Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b00015 • Publication Date (Web): 23 Mar 2016

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery, Optimization and Biological Evaluation of Sulfonamidoacetamides as an Inducer of Axon Regeneration

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Abstract

The axon regeneration after injury in the central nervous system is hampered due in part to an age-dependent decline in the intrinsic axon growth potential, and one of the strategies to stimulate axon growth in injured neurons involves pharmacological manipulation of implicated signaling pathways. Here we report phenotypic cell-based screen of chemical libraries and structure-activity guided optimization that resulted in the identification of compound **7p**, which promotes neurite outgrowth of cultured primary neurons derived from the hippocampus, cerebral cortex, and retina. In an animal model of optic nerve injury, compound **7p** was shown to induce growth of GAP-43 positive axons, indicating that the *in vitro* neurite outgrowth activity of compound **7p** translates into stimulation of axon regeneration *in vivo*. Further optimization of compound **7p** and elucidating the mechanisms by which it elicits axon regeneration *in vivo* will provide a rational basis for future efforts to enhance treatment strategies.

Introduction

The neurons in the adult central nervous system (CNS) fail to regenerate axons after injury, which accounts in part for the poor functional recovery in patients with traumatic and neurodegenerative diseases.[1] The failure of axon regeneration is attributable to a growth-inhibiting environment involving myelin-associated inhibitors (Nogo-A, myelin associated glycoprotein, and oligodendrocyte/myelin glycoprotein) and glial scar at the injury site.[2] However, neutralizing and/or removing such inhibitory factors are insufficient to promote long-distance axon regeneration.[3] Adding to the complexity of inhibitory extrinsic factors is an age-dependent decline in the intrinsic axon growth potential; while the capacity of axon to elongate during development is robust, cell intrinsic mechanisms regulating axon growth are suppressed after the formation of functional synapses in the adult CNS and remain inactive even following traumatic injury.[4, 5]

Recent years have seen some progress in understanding the mechanisms that lead to reactivation of cell intrinsic axon growth programs. The mechanisms involve an epigenetic regulation of gene transcription, development-dependent transcription factors, mTOR (mammalian target of rapamycin) and STAT3 (signal transducer and activator of transcription 3) signaling pathways, and manipulation of these cellular signaling pathways in conjunction with providing a permissive environment have been considered as therapeutic approaches to elicit axon growth in injured CNS neurons.[6-10]

Signaling pathways relevant to human diseases are likely to be amenable to small molecule intervention, and bioactive small molecules have proven to be valuable tools for exploring complex cellular processes.[11, 12] Discovering such small molecules using unbiased screening approaches and identification of protein targets of the compounds should provide a rational basis for elucidating new drug targets and signaling pathways relevant to human diseases.[13, 14] In this regards, phenotype-based small molecule screens involving axon

elongation can provide an opportunity for pharmacological manipulation of previously unknown signaling factors which are implicated in cell intrinsic control of axon growth.[15] These endeavors could bring forth novel therapeutic agents for the treatment of clinical conditions associated with a loss of axon.

In this study, in an effort to discover new pharmacological modalities to aid in axon regeneration, we employed phenotypic cell-based screens that allow visual assessment and quantitative measurement of neurite outgrowth *in vitro*. The phenotypic screening campaign and chemical modification efforts led to identification of compound **7p** that enhances neurite outgrowth in cultured primary neurons derived from the hippocampus, cerebral cortex, and retina and that induce optic nerve regeneration in an animal model of optic nerve injury. While it need to be determined how the compound stimulates axon growth *in vivo*, our results should provide further insight into the treatment strategies for clinical conditions associated with a loss of axon.

Results and Discussion

Phenotypic screens for small molecules that stimulate neurite outgrowth

In an effort to discover synthetic small molecules that stimulate axon growth by targeting cell intrinsic mechanisms of axon elongation, we made use of neuronal differentiation of P19 embryonic carcinoma cells by neurogenic transcription factor NeuroD2 in the primary screen.[16, 17] Upon expression of NeuroD2, P19 cells begin to sprout neurites which can be visually detected by indirect immunofluorescence with an antibody against neuron-specific class III β-tubulin.[18] We envisioned that the intrinsic mechanisms of axon growth in primary neurons can be recapitulated in the neurite outgrowth of P19-derived neuron-like cells.

We screened commercially available libraries of 170,000 synthetic small molecules from the DIVERSet libraries, kinase directed libraries, PremiumSet library, and GPCR library (ChemBridge). The compounds were tested at a concentration of 10 μM in 384-well format for their ability to promote neurite outgrowth in P19 cells which were transiently transfected with a NeuroD2 cDNA construct. The compounds that enhance neurite outgrowth greater than 2 fold as compared with neurite outgrowth in DMSO-treated cells were selected, and we were able to confirm the activity of 185 compounds (0.11% hit rate). Then, the hit compounds were subject to a secondary screen which involves axon elongation in rat primary hippocampal neurons. The primary hippocampal neurons have been extensively used as a cellular system to study neuritogenesis,[19, 20] and we tested the hit compounds that enhance neurite outgrowth in P19 cells to see whether they can stimulate neurite elongation in the neuronal context. Interestingly, the secondary screen yielded a set of compounds that share a common chemical scaffold containing a core substructure of sulfonamido acetamide, and compound **1** was most potent among the analogs (Figure 1). The neurite elongation activity of compound **1** reached a plateau at concentrations between 10 and 20 μM (~ 3 fold higher than control), and it lost neurite elongation activity below 1 μM. Similarly, compound **1** was

able to enhance neurite elongation in assay with primary cortical neurons with a similar potency as shown in primary hippocampus neurons (data not shown).

Chemistry

Compound **1** exhibited limited water solubility and poor metabolic stability (half-life in liver S9 fraction) *in vitro*, indicating that compound **1** is not an appropriate candidate for evaluation *in vivo*. In order to address these deficiencies, we initiated structure-activity guided optimization using the neurite elongation of primary cortical neurons.

We sectioned the chemical structure of the compound for chemical modification as shown in Figure 2 and planned three synthetic routes in order to synthesize the derivatives of compound **1** as shown in Figure 3. In route A, compound **A** bearing R_1 and R_2 was used to introduce an aromatic ring or a heteroaromatic ring which include various substituents in section 3 of compound **1**. Compound **B** was obtained through hydrolysis of compound **A** and compound **B** reacted with diverse amine (**C**) to synthesize compound **D**. Synthetic route B was advantageous for fixing R_1 and R_3 and changing the structure of R_2. Compound **E** was synthesized by substitution reaction, and then reacted with various sulfonyl chloride compounds (**F**) along with the diversity of R_2. There is a limitation that secondary amine (-NH-) and nitrogen (-NHCO-) of amide in compound **E** competitively reacted with alkyl halide or acyl halide to produce mixtures. Alternatively, we chose route C to minimize production of the mixtures (tertiary amine and secondary amide) as mentioned above. Amine compounds bearing various substituents (R_1) reacted with arylsulfonyl chloride, acyl chloride and alkyl chloride for compound **G**, which was substituted with compound **H** to afford the compound **D**. As mentioned above, we could avoid competitive reactions of secondary amine (–NH-) and amide (–NHCO-) of compound **E** because R_2 was introduced in advance.

The synthesis of compounds **7a-x** through synthetic route A is described in Scheme 1. Compounds **2a-g** were selected as starting materials and reacted with toluenesufonyl chloride to synthesize N-toluenesulfonamides (compounds **3a-g**). Substitutions of compounds **3a-g** with ethyl bromoacetate afforded compounds **4a-g**, which was followed base catalyzed hydrolysis to obtain compounds **5a-g**. HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) was chosen to produce active ester to conduct amide coupling of various amines (compounds **6a-t**) and carboxylic acid (compound **5a-g**) for compounds **7a-x**.

Compounds **12a-g** bearing 2-methoxy in R_3 and 2,4-dimethoxyphenyl or 4-methoxy-pyridin-3-yl in R_6 were synthesized by reaction of compounds **10a** and **10b** with various benzenesulfonylchloride through synthetic route B. Substitution reaction of compound **2a** with compounds **8** and **9** were carried out for compounds **10a** and **10b**, which was followed by reaction with arylsufonyl chlorides (**11a-e**) to synthesize compounds **12a-g** having diverse substituents in the C ring (Scheme 2).

Compounds **17** and **18** were synthesized for substitution of sulfonamide into amide (-CONH-) or alkyl (-CH_2-) as linkers in section 2 via synthetic route C. Compound **2a** was reacted with chlorobenzoyl chloride (**13**) and 4-methylbenzyl chloride (**14**), respectively, afforded N-acylated compound **15** and *N*-alkylated compound **16**, which were reacted with compound **8** to synthesize compounds **17** and **18**. At the beginning, we tried to synthesize compounds **17** and **18** through synthetic route B, however the chemical yields were low due to a competitive reaction of two nucleophilic nitrogens of secondary amine and amide in compound **E**. Alternatively, the synthetic route C were required and showed no undesired products (Scheme 3).

The neurite outgrowth activities of the compounds bearing the various substituents of ring C and heteroaromatic system in section 3 were summarized in Table 1. First, we modified section 3 to find the effective functional group on ring C. The directing effect of the substituents on ring C was mostly shown in the ortho position (Table 1, entries 1 and 2) and the ethoxy group, an electron donating group, confer better outgrowth activity, compared to an electron withdrawing group, such as halogen and methoxycarbonyl (Table 1, entries 1-8). Replacement of the 2-ethoxyphenyl on ring C of compound **1** with 2-ethoxybenzyl yielded compound **7i** where ring C was placed far away from the basic frame due to insertion of methylene. However replacement of the 2-ethoxyphenyl with imidazole or thiazole yielded compounds which exhibited cytotoxicity in neurite outgrowth assay with primary cortical neurons (Table 1, entries 9, 10 and 11).

We next investigated the structure-activity relationship between substituents in R_4 of ring A and R_5 of ring B along with 2-ethoxy or 2,4-dimethoxy in R_6 of ring C (Table 2). First, the activity of the compounds which adopted a methoxy group in ortho or meta position in R_4 was compared, and it was revealed that the meta substituted compounds exhibited less activity as compared to compound **1** (Table 2, entries 1-2). There was no significant difference in neurite outgrowth activity depending on electron donating/withdrawing groups on ring A (Table 2, entries 1 and 3-6). Because 2,4-dimthoxy group in R_6 presented similar activity with compound **1**, chemical modification was shifted to R_5 in ring B. The activities of halogen substituted compounds were higher than that of compound **12d** in that 4-methoxy was substituted for methyl of R_5 (Table 2, entries 8-11). Among them, the neurite outgrowth activity of 4-bromo substituted compound **12c** was five-fold higher than that of DMSO (Table 2, entry 10). We synthesized compounds **17** and **18** bearing 4-chlorobenzoyl and

methylbenzyl in order to test whether the sulfonamide in section 2 is a structural feature necessary to impart activity; however the results showed that replacements of the sulfonamide residue led to a complete loss of neurite outgrowth activity or yielded compounds that exhibits cytotoxicity (Table 2, entries 14 and 15).

