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RESEARCH ARTICLE

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Synthesis and anti-diabetic activity of novel biphenylsulfonamides as glucagon receptor antagonists

Chang-Yong Lee[1] | **Hojung Choi**[1,2] | **Eun-Young Park**[3] | **Thi-Thao-Linh Nguyen**[1] | **Han-Joo Maeng**[1] | **Kyoung Mee Lee**[4] | **Hee-Sook Jun**[1,2,5] | **Dongyun Shin**[1]

[1]College of Pharmacy, Gachon Institute of Pharmaceutical Science, Gachon University, Incheon, Korea

[2]Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon, Korea

[3]College of Pharmacy, Mokpo National University, Muan-gun, Jeollanam-do, Korea

[4]Kwangdong Pharmaceutical Co., Ltd, Seoul, Korea

[5]Gachon Medical Research Institute, Gil Hospital, Incheon, Korea

Correspondence
Dongyun Shin and Hee-Sook Jun, College of Pharmacy, Gachon Institute of Pharmaceutical Science, Gachon University, Yeonsu-gu, Incheon, Korea.
Emails: dyshin@gachon.ac.kr; hsjun@gachon.ac.kr

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Abstract

Type 2 diabetes is characterized by chronic hyperglycemia. Insulin, a hormone secreted from pancreatic β-cells, decreases blood glucose levels, and glucagon, a hormone secreted from pancreatic α-cells, increases blood glucose levels by counter-regulation of insulin through stimulation of hepatic glucose production. In diabetic patients, dysregulation of glucagon secretion contributes to hyperglycemia. Thus, inhibition of the glucagon receptor is one strategy for the treatment of hyperglycemia in type 2 diabetes. In this paper, we report a series of biphenylsulfonamide derivatives that were designed, synthesized, and then evaluated by cAMP and hepatic glucose production assays as glucagon receptor antagonists. Of these, compound **7aB-3** decreased glucagon-induced cAMP production and glucagon-induced glucose production in the in vitro assays. Glucagon challenge tests and glucose tolerance tests showed that compound **7aB-3** significantly inhibited glucagon-induced glucose increases and improved glucose tolerance. These results suggest that compound **7aB-3** has therapeutic potential for the treatment of type 2 diabetes.

KEYWORDS

biphenyl, glucagon receptor antagonist, sulfonamide, Type 2 diabetes

1 | INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases caused by chronic hyperglycemia resulting from defects in insulin action and/or insulin secretion (Kharroubi & Darwish, 2015). Excess glucose production in the liver and morbidly increased glucose reabsorption in the kidneys induce hyperglycemia (DeFronzo, 2009; DeFronzo et al., 2014).

Glucagon, a 29 amino acid peptide hormone secreted from pancreatic α cells (Authier & Desbuquois, 2008), increases plasma glucose levels (Christensen et al. 2011). When glucagon binds to the glucagon receptor (GCGR), Gsα activation leads to activation of adenylate cyclase, increases in intracellular cAMP levels, and subsequent activation of protein kinase A (PKA) (Bagger et al., 2011; Jiang & Zhang, 2003; Li & Zhuo, 2013; Quesada et al., 2008). Activation of glucagon-signaling results in increased glycogenolysis and gluconeogenesis, which are responsible for increased hepatic glucose production (Qureshi et al., 2004).

Small molecules involved in GCGR antagonism are recognized as novel approaches for better glycemic control in the diabetic state by inhibiting glucagon-induced hepatic

Chang-Yong Lee and Hojung Choi are contributed equally to this work.

glucose output (Sammons & Lee, 2015). Recently, many pharmaceutical companies have designed a number of GCGR antagonists and evaluated their biological activities. Representative compounds are BAY 27–9955 (Petersen & Sullivan, 2001), MK-0893 (Xiong et al., 2012), PF-06291874 (Bergman et al., 2017), LY-2409021 (Guzman et al., 2017; Kelly et al., 2015), and LGD-6972 (Vajda et al., 2012, 2017). All of these compounds have phenyl ether and *β*-alanine benzamide moieties in common. Moreover, the crystal structure of GCGR complexed with MK-0893 revealed the detailed binding mode of the β-alanine benzamide moiety and other aryl groups (Figure 1; Jazayeri et al., 2016).

Since Novo-Nordisk first discovered *β*-alanine benzamide as an important pharmacophore of GCGR antagonism (Kodra et al., 2008; Lau et al., 2007; Ling et al. 2000; Madsen et al., 2009), other research groups have adopted this moiety or its sulfonic acid congener (e.g., LGD-6972) as an essential structure and performed lead optimization by modifying the hydrophobic region to generate novel antagonists. By analyzing known potent antagonists, we determined that their hydrophobic parts have a rotatable carbon (Lee et al., 2014) and most of the reported compounds contain a stereogenic carbon center at the benzylic position, which could be disadvantageous during the drug discovery process in terms of eutomer purity and cost of synthesis. Considering the structural features of known compounds and novelty of our designed antagonists, we predicted that biphenylsulfonamides would be strong candidates, as shown in Figure 2. In this planned structure, Novo-Nordisk's *β*-alanine benzamide was preserved and a hydrophobic biphenyl group was introduced at the para position of the benzamide via sulfonamide bond. This compound is conformationally more rigid than the carbon-linked compounds and has no stereogenic carbon (Figure 2).

2 | METHODS AND MATERIALS

2.1 | Chemistry

The reaction solvents used for the synthesis were dried over appropriate drying agents or purchased as anhydrous grade or reagent grade. The reactions were performed under an N_2 atmosphere or under designated conditions. Purification of products was carried out using flash column chromatography with silica gel (40–63 μm) purchased from ZEOprep or using medium-performance liquid chromatography (Isolera™ by Biotage (Uppsala, Sweden)). Melting points were determined on an OptiMelt-automated melting point system (Stanford Research Systems) and were uncorrected. Proton and carbon 13 magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker 600 (^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz) spectrometer with solvent resonance as the internal standard (^{1}H NMR: $CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.5 ppm; ^{13}C NMR: $CDCl_3$ at 77.0 ppm, DMSO-d_6 at 39.5 ppm). Mass spectra were obtained using a Waters 3100 Mass Detector spectrometer using electrospray ionization (Waters Technologies, Accurate–Mass QTOF LC-MS, 1200 series LC with dual spray ESI source). Optical rotation was determined on the Optical Activity Ltd. AA-10R automatic polarimeter in CH_2Cl_2.

2.1.1 | Ethyl 3-(4-nitrobenzamido)propanoate (**2**)

To a stirred solution of *β*-alanine ethyl ester hydrochloride (4.1 g, 26.9 mmol) and TEA (8.3 ml, 59.3 mmol) in $CHCl_3$ (135 ml) at 0°C, 4-nitrobenzoyl chloride (5 g, 26.9 mmol) was successively added, and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc. The organic layer was washed with H_2O and brine

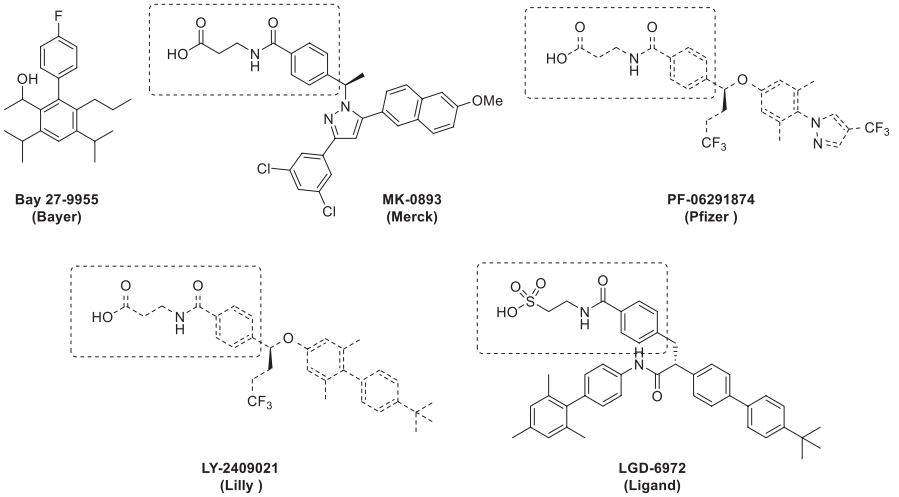


FIGURE 1 GCGR antagonists

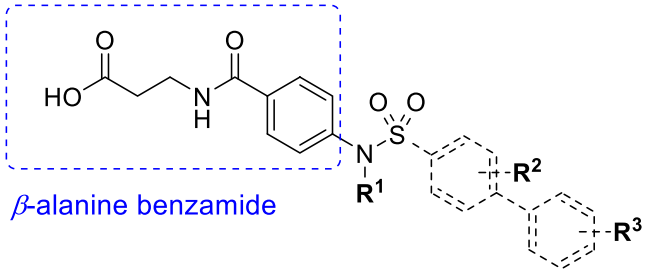


FIGURE 2 Design of biphenylsulfonamide as novel GCGR antagonist [Colour figure can be viewed at wileyonlinelibrary.com]

and then dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography. The synthesis yielded 6.8 g (94%) of 3-(4-nitrobenzamido)propanoic acid as a white solid. Mp: 86–87°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.26 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H), 7.37 (s, 1H), 4.17 (q, J = 7.2 Hz, 2H), 3.75 (q, J = 5.4 Hz, 2H), 2.68 (t, J = 6 Hz, 2H), 1.28 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 172.8, 165.4, 149.5, 139.9, 128.2, 123.7, 61.0, 35.7, 33.6, 14.1; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{12}H_{15}N_2O_5^+$ 267.0903, found 267.0913.

2.1.2 | Ethyl 3-(4-aminobenzamido) propanoate (**3**)

To a stirred solution of compound **2** (6.15 g, 23.1 mmol) in MeOH (75 ml), palladium (10% on carbon, 250 mg, 0.231 mmol) was added, and the mixture was stirred for 5 hr under a hydrogen atmosphere at room temperature. The reaction mixture was filtered and washed with MeOH. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography. The synthesis yielded 5.3 g (97%) of ethyl 3-(4-aminobenzamido)propanoate as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.59 (d, J = 8.4 Hz, 2H), 6.72 (s, 1H), 6.64 (d, J = 8.4 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 3.99 (bs, 2H), 3.68 (q, J = 6.6 Hz, 2H), 2.61 (t, J = 6 Hz, 2H), 1.26 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.1, 167.1, 149.6, 128.7, 123.9, 114.1, 60.7, 35.2, 34.1, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{12}H_{17}N_2O_3^+$ 237.1161, found 237.1153.

