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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro activities on anti-platelet aggregation of 4-methoxyisophthalamides

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Abstract

A series of 4-methoxyisophthalamides (1d–1w) were designed and synthesized and their chemical structures were confirmed by IR, MS, ^{1}H-NMR, and ^{13}C-NMR. The in vitro on anti-platelet aggregation activities of these compounds were assessed by using Born method. Compounds with higher activities were selected to continue research via Cell Counting Kit-8 (CCK-8) assays of their cytotoxicities. Biological screening results revealed four compounds 1h, 1i, 1q, and 1v exhibited higher activities than the control drugs on against the platelet aggregation induced by adenosine triphosphate (ADP). Moreover, compounds 1p and 1q exhibited higher in vitro activities than picotamide induced by collagen at the concentration of 1.3 μM. Compound 1p also possessed anti-platelet aggregation activity superior to the control drug picotamide induced by arachidonic acid (AA) at the concentration of 1.3 μM. At the same time, the result of cytotoxicities exhibited that none of the compounds have significant cytotoxicities. Therefore, 4-methoxyisophthalamides are potential to become novel anti-platelet drugs with high activities and minimum toxicities.

Keywords 4-Methoxyisophthalamides · Anti-platelet aggregation · Synthesis · Picotamide · Structure activity relationship · Cytotoxicity assay

Abbreviations

ADP	Adenosine triphosphate
AA	Arachidonic acid
CCK-8	Cell Counting Kit-8
DMSO	Dimethyl sulfoxide
PAD	Peripheral arterial disease
PGI_2	Prostaglandin I_2
SAR	Structure activity relationship
TLC	Thin layer chromatography
TXA_2	Thromboxane A_2.

Introduction

Anti-platelet drugs are typically used in the clinical treatment of anti-thrombosis and prevention of thrombosis. Thrombosis that is caused secondary to disrupted atherosclerotic plaques is the initiator of most cardiovascular diseases including heart attacks and strokes. It is well-known that platelets play a major role in the pathogenesis of atherothrombosis (Brito et al. 2010; Eskandariyan et al. 2014). Picotamide (Figs. 1–1) is an anti-platelet drug with a dual inhibitory action, which inhibits both thromboxane A_2 (TXA_2) receptors and TXA_2 synthase (Balsano and Violi 1993; Berrettini et al. 1990; Violi et al. 1988; Yip and Benavente 2011). Meanwhile, compared with aspirin, picotamide has the benefit of not interfering with endothelial prostacyclin (PGI_2) production (Celestini and Violi 2007; Sartori et al. 1993). Recent research had indicated that picotamide is more effective than aspirin in reducing total mortality in patients with type II diabetes associated

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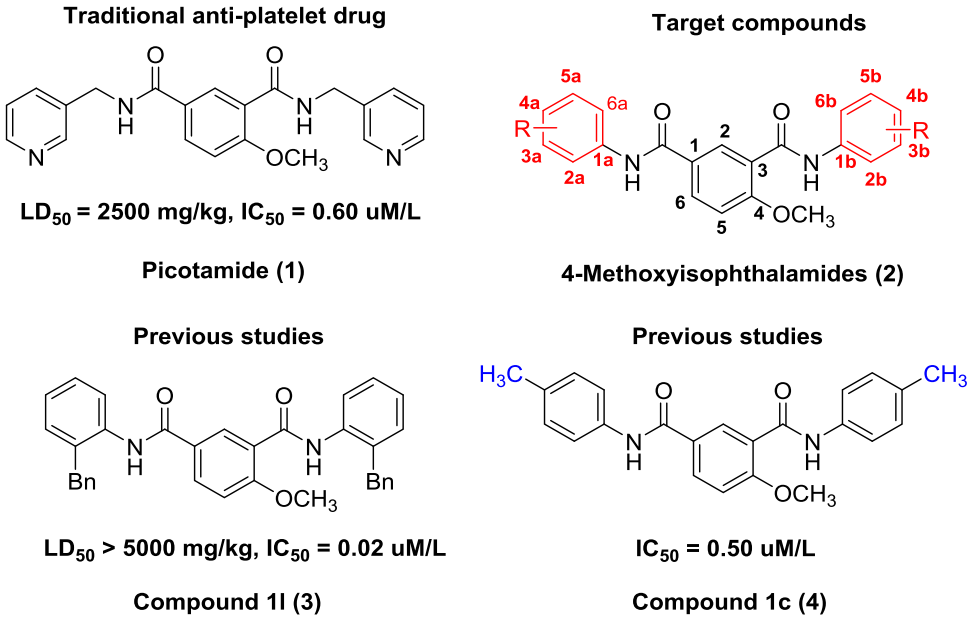


Fig. 1 Structures of Picotamide (1), target compounds (2), compound **1l** (3) and compound **1c** (4)

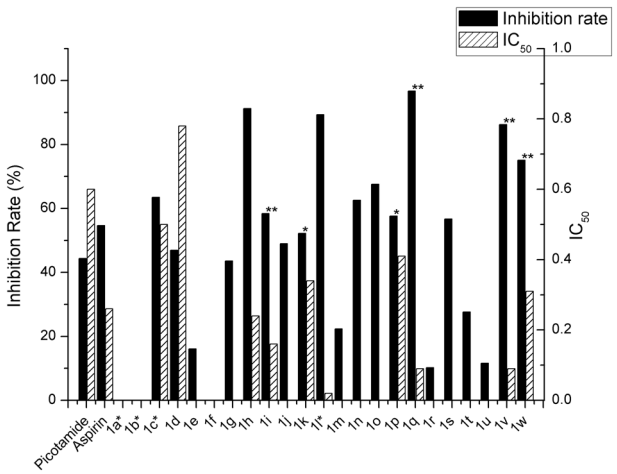


Fig. 2 The Inhibition Rate and IC_{50} of Compounds

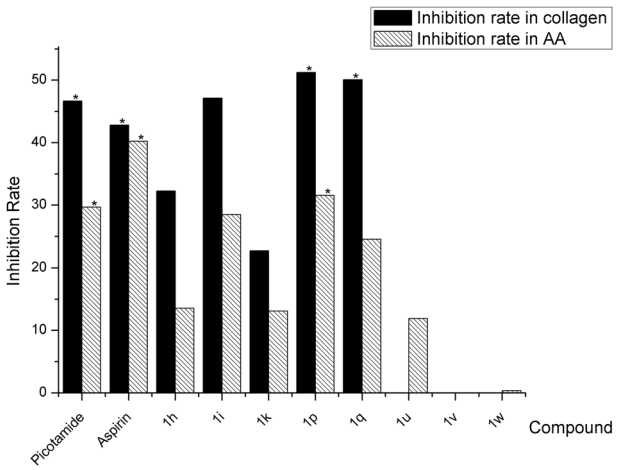


Fig. 3 The Inhibition Rate and IC_{50} of Compounds for collagen and AA

with peripheral arterial disease (PAD) (Neri Serneri et al. 2005).

Since 2000, we have concerned on the anti-platelet aggregating activity studies of various series of 4-methoxyisophthalamides (Figs. 1–2) and stated the relevant report (Liu et al. 2006, 2011, 2012). Among those compounds, nearly 30% compounds exhibited significant anti-platelet aggregation activities than picotamide. In particularly, compound **1l** (Figs. 1–3) with benzyl groups attached to the phenyl rings in the parent compound showed excellent activity against the platelet aggregation induced by ADP, and its IC_{50} value is as low as 0.02 μM/L and its LD_{50} is over 5000 mg/kg. It is 30 times as pictoamide's ($IC_{50} = 0.60$ μM/L, $LD_{50} = 2500$ mg/kg) (Liu et al. 2012, 2017a). All the data justify that 4-methoxyisophthalamides have the potential of becoming new anti-platelet drugs. Nonetheless, researches of alkyl substituted 4-methoxyisophthalamide are insufficient. And compound **1c** (Figs. 1–4) with methyl groups in the *p*-position of the side chain phenyl rings showed moderate anti-platelet activity, which aroused our interest to continue to research.

To search for the new anti-platelet agents and perfect the SAR of 4-methoxyisophthalamides, the depth and breadth of research for the 4-methoxyisophthalamides have been enhanced and expanded. Among which in the alkyl, the original limited to methyl research extended to ethyl, isopropyl, n-/t-butyl and for unsaturated ethynyl and cyano synthesis and research for the first time. Total 19 novel compounds (**1d–1w**) were designed and synthesized. Compounds **1d–1k** with alkyl ($-CH_2CH_3$ $-CH(CH_3)_2$ –

