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ORIGINAL RESEARCH

Design, synthesis, and biological evaluation of sulfonic acid ester and benzenesulfonamide derivatives as potential CETP inhibitors

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Abstract Epidemiological studies have established an inverse relationship between plasma high-density lipoprotein (HDL) cholesterol concentration, and incidence of coronary artery disease (CAD); thus, the development of novel therapies that attempt to exploit the atheroprotective functions of HDL is a major goal. Inhibition of cholesteryl ester transfer protein (CETP) is one of the approaches targeted to increase HDL cholesterol concentration. CETP is a glycoprotein involved in transporting lipoprotein particles and neutral lipids between HDL and low-density lipoproteins (LDL), and therefore CETP inhibitors could be useful agents in the future for treating dyslipidemia and related disorders. Guided by our previously reported pharmacophore and QSAR models for CETP inhibition, we synthesized and bioassayed a series of sulfonic acid ester and benzenesulfonamide derivatives that can serve as a promising lead compounds for the development of potential and selective CETP inhibitors. The most potent compound **6k** illustrated an IC_{50} of 3.4 μM.

Keywords CETP inhibitors · High-density lipoprotein · Pharmacophore modeling · Benzenesulfonamide · Sulfonic acid ester

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Introduction

Cardiovascular disease continues to be a leading cause of death worldwide. Dyslipidemia, a major risk factor for cardiovascular disease, represents a multifactorial disease and current antidyslipidemic medicines focus on either lowering low-density lipoproteins (LDL) cholesterol or raising high-density lipoprotein (HDL) cholesterol (Grass *et al.*, 1995; Paigen *et al.*, 1990; Hansson, 2005).

A number of epidemiological studies have established an inverse relationship between serum HDL cholesterol levels and the incidence of ischemic heart disease (Julve *et al.*, 2011; Lamarche *et al.*, 1996). HDL removes excess cholesterol from peripheral tissues to the liver for biliary elimination (Cuchel *et al.*, 2010; Lewis, 2006; Tall *et al.*, 2001). CETP, a 476-residue glycoprotein, is engaged in interchanging lipoprotein particles and neutral lipids, including cholesteryl esters, phospholipids, and triglycerides between HDL and LDL (Chapman *et al.*, 2010). CETP, as revealed by X-ray crystallography (PDB code: 2OBD, resolution 2.2 Å), has a lipophilic binding site capable of binding up to four lipid molecules (Qiu *et al.*, 2007). In human plasma, CETP plays a proatherogenic task by moving cholesteryl esters from HDL to very low-density lipoprotein (VLDL) and LDL particles, thereby lowering atheroprotective HDL cholesterol and raising proatherogenic VLDL and LDL cholesterols. Obviously, the risk of coronary artery disease (CAD) is proportional to the plasma levels of CETP (Vasan *et al.*, 2009; Boekholdt *et al.*, 2004). Actually, It is rather frequent within the CAD population to have elevated CETP plasma protein levels that are 2- to 3-fold higher than concentrations typically found in the plasma of normal subjects (1–3 μg/ml) (McPherson *et al.*, 1991).

Indication exists that the outcomes of CETP activity may depend on the metabolic setting, particularly on

triglyceride levels. Therefore, pharmacological CETP inhibition may reduce the risk of CAD in humans, but only in those with high triglyceride levels (Vasan *et al.*, 2009; Boekholdt *et al.*, 2004).

The inaccessibility of a reasonable high-resolution crystallographic structures for CETP combined with its large binding pocket locked up most modeling-related discovery projects to ligand-based approaches particularly quantitative structure–activity relationship analysis (QSAR) (Castilho *et al.*, 2007; Hanumantharao *et al.*, 2005; Kelkar *et al.*, 2004; Cronin and Schultz, 2003; Akamatsu, 2002).

Earlier, we have developed ligand-based three-dimensional (3D) pharmacophores integrated within self-consistent QSAR model for CETP inhibitors. The pharmacophore models were used as 3D search queries to mine 3D libraries for new CETP inhibitors, while the QSAR model predicted their biological activities and therefore prioritize them for in vitro evaluation (Abu Khalaf *et al.*, 2010; Abu Sheikha *et al.*, 2010). Our shape-constrained Hypo4/8 captured the most potent hit (**1**) with IC_{50} value of 1.9 μM (see Fig. 1) (Abu Khalaf *et al.*, 2010).

However, optimization based upon the pharmacophoric features of hit **1** and the lipophilic binding site of CETP target guided us to design and synhesize a novel series of derivatives of *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamide (**6a–6g**, Scheme 1), *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide (**6h–6l**, Scheme 1), *N*-(4-benzylaminophenyl)-toluene-4-sulfonic acid ester (**8a–8c**, Scheme 2), and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid ester (**8d–8l**, Scheme 2) that have CETP inhibitory activities. In the newly synthesized compounds, we explore the electronic effect of different substituents such as CF_3, Br, NO_2, Cl, OCH_3, and CH_3 together with the compound lipophilicity effect on the CETP inhibitory activity.

Materials and methods

General methods

The proposed structures for compounds **6a–6l** and **8a–8l** were confirmed via elemental analyses, IR spectroscopy, mass spectroscopy, ^{1}H- and ^{13}C-NMR spectra.

Melting points were measured using Gallenkampf melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were collected on a Varian Oxford NMR^{300} spectrometer. The samples were dissolved in $CDCl_3$. Mass spectrometry was performed using LC Mass Bruker Apex-IV mass spectrometer utilizing an electrospray interface.

Infrared spectra were recorded using Shimadzu IRAffinity-1 spectrophotometer. The samples were dissolved in $CHCl_3$ and analyzed as thin solid films using NaCl plates. Analytical thin layer chromatography (TLC) was carried out using pre-coated aluminum plates and visualized by UV light (at 254 and/or 360 nm). Elemental analysis was performed using EuroVector elemental analyzer.

Chemicals and solvents were purchased from corresponding companies (Sigma-Aldrich, Riedel-de Haen, Fluka, BDH Laboratory Supplies and Promega Corporation) and were used in the experimentation without further purification.

