ORIGINAL RESEARCH

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N, N'-disubstitutedphenyl-4-ethoxyl benzene-1, 3-disulfonamides: design, synthesis, and evaluation of anti-platelet aggregation activity

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Abstract

According to the bio-isosterism theory, a series of N, N'-disubstitutedphenyl-4-ethoxylbenzene-1, 3-disulfonamides (**5a-p**) were designed and synthesized by two steps of reactions including chlorosulfonation and ammonolysis. The structures of all compounds have been confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and ESI-MS spectra. The in vitro anti-platelet aggregation activities were evaluated by Born's test induced by adenosine diphosphate (ADP) and arachidonic acid (AA), respectively. The biological evaluation results revealed that compound **5h** had the lowest IC_{50} value (0.32 μM) and the highest inhibition rate (40.9 %) that of three positive control agents clopidogrel (0.41 μM, 23.5 %), aspirin (0.53 μM, 28.9 %), and picotamide (0.76 μM, 32.7 %). Afterwards, compounds with higher activities were selected to further study in vitro cytotoxicity via cell counting kit-8 (CCK-8) assay. The cytotoxicity results indicated that compound **5h** had simultaneously the lowest cytotoxicity, while other compounds had no significant relationship between the anti-platelet activities and cytotoxicities. Based on above in vitro anti-platelet activity data, the SAR (Structure Activity Relationship) of the target compounds was preliminarily summarized. In general, N, N'-disubstitutedphenyl-4-ethoxylbenzene-1, 3-disulfonamides have the potential of further study and very likely become safer and more effective anti-platelet agents.

Keywords N, N'-disubstitutedphenyl-4-Ethoxyl Benzene-1, 3-Disulfonamides · Anti-platelet aggregation · Structure activity relationship · Cytotoxicity

Abbreviations

ADP	Adenosine diphosphate
AA	Arachidonic acid
SAR	Structure activity relationship
COX-I	Cyclooxygenase-I
TXA_2	Thromboxane A_2
PGI_2	Prostaglandin I_2
TLC	Thin layer chromatography
CCK-8	Cell Counting Kit-8

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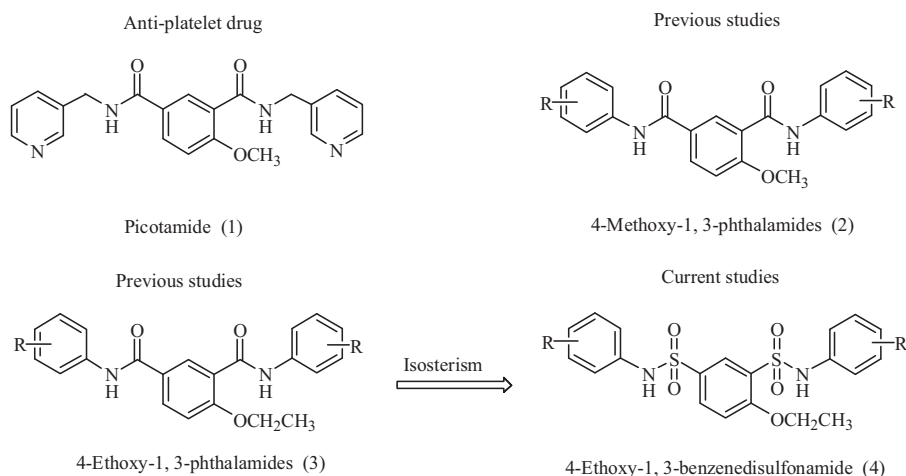
Introduction

Thrombosis disorders are key factors responsible for morbidity and mortality all over the world (Reddy et al. 2011). Platelet aggregation is a significant reason in thrombosis, which may bring about atherosclerotic plaques that is the initiator of most thrombosis disorders (Brito et al. 2010). Therefore, anti-platelet therapy is an effective method to prevent and treat thrombus-related diseases (Mirfazli et al. 2014). Anti-platelet agents can potently hold back thrombosis through the inhibition of platelet adhesion and aggregation (Liu et al. 2017). Aspirin and clopidogrel are well-established anti-platelet agents clinically. Aspirin is cyclooxygenase-I (COX-I) inhibitor that can concurrently inhibits production of thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2) (Jayakumar et al. 2016; Mclewee et al. 2017). Clopidogrel is ADP receptor antagonist, which may selectively inhibit platelet aggregation by blocking ADP-mediated activation of the glycoprotein IIb/IIIa pathway (Peng et al. 2018; Metil et al. 2018). But these anti-platelet agents still have some serious limitations including the high

Fig. 1 Structures of Picotamide (**1**), 4-Methoxy-1, 3-phthalamides (**2**), 4-Ethoxy-1, 3-phthalamides (**3**) & 4-Ethoxy-1, 3-benzenedisulfonamides (**4**)

Anti-platelet drug

Picotamide (**1**)

Previous studies

4-Methoxy-1, 3-phthalamides (**2**)

Previous studies

4-Ethoxy-1, 3-phthalamides (**3**)

Isosterism

Current studies

4-Ethoxy-1, 3-benzenedisulfonamide (**4**)

incidence of bleeding events (aspirin and clopidogrel) (Guthrie 2011), irreversibly inhibition of platelet function (aspirin) (Mclewee et al. 2017), slow onset of action (clopidogrel) (Eskandariyan et al. 2014). On this background, we attempt to find new anti-platelet agents, which are safer and more effective are very momentous (Siwek et al. 2011).

Picotamide (1, Fig. 1) is an effective platelet aggregation inhibitor with a dual inhibitory action, which can concurrently inhibit both TXA_2 receptors and TXA_2 synthesis (Pogliani and Milani 1996; Yip and Benavente 2011). In contrast to aspirin, it has the advantage of not interfere with PGI_2 production (Celestini and Violi 2007; Wang et al. 2018). Since 2000, through the structural transformation of picotamide, our research group envisaged that replacing the two 3-pycolyl groups of picotamide with two substituted phenyl groups might open another door for anti-platelet agents. A series of 4-methoxyl-1, 3-phthalamides (2, Fig. 1) which are the structural analogs of picotamide were designed and synthesized (Liu et al. 2006, 2011, 2012). We wondrously found that among them, nearly 30% compounds exhibited higher in vitro anti-platelet activities than picotamide. It is well-known that sulfonamides have wide pharmacological activities including anti-inflammatory, anti-viral, anti-fungal and anti-neoplastic, and others (Khalid et al. 2018). Moreover, sulfonamide analogues have also shown higher inhibitory activity against platelet aggregation (Sharma et al. 2018). In view of the significance of sulfonamides in medicinal chemistry and according to the bio-isomerism theory, a series of 4-methoxy-1, 3-benzenedisulfonamides were designed and synthesized by replacing the two amide structures of picotamide with two sulfamide structures (Liu et al. 2006, 2011; Li et al. 2015).

During the period, we made the contour maps of steric QSAR from CoMFA. The results indicated that the anti-platelet activity might be influenced by steric factors. In order to confirm the steric model hypotheses as above, we further synthesized 4-ethoxy-1, 3-phthalamides (3, Fig. 1) (Liu et al. 2015) and 4-ethoxy -1, 3-benzenedisulfonamides (4, Fig. 1).
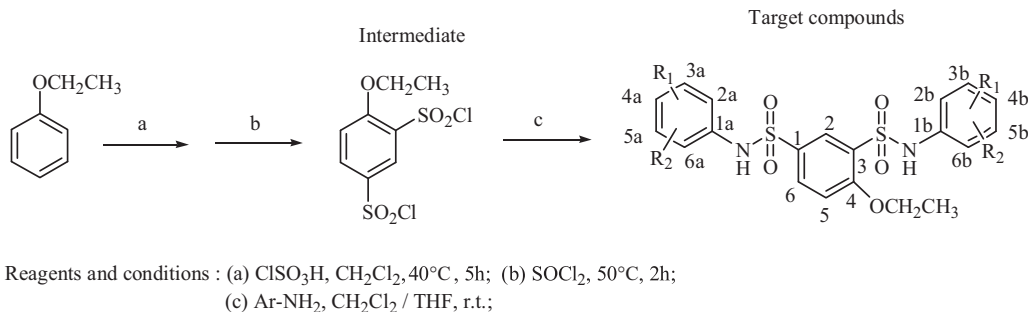
It was amazing for us the anti-platelet activities of above compounds are underestimated in our previous study. On the purpose of releasing the full potential of 4-ethoxy-1, 3-benzenedisulfonamides as anti-platelet agents, we carried out more detailed studies in SAR. We introduced six previous reported mono-substituted compounds (**4a***–**f***). The order of anti-platelet aggregation activity of the different substituents at same position was: In 2-position: **4a*** (2-F) > **4b*** (2-Cl) > **4c*** (2-Br) > clopidogrel, aspirin, picotamide > **4d*** (2-CH₃). The result indicated that the derivatives bearing a strong electron-withdrawing group in the 2-position turned out to have remarkable the anti-platelet activity, which may be related to the electron-withdrawing inductive effect.

Encouraged by results of our previous studies on mono-substituted compounds (**4a***–**f***) and based on the above mentioned reports, fluorine, chlorine, and methyl groups are, respectively, retained at the 2-position of the phenyl rings, and fluorine, chlorine, bromine, and methyl groups are continuously introduced at the 4-position of phenyl rings, eight novel 2, 4-disubstituted compounds (**5a**–**h**) were designed and synthesized in this study. Subsequently, in order to compare the similarities and differences on anti-platelet activity between the 2, 4-disubstituted compounds (**5a**-**h**) and 3, 4-disubstituted compounds (**5i**–**p**), 2-position are replaced with 3-position and introduced same substituent groups at the 4-position of phenyl rings, eight novel 3, 4-disubstituted compounds (**5i**–**p**) were designed and synthesized.

