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Discovery of Highly Potent and Selective Small Molecule ADAMTS-5 Inhibitors That Inhibit Human Cartilage Degradation via Encoded Library Technology (ELT)

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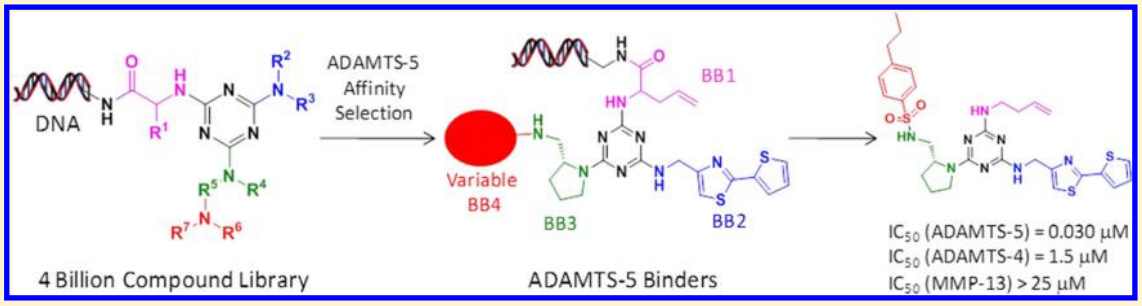
Supporting Information



ABSTRACT: The metalloprotease ADAMTS-5 is considered a potential target for the treatment of osteoarthritis. To identify selective inhibitors of ADAMTS-5, we employed encoded library technology (ELT), which enables affinity selection of small molecule binders from complex mixtures by DNA tagging. Selection of ADAMTS-5 against a four-billion member ELT library led to a novel inhibitor scaffold not containing a classical zinc-binding functionality. One exemplar, (*R*)-*N*-((1-(4-(but-3-en-1-ylamino)-6-(((2-(thiophen-2-yl)thiazol-4-yl)methyl)amino)-1,3,5-triazin-2-yl)pyrrolidin-2-yl)methyl)-4-propylbenzenesulfonamide (**8**), inhibited ADAMTS-5 with IC_{50} = 30 nM, showing >50-fold selectivity against ADAMTS-4 and >1000-fold selectivity against ADAMTS-1, ADAMTS-13, MMP-13, and TACE. Extensive SAR studies showed that potency and physicochemical properties of the scaffold could be further improved. Furthermore, in a human osteoarthritis cartilage explant study, compounds **8** and **15f** inhibited aggrecanase-mediated ^{374}ARGS neoepitope release from aggrecan and glycosaminoglycan in response to IL-1β/OSM stimulation. This study provides the first small molecule evidence for the critical role of ADAMTS-5 in human cartilage degradation.

INTRODUCTION

Human osteoarthritis (OA) is a degenerative joint disease affecting tens of millions of people worldwide. Current treatments are mostly confined to reducing inflammation and pain associated with the disease using steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) with few available agents that can modify the progression of the disease.[1] OA is characterized by the degradation of articular cartilage as a result of uncontrolled proteolytic destruction of extracellular matrix.[2]

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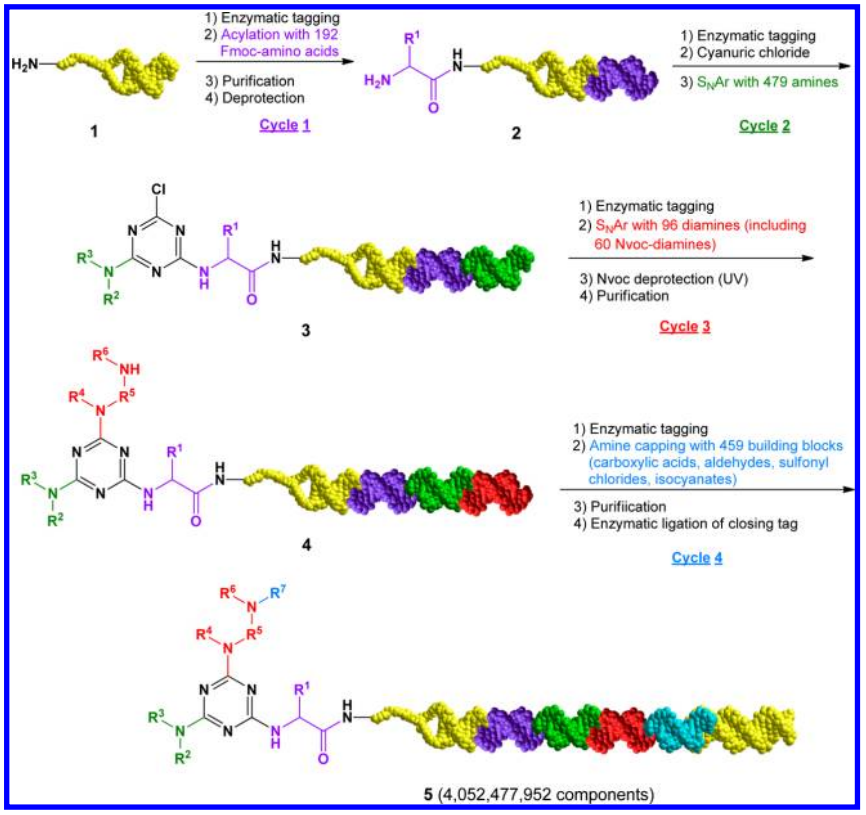
Scheme 1. Synthesis of the Four Cycle DNA Encoded Triazine Library



The major components of the cartilage extracellular matrix are type II collagen and aggrecan.[3] Aggrecan is a large multidomain proteoglycan that provides cartilage with elasticity and compressibility; thus, its loss from the matrix is considered a critical event in the early stages of cartilage destruction.[4] Aggrecanases including ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) are members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family of zinc metalloproteases that cleave the N-terminal interglobular domain of aggrecan at the Glu^{373}-Ala^{374} peptide bond.[3,5,6] Inhibition of aggrecanases reduces aggrecan degradation and leads to overall cartilage protection.[7,8] However, the identity of the aggrecanase isozyme playing the dominant role in cartilage destruction remains controversial. On the one hand, ADAMTS-5 knockout mice and ADAMTS-4/-5 double knockout mice, but not ADAMTS-4 knockout mice, were shown to be significantly protected from degradation,[9−11] suggesting that ADAMTS-5 is the crucial enzyme in aggrecanolysis. On the other hand, small interfering RNA (siRNA) specifically targeting either ADAMTS-4 or ADAMTS-5 attenuated the degradation of aggrecan in both cytokine-stimulated normal cartilage and unstimulated human OA cartilage, suggesting that both aggrecanases participate in cartilage degradation.[12] To further elucidate these enzymes' respective roles in cartilage degradation and validate them as targets for OA prevention and treatment, we launched a campaign to identify specific small molecule inhibitors for either ADAMTS-4 or ADAMTS-5 using our proprietary encoded library technology (ELT). Most of the known aggrecanase inhibitors contain known zinc binding groups (carboxylic acids, hydroxamic acids, tartrates, hydantoins, etc.) and possess limited selectivity over other zinc metalloproteases as well as poor physicochemical properties for systemic exposure.[1,13] In this paper we report the discovery of potent and selective novel ADAMTS-5 inhibitor scaffold lacking a classical zinc binding motif that inhibits human cartilage degradation in an ex vivo human OA cartilage explant experiment.

RESULTS AND DISCUSSION

ELT is a novel hit identification technology that has recently attracted significant attention from both academia and industry.[14−16] The technology encompasses four critical components: (1) the design and synthesis of DNA encoded small molecule libraries as combinatorial mixtures of 10^6−10^{10} members, (2) affinity selection of libraries against therapeutic targets, (3) hit structure deconvolution through DNA sequencing, and (4) off-DNA hit resynthesis and activity confirmation. The advantages of this approach include high diversity, minimal target protein consumption, and rapid execution; following library synthesis (on a scale amenable to several thousand target screens), the complete process of affinity selection, deconvolution, and hit confirmation may be accomplished in 6 weeks. Since its inception, ELT has identified tractable hit series for several dozen targets including both soluble and integral membrane proteins.[17−20] Application of ELT to ADAMTS-4 has been previously reported.[20,21] Here we describe the identification of ADAMTS-5 inhibitors from a four-billion-member DNA encoded triazine library.

The library scaffold for the present ADAMTS-5 work consists of a 1,3,5-triazine core elaborated with four synthetic cycles to create diversity. As we previously reported for two related libraries,[17] it was synthesized using the split-and-pool strategy, beginning from a short sequence of duplex DNA stabilized by a synthetic hairpin (the "headpiece"). The design of the headpiece allows buildup of a small molecule warhead

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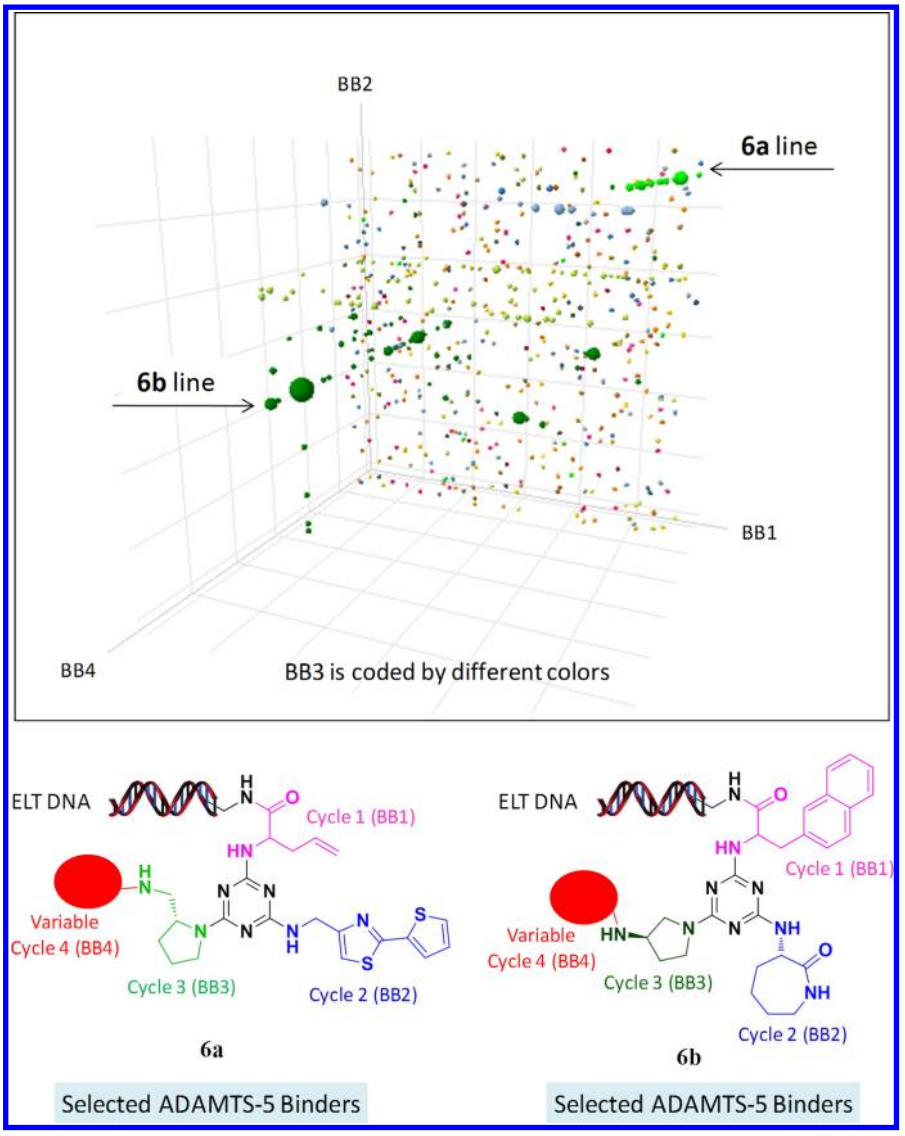