We measured the metabolic stability in mouse liver S9 fraction of compounds **1**, **7g**, **12b** and **12d** whose neurite outgrowth activity was higher than that of DMSO. Unfortunately, when the selected compounds were incubated in the presence of isolated mouse liver S9 fraction for 30 minutes, the residual rates were 0.3 ~ 0.7%, suggesting very low metabolic stability. One of strategies for improving the metabolic stability of compound is to reduce the overall lipophilicity (e.g. logP and logD). Because the binding site of metabolizing enzyme is lipophilic, lipophilic molecules could be accepted more readily to the binding site of enzymes.[21] Thus, we designed compounds with higher metabolic stability and synthesized compounds introducing pyridine or quinoline, as shown in Table 3. The neurite outgrowth activities were different depending on the substituted positions of quinoline; in particular, compound **7l** showed the highest activity (Table 3, entries 1-3). Replacement of R_3 with pyridine derivatives resulted in compounds with varied potency depending on the position of nitrogen; the most potent one was compound **7p** (Table 3, entries 4-6). As expected, the lipophilicity of compound **7p** (logP = 2.43) bearing the pyridyl amide was reduced when compared to that of compound **1** (logP = 3.76), and hence the metabolic stability of the compound **7p** (remaining compound, 61.2%) was remarkably enhanced relative to compound **1** (remaining compound, 0.7%) (Table 4). In addition, it was assumed that the small alkoxy group on pyridine was more adequate with regards to the neurite outgrowth activity (Table 3, entries 6-8). Further modification of compound **7p** by introducing 4-methylthio or 4-bromo substituent in section 2 did not adversely affect its ability to enhance neurite outgrowth of primary cortical neurons

(Table 3, entries 9-10).

Taken together, the structure-activity guided optimization efforts led to identification of a subset of compounds that exhibit improved neurite outgrowth activity and water solubility as compared to compound **1**; compound **7p** was best among the derivatives as it also exhibited improved metabolic stability relative to compound **1**.

The effect of compound 7p on optic nerve regeneration

We discovered compound **1** and its derivatives that demonstrated the ability to stimulate neurite outgrowth of cultured primary neurons derived from hippocampus and cerebral cortex in the brain of rat E18. The *in vitro* results raised the question of whether such compounds can stimulate axon outgrowth and produce desired biological outcome such as axon regeneration in CNS injury site. To address the question, we first tested compound **7p** for its ability to stimulate axon growth of retina neuronal cells *in vitro*, and then employed an animal model of optic nerve injury to test compound **7p** *in vivo*.

The retina neuronal cells prepared as described in Experimental Section were treated with compound **7p** at concentrations between 2 and 20 μM. After a 72 hr incubation, the cells were fixed with paraformaldehyde and stained with an anti-neurofilament antibody (SMI-312) to detect axons (Figure 4A and 4B). Axonal outgrowth was quantified by counting the number of neuronal cells with axon length less than either 50 μm, 50 ~ 100 μm, 100 ~ 200 μm, or longer than 200 μm. Although the cultures were purified partially, we were able to select retinal ganglion cells (RGCs) by cell size and morphology, and the cells bigger than 10 μm in cell body diameter were counted. As shown in Figure 4C, the axonal growth stimulatory effect of compound **7p** was evident in all range of length, and was most prominent at 10 μM.

In order to examine whether compound **7p** was able to stimulate axonal outgrowth of RGCs in an animal model of optic nerve injury, the crushed optic nerves in the injury model were treated with either PBS or compound **7p** (0.22 μg per eye) for three weeks. Then the animals were sacrificed, and optic nerve sections were stained with an anti-GAP43 antibody to visualize growing axons. As shown in Figure 5A, the axons of optic nerves from animals treated with compound **7p** were significantly extended beyond the lesion epicenter as compared to those from the sham-control animals treated with PBS. The differences in the number of GAP-43 positive axons were evident in all cases of measurements at 500, 1000 and 1500 μm distal to the crush center (Figure 5B) but was statistically significant beyond the 1500 μm (injured, 0.22 ± 0.081; injured + **7p,** 0.70 ± 0.20) as determined by quantification of GAP-43 positive axons.

Conclusion

An approach to activate cell intrinsic mechanisms regulating axon growth in the adult CNS is likely to contribute to functional recovery following traumatic injury. Upon screening chemical libraries of 170,000 synthetic small molecules for those that stimulate neurite outgrowth of P19-derived neuron-like cells, we discovered a promising hit (compound **1**) that is also capable of stimulating neurite outgrowth of cultured primary neurons. The structure-activity guided optimization and the efforts to improve metabolic stability of compound **1** resulted in the identification of compound **7p** with properties more suitable for use in *in vivo* studies. It is of note that the *in vitro* neurite outgrowth activity of compound **7p** translates into stimulation of axon regeneration in an animal model of optic nerve injury. Further optimization of compound **7p** and elucidating the mechanisms by which compound **7p** elicits

axon regeneration *in vivo* will provide a rational basis for future efforts to enhance treatment strategies.

Experimental Section

P19 cell culture and chemical treatment

P19 embryonic carcinoma cells (6 x 10^6/10-cm dish) were transfected with 10 μg of DNA construct encoding NeuroD2 by using FuGENE HD. Twelve hr after transfection, the cells were trypsinized and replated at a density of 8,000 cells/well to a 384-well plate in MEM (minimum essential medium) containing 5% FBS, 1 mM sodium pyruvate, and 2 mM glutamate. After a 24 hr incubation, the cells were treated with compounds for 48 hr, and then beta III tubulin was visualized by indirect immunofluorescence. The chemical libraries used in this work were purchased from ChemBridge Corporation (San Diego, CA, USA).

Animal Housing and Ethics

The animal study was approved by the Animal Ethical Committee of Gyeonggi Institute of Science & Technology Promotion and Dankook University, and carried out in accordance with guidelines from KFDA (Korea Food and Drug Administration) and Animal and Plant Quarantine Agency in Korea.

Cell culture of primary neurons

Entire forebrains were dissected from embryonic day 18 (E18) embryos of Sprague-Dawley (SD) rats as described with minor modification.[22] In brief, skull covering the brain was cut and opened with microscissors, and the brain was transferred to a clean dish with ice-cold Hank's buffered salt solution (HBSS). The meninges were teased off with microforceps, and hippocampus and cerebral cortex were collected separately in HBSS. After being centrifuged at 100 x g for 1 min, they were resuspended in Neurobasal medium containing serum-free supplement (B27) and 2 mM glutamate and then were triturated approximately 30 times with a 1 mL micropipette tip to achieve a single-cell suspension.The cells were washed twice with

Neurobasal medium containing B27 and 2 mM glutamate, passed through a 40-μm cell strainer, and then seeded onto 384-well plates at a density of 2,000 cells/well. After a 24 hr incubation, the cells were treated with compounds for 48 hr, and then beta III tubulin was visualized by indirect immunofluorescence.

RGCs were obtained from the postnatal day 7 (P7) SD rats and maintained in Dulbecco's Phosphate-Buffered Saline (DPBS) with 0.5% Bovine Serum Albumin (MP, Cellect^{TM} BSA). The RGCs separation was done by using a RGC isolation kit (Miltenyi Biotec; Auburn, CA). The RGCs were seeded on 18 mm cover glass (Deckglaser) in 12- well plates (Costar, Corning, NY) which had been pre-coated with poly-D-lysine (20 μg/mL) followed by laminin (10 μg/mL) in Dulbecco's Modified Eagle's Medium (DMEM) at a density of 200,000 cells/well. Two hour after plating, RGCs were treated with various doses (0, 2, 10, 20 μM) of compound **7p** and maintained for 3 days.

Indirect immunofluorescence

The fixed cells were washed with PBS and permeabilized with 0.25% Triton X-100 in PBS for 8 min. Primary and secondary antibodies were diluted in PBS containing 5% bovine serum album and used for staining. The antibodies are as follows: 0.5 μg/mL of mouse anti-β III tubulin (Covance); 2 μg/mL Alexa Fluor® 488 goat anti-mouse IgG (Invitrogen). 4',6-Diamidino-2-phenylindole (DAPI) was used to stain the nucleus. Confocal fluorescence images were acquired using a LSM700 with Zen software (Carl Zeiss).

The cultured retina neuronal cells were washed with 0.1 M PBS, fix with 4% paraformaldehyde (PFA) prepared in 0.1 M PBS for 15 min at room temperature and washed again with 0.1 M PBS 2 times for 5 min. Non-specific protein binding was blocked by incubation with 1% BSA and 3% Goat Serum (Vector Laboratories, Burlingame, CA)

prepared in 0.1 M PBS containing 0.1% Triton X-100 (Sigma) for 60 min. Then cells were incubated with primary anti-neurofilament antibody (SMI312 Monoclonal, 1:400, Covance) antibody for overnight at 4℃ and then with secondary anti-rabbit IgG conjugated with Alexa 594 (diluted 1:500 in PBS, Life Technologies) for 90 min at room temperature. Cells were mounted with Vectashield kit (Vector Laboratories) and the images were photographed using a Zeiss microscope (Zeiss, Immersol® 518F, ZEN 2012, blue edition). Using Image J program (NIH Image, Bethesda, MD), the numbers of SMI312 positive neurons were counted.

Quantification of neurite outgrowth

In order to measure the neurite outgrowth of P19, primary hippocampal neurons, and primary cortical neurons, wide field fluorescence images from four fields in each well of 384-well plate were acquired on the ArrayScan® V^{TI} HCS reader (Thermo Fisher Scientific) using a 10 x objective lens, and the images were analyzed with the Neuronal Profiling BioApplication software (Thermo Fisher Scientific).

For the measurement of retina neuronal cells, the number and axonal length of neurons in nine consecutive non-overlapping microscopic fields with areas of 13.55 mm^2 (total area 40.65 mm^2) were counted. Both the total number of cells and the numbers of cells which contains axon length less than 50 μm, 50 ~ 100 μm, 100 ~ 200 μm and longer than 200 μm were counted.