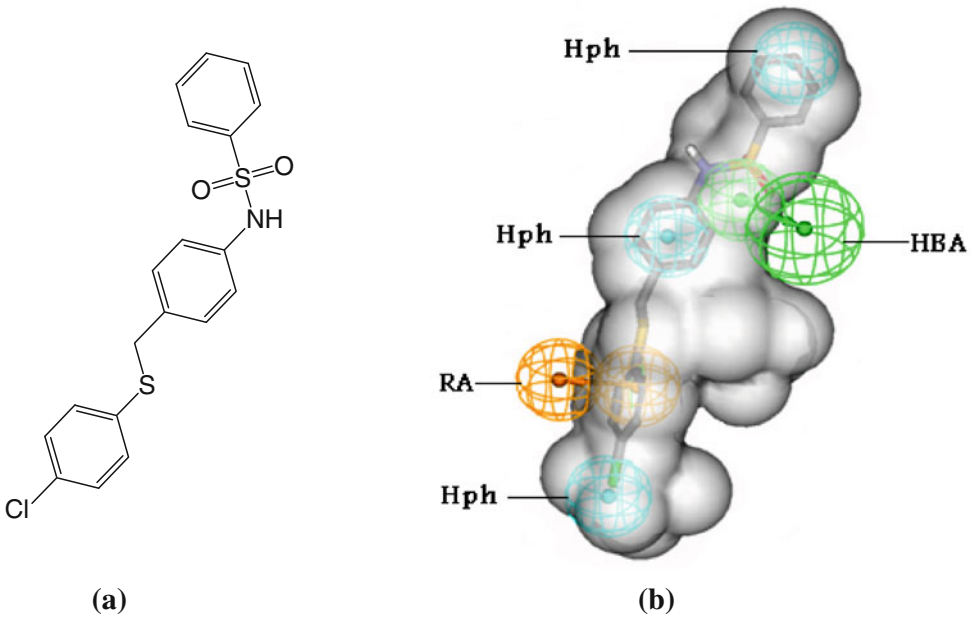
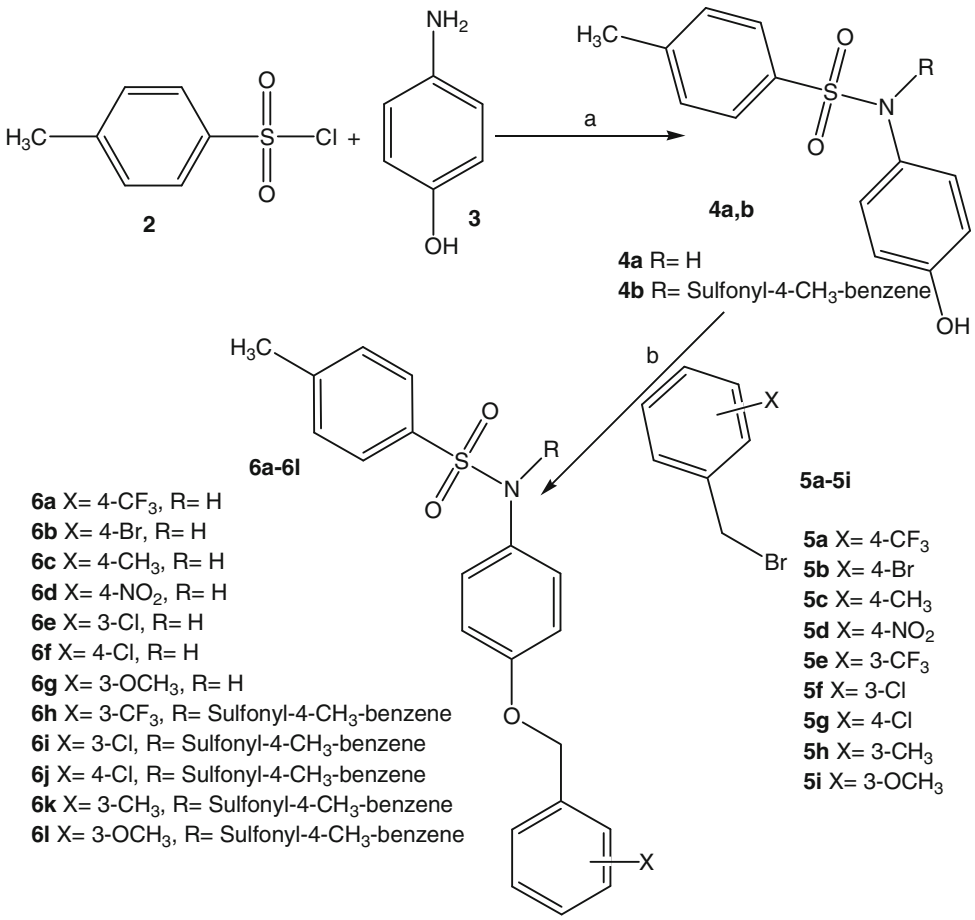


Fig. 1 The mapping of shape-complemented Hypo4/8 pharmacophore hypotheses against hit **1** ((HBA) Hydrogen bond acceptor, (Hph) hydrophobic features, (RA) ring aromatic): **a** Inhibitor **1**, **b** Hypo4/8 fitted against **1**



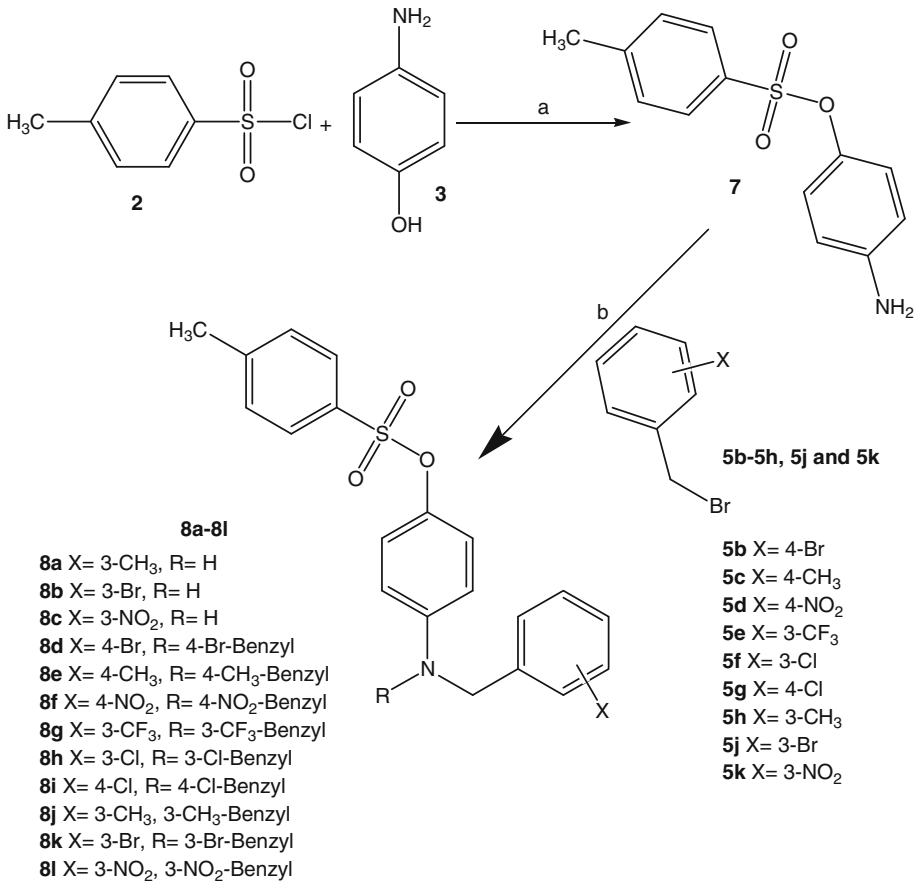
Scheme 1 Synthesis of *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamide (**6a–6g**) and *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide (**6h–6l**) derivatives. Reagents and conditions: (*a*) DCM, TEA, RT, 2 h; (*b*) DMF, NaOH, RT, 3 h

General procedure for the synthesis of *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamide and *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide derivatives (**6a–6l**)

Tosyl chloride **2** (0.953 g, 5 mmol) was dissolved in CH_2Cl_2 (10 ml) in an ice bath and *p*-aminophenol **3** (0.655 g, 6 mmol) was added. Then, triethylamine (0.83 ml, 6 mmol) was added. The mixture was stirred at room temperature for 2 h and then extracted with water (10 ml). The organic extract was dried on anhydrous Na_2SO_4 and filtered. The residue, after evaporation of the solvent, was purified by column chromatography eluting with CH_2Cl_2/MeOH (95:5) to give *N*-(4-hydroxy-phenyl)-4-methyl-benzenesulfonamide **4a** (0.397 g, 30%) and **4b** (0.167 g, 16%).

Subsequently, *N*-(4-hydroxy-phenyl)-4-methyl-benzenesulfonamide **4a** (0.741 g, 2.8 mmol) was dissolved in DMF (5 ml) in an ice bath and one of the substituted benzyl bromides **5a–5i** (3.6 mmol) was added. Then, NaOH (0.24 g, 6 mmol) was added. The mixture was stirred at room temperature for 3 h and then DMF was evaporated. The residue was dissolved in CH_2Cl_2 (10 ml) and extracted with water (10 ml). The organic extract was dried on anhydrous Na_2SO_4 and filtered. The residue, after evaporation of the solvent, was purified by column chromatography eluting with CH_2Cl_2/cyclohexane (85:15) to give *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamide derivatives **6a–6g**.

In addition, *N*-(4-hydroxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide **4b** (1.169 g, 2.8 mmol) was dissolved in DMF (5 ml) in an ice bath and one of the substituted benzyl bromides **5a–5i** (3.6 mmol) was added. Then, NaOH (0.24 g, 6 mmol) was added. The mixture was stirred at room temperature for 3 h and then DMF was evaporated. The residue was dissolved in CH_2Cl_2 (10 ml) and extracted with water (10 ml). The organic extract was dried on anhydrous Na_2SO_4 and filtered. The residue, after evaporation of the solvent, was purified by column chromatography eluting with CH_2Cl_2/cyclohexane (85:15) to give *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide derivatives **6h–6l**.