2.1.3 | General procedure for the synthesis of (**4**)

To a stirred solution of compound **3** (2 g, 8.46 mmol) in MeOH (42 ml), palladium (10% on carbon, 450 mg, 0.846 mmol) and propanal (0.909 ml, 12.7 mmol) were added, and the mixture was stirred overnight under a hydrogen atmosphere at room temperature. The reaction mixture was filtered and washed with MeOH. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography.

*Ethyl 3-(4-(propylamino)benzamido)propanoate (**4a**)*

Yield: 68%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.61 (d, J = 9 Hz, 2H), 6.71 (bs, 1H), 6.54 (d, J = 9 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 4.11 (bs, 1H), 3.69 (q, J = 6 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 6 Hz, 2H), 1.67–1.61 (m, 2H), 1.26 (t, J = 7.2 Hz, 3H), 0.99 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 167.2, 151.1, 128.6, 122.1, 111.6, 60.7, 45.2, 35.1, 34.2, 22.5, 14.2, 11.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{15}H_{23}N_2O_3^+$ 279.1530, found 279.1541.

*Ethyl 3-(4-(butylamino)benzamido)propanoate (**4b**)*

Yield: 73%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.61 (d, J = 8.4 Hz, 2H), 6.66 (bs, 1H), 6.55 (d, J = 9 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 4.00 (bs, 1H), 3.69 (q, 6.6 Hz, 2H), 3.14 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 6 Hz, 2H), 1.63–1.58 (m, 2H), 1.46–1.40 (m, 2H), 1.27 (t, J = 6.6 Hz, 3H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.1, 167.2, 151.1, 128.6, 122.2, 111.6, 60.7, 43.2, 35.1, 34.2, 31.4, 20.2, 14.2, 13.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{16}H_{25}N_2O_3^+$ 293.1787, found 293.1772.

*Ethyl 3-(4-((3,3,3-trifluoropropyl)amino)benzamido) propanoate (**4c**)*

Yield: 75%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.64 (d, J = 8.4 Hz, 2H), 6.69 (bs, 1H), 6.58 (d, J = 8.4 Hz, 2H), 4.17 (q, J = 7.2 Hz, 2H), 3.70 (q, J = 6 Hz, 2H), 3.49 (q, J = 6.6 Hz, 2H), 2.63 (t, J = 6 Hz, 2H), 2.46–2.39 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.1, 166.9, 149.6, 128.8, 123.5, 111.9, 60.7, 36.7, 35.1, 34.1, 33.6, 33.4, 33.2, 33.1, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{15}H_{20}F_3N_2O_3^+$ 333.1348, found 333.1350.

2.1.4 | General procedure for the synthesis of (**5**)

To a stirred solution of compound **4a** (750 mg, 2.69 mmol) in CH_2Cl_3 (15 ml) and TEA (0.6 ml, 4.04 mmol) in CH_2Cl_3 (15 ml) at 0°C, 4-iodobenzenesulfonyl chloride (978 mg, 3.23 mmol) was successively added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc. The organic layer was washed with H_2O and brine and then dried over $MgSO_4$. After filtration,

the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography.

*Ethyl 3-(4-((4-iodo-N-propylphenyl)sulfonamido) benzamido)propanoate (**5aA**)*

Yield: 60%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.81 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 9 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 9 Hz, 2H), 7.00 (t, J = 6 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.72 (q, J = 6 Hz, 2H), 3.51 (t, J = 6.6 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.45–1.69 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 172.8, 166.4, 141.6, 138.2, 137.6, 133.8, 128.9, 128.5, 127.6, 100.3, 60.8, 60.4, 52.0, 35.4, 33.8, 21.4, 21.1, 14.2, 10.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{21}H_{26}IN_2O_5S^+$ 545.0529, found 545.0512.

*Ethyl 3-(4-((4-iodo-N-(3,3,3-trifluoropropyl)phenyl) sulfonamido)benzamido)propanoate (**5cA**)*

Yield: 63%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.84 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 9 Hz, 2H), 7.27–7.25 (m, 3H), 7.14 (d, J = 8.4 Hz, 2H), 6.89 (t, J = 5.4 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.79 (t, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H) 2.65 (t, J = 6 Hz, 2H), 2.40–2.33 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.1, 141.2, 138.4, 136.8, 134.5, 128.9, 128.5, 128.2, 101.0, 61.0, 44.4, 35.4, 33.8, 33.7, 33.5, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{21}H_{23}F_3IN_2O_5S^+$ 598.0246, found 598.0250.

*Ethyl 3-(4-((4-bromo-3,5-dimethyl-N-propylphenyl) sulfonamido)benzamido)propanoate (**5aB**)*

Yield: 58%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 8.4 Hz, 2H), 7.25 (s, 2H), 7.16 (d, J = 9 Hz, 2H), 6.88 (t, J = 6Hz, 1H), 4.18 (q, J = 6.6 Hz, 2H), 3.73 (q, J = 5.4 Hz, 2H), 3.51 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 5.4 Hz, 2H), 2.42 (s, 6H), 1.46–1.40 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.4, 141.9, 139.6, 136.2, 133.7, 132.9, 128.6, 127.7, 126.6, 60.9, 52.0, 35.4, 33.8, 24.0, 21.4, 14.2, 10.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{23}H_{30}BrN_2O_5S^+$ 525.0981, found 525.0968.

*Ethyl 3-(4-((4-bromo-N-butyl-3,5-dimethylphenyl) sulfonamido)benzamido)propanoate (**5bB**)*

Yield: 67%, a colorless oil. 1H NMR (600MHz, CDCl3) δ(ppm): 7.73 (d, J = 8.6 Hz, 2H), 7.25 (s, 2H), 7.16 (d, J = 8.6 Hz, 2H), 6.88 (t, J = 5.7 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.73 (dd, J = 11.8, 6.0 Hz, 2H), 3.53–3.48 (m, 2H), 2.67–2.63 (m, 2H), 2.42 (s, 6H), 1.46–1.39 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); 13C NMR (150MHz, CDCl3) δ(ppm): 173.0, 142.0, 139.6, 133.7, 128.6, 127.7, 126.6, 60.9, 52.0, 35.4, 33.8, 24.1, 21.4, 14.2, 11.0; HRMS (ESI-TOF) *m/z* [M + H]+calculated for C23H29BrN2O5S+ 524.0981, found 524.0966.

*Ethyl 3-(4-((4-bromo-3,5-dimethyl-N-(3,3,3-trifluoropropyl)phenyl)sulfonamido)benzamido) propanoate (**5cB**)*

Yield: 64%, a colorless oil. ^{1}H NMR (600MHz, $CDCl_3$) δ(ppm): 7.84 (d, J = 8.9 Hz, 1H), 7.76 (d, J = 8.6 Hz, 1H), 7.48 (d, J = 8.9 Hz, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 7.17 (d, J = 8.7 Hz, 1H), 6.92–6.84 (m, 1H), 4.19 (qd, J = 7.1, 1.0 Hz, 2H), 3.75 (dd, J = 15.9, 9.4 Hz, 2H), 2.66 (dd, J = 11.6, 5.6 Hz, 2H), 2.52–2.37 (m, 6H), 1.33–1.24 (m, 3H); ^{13}C NMR (150MHz, $CDCl_3$) δ(ppm): 128.5, 128.1, 126.6, 121.2, 115.7, 60.9, 33.8, 31.8, 24.0, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{23}H_{26}BrF_3N_2O_5S^+$ 578.0698, found 578.0686.

2.1.5 | General procedure for the synthesis of (**6**)

Compound **5a** (50 mg, 0.092 mmol), boronic acid (17 mg, 0.138 mmol), and 20% K_2CO_3 (2.2 ml) were dissolved in anhydrous THF (2.2 ml). The mixture was purged with nitrogen for 5 min. $Pd(PPh_3)_4$ (5 mg, 4.6 μmol) was added, and the mixture was stirred at 80°C overnight. The mixture was partitioned between EtOAc and water, and the aqueous layer was extracted with EtOAc. The pooled organic layer was washed with H_2O and brine and then dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography.

*Ethyl 3-(4-(N-propyl-[1,1′-biphenyl]-4-sulfonamido) benzamido)propanoate (**6aA-1**)*

Yield: 73%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 8.4, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.63–7.61 (m, 4H), 7.48 (t, J = 7.4 Hz, 2H), 7.42 (t, J = 7.4 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.87 (t, J = 5.4 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H), 3.56 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.48–1.42 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.4, 141.9, 139.6, 136.2, 133.7, 132.9, 128.6, 127.7, 126.6, 60.9, 52.0, 35.4, 33.8, 24.0, 21.4, 14.2, 10.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{31}N_2O_5S^+$ 495.1875, found 495.1887.

*Ethyl 3-(4-((4′-chloro-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6aA-2**)*

Yield: 65%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.72 (d, J = 8.4 Hz, 2H), 7.64–7.61 (m, 4H), 7.54 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 6.87 (t, J = 5.4 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H), 3.56 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.48–1.42 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 0.91 (t,

J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 129.3, 128.6, 128.2, 127.8, 127.3, 60.9, 52.0, 35.4, 33.8, 21.4, 14.2, 10.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{30}ClN_2O_5S^+$ 529.1486, found 529.1466.

*Ethyl 3-(4-((N-propyl-4′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6aA-3**)*

Yield: 80%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (m, 6H), 7.67 (q, J = 7.8 Hz, 4H), 7.18 (d, J = 8.5 Hz, 2H), 6.91 (t, J = 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 3.57 (t, J = 7.1 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 1.46 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.4, 144.0, 142.7, 141.9, 137.6, 133.8, 128.6, 128.3, 127.8, 127.7, 126.0, 60.9, 52.0, 35.4, 33.8, 21.5, 14.2, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{30}F_3N_2O_5S^+$ 563.1749, found 563.1741.

*Ethyl 3-(4-((4′-(tert-butyl)-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6aA-4**)*

Yield: 60%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.72 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 9 Hz, 2H), 7.60 (d, J = 9 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 6.86 (t, J = 5.4 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H), 3.56 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.47–1.41 (m, 2H), 1.37 (s, 8H), 1.28 (t, J = 6.6 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.5, 145.4, 142.1, 136.2, 133.6, 128.6, 128.1, 127.7, 127.2, 126.9, 126.0, 60.9, 51.9, 35.4, 34.7, 33.9, 31.3, 21.4, 14.2, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{39}N_2O_5S^+$ 551.2501, found 551.2600.

*Ethyl 3-(4-((3′,5′-dichloro-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6aA-5**)*

Yield: 71%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 8.4 Hz, 2H), 7.63 (q, J = 9 Hz, 4H), 7.47 (d, J = 1.8 Hz, 2H), 7.42 (t, J = 1.8 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 6.88 (t, J = 5.4 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H), 3.56 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.48–1.42 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.4, 142.8, 142.1, 141.8, 137.9, 135.7, 133.8, 128.6, 128.4, 128.3, 127.8, 127.5, 125.9, 60.9, 52.0, 35.4, 33.8, 21.5, 14.2, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{29}Cl_2N_2O_5S^+$ 563.1095, found 563.1088.

