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N-substituted sultam carboxylic acids as novel glycogen synthase activators

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Decreased glycogen synthesis and turnover is a common defect in type 2 diabetic patients. Activating glycogen synthase, the enzyme that catalyses the transfer of glucose from UDP-glucose to a glycogen polymer chain, could be a potential therapeutic target for the treatment of diabetes. We discovered a series of *N*-substituted sultam carboxylic acids as potent glycogen synthase activators. Treatment of human skeletal muscle cells with these compounds resulted in an increase in glycogen synthesis. Compound **4** displayed good oral bioavailability and therefore may be a useful tool molecule to study GS as a potential anti-diabetic target.

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Type 2 diabetes (T2D) is a common and serious disorder affecting a large population around the globe. The disease pathology is characterized by an abnormal increase in hepatic glucose production and a decrease in glucose utilization in peripheral tissues. Although several classes of anti-diabetic agents are available, there is still a strong need to identify efficacious treatments without serious side effects.[1]

The two major pathways of glucose utilization in the liver and skeletal muscles are glycolysis, or oxidation of glucose to pyruvate, and glycogenesis, or storage of glucose as glycogen. One of the key enzymes in glycogen synthesis is glycogen synthase (GS). GS catalyses the transfer of glucose from UDP-glucose to the growing glycogen chain through a 1,4-α linkage, and this step is the rate-limiting step under most circumstances.[2,3]

Two forms of GS exist in mammals, encoded by the genes GYS1 and GYS2. The gene products share 69% homology, where GYS2 encodes the liver specific form and GYS1 encodes the form expressed in all other tissues including muscle.[4,5] Both genetic and clinical data suggest the involvement of GS in T2D. It has been reported that a polymorphism of the muscle gene GYS1 is more prevalent in T2D patients than in non-diabetic controls.[6] Furthermore, basal and insulin-stimulated GS activities in muscle cells from diabetic subjects are significantly decreased relative to cells from non-diabetic subjects.[7] Using ^{13}C-NMR spectroscopy in hyperglycemic–hyperinsulinemic clamp studies with ^{13}C-glucose tracer, a greater than 50% decrease in muscle glycogen synthesis in T2D patients compared to normal subjects was found.[8] Therefore, activating GS by GS activators may provide a potential therapeutic target for T2D treatment.

The success of our discovery of glucokinase (GK) activators as potential anti-diabetic agents prompted us to investigate other targets in the glucose homeostasis pathway.[9,10] Since GS is the key regulatory enzyme in glucose storage in peripheral tissues, we were interested in identifying small molecule GS activators. Screening of our compound library provided us a single hit **1** shown in Fig. 1 (GYS1 EC_{50} = 15.1 μM). Our initial hit expansion was focused on the left-hand bi-phenyl and the central furan ring, and a significant increase in GS activation potency was achieved as exemplified by **2** (GYS1 EC_{50} = 0.14 μM).[11] Subsequently, the modification of the proline moiety on the right-hand side led to the discovery of *N*-alkylglycine analogue **3** (GYS1 EC_{50} = 0.1 μM).[12] To further explore the chemical diversity of GS activators from this biphenyl ether chemical class, we took a bioisostere approach to replace the proline or glycine fragments in **2** and **3** and identified *N*-alkylsultam carboxylic acid **4**, a novel GS activator with oral bioavailability. Herein we report the discovery and biological evaluation of these sultam-derived GS activators.

To further explore the structure–activity relationship of **3**, we replaced the amide carbonyl group in **3** with a bioisosteric methylene group and prepared the corresponding amide, carbamate and sulfonamide analogues (**5–12**, in Table 1). In order to reduce the number of rotatable bonds in **7** and **12**, we rigidified the molecule and prepared the corresponding lactam **13** and sultam **14**. Compounds **5–14** were synthesized according to Schemes 1–3.[19]