Scheme 2 Synthesis of 4-benzylaminophenyl-toluene-4-sulfonic acid ester (**8a–8c**) and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid ester (**8d–8l**) derivatives. Reagents and conditions: (*a*) DCM, TEA, RT, 2 h; (*b*) DMF, RT, 3 h

*N-(4-Hydroxy-phenyl)-4-methyl-benzenesulfonamide (**4a**)*

Offwhite powder (30%): $R_f = 0.77$ ($CHCl_3$–MeOH, 95:5); mp. 162–163°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.3 (s, 1H, O*H*), 7.62 (d, $J = 8.25$ Hz, 2H), 7.40 (d, $J = 8.33$ Hz, 2H), 6.95 (dd, $J = 8.94$, 3.0 Hz, 2H), 6.85 (dd, $J = 8.96$, 2.98 Hz, 2H), 2.35 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.25 (1C), 145.53 (1C), 137.32 (1C), 131.68 (1C), 130.21 (2C), 127.16 (2C), 123.41 (2C), 121.51 (2C), 21.66 ppm (1C); IR (thin film) cm^{-1} 3465, 3021, 2963, 1721, 1603, 1501, 1377, 1215.

*N-(4-Hydroxyphenyl)-N-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide (**4b**)*

White powder (16%): $R_f = 0.85$ ($CHCl_3$–MeOH, 95:5); mp. 150–151°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.4 (s, 1H, O*H*), 7.59 (d, $J = 8.34$ Hz, 2H), 7.44 (d, $J = 8.28$ Hz, 2H), 6.95–7.10 (m, 4H), 6.75–6.89 (m, 4H), 2.43 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.68 (1C), 145.65 (1C), 134.50 (1C), 134.31 (1C), 131.91 (2C), 131.30 (2C), 129.80 (2C), 129.72 (2C), 128.54 (2C), 128.19 (2C), 123.03 (2C), 21.78 (1C), 21.65 ppm (1C); IR (thin film) cm^{-1} 3512, 3027, 2959, 1725, 1601, 1500, 1368, 1221.

*4-Methyl-N-[4-(4-trifluoromethyl-benzyloxy)-phenyl]-benzenesulfonamide (**6a**)*

White powder (33%): $R_f = 0.85$ ($CHCl_3$–MeOH, 98:2); mp. 115–116°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.58 (d, $J = 7.82$ Hz, 2H), 7.48 (dd, $J = 12.09$, 6.96 Hz, 2H), 7.25 (m, 4H), 7.85 (m, 2H), 6.72 (d, $J = 8.75$ Hz, 1H), 6.42 (d, $J = 8.76$ Hz, 1H), 4.70 (s, 2H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.76 (1C), 145.71 (1C), 144.18 (1C), 140.03 (1C), 137.53 (1C), 135.13 (1C), 129.94 (2C), 129.76 (2C), 127.47 (2C), 125.43(2C), 123.08 (2C), 113.06 (2C), 54.21 (1C), 26.96 (1C), 21.74 ppm (1C); IR (thin film) cm^{-1} 3399, 3032, 2963, 1597, 1497, 1454; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 444.08517 ($C_{21}H_{18}F_3NNaO_3S$ requires 444.43377); Anal. Calcd for $C_{21}H_{18}F_3NO_3S$: C 59.85, H 4.31, F 13.52, N 3.32, S 7.61, found: C 59.57, H 4.93, F 13.52, N 3.25, S 7.81.

*N-[4-(4-Bromo-benzyloxy)-phenyl]-4-methyl-benzenesulfonamide (**6b**)*

Off-white powder (63%): $R_f = 0.75$ ($CHCl_3$); mp. 129–130°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.66 (d, $J =$

8.27 Hz, 2H), 7.42 (d, $J = 8.32$ Hz, 2H), 7.28 (d, $J = 8.25$ Hz, 2H), 7.18 (d, $J = 8.28$ Hz, 2H), 6.73 (dd, $J = 6.89$, 1.94 Hz, 2H), 6.43 (dd, $J = 6.95$, 1.86 Hz, 2H), 4.23 (s, 2H), 2.45 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.46 (1C), 145.13 (1C), 141.20 (1C), 137.80 (1C), 132.52 (1C), 131.82 (2C), 129.68 (2C), 129.11 (2C), 128.62 (2C), 123.32 (2C), 121.20 (1C), 113.19 (2C), 47.86 (1C), 21.79 ppm (1C); IR (thin film) cm^{-1} 3399, 3030, 2967, 1605, 1520, 1489, 1474; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 455.00830 ($C_{20}H_{18}BrNNaO_3S$ requires 455.33186); Anal. Calcd for $C_{20}H_{18}BrNO_3S$: C 55.56, H 4.20, Br 18.48, N 3.24, S 7.42, found: C 55.86, H 4.37, Br 18.48, N 3.06, S 7.31.

*4-Methyl-N-[4-(4-methyl-benzyloxy)-phenyl]-benzenesulfonamide (**6c**)*

Off-white powder (15%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); mp. 119–120°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.27$ Hz, 2H), 7.28 (d, $J = 8.30$ Hz, 2H), 7.15 (dd, $J = 13.19$, 8.01 Hz, 4H), 6.72 (dd, $J = 8.92$, 3.34 Hz, 2H), 6.45 (dd, $J = 8.93$, 1.99 Hz, 2H), 4.20 (s, 2H), 2.45 (s, 3H), 2.30 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.92 (1C), 145.01 (1C), 140.99 (1C), 137.17 (1C), 135.86 (1C), 132.64 (1C), 129.64 (2C), 129.42 (2C), 128.64 (2C), 127.56 (2C), 123.24 (2C), 113.02 (2C), 48.28 (1C), 21.75 (1C), 21.14 ppm (1C); IR (thin film) cm^{-1} 3422, 3041, 2924, 1605, 1508, 1447; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 390.11344 ($C_{21}H_{21}NNaO_3S$ requires 390.46238); Anal. Calcd for $C_{21}H_{21}NO_3S$: C 68.64, H 5.76, N 3.81, S 8.73, found: C 68.64, H 5.70, N 3.67, S 8.64.

*4-Methyl-N-[4-(4-nitro-benzyloxy)-phenyl]-benzenesulfonamide (**6d**)*

Yellow powder (12%): $R_f = 0.63$ ($CHCl_3$); mp. 118–119°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.25 (d, $J = 7.95$ Hz, 2H), 7.78 (d, $J = 7.47$ Hz, 2H), 7.55 (d, $J = 8.02$ Hz, 2H), 7.30 (d, $J = 7.5$ Hz, 2H), 6.78 (d, $J = 8.27$ Hz, 2H), 6.45 (d, $J = 7.97$ Hz, 2H), 4.48 (s, 2H), 2.43 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.32 (1C), 146.66 (1C), 146.03 (1C), 145.20 (1C), 141.46 (1C), 132.53 (1C), 129.70 (2C), 129.31 (2C), 127.81 (2C), 124.00 (2C), 123.43 (2C), 113.27 (2C), 47.83 (1C), 21.78 ppm (1C); IR (thin film) cm^{-1} 3395, 3063, 2843, 1605, 1516, 1474; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 421.08286 ($C_{20}H_{18}N_2NaO_5S$ requires 421.43340); Anal. Calcd for $C_{20}H_{18}N_2O_5S$: C 60.29, H 4.55, N 7.03, S 8.05, found: C 60.40, H 4.25, N 6.94, S 8.54.

*N-[4-(3-Chloro-benzyloxy)-phenyl]-4-methyl-benzenesulfonamide (**6e**)*

White oil (13.6%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.7 (d, $J = 8.31$ Hz, 2H), 7.15–7.40 (m, 6H), 6.7 (dd, $J = 6.83$, 2.19 Hz, 2H), 6.4 (dd, $J = 6.86$, 2.2 Hz, 2H), 4.15 (s, 2H), 2.4 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.60 (1C), 145.09 (1C), 141.15 (1C), 141.13 (1C), 134.62 (1C), 132.56 (2C), 130.01 (2C), 129.67 (2C), 128.62 (2C), 127.56 (1C), 127.39 (1C), 125.42 (1C), 123.30 (2C), 113.05 (2C), 47.85 (1C), 21.75 ppm (1C); IR (thin film) cm^{-1} 3426, 3063, 2924, 1601, 1512, 1431, 1177; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 410.05881 ($C_{20}H_{18}ClNNaO_3S$ requires 410.06959).