*Ethyl 3-(4-((2′-fluoro-N-propyl-5′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6aA-6**)*

Yield: 76%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75–7.73 (m, 3H), 7.69–7.64 (m, 5H), 7.32 (t, J = 9 Hz, 1H), 7.19 (d, J = 8.4 Hz, 2H), 6.88 (t, J = 6 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 6 Hz, 2H), 3.57 (t, J = 6.6 Hz, 2H), 2.65 (t, J = 8.4 Hz, 2H), 1.49–1.43 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.4, 141.9, 138.7, 137.9, 133.8, 129.5, 128.6, 128.1, 127.1, 127.8, 117.2, 117.1, 60.9, 52.0, 35.4, 33.8, 21.5, 14.2, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{29}F_4N_2O_5S^+$ 581.1655, found 581.1650.

*Ethyl 3-(4-((4′-chloro-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cA-1**)*

Yield: 66%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.76 (d, J = 8.6 Hz, 2H), 7.63 (q, J = 9.5 Hz, 4H), 7.54 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 6.89 (t, J = 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.83 (t, J = 7.6 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.43–2.35 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.2, 144.9, 141.5, 137.4, 136.1, 135.0, 134.4, 129.3, 128.6, 128.5, 128.3, 128.1, 127.5, 60.9, 44.3, 35.4, 33.8, 30.9, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{27}ClF_3N_2O_5S^+$ 583.1203, found 583.1199.

*Ethyl 3-(4-((4′-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cA-2**)*

Yield: 72%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.77–7.65 (m, 10H), 7.19 (d, J = 8.6 Hz, 2H), 6.91 (t, J = 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.85 (t, J = 7.6 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.44–2.36 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.1, 144.6, 142.5, 141.4, 136.8, 134.4, 128.5, 128.4, 128.2, 127.9, 127.7, 126.1, 124.9, 60.9, 60.4, 44.4, 35.4, 33.8, 33.7, 33.5, 21.1, 14.2, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{27}F_6N_2O_5S^+$ 617.1467, found 617.1417.

*Ethyl 3-(4-((4′-(tert-butyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cA-3**)*

Yield: 66%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.88 (t, J = 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.83 (t, J = 7.6 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.43–2.35 (m, 2H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 128.6, 128.2, 128.1, 127.4, 127.0, 126.1, 60.9, 35.4, 33.8, 31.3, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{36}F_3N_2O_5S^+$ 605.2219, found 605.2111.

*Ethyl 3-(4-((N-butyl-4′-chloro-2,6-dimethyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6bB-1**)*

Yield: 75%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.30 (s, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 5.5 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 3.60 (t, J = 6.9 Hz, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.01 (s, 6H), 1.43–1.37 (m, 2H), 1.36–1.31 (m, 2H), 1.28 (t, J = 7.1 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 207.0, 173.0, 166.5, 145.2, 142.2, 137.8, 137.3, 136.7, 133.5, 129.8, 129.1, 128.6, 127.7, 126.3, 60.9, 50.9, 50.0, 35.4, 33.8, 30.9, 30.2, 24.0, 20.9, 19.6, 14.2, 13.6, 13.5; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{30}H_{36}ClN_2O_5S^+$ 571.1955, found 571.1910.

*Ethyl 3-(4-((N-butyl-2,6-dimethyl-4′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6bB-2**)*

Yield: 69%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.77–7.23 (m, 4H), 7.32 (s, 2H), 7.26–7.23 (m, 4H), 6.91 (t, J = 5.9 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 3.62 (t, J = 6.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.01 (s, 6H), 1.44–1.38 (m, 2H), 1.37–1.32 (m, 3H), 1.28 (t, J = 7.1 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.5, 144.9, 143.2, 142.2, 137.1, 133.5, 128.9, 128.6, 127.7, 126.6, 126.4, 125.8, 60.9, 53.4, 50.9, 50.0, 35.4, 33.8, 30.2, 30.1, 24.0, 20.9, 19.6, 14.2, 13.6, 13.5; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{36}F_3N_2O_5S^+$ 605.2219, found 605.2185.

*Ethyl 3-(4-((4′-(tert-butyl)-N-butyl-2,6-dimethyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6bB-3**)*

Yield: 74%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.29 (s, 2H), 7.24 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.87 (t, J = 5.9 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 3.60 (t, J = 6.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.03 (s, 6H), 1.42–1.33 (m, 12H), 1.28 (t, J = 7.1 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.5, 150.2, 146.7, 142.4, 137.6, 136.3, 136.1, 133.4, 128.6, 128.0, 127.6, 126.1, 125.5, 60.9, 49.9, 35.3, 34.6, 33.9, 31.4, 30.2, 21.1, 19.6, 14.2, 13.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{34}H_{45}N_2O_5S^+$ 593.2971, found 593.2966.

*Ethyl 3-(4-((4′-chloro-2,6-dimethyl-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cB-1**)*

Yield: 65%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.78 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.30 (s, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 6.91 (t, J = 5.7 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.84 (t, J = 7.6 Hz, 2H), 3.73 (q, J = 5.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.42–2.36 (m, 2H), 2.03 (s, 6H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.1, 145.8, 137.7, 137.6, 134.2, 133.6, 129.7, 129.1, 128.5, 128.1, 126.3, 60.9, 35.4, 33.8, 21.0, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{29}H_{31}ClF_3N_2O_5S^+$ 611.1516, found 611.1415.

*Ethyl 3-(4-((2,6-dimethyl-4′-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cB-2**)*

Yield: 63%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.80–7.74 (m, 3H), 7.33 (s, 2H), 7.27–7.24 (m, 4H), 7.17 (d, J = 8.5 Hz, 1H), 6.99 (t, J = 6 Hz, 1H), 4.20–4.16 (m, 2H), 3.86 (t, J = 7.6 Hz, 2H), 3.79 (t, J = 7.5 Hz, 1H), 3.73 (q, J = 5.9 Hz, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.41–2.34 (m, 2H), 2.02 (s, 5H), 1.30–1.27 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 166.2, 145.5, 143.0, 141.7, 141.4, 140.0, 137.5, 136.4, 135.4, 134.3, 134.2, 133.5, 130.0, 129.8, 128.8, 128.5, 128.1, 126.6, 126.4, 125.9, 125.0, 123.2, 60.9, 53.4, 50.8, 44.3, 35.4, 33.9, 33.8, 33.7, 33.5, 33.4, 29.7, 24.1, 22.7, 20.9, 14.2, 14.1; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{30}H_{31}F_6N_2O_5S^+$ 645.1780, found 645.1654.

*Ethyl 3-(4-((4′-(tert-butyl)-2,6-dimethyl-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoate (**6cB-3**)*

Yield: 73%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.79 (d, J = 8 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 7.29 (s, 2H), 7.25 (d, J = 8.2 Hz, 2H), 7.02 (d, J = 7.7 Hz, 2H), 6.90 (t, J = 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.85 (t, J = 7.6 Hz, 2H), 3.74 (q, J = 5.8 Hz, 2H), 2.65 (t, J = 5.7 Hz, 2H), 2.44–2.36 (m, 2H), 2.04 (s, 5H), 1.37 (s, 7H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 173.0, 147.3, 141.9, 138.0, 136.1, 134.1, 128.6, 128.0, 127.9, 126.1, 125.6, 60.9, 35.4, 34.6, 33.8, 31.4, 30.9, 21.1, 14.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{33}H_{40}F_3N_2O_5S^+$ 633.2532, found 633.2510.

2.1.6 | General procedure for the synthesis of (**7**)

$LiOH{\cdot}H_2O$ (6.0 mg, 0.135 mmol) was added to a solution of compound **6a** (33 mg, 0.068 mmol) in THF/H_2O (0.3 ml/0.2 ml). The reaction mixture was stirred at room temperature overnight. After completion, the reaction mixture was diluted with H_2O and acidified to pH 2 with 1 N HCl. The mixture was diluted with EtOAc, and the organic layer was washed with H_2O and brine and then dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography.

*3-(4-(N-propyl-[1,1′-biphenyl]-4-sulfonamido) benzamido)propanoic acid (***7aA-1***)*

Yield: 93% (29 mg) of 3-(4-(N-propyl-[1,1′-biphenyl]-4-sulfonamido)benzamido)propanoic acid as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.72 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.62–7.60 (m, 3H), 7.48 (t, J = 7.5 Hz, 2H), 7.43–7.41 (m, 1H), 7.19 (d, J = 8.6 Hz, 2H), 6.77 (t, J = 6 Hz, 1H), 3.75 (q, J = 5.9 Hz, 2H), 3.56 (t, J = 7.1 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 1.48–1.42 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 129.1, 128.6, 128.1, 127.8, 127.5, 127.3, 51.9, 35.2, 33.3, 21.4, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{33}H_{40}F_3N_2O_5S^+$ 467.1562, found 467.1506.

*3-(4-((4′-chloro-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7aA-2***)*

Yield: 87%, a colorless oil. ^{1}H NMR (600 MHz, DMSO-*d*6) δ (ppm): 8.58 (t, J = 5.3 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.79 (q, J = 7.9 Hz, 4H), 7.61 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 3.58 (t, J = 6.9 Hz, 2H), 3.45 (q, J = 6.5 Hz, 2H), 1.32 (q, J = 7.1 Hz, 2H), 1.22 (s, 1H), 0.83 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-*d*6) δ (ppm): 173.4, 165.9, 143.5, 141.5, 137.5, 137.0, 134.1, 133.9, 129.6, 129.3, 128.4, 128.4, 127.8, 51.6, 36.1, 34.2, 21.4, 11.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{25}H_{26}ClN_2O_5S^+$ 501.1173, found 501.1123.

*3-(4-((N-propyl-4′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7aA-3***)*

Yield: 78%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75–7.70 (m, 5H), 7.66 (q, J = 7.9 Hz, 4H), 7.19 (d, J = 8.5 Hz, 2H), 6.79 (t, J = 5.8 Hz, 1H), 3.75 (q, J = 5.9 Hz, 2H), 3.56 (t, J = 7.1 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 1.48–1.42 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.7, 144.1, 142.1, 137.5, 128.6, 128.3, 127.8, 127.7, 126.0, 52.0, 35.2, 33.4, 21.4, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{26}H_{26}F_3N_2O_5S^+$ 535.1436, found 535.1331.

*3-(4-((4′-(tert-butyl)-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7aA-4***)*

Yield: 89%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.72 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.78 (t, J = 5.4 Hz, 1H), 3.74 (q, J = 5.9 Hz, 2H), 3.55 (t, J = 7.1 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 1.47–1.41 (m, 2H), 1.37 (s, 9H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 151.8, 142.3, 136.1, 128.6, 128.1, 127.7, 127.2, 126.9, 126.1, 51.9, 35.2, 34.7, 33.4, 31.3, 21.4, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{29}H_{35}N_2O_5S^+$ 523.2188, found 523.2149.