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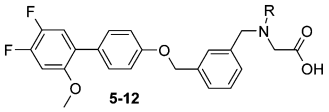
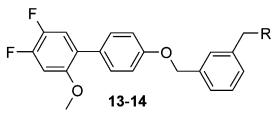
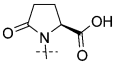
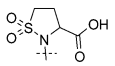
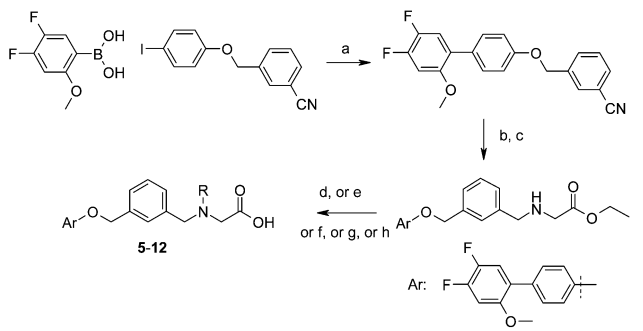
Table 1 Activation potency of GS activators **5–14** in GS enzymatic and cellular assays[a]



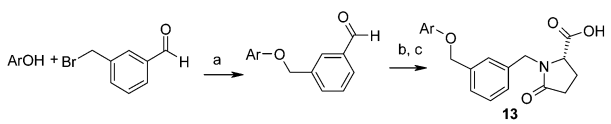


Compound	R	GS enzyme EC_{50} (μM)	GS enzyme SC_{200} (μM)	GS enzyme fold increase @ 75 μM	GS cellular SC_{250} (μM)	GS cellular fold increase @ 75 μM
2		0.14 ± 0.04	0.02 ± 0.01	5.4 ± 1.6	0.20 ± 0.10	32.5 ± 10.5
3		0.10 ± 0.01	0.02 ± 0.01	6.8 ± 1.5	0.16 ± 0.10	33.2 ± 5.0
5	H	4.9	0.38	5.5	6.1	3.9
6		1.93	0.50	7.6	3.7	6.5
7		0.37	0.10	5.4	10.3	7.9
8		0.72	0.18	6.6	2.4	8.6
9		1.34	0.21	6.4	5.5	9.1
10		1.82	0.42	3.5	0.71	14.9
11		0.47	0.03	7.9	0.37 ± 0.05	15.6 ± 0.2
12		0.31 ± 0.10	0.04 ± 0.01	5.7 ± 0.4	0.28 ± 0.07	28.2 ± 6.0
13		0.12	0.02	6.0	0.70	25.7
14		0.13 ± 0.05	0.02 ± 0.01	5.2 ± 1.2	0.44	30.7

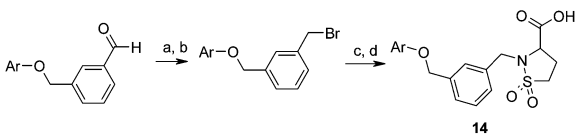
[a] Numbers with ±SEM are from at least three independent biological assays and numbers without ±SEM are the average from dose-dependent enzymatic or cellular assays in triplicate with the overall CV% less than 30%.



Scheme 1 *Reagents and conditions*: (a) K_2CO_3, $PdCl_2$(dppf) cat., DMF/H_2O, 60 °C, 15 h, 92%; (b) $LiAlH_4$, ether/THF, rt, 2 h, 98%; (c) $BrCH_2COOEt$, K_2CO_3, CH_3CN, reflux, 15 h, 69%; (d) LiOH (aq.)/THF, 63%; (e) EtI, TEA, CH_2Cl_2, then (d), 54%; (f) acyl chloride, TEA, CH_2Cl_2, then (d), 95%; (g) alkyl chloroformate, TEA, CH_2Cl_2, then (d), 90%; (h) $EtSO_2Cl$, TEA, CH_2Cl_2, then (d), 45%.



Scheme 2 *Reagents and conditions*: (a) K_2CO_3, acetone, reflux, 15 h, 51%; (b) L-glutamic acid diethyl ester hydrochloride, MeOH/THF/HOAc, $NaBH(OAc)_3$, 69%; (c) LiOH (aq.)/THF, 97%.



Scheme 3 *Reagents and conditions*: (a) $NaBH_4$, 100%; (b) PBr_3, CH_2Cl_2, 0 °C to rt, 42%; (c) 1,1-dioxo-isothiazolidine-3-carboxylic acid methyl ester, K_2CO_3, DMF, rt, 3 h, 73%; (d) LiOH (aq.)/THF, 98%.