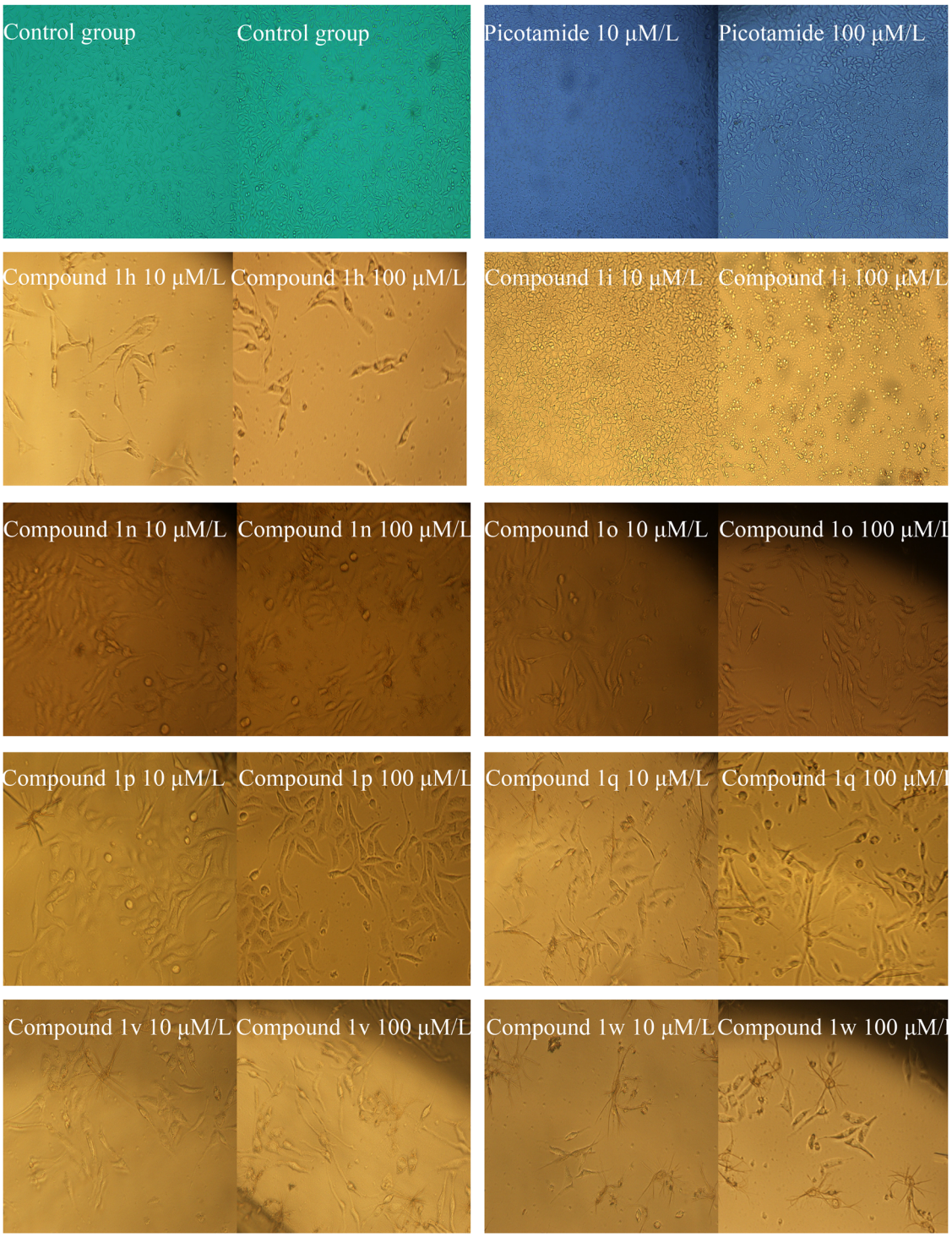


Fig. 4 The cell morphology of L-929 following 48 h incubation

$(CH_2)_3CH_3$ $-C(CH_3)_3$) in different positions were synthesized to investigate the effect of increasing the steric hindrance of the substitutions on anti-platelet aggregation activity. Compound **1m** with ethynyl (–C≡CH) was synthesized to investigate the effect of different hybridization substituent having an unsaturated bond on anti-platelet activity. We also try to increase the electron-withdrawing of the substitutions, such as cyano (–C≡N) and trifluoromethyl ($-CF_3$) to synthesize the compounds **1n**, **1o**, **1p**, **1q**, **1r**, **1s** to investigate the effect of their introducing on anti-platelet aggregation. In order to investigate the effect of the number of substitution on anti-platelet aggregation activity,

compounds **1t** and **1u** were designed and synthesized by increasing the number of alkyl on the phenyl ring. In order to investigate the effect of introducing chlorine on anti-platelet aggregation, we try to introduce methyl and chlorine on the phenyl ring to synthesize the compounds **1v** and **1w**.

The chemical structures of target compounds were confirmed by IR, MS, ^{1}H-NMR, and ^{13}C-NMR. Their inhibitory effects on platelet aggregation were tested and assessed by Born method for ADP inducer. Ten compounds that were significantly more active in vitro than control drug picotamide were selected and continued to test and assess for two inducers (collagen and arachidonic acid). And the compounds with higher activities on anti-platelet aggregation were selected to continue research of their cytotoxicities.

Materials and methods

General experimental techniques

All reagents for the synthesis of 4-methoxyisophthalamides were purchased (purity > 99%) and used without further purification. The melting points were determined with X-4 digital micro melting point apparatus (thermometer was uncorrected); Mass spectra (MS) were measured on Xevo GZQ-Tof VHRS mass spectrometer; IR spectra were recorded on Avatar 370 spectrophotometer; ^{1}H-NMR spectra were determined with AVANCE III (400 MHz) spectrometer (Tetramethyl silane as an internal standard); ^{13}C-NMR spectra were determined with AVANCE III (101 MHz) spectrometer.

Chemistry

Synthetic routes of 4-methoxylisophthaloyl dichloride

Route a The reaction of phenol **1** with formaldehyde and hydrochloric acid in benzene gave 2,4-bis (chloromethyl) phenol **2**, which reacted with dimethyl sulfate in water gave 2,4-bis (chloromethyl) -1-methoxybenzene **3**. Then compound **3** was oxidized with potassium permanganate, 4-methoxyisophthalic acid **4** was obtained. Finally, using compound **4** reacted with sulfoxide chloride, a white solid 4-methoxyisophthaloyl dichloride **5** was obtained.

Route b 4-Methylphenol **1'** was used as starting material. Acetylation of **1'** with acetic anhydride gave 4-methylphenyl acetate **2'**. And then compound **2'** was subjected to Fries rearrangement to 1- (2-hydroxy-5-methylphenyl) ethan-1-one **3'** in the presence of $AlCl_3$ (Shaikh et al. 2016). The reaction of compound **3'** with dimethyl sulfate gave 2,4-bis (chloromethyl) -1-methoxybenzene **4'**. Then the methyl and acetyl of compound **4'** was oxidized with potassium permanganate, 4-methoxyisophthalic acid 4 was obtained (Liu et al. 2017b). At least, compound **4** reacted with sulfoxide chloride, a white solid 4-methoxyisophthaloyl dichloride **5** was obtained.

Synthetic route of 4-methoxyisophthalamides

N^1,N^3-bis(2-ethylphenyl)-4-methoxyisophthalamide (1d) A round-bottomed flask (100 ml) equipped with a magnetic stirrers was charged with 2-ethylaniline (10.0 mmol, 1.2 g) in anhydrous tetrahydrofuran (15 ml). The fresh 4-methoxyisophthaloyl dichloride **5** (5.0 mmol, 1.2 g) in dry tetrahydrofuran (15 ml) was added drop-wise to the above solution at room temperature. Ten hours after the addition of compound **5**, dry triethylamine (1 ml) was added drop-wise to the reaction mixture. The resultant mixture reaction was stirred for 24 h at room temperature. After the reaction completed as monitored by thin layer chromatography (TLC). The solvent was removed under reduced pressure to obtain the crude product. The crude product was further purified by recrystallization from anhydrous ethanol to yield the desired product **1d** as white solid. Yield: 55.00%, m.p.: 175.0~176.0 °C.

Compounds **1e**–**1w** were prepared in the similar manner.

N^1,N^3-bis(2-methylphenyl)-4-methoxyisophthalamide (1a) White solid (The residue was recrystallized by acetone.) Yield: 41.00%; m.p.: 218.0–220.0 °C; IR (KBr) υ_{max} 3347, 1677, 1605, 1551, 1319, 1091, 816 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.77 (1H, s, CONH), 8.80 (1H, d, J = 2.2 Hz, 2-H), 8.24 (2H, m, Ar–H), 7.99 (1H, s CONH), 7.82 (1H, s, 2a-H), 7.30 (1H, s, 2b-H), 7.19 (6H, m, Ar–H), 4.16 (3H, s, OCH_3), 2.36 (3H, s, Ar–$\underline{CH_3}$), 2.38 (3H, s, Ar–$\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.2 (C, C = O), 159.5 (C, 4-C), 136.1 (C, 1a-C), 135.5 (C, 1b-C), 134.8 (CH, 6-C), 133.9 (C, 2a-C), 133.8(C, 2b-C), 130.1 (C, 1-C), 128.6 (CH, 3a-C), 128.4 (CH, 3b-C), 128.0 (C, 3-C), 126.7 (CH, 4a-C), 126.5 (CH, 4b-C), 125.8 (CH, 2-C), 125.0(CH, 5a-C), 124.3 (CH, 5b-C), 122.9 (CH, 6a-C), 121.4 (CH, 6b-C), 112.1 (CH, 5-C), 58.3 (CH_3, OCH_3), 14.1 (CH_3, Ar–$\underline{CH_3}$), 13.8 (CH_3, Ar–$\underline{CH_3}$); MS (*m/z*): 375.1063 $[M+H]^+$ (Liu et al. 2012).

N^1,N^3-bis(3-methylphenyl)-4-methoxyisophthalamide (1b) White solid (The residue was recrystallized by acetone.) Yield: 51.00%; m.p.: 179.0–180.0 °C; IR (KBr) υ_{max} 3264, 1618, 1593, 1521, 1487, 1236, 1070, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.72 (1H, s, CONH), 8.71 (1H, d, J = 1.9 Hz, 2-H), 8.24 (1H, dd, J = 1.0, 4.0 Hz, 6-H), 7.99 (1H, d J = 1.0 Hz, 2-H), 7.26 (1H, d, J = 4.0 Hz, 5-H), 7.55 (4H, m, 2a-H, 2b-H, 6a-H, 6b-H), 7.20 (4H, m, 3a-H, 3b-H, 5a-H, 5b-H), 4.14 (3H, s, OCH_3), 2.35 (6H, s, 2 × Ar–$\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.2 (C,

C = O), 162.3 (C, C = O), 159.4 (C, 4-C), 140.7 (C, 3a-C), 140.4 (C, 3b-C), 135.6 (C, 1a-C), 135.5 (C, 1b-C), 133.7 (CH, 6-C), 130.0 (C, 1-C), 128.3 (CH, 5a-C), 128.2 (CH, 5b-C), 128.1 (CH, 2-C), 121.2 (CH, 2a-C, 2b-C), 120.7 (CH, 4a-C, 4b-C), 120.4 (CH, 6a-C, 6b-C), 112.1 (C, 3-C), 58.4 (CH_3, OCH_3), 15.6 (CH_3, Ar–$\underline{CH_3}$), 15.6 (CH_3, Ar–$\underline{CH_3}$); MS (*m/z*): 375.1065 $[M+H]^+$ (Liu et al. 2012).