*N-[4-(4-Chloro-benzyloxy)-phenyl]-4-methyl-benzenesulfonamide (**6f**)*

White powder (30.4%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); mp. 120–121°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.82$ Hz, 2H), 7.20-7.32 (m, 6H), 6.72 (dd, $J = 8.93$, 2.08 Hz, 2H), 6.43 (dd, $J = 8.95$, 2.08 Hz, 2H), 4.25 (s, 2H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.61 (1C), 145.11 (1C), 141.12 (1C), 137.35 (1C), 133.12 (1C), 132.53 (1C), 129.68 (2C), 128.86 (1C), 128.80 (1C), 128.78 (2C), 128.62 (2C), 123.30 (2C), 113.08 (2C), 47.76 (1C), 21.79 ppm (1C); IR (thin film) cm^{-1} 3399, 3032, 2963, 1605, 1512, 1493, 1447, 1173; MS (ESI, positive mode) *m/z* $[M + H]^+$ 388.07687 ($C_{20}H_{19}ClNO_3S$ requires 388.06959); Anal. Calcd for $C_{20}H_{18}ClNO_3S$: C 61.93, H 4.68, Cl 9.14, N 3.61, S 8.27, found: C 60.14, H 4.84, Cl 9.14, N 3.32, S 8.91.

*N-[4-(3-Methoxy-benzyloxy)-phenyl]-4-methyl-benzenesulfonamide (**6g**)*

Off-white powder (17.6%): $R_f = 076$ ($CHCl_3$–MeOH, 98:2); mp. 97–98°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.32$ Hz, 2H), 7.18–7.31 (m, 4H), 6.78–6.92 (m, 2H), 6.72 (dd, $J = 8.93$, 2.13 Hz, 2H), 6.44 (dd, $J = 8.96$, 2.19 Hz, 2H), 4.26 (s, 2H), 3.80 (s, 3H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 159.98 (1C), 146.90 (1C), 145.03 (1C), 141.02 (1C), 140.53 (1C), 132.62 (1C), 129.78 (1C), 129.65 (2C), 128.64 (2 C), 123.25 (2C), 119.71 (1C), 113.12 (1C), 113.02 (2C), 112.73 (1C), 55.27 (1C), 48.44 (1C), 21.75 ppm (1C); IR (thin film) cm^{-1} 3422, 3027, 2963, 1601, 1512, 1454, 1173; MS (ESI, positive mode) *m/z* $[M + H]^+$ 384.12641 ($C_{21}H_{22}NO_4S$ requires 384.11913); Anal. Calcd for $C_{21}H_{21}NO_4S$: C 65.78, H 5.52, N 3.65, S 8.36, found: C 65.36, H 5.62, N 3.27, S 8.15.

*4-Methyl-N-[4-(3-trifluoromethyl-benzyloxy)-phenyl]-N-(toluene-4-sulfonyl)-benzenesulfonamide (**6h**)*

Off-white oil (29.5%): $R_f = 0.85$ ($CHCl_3$–MeOH, 98:2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.58 (d, $J = 8.32$ Hz, 2H), 7.46 (d, $J = 8.27$ Hz, 3H), 7.35 (m, 3H), 7.25 (m, 4H), 6.85 (m, 4H), 4.75 (s, 2H), 2.43 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.88 (1C), 145.55 (1C), 144.07 (1C), 137.59 (1C), 136.90 (1C), 135.18 (1C), 132.28 (1C), 131.81 (1C), 129.97 (2C), 129.69 (2C), 129.02 (1C), 128.44 (2C), 127.72 (2C), 125.17 (2C), 125.12 (2C), 124.65 (1C), 124.60 (1C), 122.98 (2C), 54.28 (1C), 21.60 (1C), 21.52 ppm (1C); IR (thin film) cm^{-1} 3032, 2963, 1597, 1497, 1451, 1161; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 598.09609 ($C_{28}H_{24}F_3NNaO_5S_2$ requires 598.10480).

*4-Methyl-N-[4-(3-chloro-benzyloxy)-phenyl]-N-(toluene-4-sulfonyl)-benzenesulfonamide (**6i**)*

White oil (48.8%): $R_f = 0.79$ ($CHCl_3$–MeOH, 98:2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.58 (d, $J = 8.35$ Hz, 2H), 7.46 (d, $J = 8.29$ Hz, 2H), 7.22–7.30 (m, 4H), 7.05–7.18 (m, 4H), 6.78–6.90 (m, 4H), 4.60 (s, 2H), 2.40 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.73 (1C), 145.64 (1C), 144.08 (1C), 137.69 (1C), 134.90 (1C), 134.31 (1C), 131.91 (2C), 130.0 (2C), 129.80 (2C), 129.78 (2C), 129.72 (2C), 128.54 (2C), 128.50 (2C), 128.01 (1C), 126.69 (1C), 123.03 (2C), 54.12 (1C), 21.78 (1C), 21.65 ppm (1C); IR (thin film) cm^{-1} 3032, 2924, 1597, 1497, 1449, 1157; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 564.06795 ($C_{27}H_{24}ClNNaO_5S_2$ requires 564.07844).

*4-Methyl-N-[4-(4-chloro-benzyloxy)-phenyl]-N-(toluene-4-sulfonyl)-benzenesulfonamide (**6j**)*

White powder (51.5%): $R_f = 0.85$ ($CHCl_3$-MeOH, 98:2); mp. 119.5-120.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.56 (d, $J = 8.32$ Hz, 2H), 7.45 (d, $J = 8.26$ Hz, 2H), 7.22–7.30 (m, 4H), 7.05–7.18 (m, 4H), 6.78–6.84 (m, 4H), 4.60 (s, 2H), 2.44 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.70 (1C), 145.67 (1C), 144.04 (1C), 137.47 (1C), 134.90 (1C), 134.31 (1C), 130.07 (2C), 130.0 (2C), 129.76 (2C), 129.72 (2C), 129.65 (2C), 128.72 (2C), 128.66 (2C), 128.01 (1C), 127.71 (1C), 122.99 (2C), 54.03 (1C), 21.79 (1C), 21.67 ppm (1C); IR (thin film) cm^{-1} 3028, 2963, 1597, 1497, 1447, 1262; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 564.05881 ($C_{27}H_{24}ClNNaO_5S_2$ requires 564.07844); Anal. Calcd for $C_{27}H_{24}ClNO_5S_2$: C 59.82, H 4.46, Cl 6.54, N 2.58, S 11.83, found: C 59.07, H 5.05, Cl 6.14, N 2.19, S 11.37.

*4-Methyl-N-[4-(3-methyl-benzyloxy)-phenyl]-N-(toluene-4-sulfonyl)-benzenesulfonamide (**6k**)*

White powder (32.1%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); mp. 125.5–126.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.58 (d, $J = 8.36$ Hz, 2H), 7.33–7.43 (m, 6H), 6.82–7.12 (m, 8H), 4.65 (s, 2H), 2.35 (s, 6H), 2.15 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.12 (1C), 146.37 (1C), 144.27 (1C),137.98 (1C), 137.95 (1C), 136.24 (1C), 134.86 (1C), 131.42 (2C), 130.62 (2C), 130.31(1C), 130.29 (1C), 129.13 (2C), 128.70 (1C), 128.64 (3C), 127.83 (2C), 125.71 (1C), 122.89 (2C), 53.54 (1C), 21.68 (1C), 21.56 (1C), 21.41 ppm (1C); IR (thin film) cm^{-1} 3028, 2955, 1597, 1497, 1451, 1165; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 544.12091 ($C_{28}H_{27}NNaO_5S_2$ requires 544.13306); Anal. Calcd for $C_{28}H_{27}NO_5S_2$: C 64.47, H 5.22, N 2.69, S 12.29, found: C 64.70, H 5.28, N 3.49, S 11.64.