Compounds **5–14** were studied in the biochemical assay for their activities as activators of human muscle glycogen synthase (GYS1). The recombinant human muscle GYS1 expressed and partially purified from sf9 cells was used as glycosyltransferase and the assay was carried out by coupling with pyruvate kinase and lactate dehydrogenase.[13] Dose-dependent activation (in comparison with the same assay without activators) of GYS1 was observed and compounds were characterized with three parameters as listed in Table 1: fold-increase in enzyme activity at the highest compound concentration tested (75 μM), EC_{50} (compound concentration required to achieve half of the maximum activation) and SC_{200}

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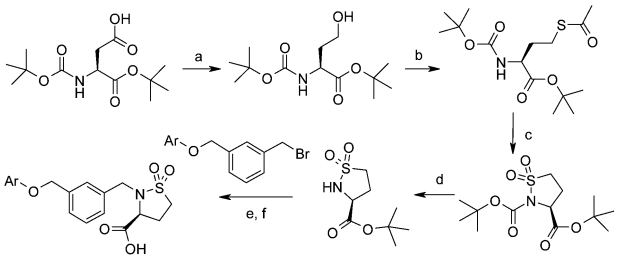
(compound concentration required to achieve a 2-fold enzyme activity). We next tested these activators in activating GYS1 in human muscle cells. Primary human myoblasts were fully differentiated into polynucleated myotubes and cells were treated with D-glucose with ^{14}C-labelled tracer. Following insulin stimulation (50 nM), the incorporation of ^{14}C-glucose into glycogen was measured.[14] Dose-dependent cellular GS activation was observed by comparing the same assay without activator addition. Cellular GS activation was characterized by two parameters as listed in Table 1: fold-increase in GS activity at the highest compound concentration tested (75 μM) and SC_{250} (compound concentration required to achieve a 2.5-fold cellular GS activity). As shown in Table 1, compounds **2**, **3**, and **10–14** displayed significantly higher levels of enzyme activation in the cellular assay than in the corresponding biochemical assay. There is a clear synergistic effect between insulin and GS activators (augmentation of the activator's capability of activating GYS1 in the presence of insulin stimulation). In comparison with **2** and **3**, compounds **5–9** displayed a significant loss of cellular GS activation potency (SC_{250} and fold-increase), while the two carbamates (**10** and **11**) showed medium cellular GS activation (fold-increase). The sulfonamide **12**, lactam **13** and sultam **14** showed comparable activation potency when compared with **2** and **3** in both biochemical and cellular GS activation assays. When lactam **13** was orally dosed to C57 mice, poor oral exposure was observed at both 2 h and 4 h time points, while the sulfonamide **12** and sultam **14** displayed higher oral exposure (data not shown).

Since **14** is a mixture of enantiomers, we used super-critical fluid chromatography (SFC) with a chiral column to separate the racemate. As listed in Table 2, the (*S*)-enantiomer is significantly more active than the corresponding (*R*)-enantiomer. To confirm the absolute stereochemistry of two enantiomers from SFC separation, pure enantiomers were synthesized as described in Scheme 4 using the literature route with our modifications,[15]

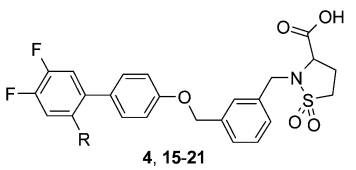


Scheme 4 (a) (1) Isobutyl chloroformate, *N*-methylmorpholine, THF, −15 °C, 15 min; (2) $NaBH_4$, THF, MeOH, 81%; (b) thioacetic acid, PPh_3, DIAD, 0 °C to rt, 62%; (c) HOAc, H_2O, NaOAc, Cl_2, 0 °C, 100%; (d) CH_2Cl_2, TFA, 0 °C to rt, 50%; (e) K_2CO_3, DMF, rt, 3 h, 73%; (f) CH_2Cl_2, TFA, 0 °C to rt, 80%.

and each enantiomer with defined stereochemistry was matched with components separated from chiral SFC.