N^1,N^3-bis(4-methylphenyl)-4-methoxyisophthalamide (1c)

White solid (The residue was recrystallized by acetone); Yield: 30.00%; m.p.: 246.0–247.0 °C; IR (KBr) υ_{max} 3264, 1618, 1593, 1521, 1487, 1236, 1070, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.68 (1H, s, CONH), 8.71 (1H, s, 2-H), 8.25 (1H, dd, *J* = 1.9, 3.8 Hz, 6-H), 7.99 (1H, s CONH), 7.53 (2H, s, 2a-H, 2b-H), 7.45 (2H, m, 6a-H, 6b-H), 7.30 (1H, d, *J* = 3.8 Hz, 5-H), 7.27 (1H, m, 5a-H), 7.18 (1H, m, 5b-H), 6.99 (2H, m, 4a-H, 4b-H), 4.16 (3H, s, OCH_3), 2.39 (6H, s, 2 × Ar–$\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.2 (C, C = O), 162.3 (C, C = O), 159.0 (C, 4-C), 140.5 (C, 4a-C), 140.4 (C, 4b-C), 135.8 (C, 1a-C), 135.6 (C, 1b-C), 133.7 (CH, 6-C), 129.9 (C, 1-C), 128.4 (2 × CH, 3a-C, 5a-C), 128.3 (2 × CH, 3b-C, 5b-C), 127.9 (CH, 2-C), 121.0 (C, 3-C), 120.4 (2 × CH, 2a-C, 6b-C), 120.2 (2 × CH, 2b-C, 6b-C), 112.9 (CH, 5-C), 65.5 (CH_3, OCH_3), 15.6 (2 × CH_3, Ar–$\underline{CH_3}$); MS (m/z): 375.1064 $[M+H]^+$ (Liu et al. 2012).

N^1,N^3-bis(2-ethylphenyl)-4-methoxyisophthalamide (1d)

White solid (The residue was recrystallized by anhydrous ethanol.) Yield: 55.00%; m.p.: 175.0–176.0 °C; IR (KBr) υ_{max} 3354.80, 2960.69, 1659.50, 1597.94, 1530.14, 1463.90, 1256.00, 753.97, 627.80 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.76 (1H, s, CONH), 8.80 (1H, d, *J* = 2.4 Hz, 2-H), 8.24 (1H, dd, *J* = 8.7, 2.4 Hz, 6-H), 8.19 (1H, d, *J* = 8.0 Hz, 5-H), 7.95 (1H, s, CONH), 7.83 (1H, d, *J* = 7.8 Hz, Ar–H), 7.29–7.23 (3H, m, Ar–H), 7.21–7.10 (4H, m, Ar–H), 4.14 (3H, s, OCH_3), 2.71 (4H, m, 2 × $\underline{CH_2}CH_3$), 1.34–1.24 (6H, m, 2 × $CH_2\underline{CH_3}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.7 (C, C = O), 162.5 (C, C = O), 159.8 (C, 4-C), 136.3 (C, 1a-C), 135.8 (C, 1b-C), 135.1 (CH, 6-C), 134.2 (2 × C, 2a-C, 2b-C), 130.4 (C, 1-C), 128.8 (CH, 4a-C), 128.2 (CH, 4b-C), 126.9 (CH, 5a-C), 126.1 (CH, 5b-C), 125.3 (2 × CH, 3a-C, 3b-C), 124.5 (CH, 2-C), 123.2 (CH, 5-C), 121.6 (C, 3-C), 112.4 (2 × CH, 6a-C, 6b-C), 56.8 (CH, O-CH_3), 25.0 (CH_2, Ar–$\underline{CH_2}CH_3$), 24.5 (CH_2, Ar–$\underline{CH_2}CH_3$), 14.4 (CH_3, Ar–$CH_2\underline{CH_3}$), 14.1 (CH_3, Ar–$CH_2\underline{CH_3}$); MS (*m/z*): 403.2042 $[M+H]^+$.

N^1,N^3-bis(3-ethylphenyl)-4-methoxyisophthalamide (1e)

White solid (The residue was recrystallized by 75% ethanol.) Yield: 82.85%; m.p.: 219.2–221.7 °C; IR (KBr) υ_{max} 3368.70, 2958.00, 2863.60, 1665.00, 1553.00, 1441.09, 1262.70, 1013.88, 785.75 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.69 (1H, s, CONH), 8.70 (1H, d, *J* = 2.3 Hz, 2-H), 8.22 (1H, dd, *J* = 8.7, 2.3 Hz, 6-H), 8.17 (1H, s, CONH), 7.57 (4H, dd, *J* = 8.2, 3.4 Hz, Ar–H), 7.21 (4H, dd, *J* = 8.2, 3.2 Hz, Ar–H), 7.14 (1H, d, *J* = 8.7 Hz, 5-H), 4.12 (3H, s, OCH_3), 2.69–2.61 (4H, m, 2 × $\underline{CH_2}CH_3$), 1.25 (6H, td, *J* = 7.6, 2.7 Hz, 2 × $CH_2\underline{CH_3}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.3 (C, C = O), 162.5 (C, C = O), 159.6 (C, 4-C), 140.8 (C, 1a-C), 140.6 (C, 1b-C), 135.8 (C, 3a-C), 135.7 (C, 3b-C), 133.9 (CH, 6-C), 130.1 (C, 1-C), 128.5 (2 × CH, 5a-C, 5b-C), 128.4 (2 × CH, 2a-C, 2b-C), 128.3 (CH, 2-C), 121.4 (2 × CH, 2a-C, 2b-C), 120.9 (2 × CH, 6a-C, 6b-C), 120.6 (C, 3-C), 112.3 (C,5-C), 56.7 (CH_3, O-CH_3), 28.4 (2 × CH_2, Ar$\underline{CH_2}CH_3$), 15.7 (2 × CH_3, Ar$CH_2\underline{CH_3}$); MS: (*m/z*): 403.2031 $[M+H]^+$.

N^1,N^3-bis(4-ethylphenyl)-4-methoxyisophthalamide (1f)

Compound (1f): White solid (The residue was recrystallized by anhydrous ethanol.) Yield: 65.30%; m.p.: 221.0–222.5 °C; IR (KBr) υ_{max} 3358.42, 2961.45, 2929.90, 2871.20, 1674.39, 1652.30, 1601.80, 1517.70, 1319.96, 1264.69, 1118.60 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.65 (1H, s, CONH), 8.65 (1H, d, *J* = 2.0 Hz, 2-H), 8.33 (1H, s, CONH), 8.15 (1H, dd, *J* = 8.6, 2.0 Hz, 6-H), 7.55 (4H, dd, *J* = 14.3, 8.3 Hz, 2a-H, 6a-H, 2b-H, 6b-H), 7.18 (4H, d, *J* = 8.2 Hz, 3a-H, 5a-H, 3b-H, 5b-H), 7.06 (1H, d, *J* = 8.7 Hz, 5-H), 4.05 (3H, s, OCH_3), 2.64 (4H, q, *J* = 7.4 Hz, 2 × Ar–$\underline{CH_2}CH_3$), 1.24 (6H, t, *J* = 7.6 Hz, 2 × $CH_2\underline{CH_3}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.4 (C, C=O), 162.4 (C, C=O), 159.1 (C, 4-C), 140.6 (C, 1a-C), 140.6 (C, 1b-C), 135.9 (C, 4a-C), 135.8 (C, 4b-C), 133.9 (CH, 6-H), 130.1 (C, 1-C), 128.6 (2 × CH, 2a-C, 2b-C), 128.4 (2 × CH, 6a-C, 6b-C), 128.1 (CH, 2-H), 121.2 (C, 3-C), 120.6 (2 × CH, 3a-C, 3b-C), 120.4 (2 × CH, 5a-C, 5b-C), 113.0 (CH, 5-C), 65.7 (CH_3, OCH_3), 28.5 (CH_2, Ar–$\underline{CH_2}CH_3$), 28.5 (CH_2, Ar–$\underline{CH_2}CH_3$), 15.7 (CH_3, Ar–$CH_2\underline{CH_3}$), 14.9 (CH_3, Ar–$CH_2\underline{CH_3}$); MS (*m/z*): 403.2031 $[M+H]^+$.

N^1,N^3-bis(2-isopropylphenyl)-4-methoxyisophthalamide (1g)

White needles (The residue was recrystallized by anhydrous ethanol.) Yield: 70.83%; m.p.: 154.8–156.0 °C; IR (KBr) υ_{max} 3376.10, 3270.50, 2961.18, 1656.57, 1527.50, 1467.14, 1265.48 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.76 (1H, s, CONH), 8.82 (1H, d, *J* = 2.3 Hz, 2-H), 8.29 (1H, dd, *J* = 8.7, 2.1 Hz, 6-H), 8.05 (1H, d, *J* = 7.8 Hz, 5-H), 7.97 (1H, s, CONH), 7.73 (1H, m, 6a-H), 7.34 (2H, m, 3a-H, 3b-H), 7.29 (1H, dd, *J* = 7.6, 1.4 Hz, 6b-H), 7.27–7.19 (4H, m, 4a-H, 5a-H, 4b-H, 5b-H), 4.16 (3H, s,

OCH_3), 3.20 (2H, m, 2 × Ar-CH(CH_3)$_2$), 1.34 (6H, d, J = 6.8 Hz, Ar-CH(CH_3)$_2$), 1.29 (6H, d, J = 6.8 Hz, Ar-CH(CH_3)$_2$); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 162.7 (2 × C, C=O), 159.8 (C, 4-C), 141.8 (C, 1a-C), 139.6 (C, 1b-C), 134.8 (C, 1-C), 134.2 (C, 2a-C), 134.2 (C, 2b-C), 130.4 (CH, 6-C), 128.2 (CH, 2-C), 126.6 (CH, 3a-C), 126.6 (CH, 3b-C), 125.9 (CH, 5a-C), 125.8 (CH, 5b-C), 125.6 (2 × CH, 4a-C, 4b-C), 124.3 (CH, 2-C), 121.6 (CH, 5-C), 112.4 (2 × CH, 6a-C, 6b-C), 56.8 (CH_3, O-CH_3), 28.4 (CH, Ar–CH(CH_3)$_2$), 28.3 (CH, Ar–CH(CH_3)$_2$), 23.3 (2 × CH_3, Ar–CH(CH_3)$_2$), 23.2 (2 × CH_3, Ar–CH(CH_3)$_2$); MS (*m/z*): 431.2354 $[M+H]^+$.