Material and methods

General experimental instrument and reagent

All chemical reagents were purchased from Tianjin Hengshan (P.R. China), Aladdin Industrial Corporation (P.R. China) and Energy Chemical (P.R. China), and used without further purification. The reagents of cell viability were

Target compounds

Intermediate

Reagents and conditions : (a) ClSO₃H, CH₂Cl₂, 40 °C, 5 h; (b) SOCl₂, 50 °C, 2 h; (c) Ar-NH₂, CH₂Cl₂ / THF, r.t.;

Scheme 1 Synthesis route of target compounds (**5a–p**)

purchased from Beyotime Biotechnology (P.R. China), other biological reagents ADP was purchased from Solarbio life sciences and AA was purchased from Sigma.

The melting points were veritably measured on X-4 digital display micro melting point apparatus and were uncorrected. The IR spectra were confirmed by Fourier transfrom infrared (FTIR) 1700 infrared spectrophotometer with KBr as diluents. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were obtained by Bruker spectrometers (Bruker, Rheinstetten, Germany), tetramethylsilane (TMS, 0.05 % v/v) as internal standard, shift chemical are illustrated in parts per million (δ_{ppm}) in deuterated solvents (DMSO-d_6), coupling constant values (J) are expressed in Hertz. Signal multiplicities are reported by: s (singlet), d (doublet), t (triplet), dd (double doublet), q (quartet), m (multiplet), and brs (broad signal). Thin-layer chromatography (TLC) analysis was accomplished with Silica gel plates GF254 and observed with UV irradiation (254 nm). Electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on an Agilent 6520B UPLC-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The physicochemical parameters including Clog P value, refractivity, polarizability, molecular volume, and surface area were calculated by MarvinSketch 17.13 software.

Chemistry

The synthetic pathway of the target compounds (**5a–p**) was depicted in Scheme 1 and their structure were shown in Table 1. Reaction of phenetole with chlorosulfonic acid and thionyl chide to produce the intermediate 4-ethoxybenzene-1,3-disulfonyl chloride, using dichloromethane as solvent. The intermediate was reacted with the different disubstituted anilines in tetrahydrofuran (THF) or dichloromethane (CH₂Cl₂) to obtain the target compounds with excellent yield.

General procedure for the preparation of intermediate

4-Ethoxy-1, 3-benzenedisulfonyl chloride was synthesized according to the literature method of 4-methoxy-1, 3-benzenedisulfonyl chloride (Liu et al. 2006).

Table 1 Structures of 4-Ethoxy-1, 3-benzenedisulfonamides (**5a–p**)

Compound	R₁	R₂	Compound	R₁	R₂
4a[*]	1-H	2-F	**5f**	2-CH₃	4-Cl
4b[*]	1-H	2-Cl	**5g**	2-CH₃	4-Br
4c[*]	1-H	2-Br	**5h**	2-CH₃	4-CH₃
4d[*]	1-H	2-CH₃	**5i**	3-F	4-F
4e[*]	1-H	3-CH₃	**5j**	3-F	4-Cl
4f[*]	1-H	4-CH₃	**5k**	3-Cl	4-F
5a	2-F	4-F	**5l**	3-Cl	4-Cl
5b	2-F	4-Cl	**5m**	3-CH₃	4-F
5c	2-Cl	4-F	**5n**	3-CH₃	4-Cl
5d	2-Cl	4-Cl	**5o**	3-CH₃	4-Br
5e	2-CH₃	4-F	**5p**	3-CH₃	4-CH₃

Note: These compounds (4a*–4f*) were synthesized by member of our laboratory and published in the degree dissertation. Zhang JF (2018) Synthesis and Study on Antiplatelet Activity & Toxicity of 4-Ethoxydisulfonamides. China, Tianjin university of technology

Phenetole (2.40 mL; 0.02 mol) was dropwise added to a solution of chlorosulfonic acid (9.20 mL; 0.14 mol) in anhydrous dichloromethane (15 mL) at 0 °C under mechanical stirring and the reaction mixture was stirred at 40 °C for about 5 h. Subsequently, thionyl chide (2.90 mL; 0.04 mol) was dropwise added to the reaction mixture and the reaction mixture was stirred at 50 °C for about 2 h. The reaction was monitored by TLC (PE : EA = 1:1, Rf = 0.67). After that, the mixture was poured into a mixture of water and ice with stirring. The white solid was filtered with excellent yield. The crude product was purified by recrystallization from cyclohexane five times to obtain the high purity intermediate. Yield: 65.4 %; m.p.: 99.3–100.2 °C.

General procedure for the preparation of target compounds (5a–p)

N¹, N³-bis (2, 4-difluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide (5a)

The intermediate 4-ethoxy-1, 3-benzenedisulfo chloride (1.2 g, 3.7 mmol) was dropwise added to a solution of 2, 4-difluoro aniline (1.0 g, 7.5 mmol) with dichloromethane

(15 mL) as solvent at room temperature under magnetic stirring. After 10 min, triethylamine (0.5 mL) as acid-binding agent was dropwise added to the reaction mixture. The mixture was stirred under reflux at 35 °C for about 8 h. After the reaction completed as monitored by TLC, the solvent was removed under reduced pressure. Under the condition of ice bath, the mixture was dissolved in cold 5% NaOH aqueous solution to obtain filtrate. The solid was obtained by adjusting the pH of filtrate with hydrochloric acid (V_{HCl}: V_{water} = 1:1) in the condition of ice salt bath. After filtrating and drying, crude product was prepared. The crude product was purified by recrystallization from ethanol with excellent yield (Scheme 1).

Other target compounds (**5b–p**) were prepared by the same process. The results of each compound are described as follows.

N^1, N^3-bis (2-fluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(**4a**[*]): Yellow solid (The crude product was recrystallized by ethanol.) Yield: 70.1%; m.p.: 201.2–207.3 °C; IR (KBr) cm^{-1}: 3278.67 (υ_{NH}), 2898.25 (υ_{CH}), 1417.35, 1348.66 (υ_{SO2}), 1290.12, 1258.09 (υ_{C-O-C}), 920.38, 797.62, 759.45 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.18 (1H, s, –SO$_2$NH–), 9.85 (1H, s, –SO$_2$NH–), 8.42 (1H, d, J = 2.5 Hz, 2-H), 7.82 (1H, dd, J = 8.9, 2.5 Hz, 6-H), 7.35 (1H, d, J = 8.9 Hz, 5-H), 7.24 (4H, m, 3A-H, 5A-H, 3B-H, 5B-H), 7.05 (2H, m, 6A-H, 6B-H), 6.92 (2H, m, 4A-H, 4B-H), 4.23 (2H, q, J = 7.0 Hz, –OCH$_2$–), 1.29 (3H, t, J = 7.0 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS) δ (ppm): 159.31 (C, 4-C), 157.56 (C, 2a-C), 157.51 (C, 2b-C), 133.90 (C, 1a-C), 133.28 (C, 1b-C), 132.16 (C, 1-C), 129.15 (CH, 6-C), 125.11 (CH, 5a-C), 125.05 (CH, 5b-C), 123.87 (CH, 4a-C), 123.35 (CH, 4b-C), 122.66 (CH, 2-C), 120.12 (C, 3-C), 117.64 (CH, 5a-C), 117.45 (CH, 5b-C), 115.50 (CH, 3a-C), 115.34 (CH, 3b-C), 112.28 (CH, 5-C), 65.87 (CH$_2$, –OCH$_2$–), 14.27 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 469.0700 [M + H]$^+$.

N^1, N^3-bis (2-chlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(**4b**[*]): White solid (The crude product was recrystallized by ethanol.) Yield: 72.1%; m.p.: 213.3–215.0 °C; IR (KBr) cm^{-1}: 3277.54 (υ_{NH}), 2946.12 (υ_{CH}), 1470.26, 1328.25 (υ_{SO2}), 1289.56, 1250.37 (υ_{C-O-C}), 923.14, 792.13 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.38 (1H, s, -SO$_2$NH-), 10.27 (1H, s, –SO$_2$NH–), 8.35 (1H, d, J = 2.5 Hz, 2-H), 7.87 (1H, dd, J = 8.6, 2.4 Hz, 6-H), 7.36 (1H, d, J = 8.0 Hz, 5-H), 7.28 (2H, m, 3A-H, 3B-H), 7.15 (2H, m, 5A-H, 5B-H), 6.99 (4H, m, 4A-H, 4B-H, 6A-H, 6B-H),

4.21 (2H, q, J = 7.0 Hz, -OCH$_2$-), 1.40 (3H, t, J = 6.9 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS) δ (ppm): 158.58 (C, 4-C), 131.89 (C, 1-C), 129.80 (CH, 3a-C), 129.56 (CH, 3b-C), 128.89 (CH, 6-C), 127.66 (2 × C, 1a-C, 5a-C), 127.09 (2 × C, 1b-C, 5b-C), 125.65 (2 × C, 2a-C, 4a-C), 125.20 (2 × C, 2b-C, 4b-C), 124.66 (CH, 6a-C), 124.21 (CH, 6b-C), 122.39 (CH, 2-C), 121.39 (C, 3-C), 114.35 (C, 5H-C), 65.72 (CH$_2$, –OCH$_2$–), 14.55 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 531.0426 [M + H]$^+$.

N^1, N^3-bis (2-bromophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(**4c**[*]): White solid (The crude product was recrystallized by ethanol.) Yield: 80.3%; m.p.: 167.5–168.9 °C; IR (KBr) cm^{-1}: 3281.06 (υ_{NH}), 2890.33 (υ_{CH}), 1417.23, 1327.02 (υ_{SO2}), 1288.24 (υ_{C-O-C}), 1163.33, 923.29, 758.14 (υ_{ph-H}); ^{1}H NMR (400-MHz, DMSO-d_6, TMS), δ (ppm): 10.34 (1H, s, –SO$_2$NH–), 10.18 (1H, s, –SO$_2$NH), 8.53 (1H, d, J = 2.2 Hz, 2-H), 7.92 (1H, dd, J = 8.7, 2.3 Hz, 6-H), 7.55 (2H, m, 3A-H, 3B-H), 7.42 (1H, d, J = 7.6 Hz, 5-H), 7.13 (2H, m, 5A-H, 5B-H), 6.79 (4H, m, 4A-H, 4B-H, 6A-H, 6B-H), 4.25 (2H, q, J = 6.8 Hz, –OCH$_2$–), 1.41 (3H, t, J = 7.5 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS) δ (ppm): 158.65 (C, 4-C), 137.30 (C, 1a-C), 136.69 (C, 1b-C), 132.81 (CH, 3a-C), 132.63 (CH, 3b-C), 131.90 (C, 1-C), 130.32 (CH, 6-C), 128.81 (CH, 5a-C), 128.63 (CH, 5b-C), 126.63 (CH, 4a-C), 126.52 (CH, 4b-C), 124.88 (CH, 2-C), 123.27 (CH, 6a-C), 123.06 (CH, 6b-C), 120.15 (CH, 2-C), 117.81 (C, 2a-C), 117.63 (C, 2b-C), 114.4 (CH, 5-C), 65.70 (CH$_2$, –OCH$_2$–), 14.62 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 587.9082 [M + H]$^+$.