Figure 1. Spotfire cube data view of ADAMTS-5 selection features: BB1, cycle 1 building blocks; BB2, cycle 2 building blocks; BB4, cycle 4 building blocks; BB3, cycle 3 building blocks. The BB3 building blocks are represented by different colors.

starting from the free amino group, while DNA tags encoding the building blocks (BBs) are enzymatically ligated at the opposite end. In this instance, cycle 1 incorporated 192 Fmoc-amino acids via acylation onto the AOP-headpiece (DNA) starting material (**1**, Scheme 1; refer to Supporting Information Figure 2 for detailed AOP-headpiece structure). After Fmoc-deprotection (**2**), the triazine scaffold was installed by the addition of cyanuric chloride. In cycle 2, a set of 479 amine building blocks were incorporated (**3**), followed by a set of 96 diamines (including 60 *o*-nitroveratryloxycarbonyl (Nvoc) diamines) at cycle 3 (**4**). After removal of the photolabile Nvoc protecting group, the free amines were then further elaborated in cycle 4 by acylation with 173 carboxylic acids, reductive alkylation with 94 aldehydes, sulfonylation with 107 sulfonyl chlorides, and urealaytion with 85 isocyanates (**5**). The DNA sequence was then "closed" with a final tag encoding a library identifier, a priming region for PCR amplification, and a degenerate sequence to control for PCR amplification biases.

The affinity selections were performed on a chemically biotinylated human ADAMTS-5(262−624). The target protein construct (~10 μg total) was immobilized on Phynexus affinity columns derivatized with streptavidin. An aliquot (~5 nmol) of library was incubated on the tips for an hour, and the tips were then washed extensively to remove nonbinders. The bound molecules were eluted by heat denaturation of the protein at 80 °C. The eluant was then incubated with fresh immobilized protein to start the second round of affinity selection. Three rounds of selection were performed. In order to exclude molecules whose enrichment was due to binding to the resin matrix or streptavidin, a parallel selection performed in an identical fashion without the addition of the protein target was used as a no target control (NTC). In order to add priming sites for DNA sequencing and to ensure enough molecules for sequencing, the final eluant was amplified by PCR from 3×10^5 to 1×10^7 molecules. DNA sequencing was then performed using Roche/454 technology, and the sequences were translated to identify the structures of the putative ADAMTS-5 binders.

Further analysis of the affinity selection output could be conveniently performed in a Spotfire cube view. In Figure 1, warheads enriched at the level of two independent DNA sequences or greater are shown with the cube axes representing

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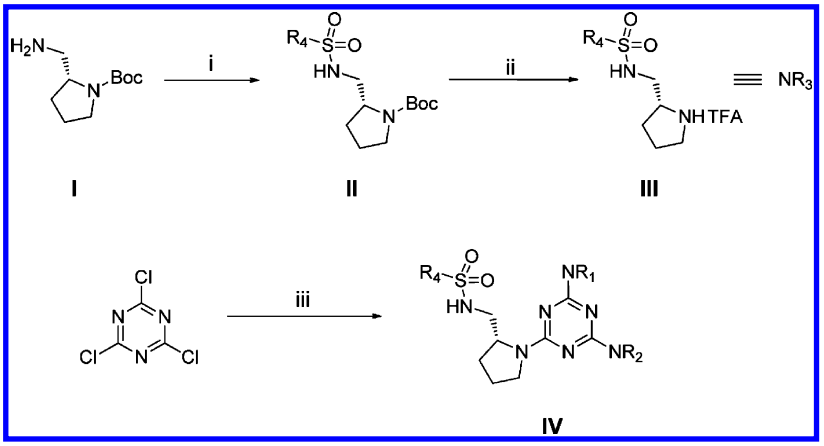
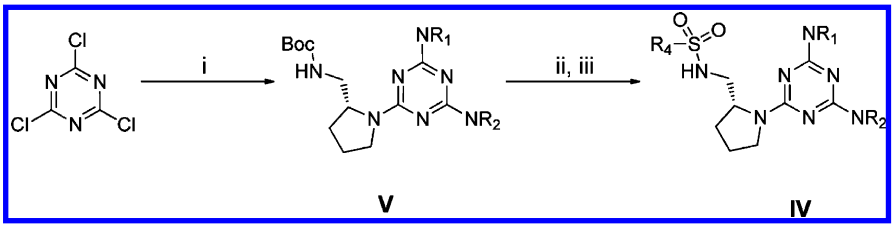
Scheme 2. Synthesis of ADAMTS-5 Off-DNA Hits[a]



[a]Reagents and conditions: (i) sulfonyl chlorides (R_4SO_2Cl), triethylamine, dichloromethane; (ii) 20% trifluoroacetic acid in dichloromethane; (iii) (a) cycle 2 amines (NR_2, 1.0 equiv), DIPEA (1.0 equiv), acetonitrile, 0 °C, 30 min; (b) cycle 3 amines (NR_3, 1.0 equiv), DIPEA (3.0 equiv), acetonitrile, room temperature, 1 h; (c) cycle 1 amines (NR_1, 3 equiv), DIPEA (3 equiv), acetonitrile, 80 °C, 3 h to overnight.

Scheme 3. Alternative Synthesis of ADAMTS-5 Off-DNA Hits[a]



[a]Reagents and conditions: (i) (a) cycle 2 amines (NR_2, 1.0 equiv), DIPEA (1.0 equiv), acetonitrile, 0 °C, 30 min; (b) (*R*)-*tert*-butyl (pyrrolidin-2-ylmethyl)carbamate (1.0 equiv), DIPEA (3.0 equiv), acetonitrile, room temperature, 1 h; (c) cycle 1 amines (NR_1, 3 equiv), DIPEA (3.0 equiv), acetonitrile, 80 °C, 3 h to overnight; (ii) 20% trifluoroacetic acid in dichloromethane; (iii) sulfonyl chlorides (R_4SO_2Cl), triethylamine, dichloromethane.

building blocks used in cycle 1, cycle 2, and cycle 4 chemistry, respectively, and with cycle 3 building blocks indicated by different colors. As discussed in the earlier report,[17] families of related warheads are of particular interest because they contain one or more building blocks in common and are easily recognized in these views as lines or planes. Here, two such features were evident: the bright green line corresponding to scaffold **6a** and the dark green line corresponding to scaffold **6b**. A detailed examination of the data set revealed that all of the putative binders on the **6a** line had the same BB1 (2-aminopent-4-enoic acid) and BB2 ([2-(thiophen-2-yl)thiazol-4-yl]methanamine) moieties. In addition, all of the binders on the **6a** line also shared a common BB at cycle 3, ((*R*)-pyrrolidin-2-ylmethanamine). These families varied in the nature of the BB4 building blocks. A closer check of these BB4s, however, found that all 23 groups selected at this site were sulfonyl chlorides, whereas sulfonyl chlorides comprised 107 of the 459 building blocks available. Therefore, the selection for this site was also specific. The dark green line (line **6b**) in this cube view was analyzed in the same fashion, showing that 2-amino-3-(naphthalen-2-yl)propanoic acid, (*S*)-3-aminoazepan-2-one, and (*R*)-pyrrolidin-3-amine were selected at cycles 1, 2, and 3, respectively.

The above analysis suggested that at least two series of compounds were selected from the library by ADAMTS-5 through affinity interaction. To confirm this analysis, we synthesized representative compounds from each of the two different series without the DNA attachment by standard medicinal chemistry methods. Compounds were prepared using two similar synthetic routes as outlined in Schemes 2 and 3. In Scheme 2, the N^1-Boc protected 2-(aminomethyl)pyrrolidine (**I**) was reacted with various sulfonyl chlorides followed by removal of the Boc group to give corresponding amines (**III**, NR_3). Three sequential amine replacements of cyanuric chloride under different conditions afforded final compounds **IV** in reasonable yields (60−80%). Alternatively, as in Scheme 3, the cyanuric chloride was sequentially reacted with three different amines under different conditions to give intermediate compounds **V**, which were then deprotected and reacted with various sulfonyl chlorides to yield final compounds **IV** in 25−65% overall yield.

Next, we tested these off-DNA compounds for their ability to inhibit ADAMTS-5 in a fluorescence resonance energy transfer (FRET) assay using human ADAMTS-5 protein and WAAG-3R substrate.[22] As shown in Figure 2, three compounds from the **6a** series including **7**, **8**, and **9**, although having different cycle 4 groups, all showed potent inhibitory activity against ADAMTS-5 with IC_{50} values of 50, 30, and 40 nM, respectively. Off-DNA compounds **10** and **11** from the **6b** series were inactive (both having IC_{50} > 10 uM), suggesting that these binders might bind to the protein at a site that does not affect the protein's enzymatic activity (since ELT selections are based on binding affinity between library compounds and a protein target without a functional readout, it is possible to find good binders with no functional activity). This result was very encouraging, especially considering that among more than four-