N^1,N^3-bis(3-isopropylphenyl)-4-methoxyisophthalamide (1h)

White solid (The residue was recrystallized by cyclohexane.) Yield: 61.58%; m.p.: 152.0–154.0 °C; IR (KBr) υ_{max} 3248.30, 1647.45, 1610.23, 1548.99, 1487.90, 1463.93, 2958.72 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.74 (1H, s, CONH), 8.75 (1H, d, J = 2.1 Hz, 2-H), 8.26 (1H, dd, J = 2.2, 8.6 Hz, 6-H), 8.14 (1H, s, CONH), 7.61 (1H, s, Ar–H), 7.55 (2H, d, J = 11.0 Hz, Ar–H), 7.45 (1H, d, J = 8.0 Hz, Ar–H), 7.31 (2H, td, J = 7.8, 4.5 Hz, Ar–H), 7.19 (1H, d, J = 8.7 Hz, 5-H), 7.05 (2H, t, J = 8.4 Hz, Ar–H), 4.16 (3H, s, OCH_3), 2.94 (2H, dq, J = 13.4, 6.8 Hz, 2 × Ar–CH(CH_3)$_2$), 1.32–1.26 (12H, m, 2 × Ar–CH(CH_3)$_2$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.1 (C, C=O), 162.5 (C, C=O), 159.8 (C, 4-C), 150.2 (C, 3a-C), 150.1 (C, 3b-C), 138.1 (C, 1a-C), 138.0 (C, 1b-C), 134.2 (CH, 5-C), 129.9 (C, 1-C), 129.1 (CH, 5a-C), 129.1 (CH, 5b-C), 128.3 (CH, 2-C), 122.9 (CH, 4a-C), 122.9 (CH, 4b-C), 121.5 (C, 3-C), 119.0 (CH, 6a-C), 118.4 (CH, 6b-C), 118.3 (CH, 2a-C), 117.9 (CH, 2b-C), 112.5 (CH, 5-C), 56.8 (CH_3, O-CH_3), 34.4 (2 × CH, Ar–CH(CH_3)$_2$), 24.1 (2 × CH_3, Ar–CH(CH_3)$_2$); MS (*m/z*): 431.2332 $[M+H]^+$.

N^1,N^3-bis(4-isopropylphenyl)-4-methoxyisophthalamide (1l)

White flocculent (The residue was recrystallized by acetone.) Yield: 82.90%; m.p.: 221.0–224.0 °C; IR (KBr) υ_{max} 3429.6, 2954.2, 1656.9, 1526.9, 1530.1, 1316.3, 1256.0, 1030.7 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.67 (1H, s, CONH), 8.69 (1H, d, J = 2.3 Hz, 2-H), 8.24 (1H, s, CONH), 8.20 (1H, dd, J = 8.7, 2.3 Hz, 6-H), 7.58 (4H, t, J = 8.0 Hz, 2a-H, 2b-H, 6a-H, 6b-H), 7.23 (4H, dd, J = 8.4, 3.2 Hz, 3a-H, 3b-H, 5a-H, 5b-H), 7.12 (1H, d, J = 8.7 Hz, 5-H), 4.09 (3H, s, OCH_3), 2.91 (2H, qd, J = 10.8, 6.8 Hz, Ar–CH(CH_3)$_2$), 1.27 (6H, d, J = 2.7 Hz, Ar–CH(CH_3)$_2$), 1.25 (6H, d, J = 2.7 Hz, Ar–CH(CH_3)$_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 164.3 (C, C=O), 162.4 (C, C=O), 159.2 (C, 4-C), 145.3 (C, 4a-C), 145.3 (C, 4b-C), 136.1 (C, 1a-C), 135.8 (C, 1b-C), 134.0 (C, 6-C), 130.0 (C, 1-C), 128.1 (CH, 2-C), 127.2 (2 × CH, 2a-C, 6a-C), 127.1 (2 × CH, 2a-C, 6a-C), 121.2 (C, 3-C), 120.6 (2 × CH, 3a-C, 5a-C), 120.4 (2 × CH, 5b-C, 5b-C), 113.1 (CH, 5-C), 65.7 (CH_3, O-CH_3), 33.8 (2 × CH, Ar–CH(CH_3)$_2$), 24.2 (4 × CH_3,Ar–CH(CH_3)$_2$); MS (*m/z*):431.2361 $[M+H]^+$.

N^1,N^3-bis(4-butylphenyl)-4-methoxyisophthalamide (1j)

White solid (The residue was recrystallized by 75% ethanol.) Yield: 73.58%; m.p.: 254.0–257.0 °C; IR (KBr) υ_{max} 3240.13, 2959.03, 1591.35, 1507.43, 1485.28, 1461.83, 2927.05 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.64 (1H, s, CONH), 8.65 (1H, d, J = 1.7 Hz, 2-H), 8.35 (1H, s, CONH), 8.14 (1H, dd, J = 8.5, 1.7 Hz, 6-H), 7.54 (4H, dd, J = 16.2, 8.1 Hz, 2a-H, 6a-H, 2b-H, 6b-H), 7.15 (4H, d, J = 8.1 Hz, 3a-H, 5a-H, 3b-H, 5b-H), 7.05 (1H, d, J = 8.7 Hz, 5-H), 4.04 (3H, s, OCH_3), 2.59 (4H, t, J = 7.4 Hz, Ar–$CH_2CH_2CH_2CH_3$), 1.59 (4H, dt, J = 15.2, 7.6 Hz, Ar–$CH_2CH_2CH_2CH_3$), 1.36 (4H, dq, J = 14.5, 7.2 Hz, Ar–$CH_2CH_2CH_2CH_3$), 0.94 (6H, t, J = 7.2 Hz, Ar–$CH_2CH_2CH_2CH_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.4 (C, C=O), 162.4 (C, C=O), 159.5 (C, 4-C), 139.4 (C, 4a-C), 139.2 (C, 4b-C), 135.7 (2 × C, 1a-C, 1b-C), 133.8 (CH, 6-C), 130.1 (C, 1-C), 130.0 (4 × CH, 2a-C, 6a-C, 2b-C, 6b-C), 128.2 (CH, 2-C), 121.3 (C, 3-C), 120.7 (2 × CH, 3a-C, 5a-C), 120.4 (2 × CH, 3b-C, 5b-C), 112.1 (CH, 5-C), 56.6 (CH_3, O-CH_3), 35.2 (2 × CH_2, Ar–$CH_2CH_2CH_2CH_3$), 33.8 (2 × CH_2, Ar–$CH_2CH_2CH_2CH_3$), 22.4 (2 × CH_2, Ar–$CH_2CH_2CH_2CH_3$), 14.1 (2 × CH_3, Ar–$CH_2CH_2CH_2CH_3$); MS (*m/z*): 459.2656 $[M+H]^+$.

N^1,N^3-bis(4-(tert-butyl)phenyl)-4-methoxyisophthalamide (1k)

White solid (The residue was recrystallized by anhydrous ethanol.) Yield: 55.43%; m.p.: 259.5–261.0 °C; IR (KBr) υ_{max} 3339.43, 2953.56, 1659.56, 1606.20, 1529.48, 1252.80, 1100.98, 952.2, 829.8 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.66 (1H, s, CONH), 8.69 (1H, s, CONH), 8.20 (2H, m, 6-H, 2-H), 7.58 (4H, m, 2a-H, 6a-H, 2b-H, 6b-H), 7.36 (4H, m, 3a-H, 5a-H, 3b-H, 5b-H), 7.13 (1H, s, 5-H), 4.10 (3H, s, OCH_3), 1.32–1.33 (18H, s, 2 × Ar–C(CH_3)$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.3 (C, C=O), 162.4 (C, C=O), 159.2 (C, 4-C), 147.6 (C, 4a-C), 147.5 (C, 4b-C), 135.8 (C, 1a-C), 135.5 (C, 1b-C), 133.9 (CH, 6-H), 130.0 (C, 1-C), 128.1 (CH, 2-C), 126.1 (2 × CH, 3a-C, 5a-C), 126.0 (2 × CH, 3b-C, 5b-C), 121.2 (C, 3-C), 120.1 (2 × CH, 2a-C, 6a-C), 113.1 (2 × CH, 2b-C, 6b-C), 112.9 (CH, 5-C), 65.7 (CH_3, O-CH_3), 34.5 (2 × C, Ar–C(CH_3)$_3$), 31.5 (6 × CH_3, Ar–C(CH_3)$_3$); MS (*m/z*): 459.2649 $[M+H]^+$.

N^1,N^3-bis(2-benzylphenyl)-4-methoxyisophthalamide (1l)

White solid (The residue was recrystallized by acetone.) Yield: 59.00%; m.p.: 238.0–240.0 °C; IR (KBr) υ_{max} 3301, 3025, 2922, 1653, 1606, 1587, 1493, 1451, 1300, 1262, 1027, 825 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 9.96 (1H, s, CONH), 9.79 (1H, s, CONH), 8.40 (1H, dd, J = 4.0, 12.0 Hz, 6-H), 8.04 (1H, d, J = 12.0 Hz, 2-H), 7.75 (1H, d, J = 4.0 Hz, 5-H), 7.11–7.36 (18H, m, Ar–H), 4.07 (2H, s, Ar–$\underline{CH_2}$–Ph), 4.00 (2H, s, Ar–$\underline{CH_2}$–Ph), 3.85 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.7 (C, C=O), 163.5 (C, C=O), 160.8 (C, 4-C), 140.2 (C, 1a-C), 140.0 (C, 1b-C), 138.3 (C, 2a-C), 138.2 (C, 2b-C), 131.9 (CH, 6-H), 131.0 (2 × C, 1a'-C, 1b'-C), 128.5 (2 × CH, 3a'-C, 5a'-C), 128.4 (2 × CH, 3b'-C, 5b'-C), 128.2 (2 × CH, 2a'-C, 6a'-C), 128.0 (4 × CH, 2b'-C, 6b'-C, 4a-C, 4b-C), 126.6 (C, 1-C), 126.0 (C, 2-C), 125.5 (2 × CH, 3a-C, 3b-C), 125.4 (2 × CH, 4a'-C, 4b'-C), 120.4 (2 × CH, 6a-C, 6b-C), 116.5 (C, 3-C), 112.3 (C, 5-C), 60.3 (CH_3, OCH_3), 34.5 (CH_2, Ar–$\underline{CH_2}$–Ph), 32.7 (CH_2, Ar–$\underline{CH_2}$–Ph); MS (*m/z*): 527.6063 $[M+H]^+$ (Liu et al. 2012).