N^1, N^3-bis (2-methylphenyl)-4-ethoxybenzene-1, 3-disulfonamide

(**4d**[*]): White solid (The crude product was recrystallized by ethanol.) Yield: 77.8%; m.p.: 170.3–171.0 °C; IR (KBr) cm^{-1}: 3319.63 (υ_{NH}), 2912.35 (υ_{CH}), 1498.12, 1432.11 (υ_{SO2}), 1234.53 (υ_{C-O-C}), 854.34, 752.12, 712.36 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 9.69 (1H, s, –SO$_2$NH–), 9.58 (1H, s, –SO$_2$NH–), 8.47 (1H, d, J = 2.4 Hz, 2-H), 7.95 (1H, dd, J = 8.8, 2.4 Hz, 6-H), 7.30 (1H, d, J = 8.9 Hz, 5-H), 7.11 (4H, m, 3A-H, 3B-H, 5A-H, 5B-H), 6.85 (4H, m, 4A-H, 4B-H, 6A-H, 6B-H), 4.23 (2H, q, J = 7.0 Hz, –OCH$_2$–), 3.79 (3H, s, 2a-CH$_3$), 3.62 (3H, s, 2b-CH$_3$), 1.36 (3H, t, J = 7.3 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS), δ (ppm): 160.15 (C, 4-C), 139.65 (C, 1a-C), 139.23 (C, 1b-C), 133.20 (C, 2a-C), 133.07 (C, 2b-C), 130.11 (C, 1-C), 129.66 (CH, 3a-C), 129.24 (CH, 3b-C), 128.35 (CH, 6-C), 127.82 (CH, 5a-C), 127.08 (CH, 5b-C), 125.34 (CH, 6a-C), 125.01 (CH, 6b-C),

123.65 (CH, 4a-C), 123.33 (CH, 4b-C), 122.54 (CH, 2-C), 119.66 (C, 3-C), 113.57 (CH, 5-C), 65.87 (CH$_2$, –OCH$_2$–), 15.12 (CH$_3$, 2a–CH$_3$), 14.96 (CH$_3$, 2b–CH$_3$), 14.27 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 461.1203 [M + H]$^+$.

N^1, N^3-bis (3-methylphenyl)-4-ethoxybenzene-1, 3-disulfonamide

(4e*): White solid (The crude product was recrystallized by ethanol.) Yield: 80.9%; m.p.: 177.1–178.0 °C; IR (KBr) cm^{-1}: 3302.54 (υ_{NH}), 2978.21 (υ_{CH}), 1398.22, 1342.56 (υ_{SO2}), 1184.43 (υ_{C-O-C}), 952.44, 753.52, 708.45 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 10.58 (1H, s, –SO$_2$NH–), 9.68 (1H, s, –SO$_2$NH–), 9.01 (1H, d, J = 2.4 Hz, 2-H), 7.76 (1H, dd, J = 8.8, 2.4 Hz, 6-H), 7.31 (1H, d, J = 8.9 Hz, 5-H), 7.04 (2H, m, 5A-H, 5B-H), 6.84 (2H, s, 2A-H, 2B-H), 6.73 (4H, m, 4A-H, 4B-H, 6A-H, 6B-H), 4.21 (2H, q, J = 6.8 Hz, –OCH$_2$–), 3.72 (3H, s, 3a-CH$_3$), 3.54 (3H, s, 3b-CH$_3$), 1.58 (3H, t, J = 7.1 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS), δ (ppm): 158.12 (C, 4-C), 142.69 (C, 3a-C), 142.01 (C, 3b-C), 138.69 (C, 1a-C), 138.14 (C, 1b-C), 131.61 (C, 1-C), 129.33 (CH, 5a-C), 129.05 (CH, 5b-C), 129.16 (CH, 6-C), 124.28 (CH, 2-C), 120.16 (C, 3-C), 119.25 (2 × CH, 2a-C, 2b-C), 118.78 (2 × CH, 4a-C, 4b-C), 117.44 (2 × CH, 6a-C, 6b-C), 116.30 (CH, 5-C), 67.43 (CH$_2$, –OCH$_2$–), 15.43 (CH$_3$, 3a–CH$_3$), 15.26 (CH$_3$, 3b–CH$_3$), 14.6 (CH$_3$, -CH$_3$); HR-ESI-MS (m/z): 461.1200 [M + H]$^+$.

N^1, N^3-bis (4-methylphenyl)-4-ethoxybenzene-1, 3-disulfonamide

(4f*): White solid (The crude product was recrystallized by ethanol.) Yield: 89.1%; m.p.: 214.5–215.3 °C; IR (KBr) cm^{-1}: 3269.49 (υ_{NH}), 2892.05 (υ_{CH}), 1394.17, 1382.21 (υ_{SO2}), 1064.44 (υ_{C-O-C}), 824.31, 722.33, 702.58 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 11.09 (1H, s, –SO$_2$NH–), 10.81 (1H, s, –SO$_2$NH–), 7.99 (1H, d, J = 2.4 Hz, 2-H), 7.72 (1H, dd, J = 8.8, 2.4 Hz, 6-H), 7.39 (1H, d, J = 8.9 Hz, 5-H), 7.05 (4H, m, 3A-H, 3B-H, 5A-H, 5B-H), 6.98 (4H, 2A-H, 2B-H, 6A-H, 6B-H), 4.25 (2H, q, J = 7.0 Hz, –OCH$_2$–), 3.58 (3H, s, 4a-CH$_3$), 3.76 (3H, s, 4b-CH$_3$), 1.69 (3H, t, J = 7.3 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6, TMS), δ (ppm): 159.92 (C, 4-C), 136.75 (C, 1a-C), 136.13 (C, 1b-C), 133.78 (C, 4a-C), 133.35 (C, 4b-C), 131.60 (C, 1-C), 129.33 (2 × CH, 3a-C, 5a-C), 129.12 (2 × CH, 3b-C, 5b-C), 128.57 (CH, 6-C), 122.6 (CH, 2-C), 119.5 (C, 3-C), 116.5 (2 × CH, 2a-C, 6a-C), 116.2 (2 × CH, 2b-C, 6b-C), 112.6 (CH, 5-C), 66.8 (CH$_2$, -OCH$_2$), 22.8 (2 × CH$_3$, 4a-CH$_3$, 4b-CH$_3$), 14.7 (CH$_3$, -CH$_3$); HR-ESI-MS (m/z): 461.1212 [M + H]$^+$.

N^1, N^3-bis (2, 4-difluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5a): White solid (The crude product was recrystallized by ethanol.) Yield: 37.5%; m.p.: 189.3–191.2 °C; IR (KBr) cm^{-1}: 3126.44 (υ_{NH}), 2983.11 (υ_{CH}), 1400.87, 1336.40 (υ_{SO2}), 1287.53, 1170.76 (υ_{C-O-C}), 967.47, 847.63, 732.06 (υ_{ph-H}); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ (ppm): 10.97 (2H, s, 2 × –SO$_2$NH–), 8.31 (1H, d, J = 2.4 Hz, H-2), 7.91 (1H, dd, J = 10.8, 2.0 Hz, H-6), 7.37 (1H, d, J = 7.2 Hz, H-5), 6.78 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 6.56 (2H, s, 3A-H, 3B-H), 4.25 (2H, q, J = 6.9 Hz, –OCH$_2$–), 1.31 (3H, t, J = 6.8 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 161.21 (C, 4-C), 159.56 (2 × C, 2a-C, 2b-C), 154.53 (2 × C, 4a-C, 4b-C), 131.39 (C, 1-C), 129.65 (C, 1a-C), 129.12 (C, 1b-C), 128.23 (CH, 6-C), 123.74 (CH, 2-C), 121.10 (C, 3-C), 119.36 (2 × CH, 5a-C, 5b-C), 119.03 (2 × CH, 6a-C, 6b-C), 112.06 (CH, 5-C), 104.80 (2 × CH, 3a-C, 3b-C), 66.29 (CH$_2$, –OCH$_2$–), 14.56 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 505.0512 [M + H]$^+$.

N^1, N^3-bis (2-fluoro-4-chlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5b): White solid (The crude product was recrystallized by ethanol.) Yield: 42.2%; m.p.: 176.3–178.0 °C; IR (KBr) cm^{-1}: 3260.59 (υ_{NH}), 2932.12 (υ_{CH}), 1387.66, 1325.78 (υ_{SO2}), 1276.33, 1159.74 (υ_{C-O-C}), 1032.77, 987.32, 855.43, 759.61 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.54 (1H, s, –SO$_2$NH–), 10.26(1H, s, –SO$_2$NH–), 8.44 (1H, d, J = 3.4 Hz, H-2), 7.93 (1H, dd, J = 8.8, 2.7 Hz, H-6), 7.55 (2H, s, 3A-H, 3B-H), 7.23 (1H, d, J = 8.9 Hz, H-5), 6.75 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 4.43 (2H, q, J = 6.8 Hz, –OCH$_2$–), 1.37 (3H, t, J = 6.5 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 158.21 (C, 4-C), 156.79 (C, 2a-C), 156.22 (C, 2b-C), 133.88 (C, 1a-C), 133.63 (C, 1b-C), 131.66 (C, 1-C), 128.79 (CH, 6-C), 127.53 (2 × C, 4a-C, 4b-C), 125.76 (2 × CH, 5a-C, 5b-C), 123.98 (CH, 2-C), 120.55 (C, 3-C), 119.32 (2 × CH, 6a-C, 6b-C), 117.76 (CH, 5-C), 113.24 (2 × CH, 3a-C, 3b-C), 68.87 (CH$_2$, –OCH$_2$–), 15.65 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 538.3738 [M + H]$^+$.