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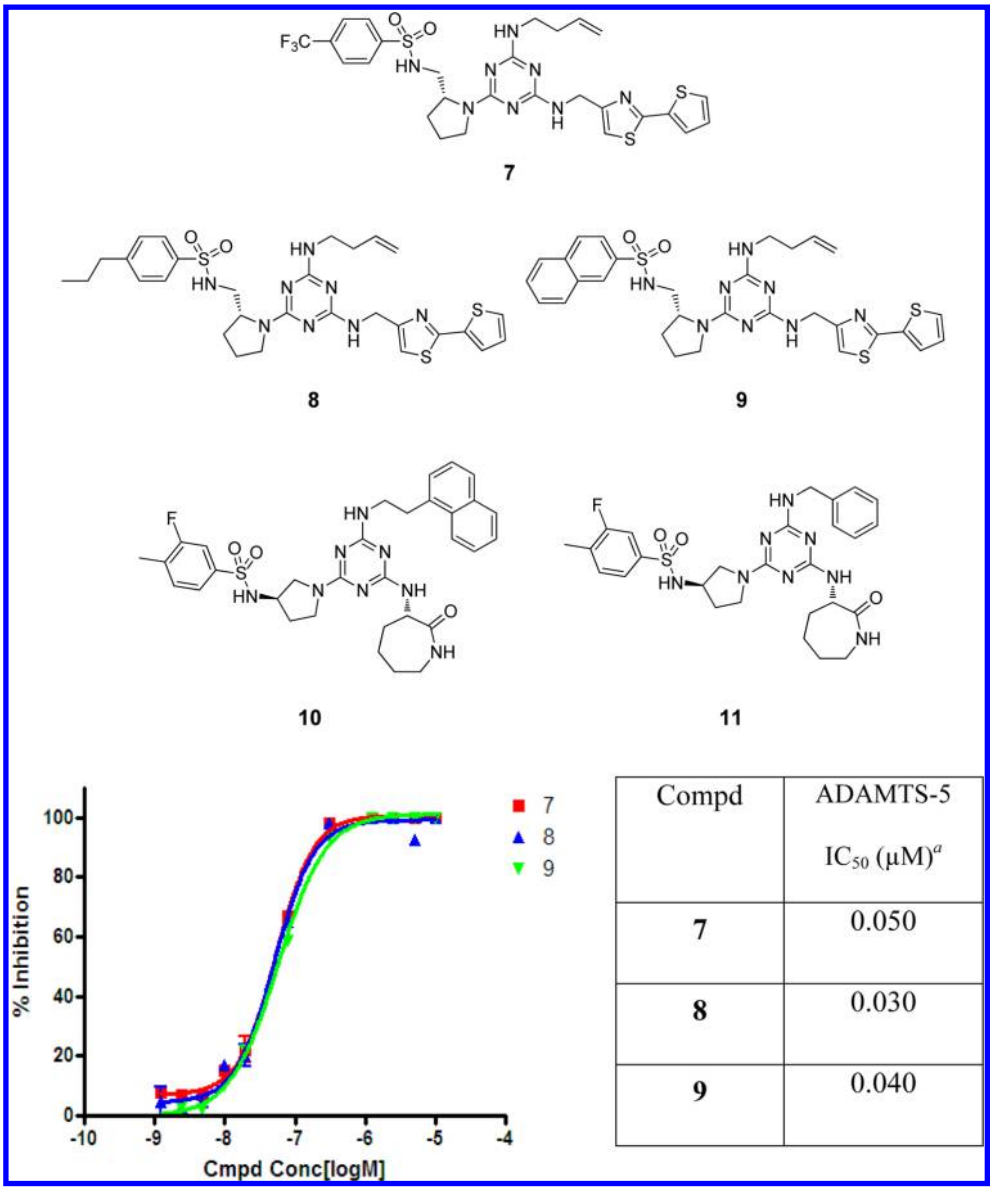

Compd	ADAMTS-5 IC_{50} (μM)[a]
7	0.050
8	0.030
9	0.040

Figure 2. Structures of initially synthesized off-DNA compounds and the corresponding activity against ADAMTS-5. Superscript "a" indiates that all IC_{50} values are the mean of at least three independent experiments. IC_{50} > 10 μM for compounds **10** and **11**.

billion diversified compounds in the library, only a small number of compounds were selected based on specific binding interactions with ADAMTS-5. In addition, this novel and potent ADAMTS-5 inhibitor scaffold contains no classical zinc binding groups typically seen in known metalloprotease inhibitors and is therefore an attractive starting point for achieving high specificity and druglike properties.[23,24]

The affinity selection data afford an initial insight into structure−activity relationships (SARs) for this novel ADAMTS-5 inhibitor scaffold. To extend our understanding beyond the building blocks contained within the library and also to deconvolute any biases that could be introduced by the DNA linkage of the warheads, we undertook further SAR studies beginning with each of the attached building blocks in turn. In the absence of X-ray cocrystal structures, this effort was carried out in traditional medicinal chemistry fashion.

The SAR around the triazine 2-position (BB1 site) was explored first. As can be seen from Table 1, the but-3-en-1-amine group in compound **7** was initially replaced by *n*-butylamine, 1-cyclopropylmethanamine, methylamine, hydroxyl, or hydrogen (R_1 in **12**) to map the scope of tolerance to structural changes at this site. From Table 1 we can see that replacing the 3-butene-1-amine group with *n*-butylamine (**12a**) or 1-cyclopropylmethanamine (**12b**) decreased the activity by 5- and 2-fold correspondingly. Truncation of this side chain to methylamine (**12c**), hydroxyl group (**12d**), and hydrogen (**12e**) also decreased the activity significantly, suggesting the necessity of a small alkyl or alkenyl amine substitution at this site. This initial observation was confirmed with additional SAR studies as shown in the second part of Table 1, using **13** as a generic structure where a *n*-propylamine (**13a**) or a cyclopropyl substitution (**13e**) retained the activity and an isobutylamine substitution (**13c**) gave similar activity as the 1-cyclopropylmethanamine substitution (**12b**). We note that at the triazine 4-position, the 2-thiophenythiazole group and the 2-phenylthiazole group gave equal potency as shown in Table 2, compounds **7** and **14a**. Further increase of the ring size to a cyclopentyl group (**13f**) decreased the activity by 30-fold, and

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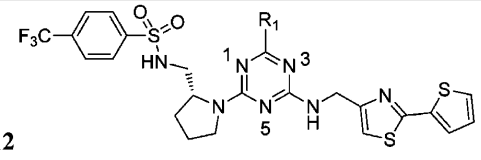
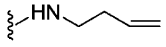
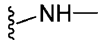
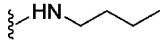
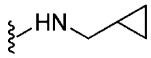
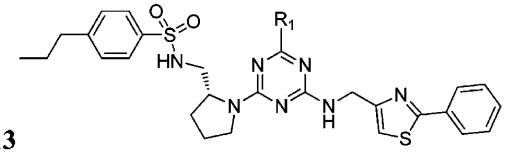
Table 1. SAR for Triazine 2-Position (BB1 Site)[a]

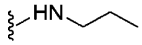
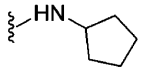
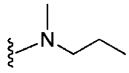
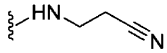
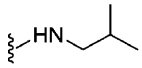
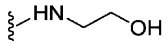
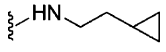
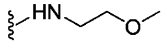
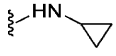
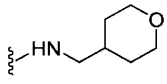


12

Compd	R_1	TS-5 IC_{50} (μM)	Compd	R_1	TS-5 IC_{50} (μM)
7		0.05	**12c**		0.39
12a		0.24	**12d**		5.30
12b		0.11	**12e**		3.50



13

Compd	R_1	TS-5 IC_{50} (μM)	Compd	R_1	TS-5 IC_{50} (μM)
13a		0.05	**13f**		1.60
13b		> 10	**13g**		0.09
13c		0.12	**13h**		0.26
13d		3.8	**13i**		0.21
13e		0.07	**13j**		2.14

[a]All IC_{50} values are the mean of at least three independent experiments.

changing the primary amine to a secondary amine (**13b**) completely abolished the activity. While certain small sized aminoalkyl or aminoalkenyl substitutions were preferred, an appropriate heteratom/hydrophilic group at the distal end of a substituent was acceptable. For example, the 3-aminopropanenitrile substitution (**13g**) at this site gave potency comparable to that of compound 7. But the hydroxyl (**13h**) and methoxy groups (**13i**) both decreased the potency by 5-fold, and the (tetrahydro-2*H*-pyran-4-yl)methyl substitution (**13j**) rendered the compound 40-fold less active.

We then explored the triazine 4-position (BB2) as shown in Table 2. As can be seen, the 2-thiophenylthiazolemethyl group can be replaced by the phenylthiazolemethyl group with good retention of activity (**14a**). However, further replacement of the thiazole ring with a second phenyl ring, such as appeared in 4- or 3-biphenylmethyl groups (**14b** and **14c**) rendered the compounds totally inactive. Removal of the distal thiophene ring decreased the activity (**14d**) about 8-fold. Replacement of the 2-methylthiazolemethyl with a simple benzyl group gave further decreased activity (**14e**). These observations led us to consider whether it was the thiazole ring at this position or rather the basic nitrogen in the thiazole ring that was crucial for the activity. This issue was further explored with additional single ring systems as shown in Table 2, using **15** as generic structure. Using compound **12a** as a reference, we found that simple thiophene (**15a**) and substituted thiophenes (**15b**, **15c**) were much less active (or inactive) than the original thiazole derivatives. On the other hand, the 2-(6-methyl)pyridyl group

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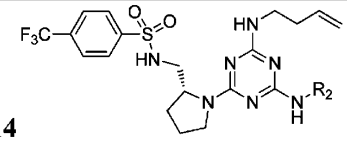
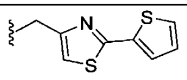
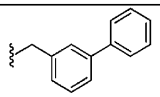
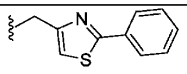
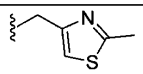
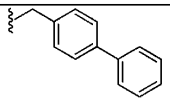
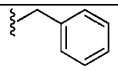
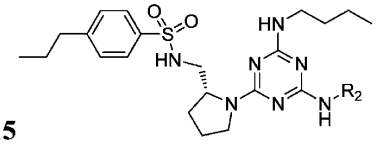
Table 2. SAR for Triazine 4-Position (BB2 Site)[a]

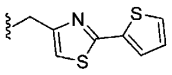
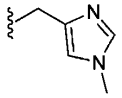
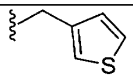
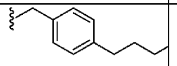
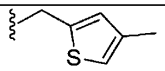
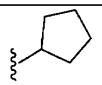
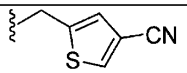
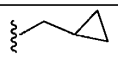
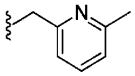
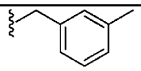


14

Compd	R_2	TS-5 IC_{50} (μM)	Compd	R_2	TS-5 IC_{50} (μM)
7		0.05	**14c**		> 10
14a		0.06	**14d**		0.43
14b		> 10	**14e**		0.87



15

Compd	R_2	TS-5 IC_{50} (μM)	Compd	R_2	TS-5 IC_{50} (μM)
12a		0.24	**15f**		0.03
15a		1.17	**15g**		> 10
15b		> 10	**15h**		> 10
15c		4.1	**15i**		6.5
15d		0.41	**15j**		5.8
15e		> 10			

[a]All IC_{50} values are the mean of at least three independent experiments.