N^1,N^3-bis(3-ethynylphenyl)-4-methoxyisophthalamide (1m)

White needles (The residue was recrystallized by ethanol.) Yield: 67.40%; m.p.: 174.1–175.7 °C; IR (KBr) υ_{max} 3346.23, 32837, 2955.33, 1671.50, 649.38, 1582.54, 1554.22, 1431.70, 2104.20, 1185.81, 1017.99, 840, 790 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.69 (1H, s, CONH), 8.63 (1H, d, J = 2.3 Hz, 2-H), 8.58 (1H, s, CONH), 8.16 (1H, dd, J = 8.7, 2.3 Hz, 6-H), 7.86 (1H, s, Ar–H), 7.73–7.63 (3H, m, Ar–H), 7.32–7.28 (2H, m, Ar–H), 7.25 (2H, d, J = 8.6 Hz, 5a-H, 5b-H), 7.08 (1H, d, J = 8.8 Hz, 5-H), 4.09 (3H, s, OCH_3), 3.11 (1H, s, C≡$\underline{CH}$), 3.09 (1H, s, C≡$\underline{CH}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.4 (C, C = O), 162.5 (C, C = O), 159.8 (C, 4-C), 138.2 (C, 1a-C), 138.1 (C, 1b-C), 134.3 (CH, 6-C), 130.3 (C, 1-C), 129.3 (CH, 5a-C), 129.2 (CH, 5b-C), 128.4 (CH, 4a-C), 128.3 (CH, 4b-C), 128.0 (CH, 2-C), 124.2 (CH, 2a-C), 123.9 (CH, 2b-C), 123.0 (C, 4a-C), 130.0 (C, 4b-C), 121.4 (CH, 6a-C), 121.1 (CH, 6b-C), 120.9 (C, 3-C), 112.4 (C, 5-C), 83.4 (2 × C, Ar–$\underline{C}$≡CH), 77.7 (2 × CH, Ar–C≡$\underline{CH}$), 56.8 (CH_3, O–CH_3); MS (*m/z*): 395.1388 $[M+H]^+$.

N^1,N^3-bis(3-cyanophenyl)-4-methoxyisophthalamide (1n)

Brown solid (The residue was recrystallized by DMF: H_2O = 1.5: 1); Yield: 56.70%; m.p.: 263.6–265.2 °C; IR (KBr) υ_{max} 3356.13, 2952.32, 1668.50, 1546.26, 1420.47, 2228.49, 1179.80, 1086.95, 830, 792, 680 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.85 (1H, s, CONH), 8.73 (1H, s, 2-H), 8.65 (1H, s, CONH), 8.23 (1H, d, J = 8.7 Hz, 6-H), 8.02 (1H, s, 3a-H), 7.91 (1H, s, 3b-H), 7.84 (1H, d, J = 8.0 Hz, 4a-H), 7.78 (1H, d, J = 7.8 Hz, 4b-H), 7.44 (2H, t, J = 6.7 Hz, 5a-H, 5b-H), 7.38 (2H, t, J = 7.5 Hz, 6a-H, 6b-H), 7.16 (1H, d, J = 8.8 Hz, 5-H), 4.14 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6) δ = 166.4 (C, C=O), 164.5 (C, C=O), 164.3 (C, 4-C), 159.9 (C, 1a-C), 159.2 (C, 1b-C), 140.0 (CH, 4a-C), 139.6 (CH, 4b-C), 133.6 (CH, 2a-C), 132.2 (CH, 2b-C), 131.0 (CH, 6-C), 130.2 (CH, 5a-C), 129.3 (CH, 5b-C), 127.2 (C, 1-C), 126.1 (CH, 2-C), 124.8 (CH, 6a-H), 124.5 (CH, 6b-H), 124.4 (CH, 3-C), 123.0 (C, Ar–$\underline{C}$≡N), 122.5 (C, Ar–$\underline{C}$≡N), 118.6 (CH, 5-C), 112.1 (C, 3a-C), 111.6 (C, 3b-C), 56.4 (CH_3, O–CH_3); MS (*m/z*): 419.1131 $[M + Na]^+$.

N^1,N^3-bis(4-cyanophenyl)-4-methoxyisophthalamide (1o)

White solid (The residue was recrystallized by ethanol.) Yield: 62.51%; m.p.: 271.5–273.3 °C; IR (KBr) υ_{max} 3339.20, 2997.36, 1678.58, 1592.21, 1493.40, 2224.40, 1176.00, 1014.90, 823, 753 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.70 (1H, s, CONH), 10.62 (1H, s, CONH), 8.26 (1H, d, J = 2.2 Hz, 2-H), 8.18 (1H, dd, J = 8.7, 2.2 Hz, 6-H), 8.00 (2H, d, J = 8.7 Hz, 2a-H, 2b-H), 7.94 (2H, d, J = 8.7 Hz, 6a-H, 6b-H), 7.83 (4H, dd, J = 8.7, 4.7 Hz, 3a-H, 5a-H, 3b-H, 5b-H), 7.36 (1H, d, J = 8.9 Hz, 5-H), 3.96 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.8 (C, C=O), 164.6 (C, C=O), 159.3 (C, 4-C), 143.5 (C, 1a-C), 143.1 (C, 1b-C), 133.3 (2 × CH, 3a-C, 5a-C), 133.1 (2 × CH, 3b-C, 5b-C), 132.3 (CH, 6-C), 129.2 (C, 1-C), 126.0 (CH, 2-C), 124.8 (C, 3-C), 120.2 (2 × CH, 2a-C, 6a-C), 119.6 (2 × CH, 2b-C, 6b-C), 119.0 (2 × C, Ar–$\underline{C}$≡N), 112.0 (CH, 5-C), 105.4 (C, 4a-C), 105.3 (C, 4b-C), 56.4 (CH_3, O-CH_3); MS (*m/z*): 419.1129 $[M + Na]^+$.

N^1,N^3-bis(2-(trifluoromethyl)phenyl)-4-methoxyisophthalamide (1p)

White solid (The residue was recrystallized by acetonitrile.) Yield: 74.71%; m.p.: 172.6–174.3 °C; IR (KBr) υ_{max} 3354.20, 2957.38, 1679.53, 1645.22, 1535.50, 1134.10, 1058.90, 824, 764 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 10.27 (2H, s, CONH), 8.63 (1H, d, J = 1.7 Hz, 2-H), 8.23–8.14 (2H, m, Ar–H), 7.80 (2H, dd, J = 2.4, 10.8 Hz, 3a-H, 3b-H), 7.75 (2H, t, J = 7.7 Hz, 4a-H, 4b-H), 7.55 (2H, t, J = 6.5 Hz, 5a-H, 5b-H), 7.43 (2H, t, J = 8.5 Hz, 6a-H, 6b-H), 4.09 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 165.2 (C, C=O), 162.9 (C, C=O), 159.6 (C, 4-C), 135.8 (CH, 5a-C), 135.3 (CH, 5b-C), 133.3 (C, 1a-C), 133.1 (C, 1b-C), 131.3 (CH, 6-C), 127.4 (2 × CH, 3a-C, 3b-C), 126.7 (C, 1-C), 126.6 (2 × C, Ar–$\underline{CF_3}$), 126.3 (2 × CH, 4a-C, 4b-C), 126.2 (C, 2-C), 125.4 (2 × C, 2a-C, 2b-C), 122.3 (C, 3-C), 121.0 (CH, 5-C), 112.6 (2 × CH, 6a-C, 6b-C), 56.7 (CH_3, O-CH_3); MS (*m/z*): 483.1153 $[M+H]^+$.

N^1,N^3-bis(3-(trifluoromethyl)phenyl)-4-methoxyisophthalamide (1q)

White solid (The residue was recrystallized by ethanol.) Yield: 81.60%; m.p.: 201.1–202.6 °C; IR (KBr) υ_{max} 3335.26, 2961.30, 1686.50, 1568.21, 1496.48, 1441.10, 1337.38, 1226.16, 1014.30, 829, 790, 698 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.60 (1H, s, CONH), 10.54 (1H, s, CONH), 8.31 (1H, d, J = 2.1 Hz, 2-H), 8.25 (2H, s, 2a-H, 2b-H), 8.19 (1H, dd, J = 8.8, 2.1 Hz, 6-H), 8.09 (1H, d, J = 8.4 Hz, 4a-H), 7.97 (1H, d, J = 8.1 Hz, 4b-H), 7.60 (2H, td, J = 8.0, 2.7 Hz, 5a-H, 5b-H), 7.46 (2H, t, J = 7.4 Hz, 6a-H, 6b-H), 7.36 (1H, d, J = 8.8 Hz, 5-H), 3.97 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.7 (C, C = O), 164.5 (C, C=O), 159.1 (C, 4-C), 140.0 (C, 1a-C), 139.7 (1, 1b-C), 132.1 (CH, 6-C), 130.0 (C, 3a-C), 129.8 (C, 3b-C), 129.5 (CH, 5a-C), 129.3 (CH, 5b-C), 129.1 (C, 1-C), 126.1 (CH, 2-C), 125.5 (2 × CH, 2a-C, 2b-C), 124.8 (2 × CH, 6a-C, 6b-C), 123.8 (2 × C, Ar–$\underline{CF_3}$), 123.2 (C, 3-C), 120.0 (CH, 4a-C), 116.4 (CH, 4b-C), 112.0 (CH, 5-H), 56.4 (CH_3, O-CH_3); MS (*m/z*): 483.1141 $[M+H]^+$.