*4-Methyl-N-[4-(3-methoxy-benzyloxy)-phenyl]-N-(toluene-4-sulfonyl)-benzenesulfonamide (**6l**)*

White powder (18.0%): $R_f = 0.88$ ($CHCl_3$–MeOH, 98:2); mp. 148–149°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.55 (d, $J = 8.28$ Hz, 2H), 7.47 (d, $J = 8.22$ Hz, 2H), 7.08–7.28 (m, 6H), 6.68–6.89 (m, 6H), 4.60 (s, 2H), 3.74 (s, 3H), 2.43 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 159.66 (1C), 148.57 (1C), 145.57 (1C), 143.90 (1C), 137.00 (1C), 135.04 (1C), 131.89 (1C), 130.04 (2C), 129.75 (2C), 129.67 (2C), 129.44 (2C), 128.53 (2C), 127.73 (2C), 122.88 (2C), 120.91 (1C), 113.88 (1C), 113.45 (1C), 55.22 (1C), 54.56 (1C), 21.79 (1C), 21.66 ppm (1C); IR (thin film) cm^{-1} 3029, 2924, 1597, 1497, 1458, 1165; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 560.10835 ($C_{28}H_{27}NNaO_6S_2$ requires 560.12798); Anal. Calcd for $C_{28}H_{27}NO_6S_2$: C 62.55, H 5.06, N 2.61, S 11.93, found: C 61.85, H 5.03, N 2.39, S 12.04.

General procedure for synthesis of *N*-(4-benzylaminophenyl)-toluene-4-sulfonic acid ester and *4-(N,N)*-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid ester derivatives (**8a**–**8l**)

Tosyl chloride **2** (0.953 g, 5 mmol) was dissolved in CH_2Cl_2 (10 ml) in an ice bath and *p*-aminophenol **3** (0.655 g, 6 mmol) was added. Then, triethylamine (0.83 ml, 6 mmol) was added. The mixture was stirred at room temperature for 2 h and then extracted with water (10 ml). The organic extract was dried on anhydrous Na_2SO_4 and filtered. The residue, after evaporation of the solvent, was purified by column chromatography eluting with CH_2Cl_2/MeOH (95:5) to give toluene-4-sulfonic acid 4-amino-phenyl ester **7** (0.503 g, 38%).

Subsequently, 4-aminophenyl-4-toluene-sulfonic acid ester **7** (0.741 g, 2.8 mmol) was dissolved in DMF (5 ml) in an ice bath and one of the substituted benzyl bromides **5b**–**5h**, **5j**, and **5k** (3.6 mmol) was added. Then, NaOH (0.24 g, 6 mmol) was added. The mixture was stirred at room temperature for 3 h and then DMF was evaporated. The residue was dissolved in CH_2Cl_2 (10 ml) and extracted with water (10 ml). The organic extract was dried on anhydrous Na_2SO_4 and filtered. The residue, after evaporation of the solvent, was purified by column chromatography eluting with CH_2Cl_2/cyclohexane (85:15) to give *N*-(4-benzylaminophenyl)-toluene-4-sulfonic acid ester and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid ester derivatives **8a**–**8l**.

*4-Aminophenyl-4-toluene-sulfonic acid ester (**7**)*

White powder (38%): $R_f = 0.80$ ($CHCl_3$–MeOH, 95:5); mp. 140–141°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.64 (d, $J = 8.33$ Hz, 2H), 7.41 (d, $J = 8.01$ Hz, 2H), 6.57 (dd, $J = 8.90$, 2.19 Hz, 2H), 6.38 (dd, $J = 8.94$, 2.19 Hz, 2H), 5.18 (br s, 2H, NH_2), 2.38 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.37 (1C), 145.80 (1C), 139.47 (1C), 132.22 (1C), 130.50 (2C), 128.73 (2C), 122.96 (2C), 114.39 (2C), 21.64 ppm (1C); IR (thin film) cm^{-1} 3378, 3021, 2967, 1721, 1610, 1505, 1424, 1215.

*Toluene-4-sulfonic acid 4-(3-methyl-benzylamino)-phenyl ester (**8a**)*

Off-white oil (14.9%): $R_f = 0.85$ ($CHCl_3$–MeOH, 98:2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.31$ Hz, 2H), 7.05–7.32 (m, 6H), 6.75 (dd, $J = 8.91$, 2.12 Hz, 2H), 6.44 (dd, $J = 8.94$, 2.14 Hz, 2H), 4.27 (s, 2H), 3.96 (br s, 1H, N*H*), 2.44 (s, 3H), 2.30 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.04 (1C), 145.03 (1C), 140.93 (1C), 138.77 (1C), 138.46 (1C), 132.61 (1C), 129.65 (2C), 128.65 (3C), 128.31 (1C), 128.22 (1C), 124.59 (1C), 123.25 (2C), 112.93 (2C), 48.47 (1C), 21.76 (1C), 21.47 ppm (1C); IR (thin film) cm^{-1} 3426, 3028, 2924, 1605, 1512, 1470, 1451; MS (ESI, positive mode) *m/z* $[M + H]^+$ 368.13149 ($C_{21}H_{22}NO_3S$ requires 368.12421).

*Toluene-4-sulfonic acid 4-(3-bromo-benzylamino)-phenyl ester (**8b**)*

Off-white oil (26%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.66 (d, $J = 8.31$ Hz, 2H), 7.48 (s, 1H), 7.38 (d, $J = 7.49$ Hz, 1H), 7.28 (d, $J = 8.05$ Hz, 2H), 7.24 (d, $J = 6.24$ Hz, 1H), 7.19 (m, 1H), 6.74 (dd, $J = 8.98$, 2.18 Hz, 2H), 6.43 (dd, $J = 8.98$, 2.15 Hz, 2H), 4.10 (br s, 1H, N*H*), 4.30 (s, 2H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 146.57 (1C), 145.08 (1C), 141.37 (1C), 141.17 (1C), 132.54 (1C), 130.52 (1C), 130.32 (2C), 129.67 (2C), 128.64 (2C), 125.90 (1C), 123.33 (2C), 122.86 (1C), 113.04 (2C), 47.81 (1C), 21.77 ppm (1C); IR (thin film) cm^{-1} 3422, 3059, 2924, 1605, 1508, 1483; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 455.00830 ($C_{20}H_{18}BrNNaO_3S$ requires 455.33186).

*Toluene-4-sulfonic acid 4-(3-nitro-benzylamino)-phenyl ester (**8c**)*

Pale green oil (18.2%): $R_f = 0.76$ ($CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.67 (d, $J = 8.26$ Hz, 2H), 7.25–7.53 (m, 6H), 6.82 (d, $J = 9.07$ Hz, 2H), 6.64 (d, $J = 9.03$ Hz, 2H), 4.95 (br s, 1H, N*H*), 4.50 (s, 2H), 2.44 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.24 (1C), 145.42 (1C), 140.64 (1C), 139.23 (1C), 138.78 (1C), 132.18 (1C), 129.88 (2C), 129.74 (2C), 128.60 (1C), 127.49 (1C), 122.66 (2C), 121.95 (2C), 110.93 (2C), 47.46 (1C), 21.77 ppm (1C); IR (thin film) cm^{-1} 3449, 3071, 2924, 1601, 1528, 1508, 1346; MS (ESI, positive mode) *m/z* $[M + H]^+$ 399.16944 ($C_{20}H_{19}N_2O_5S$ requires 399.09364).