N^1, N^3-bis (2-chloro-4-fluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5c): White solid (The crude product was recrystallized by ethanol) Yield: 44.1%; m.p.: 202.1–204.0 °C; IR (KBr) cm^{-1}: 3126.50 (υ_{NH}), 2916.64 (υ_{CH}), 1400.80, 1336.51 (υ_{SO2}), 1287.34, 1170.82(υ_{C-O-C}), 907.55, 847.76, 732.28 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.14 (1H, s, -SO$_2$NH-), 9.83 (1H, s, -SO$_2$NH-), 8.34 (1H,

d, $J = 3.6$ Hz, H-2), 7.89 (1H, dd, $J = 7.2$, 2.4 Hz, H-6), 7.63 (2H, s, 3A-H, 3B-H), 7.48 (1H, d, $J = 12.0$ Hz, H-5), 6.81 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 4.25 (2H, q, $J = 6.5$ Hz, –OCH$_2$–), 1.31 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.14 (C, 4-C), 153.79 (C, 4a-C), 153.36 (C, 4b-C), 131.22 (C, 1-C), 129.35 (CH, 6-C), 127.21 (2 × C, 2a-C, 2b-C), 124.93 (C, 1a-C), 124.29 (C, 1b-C), 122.01 (CH, 2-C), 119.87 (C, 3-C), 118.99 (CH, 6a-C), 118.56 (CH, 6b-C), 117.87 (2 × CH, 3a-C, 3b-C), 115.66 (CH, 5-C), 113.87 (2 × CH, 5a-C, 5b-C), 65.96 (CH$_2$,–OCH$_2$–), 14.30 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 538.4125 [M + H]$^+$.

N^1, N^3-bis (2, 4-dichlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5d): White solid (The crude product was recrystallized by ethanol.) Yield: 42.2%; m.p.: 176.3–178.0 °C; IR (KBr) cm^{-1}: 3131.84 (υ_{NH}), 2826.07 (υ_{CH}), 1400.72, 1341.16 (υ_{SO2}), 1285.55, 1146.34(υ_{C-O-C}), 1066.78, 997.93, 844.78, 742.19 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.97 (1H, s, –SO$_2$NH–), 10.78 (1H, s, –SO$_2$NH–), 8.31 (1H, d, $J = 3.6$ Hz, H-2), 7.99 (1H, dd, $J = 9.0$, 2.5 Hz, H-6), 7.56 (2H, s, 3A-H, 3B-H), 7.37 (1H, d, $J = 8.7$ Hz, H-5), 6.90 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 4.29 (2H, q, $J = 6.8$ Hz, –OCH$_2$–), 1.29 (3H, t, $J = 6.5$ Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.21 (C, 4-C), 134.24 (C, 1-C), 130.39 (2 × CH, 3a-C, 3b-C), 128.56 (CH, 6-C), 126.87 (CH, 5a-C), 126.38 (CH, 5b-C), 126.06 (2 × C, 2a-C, 2b-C), 124.80 (C, 1a-C), 124.67 (C, 1b-C), 124.26 (2 × C, 4a-C, 4b-C), 122.03 (CH, 2-C), 120.23 (C, 3-C), 117.18 (2 × CH, 6a-C, 6b-C), 115.97 (CH, 5-C), 66.09 (CH$_2$, –OCH$_2$–), 14.33 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 571.3266 [M + H]$^+$.

N^1, N^3-bis (2-methyl-4-fluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5e): Red solid (The crude product was recrystallized by isopropanol.) Yield: 54.0%; m.p.: 188.3–190.0 °C; IR (KBr) cm^{-1}: 3133.45 (υ_{NH}), 2887.62 (υ_{CH}), 1401.59, 1337.53 (υ_{SO2}), 1284.83, 1166.48 (υ_{C-O-C}), 997.08, 834.44, 723.16 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 9.58 (2H, s, 2 × -SO$_2$NH-), 7.99 (1H, d, $J = 2.0$ Hz, H-2), 7.73 (1H, dd, $J = 11.2$, 2.4 Hz, H-6), 7.39 (1H, d, $J = 8.8$ Hz, H-5), 6.92 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 6.70 (2H, s, 3A-H, 3B-H), 4.27 (2H, q, $J = 6.4$ Hz, –OCH$_2$–), 2.12 (3H, s, 2a-CH$_3$), 1.99 (3H, s, 2b-CH$_3$), 1.33 (3H, t, $J = 6.6$ Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.61 (C, 4-C), 159.57 (C, 4a-C), 159.13 (C, 4b-C), 135.41 (C, 1a-C), 135.21 (C, 1b-C), 131.09 (C, 1-C), 130.71 (C, 2a-C), 130.26 (C, 2b-C), 128.77 (CH, 6-C), 124.58 (CH, 2-C), 120.69 (C, 3-C), 117.67 (CH, 6a-C),

117.52 (CH, 6b-C), 115.45 (CH, 3a-C), 115.30 (CH, 3b-C), 114.33 (CH, 5-C), 112.66 (CH, 5a-C), 112.38 (CH, 5b-C), 65.94 (CH, –OCH$_2$–), 18.01 (2×-CH$_3$, 2a-CH$_3$, 2b-CH$_3$), 14.35(CH$_3$,–CH$_3$); HR-ESI-MS (m/z): 497.1031 [M + H]$^+$.

N^1, N^3-bis (2-methyl-4-chlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5f): White solid (The crude product was recrystallized by ethanol.) Yield: 51.5%; m.p.: 194.3–195.8 °C; IR (KBr) cm^{-1}: 3133.95 (υ_{NH}), 2818.41 (υ_{CH}), 1400.25, 1336.66 (υ_{SO2}), 1165.30 (υ_{C-O-C}), 923.53, 872.28, 713.78 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 9.72 (1H, s, –SO$_2$NH–), 9.57 (1H, s, –SO$_2$NH–), 7.94 (1H, d, $J = 2.2$ Hz, H-2), 7.75 (1H, dd, $J = 12.8$, 2.4 Hz, H-6), 7.39 (1H, d, $J = 8.8$ Hz, H-5), 7.19 (2H, s, 3A-H, 3B-H), 6.89 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 4.25 (2H, q, $J = 6.7$ Hz, –OCH$_2$–), 2.11 (3H, s, 2a-CH$_3$), 1.97 (3H, s, 2b-CH$_3$), 1.31 (3H, t, $J = 7.0$ Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.15 (C, 4-C), 137.26 (C, 1a-C), 137.20 (C, 1b-C), 135.63 (CH, 3a-C), 135.03 (CH, 3b-C), 131.77 (C, 1-C), 130.33 (C, 2a-C), 130.19 (C, 2b-C), 128.74 (CH, 6-C), 126.74 (CH, 5a-C), 126.72 (CH, 5b-C), 124.89 (C, 4a-C), 124.80 (C, 4b-C), 122.64 (CH, 2-C), 119.22 (C, 3-C), 117.97 (CH, 6a-C), 117.73 (CH, 6b-C), 113.69 (CH, 5-C), 65.87 (CH$_2$, –OCH$_2$–), 17.76 (2 × –CH$_3$, 2a-CH$_3$, 2b-CH$_3$), 14.31(CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 530.0676 [M + H]$^+$.

N^1, N^3-bis (2-methyl-4-bromophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5g): Red solid (The crude product was recrystallized by methanol.) Yield: 41.3%; m.p.: 207.0–209.0 °C; IR (KBr) cm^{-1}: 3249.19 (υ_{NH}), 2889.18 (υ_{CH}), 1339.13, 1281.83 (υ_{SO2}), 1170.15 (υ_{C-O-C}), 1107.85 (υ_{C-N}), 922.61, 816.70, 729.89 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 9.72 (1H, s, –SO$_2$NH–), 9.56 (1H, s, –SO$_2$NH–), 7.95 (1H, d, $J = 2.0$ Hz, H-2), 7.75 (1H, dd, $J = 11.2$, 2.4 Hz, H-6), 7.41 (1H, d, $J = 8.4$ Hz, H-5), 7.17 (4H, m, 3A-H, 3B-H, 5A-H, 5B-H), 6.79 (2H, m, 6A-H, 6B-H), 4.25 (2H, q, $J = 6.8$ Hz, -OCH$_2$-), 2.11 (3H, s, 2a-CH$_3$), 1.97 (3H, s, 2b-CH$_3$), 1.31 (3H, t, $J = 6.5$ Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.15 (C, 4-C), 137.51 (C, 1a-C), 137.48 (C, 1b-C), 135.71 (CH, 3a-C), 135.68 (CH, 3b-C), 132.67 (C, 2a-C), 132.55 (C, 2b-C), 131.77 (C, 1-C), 129.96 (CH, 5a-C), 129.61 (CH, 5b-C), 127.54 (CH, 6-C), 124.59 (CH, 2-C), 122.89 (C, 3-C), 118.03 (CH, 6a-C), 117.98 (CH, 6b-C), 114.69 (CH, 5-C), 112.62 (C, 4a-C), 112.38 (C, 4b-C), 65.88 (CH$_2$, –OCH$_2$–), 17.70 (–CH$_3$, 2a-CH$_3$), 17.68 (-CH$_3$, 2b-CH$_3$), 14.31 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 619.4814 [M + H]$^+$.