By using the chiral synthesis of the sultam and the chiral SFC separation of the racemate, we obtained 4 pairs of enantiomers (**4** and **15–21**). These 8 compounds were studied in both biochemical and cellular assays for their GS activation potency and results are listed in Table 2. When comparing each pair of enantiomers, the (*S*)-isomer is significantly more potent than the corresponding (*R*)-isomer. The stereochemical effect on the GS enzyme activation potency is most significant for fluorine substitution (**4**), while the methoxy substitution displayed only a 3-fold difference in the EC_{50} value in the enzyme assay. It is also important to note that the more potent (*S*)-isomer demonstrated higher potency in the GS cellular assay as measured by both the SC_{250} value and the fold-increase of glycogen synthesis at the highest concentration. Interestingly, the sultam analogue with methoxy substitution (**16**) demonstrated the highest cellular potency, which is consistent with our observation for compounds in the proline and *N*-alkylglycine chemical series (**2** and **3**). Data from Table 2 clearly suggest a good correlation between our GS biochemical assay and the human skeletal muscle cellular assay.

Table 2 Activation potency of GS activators **4**, **15–21** in GS enzymatic and cellular assays[a]



4, **15-21**

Compd	R	(*R*) or (*S*)	GS enzyme EC_{50} (μM)	GS enzyme SC_{200} (μM)	GS enzyme fold increase @ 75 μM	GS cellular SC_{250} (μM)	GS cellular fold increase @ 75 μM
4	F	(*S*)	0.09 ± 0.03	0.01 ± 0.0	5.7 ± 0.4	2.2 ± 0.5	34.1 ± 4.0
15	F	(*R*)	1.82 ± 0.20	0.65 ± 0.27	5.4 ± 0.7	30.8 ± 10.0	4.5 ± 0.5
16	OCH_3	(*S*)	0.12 ± 0.02	0.01 ± 0.0	5.9 ± 0.5	0.30	46.5
17	OCH_3	(*R*)	0.39 ± 0.06	0.10 ± 0.01	5.8 ± 0.5	11.2	10.9
18	Cl	(*S*)	0.10	0.03	5.3	1.0	24.7
19	Cl	(*R*)	0.84	0.42	5.0	12.1	11.2
20	CH_3	(*S*)	0.11	0.01	6.1	1.4	39.1
21	CH_3	(*R*)	0.90 ± 0.10	0.32 ± 0.11	5.2 ± 1.1	8.2 ± 0.1	11.1 ± 1.1

[a] Numbers with ±SEM are from at least three independent biological assays and numbers without ±SEM are the average from dose-dependent enzymatic or cellular assays in triplicate with the overall CV% less than 30%.

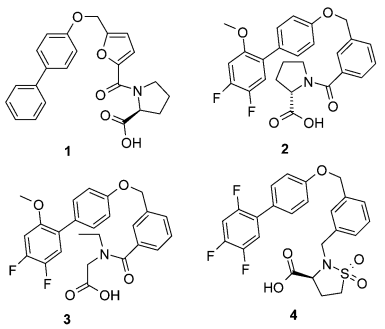


Fig. 1 Structures of GS activators **1–4**.

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Table 3 Pharmacokinetic properties of **4** in rats ($n = 3$)[a]

Route	iv	po
Dose (mg kg^{-1})	5	10
Cl (mL min^{-1} kg^{-1})	4.26	
V_{dss} (L kg^{-1})	0.98	
$AUC_{0\text{-inf}}$ (ng h mL^{-1})	19 855	20 640
C_{max} (ng mL^{-1})		3327
F (%)		52

[a] The observed CV% for the iv route was less than 20%.

Comparing the structural features of the sultam analogues described in this report with proline derivatives (Fig. 1), it is clear that they share the common fragment of the left hand biphenyl ether and the carboxylic acid functional group. We carried out extensive enzyme activation kinetic studies for both HTS hit (compound **1**) and highly potent GS activators in the proline chemical series (chemical class of **2**). Our data revealed that they are non-competitive against the substrate UDP-glucose and the physiological activator glucose-6-phosphate (G6P). These compounds appear to bind GS at the allosteric site and synergize with G6P. Although we did not further characterize the kinetic profiles of sultam analogues, it is likely that these sultam carboxylic acids share the same allosteric binding mode as the proline derivatives based on the SAR and the common structural fragments.