N^1,N^3-bis(4-(trifluoromethyl)phenyl)-4-methoxyisophthalamide (1r)

White solid (The residue was recrystallized by ethanol.) Yield: 45.37%; m.p.: 244.7–245.0 °C; IR (KBr) υ_{max} 3339.20, 1661.96, 1605.88, 1549.44, 1493.29, 1408.30, 1321.85, 1260.93, 1067.12 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.64 (1H, s, CONH), 10.58 (1H, s, CONH), 8.28 (1H, d, J = 1.2 Hz, 2-H), 8.19 (1H, d, J = 8.7 Hz, 6-H), 8.03 (2H, d, J = 8.5 Hz, 3a-H, 3b-H), 7.97 (2H, d, J = 8.3 Hz, 3b-H, 5b-H), 7.73 (4H, dd, J = 8.4, 2.6 Hz, 2a-H, 6a-H, 2b-H, 6b-H), 7.36 (1H, d, J = 8.8 Hz, 5-H), 3.96 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.8 (C, C = O), 164.6 (C, C = O), 159.2 (C, 4-C), 142.9 (C, 1a-C), 142.6 (C. 1b-C), 132.3 (2 × CH, 4a-C, 4b-C), 129.2 (CH, 6-H), 126.2 (C, 1-C), 126.0 (2 × CH, 3a-C, 5a-C), 125.9 (2 × CH, 3b-C, 5b-C), 125.8 (CH, 2-C), 125.8 (C, Ar–$\underline{CF_3}$), 125.0 (C, Ar–$\underline{CF_3}$), 123.9 (C, 3-C), 123.6 (CH, 2a-C), 123.1 (CH, 2b-C), 120.2 (CH, 6a-C), 119.6 (CH, 6b-C), 112.0 (CH, 5-C), 56.4 (CH_3, O-CH_3); MS (*m/z*): 483.1152 $[M+H]^+$.

N^1,N^3-bis(2-chloro-4-(trifluoromethyl)phenyl)-4-methoxyisophthalamide (1s)

White solid (The residue was recrystallized by acetone.) Yield: 63.21%; m.p.: 226.0–229.0 °C; IR (KBr) υ_{max} 3421.20, 3312.13, 1685.96, 1595.88, 1542.74, 1500.79, 1477.30, 1324.85, 1261.93, 1081.12 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.76 (1H, s, CONH), 10.43 (1H, s, CONH), 8.74 (1H, d, J = 2.1 Hz, 2-H), 8.71 (1H, d, J = 8.8 Hz, 6a-H), 8.26 (1H, dd, J = 8.7, 2.2 Hz, 6-H), 8.00 (2H, d, J = 6.0 Hz, 3a-H, 3b-H), 7.90 (1H, d, J = 8.4 Hz, 6b-H), 7.79 (2H, t, J = 7.3 Hz, 5a-H, 5b-H), 7.48 (1H, d, J = 8.8 Hz, 5-H), 4.18 (3H, s, OCH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.2 (C, C=O), 162.5 (C, C=O), 160.0 (C, 4-C), 139.1 (C, 1a-C), 138.7 (C, 1b-C), 133.8 (2 × C, 4a-C, 4b-C), 131.7 (CH, 6-C), 129.5 (2 × C, 2a-C, 2b-C), 128.3 (C, 1-C), 126.7 (CH, 3a-C), 126.4 (CH, 3b-C), 125.3 (C, 1-C), 124.8 (2 × CH, 6a-C, 6b-C), 124.6 (2 × CH, 5a-C, 5b-C), 123.3 (CH, 2-C), 122.0 (2 × C, Ar–$\underline{CF_3}$), 120.4 (C, 3-C), 113.0 (CH, 5-H), 57.4(CH_3, O–CH_3); MS (*m/z*): 551.0373 $[M+H]^+$.

N^1,N^3-bis(2,3-dimethylphenyl)-4-methoxyisophthalamide (1t)

White solid (The residue was recrystallized by acetone.) Yield: 61.58%; m.p.: 173.0–174.0 °C; IR (KBr) υ_{max} 3369.52, 3273.61, 1665.90, 1637.69, 1607.32, 1526.80, 2821.76 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.68 (1H, s, CONH), 8.81 (1H, d, J = 1.8 Hz, 2-H), 8.26 (1H, dd, J = 8.5, 1.4 Hz, 6-H), 7.98 (1H, s, CONH), 7.89 (1H, d, J = 8.0 Hz, 5-H), 7.49 (1H, d, J = 7.9 Hz, Ar–H), 7.17 (1H, ddd, J = 19.7, 10.2, 5.5 Hz, Ar–H), 7.06 (2H, t, J = 8.1 Hz, Ar–H), 4.15 (3H, s, OCH_3), 2.35 (3H, s, Ar–$\underline{CH_3}$), 2.33 (3H, s, Ar–$\underline{CH_3}$), 2.27 (3H, s, Ar–$\underline{CH_3}$), 2.22 (3H, s, Ar–$\underline{CH_3}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 162.6 (2 × C, C=O), 159.8 (C, 4-C), 137.7 (C, 3a-C), 137.4 (C, 3b-C), 136.2 (C, 1a-C), 135.4 (C, 1b-C), 134.1 (CH, 6-C), 130.4 (2 × C, 2a-C, 2b-C), 128.1 (C, 1-C), 128.0 (CH, 2-C), 127.9 (CH, 4a-C), 127.1 (CH, 4b-C), 126.2 (CH, 5a-C), 126.0 (CH, 5b-C), 122.8 (C, 3-C), 121.6 (CH, 6a-C), 121.4 (CH, 6b-C), 112.3 (CH, 5-C), 56.8 (CH_3, O–CH_3), 20.8 (CH_3, 3a-CH_3), 20.7 (CH_3, 3b-CH_3), 14.2 (CH_3, 2a-CH_3), 13.9 (CH_3, 2b-CH_3); MS (*m/z*): 403.2015 $[M+H]^+$.

N^1,N^3-bis(3,4-dimethylphenyl)-4-methoxyisophthalamide (1u)

White floccule (The residue was recrystallized by 70% ethanol.) Yield: 58.00%; m.p.: 172.0–173.0 °C; IR (KBr) υ_{max} 3133.96, 1666.73, 1543.65, 1402.17, 923.32, 840.95, 727.79 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.52 (1H, s, CONH), 8.60 (1H, s, CONH), 8.55 (1H, s, 2-H), 8.06 (1H, d, J = 7.9 Hz, 6-H), 7.44 (1H, s, Ar-H), 7.41–7.33 (2H, m, 2a-H, 2b-H), 7.30 (1H, d, J = 8.1 Hz, Ar–H), 7.04 (2H, dd, J = 8.0, 2.6 Hz, 5a-H, 5b-H), 6.95 (1H, d, J = 8.7 Hz, 5-H), 3.97 (3H, s, OCH_3), 2.20 (12H, s, 4 × Ar–CH_3); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 164.5 (2 × C, C=O), 159.3 (C, 4-C), 137.2 (C, 3a-C), 137.0 (C, 3b-C),

136.0 (C, 1a-C), 135.7 (C, 1b-C), 133.4 (CH, 6-C), 132.8 (C, 4a-C), 132.6 (4b-C), 130.3 (C, 1-C), 129.9 (CH, 5a-C), 129.9 (CH, 5b-C), 128.2 (CH, 2-C), 122.0 (CH, 2a-C), 121.8 (CH, 2b-C), 121.3 (C, 3-C), 118.2 (CH, 6a-C), 118.0 (CH, 6b-C), 111.8 (CH, 5-C), 56.5 (CH_3, O–CH_3), 19.9 ($2 \times CH_3$, 3a-CH_3, 3b-CH_3), 19.2 ($2 \times CH_3$, 4a-CH_3, 4b-CH_3); MS (*m/z*): 403.2039 $[M+H]^+$.

N^1,N^3-bis(3-chloro-2-methylphenyl)-4-methoxyisophthalamide (1v)

White solid (The residue was recrystallized by dioxane.) Yield: 85.10%; m.p.: 232.7–234.3 °C; IR (KBr) υ_{max} 3375.13, 3255.68, 1667.20, 1524.26, 1437.48, 1180.20, 1047.42, 2948.29, 821, 779 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.27 (1H, s, CONH), 10.03 (1H, s, CONH), 8.45 (1H, d, *J* = 2.0 Hz, 2-H), 8.19 (1H, dd, *J* = 9.2, 1.5 Hz, 6-H), 7.68 (1H, d, *J* = 7.9 Hz, 4a-H), 7.55 (1H, d, *J* = 8.0 Hz, 4b-H), 7.50 (1H, d, *J* = 7.9 Hz, 5-H), 7.38 (1H, d, *J* = 8.8 Hz, 5a-H), 7.33 (1H, d, *J* = 8.2 Hz, 5b-H), 7.20 (2H, td, *J* = 7.8, 4.0 Hz, 6a-H, 6b-H), 4.05 (3H, s, OCH_3), 2.40 (3H, s, 2a-CH_3), 2.28 (3H, s, 2b-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.4 (C, C=O), 163.4 (C, C=O), 159.3 (C, 4-C), 138.0 (C, 1a-C), 137.8 (C, 1b-C), 134.0 (C, 2a-C), 132.2 (C, 2b-C), 131.3 (CH, 6-C), 130.0 (C, 3a-C), 129.2 (C, 3b-C), 127.4 (CH, 5a-C), 127.3 (CH, 5b-C), 126.6 (C, 1-C), 126.3 (CH, 2-C), 124.7 (CH, 4a-C), 124.6 (CH, 4b-C) 124.4 (C, 3-C), 123.3 (CH, 5-C), 112.1 ($2 \times$CH, 6a-C, 6b-C), 56.6 (CH_3, O–CH_3), 18.4 (CH_3, 2a-CH_3), 18.0 (CH_3, 2b-CH_3); MS (*m/z*): 443.0937 $[M+H]^+$.