*3-(4-((3′,5′-dichloro-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7aA-5***)*

Yield: 85%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 8.5 Hz, 2H), 7.62 (q, J = 7.8 Hz, 4H), 7.47 (d, J = 1.9 Hz, 2H), 7.41 (t, J = 1.8 Hz, 1H), 7.17 (d, J = 8.6 Hz, 2H), 6.78 (bs, 1H), 3.75 (q, J = 5.9 Hz, 2H), 3.56 (t, J = 7.1 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 1.48–1.42 (m,2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 128.6, 128.3, 127.8, 127.6, 125.9, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{25}H_{25}Cl_2N_2O_5S^+$ 535.0783, found 535.0775.

*3-(4-((2′-fluoro-N-propyl-5′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7aA-6***)*

Yield: 81%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75–7.72 (m, 3H), 7.67–7.64 (m, 5H), 7.32 (t, J = 9.2 Hz, 1H), 7.19 (d, J = 8.6 Hz, 2H), 6.82 (t, J = 6 Hz, 1H), 3.74 (q, J = 5.9 Hz, 2H), 3.57 (t, J = 7.1 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 1.48–1.42 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.8, 142.0, 138.8, 137.9, 133.5, 129.5, 129.5, 128.6, 128.1, 127.9, 127.9, 117.3, 117.1, 52.0, 35.3, 33.5, 21.4, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{26}H_{25}F_4N_2O_5S^+$ 553.1342, found 553.1355.

*3-(4-((4′-chloro-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7cA-1***)*

Yield: 89%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.76 (d, J = 8.5 Hz, 2H), 7.63 (q, J = 9.8 Hz, 4H), 7.54 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.80 (t, J = 5.8 Hz, 1H), 3.83 (t, J = 7.5 Hz, 2H), 3.75 (q, J = 5.9 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 2.43–2.35 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 129.3, 128.6, 128.3, 128.2, 127.5, 30.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{25}H_{23}ClF_3N_2O_5S^+$ 555.0890, found 555.0873.

*3-(4-((4′-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (***7cA-2***)*

Yield: 91%, a colorless oil. ^{1}H NMR (600 MHz, DMSO-*d*6) δ (ppm): 8.59 (t, J = 5.4 Hz, 1H), 7.96 (q, J = 4.2 Hz, 4H), 7.86 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.5 Hz, 2H), 3.92 (t, J = 6.6 Hz, 2H), 3.44 (q, J = 6.5 Hz, 2H), 3.32 (s, 2H), 2.43 (m, 2H); ^{13}C NMR (150 MHz, DMSO-*d*6) δ (ppm): 173.3, 165.8, 143.6, 142.6, 140.9, 137.0, 134.3, 128.6, 128.5, 128.5, 126.5, 126.5, 36.1, 34.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{26}H_{23}F_6N_2O_5S^+$ 589.1154, found 589.1132.

*3-(4-((4′-(tert-butyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7cA-3**)*

Yield: 84%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.76 (m, 2H), 7.72–7.65 (m, 3H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.19 (q, *J* = 4.7 Hz, 2H), 6.81 (t, *J* = 6.2 Hz, 1H), 3.83 (q, *J* = 7.9 Hz, 2H), 3.75 (q, *J* = 5.7 Hz, 2H), 2.74 (t, *J* = 5.5 Hz, 2H), 2.43–2.35 (m, 2H), 1.37 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 128.6, 128.3, 128.2, 128.1, 127.7, 127.4, 127.0, 126.1, 34.7, 31.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{36}F_3N_2O_5S^+$ 605.2219, found 605.2200.

*3-(4-((4′-chloro-2,6-dimethyl-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7aB-1**)*

Yield: 87%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.14 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.30 (s, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.80 (t, *J* = 6 Hz, 1H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.01 (s, 6H), 1.49–1.43 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 137.4, 137.0, 135.0, 129.8, 129.1, 128.6, 128.5, 127.7, 126.3, 21.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{30}ClN_2O_5S^+$ 529.1486, found 529.1770.

*3-(4-((2,6-dimethyl-N-propyl-4′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7aB-2**)*

Yield: 83%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.76–7.73 (m, 3H), 7.68–7.65 (m, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.49–7.46 (m, 2H), 7.32 (s, 2H), 7.24 (d, *J* = 8.6 Hz, 1H), 6.88 (t, *J* = 5.8 Hz, 1H), 3.74 (q, *J* = 5.9 Hz, 2H), 3.58 (t, *J* = 7.1 Hz, 2H), 2.72 (t, *J* = 5.7 Hz, 2H), 2.01 (s, 6H), 1.49–1.43 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.7, 137.1, 132.2, 132.1, 132.1, 128.9, 128.6, 127.7, 126.4, 125.8, 52.0, 35.3, 33.4, 21.5, 20.9, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{30}F_3N_2O_5S^+$ 563.1749, found 563.1724.

*3-(4-((4′-(tert-butyl)-2,6-dimethyl-N-propyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7aB-3**)*

Yield: 86%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.29 (s, 2H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.82 (t, *J* = 6.1 Hz, 1H), 3.74 (q, *J* = 5.9 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 2.73 (t, *J* = 5.8 Hz, 2H), 2.02 (s, 6H), 1.48–1.44 (m, 2H), 1.37 (s, 9H), 0.91 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.8, 146.7, 142.5, 137.6, 136.2, 136.1, 135.6, 128.6, 127.9, 127.7, 126.1, 125.5, 125.0, 51.9, 35.2, 34.6, 33.5, 31.4, 31.2, 21.5, 21.1, 11.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{39}N_2O_5S^+$ 551.2501, found 551.2500.

*3-(4-((N-butyl-4′-chloro-2,6-dimethyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7bB-1**)*

Yield: 85%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.30 (s, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.82 (t, *J* = 6 Hz, 1H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.60 (t, *J* = 6.9 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.01 (s, 6H), 1.43–1.38 (m, 2H), 1.37–1.31 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 137.7, 137.4, 136.7, 133.5, 133.2, 129.8, 129.1, 128.6, 127.7, 126.3, 49.9, 35.2, 30.2, 21.0, 19.6, 13.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{32}ClN_2O_5S^+$ 543.1642, found 543.1622.

*3-(4-((2,6-dimethyl-N-propyl-4′-(trifluoromethyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7bB-2**)*

Yield: 90%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.77–7.73 (m, 4H), 7.32 (s, 2H), 7.24 (d, *J* = 8.5 Hz, 4H), 6.82 (t, *J* = 5.9 Hz, 1H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.61 (t, *J* = 7 Hz, 2H), 2.74 (t, *J* = 5.7 Hz, 2H), 2.01 (s, 6H), 1.43–1.39 (m, 2H), 1.36–1.31 (m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 137.1, 133.3, 128.9, 128.6, 127.7, 126.4, 125.8, 49.9, 35.2, 30.2, 24.0, 20.9, 19.6, 13.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{29}H_{32}F_3N_2O_5S^+$ 577.1906, found 577.1886.

*3-(4-((4′-(tert-butyl)-N-butyl-2,6-dimethyl-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7bB-3**)*

Yield: 88%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.75 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.29 (s, 2H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 2H), 6.81 (t, *J* = 5.9 Hz, 1H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.60 (t, *J* = 7 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.02 (s, 6H), 1.43–1.31 (m, 12H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 137.6, 136.0, 128.6, 127.9, 127.7, 126.1, 125.5, 49.9, 35.2, 34.6, 31.4, 30.2, 21.1, 19.6, 13.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{32}H_{41}N_2O_5S^+$ 565.2658, found 565.2666.

*3-(4-((4′-chloro-2,6-dimethyl-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido)benzamido)propanoic acid (**7cB-1**)*

Yield: 85%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.78 (d, *J* = 8.5 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.30 (s, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.3 Hz, 2H), 6.84 (t, *J* = 5.8 Hz, 1H), 3.84 (t, *J* = 7.5 Hz, 2H), 3.75 (q, *J* = 5.7 Hz, 2H), 2.74 (t, *J* = 5.7 Hz, 2H), 2.40–2.36 (m, 2H), 2.02 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 141.9, 137.7, 137.5, 136.0, 129.7, 129.2, 128.6, 128.1, 126.3, 44.3, 35.3, 33.4, 29.7, 21.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{27}H_{27}ClF_3N_2O_5S^+$ 583.1203, found 583.1213.

*3-(4-((2,6-dimethyl-4′-(trifluoromethyl)-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido) benzamido)propanoic acid (**7cB-2**)*

Yield: 89%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.79 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8 Hz, 2H), 7.32 (s, 2H), 7.26–7.24 (m, 4H), 6.84 (bs, 1H), 3.85 (q, J = 5 Hz, 2H), 3.75 (d, J = 5.6 Hz, 2H), 2.74 (s, 2H), 2.40–2.36 (m, 2H), 2.02 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 137.5, 128.8, 128.6, 128.1, 126.4, 125.9, 20.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{28}H_{27}F_6N_2O_5S^+$ 617.1467, found 616.1450.

*3-(4-((4′-(tert-butyl)-2,6-dimethyl-N-(3,3,3-trifluoropropyl)-[1,1′-biphenyl])-4-sulfonamido) benzamido)propanoic acid (**7cB-3**)*

Yield: 81%, a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.78 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.29 (s, 2H), 7.25 (s, 2H), 7.01 (d, J = 8.3 Hz, 2H), 6.82 (t, J = 5.6 Hz, 1H), 3.84 (t, J = 5 Hz, 2H), 3.75 (q, J = 5.9 Hz, 2H), 2.74 (t, J = 5.7 Hz, 2H), 2.44–2.36 (m, 2H), 2.03 (s, 5H), 1.37 (s, 8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 128.6, 128.0, 127.9, 126.1, 125.6, 34.6, 31.4, 21.1; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{31}H_{36}F_3N_2O_5S^+$ 605.2219, found 605.2201.

2.1.7 | Synthesis of 4-bromo-3,5-dimethylbenzenesulfonyl chloride

*4-bromo-3,5-dimethylaniline (**9**)*

3,5-Dimethylaniline (5 ml, 40 mmol) was dissolved in acetonitrile (60 ml). NBS (7 g, 40 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then quenched with H_2O and diluted with EtOAc. The organic phase was separated, washed with H_2O and brine, and then dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography. The reaction yielded 6 g (75%) of 4-bromo-3,5-dimethylaniline. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 6.44 (s, 2H), 3.53 (bs, 2H), 2.31 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 144.9, 138.8, 115.9, 115.1, 23.8; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_8H_{11}BrN^+$ 199.9997, found 199.9999.