*Toluene-4-sulfonic acid 4-[bis-(4-bromo-benzyl)-amino]-phenyl ester (**8d**)*

White powder (50%): $R_f = 0.82$ ($CHCl_3$); mp. 129–130°C; ^{1}H-NMR (300 MHz, CDCl3) δ 7.68 (d, $J = 8.27$ Hz, 2H), 7.42 (d, $J = 8.36$ Hz, 4H), 7.25 (d, $J = 8.04$ Hz, 2H), 7.05 (d, $J = 8.31$ Hz, 4H), 6.73 (d, $J = 9.14$ Hz, 2H), 4.52 (s, 4H), 2.45 ppm (s, 3H); ^{13}C-NMR (300 MHz, CDCl3) δ 147.43 (1C), 145.16 (1C), 140.93 (2C), 136.75 (2C), 132.68 (1C), 131.90 (4C), 129.69 (2C), 128.57 (4C), 128.43 (1C), 123.26 (2C), 121.01 (2C), 113.19 (2C), 54.22 (2C), 21.79 ppm (1C); IR (thin film) cm^{-1} 3051, 2963, 1601, 1508, 1455; MS (ESI, positive mode) *m/z* $[M + H]^+$ 602.97937 ($C_{27}H_{24}Br_2NO_3S$ requires 602.35046); Anal. Calcd for $C_{27}H_{23}Br_2NO_3S$: C 53.93, H 3.86, Br 26.57, N 2.33, S 5.33, found: C 53.15, H 3.48, Br 26.48, N 2.62, S 5.64.

*Toluene-4-sulfonic acid 4-[bis-(4-methyl-benzyl)-amino]-phenyl ester (**8e**)*

Off-white powder (19%): $R_f = 0.82$ ($CHCl_3$–MeOH, 98:2); mp. 149–150°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.06$ Hz, 2H), 7.26 (d, $J = 9.74$ Hz, 2H), 7.08 (dd, $J = 8.79$, 7.94 Hz, 8H), 6.70 (d, $J = 8.98$ Hz, 2H), 6.53 (d, $J = 9.04$ Hz, 2H), 4.55 (s, 4H), 2.45 (s, 3H), 2.30 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.13 (1C), 144.98 (1C), 140.82 (1C), 136.70 (2C), 134.95 (2C), 132.97 (1C), 129.64 (2C), 129.64 (4C), 128.60 (2C), 126.56 (4C), 123.01 (2C), 112.69 (2C), 54.19 (2C), 21.76

(1C), 21.13 ppm (2C); IR (thin film) cm^{-1} 3062, 2974, 1601, 1501, 1462; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 494.17604 ($C_{29}H_{29}NNaO_3S$ requires 494.61150); Anal. Calcd for $C_{29}H_{29}NO_3S$: C 73.86, H 6.20, N 2.97, S 6.80, found: C 73.94, H 6.36, N 2.63, S 6.23.

*Toluene-4-sulfonic acid 4-[bis-(4-nitro-benzyl)-amino]-phenyl ester (**8f**)*

Pale-green oil (61%): $R_f = 0.61$ ($CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.18 (d, $J = 8.7$ Hz, 4H), 7.68 (d, $J = 8.3$ Hz, 2H), 7.38 (d, $J = 8.62$ Hz, 4H), 7.28 (d, $J = 8.05$ Hz, 2H), 6.76 (dd, $J = 9.15$, 2.17 Hz, 2H), 6.53 (dd, $J = 9.18$, 2.13 Hz, 2H), 4.68 (s, 4H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.42 (1C), 146.73 (1C), 145.30 (2C), 141.50 (2C), 132.62 (2C), 129.74 (2C), 128.51 (2C), 127.38 (4C), 124.21 (4C), 123.57 (2C), 113.32 (2C), 54.68 (2C), 21.79 ppm (1C); IR (thin film) cm^{-1} 3055, 2963, 1597, 1512, 1478; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 556.11513 ($C_{27}H_{23}N_3NaO_7S$ requires 556.55354).

*Toluene-4-sulfonic acid 4-[bis-(3-trifluoromethyl-benzyl)-amino]-phenyl ester (**8g**)*

Off-white oil (45.6%): $R_f = 0.74$ ($CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.65 (d, $J = 8.03$ Hz, 2H), 7.5 (d, $J = 7.61$ Hz, 2H), 7.32-7.45 (m, 7H), 7.25 (d, $J = 8.09$ Hz, 2H), 6.75 (dd, $J = 9.13$, 2.15 Hz, 2H), 6.53 (dd, $J = 9.18$, 2.16 Hz, 2H), 4.65 (s, 4H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.36 (1C), 145.22 (1C), 141.17 (1C), 138.85 (2C), 132.40 (1C), 131.42 (1C), 130.00 (2C), 129.66 (2C), 129.37 (2C), 128.61 (2C), 124.32 (1C), 124.27 (2C), 124.22 (2C), 124.17 (1C), 123.36 (1C), 123.23 (1C), 122.23 (1C), 113.20 (2C), 54.50 (2C), 21.74 ppm (1C); IR (thin film) cm^{-1} 3051, 2963, 1601, 1512, 1451; MS (ESI, positive mode) *m/z* $[M + H]^+$ 580.13669 ($C_{29}H_{24}F_6NO_3S$ requires 580.13028).

*Toluene-4-sulfonic acid 4-[bis-(3-chloro-benzyl)-amino]-phenyl ester (**8h**)*

White oil (14.6%): $R_f = 0.68$ ($CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.33$ Hz, 2H), 7.2-7.32 (m, 6H), 7.15 (s, 2H), 7.05 (m, 2H), 6.75 (dd, $J = 9.21$, 2.28 Hz, 2H), 6.51 (dd, $J = 9.23$, 2.29 Hz, 2H), 4.55 (s, 4H), 2.43 ppm (s, 3 H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.37 (1C), 145.13 (1C), 140.98 (1C), 140.05 (2C), 134.79 (2C), 132.60 (1C), 130.15 (2C), 129.67 (2C), 128.60 (2C), 127.49 (2C), 126.65 (2C), 124.78 (2C), 123.29 (2C), 113.04 (2C), 54.28 (2C), 21.75 ppm (1C); IR (thin film) cm^{-1} 3055, 2963, 1597, 1574, 1508, 1431; MS (ESI, positive mode) *m/z* $[M + Na]^+$ 534.06679 ($C_{27}H_{23}Cl_2NNaO_3S$ requires 534.07757).

*Toluene-4-sulfonic acid 4-[bis-(4-chloro-benzyl)-amino]-phenyl ester (**8i**)*

White powder (10.4%): $R_f = 0.70$ ($CHCl_3$); mp. 151–152°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.67 (d, $J = 8.26$ Hz, 2H), 7.25-7.32 (m, 6H), 7.10 (d, $J = 8.31$ Hz, 4H), 6.75 (dd, $J = 9.07$, 1.99 Hz, 2H), 6.51 (dd, $J = 9.13$, 2.19 Hz, 2H), 4.50 (s, 4H), 2.40 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.56 (1C), 145.08 (1C), 140.93 (1C), 136.29 (1C), 132.98 (2C), 132.83 (2C), 129.64 (2C), 128.93 (4C), 128.55 (2C), 128.06 (4C), 123.21 (2C), 113.22 (2C), 54.15 (2C), 21.72 ppm (1C); IR (thin film) cm^{-1} 3048, 2963, 1601, 1508, 1489, 1443; MS (ESI, positive mode) *m/z* $[M + H]^+$ 512.08485 ($C_{27}H_{24}Cl_2NO_3S$ requires 512.07757); Anal. Calcd for $C_{27}H_{23}Cl_2NO_3S$: C 63.28, H 4.52, Cl 13.84, N 2.73, S 6.26, found: C 62.19, H 4.92, Cl 13.56, N 2.51, S 6.88.