Optic nerve crush

Male Sprague Dawley (SD) rats (8 weeks of age, weighing 200 ~ 250 g) were purchased from Samtako Bio Korea, Inc. (Osan, GyeongGi-Do, Korea). Rats were housed in a temperature (22 ~ 24℃) and humidity-controlled environment under a 12 hour light/dark

cycle (light on at 08:00 and off at 20:00). Rats were given ordinary laboratory food and water ad libitum. Rats were randomly divided into two groups: sham-operated normal control (n=3) and compound **7p** after optic nerve crush (n=4). The animals were anesthetized by intraperitoneal injection of 50 mg/kg tiletamine plus zolazepam (Zoletil; Virbac, Carros, France) and 15 mg/kg xylazine hydrochloride (Rompun; Bayer, Leuverkeusen, Germany). The pupils were dilated with 1% tropicamide eye drops. Dissection of the conjunctiva was made with forcep towards the back of the eye to expose the retrobulbar optic nerve. The optic nerve was crushed with sharp forceps for 15 seconds at 2 mm behind connected optic head. A sham-operated normal control group was injected with saline using 30G needle intra-vitreously once in 4 days. The crushed group was injected with compound **7p** (0.22 μg in 5 μL per eye) prepared in 100 mM potassium phosphate buffer with 0.01 N HCl once in four days for 3 weeks.

Preparation of optic nerve sections and immunohistochemistry

The anterior segments of the eyeballs with optic nerve and the vitreous humor were removed. The eyeballs with optic nerve were isolated and fixed by soaking in 4% paraformaldehyde for 2 h. The fixed retinas were soaked in 30% sucrose solution prepared in PBS, pH 7.4 at 4 ℃ overnight for cryoprotection and embedded in O.C.T. compound (Tissue-tek) before freezing at -80 ℃. Leica cryostat was used to section the retina (12 μm). The sections were washed with PBS three times and were incubated with 1% BSA (MP, Cellect^{TM} BSA) and 3% Goat Serum (Vector) in PBS (1% BSA) (blocking solution) for 1 hour at room temperature to block nonspecific protein binding. Then the sections were incubated with primary anti-GAP-43 BRN3α (Polyclonal, 1:200, Novus Biologicals) antibody for overnight at 4℃ and then with secondary anti-rabbit IgG conjugated with Vector 488 (diluted 1:500 in PBS, Life

technologies) for 90 min at room temperature. Sections were mounted with a Vecta shield kit (Vector Laboratories, Inc. Burlingame, CA) and the images were photographed usinga Zeiss microscope (Zeiss, Immersol® 518F, ZEN 2012, blue edition). Using Image J program (NIH Image, Bethesda, MD), the numbers of GAP-43 positive axons extending 0.5 and 1 mm from the end of the crush site were counted.

Quantification of axon growth

For analysis of axon length, the number of GAP-43 positive axon was counted in the 0.5, 1 and 1.5 mm from the end of the crush site in sections. The axons were measured by using formula at which the counts were used to calculate and group average number of axons per millimeter of nerve width (100 μm box size). Then neuronal axon length was measured by formula as described.[23]

$\Sigma\alpha_d = \pi r^2 X[\text{average axon/mm}]/t$

$\Sigma\alpha_d$: The total number of axon extending distance

t: thickness, r: radius of nerve

Statistics

Significant differences among groups were determined using a program, Graphpad prism (version 5, GraphPad Software, Inc, La Jolla, California). All values are reported as mean ± standard error of the mean. Statistical significance was set at $p < 0.05$.

General Methods

All substances were purchased from Sigma–Aldrich and used as received. Purchased anhydrous MeOH was used without further purification. Analytical thin-layer

chromatography was conducted on E. Merck TLC plates (silica gel 60 F_{254}, aluminum back). Silica gel 60 (230–400 mesh) for column chromatography was purchased from E. Merck. Melting points were measured with a Kofler block or Büchi B-545 melting point apparatus. The ^{1}H and ^{13}C NMR spectroscopic data were recorded in $CDCl_3$, DMSO-d_6, CD_3OD solutions at ambient temperature with Avance II 400 [Bruker Biospin, Germany] (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and Ascend III 700 [Bruker Biospin] (700 MHz for ^{1}H NMR and 175 MHz for ^{13}C NMR). Chemical shifts were recorded as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane by the solvent signal. Coupling constants (*J*) are reported in Hertz. Infrared spectra were recorded on a Nicolet 6700 FTIR spectrophotometer. Low resolution mass spectra were measured on a Micromass Quattro Micro API (Waters, USA), whereas high resolution mass spectra were recorded on LTQ Orbitrap XL (Thermo Scientific, USA) spectrometer. Characterization data for known compounds were consistent with literature values.

Representative compounds and their synthetic procedures

All of the compounds were synthesized via route A (**7i-k**, **7l-p** and **7r-g**), route B (**l**, **7c-f** and **7t-x**), and route C (**7q**, **12e**, **17** and **18**) except commercially available compounds (**7b**, **7g** and **7h**).

2-(*N*-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-*N*-(4-methoxypyridin-3-yl)acetamide (7p) via route A

To a stirred compound **2a** (10.0 g, 81.2 mmol) and K_2CO_3 (16.8 g, 122 mmol) in DMF (150 mL) was added tosyl chloride (16.3 g, 85.3 mmol). After being stirred at rt for 10 min, the reaction solution was quenched with water and extracted with EtOAc. The combined organic phases were washed with water and brine, and dried over Na_2SO_4, concentrated. The

resulting solid was tritured with hexane, providing 15.0 g (67%) of *N*-(2-methoxyphenyl)-4-methylbenzenesulfonamide (**3a**) as a white solid. To a stirred compound **3a** (15.0 g, 54.1 mmol) and K_2CO_3 (18.6 g, 135 mmol) was added dropwise ethyl bromoacetate (5.98 mL, 85.3 mmol). After stirring at rt for 2 h, the reaction solution was quenched with water and extracted with EtOAc. The organic layer was washed with water and brine three times, dried over Na_2SO_4, filtered, and concentrated to give 19.0 g (96%) of ethyl 2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetate (**4a**) as a white solid.

To a solution of compound **4a** (19.0 g, 52.3 mmol) was added 2.5 N NaOH (63 μL, 157 mmol) at rt. The reaction mixture was heated to reflux for 6 h. After cooled to rt, the mixture was concentrated and diluted with EtOAc and H_2O. The aqueous layer was adjusted to pH 2 with 1N HCl (aq), then the product was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated. The resulting residue was recrystallized from EtOAc and hexane to obtain 15.0 g (86%) of 2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetic acid (**5a**) as a white solid, To a mixture of compound **5a** (0.5 g, 1.49 mmol), compound **6p** (185 mg, 1.49 mmol) and HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) in DMF (20 mL) was added triethylamine (416 μL, 2.98 mmol) at 0 ^oC. The reaction solution was allowed to stir at rt for 2 h. The reaction was quenched with H_2O, diluted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, concentrated. The residue was purified by flash chromatography (EtOAc/Hexane, 1:1, v:v) to give 250 mg (38%) of compound **7p** as a white solid, mp 153.0 ^oC; ^1H NMR (700 MHz, $CDCl_3$) δ 9.45 (s, 1H), 9.32 (s, 1H), 8.36 (d, *J* = 6.3 Hz, 1 H), 7.58 (d, *J* = 7.7 Hz, 2 H), 7.35 (t, *J* = 7.0 Hz, 1 H), 7.31-7.29 (m, 3H), 6.99-6.95 (m, 2H), 6.86 (d, *J* = 8.4 Hz, 1 H), 4.36 (s, 2H), 4.12 (s, 3H), 3.47 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.59, 155.45, 154.21, 146.37, 143.78, 141.43, 134.96, 131.46, 130.34, 129.10, 127.74, 126.89, 124.23,

120.75, 111.92, 105.55, 55.95, 55.02, 54.70, 21.61; LRMS (ESI) m/z: 442.1 [M+H]+; HRMS (ESI) m/z: Calcd. for [M+H]+ $C_{22}H_{24}N_3O_5S$: 442.1431; found: 442.1435.

_N_-(2,5-Dimethoxyphenyl)-2-(4-fluoro-_N_-(2-methoxyphenyl)phenylsulfonamido)acetamide (12a) via route B

A mixture of compound **2a** (0.98 mL, 8.7 mmol), compound **8** (2.0 g, 8.7 mmol) and K_2CO_3 (1.8 g, 13.1 mmol) in DMF (17.4 mL) was heated at 80 ^oC for 2 h. The reaction was quenched with H_2O and the solution was diluted with EA and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash chromatography (EtOAc/Hexane, 1:2, v:v) to give compound **1a** as a brown solid. To a solution of compound **10a** (20 mg, 0.06 mmol) and compound **11a** (13 mg, 0.07 mmol) in DCM (0.3 mL) was added pyridine (25.0 μL, 0.32 mmol). After 3 h at rt, the reaction was quenched with H_2O, and then the solution was diluted with DCM. The organic layer was washed with $CuSO_4$ (aq), H_2O and brine, dried over Na_2SO_4, filtered, concentrated. The residue was purified by flash chromatography (EtOAc/Hexane, 1:4, v:v) to obtain 22 mg (78%) of compound **12a** as a yellow solid, mp 148.4 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1 H), 7.75-7.72 (m, 3 H), 7.46-7.33 (m, 4 H), 7.01-6.98 (m, 3 H), 6.64 (dd, J = 8.8, 2.8 Hz, 1 H), 4.42 (s, 2 H), 3.84 (s, 3 H), 3.68 (s, 3 H), 3.41 (s, 3 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 167.01, 165.79, 164.36, 156.03, 153.52, 143.29, 135.89, 135.89, 132.56, 130.93, 128.15, 127.15, 120.91, 116.61, 112.97, 112.44, 108.55, 107.34, 56.95, 56.18, 55.86; LRMS (ESI) *m/z*: 475.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{24}FN_2O_6S$: 475.1334; found: 475.1336.