*4-bromo-3,5-dimethylbenzenesulfonyl chloride (**10**)*

(A) Thionyl chloride (5 ml, 70 mmol) was added dropwise to copper chloride (l) (99 mg, 1 mmol) dissolved in H_2O (20 ml) on ice. The reaction temperature was raised slowly to room temperature, and the mixture was stirred overnight. (B) Sodium nitrite (759 mg, 11 mmol) in H_2O (16 ml) was added dropwise to a mixture of 4-bromo-3,5-dimethylaniline (2 g, 10 mmol), concentrated HCl (10 ml, 330 mmol), and H_2O (14 ml) at −5°C. After stirring for 30 min at −5°C, the solution prepared in (A) was added dropwise at the same temperature. The reaction temperature was increased slowly, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O and brine, and then dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by SiO_2 flash column chromatography. The synthesis yielded 1.5 g (52%) of 4-bromo-3,5-dimethylbenzenesulfonyl chloride. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.73 (s, 2H), 2.54 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 144.3, 140.8, 135.8, 125.7, 24.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_8H_9BrClO_2S^+$ 282.9117, found 282.9079.

2.2 | Animals

Five-week-old male C57BL/6 mice were purchased from Orient Bio Inc. (Seongnam, Kyounggido, Korea). The mice were maintained under specific pathogen-free conditions in a temperature-controlled room under a 12 hr light/dark cycle with ad libitum access to food and water at the Animal Care Center, Lee Gil Ya Cancer and Diabetes Institute, Gachon University, South Korea. The mice were fed HFD (60% fat composed primarily of lard, Research Diet, Inc., New Brunswick, NJ, USA, #D12492). After 8 weeks (at 14 weeks of age), the mice were randomly divided into three groups: 9% Kolliphor® HS 15 with 10% DMSO-treated group (Vehicle), **7aB-3**-treated group (**7aB-3**), and **LGD-6972**-treated group (**LGD-6972**). Fifty milligrams per kilogram of **7aB-3** or **LGD-6972** (dissolved in 9% Kolliphor® HS 15 with 10% DMSO in sterilized water) was administered orally by gavage once daily for 6 weeks. **LGD-6972** was synthesized in the laboratory. Body weight and food intake were measured daily. Fasting blood glucose level was measured every two weeks, and non-fasting blood glucose level was measured at weeks 3 and 4 after administration. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Lee Gil Ya Cancer and Diabetes Institute (LCDI-2015-0006).

2.3 | Cell culture and isolation of hepatocytes

The cAMP Hunter™ CHO-K1 GCGR Gs cell line (CHO-K1 GCGR Gs cells) expressing human GCGR were purchased from DiscoverX (Fremont, USA). The cells were maintained in CHO-K1 medium (DiscoverX). Isolation of hepatocytes was performed as previously described (Yoon et al., 2017). Briefly, ten-week-old male C57BL/6N mice were anesthetized with ketamine. The liver was perfused through the

portal vein using perfusion buffer I (142 mM NaCl, 6.7 mM KCl, 0.01 M HEPES, and 2.5 mM EGTA, pH 7.4) for 10 min and then perfusion buffer II (66.7 mM NaCl, 6.7 mM KCl, 10 mM HEPES, and 4.8 mM $CaCl_2$, pH 7.6) with 0.5 mg/ml of Collagenase IV for 5 min. The liver was excised and dissected in cold high-glucose DMEM to separate the hepatic cells. The cells were centrifuged at 50 g for 5 min at 4°C, washed using cold high-glucose DMEM, and then centrifuged again at 50 g for 5 min at 4°C. Hepatocytes were isolated by Percoll gradient centrifugation (250 g for 5 min without brake at 4°C). The protocol was approved by the Institutional Animal Care and Use Committee of the Lee Gil Ya Cancer and Diabetes Institute (LCDI-2015–0006). The isolated primary mouse hepatocytes were grown in HepatoZYME-SFM (Gibco-BRL, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; Gibco-BRL) and 1% antibiotics (100 unit/ml penicillin and 100 μg/ml streptomycin; Gibco-BRL). All cells were maintained under subconfluent conditions in a humidified incubator at 37°C with 95% ambient air and 5% CO_2.

2.4 | Measurement of cAMP production

Briefly, 1×10^4 CHO-K1 GCGR Gs cells/well were seeded in 96-well plates in CHO-K1 medium (DiscoverX). The next day, the cells were treated with GCGR antagonist candidates in phosphate-buffered saline for 15 min at 37°C in a 5% CO_2 incubator. After incubation, 0.1 nM glucagon and 10 μM forskolin were added, and the cells were incubated for 30 min at 37°C in a 5% CO_2 incubator. As a positive control, the CHO-K1 GCGR Gs cells were treated with 10 μM of GA (GCGR antagonist I, Santa Cruz Biotechnology, sc-203972). cAMP was measured using the HitHunter® cAMP Assay for Small Molecules Kit (DiscoverX) according to the manufacturer's instructions. Luminescence was measured using a Victor3 multilabel plate reader (PerkinElmer, Waltham, MA, USA).

2.5 | Measurement of hepatic glucose production

Mouse primary hepatocytes were seeded at 2.5×10^5 cells/well in 12-well plates. After 24 hr, the cells were washed twice in pre-warmed glucose-free DMEM and incubated for 3 hr in glucose-free DMEM. The cells were treated with various concentrations of GCGR antagonist candidates, gluconeogenic substrates (20 mM sodium lactate and 2 mM sodium pyruvate), and 10 nM glucagon. After 30 min, glucose in the medium was quantified using a glucose assay kit (GAGO-20, Sigma-Aldrich, St. Louis, MO, USA) and normalized to total cellular protein concentration (Choi et al., 2018; J. Y. Yoon et al., 2017). As a positive control, mouse primary hepatocytes were treated with 20 μM of GA (GCGR antagonist I, Santa Cruz Biotechnology, sc-203972). Five independent experiments were performed in triplicate.

2.6 | Glucagon-induced glucose excursion and intraperitoneal glucose tolerance test (ipGTT)

For the glucagon-induced glucose excursion test, after 5 weeks of treatment with **7aB-3** or **LGD-6972**, the mice were fasted for 5 hr, and then, 50 mg/kg of **7aB-3** or **LGD-6972** was orally administered by gavage. After 1 hr, glucagon was injected intraperitoneally at a dose of 15 μg/kg. Blood glucose levels were measured at 15, 30, 45, and 60 min after glucagon injection. For the ipGTT, after 6 weeks of treatment with **7aB-3** or **LGD-6972**, the mice were fasted for 14 hr, and then, 50 mg/kg of **7aB-3** or **LGD-6972** was orally administered by gavage. After 1 hr, glucose (2 g/kg) was injected intraperitoneally, and blood glucose levels were measured at 30, 60, 90, and 120 min afterward.

2.7 | In vivo pharmacokinetic studies and bioanalytical assessment by LC-MS/MS

Rats were fasted overnight prior to the pharmacokinetic studies but were allowed free access to water. Briefly, after cannulation with a PE50 tube of the femoral vein for IV administration and femoral artery for blood collection under anesthesia, similar to our previous studies (Jeong et al., 2019; Song et al., 2020), the systemic and oral pharmacokinetics of the test compounds were investigated at doses of 2 and 5 mg/kg, respectively. After recovering from anesthesia, the fasted rats were IV or PO administered compound **7aB-3**. At predetermined time intervals (i.e., 1 (IV only), 5, 15, 30, 60, 120, 240, and 480 min), ~100 μl of blood was collected from the femoral artery cannula and replaced with an equal volume of normal saline to compensate for blood loss. Plasma samples were prepared by centrifugation of the blood at 12,000 *g* and 4°C for 10 min, and then, the supernatant was stored at −20°C until the LC-MS/MS assay. A simple deproteinization method was used by adding a 2-fold volume of methanolic internal standard solution. The internal standard was used with the other test compounds. After vigorous vortexing, followed by centrifugation at 12,000 *g* and 4°C for 15 min, the supernatant was injected into the LC-MS/MS system. The LC-MS/MS consisted of an Agilent HPLC system (1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) and Agilent 6490 QQQ mass spectrometer with a positive electrospray ionization (ESI^+) Agilent Jet Stream ion source (Agilent Technologies). To separate the test compounds from endogenous substances in plasma, a Synergi™ 4 μm polar-RP 80A column (150 mm × 2.0 mm, 4 μm, Phenomenex, Torrance, CA, USA) was utilized. The

mobile phase was a mixture of 0.1% formic acid and acetonitrile (65:35, v/v) at a flow rate of 200 μl/s. Multiple reaction monitoring (MRM) mode was chosen as follows: **7aB-3,** *m/z* 551.2 → 462.0; internal standard (**IS),** *m/z* 403.4 → 138.2. The calibration curves were linear with a range from 5 to 10,000 ng/ml (coefficient of determination $R^2 = 0.9986$) with weighing of 1/x. For the calculation of pharmacokinetic parameters, non-compartmental analysis was performed using WinNonlin 5.0.1 (Pharsight, Cary, NC, USA). The absolute oral bioavailability (BA) was calculated by dividing the dose-normalized total area under the plasma concentration-time curve from time zero to time infinity (AUC) after PO administration using the dose-normalized AUC after IV administration (Han et al., 2021; Maeng et al., 2019; Yoon et al., 2020). All data are expressed as the mean ± standard deviation (*SD*).