*Toluene-4-sulfonic acid 4-[bis-(3-methyl-benzyl)-amino]-phenyl ester (**8j**)*

Off-white powder (6.4%): $R_f = 0.68$ ($CHCl_3$); mp. 90–91°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.28$ Hz, 2H), 7.18–7.32 (m, 6H), 7.08 (d, $J = 7.52$ Hz, 2H), 6.95 (d, $J = 6.29$ Hz, 4H), 6.70 (dd, $J = 9.17$, 2.11 Hz, 2H), 6.52 (dd, $J = 9.2$, 2.16 Hz, 2H), 4.52 (s, 4H), 2.42 (s, 3H), 2.3 ppm (s, 6H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.12 (1C), 145.01 (1C), 140.27 (1C), 138.46 (2C), 138.07(2C), 132.77 (1C), 129.64 (1C), 128.66 (3C), 128.60 (2C), 127.84 (2C), 127.20 (2C), 123.59 (2C), 123.03 (2C), 112.59 (2C), 54.44 (2C), 21.77 (1C), 21.59 ppm (2C); IR (thin film) cm^{-1} 3047, 2963, 1605, 1512, 1447; MS (ESI, positive mode) *m/z* $[M + H]^+$ 472.19409 ($C_{29}H_{30}NO_3S$ requires 472.18681); Anal. Calcd for $C_{29}H_{29}NO_3S$: C 73.86, H 6.20, N 2.97, S 6.80, found: C 73.73, H 6.60, N 2.04, S 6.88.

*Toluene-4-sulfonic acid 4-[bis-(3-bromo-benzyl)-amino]-phenyl ester (**8k**)*

Off-white oil (11%): $R_f = 0.69$ ($CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.3$ Hz, 2H), 7.38 (d, $J = 7.9$ Hz, 2H), 7.38 (dd, $J = 14.44$, 8.76 Hz, 4H), 7.18 (d, $J = 7.77$ Hz, 2H), 7.08 (d, $J = 7.83$ Hz, 2H), 6.74 (d, $J = 9.16$ Hz, 2H), 6.50 (d, $J = 9.20$ Hz, 2H), 4.54 (s, 4H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 147.02 (1C), 146.53 (1C), 145.42 (2C), 140.30 (2C), 130.43 (2C), 129.68 (2C), 129.56 (2C), 128.61 (4C), 125.22 (4C), 123.32 (2C), 113.00 (2C), 54.19 (2C), 29.75 ppm (1C); IR (thin film) cm^{-1} 3048, 2963, 1597, 1508, 1465; MS (ESI,

positive mode) *m/z* $[M + Na]^+$ 624.96507 ($C_{27}H_{23}Br_2NNaO_3S$ requires 624.35046).

Toluene-4-sulfonic acid 4-[bis-(3-nitro-benzyl)-amino]-phenyl ester ***(8l)***

Yellow powder (17.6%): $R_f = 0.64$ ($CHCl_3$); mp. 133–134°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.13 (m, 2H), 8.02 (s, 2H), 7.65 (d, $J = 8.3$ Hz, 2H), 7.5 (m, 4H), 7.25 (d, $J = 8.14$ Hz, 2H), 6.78 (dd, $J = 9.23$, 2.12 Hz, 2H), 6.5A3 (dd, $J = 9.2$, 2.13 Hz, 2H), 4.75 (s, 4H), 2.42 ppm (s, 3H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 148.77 (1C), 146.77 (1C), 145.28 (1C), 145.20 (1C), 141.60 (1C), 141.58 (1C), 140.02 (2C), 132.75 (2C), 132.41 (1C), 129.99 (2C), 129.72 (2C), 128.57 (2C), 123.59 (2C), 122.60 (2C), 121.58 (2C), 113.50 (2C), 54.57 (2C), 21.75 ppm (1C); IR (thin film) cm^{-1} 3074, 2928, 1603, 1530, 1506, 1350; MS (ESI, positive mode) *m/z* $[M + H]^+$ 534.13044 ($C_{27}H_{24}N_3O_7S$ requires 534.12567); Anal. Calcd for $C_{27}H_{23}N_3O_7S$: C 60.78, H 4.34, N 7.88, S 6.01, found: C 60.33, H 4.56, N 7.76, S 6.39.

CETP inhibition assay

CETP inhibitory bioactivities were assayed by fluorescent-CE transfer employing commercially available kit (Bio-Vision, Linda Vista Avenue, USA). The assay kit is based on donor molecule containing fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP (from rabbit serum). CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in increase in fluorescence. Inhibition of CETP will prevent lipid transfer and therefore decrease fluorescence intensity.

The assay procedure can be described briefly as follows. An aliquot of 1.5 µl of rabbit serum (CETP) was added to 160 µl of testing sample (12.8 µM). Then, 20 µl of the master mix, provided in the assay kit (5 µl donor molecule, 5 µl acceptor molecule and 10 µl assay buffer), was added, mixed well, and the volume was made up to 203 µl with the provided assay buffer. The assay is carried out using a black 96-well plate with led. After incubation at 37°C for 1 h, fluorescence intensity (Excitation λ: 465 nm; Emission λ: 535 nm) was read in a FLX800TBI Microplate Fluorimeter (BioTek Instruments, Winooski, USA).

The tested compounds were initially dissolved in DMSO to yield 10 mM stock solutions and subsequently diluted to the required concentrations using distilled deionized water (12.8 µM). The final concentration of DMSO was adjusted to 0.1%. The percentage of residual activity of CETP was determined for each compound by comparing the activity of CETP in the presence and absence of the tested compound. Positive controls were tested to assess the degree of CETP inhibition by 0.1% DMSO. CETP was not affected by DMSO. The increase in fluorescence intensity with CETP (rabitt serum) is usually 1.5- to 2-fold over 0.1% DMSO. Negative controls lacking rabbit serum were used as background. All measurements were conducted in duplicates.

Table 1 The synthesized *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamide and *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide derivatives with their fit values against Hypo4/8 and in vitro CETP bioactivities

Compound	Fit values against Hypo4/8	In vitro % inhibition of CETP at 10 µM	In vitro IC_{50} (µM)
6a	9.1	9.9 ± 1.3	–
6b	8.9	10.2 ± 0.7	–
6c	8.6	0.0 ± 0.2	–
6d	0.0	15.5 ± 1.1	–
6e	9.2	29.6 ± 0.2	–
6f	8.7	23.0 ± 1.4	–
6g	9.4	22.8 ± 2.7	–
6h	9.0	39.0 ± 2.6	11.1 (0.99)[a]
6i	9.2	42.6 ± 0.2	10.5 (0.99)[a]
6j	8.8	37.5 ± 2.8	13.2 (0.99)[a]
6k	9.1	66.9 ± 1.5	3.4 (0.99)[a]
6l	9.5	65.3 ± 2.8	3.9 (0.99)[a]

[a] This value represents the correlation coefficient of the corresponding dose–response line at three concentrations

Table 2 The synthesized 4-benzylaminophenyl toluene-4-sulfonic acid ester and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid ester derivatives with their fit values against Hypo4/8 and in vitro CETP bioactivities

Compound	Fit values against Hypo4/8	In vitro % inhibition of CETP at 10 µM	In vitro IC_{50} (µM)
8a	7.9	31.3 ± 0.4	30.1 (0.99)[a]
8b	8.3	16.1 ± 1.3	–
8c	4.3	29.9 ± 2.9	–
8d	8.9	17.5 ± 1.1	–
8e	8.5	22.3 ± 0.6	–
8f	0.0	9.3 ± 0.5	–
8g	8.7	24.7 ± 1.0	–
8h	8.3	31.9 ± 1.4	28.5 (0.99)[a]
8i	8.9	24.9 ± 2.9	–
8j	8.1	29.0 ± 2.5	–
8k	8.4	23.1 ± 1.2	–
8l	4.6	31.4 ± 0.5	29.7 (0.99)[a]

[a] This value represents the correlation coefficient of the corresponding dose–response line at three concentrations

Results and discussion

Chemistry

In the current work, the intermediates *N*-(4-hydroxyphenyl)-4-methylbenzenesulfonamide **4a**, *N*-(4-hydroxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamide **4b,** and 4-aminophenyl-4-toluene-sulfonic acid ester **7** were prepared from the reaction of tosyl chloride **2** with *p*-aminophenol **3** in dichloromethane at room temperature and in the presence of triethylamine, as illustrated in Schemes 1 and 2.