N^1, N^3-bis (2, 4-dimethylphenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5h): White solid (The crude product was recrystallized by ethanol.) Yield: 46.3%; m.p.: 186.5–187.6 °C; IR (KBr) cm^{-1}: 3238.46 (υ_{NH}), 2910.28 (υ_{CH}), 1403.75, 1321.55 (υ_{SO2}), 1287.53, 1163.21 (υ_{C-O-C}), 954.26, 856.32, 725.14 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 9.67 (2H, s, 2 × -SO$_2$NH-), 8.15 (1H, d, J = 2.6 Hz, H-2), 7.82 (1H, dd, J = 10.5, 2.2 Hz, H-6), 7.42 (1H, d, J = 7.3 Hz, H-5), 6.94 (2H, m, 5A-H, 5B-H), 6.69 (4H, m, 3A-H, 3B-H, 6A-H, 6B-H), 4.36 (2H, q, J = 7.1 Hz, -OCH$_2$-), 2.33 (3H, s, 4a-CH$_3$), 2.30 (3H, s, 4b-CH$_3$), 2.20 (3H, s, 2a-CH$_3$), 2.15 (3H, s, 2b-CH$_3$), 1.32 (3H, t, J = 6.7 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 160.58 (C, 4-C), 138.77 (C, 4a-C), 138.25 (C, 4b-C), 134.60 (C, 1a-C), 134.11 (C, 1b-C), 132.77 (CH, 3a-C), 132.56 (CH, 3b-C), 130.12 (C, 1-C), 128.88 (CH, 6-C), 127.65 (2 × C, 2a-C, 2b-C), 125.66 (2 × CH, 5a-C, 5b-C), 124.23 (CH, 2-C), 122.01 (C, 3-C), 118.87 (CH, 6a-C), 118.45 (CH, 6b-C), 113.31 (CH, 5-C), 66.87 (CH$_2$, -OCH$_2$-), 20.78 (CH$_3$, 4a-CH$_3$), 20.31 (CH$_3$, 4b-CH$_3$), 17.52 (CH$_3$, 2a-CH$_3$), 17.03 (CH$_3$, 2b-CH$_3$), 14.34 (CH$_3$, -CH$_3$); HR-ESI-MS (*m/z*): 489.1542 [M + H]$^+$.

N^1, N^3-bis (3, 4-difluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5i): White solid (The crude product was recrystallized by ethanol.) Yield: 46.6%; m.p.: 205.1–206.3 °C; IR (KBr) cm^{-1}: 3134.29 (υ_{NH}), 2981.59 (υ_{CH}), 1401.00, 1324.29 (υ_{SO2}), 1285.69, 1156.85 (υ_{C-O-C}), 973.66, 817.17, 744.11 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.48 (1H, s, -SO$_2$NH-), 10.32 (1H, s, -SO$_2$NH-), 8.39 (1H, d, J = 2.4 Hz, H-2), 7.96 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.43 (1H, d, J = 8.8 Hz, H-5), 7.29 (2H, m, 5A-H, 5B-H), 6.84 (2H, s, 2A-H, 2B-H), 6.45 (2H, m, 6A-H, 6B-H), 4.27 (2H, q, J = 7.6 Hz, -OCH$_2$-), 1.30 (3H, t, J = 7.0 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.16 (C, 4-C), 144.70 (C,3a-C), 144.34 (C, 3b-C), 137.28 (C, 4a-C), 137.04 (C, 4b-C), 135.82 (C, 1a-C), 135.27 (C, 1b-C), 132.55 (C, 1-C), 127.59 (CH, 6-C), 124.47 (CH, 2-C), 122.87 (C, 3-C), 115.87 (CH, 5-C), 114.43 (CH, 5a-C), 114.07 (CH, 5b-C), 112.29 (CH, 6a-C), 112.09 (CH, 6b-C), 108.30 (CH, 2a-C), 108.10 (CH, 2b-C), 65.97 (CH$_2$, -OCH$_2$-), 14.34 (CH$_3$, -CH$_3$); HR-ESI-MS (*m/z*): 505.0511 [M + H]$^+$.

N^1, N^3-bis (3-fluoro-4-chlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5j): White solid (The crude product was recrystallized by acetone.) Yield: 43.3%; m.p.: 199.0−200.2 °C; IR(KBr)

cm^{-1}: 3253.33 (υ_{NH}), 2981.81 (υ_{CH}), 1383.91, 1333.20 (υ_{SO2}), 1288.11, 1155.44 (υ_{C-O-C}), 981.84, 812.25, 742.53 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.75 (1H, s, -SO$_2$NH-), 10.34 (1H, s, -SO$_2$NH-), 8.30 (1H, d, J = 4.0 Hz, H-2), 7.92 (1H, dd, J = 8.4, 3.6 Hz, H-6), 7.43 (1H, d, J = 11.6 Hz, H-5), 7.34 (2H, m, 5A-H, 5B-H), 6.92 (4H, m, 2A-H, 2B-H, 6A-H, 6B-H), 4.25 (2H, q, J = 7.3 Hz, -OCH$_2$-), 1.29 (3H, t, J = 6.5 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 162.50 (C,3a-C), 162.07 (C, 3b-C), 159.79 (C, 4-C), 136.33 (C, 1a-C), 136.27 (C, 1b-C), 133.09 (C, 1-C), 130.25 (CH, 5a-C), 130.06 (CH, 5b-C), 127.47 (CH, 6-C), 122.99 (CH, 2-C), 120.20 (C, 3-C), 117.49 (CH, 6a-C), 117.24 (CH, 6b-C), 114.9 (CH, 5-C), 111.59 (CH, 4a-C), 111.35 (CH, 4b-C), 105.49 (CH, 2a-C), 105.29 (CH, 2b-C), 66.05 (CH$_2$, -OCH$_2$-), 14.35 (CH$_3$, -CH$_3$); HR-ESI-MS (*m/z*): 538.4102 [M + H]$^+$.

N^1, N^3-bis (3-chloro-4-fluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5k): White solid (The crude product was recrystallized by ethanol.) Yield: 45.5%; m.p.: 204.0–205.3 °C; IR (KBr) cm^{-1}: 3158.20 (υ_{NH}), 2987.01 (υ_{CH}), 1400.68, 1326.62 (υ_{SO2}), 1107.21 (υ_{C-O-C}), 961.44, 774.67 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.49 (2H, s, 2 × -SO$_2$NH-), 8.40 (1H, d, J = 2.4 Hz, H-2), 7.95 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.34 (1H, d, J = 8.8 Hz, H-5), 7.20–7.02 (4H, m, 2A-H, 2B-H, 5A-H, 5B-H), 6.76 (2H, m, 6A-H, 6B-H), 4.26 (2H, q, J = 6.7 Hz, -OCH$_2$-), 1.29 (3H, t, J = 7.1 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.14 (C, 4-C), 146.93 (C, 4a-C), 146.29 (C, 4b-C), 136.00 (C, 1a-C), 135.88 (C, 1b-C), 131.21 (C, 1-C), 128.66 (CH, 6-C), 122.75 (CH, 2-C), 121.77 (C, 3-C), 120.57 (C,3a-C), 120.16 (C, 3b-C), 118.54 (CH, 2a-C), 118.21 (CH, 2b-C), 115.98 (CH, 6a-C), 115.78 (CH, 6b-C), 114.92 (CH, 5-C), 113.66 (CH, 5a-C), 113.25 (CH, 5b-C), 65.96 (CH$_2$, -OCH$_2$-), 14.30 (CH$_3$, -CH$_3$); HR-ESI-MS (*m/z*): 538.9937 [M + H]$^+$.

N^1, N^3-bis (3, 4-dichlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5l): White solid (The crude product was recrystallized by ethanol.) Yield: 45.5%; m.p.: 190.6–191.8 °C; IR (KBr) cm^{-1}: 3241.30 (υ_{NH}), 2989.38 (υ_{CH}), 1401.04, 1316.00 (υ_{SO2}), 1156.30 (υ_{C-O-C}), 933.19, 859.67, 731.92 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 9.51 (1H, s, -SO$_2$NH-), 9.35 (1H, s, -SO$_2$NH-), 7.93 (1H, d, J = 2.4 Hz, H-2), 7.68 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.35 (1H, d, J = 8.8 Hz, H-5), 7.26–7.02 (6H, m, 2 × -C$_6$H$_3$), 4.27 (2H, q, J = 9.2 Hz, -OCH$_2$-), 1.32 (3H, t, J = 6.4 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.94 (C,

4-C), 135.34 (C, 1a-C), 135.31 (C, 1b-C), 131.49 (C, 3a-C), 131.21 (C, 3b-C), 130.33 (C, 1-C), 129.76 (CH, 5a-C), 129.12 (CH, 5b-C), 128.84 (CH, 6-C), 123.66 (C, 4a-C), 123.14 (C, 4b-C), 122.65 (CH, 2-C), 121.54 (C, 3-C), 120.34 (CH, 6a-C), 120.02 (CH, 6b-C), 117.78 (CH, 2a-C), 117.15 (CH, 2b-C), 114.45 (CH, 5-C), 65.73 (CH$_2$, –OCH$_2$–), 14.42 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 570.2911 [M + H]$^+$.

N^1, N^3-bis (3-methyl-4-fluorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5m): White solid (The crude product was recrystallized by ethanol.) Yield: 50.6%; m.p.: 197.0–199.0 °C; IR (KBr) cm^{-1}: 3253.81 (υ_{NH}), 2872.32 (υ_{CH}), 1383.24, 1331.11 (υ_{SO2}), 1288.64, 1153.93 (υ_{C-O-C}), 974.75, 853.40, 741.35 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.41 (2H, s, 2 × –SO$_2$NH–), 8.26 (1H, d, J = 2.4 Hz, H-2), 7.85 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.29 (1H, d, J = 4.8 Hz, H-5), 7.06–6.78 (6H, m, 2 × -C$_6$H$_3$), 4.25 (2H, q, J = 9.1 Hz, -OCH$_2$-), 2.09 (6H, d, J = 11.2 Hz, 3a-CH$_3$, 3b-CH$_3$), 1.31 (3H, t, J = 6.2 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.06 (C, 4-C), 151.51 (C, 4a-C), 151.34 (C, 4b-C), 134.62 (C, 1a-C), 134.17 (C, 1b-C), 131.93 (C, 1-C), 127.29 (CH, 6-C), 125.67 (C, 3a-C), 125.20 (C, 3b-C), 122.77 (CH, 2-C), 120.59 (C, 3-C), 117.88 (CH, 2a-C), 117.34 (CH, 2b-C), 116.66 (CH, 5a-C), 116.49 (CH, 5b-C), 115.21 (CH, 5-C), 113.40 (CH, 6a-C), 113.14 (CH, 6b-C), 65.89 (CH$_2$, –OCH$_2$–), 14.39 (CH$_3$, –CH$_3$), 13.94 (2 × CH$_3$, 3a-CH$_3$, 3b-CH$_3$); HR-ESI-MS (m/z): 497.1052 [M + H]$^+$.

