NH), 7.53 (m, 4H, 22′, 66′2 × -NHC_6H_4), 7.12 (d, 1H, benzene-H), 6.88 (m, 4H, 33′, 55′, 2 × NHC_6H_4), 4.09 (s, 3H, OCH_3), 4.01 (m, 4H, 2 × CH_2CH_3), 1.40 (m, 6H, 2 × CH_2CH_3).

3j. Yield = 75%; mp 226–228 °C; MS (*m*/*z*) = 463 (M+1); IR (KBr σ/cm^{-1}): 3349, 2963, 2876, 1650, 1601, 1508, 1233, 1068, 828; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 9.63 (s, 1H, NH), 8.70 (s, 1H, benzene-H), 8.23 (d, 1H, benzene-H), 8.05 (s, 1H, NH), 7.55 (m, 4H, 22′, 66′2 × NHC_6H_4), 7.15 (d, 1H, benzene-H), 6.91 (m, 4H, 33′, 55′2 × -NHC_6H_4), 4.13 (s, 3H, OCH_3), 3.92 (m, 4H, 2 × OCH_2), 1.80 (m, 4H, 2 × CH_2), 1.05 (m, 6H, 2 × CH_3).

3k. Yield = 68%; mp 200–202 °C; MS (*m*/*z*) = 463 (M+1); IR (KBr σ/cm^{-1}): 3257, 2977, 1592, 1508, 1484, 1459, 1399, 1251, 824; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 9.64 (s, 1H, NH), 8.71 (d, 1H, benzene-H), 8.23 (d, 1H, benzene-H), 8.15 (s, 1H, NH), 7.56 (m, 4H, 22′, 66′2 × NHC_6H_4), 7.17 (d, 1H, benzene-H), 6.90 (m, 4H, 33′, 55′2 × NHC_6H_4), 4.54 (m, 2H, 2 × OCH), 4.14 (s, 3H, OCH_3), 1.34 (m, 12H, 4 × CH_3).

3l. Yield = 26%; mp >300 °C; MS (*m*/*z*) = 491 (M+1); IR (KBr σ/cm^{-1}): 3353, 2957, 2871, 1661, 1611, 1512, 1464, 1411, 1245, 825; ^{1}H NMR δ (*J*/Hz): (DMSO)1.01 (m, 6H, 2 × $C_3H_6CH_3$), 1.15 (m, 4H, 2 × $C_2H_4CH_2CH_3$), 1.17 (m, 4H, 2 × $CH_2CH_2C_2H_5$), 3.95 (m, 4H, 2 × $CH_2C_3H_6$), 8.25 (d, 1H, H-2), 4.10 (s, 3H, OCH_3), 8.67 (d, 1H, H-2), 8.21 (dd, 1H, H-6), 7.12 (d, 1H, H-5), 7.54 (d, 4H, 2 × C_6H_4), 6.89 (m, 4H, 2 × C_6H_4), 9.62 (s, 1H, CONH), 8.10 (s, 1H, CONH).

4a. Yield = 68%; mp 232–234 °C; MS (*m*/*z*) = 347 (M+1); IR (KBr σ/cm^{-1}): 3339, 3262, 3060, 1679, 1598, 1497, 1320, 1264, 829; ^{1}H NMR δ (*J*/Hz): (DMSO-d_6) 3.93 (s, 3H, OCH_3), 7.04–7.77 (m, 8H, 2 × C_6H_4), 7.29 (d, 1H, 5-H), 8.11 (dd, 1H, 6-H), 8.27 (d, 1H, 2-H), 10.19 (d, 2H, 2 × CONH).

4b. Yield = 65%; mp 152–153 °C; MS (*m*/*z*) = 375 (M+1); IR (KBr σ/cm^{-1}): 3399, 1649, 1638, 1534, 1492, 1274, 1100, 1004, 721; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 3.976 (s, 3H, OCH_3), 4.649 (dd, 4H, 2 × CH_2-Ar), 6.659 (s, 1H, CONH), 7.062 (d, 1H, 5-H), 7.259–7.298, 7.337–7.347 (m, 10H, 2 × C_6H_4), 8.147 (s, 1H, CONH), 8.158 (dd, 1H, 6-H), 8.542 (d, 1H, 2-H).

4c. Yield = 60%; mp 152–154 °C; MS (*m*/*z*) = 403 (M+1); IR (KBr σ/cm^{-1}): 3370, 2928, 1639, 1603, 1529, 1493, 1455, 1279, 1013, 765, 748; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$)3.79 (s, 3H, OCH_3), 2.91 (t, 4H, 2 × $NHCH_2CH_2$), 3.71 (q, 4H, 2 × $NHCH_2CH_2$), 7.23–7.37 (m, 10H, 2 × C_6H_5), 6.97 (d, 1H, H-5), 8.05 (dd, 1H, H-6), 8.42 (d, 1H, H-2), 6.38 (t, 1H, CONH), 7.86 (t, 1H, CONH).