2.8 | Statistical analysis

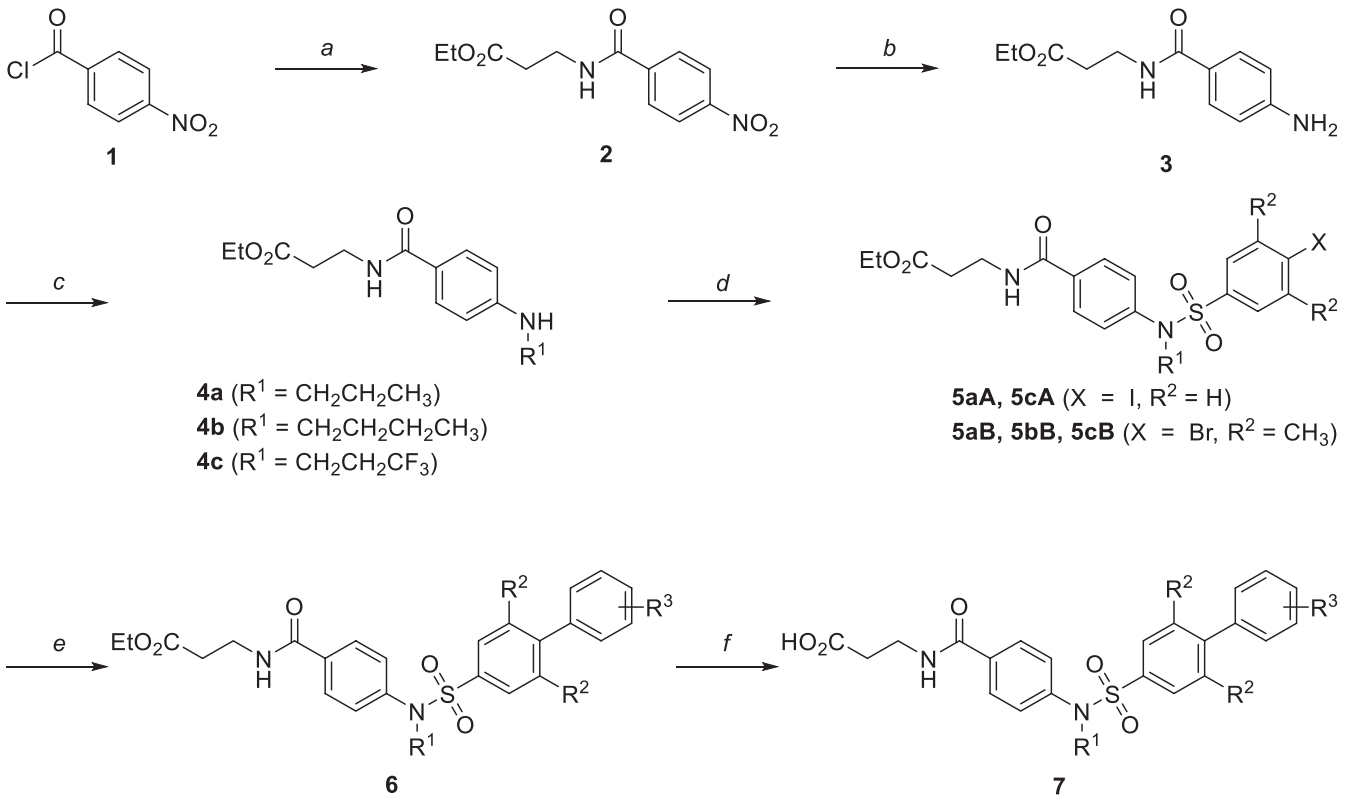
Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference test (* $p < .05$, ** $p < .01$, and *** $p < .001$) using GraphPad Prism software.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis

The synthesis of the biphenylsulfonamide derivatives is shown in Scheme 1. 4-Nitrobenzoyl chloride was treated with *β*-alanine ethyl ester to give amide **2**, of which the nitro group was reduced to form amine **3** with H_2 and Pd/C in MeOH. Several alkyl groups, such as *n*-Pr, *i*-Bu, *n*-Bu, *n*-Hexyl, and 3,3,3-trifluoropropyl, were conjugated to the amine by reductive amination to produce N-alkylanilines **4**, which were converted to 4-halosulfonamides **5A** and **5B** using 4-iodosulfonyl chloride or 4-bromo-3,5-dimethylsulfonyl chloride, respectively. The halides were exchanged with various substituted phenyl groups through the Suzuki reaction to afford biphenyl compounds **6** in good yields. Finally, the ethyl ester was hydrolyzed to obtain the corresponding carboxylic acids **7**. Eighteen analogs were synthesized and are presented in Table 1.

The first step in the synthesis of 4-bromo-3,5-dimethylben zenesulfonyl chloride (shown in Scheme 2) was carried out by bromination of commercially available 3,5-dimethylaniline in the para position with NBS (Zysman-Colman et al., 2009)



SCHEME 1 Synthesis of biphenylsulfonamides. *Reagents and conditions*: (a) *β*-alanine ethyl ester hydrochloride, TEA, $CHCl_3$, rt, 94%; (b) Pd/C, H_2, MeOH, rt, 98%; (c) alkyl aldehyde, Pd/C, H_2, MeOH, rt, 68 ~ 75%; (d) 4-iodobenzenesulfonyl chloride or 4-bromo-3,5-dimethylbenzenesulfonyl chloride, TEA, CH_2Cl_2, rt, 58 ~ 67%; (e) substituted phenylboronic acid, 20% K_2CO_3, $Pd(PPh_3)_4$, THF, 80℃, 60%–80%; (f) $LiOH{\cdot}H_2O$, THF:H_2O (1.5:1), rt, 78 ~ 93%

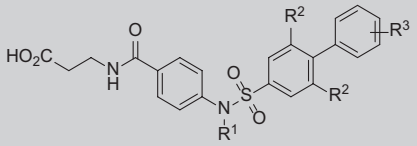
to yield compound **9**, followed by diazotization (Hogan & Cox, 2009) and treatment with thionyl chloride to afford sulfonyl chloride **10** (Rennison et al., 2013).

3.2 | Biological activity

3.2.1 | Screening of biphenylsulfonamide compounds for cytotoxicity

We synthesized 18 compounds as GCGR antagonist candidates based on the biphenylsulfonamide structure. The cytotoxic effects of the synthesized compounds were evaluated in CHO-K1 GCGR Gs cells. These cells overexpress human GCGR and were used for screening the biological activity of the compounds. The cells were treated with 20 μM of each compound for 24 hr, and cell viability was examined. As shown in Figure 3, we selected 10 compounds that produced over 95% cell viability in the CHO-K1 GCGR Gs cells.

TABLE 1 Biphenylsulfonamide derivatives



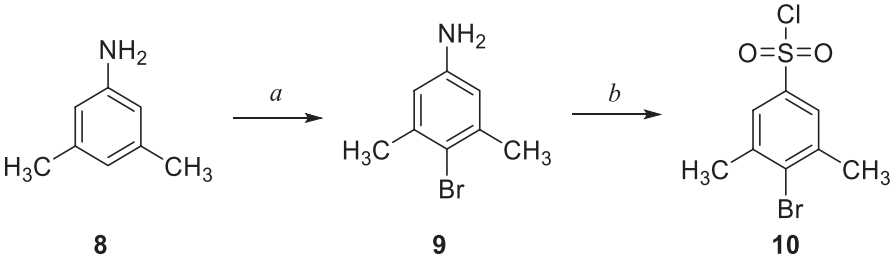
Entry	Compound	R_1	R_2	R_3
1	**7aA-1**	$CH_2CH_2CH_3$	H	H
2	**7aA-2**	$CH_2CH_2CH_3$	H	4-Cl
3	**7aA-3**	$CH_2CH_2CH_3$	H	4-CF_3
4	**7aA-4**	$CH_2CH_2CH_3$	H	4-*t*-Bu
5	**7aA-5**	$CH_2CH_2CH_3$	H	3,5-Di-Cl
6	**7aA-6**	$CH_2CH_2CH_3$	H	2-F, 5-CF_3
7	**7cA-1**	$CH_2CH_2CF_3$	H	4-Cl
8	**7cA-2**	$CH_2CH_2CF_3$	H	4-CF_3
9	**7cA-3**	$CH_2CH_2CF_3$	H	4-*t*-Bu
10	**7aB-1**	$CH_2CH_2CH_3$	CH_3	4-Cl
11	**7aB-2**	$CH_2CH_2CH_3$	CH_3	4-CF_3
12	**7aB-3**	$CH_2CH_2CH_3$	CH_3	4-*t*-Bu
13	**7bB-1**	$CH_2CH_2CH_2CH_3$	CH_3	4-Cl
14	**7bB-2**	$CH_2CH_2CH_2CH_3$	CH_3	4-CF_3
15	**7bB-3**	$CH_2CH_2CH_2CH_3$	CH_3	4-*t*-Bu
16	**7cB-1**	$CH_2CH_2CF_3$	CH_3	4-Cl
17	**7cB-2**	$CH_2CH_2CF_3$	CH_3	4-CF_3
18	**7cB-3**	$CH_2CH_2CF_3$	CH_3	4-*t*-Bu

3.2.2 | Screening of biphenylsulfonamide compounds by in vitro efficacy

When GCGR is activated by glucagon, intracellular cAMP levels are increased by stimulation of adenylate cyclase, and the cAMP-dependent protein kinase (PKA) pathway is subsequently activated (Han et al., 2015; Jiang et al., 2001; Kimball & Murlin, 1923; Li et al., 2006). We then tested the 10 selected synthetic compounds for inhibition of glucagon-induced cAMP production in the CHO-K1 GCGR Gs cells. Three compounds showed over 70% inhibition of glucagon-induced cAMP production compared to that of the vehicle-treated group (Figure 4a). Substitution of R^1 of the sulfonyl amide with *n*-propyl (-$CH_2CH_2CH_3$; **7aB-3**) and 3,3,3-trifluoropropyl (-$CH_2CH_2CF_3$; **7cA-3** and **7cB-3**) moieties was found to enhance potency in the cAMP assay. The introduction of a *t*-butyl (4-*t*-Bu) group at the para position of R^3 or methyl (CH_3) substitution at R^2 resulted in excellent inhibitory activity (Figure 4a). To examine the dose-dependent effects on glucagon-induced cAMP production, we treated the CHO-K1 GCGR Gs cells with various concentration of each compound (5, 10, or 20 μM of **7cA-3, 7aB-3,** or **7cB-3**) in the presence of glucagon (0.1 nM). Except for compound **7cA-3**, the other compounds showed concentration-dependent inhibition of glucagon-induced cAMP production. Compound **7aB-3** showed the lowest cAMP production among the three compounds at 20 μM (Figure 4b).

3.2.3 | Effect of the three selected biphenylsulfonamide compounds on glucagon-induced glucose production in primary hepatocytes

Activation of GCGR by glucagon promotes glucose production by inducing both glycogenolysis and gluconeogenesis in the liver (Brubaker & Drucker, 2002; Christensen et al. 2011). We then tested the selected three compounds for glucagon-induced glucose production in mouse primary hepatocytes.