N^1,N^3-bis(3-chloro-4-methylphenyl)-4-methoxyisophthalamide (1w)

White solid (The residue was recrystallized by ethanol.) Yield: 84.30%; m.p.: 195.8–197.6 °C; IR (KBr) υ_{max} 3361.19, 3327.30, 1645.22, 1592.26, 1400.48, 1258.40, 1181.27, 2947.25, 817, 578 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 10.32 (2H, s, $2 \times$CONH), 8.25 (1H, d, *J* = 2.2 Hz, 2-H), 8.26 (1H, dd, *J* = 2.4, 8.8 Hz, 6-H), 8.18–8.11 (1H, m, Ar-H), 7.99–7.90 (1H, m, Ar-H), 7.63 (1H, dd, *J* = 8.3, 1.9 Hz, Ar-H), 7.58–7.51 (1H, m, Ar-H), 7.32 (3H, dd, *J* = 8.3, 1.9 Hz, Ar-H), 7.19–7.24 (3H, m, Ar–H), 3.96 (3H, s, OCH_3), 2.29 (6H, s, $2 \times$Ar-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 164.42 ($2 \times$C, C = O), 159.0 (C, 4-C), 138.3 (C, 1a-C), 138.1 (C, 1b-C), 133.0 (C, 3a-C), 132.8 (C, 3b-C), 131.8 (C, 6-C), 131.2 (C, 4a-C), 131.0 (C, 4b-C), 130.3 (C, 5a-C), 130.1 (C, 5b-C), 129.0 (C, 1-C), 126.3 (CH, 2-C), 124.8 (C, 3-C), 120.2 (CH, 2a-C), 119.5 (CH, 2b-C), 118.8 (CH, 6a-C), 118.3 (CH, 6b-C), 111.9 (CH, 5-C), 56.3 (CH_3, O–CH_3), 19.0 ($2 \times CH_3$, Ar–CH_3); MS (*m/z*): 443.0924 $[M+H]^+$.

Pharmacology

In vitro anti-platelet aggregation activity

The in vitro activity studies on anti-platelet aggregation of the target compounds have been done by using turbidimetric test (Born 1962): fresh venous blood was taken from the ear vein of rabbits, with 3.8% sodium citrate as anticoagulant (blood in proportion to anticoagulant agent were 9: 1), and then centrifuged at room temperature for 10 min (500–800 r/min) to obtain the platelet-rich plasma (PRP). The platelet-poor plasma (PPP) was obtained by centrifuging of PRP at room temperature for 15 min (3000 r/min). The target compounds were dissolved in DMSO (5 μl), and then, the solution was added into PRP (20 μl), and the same volume of DMSO without target compounds was added as a control group. After 2 min incubation, using ADP as the active inducer, platelet aggregation rate was measured. Using the same test method, the platelet aggregation rates of eight compounds induced by collagen and AA were also tested. The inhibition rates for the compounds were calculated according to the following formulas:

$$\text{Aggregation}\% = \frac{\text{PPP}-\text{PRP}}{\text{PPP}} \times 100\%$$

$$\text{Inhibition}\% = \frac{\text{S}-\text{D}}{\text{S}} \times 100\%$$

S: the platelet aggregation in the presence of solvent.

D: the platelet aggregation in the presence of test compounds.

Cytotoxicity assay

Mouse fibroblast cells (L-929) was chosen to evaluate the in vitro cytotoxicity of the target compounds via Cell Counting Kit-8 (CCK-8) assays. Experiment is mainly divided into the following steps:

1. Preparation for experiment: through cell thawing, cell culture and cell passage cultivation to obtain healthy cells. Target compounds were diluted into 10 μM/L and 100 μM/L with DMSO, respectively.
2. Cell inoculation: L-929 was added into 96-well microplates (1×10^4 cell/well), cultivated in a humidified 5% CO_2 atmosohere at 37 °C for 24 h to allow cells to attach.
3. Cell to medicine: cells were exposed to target compounds at the concentrations of 10 μM/L and 100 μM/L and incubated at 37 °C for 48 h.
4. Measuring absorbance: After incubation at 37 °C for 48 h, the medium was removed and replaced with fresh complete medium of RPMI-1640, then CCK-8 solution was added to the 96-well plates at 10 μL per well and the absorbance of the solution was monitored at 450 nm using a microplate reader (Bio-Tek Flx800

fluorescence microplate reader) after 2 h of incubation (Xu et al. 1991).

Their cytotoxicity effect on L-929 cells were calculated by the following formulas:

$$\text{Cell viability}(\%) = \frac{\text{Abs(test cell)} - \text{Abs(blank cell)}}{\text{Abs(controlled cell)} - \text{Abs(blank cell)}} \times 100\%$$

Results and discussion

Chemistry

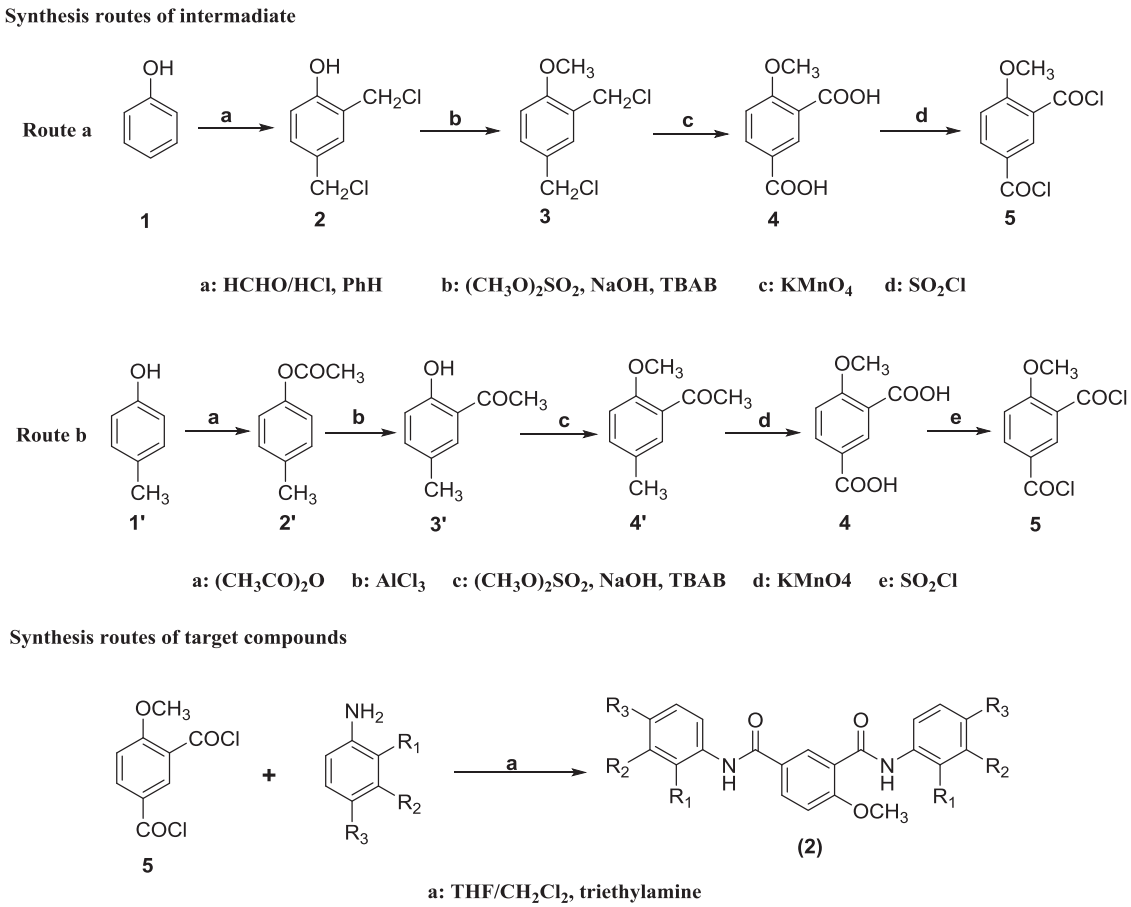
The synthetic pathway was disclosed in Scheme 1 and the structure of target compounds were shown in Table 1. Key intermediate 4-methoxylisophthaloyl dichloride was synthesized in two routes (**Route a** and **Route b**). The intermediate of compounds (**1m**, **1n**, **1o**, **1p**, **1q**, **1r**, **1s**, **1v**, **1w**) were prepared by **Route a** and the intermediate of compounds (**1d**, **1e**, **1f**, **1g**, **1h**, **1i**, **1j**, **1k**, **1t**, **1u**) were synthesized by **Route b**.

At the onset of the experiments, the laboratory's traditional method (**Route a**) was used to synthesize the intermediates. The drawback of this method is the use of volatile hydrogen chloride, it is not only difficult to handle but it also corrode the equipment. Moreover, it is dangerous for an industrial process. Considering the above question, a safer method (**Route b**) was used to synthesize the intermediates in subsequent experiments. **Route b** use Fries rearrangement as the key step, avoiding the use of large doses of hydrochloric acid, which is beneficial for the environment and equipment.