To further evaluate the potential of our novel sultam-derived GS activators as tool compounds, we investigated the pharmacokinetic properties of **4** in male Wistar rats.[18] As listed in Table 3, following intravenous dosing at 5 mg kg^{-1}, the mean clearance was low (4.26 mL min^{-1} kg^{-1}) and a moderate volume of distribution was observed (967 mL kg^{-1}). When **4** was orally dosed to rats at 10 mg kg^{-1} (aqueous suspension containing 10% PEG400 in phosphate buffer), a good oral bioavailability (52%) was observed. To answer the question whether these chiral sultam carboxylic acids can undergo *in vivo* racemization, we analysed plasma samples (using a chiral column) from mice orally dosed with **4** and **15**, and no *in vivo* racemization was observed.

Conclusions

In summary, a novel class of sultam-derived carboxylic acids were identified as potent glycogen synthase activators. These compounds demonstrated activation potency in both biochemical and cellular assays. The stereochemistry of these sultam analogues significantly affected their GS activation potency. Compound **4** further showed good oral bioavailability. Apart from glucose-6-phosphate, an endogenous GS activator, currently there are no small molecule GS activators reported in the literature except for our biphenyl ether chemical class.[16,17] The sultam carboxylic acids described here may provide tool compounds to evaluate GS activation as a potential anti-diabetic target.

Acknowledgements

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13 The enzymatic activity of GS was measured in 384-well polystyrene plates (BD Biosciences) using a coupled enzyme assay in a total volume of 32 μL per well. An 8 μL aliquot of activator solution (diluted compounds in 30 mM glycylglycine, pH 7.3, 40 mM KCl, 20 mM $MgCl_2$, 9.2% DMSO, with or without 20 mM glucose-6-phosphate) was added to 12 μL aliquot of substrate solution containing glycogen (4.32 mg mL^{-1}), 2.67 mM UDP-glucose, 21.6 mM phospho(enol)pyruvate and 2.7 mM NADH in 30 mM glycylglycine, pH 7.3 buffer. The reaction was then started by adding 12 μL of enzyme solution containing glycogen synthase (16.88 μg mL^{-1}), pyruvate kinase (0.27 mg mL^{-1}), and lactate dehydrogenase (0.27 mg mL^{-1}) in 50 mM Tris–HCl, pH 8.0, 27 mM DTT and bovine serum albumin (BSA, 0.2 mg mL^{-1}), mixed and incubated at room temperature. The conversion rate of NADH to NAD was measured every 3 min over a period of 15 minutes at abs. 340 nm on an Envision reader (Perkin Elmer). The enzyme activity (with or without compound) was calculated by the reaction rate per minute.