(**15d**) gave potency comparable to that of the 2-thiophenylthiazole biaryl ring system at this position, which suggests that the nitrogen in the two ring system could be the key to the observed activity. This notion was further supported by the comparison between compounds **15d** and **15e**, where the two ring systems should have the same steric effect and the missing nitrogen in **15e** rendered the compound inactive. More importantly, the potency was significantly boosted (more than 8-fold) when the (1-methyl-1*H*-imidazole)-4-methyl group (**15f**), a small but nitrogen containing aryl ring, was adopted at this position. An additional benefit of this modification is that it significantly reduced the compound's molecular weight and cLogP (by ∼80 and 2.5, respectively). Being able to improve physicochemical properties while maintaining a compound's potency opened the window for productive lead optimization. The last three compounds in this table with an alkyl or cycloalkyl group (**15h**, **15i**, and **15j**) at the triazine 4-position proved to be much less active.

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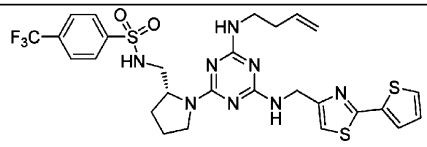
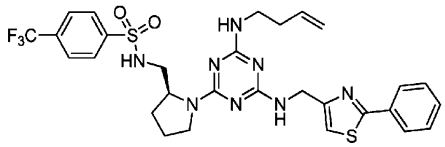
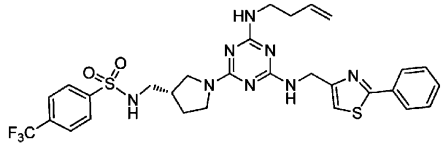
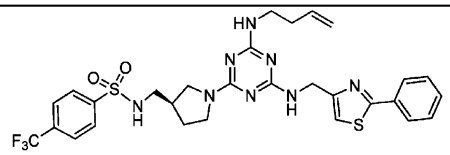
Table 3. SAR for Triazine 6-Position (BB3 Site)[a]

Compd	Structure	TS-5 IC_{50} (μM)
7		0.05
16		1.56
17		> 10
18		> 10

[a]All IC_{50} values are the mean of at least three independent experiments.

The structural exploration around triazine 6-position (BB3 site) was summarized in Table 3. At this position, we investigated the pyrrolidine ring for its substitution position and stereochemistry. As can be seen, switching the active configuration of the (*R*)-pyrrolidin-2-ylmethanamine group (**7**) to its enantiomer (*S*)-pyrrolidin-2-ylmethanamine group (**16**) caused a 30-fold drop in potency. Meanwhile, moving the substitution from the pyrrolidine 2-postion to 3-position (**17** and **18**) led to a complete loss of activity, suggesting that the (*R*)-pyrrolidin-2-ylmethanamine group at this position is highly specific.

Lastly, we explored SAR around the sulfonamide moiety. An examination of the initial three off-DNA compounds **7**, **8**, and **9** (Figure 2) suggests that this site tolerated some variations, with both *β*-naphthalenyl and 4-trifluoromethyl or 4-propyl substituted phenylsulfonamides giving almost equal potency. Because of the simplicity of the phenyl ring system, our SAR study focused on investigating different substitution effects on the phenyl ring as shown in Table 4. When a phenyl group was adopted, *p*-CF_3 and *p*-OCH_3 substitutions (**14a** and **19c**) gave 4-fold increase in potency compared to no substitution (**19d**). Both *m*- and *o*-CF_3 substitutions yielded inactive compounds (**19a**, **19b**). Further studies varied substituent sizes, electronic nature, and hydrogen-bonding capacities as represented by generic structure **20** in Table 4. As can be seen, chloro substitutions gave potency comparable to that of compound **12a**, with the para-position (**20a**) being slightly better than the meta-position (**20b**). A similar activity was seen for 3,4-dimethyl substitution (**20c**). A second phenyl group substitution at the para-position (**20d**) decreased the activity further, as did the fluoro (**20h**) and some hydrophilic substitutions including $MeSO_2$, CN, and OH (**20e**, **20f**, **20g**, **20i**, and **20j**). Overall, an appropriate lipophilic group at the phenyl para-position favored activity. When the phenyl ring was replaced by a cyclopropyl group (**19e**), the compound became inactive.

As demonstrated above, the scaffold has defined SAR and tolerates specific structural variations. We next profiled these compounds for ADAMTS-5 selectivity over other related zinc metalloproteinases as illustrated in Table 5. All of the tested compounds were more than 10-fold selective for ADAMTS-5 over ADAMTS-4 and more than 88-fold selective for ADAMTS-5 over other related enzymes including TACE, ADAMTS-1, and MMP-13. The selectivity demonstrated by these novel ADAMTS-5 inhibitors is very important, since, so far, few selective ADAMTS-5 small molecular inhibitors have been reported, especially relative to ADAMTS-4 because of the high sequence homology and conformational similarity of their active sites revealed by X-ray crystallography.[25] This, together with the unprecedented scaffold structure, renders the inhibitory mechanism of these molecules of considerable interest. The inhibitory potency of two exemplars, **8** and **14a** was essentially unaffected by added zinc over the range 1−100 μM (Supporting Information), in agreement with the lack of obvious zinc binding moiety. In addition, an enzyme kinetic study with compound **8** revealed that it inhibits ADAMTS-5 in a peptide substrate competitive manner (Supporting Information). Further discernment of the mechanism of action will be enabled by crystallographic and biophysical approaches. Meanwhile, the identification of these potent and selective ADAMTS-5 inhibitors in a very short period of time

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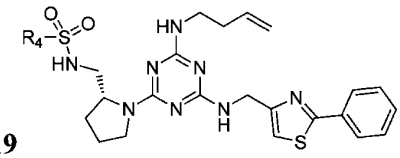
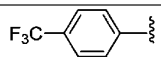
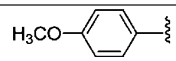
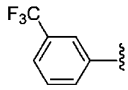
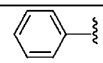
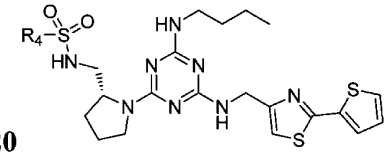
Table 4. SAR for Sulfonamide Moiety (BB4 Site)[a]

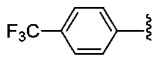
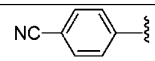
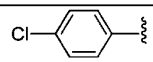
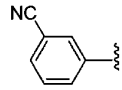
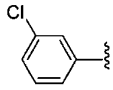
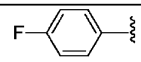
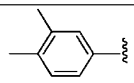
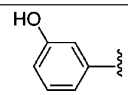
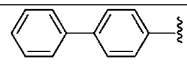
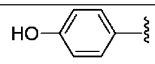
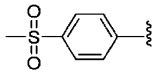


19

Compd	R_4	TS-5 IC_{50} (μM)	Compd	R_4	TS-5 IC_{50} (μM)
14a		0.06	**19c**		0.05
19a		>10	**19d**		0.23
19b		>10	**19e**		> 10



20

Compd	R_4	TS-5 IC_{50} (μM)	Compd	R_4	TS-5 IC_{50} (μM)
12a		0.24	**20f**		1.80
20a		0.46	**20g**		4.10
20b		0.70	**20h**		1.5
20c		0.72	**20i**		1.60
20d		1.25	**20j**		2.60
20e		5.30			

[a]All IC_{50} values are the mean of at least three independent experiments.

demonstrated ELT's utility as a powerful technology for rapid and efficient hit/lead identification.

In vitro profiling of three representative compounds **15f**, **13g**, and **13e** showed low solubility (16, 6, and 2 μM, respectively), high to low permeability (3.5×10^{-5}, $<5 \times 10^{-6}$, $< 5 \times 10^{-6}$ cm/s, respectively) and low microsomal stability ($Cl_{int,apparent}$ of 2930, 1799, and 1596 mL min^{-1} kg^{-1}, respectively, rat microsomes).[26] An in vivo rat pharmacokinetic (PK) study was also performed. As shown in Table 6, these compounds demonstrated a range of C_{max}, AUC, clearance, Vd_{ss}, $T_{1/2}$, and

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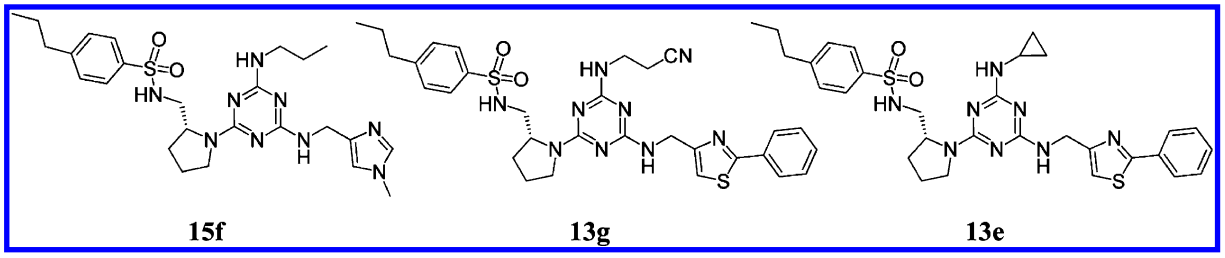
Table 5. Selectivity Data for Selected Compounds[a]

compd	IC_{50}, μM ADAMTS-5	IC_{50}, μM (fold of selectivity)			
		ADAMTS-4	TACE	ADAMTS-1	MMP-13
7	0.050	1.87 (37)	>50 (>1000)	>50 (>1000)	>50 (>1000)
8	0.030	1.50 (50)	>50 (>1000)	>50 (>1000)	>25 (>833)
9	0.040	6.0 (150)	>50 (>1000)	>50 (>1000)	>25 (>625)
13e	0.070	8.3 (118)	12.6 (180)	>50 (>1000)	7.9 (113)
13g	0.090	1.2 (13)	19.9 (221)	>50 (>1000)	7.9 (88)
15f	0.030	1.3 (43)	7.9 (263)	>50 (>1000)	25 (833)

[a]All IC_{50} values are the mean of at least three independent experiments.

Table 6. Rat PK Data Summary for Selected Compounds[a]



compd	dose, iv/po (mg/kg)	C_{max}, iv (ng/mL)	AUC_{0-t}, iv (ng·h/mL)	Cl, iv (mL h^{-1} kg^{-1})	Vd_{ss}, iv (L/kg)	$T_{1/2}$, iv (h)	F (%)
15f	0.9/2.0	207	144	6537	2.4	0.6	1.5
13g	0.8/0.5	644	422	1998	1.4	2.0	8.4
13e	0.9/0.6	1797	1357	643	2.0	7.9	8.0

[a]Rat cassette dosing, and all values are averages of three rats. F% indicates oral bioavailability.