4-Chloro-_N_-(2-((2,5-dimethoxyphenyl)amino)-2-oxoethyl)-_N_-(2-methoxyphenyl)benzamide (17) via route C

To a mixture of compound **2a** (246 mg, 2.0 mmol) and K_2CO_3 (345 mg, 2.5 mmol) in DMF (10 mL) was added compound **13** (417 mg, 2.4 mmol) at rt. The solution was allowed to stir at rt for 2 h. The reaction was quenched with H_2O and the solution was diluted with EA and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash chromatography (EtOAc/Hexane, 1:2, v:v) to give 497 mg (95%) of intermediate **15** as a white solid. After LC/MS analysis of intermediate 15, to a solution of compound **15** (26.1 mg, 0.1 mmol) and compound **8** (25.7 mg, 0.1 mmol) in DMF (5 mL) was added K_2CO_3 (20.7 mg, 0.15 mmol). The solution was heated to 80 ^oC for 2 h. The reaction was quenched with H_2O, and then the solution was diluted with DCM. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated. The residue was purified by flash chromatography (EtOAc/Hexane, 1:4, v:v) to obtain 40 mg (83%) of compound **17** as a white solid, mp 161.8 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1 H), 7.81 (s, 1 H), 7.33-7.21 (m, 6 H), 7.02-6.96 (m, 2 H), 6.85 (t, J = 7.6 Hz, 1 H), 6.64 (dd, J = 8.8, 2.8 Hz, 1 H), 4.77-4.74 (m, 1 H), 4.25-4.20 (m, 1 H), 3.81 (s, 3 H), 3.73 (s, 3 H), 3.69 (s, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.43, 155.50, 153.99, 142.77, 139.52, 137.16, 132.45, 130.70, 129.31, 128.88, 128.04, 126.62, 121.08, 111.98, 111.53, 109.04, 106.20, 56.85, 55.82, 54.93, 54.82; LRMS (ESI) *m/z*: 455.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{24}ClN_2O_5$: 455.1368; found: 455.1369.

_N_-(2-Ethoxyphenyl)-2-(_N_-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (1)

mp 178.3 ^oC; ^1H NMR (400MHz, $CDCl_3$): δ 9.56 (S, 1 H), 8.36 (d, J = 8.0 Hz, 1 H), 7.57 (d, J = 8.0 Hz, 1 H), 7.51 (d, J = 7.6 Hz, 2 H), 7.34-7.27 (m, 3 H), 7.09 (t, J = 6.4 Hz, 1 H), 6.99-6.97 (m, 3 H), 6.79 (d, J = 1.2 Hz, 1 H), 4.35 (s, 2 H), 4.26 (q, J = 8.8, 6.8 Hz, 2 H), 3.36 (s, 3 H), 2.45 (s, 3 H), 1.69 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.36, 155.54, 147.60, 143.62, 135.66, 132.08, 130.35, 129.36, 127.41, 126.96, 126.74,

124.15, 120.41, 120.32, 119.40, 112.56, 111.96, 64.22, 55.10, 54.12, 20.97, 14.63; LRMS (ESI) *m/z*: 455.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_5S$: 455.1635; found: 455.1635.

_N_-(3-Methoxyphenyl)-2-(_N_-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7b)

mp 206.7 ^oC; ^1H NMR (700MHz, CD_3OD): δ 7.60 (d, *J* = 7.7 Hz, 2 H), 7.39-7.35 (m, 3 H), 7.31 (dd, *J* = 7.7, 1.4 Hz, 1 H), 7.24-7.22 (m, 2 H), 7.02 (t, *J* = 7.7 Hz, 2 H), 6.97 (t, *J* = 7.7 Hz, 1 H), 6.71 (dd, *J* = 8.4, 2.8 Hz, 1 H), 4.38 (s, 2 H), 3.80 (s, 3 H), 3.60 (s, 3 H), 2.46 (s, 3 H); ^{13}C NMR (175 MHz, CD_3OD) δ 167.71, 160.21, 156.30, 144.11, 138.80, 136.02, 131.63, 130.15, 129.23, 129.12, 127.60, 120.63, 112.08, 112.03, 109.81, 105.71, 54.73, 54.33. 54.13, 20.05; LRMS (ESI) *m/z*: 441.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{25}N_2O_5S$: 441.1479; found: 441.1482.

2-(_N_-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-_N_-phenylacetamide (7c)

mp 167.9 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 9.35 (S, 1 H), 7.61 (dd, *J* = 21.2, 8.4 Hz, 4 H), 7.38-7.33 (m, 5 H), 7.15 (t, *J* = 7.2 Hz, 1 H), 7.02 (d, *J* = 8.4 Hz, 1 H), 6.95-6.89 (m, 2 H), 4.25 (s, 2 H), 3.87 (s, 3 H), 2.48 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.36, 155.74, 143.10, 138.54, 136.84, 132.93, 129.91, 129.22, 128.72, 127.17, 126.91, 123.36, 123.36, 120.18, 119.03, 112.24, 55.21, 52.84, 20.96; LRMS (ESI) *m/z*: 411.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{23}N_2O_4S$: 411.1373; found: 411.1386.

_N_-(2-Ethylphenyl)-2-(_N_-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7d)

mp 152.2 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.27 (S, 1 H), 7.57 (d, *J* = 8.0 Hz, 2 H), 7.40

(d, *J* = 8.0 Hz, 2 H), 7.35-7.28 (m, 3 H), 7.22-7.13 (m, 3 H), 7.01 (d, *J* = 8.0 Hz, 1 H), 6.93 (t, *J* = 7.6 Hz, 1 H), 4.38 (s, 2 H), 3.32 (s, 3 H), 2.50-2.44 (m, 2 H), 2.41 (s, 3 H), 1.03 (t, *J* = 7.6 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.19, 157.62, 145.59, 139.98, 137.14, 135.61, 133.10, 131.66, 130.57, 130.02, 129.07, 128.89, 127.92, 127.43, 127.00, 121.99, 113.50, 55.97, 55.51, 25.30, 21.50, 15.00; LRMS (ESI) *m/z*: 439.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_4S$: 439.1686; found: 439.1695.

***N*-(2-Fluorophenyl)-2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7e)**

mp 141.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (S, 1 H), 7.90-7.86 (m, 1 H), 7.56 (d, *J* = 8.4 Hz, 2 H), 7.40 (d, *J* = 8.0 Hz, 2 H), 7.34-7.23 (m, 3 H), 7.17-7.14 (m, 2 H), 7.00 (d, *J* = 7.6 Hz, 1 H), 6.95 (t, *J* = 7.6 Hz, 1 H), 4.43 (s, 2 H), 3.43 (s, 3 H), 2.41 (s, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.98, 156.42, 153.49, 144.17, 135.03, 130.48, 130.27, 129.46, 128.23, 126.05, 124.77, 124.44, 122.11, 121.22, 115.02, 114.91, 112.36, 55.57, 55.55, 21.54; LRMS (ESI) *m/z*: 429.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{22}FN_2O_4S$: 429.1279; found: 429.1283.

***N*-(2-Bromophenyl)-2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7f)**

mp 186.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (S, 1 H), 7.75 (d, *J* = 8.0 Hz, 1 H), 7.68 (dd, *J* = 8.0, 1.2 Hz, 1 H), 7.56 (d, *J* = 8.4 Hz, 2 H), 7.40-7.31 (m, 5 H), 7.49-7.10 (m, 1 H), 7.01-6.93 (m, 2 H), 4.40 (s, 2 H), 3.43 (s, 3 H), 2.41 (s, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.85, 155.69, 143.41, 136.09, 135.54, 132.64, 132.40, 130.12, 129.32, 128.13, 127.38, 126.90, 126.67, 125.01, 120.31, 112.40, 55.20, 53.33, 20.98; LRMS (ESI) *m/z*: 489.0, 491.0 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{22}BrN_2O_4S$: 489.0478; found: 489.0480.

Methyl 2-(2-(N-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamido)benzoate (7g)

mp 156.7 °C, ^{1}H NMR (700 MHz, $CDCl_3$) δ 11.88 (s, 1H), 8.73 (d, *J* = 8.3 Hz, 1H), 8.08 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.92 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.54 (m, 3H), 7.31 (td, *J* = 8.3, 1.5 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 7.3 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 4.42 (s, 2H), 4.08 (s, 3H), 3.27 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 168.27, 168.06, 155.42, 143.44, 140.52, 136.05, 134.22, 133.54, 130.92, 130.11, 129.06, 128.01, 127.32, 122.94, 120.95, 120.70, 116.29, 111.47, 55.14, 54.63, 52.61, 21.48; LRMS (ESI) *m/z*: 469.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{25}N_2O_6S$: 469.1428; found: 469.1437.

***N*-(2,4-Dimethoxyphenyl)-2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7h)**

mp 163.3 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.19 (s, 1 H), 8.15 (d, *J* = 8.8 Hz, 1 H), 7.59 (d, *J* = 10.4 Hz, 2 H), 7.38 (dd, *J* = 8.0, 1.2 Hz, 1 H), 7.35-7.27 (m, 3 H), 6.97 (t, *J* = 8.0 Hz, 1 H), 6.83 (dd, *J* = 8.4, 1.2 Hz, 1 H), 6.54 (d, *J* = 2.4 Hz, 1 H), 6.48 (dd, *J* = 8.8, 2.8 Hz, 1 H), 4.34 (s, 2 H), 3.99 (s, 3 H), 3.81 (s, 3 H), 3.43 (s, 3 H), 2.45 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.37, 156.77, 155.86, 150.06, 143.75, 135.66, 132.07, 130.36, 129.24, 128.01, 127.25, 121.07, 120.89, 120.82, 111.93, 103.81, 98.91, 56.05, 55.55, 54.96, 54.71, 21.55; LRMS (ESI) *m/z*: 471.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_6S$: 471.1585; found: 471.1590.

***N*-(2-Ethoxybenzyl)-2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7i)**

mp 143.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (t, *J* = 4.0 Hz, 1 H), 7.54 (d, *J* = 8.4 Hz, 2 H), 7.38-7.27 (m, 4 H), 7.20 (t, *J* = 6.4 Hz, 1 H), 6.98-6.88 (m, 4 H), 6.82 (t, *J* = 7.2 Hz, 1

H), 4.23-4.20 (m, 4 H), 4.03-3.98 (m, 2 H), 3.36 (s, 3 H), 2.40 (s, 3 H), 1.31 (t, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.07, 157.03, 156.29, 143.91, 135.29, 135.29, 130.44, 130.23, 130.14, 128.75, 128.23, 127.99, 125.94, 121.30, 120.96, 120.33, 112.10, 111.07, 63.58, 54.97, 54.69, 54.46, 39.32, 31.59, 21.56, 14.13; LRMS (ESI) *m/z*: 469.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{25}H_{29}N_2O_5S$: 469.1792; found: 469.1796.