14 *Human Skeletal Muscle Cell Culture and Differentiation Conditions:* Normal human skeletal muscle cells were purchased from Lonza. Myoblasts were kept in culture at a concentration of 4×10^5 mL^{-1} in Lonza SkBM medium (without the addition of insulin) and 2.0% FBS, 1% glutamine in two T225 Flasks (65 mL per Flask). After 3 days they reached 70–75% confluency and were sub-cultured using 0.025% Trypsin/EDTA 0.01% (Lonza) and neutralization solution (Lonza). Myoblasts were plated in 24-well plates (pre-coated the same day with Matrigel diluted 1 : 60 in high glucose DMEM, 25 mM Hepes) at a concentration of 1.2×10^3 cells per well in Lonza medium (without the addition of insulin) and 2% FBS, 1% glutamine. After 4 days cells reached 70–75% confluency and were differentiated by the addition of differentiation medium (DMEM (high glucose), 25 mM Hepes and 2% FBS). Myoblasts reached full differentiation into myotubes 6 days later. Biological assays were run between 6–8 days after differentiation. Myotubes responded to insulin and were used up to and including passage 5.
Glycogen Synthesis in human skeletal muscle cells: Differentiated human myotubes were fed with fresh differentiation medium (as described above) the night before the assay. Prior to compound or hormone stimulation the medium was changed to DMEM (low glucose), 5 mg mL^{-1} low endotoxin BSA for 2 hours at 37 °C. For stimulation the medium was then replaced with DMEM (low glucose), 25 mM Hepes medium containing 0.8 μCi per well of D-glucose/D-[^{14}C] with 50 nM insulin (Gibco Human Insulin Catalogue # 12585-014) with or without the GS activator. After 5 h, monolayers were washed twice with PBS and residual liquid in each well was aspirated. Cells in 24 well plates were then frozen overnight at −80 °C. The cells were lysed and the ^{14}C-labeled glycogen was extracted as described below. *Radioisotope-labelled glycogen extraction*: Frozen myotubes were lysed with addition of 100 μL of 1 M NaOH and placed on a shaker for 15 min at room temp. The cell lysates were first transferred individually to a 96-well plate, this allowed rapid transfer (using a multichannel pipette) to 96-well glass-fibre filter plates (Millipore Cat. # MAFBNOB50 – Multiscreen FB) pre-loaded with cold 100% ethanol (100 μL per well). The plates were incubated at 4 °C for 2 h to ensure the glycogen was precipitated. After precipitation the free radio ligand was separated from glycogen on a vacuum manifold. The plate was dried and snapped into the filter plate adapter (Packard # PPN 6005178) of Microscint-20 and cocktail (50 μL) was added to each well. After covering with a sealing film, the plate was loaded and counted directly in the TopCount and the incorporated glucose into glycogen was calculated.

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18 Experiments in the pharmacokinetic studies were performed in compliance with the relevant laws and institutional guidelines, and approvals were obtained prior to the experiments.

19 Compounds **4–21** were characterized by ^{1}H-NMR and analyzed by LC/MS with observed purity greater than 95%. All chiral compounds were analyzed by super-critical fluid chromatography on a chiral column (diacel AD column, 35% MeOH in CO_2) in comparison with the corresponding racemate and the enantiomeric purity was greater than 95%. The spectral data of the selected compounds are listed below:
Compound **11**: ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.11–1.21 (m, 6H), 3.75 (s, 3H), 3.84 (d, J = 18.1 Hz, 2H), 4.45 (d, J = 4.5 Hz, 2H), 4.71–4.85 (m, 1H), 5.12 (s, 2H), 7.03 (d, J = 8.1 Hz, 2H), 7.18–7.28 (m, 2H), 7.30–7.46 (m, 6H); LC/MS calcd for $C_{27}H_{27}NF_2O_6$ (m/z) 499, obsd 500.1 (M + H, ES^+).
Compound **12**: ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.25 (t, J = 7.2 Hz, 3H), 3.20 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.81 (s, 2H), 4.48 (s, 2H), 5.15 (s, 2H), 7.04 (d, J = 8.7 Hz, 2H), 7.17–7.48 (m, 8H), 12.92 (br, s, 1H); LC/MS calcd for $C_{25}H_{25}NF_2O_6S$ (m/z) 505, obsd 504.1 (M − H, ES^-).
Compound **13**: ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.87–2.04 (m, 1H), 2.15–2.43 (m, 3H), 3.75 (s, 3H), 3.82–3.97 (m, 2H), 4.91 (d, J = 15.4 Hz, 1H), 5.13 (s, 2H), 7.03 (d, J = 8.7 Hz, 2H), 7.11–7.46 (m, 8H), 13.05 (br, s, 1H); LC/MS calcd for $C_{26}H_{23}NF_2O_5$ (m/z) 467, obsd 468.0 (M + H, ES^+).

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Compound **14**: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.40–2.69 (m, 2H), 3.02–3.16 (m, 1H), 3.32 (dt, J = 12.4, 7.8 Hz, 1H), 3.78–3.87 (m, 1H), 3.77 (s, 3H), 4.28 (d, J = 15.1 Hz, 1H), 4.64 (d, J = 15.1 Hz, 1H), 5.12 (s, 2H), 6.78 (dd, J = 12.1, 6.6 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 7.04–7.17 (m, 1H), 7.30–7.48 (m, 6H); LC/MS calcd for $C_{25}H_{23}NF_2O_6S$ (m/z) 503, obsd 502.0 (M − H, ES$^-$).