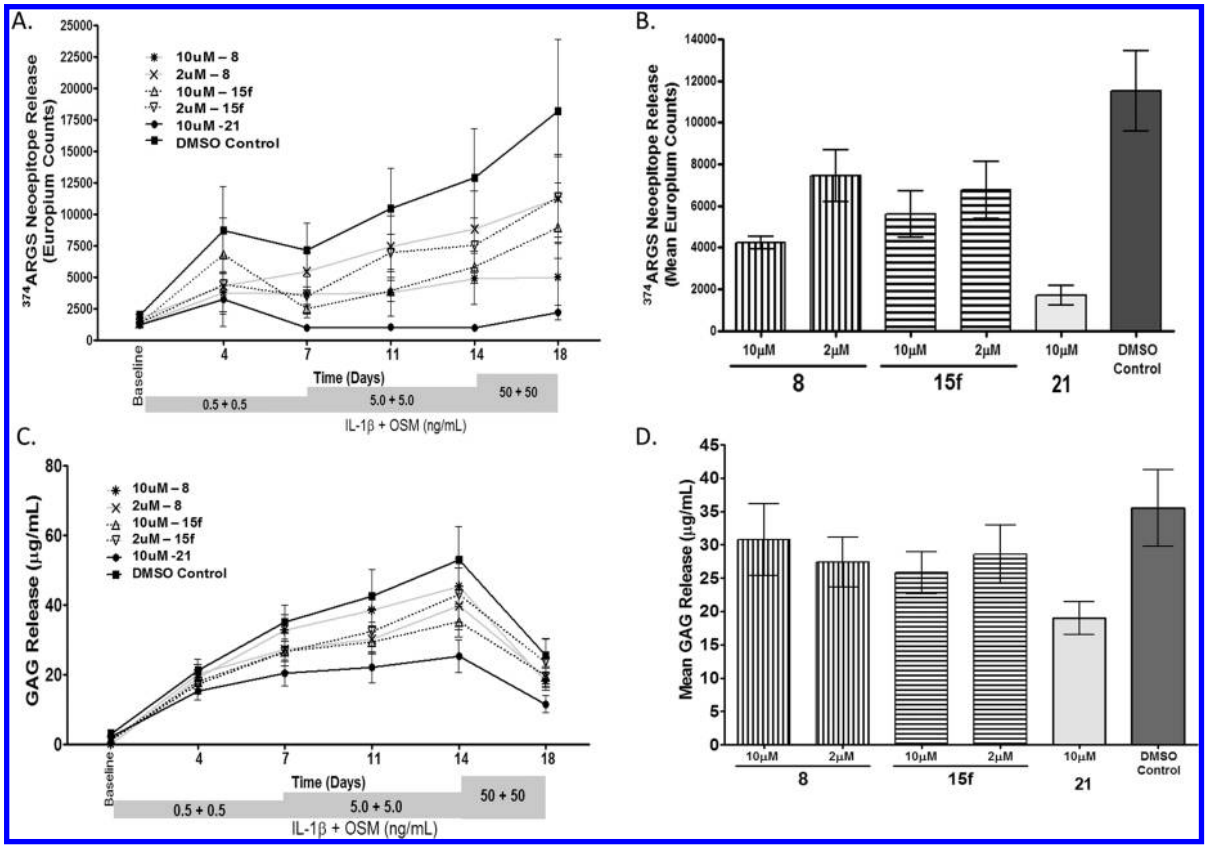


Figure 3. ADAMTS-5 inhibitors suppress cytokine mediated cartilage degradation in human osteoarthritis cartilage explants. Time course of ^{374}ARGS aggrecan neoepitope release (A) and GAG release (C) from cartilage disks treated with increasing concentrations of IL-1β and OSM is suppressed in response to inhibitor compound treatment compared to DMSO control. Mean ^{374}ARGS neoepitope (B) and GAG (D) release levels are suppressed by inhibitor compound treatment compared to DMSO control across the entire 18 days of IL-1β and OSM stimulation.

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oral bioavailability despite sharing a common core structure, triazine pyrrolidine(4-*n*-propanephenyl)sulfonamide. Compound **13g** showed high clearance (1998 mL h^{-1} kg^{-1}) and a short half-life (2 h), while compound **13e** displayed a moderate clearance (643 mL h^{-1} kg^{-1}) and a significantly increased half-life (7.9 h). The only structural difference between the two compounds is that the 2-cyanoethylamine group in **13g** is changed to the 2-cyclopropylamine group in **13e**. The potential improvement in PK properties suggests that these properties were more related to different side chains than to the central triazine scaffold.[27]

The high potency and selectivity make these inhibitors attractive pharmacological tools for investigating ADAMTS-5 protein's role in OA disease progression and treatment. Accordingly, two of the above ADAMTS-5 inhibitors were further evaluated for inhibition of cartilage degradation in an ex vivo human OA cartilage explant system.[28,29] Total knee replacement surgical discard tissue from an OA patient was obtained within 24 h following surgery, and cartilage was processed from the bone, cut into uniform disks, randomized, and placed into culture medium in 96-well plates. Following equilibration with tissue culture medium, cartilage disks were treated with ADAMTS-5 inhibitors **8** and **15f** and, for comparison, the published MMP/aggrecanase hydroxamate inhibitor (2*R*,3*R*)-1-((4-((2-chloro-4-fluorobenzyl)oxy)-phenyl)sulfonyl)-*N*,3-dihydroxy-3-methylpiperidine-2-carboxamide (**21**, Figure 4).[24] All treatments and dose levels were

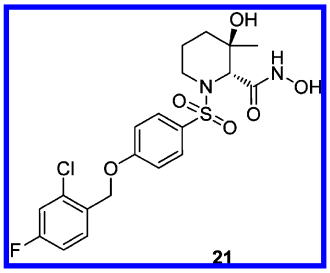

Figure 4. Structure of **21**.

conducted in replicates of seven to control for inherent cartilage plug variability. All samples were treated with increasing levels of recombinant human IL-1β (interleukin-1β)/OSM (oncostatin M, an interleukin-6 family cytokine) to stimulate cartilage degradation, and conditioned culture medium was removed at various time points and tested for cartilage degradation markers.

As shown in Figure 3, ADAMTS-5 inhibitors **8** and **15f** demonstrated a dose dependent suppression of aggrecanase specific ^{374}ARGS neoepitope release compared to DMSO control treatment over the course of the experiment (Figure 3A and Figure 3B). The two inhibitors also showed suppression of glycosaminoglycan (GAG) release over the DMSO control, although the effect was less significant (Figure 3C and Figure 3D). In both cases, the nonselective metalloprotease inhibitor **21**, which has an IC_{50} of 6.3 nM against ADAMTS-5, 3.0 nM against ADAMTS-4, 0.9 nM against MMP-13, and 1.6 nM against TACE, demonstrated the most potent inhibition of cartilage degradation.[30] The higher efficacy achieved by **21** in this experiment may be due to more effective inhibition of ADAMTS-5; alternatively, this compound may act in a nonspecific manner against other aggrecan-cleaving proteases that may have been stimulated under these conditions. The apparent decreased sensitivity of the GAG release end point compared to the ^{374}ARGS neoepitope has been consistently observed in our experience using this system and has been independently reported elsewhere[31] potentially owing to the fact that GAG release measurements cannot differentiate between GAG derived from synthetic or degradative sources. Metabolic viability of the cartilage disks at the end of the experiment, as measured by AlamarBlue fluorescent conversion assay, was unaffected by any of the treatments (data not shown), suggesting minimal toxicity.

The above pharmacological evaluation of the representative ADAMTS-5 inhibitors in the human OA cartilage explant assay showed significant inhibition of cartilage degradation as evidenced by suppressed release of ^{374}ARGS neoepitope and GAG as a consequence of aggrecan degradation triggered by IL-1β/OSM. With the caveat that the OA cartilage used here is derived from a single patient and thus imperfectly represents OA pathology, this experiment nonetheless represents, to our knowledge, the first demonstration of efficacy in a disease related model by small molecules with a high specificity for ADAMTS-5. These data further support the role of ADAMTS-5 as an important target for the treatment of human OA.

CONCLUSIONS

By use of our proprietary encoded library technology, a series of potent and selective ADAMTS-5 inhibitors were rapidly identified from a newly developed, DNA tagged, novel four-billion member small molecule compound library through affinity-based selection. These inhibitors contain neither carboxylate nor hydroxamate functional groups typically seen in zinc metalloproteinase inhibitor scaffolds. Extensive SAR study revealed the core structural requirements necessary for ADAMTS-5 inhibition but also demonstrated good potential for improving potency and physicochemical properties through further structural optimization. More importantly, these inhibitors were highly selective against other related enzymes and were capable of inhibiting cartilage degradation in an explant experiment, thus providing further support for the importance of ADAMTS-5 as a therapeutic target for OA prevention and treatment.

EXPERIMENTAL SECTION

All solvents and reagents were used as obtained. ^{1}H NMR spectra were recorded on a Varian NMR 300 M or a Varian Mercury 400 Plus. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants (J) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet), m (multiplet), br (broad). Analytical purity was ≥95% unless stated otherwise. The purity of final compounds was checked using an Agilent 1100 HPLC system coupled with a Thermo Finnigan LCQ mass spectrometer. All mass spectra were performed by electrospray ionization (ESI). Four different HPLC conditions were used to analyze compound purity. Method A involved a Phenomenex Luna 3 μm C8(2), 30 mm × 3.00 mm 100A column, running gradient of 30−98% MeCN/H_2O (+0.1% TFA) over 3.5 min with flow rate of 0.7 mL/min. Method B involved a Phenomenex Luna 3 μm C8(2), 100 mm × 3.00 mm 100A column, running gradient of 30−85% MeCN/H_2O (+0.1% TFA) over 12 min with flow rate of 0.7 mL/min. Method C involved a Phenomenex Luna 3 μm C8(2), 100 mm × 3.00 mm 100A column, running gradient of 10−85% MeCN/H_2O (+0.1% TFA) over 12 min with flow rate of 0.7 mL/min. Method D involved a SUNFIRE-C19, 50 mm × 2.1 mm, 3.5 μm column, running gradient of 5−100% MeCN/H_2O (+0.01% TFA) over 1.5 min with flow rate of 2 mL/min. High-resolution mass measurement was performed on Bruker

MicroTOF electrospray mass spectrometer coupled with an Agilent 1100 HPLC system. Purification of final compounds for biological testing was performed on a Gilson GX-281 system with either a Phenomenex Luna 5 μm C8(2), 100 mm × 21.20 mm 100A column, running gradient of 10−95% MeCN/H_2O (+0.1% TFA) over 15−20 min with flow rate of 22 mL/min (method E) or a Shimadzu, PRC-ODS, 250 mm × 21.2 mm column, running gradient 20−70% MeCN/H_2O (+0.05% TFA) over 8 min with flow rate of 30 mL/min (method F).

Synthesis of DNA Encoded Library. See Supporting Information for a detail description including headpiece DNA structure, DNA tag information, four sequential chemical conversions, and final library closing DNA sequence ligation.