_N_-(1H-Imidazol-2-yl)-2-(_N_-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7j)

mp 215.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (br, 1 H), 11.14 (br, 1 H), 7.54 (d, J = 8.4 Hz, 2 H), 7.39-7.30 (m, 4 H), 6.99-6.91 (m, 2 H), 6.72 (s, 2 H), 4.40 (s, 2 H), 3.43 (s, 3 H), 2.41 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.71, 143.11, 136.82, 132.86, 129.93, 129.24, 127.13, 126.99, 120.09, 112.22, 55.21, 52.14, 20.96; LRMS (ESI) *m/z*: 401.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{19}H_{21}N_4O_4S$: 401.1278; found: 401.1292.

2-(_N_-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-_N_-(thiazol-2-yl)acetamide (7k)

mp 187.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1 H), 7.54 (d, J = 8.0 Hz, 2 H), 7.47 (d, J = 7.6 Hz, 1 H), 7.39 (d, J = 8.0 Hz, 3H), 7.32 (t, J = 8.8 Hz, 1 H), 7.23 (d, J = 7.6 Hz, 1 H), 6.99-6.93 (m, 2 H), 4.49 (s, 2 H), 3.40 (s, 3 H), 2.41 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.95, 155.64, 143.20, 137.62, 136.63, 132.91, 129.99, 129.24, 127.13, 126.95, 120.19, 113.61, 112.22, 55.18, 52.14, 20.96; LRMS (ESI) *m/z*: 418.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{19}H_{20}N_3O_4S_2$: 418.0890; found: 418.0892.

2-(_N_-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-_N_-(quinolin-3-yl)acetamide (7l)

mp 177.0 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 10.35 (s, 1H), 9.05 (s, 1H), 8.64 (s, 1H), 7.95 (d, J = 8.4 Hz, 1 H), 7.86 (d, J = 8.4 Hz, 1 H), 7.70 (t, J = 7.7 Hz, 1 H), 7.66 (d, J = 7.7 Hz, 2

H), 7.54 (t, *J* = 7.0 Hz, 1 H), 7.34 (t, *J* = 7.0 Hz, 1 H), 7.32 (d, *J* = 7.7 Hz, 2 H), 6.97-6.93 (m, 2 H), 4.40 (s, 2H), 3.81 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 167.07, 156.34, 145.04, 144.32, 143.60, 134.34, 130.95, 130.51, 129.50, 129.03, 128.80, 128.38, 128.14, 127.93, 127.61, 127.05, 123.60, 121.63, 113.05, 56.49, 55.58, 21.68; LRMS (ESI) *m/z*: 462.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{25}H_{24}N_3O_4S$: 462.1482; found: 462.1491.

2-(*N*-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-*N*-(quinolin-5-yl)acetamide (7m)

mp 195.0 ^{o}C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.92 (s, 1H), 9.00 (s, 1H), 8.63 (d, *J* = 8.4 Hz, 1 H), 8.11 (d, *J* = 8.4 Hz, 1 H), 7.96 (d, *J* = 7.7 Hz, 1 H), 7.66 (t, *J* = 8.4 Hz, 1 H), 7.68 (d, *J* = 7.7 Hz, 2 H), 7.60-7.58 (m, 1 H), 7.41-7.37 (m, 3 H), 7.00-6.98 (m, 3 H), 4.35 (br, 2H), 3.64 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 167.27, 156.25, 150.28, 148.44, 144.27, 134.35, 132.03, 130.47, 130.10, 129.51, 129.41, 129.00, 128.48, 127.93, 127.18, 122.72, 121.38, 121.16, 121.04, 112.59, 55.88, 55.74, 21.68; LRMS (ESI) *m/z*: 462.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{25}H_{24}N_3O_4S$: 462.1482; found: 462.1493.

2-(*N*-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-*N*-(quinolin-6-yl)acetamide (7n)

mp 177.0 ^{o}C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.86 (s, 1H), 8.80 (s, 1H), 8.12 (d, *J* = 8.4 Hz, 1 H), 7.85 (d, *J* = 8.4 Hz, 1 H), 7.68 (t, *J* = 7.0 Hz, 1 H), 7.66 (d, *J* = 8.4 Hz, 2 H), 7.58 (t, *J* = 7.0 Hz, 1 H), 7.39 (t, *J* = 7.0 Hz, 1 H), 7.38 (d, *J* = 7.7 Hz, 2 H), 7.07 (d, *J* = 8.4 Hz, 1 H), 6.98 (t, *J* = 7.7 Hz, 1 H), 6.91 (d, *J* = 7.7 Hz, 1 H), 4.34 (br, 2H), 3.97 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 167.25, 156.35, 150.48, 148.94, 144.47, 134.35, 131.03, 130.47, 130.10, 129.51, 129.41, 129.00, 128.48, 127.93, 127.18, 122.72, 121.38, 121.16, 121.04, 112.59, 55.88, 55.74, 21.78; LRMS (ESI) *m/z*: 462.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{25}H_{24}N_3O_4S$: 462.1482; found: 462.1495.

2-(*N*-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-*N*-(3-methoxypyridin-2-yl)acetamide (7o)

mp 123.0 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.12 (d, *J* = 4.2 Hz, 1 H), 7.60 (d, *J* = 7.7 Hz, 2 H), 7.33-7.24 (m, 5H), 7.09 (dd, *J* = 8.4, 4.9 Hz, 1 H), 6.95 (t, *J* = 7.7 Hz, 1 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 4.44 (s, 2H), 4.04 (s, 3H), 3.47 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 155.50, 143.62, 141.27, 139.30, 131.57, 130.19, 129.03, 127.74, 126.97, 120.66, 119.68, 117.31, 111.77, 55.95, 55.09, 54.95, 21.60; LRMS (ESI) *m/z*: 442.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{24}N_3O_5S$: 442.1431; found: 442.1429.

2-(*N*-(2-Methoxyphenyl)-4-methylphenylsulfonamido)-*N*-(2-methoxypyridin-3-yl)acetamide (7q)

mp 122.0 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.54 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.90 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.38 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 2H), 6.98 (t, *J* = 7.3 Hz, 1H), 6.89 (dd, *J* = 7.7, 5.0 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 4.35 (s, 2H), 4.15 (s, 3H), 3.46 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 167.54, 155.86, 153.53, 143.93, 140.65, 135.50, 131.86, 130.48, 129.28, 128.05, 127.30, 126.88, 122.37, 121.01, 117.12, 112.08, 55.06, 54.82, 53.93, 21.52; LRMS (ESI) *m/z*: 442.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{25}N_3O_5S$: 442.1431; found: 442.1434.

***N*-(2-Ethoxypyridin-3-yl)-2-(*N*-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7r)**

mp 133.0 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.57 (d, *J* = 7.7 Hz, 1 H), 7.89 (d, *J* = 4.9 Hz, 1 H), 7.57 (d, *J* = 7.7 Hz, 2 H), 7.51 (d, *J* = 7.7 Hz, 1 H), 7.34 (t, *J* = 7.7 Hz, 1 H), 6.99 (t, *J* = 7.7 Hz, 1 H), 6.89 (t, *J* = 7.7 Hz, 1 H), 6.82 (d, *J* = 8.4 Hz, 1 H), 4.59 (q, *J* = 7.0

Hz, 2 H), 4.36 (s, 2H), 3.39 (s, 3H), 2.45 (s, 1H), 1.64 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.233, 161.24, 147.66, 143.81, 143.72, 135.42, 135.20, 129.28, 128.16, 127.69, 127.26, 124.01, 120.81, 119.73, 111.37, 106.24, 64.73, 52.89, 21.56, 15.07; LRMS (ESI) *m/z*: 456.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{26}N_3O_5S$: 456.1588; found: 456.1591.

_N_-(2-Isopropoxypyridin-3-yl)-2-(_N_-(2-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7s)

mp 124.0 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.38 (s, 1H), 8.58 (d, *J* = 7.0 Hz, 1 H), 7.89 (d, *J* = 9.1 Hz, 1 H), 7.57-7.55 (m, 1H), 7.34 (t, *J* = 7.0 Hz, 1 H), 7.29-7.28 (m, 2 H), 6.99 (t, *J* = 7.7 Hz, 1 H), 6.87 (dd, *J* = 7.7, 4.9 Hz, 1 H), 6.80 (d, *J* = 8.4 Hz, 1 H), 7.39 (t, *J* = 7.0 Hz, 1 H), 7.38 (d, *J* = 7.7 Hz, 2 H), 7.07 (d, *J* = 8.4 Hz, 1 H), 6.98 (t, *J* = 7.7 Hz, 1 H), 6.91 (d, *J* = 7.7 Hz, 1 H), 4.34 (br, 2H), 3.97 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.97, 155.25, 152.16, 143.32, 140.30, 135.57, 132.03, 130.12, 129.11, 127.16, 126.48, 126.37, 121.90, 120.06, 116.44, 112.37, 68.61, 55.08, 53.92, 21.90, 21.00; LRMS (ESI) *m/z*: 470.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{28}N_3O_5S$: 470.1744; found:470.1753.

_N_-(2-Ethoxyphenyl)-2-(_N_-(3-methoxyphenyl)-4-methylphenylsulfonamido)acetamide (7t)

mp 175.7 °C; ^{1}H NMR (700 MHz, CD_3OD) δ 8.17 (d, *J* = 8.4 Hz, 1 H), 7.55 (d, *J* = 7.7 Hz, 2 H), 7.40 (d, *J* = 7.7 Hz, 2 H), 7.13-7.09 (m, 3 H), 7.06 (d, *J* = 8.4 Hz, 1 H), 6.92 (t, *J* = 7.0 Hz, 1 H), 6.89 (d, *J* = 9.1 Hz, 2 H), 4.38 (s, 2 H), 4.24 (q, *J* = 7.0 Hz, 2 H), 3.79 (s, 3 H), 2.47 (s, 3 H), 1.61 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (175 MHz, CD_3OD) δ 166.84, 159.78, 148.14, 144.58, 134.25, 132.13, 129.45, 129.40, 127.74, 126.73, 124.44, 120.28, 119.47, 114.09, 111.33, 64.40, 55.41, 54.57, 20.10, 13.99; LRMS (ESI) *m/z*: 455.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{28}N_3O_5S$: 455.1635; found: 455.1640.

_N_-(2-Ethoxyphenyl)-2-(4-methyl-_N_-phenylphenylsulfonamido)acetamide (7u) mp 159.4 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (S, 1 H), 8.33 (d, J = 7.6 Hz, 1 H), 7.51 (d, J = 8.0 Hz, 2 H), 7.36-7.29 (m, 5 H), 7.20-7.18 (m, 2 H), 7.06 (t, J = 9.2 Hz, 1 H), 6.94 (t, J = 9.2 Hz, 2 H), 4.34 (s, 2 H), 4.24 (q, J = 8.8, 6.8 Hz, 2 H), 2.47 (s, 3 H), 1.68 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.64, 147.68, 143.98, 139.49, 134.36, 129.75, 129.11, 128.00, 127.78, 127.49, 126.81, 124.30, 120.38, 119.70, 111.99, 64.12, 54.38, 21.01, 14.64; LRMS (ESI) *m/z*: 425.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{25}N_2O_4S$: 425.1530; found: 425.1544.