4d. Yield = 38%; mp 104–105 °C; MS (*m*/*z*) = 403 (M+1); IR (KBr σ/cm^{-1}): 3394, 2974, 1641, 1604, 1530, 1491, 1451, 1265, 827; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$)8.49 (s, 1H, benzene-H), 8.15 (d, 1H, benzene-H), 8.12 (d, 1H, benzene-H), 7.30 (s, 10H, 2 × C_6H_5), 7.07 (d, 1H, –NH–), 6.55 (d, 1H, NH), 4.01 (s, 3H, OCH_3), 1.59 (t, 6H, 2 × CH_3).

4e. Yield = 39%; mp 248–250 °C; MS (*m*/*z*) = 447 (M+1); IR (KBr σ/cm^{-1}): 3366, 3265, 1655, 1600, 1557, 1486, 1272, 825; ^{1}H NMR δ (*J*/Hz): (DMSO)10.49 (s, 1H, NH), 10.38 (s, 1H, NH), 8.61 (d, 1H, benzene-H), 8.30 (d, 1H, benzene-H), 8.14 (d, 1H, naph-H), 7.81–8.01 (m, 7H, naph-H), 7.54–7.65 (m, 7H, naph-H), 7.43 (d, 1H, benzene-H), 4.14 (s, 3H, OCH_3).

4f. Yield = 77%; mp 232–234 °C; MS (*m*/*z*) = 371 (M+Na); IR (KBr σ/cm^{-1}): 3345, 1668, 1601, 1577, 1495, 1432, 1262, 829, 755; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 10.59 (s, 1H, NH), 10.43 (s, 1H, NH), 8.91 (d, 1H, benzene-H), 8.69 (d, 1H, NHC_6H_4), 8.53 (d, 1H, NHC_6H_4), 8.47 (q, 1H, benzene-H), 8.29 (m, 2H, NHC_6H_4), 7.99 (t, 1H, NHC_6H_4), 7.84 (t, 1H, NHC_6H_4), 7.21 (m, 2H, NHC_6H_4), 7.12 (m, 1H, benzene-H), 4.19 (s, 3H, OCH_3).

4g Yield = 75%; mp 136–138 °C; MS (*m*/*z*) = 355 (M+1); IR (KBr σ/cm^{-1}): 3438, 3384, 2925, 1653, 1604, 1530, 1490, 1263, 1244; ^{1}H NMR δ (*J*/Hz): (Acetone) 8.57 (d, 1H, benzene-H), 8.45 (d, 1H, NH), 8.31 (s, 1H, NH), 8.08 (dd, 1H, benzene-H), 7.47 (d, 2H, furan), 7.25 (d, 1H, benzene-H), 6.36 (m, 2H, furan), 6.29 (s, 2H, furan), 4.59 (d, 4H, 2 × CH_2), 4.06 (s, 3H, OCH_3).

4h. Yield = 37%; mp 180–182 °C; MS (*m*/*z*) = 491 (M+1); IR (KBr σ/cm^{-1}): 3375, 1643, 1602, 1531, 1489, 1249, 1041, 924, 802; ^{1}H NMR δ (*J*/Hz): ($CDCl_3$) 8.40 (d, 1H, benzene-H), 8.04 (dd, 1H, benzene-H), 7.81 (m, 1H, NH), 6.98 (d, 1H, benzene-H), 6.64–6.77 (m, 6H, Ar-H), 6.34 (s, 1H, NH), 5.91 (d, 4H, 2 × OCH_2O), 3.85 (s, 3H, OCH_3), 3.65 (m, 4H, 2 × CH_2), 2.81 (m, 4H, 2 × CH_2).

Melting points were determined with a Kofler micro melting point apparatus and the thermometer was uncorrected; All the Fourier transform infra red (FTIR) spectra were recorded on a Brukers Vector 22 spectrophotometer; ^{1}H NMR spectra were recorded with a Bruker ARX-300 spectrometer, Tetramethyl silane was used as an internal standard; MS spectra were recorded on Agilent 6310 Ion Trap and Shimadzu LCMS.