Compound **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.45–2.55 (m, 1H), 2.55–2.67 (m, 1H), 3.05–3.16 (m, 1H), 3.33 (dt, J = 12.5, 7.9 Hz, 1H), 3.84 (dd, J = 8.3, 4.5 Hz, 1H), 4.29 (d, J = 15.1 Hz, 1H), 4.65 (d, J = 15.1 Hz, 1H), 5.12 (s, 2H), 6.96–7.06 (m, 3H), 7.17–7.25 (m, 1H), 7.32–7.49 (m, 6H); LC/MS calcd for $C_{24}H_{20}NF_3O_5S$ (m/z) 491, obsd 490.0 (M − H, ES$^-$); micro analysis calcd C 58.65%, H 4.10%, N 2.85%, F 11.60%, S 6.52%; obsd C 58.87%, H 3.98%, N 2.78%, F 11.43%, S 6.55%; $[\alpha]_D$ = −26.2 (4 mg mL^{-1}, in EtOAc).

Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.44–2.55 (m, 1H), 2.55–2.68 (m, 1H), 3.05–3.16 (m, 1H), 3.28–3.39 (m, 1H), 3.84 (dd, J = 7.9, 4.3 Hz, 1H), 4.29 (d, J = 15.1 Hz, 1H), 4.65 (d, J = 14.9 Hz, 1H), 5.12 (s, 2H), 6.95–7.08 (m, 3H), 7.16–7.26 (m, 1H), 7.31–7.50 (m, 6H); LC/MS calcd for $C_{24}H_{20}NF_3O_5S$ (m/z) 491, obsd 490.0 (M − H, ES$^-$).

Compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.40–2.69 (m, 2H), 3.02–3.16 (m, 1H), 3.32 (dt, J = 12.4, 7.8 Hz, 1H), 3.78–3.87 (m, 1H), 3.77 (s, 3H), 4.28 (d, J = 15.1 Hz, 1H), 4.64 (d, J = 15.1 Hz,1H), 5.12 (s, 2H), 6.78 (dd, J = 12.1, 6.6 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 7.04–7.17 (m, 1H), 7.30–7.48 (m, 6H); LC/MS calcd for $C_{25}H_{23}NF_2O_6S$ (m/z) 503, obsd 502.0 (M − H, ES$^-$); micro analysis calcd C 59.64%, H 4.60%, N 2.78%, F 7.55%, S 6.37%; obsd C 58.81%, H 4.67%, N 2.70%, F 7.58%, S 6.25%.

Compound **18**: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.45–2.70 (m, 2H), 3.06–3.20 (m, 1H), 3.34 (dt, J = 12.4, 7.9 Hz, 1H), 3.85 (dd, J = 8.1, 4.5 Hz, 1H), 4.31 (d, J = 15.1 Hz, 1H), 4.62 (d, J = 15.1 Hz, 1H), 5.11 (s, 2H), 7.03 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 10.4, 8.6 Hz, 1H), 7.29–7.39 (m, 4H), 7.39–7.49 (m, 3H); LC/MS calcd for $C_{24}H_{20}ClNF_2O_5S$ (m/z) 507, obsd 506.0 (M − H, ES$^-$).

Compound **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.20 (s, 3H), 2.47–2.58 (m, 1H), 2.58–2.70 (m, 1H), 3.09–3.19 (m, 1H), 3.35 (dt, J = 12.3, 7.9 Hz, 1H), 3.87 (dd, J = 8.4, 4.5 Hz, 1H), 4.30 (d, J = 14.9 Hz, 1H), 4.65 (d, J = 14.9 Hz, 1H), 5.12 (s, 2H), 6.96–7.09 (m, 4H), 7.19 (d, J = 8.5 Hz, 2H), 7.33–7.50 (m, 4H); LC/MS calcd for $C_{25}H_{23}NF_2O_5S$ (m/z) 487, obsd 486.0 (M − H, ES$^-$); micro analysis calcd C 61.59%, H 4.76%, N 2.87%, F 7.79%, S 6.58%; obsd C 61.33%, H 4.78%, N 2.74%, F 8.03%, S 6.52%.

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