Synthesis of Compounds off-DNA. General Method A. To a solution of a desired sulfonyl chloride (1.0 mmol) and (*R*)-*tert*-butyl 2-(aminomethyl)pyrrolidine-1-carboxylate (**I**, 1.0 mmol) in dichloromethane (5 mL) was added triethylamine (140 μL, 1.0 equiv) dropwise. The resultant was stirred at room temperature for 1 h. The mixture was concentrated in vacuo, and the residue was dissolved in ethyl acetate and washed with 10% NH_4Cl (aqueous) and brine (3×). The organic phase was separated, dried over Na_2SO_4, and concentrated. The residue was treated with 20% TFA/dichloromethane (5 mL) at room temperature for 30 min and concentrated to dryness to provide the desired substituted pyrrolidine amine (NR_3, **III**) as a TFA salt, which was directly used in the next step of the reaction without further purification.

To a solution of cyanuric chloride (18.4 mg, 0.1 mmol) in acetonitrile (4 mL) was added the first amine (NR_2, 1.0 equiv) at 0 °C. The mixture was stirred at the same temperature for 30 min followed by addition of diisopropylethylamine (DIPEA, 1.0 equiv). The mixture was continuously stirred for another 15 min. Then to this reaction mixture were added the second amine (NR_3, 1.0 equiv) and DIPEA (3.0 equiv). The resulting reaction mixture was warmed to room temperature and stirred for 2 h followed by the addition of the third amine (NR_1, 3.0−5.0 equiv) and DIPEA (3.0 equiv). The reaction mixture was heated at 80 °C for various times as monitored by LC−MS to finish the third amine replacement. The reaction mixture was concentrated in vacuo and the residue was purified by preparative HPLC (method E) to afford the final compounds (**IV**) in reasonable yields (60−80%).

General Method B. By use of (*R*)-*tert*-butyl 2-(aminomethyl)-pyrrolidine-1-carboxylate as the second amine, the triazine intermediate (**V**) was prepared using the same procedure as described in general method A. The intermediate **V** was treated with 20% TFA/dichloromethane at room temperature for 30 min and then condensed. The residue was redissolved in dichloromethane and treated with sulfonyl chloride (1.0 equiv) and triethylamine (3.0 equiv). The mixture was stirred at room temperature for 2 h and then concentrated in vacuo. The residue was purified by preparative HPLC (method E) to afford compounds (**IV**) in 20−65% yields.

***N*-{{(2*R*)-1-[4-(3-Buten-1-ylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (7).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + H]^+$ = 651.2, t_R = 7.55 min (HPLC method B); ^1H NMR (400 MHz, CD_3OD) δ 7.99 (d, J = 8.0 Hz, 1H), 7.91 (m, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 3.6 Hz, 1H), 7.56 (d, J = 5.6 Hz, 1H), 7.36 (s, 1H), 7.12 (t, J = 4.0 Hz, 1H), 5.80 (m, 1H), 5.07 (m, 2H), 4.78−4.68 (m, 3H), 4.26 (br, 1H), 3.65−3.47 (m, 3H), 3.25−3.05 (m, 3H), 2.38−2.28 (m, 2H), 2.10−1.86 (m, 5H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{29}F_3N_8O_2S_3 + H]$ 651.1600; found 651.1577.

***N*-{{(2*R*)-1-[4-(3-Buten-1-ylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-propylbenzenesulfonamide (8).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + H]^+$ = 625.3, t_R = 7.75 min (HPLC method B). ^1H NMR (400 MHz, CD_3OD) δ 7.69 (d, J = 8.4 Hz, 1H), 7.62−7.55 (m, 3H), 7.37 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.16−7.11 (m, 1H), 5.80 (m, 1H), 5.13−5.02 (m, 2H), 4.68 (m, 2H), 4.25 (m, 1H), 3.63 (m, 1H), 3.48 (m, 3H), 3.19−3.01 (m, 2H), 2.61 (m, 2H), 2.37 (m, 1H), 2.30 (m, 1H), 2.04 (m, 3H), 1.91 (m, 1H), 1.61 (m, 2H), 0.91 (dt, J = 7.6, 3.6 Hz, 3H). HRMS $(M + H)^+$ calcd for $[C_{29}H_{36}N_8O_2S_3 + H]$ 625.2196; found 625.2152

***N*-{{(2*R*)-1-[4-(3-Buten-1-ylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-2-naphthalenesulfonamide (9).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-2-naphthalenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + H]^+$ = 633.2, t_R = 6.97 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{30}H_{32}N_8O_2S_3 + H]$ 633.1883; found 633.1853.

3-Fluoro-4-methyl-*N*-((*R*)-1-(4-((2-(naphthalen-1-yl)ethyl)-amino)-6-(((*S*)-2-oxoazepan-3-yl)amino)-1,3,5-triazin-2-yl)-pyrrolidin-3-yl)benzenesulfonamide (10). By use of (*S*)-3-amino-azepan-2-one as NR_2, (*R*)-3-fluoro-4-methyl-*N*-(pyrrolidin-3-yl)-benzenesulfonamide TFA salt as NR_3, and 2-(naphthalen-1-yl)ethanamine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + H]^+$ = 633.2, t_R = 2.31 min (HPLC method A). ^1H NMR (400 MHz, DMSO-d_6) δ 8.13 (m, 1H), 8.07 (m, 1H), 7.86 (m, 3H), 7.71 9 (m, 1H), 7.56 (m, 3H), 7.47 (m, 2H), 7.41 (m, 1H), 4.47 (m, 1H), 3.84 (m, 1H), 3.75−3.35 (m, 9H), 3.13 (m, 1H), 3.01 (m, 2H), 2.31 (s, 3H), 2.10−1.60 (m, 6H), 1.39 (m, 1H), 1.24 (m, 1H). HRMS $(M + H)^+$ calcd for $[C_{32}H_{38}N_8O_3FS + H]$ 633.2772; found 633.2773.

***N*-((*R*)-1-(4-(Benzylamino)-6-(((*S*)-2-oxoazepan-3-yl)amino)-1,3,5-triazin-2-yl)pyrrolidin-3-yl)-3-fluoro-4-methylbenzenesulfonamide (11).** By use of (*S*)-3-aminoazepan-2-one as NR_2, (*R*)-3-fluoro-4-methyl-*N*-(pyrrolidin-3-yl)benzenesulfonamide TFA salt as NR_3, and benzylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + H]^+$ = 569.3, t_R = 1.53 min (HPLC method A but with 40−98% MeCN/H_2O (+0.1% TFA) over 3.5 min gradient). ^1H NMR (400 MHz, CD_3OD) δ 7.60 (d, J = 8.0 Hz, 1H), 7.54 (m, 1H), 7.44 (m, 2H), 7.32 (m, 4H), 4.65 (m, 2H), 4.58 (m, 1H), 4.50 (m, 1H), 3.91 (m, 1H), 3.7 (m, 1H), 3.61 (m, 2H), 3.45 (m, 1H), 3.24 (m, 1H), 2.34 (s, 3H), 2.06 (m, 3H), 1.87 (m, 3H), 1.53 (m, 1H), 1.42 (m, 1H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{34}N_8O_3FS + H]$ 569.2459; found 569.2459.

***N*-{{(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]-methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (12a).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_3, the title compound was prepared by following general method A. MS (ESI) m/z $[M + 1]^+$ = 653.2, t_R = 7.84 min (HPLC method B); ^1H NMR (400 MHz, CD_3OD) δ 7.99 (d, J = 8.0 Hz, 1H), 7.91 (m, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 3.6 Hz, 1H), 7.56 (d, J = 5.6 Hz, 1H), 7.36 (s, 1H), 7.12 (t, J = 4.0 Hz, 1H), 4.82−4.68 (m, 3H), 4.27 (br, 1H), 3.65−3.35 (m, 3H), 3.25−3.05 (m, 3H), 2.06−1.92 (m, 4H), 1.62−1.28 (m, 5H), 0.98−0.86 (m, 3H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{31}F_3N_8O_2S_3 + H]$ 653.1757; found 653.1717.

***N*-{{(2*R*)-1-[4-[(Cyclopropylmethyl)amino]-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (12b).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)-benzenesulfonamide TFA salt as NR_3, and 1-cyclopropylmethanamine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + 1]^+$ = 651.3, t_R = 7.42 min (HPLC method B); ^1H NMR (400 MHz, CD_3OD) δ 7.70 (d, J = 8.4 Hz, 1H), 7.57 (m, 2H), 7.46 (d, J = 7.6 Hz, 1H), 7.29 (m, 2H), 7.07 (s, 1H), 6.83 (m, 1H), 4.40 (m, 2H), 3.96 (m, 1H), 3.30 (m, 2H), 2.97 (m, 1H), 2.87−2.83 (m, 2H), 1.76 (m, 3H), 1.62 (m, 1H), 0.81−0.74 (m, 2H), 0.26−

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0.19 (m, 2H), 0.00 (m, 2H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{29}F_3N_8O_2S_3 + H]$ 651.1600; found 651.1580.

***N*-({(2*R*)-1-[4-(Methylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (12c).** By use of {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and methylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + 1]^+$ = 611.16, t_R = 6.27 min (HPLC method B). ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, J = 8.4 Hz, 1H), 7.87 (m, 2H), 7.75 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 3.6 Hz, 1H), 7.55 (d, J = 4.8 Hz, 1H), 7.35 (s, 1H), 7.12 (t, J = 4.4 Hz, 1H), 4.81−4.77 (m, 1H), 4.67 (m, 1H), 4.26 (m 1H), 3.66−3.58 (m, 2H), 3.27−3.11 (m, 2H), 2.94−2.90 (d, J = 16.4 Hz, 2H), 2.05 (m, 3H), 1.91 (m, 1H). HRMS $(M + H)^+$ calcd for $[C_{24}H_{25}F_3N_8O_2S_3 + H]$ 611.1287; found 611.1277.

***N*-({(2*R*)-1-[4-Oxo-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,4-dihydro-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (12d).** To a solution of cyanuric chloride (18.4 mg, 0.1 mmol) in acetonitrile (4 mL) was added {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine (1.0 equiv) at 0 °C. The mixture was stirred at the same temperature for 30 min followed by addition of DIPEA (3.0 equiv). The mixture was continuously stirred for another 15 min. Then to this reaction mixture was added *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)-benzenesulfonamide TFA salt (1.0 equiv). The resulting reaction mixture was warmed to room temperature and stirred for 2 h. The mixture was then treated with CH_3CN/TFA/H_2O/anisole (2 mL, 50:40:10:1, v/v/v/v) at room temperature overnight. The reaction mixture was concentrated in vacuo and the residue was purified by preparative HPLC (method E) to afford the title compound (27 mg, 45% yield). MS (ESI) m/z $[M + 1]^+$ = 598.2, t_R = 4.85 min (HPLC method B). ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 5.2 Hz, 1H), 7.54 (dd, J = 5.2, 0.8 Hz, 1H), 7.35 (s, 1H), 7.13 (dd, J = 4.8, 4.0 Hz, 1H), 4.81 (s, 2H), 4.71 (d, J = 15.6 Hz, 1H), 4.42 (m, 1H), 3.64−3.51 (m, 2H), 3.27 (d, J = 3.2 Hz, 1H), 3.16 (dd, J = 7.2, 14.0 Hz, 1H), 2.20 (m, 1H), 2.1 (m, 2H). HRMS $(M + H)^+$ calcd for $[C_{23}H_{22}F_3N_7O_3S_3 + H]$ 598.0971; found 598.0943.