SCHEME 2 Synthesis of 4-bromo-3,5-dimethylbenzenesulfonyl chloride. *Reagents and conditions*: (a) NBS, acetonitrile, 0°C to rt, 72%; (b) CuCl, $SOCl_2$, HCl, sodium nitrite, H_2O, −5°C to rt, 52%

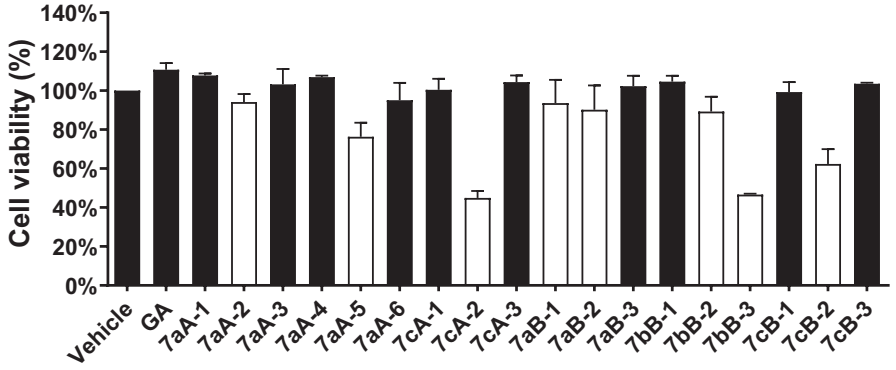


FIGURE 3 Screening of biphenylsulfonamide compounds for cytotoxicity. CHO-K1 GCGR Gs cells were treated with 20 μM of indicated compounds for 24 hr, and cell viability was determined by CCK-8 assay. As a positive control, CHO-K1 GCGR Gs cells were treated with 20 μM of GCGR antagonist I (GA). Black bars indicate the compounds selected for further analysis. $N = 3$ independent experiments. The data are presented as the mean ± standard error (SE)

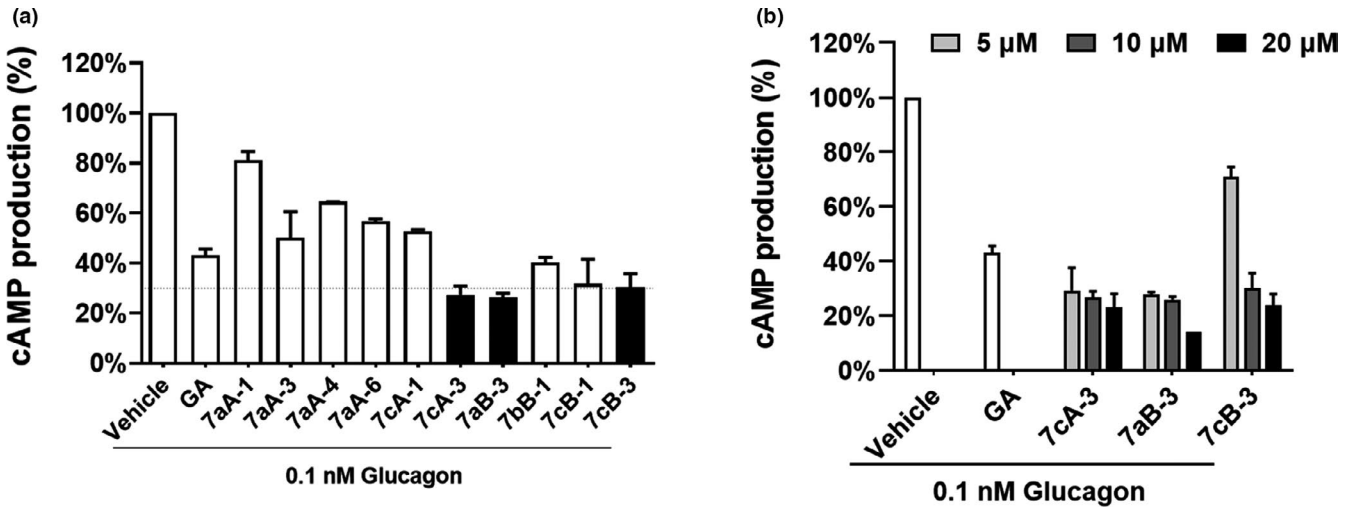


FIGURE 4 Screening of biphenylsulfonamide compounds by glucagon-induced cAMP production assay. (a). cAMP production was measured after treatment of CHO-K1 GCGR Gs cells with 10 μM of the indicated compound in the presence of 0.1 nM glucagon. (b) cAMP production was measured after treatment of CHO-K1 GCGR Gs cells with 5, 10, or 20 μM of the indicated compound in the presence of 0.1 nM glucagon. As a positive control, CHO-K1 GCGR Gs cells were treated with 10 μM of GCGR antagonist I (GA). $N = 3$ independent experiments. The data are presented as the mean ± standard error (SE)

We tested the cells with the compounds at 20 μM in the presence of glucagon. The three compounds (**7cA-3**, **7aB-3**, and **7cB-3**) produced 72%, 74%, and 71% inhibition of glucagon-induced glucose production, respectively (Figure 5a). Next, we examined the concentration-dependent effects of the compounds on glucagon-induced glucose production in mouse primary hepatocytes. We treated the cells with 1, 5, 10, or 20 μM of each compound in the presence of glucagon (10 nM). Except for compound **7cB-3**, the other compounds inhibited glucose production in a concentration-dependent manner (Figure 5b). Based on the results of the cytotoxicity, cAMP production, and glucagon-induced glucose production assays, we selected compound **7aB-3** for in vivo further experiments. We then evaluated the concentration-dependent inhibitory effect of compound **7aB-3** on glucagon-induced cAMP production in more detail (Figure 6). The obtained IC_{50} value for **7aB-3** was 8.4 μM.

3.2.4 | In vivo glucose-lowering effect of compound **7aB-3**

We selected compound **7aB-3** that inhibited glucagon-induced glucose production to the greatest extent in vitro. To evaluate the blood glucose-lowering effect of compound **7aB-3**, high-fat diet (HFD)-induced obese mice were orally administered 50 mg/kg of **7aB-3** or LGD-6972 as a control once daily for 6 weeks. To investigate whether the effect of **7aB-3** on the control of blood glucose levels is mediated by inhibition of GCGR, we performed glucagon

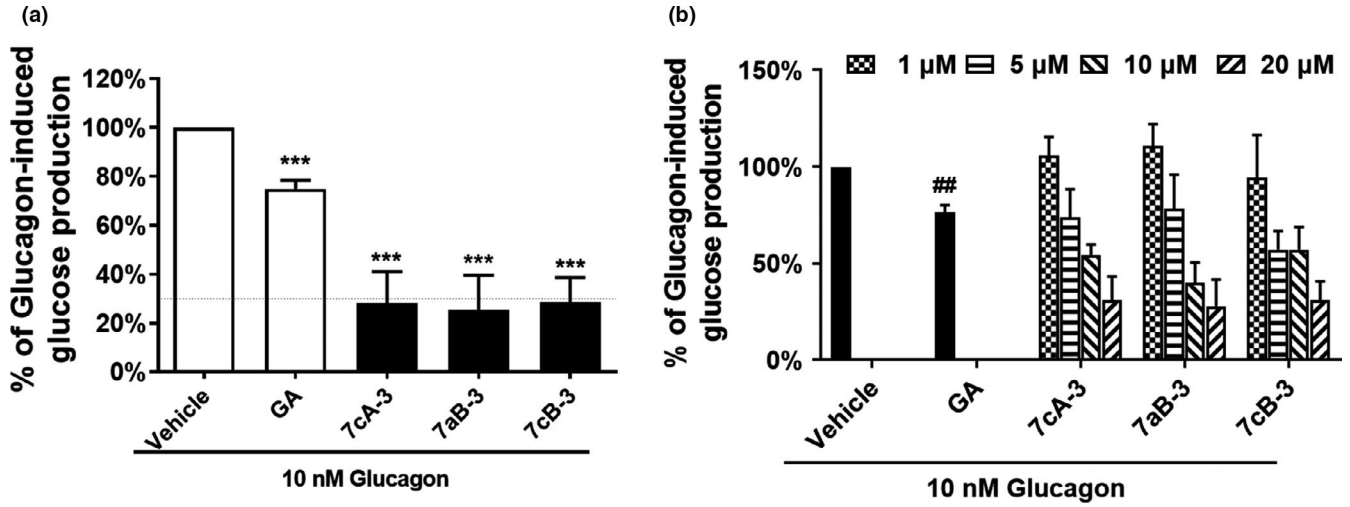


FIGURE 5 Screening of three selected biphenylsulfonamide compounds by glucagon-induced glucose production in primary hepatocytes. (a) Mouse primary hepatocytes were pretreated with 20 μM of the indicated compound in glucose-free Dulbecco's modified Eagle medium (DMEM). After 3 hr, cells were treated with 20 μM of the indicated compound in the presence of 10 nM glucagon and gluconeogenic substrates (2 mM sodium pyruvate and 20 mM sodium lactate). Glucose production was measured 30 min after glucagon treatment. (b) Mouse primary hepatocytes were treated with 1, 5, 10, or 20 μM of the indicated compound in the presence of 10 nM glucagon and gluconeogenic substrates. Twenty μM of GCGR antagonist I (GA) was used as a positive control. $N = 5$. The data are presented as the mean ± standard error (SE). $^{***}p < .001$ versus Vehicle, $^{\#\#}p < .01$ versus Vehicle

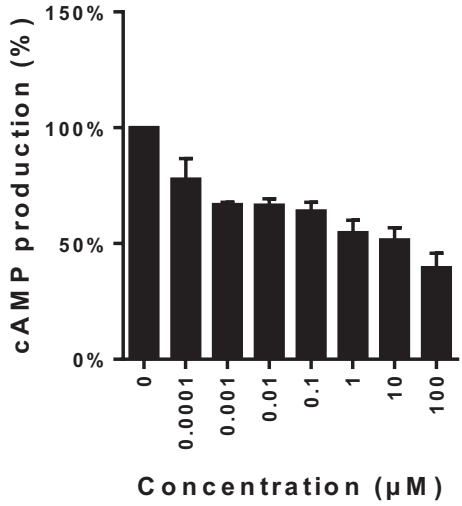


FIGURE 6 Concentration-dependent inhibition of the selected **7aB-3** compound on glucagon-induced cAMP production. cAMP production was measured after treatment of CHO-K1 GCGR Gs cells with the indicated concentration of **7aB-3** in the presence of 0.1 nM glucagon. $N = 3$. The data are presented as the mean ± standard error (SE)

challenge tests. On the first day of the experiment, treatment with **7aB-3** significantly decreased glucagon-induced blood glucose levels at all time points measured in the HFD-induced obese mice, and the effect was similar to that of LGD-6972 (Figure 7a). The area under the curve (AUC) was also significantly decreased in the **7aB-3-** and LGD-6972-treated mice (Figure 7b). Body weight was measured daily, and there was no significant change in body weight from the beginning (week 0) to the end (week 6) of the experiment (Figure 7c). We also monitored daily food intake over the 6 weeks, which did not change among all groups (Figure 7d). During the experiment, we monitored non-fasting blood glucose levels at weeks 3 and 4 during the experiment. In the group treated with 50 mg/kg **7aB-3**, the non-fasting blood glucose level decreased on weeks 3 and 4 of treatment (Figure 7e). On week 6 of treatment with **7aB-3** or **LGD-6972**, we performed a glucose tolerance test to confirm the ameliorative effect of **7aB-3** on hyperglycemia. Treatment with **7aB-3** or **LGD-6972** decreased blood glucose levels in the HFD-induced obese mice administered 2 g/kg of glucose (Figure 7f). From both the in vivo and in vitro data, we conclude that **7aB-3** is more effective than the other derivatives. Compound **7aB-3** was then recommended for further investigation.