The in vitro activity studies on anti-platelet aggregation of 41 target compounds have been done by using Born test[8,9]: Venous blood gathering from the rat eye socket vein was collected into

Table 2
The in vitro activities on anti-platelet aggregation of the synthesized compounds

Compd.	Dose (μmol L^{-1})	IC_{50} (μmol L^{-1})	Compd.	Dose (μmol L^{-1})	IC_{50} (μmol L^{-1})	Compd.	Dose (μmol L^{-1})	IC_{50} (μmol L^{-1})
Control group of solvent	–	–	**2e**	1.30	–	**3i**	1.30	–
1a	1.30	0.86	**2f**	1.30	–	**3j**	1.30	–
1b	1.30	–	**2g**	1.30	0.49	**3k**	1.30	–
1c	1.30	0.66	**2h**	1.30	–	**3l**	1.30	0.49
1d	1.30	0.30	**2i**	1.30	0.81	**4a**	1.30	0.77
1e	1.30	–	**2j**	1.30	–	**4b**	1.30	0.58
1f	1.30	–	**2k**	1.30	–	**4c**	1.30	0.24
1g	1.30	–	**3a**	1.30	0.60	**4d**	1.30	–
1h	1.30	–	**3b**	1.30	–	**4e**	1.30	–
1i	1.30	0.02	**3c**	1.30	0.51	**4f**	1.30	–
1j	1.30	0.59	**3d**	1.30	0.49	**4g**	1.30	–
2a	1.30	–	**3e**	1.30	0.75	**4h**	1.30	–
2b	1.30	–	**3f**	1.30	0.64	Picotamide	1.30	0.76
2c	1.30	–	**3g**	1.30	–			
2d	1.30	–	**3h**	1.30	0.67			

tubes which was containing 3.8% sodium citrate (1:9, v/v). Platelet aggregation was assessed in platelet-rich plasma (PRP), obtained by centrifugation of citrated whole blood at room temperature for 10 min. (500–800 rpm). The aggregation rate was measured by platelet aggregation analyzer after stimulation with ADP (5 mM.) using platelet-poor plasma (PPP) to set zero. The PPP was obtained by centrifugation of PRP at room temperature for 15 min. (3000 rpm). The solution of the new compounds dissolved in DMSO or Chloroform (5 μL) was added into PRP (200 μL), and the same volume of DMSO or Chloroform with no test compound was added as a reference sample (according to the pre-experiment, 5 μL of DMSO or Chloroform appears no significant effect on the platelet aggregation). After 2 min incubating, assessed the platelet aggregation activities and calculated the percentage inhibition of platelet aggregation using the corresponding ADP. The in vitro activities on anti-platelet aggregation of the synthesized compounds are given in Table 2.

A total of 41 4-methoxybenzene-1,3-isophthalamides were designed and synthesized, and their chemical structures were confirmed by MS, IR and ^{1}H NMR spectroscopy. The result in vitro activities on anti-platelet aggregation assessed by using Born test method on rats in Table 2 showed that thirteen compounds **1c**, **1d**, **1i**, **1j**, **2g**, **3a**, **3c**, **3d**, **3f**, **3h**, **3l**, **4b** and **4c** have significant anti-platelet aggregation activities of all target compounds, far superior than the reference drug Picotamide, and the IC_{50} value of the compound **1i** is the lowest. The SAR analysis indicated: comparison of the series 1, series 3 with series 2, the compounds with *ortho*-substituted-phenyl (series 1) and *para*-substituted-phenyl (series 3), especially the halogen groups F, Br and I, have better activity values than the *meta*-substituted-phenyl compounds of series 2.

1. The compounds **1a**, **1c**, **1d**, **1i** and **1j** of the series 1 have obvious anti-platelet aggregation activities, the order of different substituents is: CH_2Ph (**1i**) > I(**1d**) > CH_2CN(**1j**) > Br(**1c**) > F(**1a**) >the other compounds.
2. The compounds of series 2 almost have no obvious anti-platelet aggregation activities except compounds **2g** and **2i**, and the result indicated that relatively strong electron-withdrawing substituents, halogens and weak electron-donating substituents in *meta*-positions almost could not help to promote the anti-platelet aggregation activities.
3. The activity order of different substituents of series 3 is: I(**3d**) = $OCH_2CH_2CH_2CH_3$(**3l**) > Br(**3c**) > F(**3a**) > CH_3(**3f**) > $COCH_3$(**3h**) > NO_2(3e) > the other compounds. The result indicated that when strong electron-withdrawing substituents and halogens in *para*-positions, the anti-platelet aggregation activities increased significantly. The result of the comparison of different alkoxy substituted groups in series 3 indicated that each compound almost has no obvious activity except compound **3l** ($OCH_2CH_2CH_2CH_3$).
4. The activity order of different substituents of series 4 is: CH_2CH_2Ph(**4c**) > CH_2Ph(**4b**) > Ph(**4a**) > substituted compounds, of which aromatic heterocyclic substituted compounds (**4e**–**4h**) almost have no anti-platelet aggregation activity.

The preliminary SAR shows that it is favorite for the series 1 and series 3 to increase anti-platelet aggregation activities via steric hindrance groups, such as I and CH_2Ph attached to the two side chain benzene rings in *ortho*-positions. On the contrary, electrostatic factors looks as if would not contribute to the greater activities of the four series. Further work is needed to better define the role of these groups and other groups involved in the four series or other interactions.

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