***N*-({(2*R*)-1-[4-({[2-(2-Thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)-benzenesulfonamide (12e).** To a solution of 2,4-dichloro-1,3,5-triazine (5.5 mg, 0.05 mmol) in CH_3CN−THF (2:1, 3 mL) was added {[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amine (10 mg, 0.05 mmol). The mixture was stirred at room temperature for 45 min. Then *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt (0.05 mmol) and DIPEA (0.15 mmol) were added. The resultant was heated at 60 °C overnight. The reaction mixture was condensed in vacuo and the residue was purified by preparative HPLC (method E) to afford the title compound (10 mg, 34% yield). MS (ESI) m/z $[M + 1]^+$ = 582.1, t_R = 5.49 min (HPLC method B). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.34 (s, 1H), 7.12 (t, J = 4.4 Hz, 1H), 4.73 (d, J = 9.2 Hz, 2H), 4.34 (m, 1H), 3.65 (t, J = 5.4 Hz, 2H), 3.17 (m, 2H), 2.17−2.05 (m, 3H), 2.01 (m, 1H). HRMS $(M + H)^+$ calcd for $[C_{23}H_{22}F_3N_7O_2S_3 + H]$ 582.1022; found 582.1019.

***N*-({(2*R*)-1-[4-{[(2-Phenyl-1,3-thiazol-4-yl)methyl]amino}-6-(propylamino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-propylbenzenesulfonamide (13a).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]-benzenesulfonamide TFA salt as NR_3, and propylamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 607.4, t_R = 7.76 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{30}H_{38}N_8O_2S_2 + H]$ 607.2632; found 607.2593.

***N*-{[(2*R*)-1-(4-[Methyl(propyl)amino]-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13b).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *N*-methylpropan-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) m/z $[M + 1]^+$ = 621.4, t_R = 8.11 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{31}H_{40}N_8O_2S_2 + H]$ 621.2788; found 621.2763.

***N*-{[(2*R*)-1-(4-[(2-Methylpropyl)amino]-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13c).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 2-methylpropan-1-amine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 621.4, t_R = 8.36 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{31}H_{40}N_8O_2S_2 + H]$ 621.2788; found 621.2749.

***N*-{[(2*R*)-1-(4-[(2-Cyclopropylethyl)amino]-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13d).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 2-cyclopropylethanamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 633.4, t_R = 8.37 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{32}H_{40}N_8O_2S_2 + H]$ 633.2788; found 633.2753.

***N*-{[(2*R*)-1-(4-(Cyclopropylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (13e).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and cyclopropanamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 605.3, t_R = 7.26 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{30}H_{36}N_8O_2S_2 + H]$ 605.2475; found 605.2440.

***N*-{[(2*R*)-1-(4-(Cyclopentylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (13f).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and cyclopentanamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 633.4, t_R = 8.55 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{32}H_{41}N_8O_2S_2 + H]$ 633.2794; found 633.2793.

***N*-{[(2*R*)-1-(4-[(2-Cyanoethyl)amino]-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13g).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 3-aminopropanenitrile as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 618.3, t_R = 6.73 min (HPLC method B); ^{1}H NMR (400 MHz, CD_3OD) δ 7.96−7.90 (m, 2H), 7.72−7.60 (m, 2H), 7.43−7.42 (br, 3H), 7.3−7.10 (br, 3H), 4.80 (m, 2H), 3.80−3.40 (m, 4H), 3.30−2.90 (m, 2H), 2.70−2.58 (m, 4H), 2.10−1.25 (br, 7H), 0.90−0.94 (t, 3H). HRMS $(M + H)^+$ calcd for $[C_{30}H_{35}N_9O_2S_2 + H]$ 618.2428; found 618.2403.

***N*-{[(2*R*)-1-(4-[(2-Hydroxyethyl)amino]-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13h).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 2-aminoethanol as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 609.3, t_R = 6.03 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{29}H_{36}N_8O_3S_2 + H]$ 609.2425; found 609.2397.

***N*-{[(2*R*)-1-(4-{[2-(Methyloxy)ethyl]amino}-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]-methyl}-4-propylbenzenesulfonamide (13i).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and 2-methoxyethanamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) m/z $[M + 1]^+$ = 623.4, t_R = 6.89 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{30}H_{38}N_8O_3S_2 + H]$ 623.2581; found 623.2547.

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***N*-[((2*R*)-1-{4-{[(2-Phenyl-1,3-thiazol-4-yl)methyl]amino}-6-[(tetrahydro-2*H*-pyran-4-ylmethyl)amino]-1,3,5-triazin-2-yl}-2-pyrrolidinyl)methyl]-4-propylbenzenesulfonamide (13j).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and (tetrahydro-2*H*-pyran-4-yl)methanamine as NR_1, the title compound was prepared by following general method A. LC−MS (ESI) *m*/*z* $[M + 1]^+$ = 663.4, t_R = 7.13 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{33}H_{43}N_8O_3S_2 + H]$ 663.2900; found 663.2900.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-(trifluoromethyl)benzenesulfonamide (14a).** By use of (2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 645.3, t_R = 7.81 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{29}H_{31}F_3N_8O_2S_2 + H]$ 645.2036; found 645.1996.

***N*-({(2*R*)-1-[4-[(4-Biphenylylmethyl)amino]-6-(3-buten-1-ylamino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (14b).** By use of (4-biphenylylmethyl)amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 638.3, t_R = 8.60 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{32}H_{34}F_3N_7O_2S + H]$ 638.2520; found 638.2503.

***N*-({(2*R*)-1-[4-[(3-Biphenylylmethyl)amino]-6-(3-buten-1-ylamino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(trifluoromethyl)benzenesulfonamide (14c).** By use of (3-biphenylylmethyl)amine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 638.3, t_R = 8.60 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{32}H_{34}F_3N_7O_2S + H]$ 638.2520; found 638.2486.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-methyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-(trifluoromethyl)benzenesulfonamide (14d).** By use of 1-(2-methyl-1,3-thiazol-4-yl)methanamine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 583.2, t_R = 5.74 min (HPLC method B). ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 7.2 Hz, 1H), 7.85 (m, 2H), 7.08 (s, 1H), 5.82 (m, 1H), 5.13−5.00 (m, 2H), 4.82 (m, 2H), 4.25 (m, 1H), 3.66−3.46 (m, 4H), 3.26−3.02 (m, 2H), 2.39 (s, 3H), 2.34 (m, 2H), 2.06−1.91 (m, 4H). HRMS $(M + H)^+$ calcd for $[C_{24}H_{29}F_3N_8O_2S_2 + H]$ 583.1880; found 583.1856.

***N*-[((2*R*)-1-{4-(3-Buten-1-ylamino)-6-[(phenylmethyl)amino]-1,3,5-triazin-2-yl}-2-pyrrolidinyl)methyl]-4-(trifluoromethyl)-benzenesulfonamide (14e).** By use of benzylamine as NR_2, *N*-[(2*R*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 562.3, t_R = 7.48 min (HPLC method B). HRMS $(M + H)^+$ calcd for $[C_{26}H_{30}F_3N_7O_2S + H]$ 562.2207; found 562.2193.

***N*-[((2*R*)-1-{4-(Butylamino)-6-[(3-thienylmethyl)amino]-1,3,5-triazin-2-yl}-2-pyrrolidinyl)methyl]-4-propylbenzenesulfonamide (15a).** By use of thiophen-3-ylmethanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 544.3, t_R = 1.60 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (s, 1 H), 7.68−7.63 (m, 3 H), 7.25−7.18 (m, 3 H), 7.06−7.04 (m, 1 H), 6.08 (s, 1 H), 5.96 (s, 1 H), 4.55−4.53 (m, 2 H), 4.28−4.10 (m,1 H), 3.46−3.32 (m, 6 H), 2.63−2.58 (m, 2 H), 1.94 (m, 4 H), 1.67−1.56 (m, 4 H),1.36−1.33 (m, 2 H), 0.95−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{37}N_7O_2S_2 + H]$ 544.2523; found 544.2498.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(4-methyl-2-thienyl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15b).** By use of (4-methylthiophen-2-yl)-methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]-benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 558.3, t_R = 1.62 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (s, 1 H), 7.80 (s, 1 H), 7.68−7.64 (m, 3H), 7.27−7.24 (m, 2 H), 6.80−6.78 (m, 1 H), 6.75 (s, 1 H), 4.70 (m, 2 H), 4.31 (m, 1 H), 3.68−3.16 (m, 6 H), 2.64−2.61 (m, 2 H), 2.20 (m, 3 H), 1.99 (m, 4 H), 1.67−1.56 (m, 4 H),1.36−1.33 (m, 2 H), 0.94−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{39}N_7O_2S_2 + H]$ 558.2679; found 558.2671.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(4-cyano-2-thienyl)methyl]-amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15c).** By use of 5-(aminomethyl)thiophene-3-carbonitrile as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]-benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 569.2, t_R = 1.56 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (s, 1 H), 8.60 (s, 1 H), 7.81 (s, 1 H), 7.73−7.67 (m, 3 H), 7.29−7.25 (m, 2 H), 7.19−7.18 (m, 1 H), 4.89 (m, 2 H), 4.31 (m, 1 H), 3.70−3.20 (m, 6 H), 2.98 (m, 1 H), 2.65−2.62 (m, 2 H), 2.0−1.97 (m, 5 H), 1.67−1.56 (m, 4 H),1.36−1.33 (m, 2 H), 0.94−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{36}N_8O_2S_2 + H]$ 569.2475; found 569.2468.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(6-methyl-2-pyridinyl)-methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15d).** By use of 1-(6-methyl-2-pyridinyl)-methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]-benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 553.3, t_R = 1.40 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.18 (s, 1 H), 8.10 (t, 1 H), 7.72−7.70 (m, 3 H), 7.50 (m, 1 H), 7.27 (m, 3 H), 6.89 (m, 1 H), 5.24 (m, 2 H), 4.21 (m, 1 H), 3.52−3.48 (m, 4 H), 2.87 (m, 1 H), 2.86 (s, 3 H), 2.85 (m, 2 H), 2.65−2.60 (m, 2 H), 1.97 (m, 3 H), 1.67−1.56 (m, 4 H), 1.36−1.33 (m, 2 H), 0.94−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{28}H_{40}N_8O_2S + H]$ 553.3068; found 553.3086.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(3-methylphenyl)methyl]-amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15e).** By use of 1-(3-methylphenyl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 552.3, t_R = 1.63 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.89 (s, 1 H), 7.67 (m, 3 H), 7.26 (m, 3 H), 7.08 (m, 2 H), 4.53 (m, 2 H), 3.63−3.36 (m, 4 H), 3.0 (m, 2 H), 2.64−2.59 (m, 2 H), 2.34 (s, 3 H), 2.20 (m, 1 H), 1.94 (m, 4 H), 1.67−1.56 (m, 4 H), 1.36−1.33 (m, 2 H), 0.94−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{29}H_{41}N_7O_2S + H]$ 552.3115; found 552.3088.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(1-methyl-1*H*-imidazol-4-yl)-methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15f).** By use of (1-methyl-1*H*-imidazol-4-yl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]-benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 542.3, t_R = 1.27 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.02 (s, 1 H), 7.61−7.58 (d, 2 H), 7.35 (s, 1 H), 7.21−7.18 (d, 2 H), 6.92−6.78 (m, 1 H), 5.0 (s, 1 H), 5.48 (s, 1 H), 4.50 (m, 2 H), 3.63 (t, 3 H), 3.40−2.8 (m, 6 H),1.90−1.00 (m, 11 H), 0.94−0.89 (t, 6 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{39}N_9O_2S + H]$ 542.3020; found 542.3023.