_N_-(2-Ethoxyphenyl)-2-(_N_-(2-fluorophenyl)-4-methylphenylsulfonamido)acetamide (7v) mp 162.3 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) d 9.40 (s, 1 H), 8.36 (dd, J = 8.8, 2.0 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.40-7.31 (m, 4 H), 7.16 (t, J = 8.4 Hz, 1 H), 7.10-7.05 (m, 2 H), 6.97-6.93 (m, 2 H), 4.31 (s, 2 H), 4.25 (q, J = 7.2 Hz, 2 H), 2.47 (s, 3 H), 1.66 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.71, 157.91, 147.76, 144.59, 134.46, 131.85, 130.81, 129.78, 127.87, 127.29, 124.80, 124.16, 120.79, 119.48, 117.13, 116.93, 111.04, 64.58, 55.28, 21.65, 14.93; LRMS (ESI) *m/z*: 443.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{24}FN_2O_4S$: 443.1436; found: 443.1444.

2-((_N_-(2-Chlorophenyl)-4-methylphenyl)sulfonamido)-_N_-(2-ethoxyphenyl)acetamide (7w) mp 163.8 ^{o}C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.33 (dd, J = 7.9, 1.3 Hz, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.44 (dd, J = 7.7, 1.7 Hz, 1H), 7.37 (dd, J = 7.8, 1.6 Hz, 1H), 7.34-7.17 (m, 4H), 7.05 (td, J = 7.9, 1.5 Hz, 1H), 6.98 - 6.86 (m, 2H), 4.58-4.25 (m, 2H), 4.20 (s, 2H), 2.44 (s, 3H), 1.62 (t, J = 7.0 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.93, 147.77, 144.52, 136.62, 135.06, 133.85, 132.71, 131.01, 130.19, 129.77, 128.08, 127.71, 127.31, 124.15, 120.79, 119.67, 111.03, 64.54, 55.39, 21.65,

14.99; LRMS (ESI) *m/z*: 460.1 $[M+H]^+$; HRMS (ESI) *m/z*: calcd. for $[M+H]^+$ $C_{23}H_{24}ClN_2O_4S$: 459.1140; found: 459.1144.

***N*-(2-Ethoxyphenyl)-2-((4-methyl-*N*-(o-tolyl)phenyl)sulfonamido)acetamide (7x)**

Sticky oil; ^1H NMR (700 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.35 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.21 (dd, *J* = 3.6, 2.2 Hz, 2H), 7.10-7.08 (m, 1H), 7.05 (td, *J* = 7.9, 1.6 Hz, 1H), 6.95-6.90 (m, 2H), 6.82 (d, *J* = 7.9 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.44 (s, 3H), 2.30 (s, 3H), 1.60 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.11, 147.64, 144.44, 139.16, 138.44, 134.29, 131.80, 129.72, 128.84, 128.78, 128.27, 127.36, 126.75, 124.06, 120.77, 119.50, 110.97, 64.49, 56.94, 21.62, 18.67, 14.90; LRMS (ESI) *m/z*: 440.1 $[M+H]^+$; HRMS (ESI) *m/z*: calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_4S$: 439.1686; found: 439.1682.

2-(4-Chloro-*N*-(2-methoxyphenyl)phenylsulfonamido)-*N*-(2,4-dimethoxyphenyl)acetamide (12b)

mp 168.4 °C; ^1H NMR (700 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.44 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.34 (t, *J* = 7.1 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 6.48 (dd, *J* = 8.8, 2.5 Hz, 1H), 4.36 (s, 2H), 3.98 (s, 3H), 3.81 (s, 3H), 3.43 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.97, 156.85, 155.57, 149.95, 139.49, 137.21, 132.33, 130.66, 129.32, 128.88, 126.72, 121.05, 120.96, 120.76, 112.00, 103.97, 98.92, 56.05, 55.55, 54.96, 54.69; LRMS (ESI) *m/z*: 491.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{24}ClN_2O_6S$: 491.1038; found: 491.1047.

2-(4-Bromo-*N*-(2-methoxyphenyl)phenylsulfonamido)-*N*-(2,5-dimethoxyphenyl)acetamide (12c)

mp 166.9 °C; ^1H NMR (700 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.05 (d, *J* = 2.9 Hz, 1H), 7.62 (d, *J*

= 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.88 (t, *J* = 9.5 Hz, 1H), 6.80 (t, *J* = 9.7 Hz, 1H), 6.62 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.36 (s, 2H), 3.98 (s, 3H), 3.78 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.40, 155.48, 153.98, 142.77, 137.67, 132.45, 131.88, 131.60, 131.52, 131.00, 130.71, 129.38, 129.11, 128.03, 128.00, 126.59, 121.08, 111.98, 111.52, 109.04, 106.20, 56.85, 55.82, 54.94, 54.82; LRMS (ESI) *m/z*: 535.1, 537.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{23}H_{24}BrN_2O_6S$: 535.0533; found: 535.0543.

_N_-(2,5-Dimethoxyphenyl)-2-(4-methoxy-_N_-(2-methoxyphenyl)phenylsulfonamido)acetamide (12d)

mp 176.5 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.05 (d, *J* = 2.9 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.61 (dd, *J* = 8.9, 3.0 Hz, 1H), 4.34 (s, 2H), 3.98 (s, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 3.46 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.91, 163.25, 155.82, 153.97, 142.90, 132.20, 130.37, 130.33, 130.09, 128.20, 127.33, 120.91, 113.80, 111.96, 111.64, 108.99, 106.27, 56.91, 55.81, 55.65, 55.06, 54.87; LRMS (ESI) *m/z*: 487.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_7S$: 487.1534; found: 487.1536.

_N_-(2,5-Dimethoxyphenyl)-2-(_N_-(2-methoxyphenyl)-4-(methylthio)phenylsulfonamido)acetamide (12e)

mp 188.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1 H), 7.76 (s, 1 H), 7.56 (d, *J* = 8.0 Hz, 2 H), 7.43 (d, *J* = 8.8 Hz, 2 H), 7.37-7.33 (m, 2 H), 7.01-6.98 (m, 3 H), 6.64 (dd, *J* = 9.2, 3.2 Hz, 1 H), 4.38 (s, 2 H), 3.84 (s, 3 H), 3.68 (s, 3 H), 3.41 (s, 3 H), 2.55 (s, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.71, 155.73,154.00, 146.12, 142.86, 134.40, 132.28, 130.46, 128.21,

127.10, 124.93, 120.97, 111.95, 111.63, 109.06, 106.22, 56.91, 55.82, 54.99, 54.88, 14.84, 14.08; LRMS (ESI) *m/z*: 503.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{24}H_{27}N_2O_6S_2$: 503.1305; found: 503.1310.

2-(*N*-(2-Methoxyphenyl)-4-(methylthio)phenylsulfonamido)-*N*-(4-methoxypyridin-3-yl)acetamide (12f)

mp 166.0 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1 H), 8.92 (s, 1 H), 8.25 (d, J = 5.2 Hz, 1 H), 7.57 (d, J = 8.4 Hz, 2 H), 7.43 (d, J = 8.4 Hz, 2 H), 7.36 (t, J = 7.6 Hz, 2 H), 7.14 (d, J = 5.6 Hz, 1 H), 7.02-6.94 (m, 2 H), 4.43 (s, 2 H), 3.92 (s, 3 H), 3.44 (s, 3 H), 2.55 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.84, 155.67, 146.65, 145.30, 142.45, 134.87, 132.38, 130.11, 127.68, 126.92, 124.95, 123.85, 120.32, 120.32, 112.37, 106.92, 55.93, 55.23, 13.98; LRMS (ESI) *m/z*: 474.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{22}H_{24}N_3O_5S_2$: 474.1152; found: 474.1157.

2-(4-Bromo-*N*-(2-methoxyphenyl)phenylsulfonamido)-*N*-(4-methoxypyridin-3-yl)acetamide (12g)

mp 123.0 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1 H), 8.89 (s, 1 H), 8.24 (d, J = 5.6 Hz, 1 H), 7.82 (d, J = 6.8 Hz, 2 H), 7.60 (d, J = 6.4 Hz, 2 H), 7.38-7.32 (m, 2 H), 7.12 (d, J = 5.6 Hz, 1 H), 7.01-6.94 (m, 2 H), 4.46 (s, 2 H), 3.90 (s, 3 H), 2.69 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.73, 155.55, 155.40, 146.70, 142.64, 138.62, 132.61, 131.93, 130.27, 129.22, 126.83, 126.56, 123.82, 120.36, 112.35, 106.91, 55.90, 55.14, 53.08; LRMS (ESI) *m/z*: 506.0, 508.0 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{21}H_{21}BrN_3O_5S$: 506.0380; found: 506.0386.

***N*-(2,5-Dimethoxyphenyl)-2-((2-methoxyphenyl)(4-methylbenzyl)amino)acetamide (18)**

mp 142.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1 H), 8.06 (s, 1 H), 7.27 (d, *J* = 8.0 Hz, 2 H), 7.06 (d, *J* = 8.4 Hz, 4 H), 6.93 (d, *J* = 7.2 Hz, 1 H), 6.85-6.81 (m, 2 H), 6.59 (dd, *J* = 8.8, 2.8 Hz, 1 H), 4.34 (s, 2 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.78 (s, 3 H), 2.28 (s, 3 H); ^{13}C NMR (175 MHz, CD_3OD) δ 170.71, 153.88, 153.75, 142.90, 138.96, 136.67, 134.61, 128.56, 128.44, 127.64, 124.41, 121.98, 120.22, 111.36, 110.88, 107.94, 105.99, 59.05, 57.53, 55.48, 54.68, 54.54, 19.66; LRMS (ESI) *m/z*: 429.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd. for $[M+H]^+$ $C_{25}H_{29}N_2O_4$: 429.2127; found: 429.2130.

Supporting Information available:

^{1}H and ^{13}C NMR Spectra of compounds **1**, **7b-x**, **12a-g**, **17**, **18**.

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Acknowledgment

This work was supported by a grant of the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C0003). We also acknowledge the generous financial support from Gyeonggi provincial government. We thank Peter G. Schultz (The Scripps Research Institute) for providing NeuroD2 cDNA.