N^1, N^3-bis (3-methyl-4-chlorophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5n): White solid (The crude product was recrystallized by isopropanol.) Yield: 50.0%; m.p.: 202.2–203.7 °C; IR (KBr) cm^{-1}: 3252.51 (υ_{NH}), 2892.91 (υ_{CH}), 1384.07, 1327.24 (υ_{SO2}), 1148.98 (υ_{C-O-C}), 945.81, 873.15, 820.07, 734.92 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.42 (2H, s, 2 × –SO$_2$NH–), 8.26 (1H, d, J = 2.0 Hz, H-2), 7.85 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.31 (1H, d, J = 9.2 Hz, H-5), 7.29–6.94 (4H, m, 5A-H, 5B-H, 6A-H, 6B-H), 6.76 (2H, s, 2A-H, 2B-H), 4.25 (2H, q, J = 10.3 Hz, –OCH$_2$–), 2.19 (6H, d, J = 11.2 Hz, 3a-CH$_3$, 3b-CH$_3$), 1.29 (3H, t, J = 7.2 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.05 (C, 4-C), 137.55 (C, 3a-C), 137.17 (C, 3b-C), 135.36 (C, 1a-C), 135.01 (C, 1b-C), 132.25 (C, 1-C), 130.92(CH, 5a-C), 130.73 (CH, 5b-C), 128.14 (CH, 6-C), 125.99 (C, 4a-C), 125.69 (C, 4b-C), 123.36 (CH, 2-C), 121.11 (C, 3-C), 119.74 (CH, 6a-C), 119.2 (CH, 6b-C), 117.96 (CH, 2a-C),

117.35 (CH, 2b-C), 114.97 (CH, 5-C), 65.89 (CH$_2$, –OCH$_2$–), 19.23 (2 × CH$_3$, 3a-CH$_3$, 3b-CH$_3$), 14.36 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 531.0417 [M + H]$^+$.

N^1, N^3-bis (3-methyl-4-bromophenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5o): White solid (The crude product was recrystallized by isopropanol.) Yield: 31.4%; m.p.: 209.0–210.3 °C; IR (KBr) cm^{-1}: 3246.04 (υ_{NH}), 2881.73 (υ_{CH}), 1381.09, 1327.05 (υ_{SO2}), 1149.61 (υ_{C-O-C}), 1040.41, 936.84, 820.46, 722.81 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.49 (1H, s, –SO$_2$NH–), 10.34 (1H, s, –SO$_2$NH–), 8.26 (1H, d, J = 2.1 Hz, H-2), 7.96 (1H, dd, J = 7.2, 2.0 Hz, H-6), 7.31 (1H, d, J = 9.6 Hz, H-5), 7.27 (2H, m, 5A-H, 5B-H), 6.96 (4H, m, 2A-H, 2B-H, 6A-H, 6B-H), 4.25 (2H, q, J = 10.5 Hz, –OCH$_2$–), 2.19 (6H, d, J = 8.4 Hz, 3a-CH$_3$, 3b-CH$_3$), 1.29 (3H, t, J = 5.8 Hz, –OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.06 (C, 4-C), 138.54 (C, 3a-C), 138.15 (C, 3b-C), 136.99 (C, 1a-C), 136.93 (C, 1b-C), 133.69 (CH, 5a-C), 133.11 (CH, 5b-C), 130.36 (C, 1-C), 128.79 (CH, 6-C), 123.54 (CH, 2-C), 120.42 (C, 3-C), 118.26 (CH, 2a-C), 118.03 (CH, 2b-C), 116.69 (CH, 6a-C), 116.39 (CH, 6b-C), 114.91 (C, 4a-C), 114.63 (C, 4b-C), 113.66 (CH, 5-C), 65.99 (CH$_2$, –OCH$_2$–), 22.02 (2 × CH$_3$, 3a-CH$_3$, 3b-CH$_3$), 14.35 (CH$_3$, –CH$_3$); HR-ESI-MS (m/z): 619.9387 [M + H]$^+$.

N^1, N^3-bis (3, 4-dimethylphenyl)-4-ethoxybenzene-1, 3-disulfonamide

(5p): White solid (The crude product was recrystallized by ethanol.) Yield: 35.3%; m.p.: 182.3–183.9 °C; IR (KBr) cm^{-1}: 3130.75 (υ_{NH}), 2980.86 (υ_{CH}), 1400.39, 1329.35 (υ_{SO2}), 1148.86 (υ_{C-O-C}), 1018.94, 963.20, 815.27, 734.59 (υ_{ph-H}); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 10.09 (1H, s, –SO$_2$NH–), 9.91 (1H, s, –SO$_2$NH–), 8.41 (1H, d, J = 2.0 Hz, H-2), 7.96 (1H, dd, J = 11.2, 2.4 Hz, H-6), 7.46 (1H, d, J = 8.8 Hz, H-5), 7.26 (2H, m, 6A-H, 6B-H), 6.84 (4H, m, 2A-H, 2B-H, 5A-H, 5B-H), 4.25 (2H, q, J = 6.6 Hz, -OCH$_2$-), 2.64 (3H, s, 3a-CH$_3$), 2.60 (3H, s, 3b-CH$_3$), 2.43 (3H, s, 4a-CH$_3$), 2.38 (3H, s, 4b-CH$_3$), 1.32 (3H, t, J = 6.4 Hz, -OCH$_2$CH$_3$); ^{13}C-NMR (101MHz, DMSO-d_6, TMS) δ (ppm): 159.94 (C, 4-C), 137.39 (C, 3a-C), 137.20 (C, 3b-C), 135.34 (C, 1a-C), 135.31 (C, 1b-C), 131.11 (C, 1-C), 130.41 (CH, 5a-C), 130.29 (CH, 5b-C), 128.94 (CH, 6-C), 125.45 (C, 4a-C), 125.12 (C, 4b-C), 124.69 (CH, 2-C), 122.30 (C, 3-C), 116.98 (CH, 6a-C), 116.46 (CH, 6b-C), 114.54 (CH, 2a-C), 114.34 (CH, 2b-C), 113.80 (CH, 5-C), 65.73 (CH$_2$, –OCH$_2$–), 19.96 (2 × CH$_3$, 3a-CH$_3$, 3b-CH$_3$), 19.04 (2 × CH$_3$, 4a-CH$_3$, 4b-CH$_3$), 14.22 (–CH$_3$); HR-ESI-MS (m/z): 489.1539 [M + H]$^+$.

Bioassay

In vitro anti-platelet aggregation activity assay

The in vitro anti-platelet aggregation activities of the target compounds (**5a–p**) have been evaluated by Born's turbidimetry (Born 1962; Barbosa et al. 2006; Liu et al. 2018).

Rabbit were anesthetized with 10% chloral hydrate, and blood was collected from the rabbit auricle vein. Whole blood was centrifuged at room temperature for 10 min (800–1000 rpm) with 3.8% citrate as anticoagulant (9:1 by volume), platelet-rich plasma (PRP) was attained from the supernatant. Platelet-poor plasma (PPP) was obtained by centrifugation of the remaining layer at room temperature for 10–15 min (3000 rpm), which was used to set the zero (confirm 100% luminousness).

Test compounds (at the concentration of 1.3 μM) previously dissolved in DMSO (5 μl) were added into PRP (20 μl). The same volume of DMSO without target compounds were added as control sample (The aim was to prove that DMSO seems to have no remarkable effect on the anti-platelet aggregation activity). After incubating for 5 min at 37 °C, ADP and AA were added as inducers and platelet aggregation were measured. The percentage inhibition of platelet aggregation of the target compounds was calculated according to the following formula:

$$\text{congregate}\,(\%) = \frac{\text{Abs(PPP)} - \text{Abs(PRP)}}{\text{Abs(PPP)}} \times 100\%$$

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

S = the platelet aggregation in the presence of solvent. D = the platelet aggregation in the presence of test compounds.

The anti-platelet aggregation activity was denoted as percentage inhibition of platelet aggregation and the IC_{50} values of compounds with higher activities were calculated.

Cytotoxicity assay on L-929 cells

According to the results of in vitro anti-platelet aggregation activity, compounds with higher activities were chosen to further conduct in vitro cytotoxicity study. Mouse fibroblast cells (L-929) were used to assess the in vitro cytotoxicity by Cell Counting Kit-8 (CCK-8) assays (Abe et al. 2000; Xiong et al. 2007).

L-929 were added into sterilized the 96-well microplates (1×10^4 cells per well immersed in RPMI-1640 medium) and incubated in a humid 5% CO_2 atmosphere at 37 °C for 24 h. Afterwards, the cells were exposed to target compounds at a specific concentration, continued to incubate in a humid 5% CO_2 atmosphere at 37 °C for 48 h. After cultivation for 48 h, the medium was removed and replaced with fresh RPMI-1640 medium (100 mL). Ultimately, CCK-8 solution was added into the 96-well microplates (10 μL, per/well) and cultivated at 37 °C for 2 h. The absorbance was monitored and recorded on a microplate reader (Bio-Tek FLx800 fluorescence microplate reader) at 450 nm.

The cell viability rate on L-929 was calculated by the following formula:

$$\text{Cellviability}\,(\%) = \frac{\text{Abs(testcell)} - \text{Abs(blankcell)}}{\text{Abs(controlcell)} - \text{Abs(blankcell)}}$$

Docking studies

The crystal structure of P_2Y_{12} receptor (PDB code 4PXZ) was obtained from the RCSB Protein Data Bank. The 3D structures of target compounds (**5a** and **5h**) were drawn in mol2 format through ChemBio 3D. The docking studies were performed with AutoDock Vina in PyRx software. The best docked pose with the lowest energy calculated by AutoDock version 4.2 was selected and analyzed with PyMol software.