Afterward, the resulting intermediates (**4a**, **4b,** or **7**) were reacted with different substituted benzyl bromides **5a**–**5k** in DMF at room temperature and in the presence of sodium hydroxide to prepare the final *N*-(4-benzyloxy-phenyl)-4-methyl-benzenesulfonamides (**6a**–**6g**) and *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methyl-benzenesulfonamides (**6h**–**6l**). On the other hand, these intermediates were reacted with substituted benzyl bromides in DMF at room temperature to prepare *N*-(4-benzylaminophenyl)-toluene-4-sulfonic acid esters (**8a**–**8c**) and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid esters (**8d**–**8l**). The highest yield was obtained upon reacting *N*-(4-hydroxy-phenyl)-4-methyl-benzenesulfonamide with 4-bromo-benzylbromide to give **6b** in 63% yield.

Scheme 1 shows the new benzenesulfonamide derivatives (**6a**–**6l**), while Scheme 2 shows sulfonic acid ester derivatives (**8a**–**8l**).

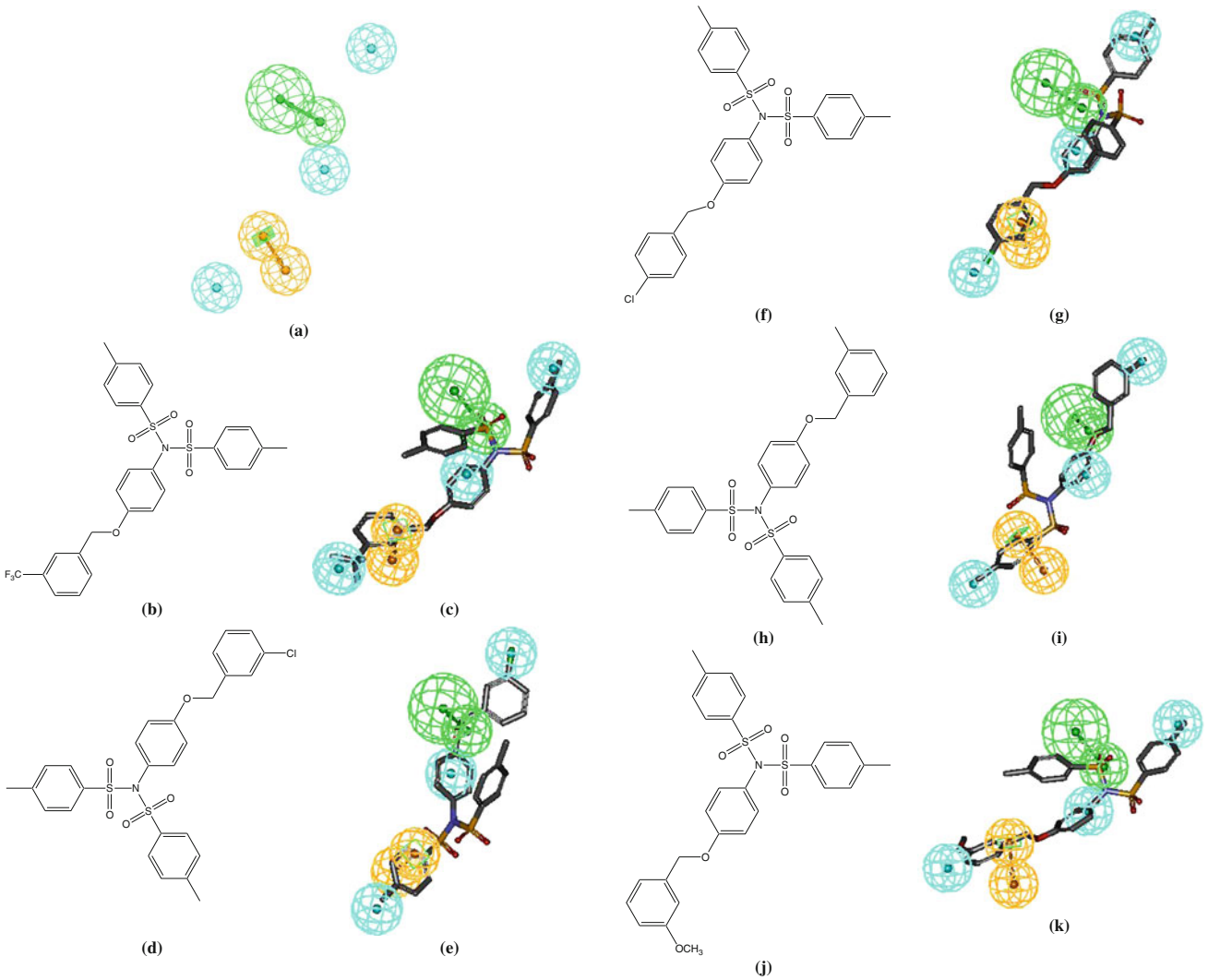


Fig. 2 The mapping of Hypo4/8 against the synthesized compounds **6h** (IC_{50} = 11.1 μM), **6i** (IC_{50} = 10.5 μM), **6j** (IC_{50} = 13.2 μM), **6k** (IC_{50} = 3.4 μM), and **6l** (IC_{50} = 3.9 μM): **a** Hypo4/8, **b** structure of **6h**, **c** Hypo4/8 mapped against **6h**, **d** structure of **6i**, **e** Hypo4/8 mapped against **6i**, **f** structure of **6j**, **g** Hypo4/8 mapped against **6j**, **h** structure of **6k**, **i** Hypo4/8 mapped against **6k**, **j** structure of **6l** and **k** Hypo4/8 mapped against **6l**

Biological evaluation

The results of CETP inhibition assay, presented in Tables 1 and 2, demonstrate that compound **6k** exhibit appreciable activity against CETP with an IC_{50} value of 3.4 μM. Although our newly synthesized compounds are of lower potency than some published CETP inhibitors, these derivatives are characterized by their novel scaffold that can serve as a promising leads for further optimization. Tables 1 and 2 show the fit values of the synthesized compounds against Hypo4/8.

Figure 2 shows how Hypo4/8 maps the most active synthesized compounds **6h–6l**. The hydrogen bond acceptor feature of Hypo4/8 fits either the sulfonamide moiety or the benzyloxy oxygen, while ring aromatic feature maps one of the aromatic rings in the structure. Furthermore, the three hydrophobic features of Hypo4/8 fit different substituted aromatic rings, i.e., substituted with methyl, trifluoromethyl, chloro, or methoxy.

The new compounds **6a–6l** and **8a–8l** were synthesized to explore the influence of bulkiness of the structure and electronic properties of the substituent (i.e., electron donating or withdrawing group) on the CETP inhibitory activity.

As a general trend, the CETP inhibitory activity for this series increases as the lipophilic character of the compound is enhanced, as can be seen in compounds having four aromatic rings, this is in accordance with the lipophilic binding pocket of CETP (Qiu *et al.*, 2007). Furthermore, the presence of donating groups on the benzyl ring, i.e., methyl and methoxy substituents, seems to contribute positively to the CETP inhibitory activity of the compound, as can be observed in compounds **6k** and **6l**, while presence of deactivating groups decreases the activity as seen in **6h–6j**.

Moreover, from the benzene sulfonamide derivatives **6a–6l**, compounds with two sulfone moieties have the best CETP inhibitory activities. These sulfone groups may be involved in significant interactions with the CETP pocket, as well as the size of the sulfur atom contributes positively to the lipophilic character of the compound. Compounds **6a–6l** and **8a–8l** were tested against CETP at 10 μM concentrations and exhibited CETP inhibitory activity up to 67%. In addition, the CETP IC_{50} values were determined for the most active synthesized compounds, **6h–6l**, **8a**, **8h**, and **8l**, where compound **6k** displayed the best activity with an IC_{50} value of 3.4 μM.

Conclusions

In conclusion, we have successfully accomplished synthetic investigation of a new series of *N*-(4-benzyloxyphenyl)-4-methyl-benzenesulfonamides, *N*-(4-benzyloxyphenyl)-*N*-(4-methylbenzenesulfonyl)-4-methylbenzenesulfonamides, *N*-(4-benzylaminophenyl)-toluene-4-sulfonic acid esters, and 4-(*N*,*N*)-[bis-(benzylaminophenyl)]-toluene-4-sulfonic acid esters as potential CETP inhibitors. Future optimization of the hydrophobic characters together with the substituent's electronic properties can lead to the discovery of more potent derivatives.

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