Pharmacology

In vitro anti-platelet aggregation activity

Taking picotamide and aspirin as positive control drugs, in vitro activities on anti-platelet aggregation were tested and assessed by using Born test for ADP inducer (Born 1962). After that, eight compounds (**1c**, **1h**, **1i**, **1k**, **1p**, **1q**, **1v**, and **1w**), they were significantly more active in vitro than control drug picotamide and their IC_{50} values for ADP



Scheme 1 Synthesis routes of intermediate (**Route a** and **b**) and target compounds (**1d**–**1w**)

Table 1 Structures of 4-Methoxylisophthalamides (**1a**–**1w**)

Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
1a[a]	CH_3	H	H	1m	H	C≡CH	H
1b[a]	H	CH_3	H	1n	H	C≡N	H
1c[a]	H	H	CH_3	1o	H	H	C≡N
1d	CH_2CH_3	H	H	1p	CF_3	H	H
1e	H	CH_2CH_3	H	1q	H	CF_3	H
1f	H	H	CH_2CH_3	1r	H	H	CF_3
1g	$CH(CH_3)_2$	H	H	1s	Cl	H	CF_3
1h	H	$CH(CH_3)_2$	H	1t	CH_3	CH_3	H
1i	H	H	$CH(CH_3)_2$	1u	H	CH_3	CH_3
1j	H	H	$(CH_2)_3CH_3$	1v	CH_3	Cl	H
1k	H	H	$C(CH_3)_3$	1w	H	Cl	CH_3
1l[a]	Bn	H	H				

[a]Reported compound

Table 2 The inhibition rate and IC_{50} of tested compounds

Compound	Does (μmol/L)	Inhibition rate (%) in ADP	IC_{50} (μM/L) in ADP	Inhibition rate (%) in collagen	Inhibition rate (%) in AA
Control group	–	–	–	–	–
Picotamide	1.3	44.38	0.60	46.67	29.67
Aspirin	1.3	54.67	0.26	42.82	40.22
1a[a]	1.3	0.00	–	–	–
1b[a]	1.3	0.00	–	–	–
1c[a]	1.3	63.50	0.50		
1d	1.3	46.96	0.78		
1e	1.3	16.12	–	–	–
1f	1.3	0.00	–	–	–
1g	1.3	43.58	–	–	–
1h	1.3	91.27	0.24	32.25	13.56
1i	1.3	58.45**	0.16	47.13**	28.47*
1j	1.3	49.02		–	–
1k	1.3	52.22*	0.34	22.76	13.10
1l[a]	1.3	89.30	0.02		
1m	1.3	22.34	–	–	–
1n	1.3	62.60	–	–	–
1o	1.3	67.63	–	–	–
1p	1.3	57.64*	0.41	51.25**	31.57*
1q	1.3	96.73**	0.09	50.07**	24.55
1r	1.3	10.27	–	–	–
1s	1.3	56.71	–	–	–
1t	1.3	27.61	–	–	–
1u	1.3	11.62	–	–	11.9
1v	1.3	86.24**	0.09	0.00	0.00
1w	1.3	75.11**	0.31	0.00	0.38

[a]Reported compound

*$p < 0.05$ VS Control group

**$p < 0.01$ VS Control group

were calculated, they were selected and continued to testing and assessing for two inducers both collagen and AA. The anti-platelet aggregation activity of the target compounds was listed in Table 2.

These results analysis for ADP and for collagen and for AA were shown in Figs. 2 and 3, respectively.

As were shown in Fig. 2, compared with positive control drugs picotamide and aspirin, 9 compounds **1h**, **1i**, **1n**, **1o**, **1p**, **1q**, **1s**, **1v**, and **1w** had superior platelet aggregation inhibition rate induced by ADP. Among them, compounds **1d**, **1j**, and **1k** were equivalent to the positive control drug picotamide, slightly lower than aspirin. Simultaneously, the IC_{50} values of compounds **1h**, **1i**, **1k**, **1p**, **1q**, **1v**, **1w** were lower than the positive control drug picotamide and the in vitro activities of four compounds **1h**, **1i**, **1q**, and **1v** with IC_{50} values of 0.24 μM/L, 0.16 μM/L, 0.09 μM/L and 0.09 μM/L, respectively, they were higher than that of two control drugs picotamide and aspirin with IC_{50} values of 0.60 μM/L and 0.26 μM/L induced by ADP (1.3 μM). Of course, among them, compound **1q**, that was a N^1,N^3-bis (3-(trifluoromethyl)phenyl) -4-methoxyisophthalamide, had the highest platelet aggregation inhibition rate with lowest IC_{50} value of 0.09 μM/L. The inhibition rate of compound **1v** was not high, but its IC_{50} value was the same as that of compound **1q**, which indicated that **1v** had high platelet inhibition rate at lower concentration.

Eight compounds (**1h**, **1i**, **1k**, **1p**, **1q**, **1u**, **1v**, and **1w**) which were significantly more active in vitro than control drug picotamide were selected and continued to test and assess for two inducers both collagen and AA. The analysis in Fig. 3 showed two compounds **1p** and **1q** showed higher in vitro activities of inhibition rates of 51.25% and 50.07% than that of the 46.67% of picotamide and 46.67% of aspirin induced by collagen, while **1p** showed higher in vitro activity of inhibition rate of 31.57% than that of the 29.67% of picotamide induced by AA at the concentration

of 1.3 μM. Compound **1p**, that was a N^1,N^3-bis (2-(trifluoromethyl)phenyl) -4-methoxyisophthalamide, had similar structure with compound **1q** and both of them had shown good results in the pharmacological tests. which is worth further researching.

Cytotoxicity assay

The effect of the compounds with higher activities on cytotoxicities was evaluated on mouse fibroblast cell (L-929) via CCK-8 assays. The survival rate of cells was set out in Table 3 and the cell morphology after exposed to target compounds were shown in Fig. 4 (Xiong et al. 2007; Abe et al. 2000).

The result analysis of cytotoxicities was shown in Fig. 5, among the eight compound, six compound (**1h**, **1n**, **1o**, **1p**, **1q**, **1v**) had higher cell survival rate than picotamide at the dose of 10 μM/L. And the cell survival rate of three compound (**1h**, **1p**, **1q**) was higher than that of picotamide at the dose of 100 μM/L. Among them, compound **1h** and **1q** had the lowest cytotoxicities at the dose of 10 μM/L and 100 μM/L.

Preliminary structure activity relationship (SAR) exploration

1. The effect of anti-platelet aggregation activity when the substitutions were saturated hydrocarbons

The order of inhibition of platelet was: in *o*-position of the phenyl rings: **1l**$^{\#a}$**>1d > 1g > 1a**^{a}. in *m*-position: **1h > 1e > 1b**^{a}. in *p*-position: **1i > 1k > 1j**. That meant the activity could be enhanced when the saturated hydrocarbons with bigger steric hindrance were introduced in the phenyl rings.

2. The effect of anti-platelet aggregation activity when the substitution were unsaturated hydrocarbons

The order of inhibition of platelet was: **1o > 1n** > aspirin > picotamide > **1m**. the result showed that cyano- substituted compounds were more potent than the alkynyl-substituted analogs. Therefore, the relatively strong electron-withdrawing substituent were introduced into the phenyl rings maybe could serve to promote the anti-platelet aggregation activity.

3. The effect of anti-platelet aggregation activity when the substitutions were trifluoromethyl

The order of inhibition of platelet was: **1q > 1p > 1s** > aspirin > picotamide > **1r**. The result indicated that when the trifluoromethyl was introduced into the *o*-position and *m*-position of phenyl rings, the anti-platelet aggregation activity increased significantly. When a electron-withdrawing group (Cl) was introduced into the *o*-position at the same time, Anti-platelet aggregation activities are significantly improved (the inhibition rate: **1s > 1r**).

Table 3 Cytotoxicity effect on L-929

Compound	Dose (μM/L)	Absorbance	Survival rate (%)
Blank group	–	0.191	–
Control group	–	0.318	–
Picotamide	10	0.275	66.14
	100	0.246	43.31
Compound **1h**	10	0.311	94.49
	100	0.285	74.02
Compound **1i**	10	0.268	60.63
	100	0.242	40.16
Compound **1n**	10	0.303	88.19
	100	0.231	31.50
Compound **1o**	10	0.290	77.95
	100	0.223	25.20
Compound **1p**	10	0.311	94.49
	100	0.287	75.59
Compound **1q**	10	0.259	53.54
	100	0.256	51.18
Compound **1v**	10	0.284	73.23
	100	0.254	49.61
Compound **1w**	10	0.245	42.52
	100	0.206	11.81

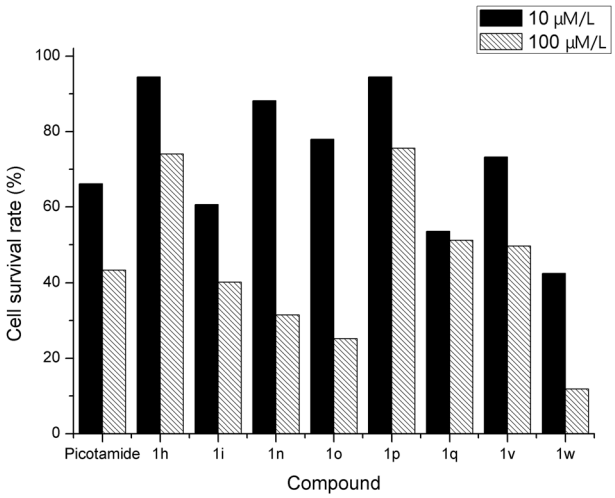


Fig. 5 Cell Survival Rate of Compounds

4. The effect of anti-platelet aggregation activity when two substitutions were introduced into the phenyl rings

Two methyl groups were introduced into the phenyl rings, compounds **1t** and **1u** were obtained. But compounds **1t** and **1u** almost had no anti-platelet aggregation activity.

Methyl and chloro groups were simultaneously introduced into the phenyl rings, compounds **1v** and **1w** were obtained. The order of inhibition of platelet was: **1v** > **1w** > aspirin > picotamide. Therefore, methyl and electron-withdrawing groups were simultaneously introduced into the phenyl rings might help to promote the anti-platelet aggregation activity.

To sum up, the anti-platelet aggregation activity will increase when the saturated hydrocarbons with bigger steric hindrance were introduced in the side chain phenyl rings. The introduction of cyano and trifluoromethyl could enhance the activity on anti-platelet aggregation. Simultaneous introduction of two methyl groups was unfavorable to the activity, while the introduction of methyl and chloro groups could significantly enhance the activity on anti-platelet aggregation.

Conclusion

In summary, 19 novel 4-methoxyisophthalamide analogs were synthesized and evaluated for anti-platelet activities, the compounds with higher activities were selected to continue research on cytotoxicities. The result exhibited that compounds **1h**, **1p**, **1q**, **1v** showed stronger anti-platelet activity and lower cytotoxicities. And, more remarkably, compound **1q**, that was a N^1,N^3-bis (3-trifluoromethylphenyl)-4-methoxyisophthalamides, had significant anti-platelet activity when the inducers were ADP and collagen and it had lower cytotoxicity at the dose of 100 μM/L. Thus, **1q** is potential to be studied and may help in developing new anti-platelet drug with elevated activity and lower toxicity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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