Results and discussion

In vitro anti-platelet aggregation activity

With picotamide, aspirin, and clopidogrel as positive control drugs, the in vitro anti-platelet activities of target compounds were assessed induced by ADP and AA. The primary screening data for target compounds (1.3 μM) in vitro activities on anti-platelet aggregation were given in Table 2 (with ADP as inducer) and Table 3 (with AA as inducer).

As were shown in Fig. 2, we surprisingly found that most 2, 4-disubstituted compounds (**5a**, **5b**, **5c**, **5d**, **5g**, **5h**) exhibited superior inhibitory activity than positive control agent picotamide while 3, 4-disubstituted compounds (**5i–p**) have no remarkable inhibitory activity induced by ADP at the concentration of 1.3 μM. The order of inhibition rate induced by ADP was: **5h** (44.5%) > clopidogrel (39.1%) > **5c** (34.86%) > **5a** (34.5%) > aspirin (32.52%) > **5b** (31.67%) > **5d** (30.45%) > **5g** (27.8%) > picotamide (26.56%). The IC_{50} values of compounds with higher activities were further calculated when ADP was used as inducer. The order of anti-platelet aggregation activity was: **5h** (0.32 μM) > Clopidogrel (0.41 μM) > **5c** (0.49 μM) > **5a** (0.51 μM) > Aspirin (0.53 μM) > **5b** (0.66 μM) > **5g** (0.73 μM) > Picotamide (0.76 μM) > **5d** (0.82 μM). Moreover, the IC_{50} value of compounds **5h** (0.32 μM), **5c** (0.49 μM), **5a** (0.51 μM) were lower than that of two positive control drugs aspirin (0.53 μM) and picotamide (0.76 μM) induced by ADP.

Table 2 The inhibition rate and IC_{50} of target compounds for ADP

Compound	Dose (μM)	Congregate rate (%)	Inhibition rate (%)	IC_{50} (μM)
DMSO	–	45.29 ± 5.34	–	
Picotamide	1.3	33.26 ± 2.73	26.56	0.76
Aspirin	1.3	30.56 ± 5.51	32.52	0.53
Clopidogrel	1.3	27.58 ± 4.06	39.1	0.41
4a[*]	1.3	20.8 ± 1.3[#]	50.89	
4b[*]	1.3	22.1 ± 0.9[#]	48.65	
4c[*]	1.3	22.8 ± 0.1[#]	47.9	
4d[*]	1.3	34.7 ± 4.9	23.76	
4e[*]	1.3	39.4 ± 10.6	14.48	
4f[*]	1.3	35.6 ± 2.0[#]	22.01	
5a	1.3	29.53 ± 4.29[#]	34.5	0.51
5b	1.3	30.92 ± 4.8[#]	31.67	0.66
5c	1.3	29.5 ± 2.5[#]	34.86	0.49
5d	1.3	31.5 ± 2.4	30.45	0.82
5e	1.3	42.5 ± 1.3[#]	6.4	
5f	1.3	36.82 ± 1.63[#]	18.74	
5g	1.3	32.69 ± 6.3[#]	27.8	0.73
5h	1.3	25.1 ± 1.3	44.5	0.32
5i	1.3	39.3 ± 2.9[#]	13.2	
5j	1.3	37.73 ± 9.8[#]	18.9	
5k	1.3	34.15 ± 2.65	24.6	
5l	1.3	34.69 ± 6.3[#]	23.5	
5m	1.3	58.5 ± 4.3[#]	–	
5n	1.3	43.9 ± 2.0[#]	3.3	
5o	1.3	45.2 ± 2.0[#]	–	
5p	1.3	35.2 ± 1.4[#]	22.28	

[*]Reported compound

[#]$p < 0.01$ VS Control group

Table 3 The inhibition rate of target compounds for AA

Compound	Dose (μM)	Congregate rate (%)	Inhibition rate (%)
DMSO	–	40.4 ± 0.6	–
Picotamide	1.3	27.2 ± 1.7	32.7
Aspirin	1.3	28.7 ± 2.6	28.9
Clopidogrel	1.3	30.9 ± 2.5	23.5
4a[*]	1.3	20.5 ± 4.5[#]	48.63
4b[*]	1.3	21.0 ± 3.2[#]	47.24
4c[*]	1.3	21.6 ± 2.5[#]	46.89
4d[*]	1.3	31.7 ± 8.6	20.85
4e[*]	1.3	33.2 ± 3.1	18.23
4f[*]	1.3	32.6 ± 1.9[#]	19.6
5a	1.3	28.6 ± 5.6[#]	29.6
5b	1.3	28.0 ± 2.5[#]	30.56
5c	1.3	32.4 ± 3.8[#]	19.8
5d	1.3	31.2 ± 1.5[#]	22.8
5e	1.3	38.5 ± 1.3[#]	4.7
5f	1.3	32.1 ± 3.3[#]	20.5
5g	1.3	28.5 ± 1.9[#]	29.4
5h	1.3	23.1 ± 1.7[#]	40.9
5i	1.3	31.3 ± 0.8[#]	22.5
5j	1.3	37.5 ± 2.1	7.2
5k	1.3	29.7 ± 1.2[#]	27.8
5l	1.3	28.9 ± 1.9[#]	28.5
5m	1.3	40.0 ± 1.3	0.9
5n	1.3	38.6 ± 2.2	4.5
5o	1.3	42.9 ± 3.1[#]	–
5p	1.3	28.1 ± 0.9[#]	30.4

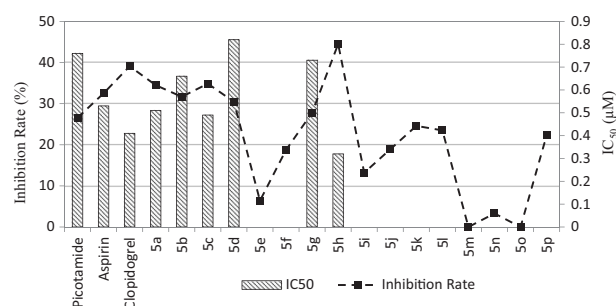[*]Reported compound

[#]$p < 0.01$ VS Control group

Especially, compound **5h** revealed the highest inhibition rate (44.5%) and the lowest IC_{50} value (0.32 μM) than three positive control drugs clopidogrel (39.1%, 0.41 μM), aspirin (32.52%, 0.53 μM), picotamide (26.56%, 0.76 μM) against ADP induced platelet aggregation.

On the other hand, as were shown in Fig. 3, 2, 4-disubstituted compounds (**5a**, **5b**, **5g**, **5h**) and 3, 4-disubstituted compounds (**5k**, **5l**, **5p**) had higher inhibition rate than positive control agent clopidogrel induced by AA at the concentration of 1.3 μM. The order of inhibition rate induced by AA was: **5h** (40.9%) > Picotamide (32.7%) > **5b** (30.56%) > **5p** (30.4%) > **5a** (29.6%) > **5g** (29.4%) > Aspirin (28.9%) > **5l** (28.5%) > **5k** (27.8%) > Clopidogrel (23.5%). Simultaneously, compounds **5h** (40.9%), **5b** (30.56%), **5p** (30.4%), **5a** (29.6%) and **5g** (29.4%) exhibited higher inhibition rates than that of two positive control agents aspirin (28.9%) and clopidogrel (23.5%). It was worth noting that compound **5h** (40.9%) displayed highest

Fig. 2 The inhibition rate and IC_{50} of target compounds for ADP

inhibition rates than three positive control drugs picotamide (32.7%), aspirin (28.9%), and clopidogrel (23.5%).

Cytotoxicity on L-929 cells

According to the results of in vitro anti-platelet activities, nine compounds were chosen to further conduct in vitro cytotoxicity study. The results analysis of in vitro
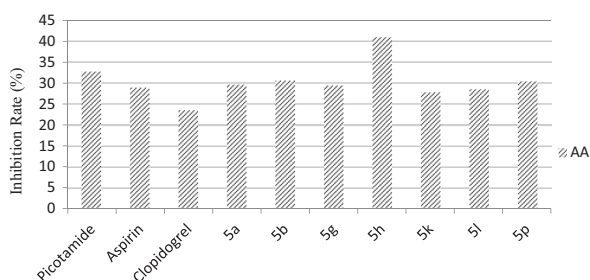
Fig. 3 The inhibition rate of target compounds with higher activities for AA

cytotoxicities were shown in Table 4. The results revealed that the cytotoxicities of the target compounds were positively correlated with the drug concentration and were negatively correlated with the IC_{50} values. The order of cytotoxicity was: **5h** (86.46 μM) < **5l** (74.73 μM) < Picotamide (72.54 μM) < **5k** (67.46 μM) < **5a** (62.13 μM) < **5g** (53.48 μM) < **5b** (52.79 μM) < **5p** (37.56 μM) < **5c** (37.30 μM) < **5d** (14.30 μM/L). Among them, the IC_{50} values of two target compounds **5h** (86.46 μM), **5l** (74.73 μM) were higher than picotamide (72.54 μM). The results displayed that compounds **5h** and **5l** had the lower cytotoxicity than picotamide.

As were shown in Fig. 4, we known that compound **5h** had the highest anti-platelet activity (44.5, 28.5 %) induced by ADP and AA and the lowest cytotoxicity (86.46 μM). What's more, other eight compounds were no significant relationship between the anti-platelet activities and cytotoxicities.

Docking studies

In order to confirm target compounds can interact with the P_2Y_{12} receptor sites on the platelet membrane, target compounds with higher activities (**5a** and **5h**) were selected to docked and analyzed the binding interaction. The molecular docking results shown that compounds **5a** had a good binding affinity represented by a total docking score of −8.93 kcal/mol (Fig. 5a). In the docking pose, compound **5a** forms four H-bonds to the binding-sites residues which including the polar and uncharged Gln-263, Asn-159, Asn-191, and the polar and positively charged Arg-256. Similarly, the docking results for compound **5h** also exhibited higher binding affinity in the total score of −11.55 kcal/mol (Fig. 5b). In the docking pose, compound **5h** forms eight H-bonds to the binding-sites residues which including the polar and uncharged Gln-263, Asn-159, Asn-191, Tyr-105, and the polar and positively charged Arg-256, Lys-179, Lys-280, and His-187. Moreover, analyzing the chemical nature of the amino acid residues, it was revealed that compound **5a** and **5h** bind to hydrophilic residues which form multiple H-bonds network.