Abbreviations used:

CNS, central nervous system; RGC, retina ganglion cell; GAP43, growth associated protein 43; SD, Sprague-Dawley; HBSS, Hank's buffered salt solution.

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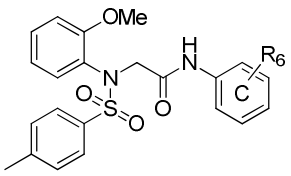
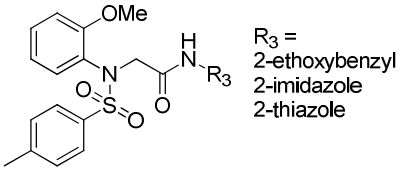
Tables

Table 1. Neurite outgrowth activity of R_3 and R_6 modified compounds.



1, 7b-h



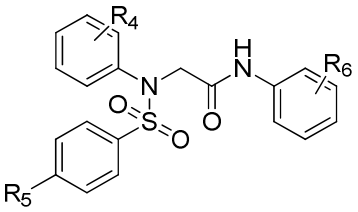
7i-k

Entry	Compound	R_6	Fold increase at 10 μM, compared to DMSO	Synthetic route
1	**1**	2-ethoxy	3.2 ± 0.4[1]	B
2	**7b**	3-ethoxy	1.8 ± 0.3	-[2]
3	**7c**	H	2.0 ± 0.1	B
4	**7d**	2-ethyl	0.8 ± 0.1	B
5	**7e**	2-fluoro	2.1 ± 0.2	B
6	**7f**	2-bromo	2.1 ± 0.2	B
7	**7g**	2-methoxycarbonyl	3.0 ± 0.4	-[2]
8	**7h**	2,4-dimethoxy	2.7 ± 0.3	-[2]
9	**7i**	Depicted above	Cytotoxic	A
10	**7j**	Depicted above	Cytotoxic	A
11	**7k**	Depicted above	Cytotoxic	A

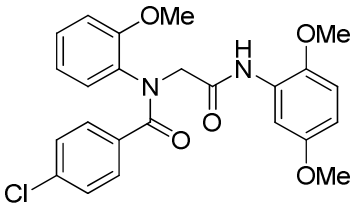
[1] The data are shown as mean ± standard deviation, n=3.

[2] Commercially available compound was used.

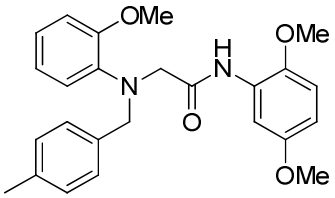
Table 2. Neurite outgrowth activities of R_4 and R_5 modified compounds



1, 7h, 7t-x, 12a-e



17

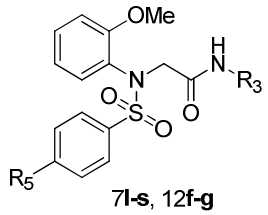


18

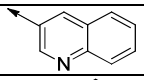
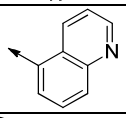
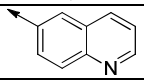
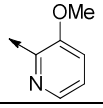
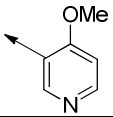
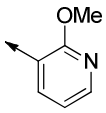
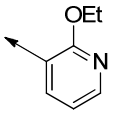
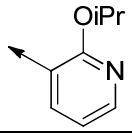
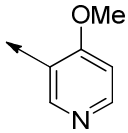
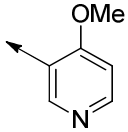
Entry	Compound	R_4	R_5	R_6	Fold increase at 10 μM, compared to DMSO	Synthetic route
1	**1**	2-methoxy	CH_3	2-ethoxy	3.2 ± 0.4[1]	B
2	**7t**	3-methoxy	CH_3	2-ethoxy	3.1 ± 0.3	B
3	**7u**	H	CH_3	2-ethoxy	2.5 ± 0.3	B
4	**7v**	2-fluoro	CH_3	2-ethoxy	2.9 ± 0.3	B
5	**7w**	2-chloro	CH_3	2-ethoxy	2.6 ± 0.2	B
6	**7x**	2-methyl	CH_3	2-ethoxy	2.6 ± 0.3	B
7	**7h**	2-methoxy	CH_3	2,4-dimethoxy	2.7 ± 0.4	C
8	**12a**	2-methoxy	F	2,4-dimethoxy	2.5 ± 0.2	C
9	**12b**	2-methoxy	Cl	2,4-dimethoxy	4.2 ± 0.5	C
10	**12c**	2-methoxy	Br	2,4-dimethoxy	5.0 ± 0.4	C
11	**12d**	2-methoxy	MeO	2,4-dimethoxy	2.3 ± 0.2	C
12	**12e**	2-methoxy	MeS	2,4-dimethoxy	3.2 ± 0.4	B
13	**17**	Depicted above			1.0 ± 0.1	B
14	**18**	Depicted above			Cytotoxic	B

[1] The data are shown as mean ± standard deviation, n=3.

Table 3. Structural modification of compound 1 and 12c for improving metabolic stability.



7l-s, 12**f-g**

Entry	Compound	R_3	R_5	Fold increase at 10 μM, compared to DMSO	Synthetic route
1	**7l**		CH_3	3.6 ± 0.3[1]	A
2	**7m**		CH_3	1.3 ± 0.1	A
3	**7n**		CH_3	1.0 ± 0.1	A
4	**7o**		CH_3	1.6 ± 0.1	A
5	**7p**		CH_3	4.2 ± 0.5	A
6	**7q**		CH_3	2.8 ± 0.3	B
7	**7r**		CH_3	2.1 ± 0.1	A
8	**7s**		CH_3	2.3 ± 0.2	A
9	**12f**		CH_3S	3.9 ± 0.3	A
10	**12g**		Br	3.7 ± 0.4	A

[1] The data are shown as mean ± standard deviation, n=3.

Table 4. Metabolic stability in mouse liver S9 fraction.

Entry	Compound	Compound remaining (%)	logP[c]
1	**1**	0.7	3.76
2	**7g**	0.3	3.37
3	**12b**	0.4	3.37
4	**12d**	0.4	2.69
5	**7p**	61.2	2.43
6	Acetaminophen[a]	97.7	-
7	Verapamil[b]	23.1	-

[a] Acetoaminophen, positive control, was not metabolized in Phase I.

[b] Verapamil, negative control, was metabolized in Phase I.

[c] logP was calculated by Chemdraw ver. 12.

Figures

Figure 1. (A) Chemical structure of compound **1**. (B) The neurite outgrowth activity of compound **1**. The primary neurons were stained with an anti-β-III tubulin antibody, and wide field fluorescence images from four fields in each well of 384-well plate were acquired. The neurite total length divided by the number of neuron is shown. Each column represents the mean ± SD, n=3.

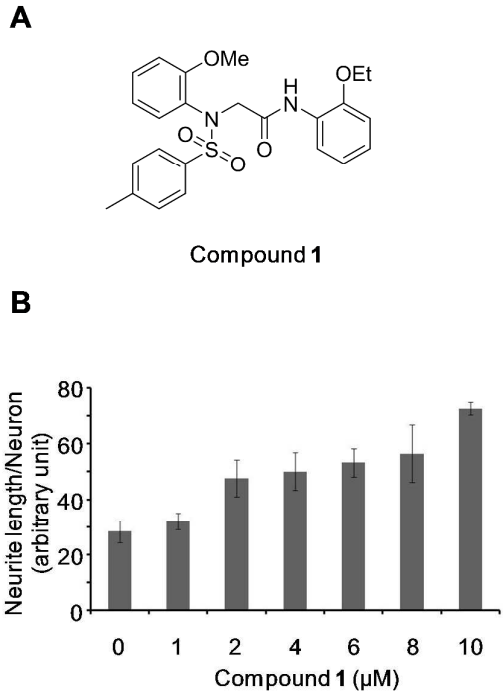


Figure 2. Strategy of structural modification of compound **1**.

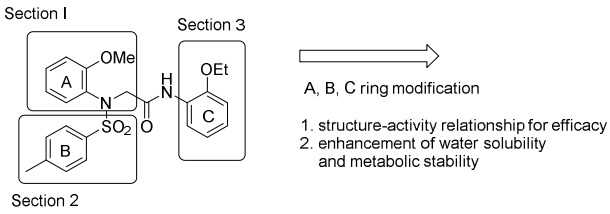


Figure 3. Synthetic routes for structural modifications of compound **1** and its derivatives.

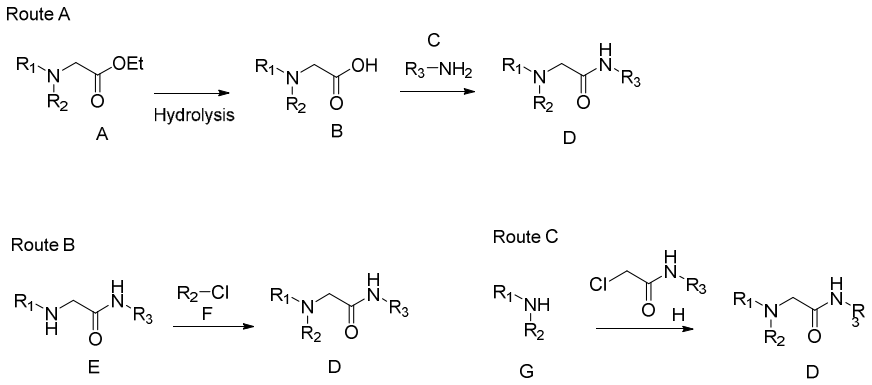


Figure 4. The effect of compound **7p** on the viability and axonal growth of cultured retina neuronal cells. The cells were treated with compound **7p** (2 ~ 20 μM) for 72 hours, and then

the viability and axonal growth were evaluated. (A) The total numbers of live cells in nine consecutive non-overlapping microscopic fields were counted. No significant difference was found (n=3). (B) The cells were immunostained with an anti-neurofilament antibody to detect axons, and representative images are shown. (C) Axonal growth was measured as described in Experimental Section after staining the culture with an anti-neurofilament antibody. Each column represents the mean ± SEM, n=9 (3 independent experiments).