3.2.5 | In vivo pharmacokinetic profiles and characteristics of **7aB-3**

To characterize the pharmacokinetic properties of **7aB-3** in vivo, we further investigated the plasma concentration-time profiles of **7aB-3** in rats following intravenous (IV) and oral (PO) administration, as shown in Figure 8 ($n = 3$–4 for each administration). The plasma concentration was measured up to 480 min for both administrations. The calculated pharmacokinetic parameters are listed in Table 2. After IV administration, **7aB-3** showed relatively moderate total clearance (CL) (i.e., 23.6 ml min^{-1} kg^{-1}) with a small

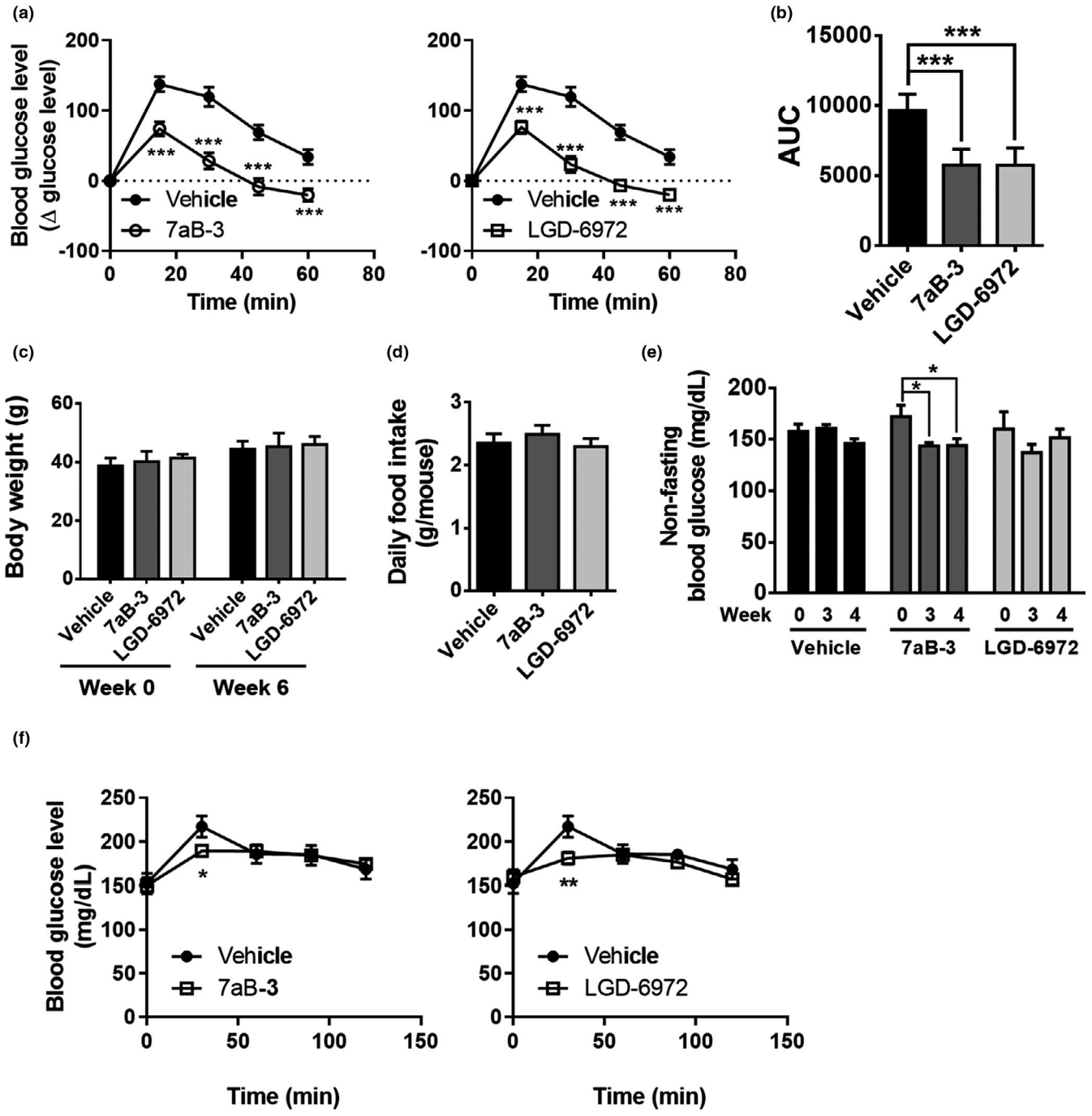


FIGURE 7 Blood glucose-lowering effect of **7aB-3** in HFD-induced obese mice. HFD-induced obese mice were orally administered 50 mg/kg of **7aB-3** or **LGD-6972** once daily for 6 weeks. A. On the first day, the mice were fasted for 5 hr and then 50 mg/kg of **7aB-3** or **LGD-6972** was orally administered. After 1 hr, 15 μg/kg of glucagon was intraperitoneally injected. Blood glucose levels were measured at the indicated time points after glucagon injection ($n = 7$/group). B. AUC of glucagon-induced glucose excursion test. C. Body weight comparison between experimental beginning (week 0) and end (week 6) ($n = 4$–6/group). D. Food intake was measured daily ($n = 4$–6/group). E. Non-fasting blood glucose levels were measured on weeks 0, 3, and 4 ($n = 4$–6/group). F. Mice were fasted for 14 hr on week 6 of the experiment and 2 g/kg of glucose was intraperitoneally injected. Blood glucose levels were measured at the indicated time points after glucose injection ($n = 4$–6/group). Data are presented as the mean ±standard error (SE). * $p < .05$, ** $p < .01$, or *** $p < .001$ versus. Vehicle group

volume of distribution at steady state (V_{ss} = 371.4 ml/kg). The elimination half-life was 90 min. However, the oral pharmacokinetics of **7aB-3** showed poor bioavailability (BA) of approximately 3.7% in vivo. Collectively, compound **7aB-3** possesses poor oral pharmacokinetic properties, although the mechanism of its low oral absorption remains unknown. Thus, pharmacokinetic optimization to enhance oral absorption may be required.

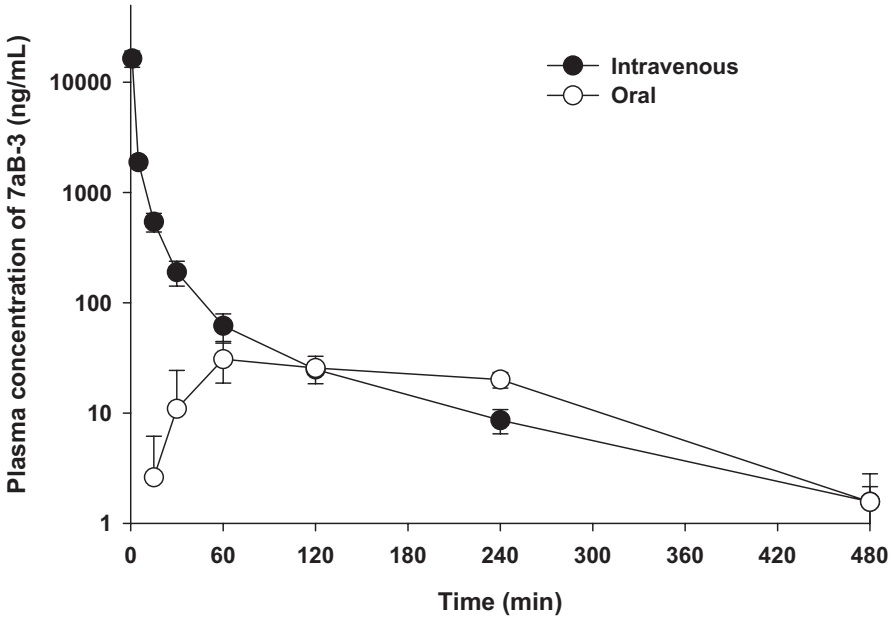


FIGURE 8 Mean plasma concentration–time profiles of **7aB-3** after IV and PO administration at doses 2 mg/kg and 5 mg/kg, respectively, in rats (n = 3–4). Vertical bars represent standard deviations

TABLE 2 Pharmacokinetic parameters of **7aB-3** after IV and PO administration in rats (n = 3–4)

Pharmacokinetic parameters	7aB-3	
	IV (2 mg/kg)	PO (5 mg/kg)
$AUC_{0\rightarrow\infty}$ (μg min^{-1} ml^{-1})	86.3 ± 13.7	8.0 ± 0.5
Terminal $t_{1/2}$ (min)	90.0 ± 15.6	–
MRT (min)	15.8 ± 2.0	183.2 ± 19.1
C_{max} (μg/ml)	–	0.0353 ± 0.0067
T_{max} (min)	–	80.0 ± 34.6
V_{ss} (ml/kg)	371.4 ± 67.1	
CL (ml min^{-1} kg^{-1})	23.6 ± 3.3	
BA (%)		3.7

4 | CONCLUSION

In summary, we synthesized 18 novel biphenylsulfonamide derivatives. Among them, several compounds significantly inhibited GCGR-dependent, glucagon-induced cAMP, and glucose production. Based on the inhibitory efficacy of glucagon-induced cAMP production in CHO-K1 GCGR Gs cells and glucagon-induced glucose production in mouse primary hepatocytes, we selected compound **7aB-3** for further study. **7aB-3** decreased glucagon-induced cAMP production and glucose production in in vivo assays. In addition, the GCGR antagonist **7aB-3** showed efficacy equivalent to that of **LGD-6972** in glucagon-signaling attenuation and blood glucose-lowering in a diabetic animal model. However, the pharmacokinetics of the GCGR antagonist, **7aB-3**, showed low oral bioavailability, requiring further pharmacokinetic optimization for oral drug development. Our results suggest that compound **7aB-3** is a potential glucose-lowering agent for treating type 2 diabetes.

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CONFLICTS OF INTEREST

There are no competing financial, professional, or personal interests that might have influenced the presentation of the work described in this manuscript.

AUTHOR CONTRIBUTIONS

Dongyun Shin and Hee-Sook Jun conceived and designed the experiments. Chang-Yong Lee, Hojung Choi, Eun-Young Park, Thi-Thao-Linh Nguyen, and Han-Joo Maeng performed the experiments and analyzed the data. In particular, Chang-Young Lee prepared all compounds. Hojung Choi and Eun-Young Park performed the biological assays and in vivo experiments. Han-Joo Maeng performed the pharmacokinetic analysis. Kyoung Mee Lee, Dongyun Shin, and Hee-Sook Jun conducted the data analyses. Chang-Yong Lee, Hojung Choi, Dongyun Shin, and Hee-Sook Jun summarized the work and wrote the manuscript.

ETHICAL APPROVAL

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Lee Gil Ya Cancer and Diabetes Institute (LCDI-2015-0006).

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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