Table 4 Cytotoxicity effect on L-929

Compound	Dose (μM)	Inhibition rate (%)	IC_{50} (μM)
picotamide	10	10.2%	72.54
	20	20.7%	
	40	30.8%	
	80	54.9%	
	100	67.7%	
5a	10	22.5%	62.13
	20	31.8%	
	40	42.2%	
	80	57.6%	
	100	78.4%	
5b	10	26.5%	52.79
	20	31.6%	
	40	45.3%	
	80	60.7%	
	100	75.5%	
5c	10	32.7%	37.30
	20	41.7%	
	40	53.3%	
	80	61.7%	
	100	73.5%	
5d	10	44.8%	14.30
	20	56.9%	
	40	63.9%	
	80	72.8%	
	100	84.3%	
5g	10	14.3%	53.48
	20	28.7%	
	40	45.5%	
	80	59.3%	
	100	79.4%	
5h	10	14.3%	86.46
	20	25.2%	
	40	37.0%	
	80	43.4%	
	100	64.9%	
5k	10	16.3%	67.46
	20	27.6%	
	40	40.8%	
	80	54.4%	
	100	72.6%	
5l	10	20.4%	74.73
	20	30.6%	
	40	42.2%	
	80	51.4%	
	100	65.7%	
5p	10	24.5%	37.56
	20	36.7%	
	40	51.8%	
	80	69.5%	
	100	83.3%	

Structure activity relationship (SAR) exploration

In order to investigate the probable correlation between the physicochemical parameters of the target compounds and
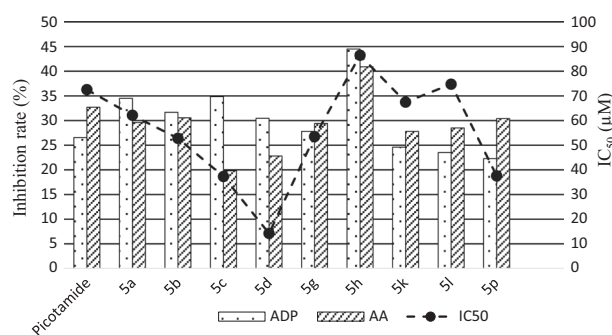
Fig. 4 Anti-platelet activity of target compounds and cytotoxicity

Fig. 5 **a** Docking results for compound 5a having total docking scores of −8.93. **b** Docking results for compound 5h having total docking scores of −11.55

their anti-platelet activity, the structure activity relationship (SAR) analysis was performed with various molecular descriptors. The hydrophobic effect has been described by mean of the octanol–water partition coefficient (Clog P). The surface area and molecular volume have been used as descriptors for the steric effect. The polarizability and refractivity have been considered as descriptor for both electronic state (London dispersive forces) properties and volume of the molecules. The physicochemical parameters of the target compounds are listed in Table 5. Analysis of the general molecular parameters does not show a significant relationship between the anti-platelet activities and physicochemical parameters of the target compounds.

However, we think that anti-platelet activity of target compounds could be influenced by the numbers and positions of the different substituents attached to the benzene ring.

1. The effect of anti-platelet aggregation activity when the two substitutions were introduced into the 2-position and 4-position of phenyl rings.

Effects of the 2, 4-disubstituted compounds on the anti-platelet activity induced by ADP and AA shows similar pattern. The order of anti-platelet aggregation activity was: **4a**[*] (2-fluoro) > **5a** (2, 4-difluoro) > **5b** (2-fluoro-4-chloro) > picotamide; **4b**[*] (2-chloro) > **5c** (2-chloro-4-fluoro) > **5d** (2, 4-dichloro) > picotamide. It was manifested that the activity of 2, 4-dihalogenated compounds were lower than that of 2-halogenated compounds, while their activity were higher than that of picotamide.

The order of anti-platelet aggregation activity was: **5h** (2, 4-dimethyl) > **5g** (2-methyl-4-bromo) > picotamide > **4d**[*] (2-methyl) > **4f**[*] (4-methyl) > **5f** (2-methyl-4-chloro) > **5e** (2-methyl-4-fluoro). That meant the activity of 2, 4-dialkyl compounds can be significantly enhanced than that of 2- or 4-alkyl. Moreover, when the derivatives bearing an electron-donating group and an electron-withdrawing group are concurrently introduced into the 2-po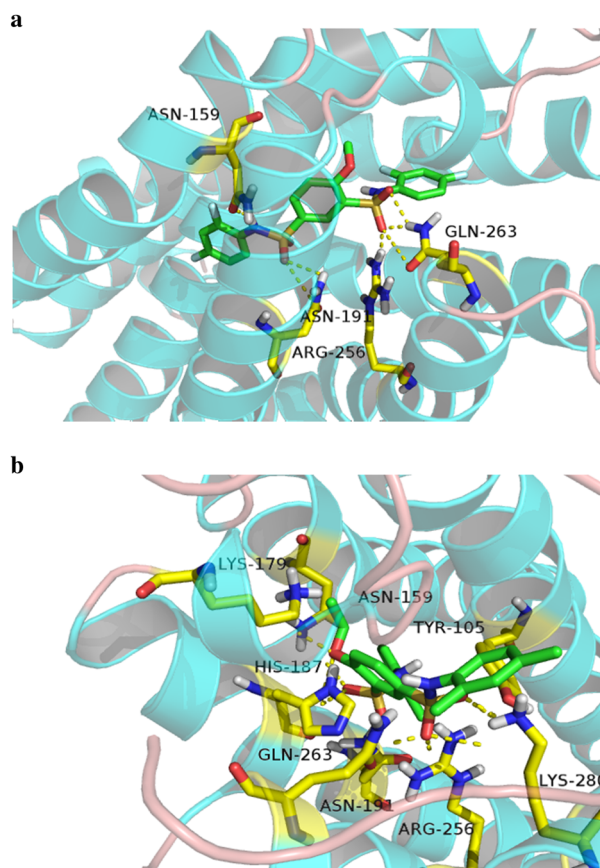sition and 4-position of phenyl rings, bromo-substituted compounds are more potent than the fluoro- and chloro-substituted analogs.

2. The effect of anti-platelet aggregation activity when the two substitutions were introduced into the 3-position and 4-position of the phenyl rings.

The order of anti-platelet aggregation activity induced by ADP was: **5a** (2, 4-difluoro) > **5b** (2-fluoro-4-chloro) > **5j** (3-fluoro-4-chloro) > **5i** (3, 4-difluoro); **5c** (2-chloro-4-fluoro) > **5d** (2, 4-dichloro) > **5k** (3-chloro-4-fluoro) > **5l** (3, 4-dichloro); **5h** (2, 4-dimethyl) > **5p** (3, 4-dimethyl); **5g** (2-methyl-4-bromo) > **5f** (2-methyl-4-chloro) > **5e** (2-methyl-4-fluoro) > **5o** (3-methyl-4-bromo), **5n** (3-methyl-4-chloro), **5m** (3-methyl-4-fluoro). In general, the result indicated that the activity of 3, 4-disubstituted compounds were lower than that of 2, 4-disubstituted compounds induced by ADP.

The order of anti-platelet aggregation activity induced by AA was similar as ADP, except four compounds **5l** (3, 4-dichloro), **5k** (3-chloro-4-fluoro), **5d** (2, 4-dichloro), and **5c** (2-chloro-4-fluoro). The order of activity of these four compounds is: **5l** > **5k** > **5d** > **5c**. The result revealed that

Table 5 General molecular parameters of the target compounds (**4a***–**f***, **5a–p**)

Compound	Clog P	Refractivity	Polarizability	Molecular volume	Surface area
4a*	3.365	111.37	43.50	370.86	607.41
4b*	4.285	120.54	46.27	389.18	624.93
4c*	4.585	126.18	50.21	397.92	632.25
4d*	3.774	121.02	47.96	395.08	657.07
4e*	4.294	121.02	47.96	394.83	658.87
4f*	4.294	121.02	47.95	394.90	658.85
5a	3.680	111.80	43.90	380.63	620.95
5b	4.820	120.98	47.80	399.01	640.16
5c	4.600	120.98	47.83	398.82	638.71
5d	5.740	130.15	52.14	417.04	657.12
5e	4.143	121.45	47.41	404.71	671.17
5f	5.283	130.63	51.75	423.23	689.97
5g	5.583	136.26	53.68	431.81	698.07
5h	4.772	131.10	51.48	429.11	722.05
5i	4.440	111.80	43.50	380.82	621.64
5j	5.720	120.98	47.84	398.85	639.60
5k	5.720	120.98	47.86	398.80	639.63
5l	6.620	130.15	52.18	417.01	657.48
5m	5.263	121.45	47.41	404.77	671.84
5n	6.403	130.63	51.75	423.01	689.87
5o	6.703	136.26	53.69	431.73	697.70
5p	5.792	131.10	51.48	428.68	723.42

Note: These compounds (4a*–4f*) were synthesized by member of our laboratory and published in the degree dissertation. Zhang JF (2018) Synthesis and Study on Antiplatelet Activity & Toxicity of 4-Ethoxydisulfonamides. China, Tianjin university of technology

some 3, 4-disubstituted compounds possibly help to promote the anti-platelet aggregation activity than that of 2, 4-disubstituted compounds induced by AA.

Conclusion

Sixteen target compounds (**5a-p**), which are the picotamide analogs, were synthesized and evaluated to the anti-platelet aggregation activity in vitro. According to the results of anti-platelet activities, compounds with higher activities were selected to continue in vitro cytotoxicity study. It has been identified that compound **5h** had the highest anti-platelet activity and the lowest cytotoxicity. The SAR study indicated that the anti-platelet activity can be changed by the introduction of different substituents in different positions of the phenyl rings. Among them, the compound bearing two methyl groups at 2- and 4-positions of the phenyl rings have remarkable in vitro anti-platelet activity than others. To sum up, *N*, *N*'-disubstitutedphenyl-4-ethoxylbenzene-1, 3-disulfonamides (**5a–p**) may help in developing novel anti-platelet agents and have the crucial significance.

Compliance with ethical standards

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