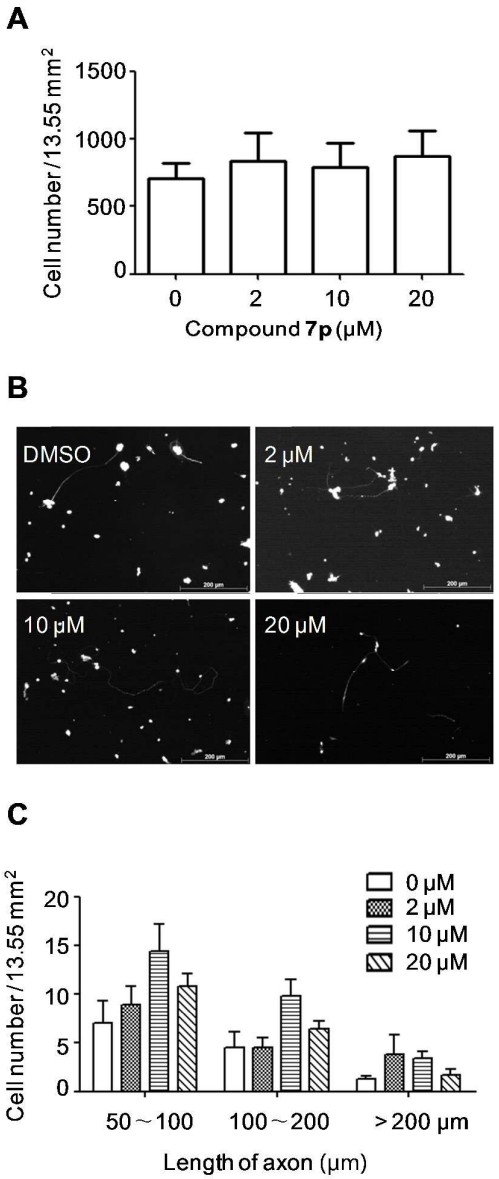
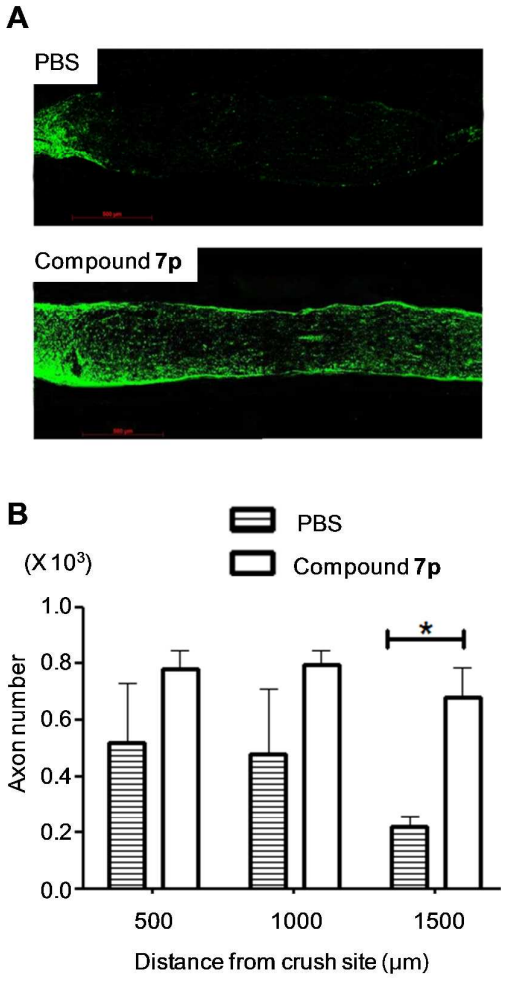


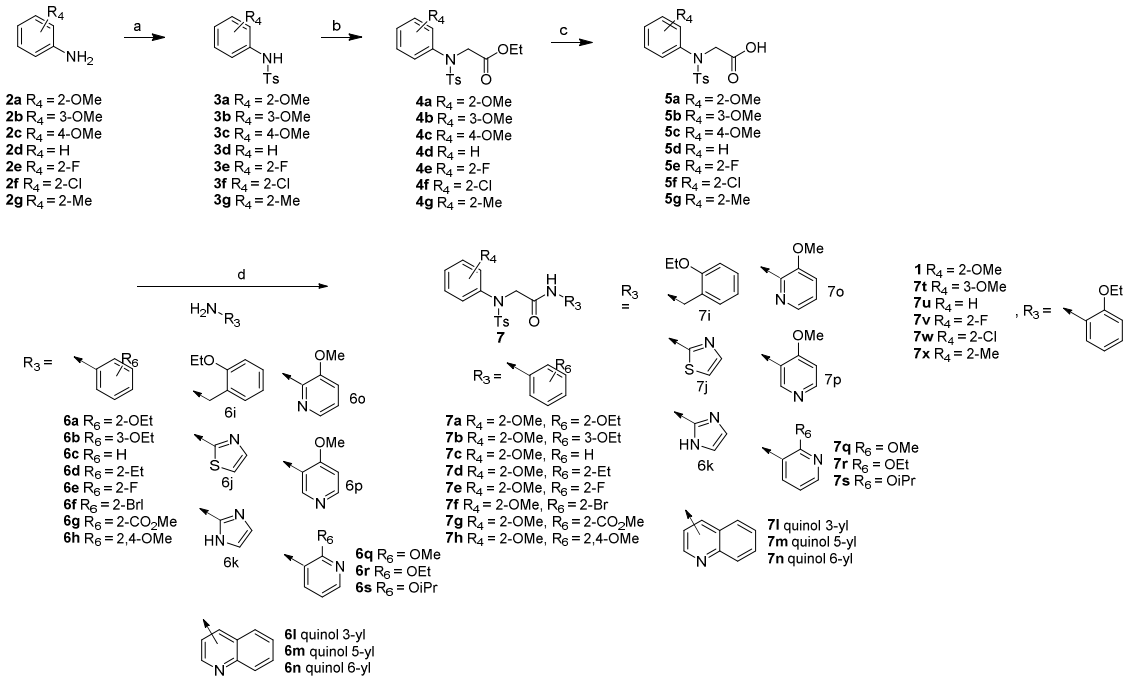
Figure 5. Compound **7p** promoted regeneration of the crushed optic nerves. (A) The rat optic nerves were crushed and treated with either PBS or Compound **7p,** and then longitudinal

sections of rat optic nerves were immunostained with an anti-GAP43 antibody. Fluorescence images show nerve fiber identified by GAP-43 immunoreactivity. (B) The numbers of axons at indicated sites were counted, and all values are shown as mean ± standard error of the mean, n=4 (11~15 sections from 4 rats, each), *p<0.05.

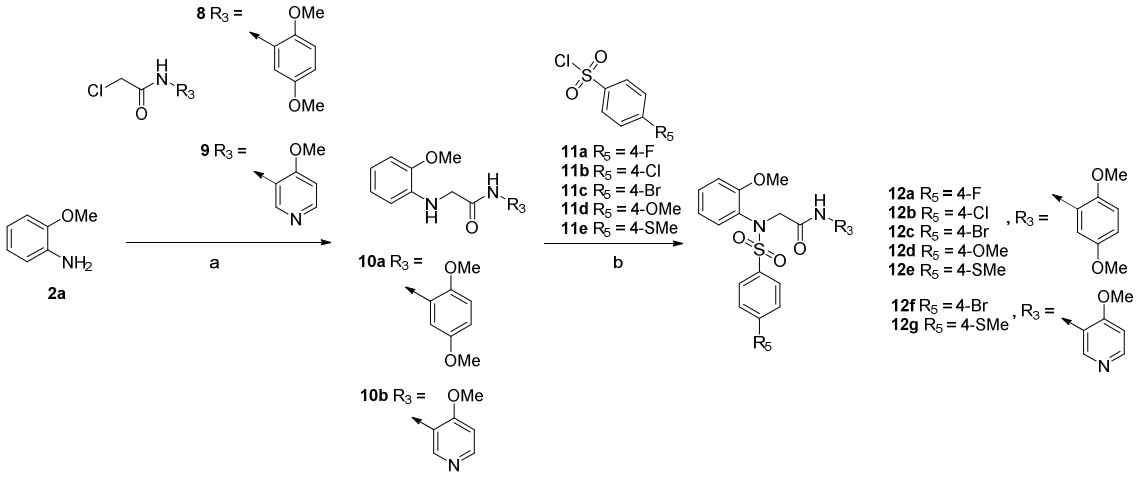


Schemes

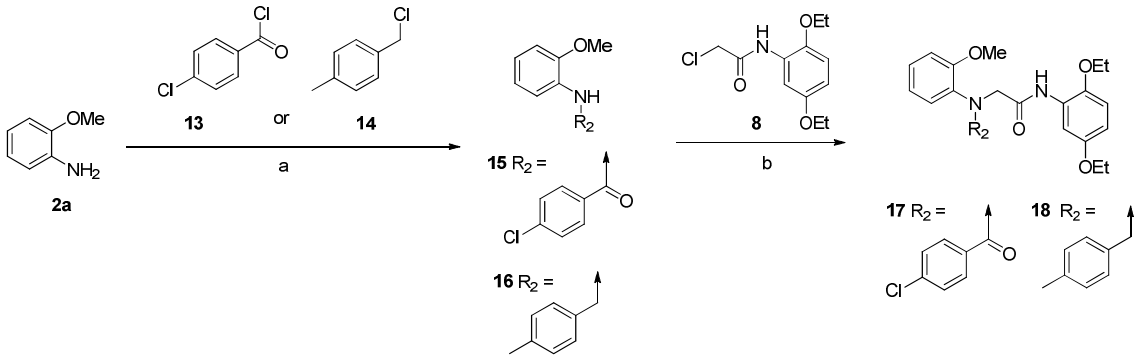
Scheme 1. (a) tosyl chloride, K_2CO_3, DMF, r.t., 2 h; (b) ethyl bromoacetate, K_2CO_3, DMF, r.t., 12 h ; (c) 2.5 M NaOH solution, THF, reflux, 6 h; (d) HATU, triethylamine, DMF, r.t., 2h.



Scheme 2. (a) **8** or **9**, K_2CO_3, DMF, 80 ^oC, 2 h; (b) pyridine, DCM, rt, 3 h


8 R3 =
9 R3 =
2a
a
10a R3 =
10b R3 =
11a R5 = 4-F
11b R5 = 4-Cl
11c R5 = 4-Br
11d R5 = 4-OMe
11e R5 = 4-SMe
b
12a R5 = 4-F
12b R5 = 4-Cl
12c R5 = 4-Br
12d R5 = 4-OMe
12e R5 = 4-SMe
, R3 =
12f R5 = 4-Br
12g R5 = 4-SMe
, R3 =

Scheme 3. (a) **13** or **14**, K_2CO_3, DMF, r.t., 2 h; (b) **8**, K_2CO_3, DMF, 80 ℃, 2 h.



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