***N*-{[(2*R*)-1-(4-(Butylamino)-6-{[(4-butylphenyl)methyl]-amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-propylbenzenesulfonamide (15g).** By use of (4-butylphenyl)methanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* $[M + 1]^+$ = 594.4, t_R = 1.74 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (s, 1 H), 7.67−7.61 (m, 2 H), 7.24−7.12 (m, 6 H), 5.70 (s, 1 H), 5.40 (s, 1 H), 4.53−4.10 (m, 3 H), 3.62−2.90 (m, 6 H), 2.64−2.56 (m, 4 H), 1.94−1.90 (m, 4 H), 1.62−1.52 (m, 6 H), 1.36−

1.33 (m, 4 H), 0.94−0.89 (t, 9 H). HRMS (M + H)$^+$ calcd for [$C_{32}H_{48}N_7O_2S$ + H] 594.3590; found 594.3589.

***N*-({(2*R*)-1-[4-(Butylamino)-6-(cyclopentylamino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-propylbenzenesulfonamide (15h).** By use of cyclopentanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 516.3, t_R = 1.62 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70−7.67 (d, 2 H), 7.30−7.29 (d, 2 H), 7.50 (s, 1 H), 7.27 (s, 1 H), 5.75 (s, 1 H), 4.35−4.20 (m, 2 H), 3.67 (m, 1 H), 3.39 (m, 2 H), 3.20 (m,1 H), 2.90 (m, 1 H), 2.65−2.63 (m, 2 H), 2.03−1.92 (m, 6 H), 1.76−1.36 (m, 11 H), 1.36 (m, 2 H), 0.98−0.92 (t, 6 H). HRMS (M + H)$^+$ calcd for [$C_{26}H_{41}N_7O_2S$ + H] 516.3115; found 516.3093.

***N*-[((2*R*)-1-{4-(Butylamino)-6-[(cyclopropylmethyl)amino]-1,3,5-triazin-2-yl}-2-pyrrolidinyl)methyl]-4-propylbenzenesulfonamide (15i).** By use of cyclopropylmethanamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 502.3, t_R = 1.57 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (s, 1 H), 7.68−7.66 (d, 2 H), 7.45 (s, 1 H), 7.27−7.25 (d, 2 H), 5.75 (s, 1 H), 4.35 (m, 1 H), 3.38−3.17 (m, 6 H), 2.66−2.61 (m, 2 H), 2.00−1.91 (m, 3 H), 1.66−1.56 (m, 4 H), 1.37−1.35 (m, 2 H), 1.09 (m, 1 H), 0.96−0.89 (t, 6 H), 0.57−0.53 (m, 2 H), 0.27−0.24 (m, 2 H). HRMS (M + H)$^+$ calcd for [$C_{25}H_{39}N_7O_2S$ + H] 502.2959; found 502.2932.

***N*-[((2*R*)-1-{4-(Butylamino)-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl}-2-pyrrolidinyl)methyl]-4-propylbenzenesulfonamide (15j).** By use of isopropylamine as NR_2, 4-propyl-*N*-[(2*R*)-2-pyrrolidinylmethyl]benzenesulfonamide TFA salt as NR_3, and *n*-butylamine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 490.3, t_R = 1.54 min (HPLC method D); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, 2 H), 7.23−7.20 (d, 2 H), 4.80 (m, 1 H), 4.10 (m, 1 H), 3.50 (m, 3 H), 3.15 (m, 2 H), 2.95 (m, 1 H), 2.64−2.59 (t, 2 H), 1.95 (m, 1 H), 1.85−1.50 (m, 7 H), 1.41 (m, 2 H),1.25 (m, 6 H), 0.94−0.89 (t, 6 H). HRMS (M + H)$^+$ calcd for [$C_{24}H_{39}N_7O_2S$ + H] 490.2959; found 490.2937.

***N*-{[(2*S*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-(trifluoromethyl)benzenesulfonamide (16).** By use of 1-(2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, *N*-[(2*S*)-2-pyrrolidinylmethyl]-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 645.3, t_R = 7.82 min (HPLC method B). ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, *J* = 8.8 Hz, 1H), 7.94−7.90 (m, 2H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.46 (m, 3H), 5.80 (m, 1H), 5.13−5.02 (m, 2H), 4.73 (m, 2H), 4.27 (m, 1H), 3.65 (m, 1H), 3.48 (m, 3H), 3.19−3.01 (m, 2H), 2.38 (m, 1H), 2.30 (m, 1H), 2.06−1.92 (m, 4H). HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2009.

***N*-{[(3*S*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-3-pyrrolidinyl]methyl}-4-(trifluoromethyl)benzenesulfonamide (17).** By use of 1-(2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, (*S*)-*N*-(pyrrolidin-3-ylmethyl)-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 645.3, t_R = 7.63 min (HPLC method B). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (m, 2H), 7.99 (m, 4H), 7.91 (d, *J* = 2.8 Hz, 1H), 7.89 (s, 1H), 7.48 (d, *J* = 5.2 Hz, 2H), 7.47 (s 1H), 5.78 (m, 1H), 5.11−4.89 (m, 2H), 4.64 (s, 2H), 3.58 (br, 2H), 3.45 (m, 5H), 3.20 (m, 1H), 2.83 (t, *J* = 5.2, 2H), 2.30 (m, 2H), 2.18 (m, 1H), 1.97 (m, 1H), 1.64 (m, 1H). HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2018. HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2007.

***N*-{[(3*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-3-pyrrolidinyl]methyl}-4-(trifluoromethyl)benzenesulfonamide (18).** By use of 1-(2-phenyl-1,3-thiazol-4-yl)methanamine as NR_2, (*R*)-*N*-(pyrrolidin-3-ylmethyl)-4-(trifluoromethyl)benzenesulfonamide TFA salt as NR_3, and 3-buten-1-amine as NR_1, the title compound was prepared by following general method A. MS (ESI) *m*/*z* [M + 1]$^+$ = 645.3, t_R = 7.64 min (HPLC method B). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (m, 2H), 7.96 (m, 4H), 7.91 (d, *J* = 2.8 Hz, 1H), 7.89 (s, 1H), 7.48 (d, *J* = 5.2 Hz, 2H), 7.47 (s 1H), 5.78 (m, 1H), 5.05 (m, 2H), 4.65 (s, 2H), 3.82 (br, 2H), 3.62 (m, 2H), 3.45−3.34 (m, 3H), 3.20 (m 1H), 2.83 (t, *J* = 5.2, 2H), 2.28 (m, 2H), 2.18 (m, 1H), 1.96 (m, 1H), 1.65 (m, 1H). HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2018.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-2-(trifluoromethyl)benzenesulfonamide (19a).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-(but-3-en-1-yl)-*N*4-((2-phenylthiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine was first made, which was then treated with 3-(trifluoromethyl)-benzene-1-sulfonyl chloride to afford the title compound in 36% yield. LC−MS (ESI) *m*/*z* [M + 1]$^+$ = 645.3, t_R = 9.66 min (HPLC method C). HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2011.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-2-(trifluoromethyl)benzenesulfonamide (19b).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-(but-3-en-1-yl)-*N*4-((2-phenylthiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine was first made, which was then treated with 2-(trifluoromethyl)-benzene-1-sulfonyl chloride to afford the title compound in 39% yield. LC−MS (ESI) *m*/*z* [M + 1]$^+$ = 645.3, t_R = 9.32 min (HPLC method C). HRMS (M + H)$^+$ calcd for [$C_{29}H_{31}F_3N_8O_2S_2$ + H] 645.2036; found 645.2007.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-4-(methyloxy)benzenesulfonamide (19c).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-(but-3-en-1-yl)-*N*4-((2-phenylthiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine was first made, which was then treated with 4-methoxylbenzene-1-sulfonyl chloride to afford the title compound in 65% yield. LC−MS (ESI) *m*/*z* [M + 1]$^+$ = 607.3, t_R = 6.56 min (HPLC method B); ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, 2H), 7.60−7.80 (m, 2H), 7.4 (m, 4H), 7.10−6.80 (dd, 2H), 5.80 (m, 1H), 5.14−5.03 (m, 2H), 4.80−4.68 (m, 2H), 4.23 (br, 2H), 3.85−3.76 (d, 3H), 3.40−3.20 (m, 2H), 2.40−2.20 (m, 2H), 2.00−1.89 (m, 4H). HRMS (M + H)$^+$ calcd for [$C_{29}H_{34}N_8O_3S_2$ + H] 607.2268; found 607.2247.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-benzenesulfonamide (19d).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-(but-3-en-1-yl)-*N*4-((2-phenylthiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine was first made, which was then treated with phenylsulfonyl chloride to afford the title compound in 23% yield. LC−MS (ESI) *m*/*z* [M + 1]$^+$ = 577.3, t_R = 8.74 min (HPLC method C). HRMS (M + H)$^+$ calcd for [$C_{28}H_{32}N_8O_2S_2$ + H] 577.2162; found 577.2150.

***N*-{[(2*R*)-1-(4-(3-Buten-1-ylamino)-6-{[(2-phenyl-1,3-thiazol-4-yl)methyl]amino}-1,3,5-triazin-2-yl)-2-pyrrolidinyl]methyl}-cyclopropanesulfonamide (19e).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-(but-3-en-1-yl)-*N*4-((2-phenylthiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine was first made, which was then treated with cyclopropanesulfonyl chloride to afford the title compound in 40% yield. LC−MS (ESI) *m*/*z* [M + 1]$^+$ = 541.3, t_R = 5.71 min (HPLC method B). HRMS (M + H)$^+$ calcd for [$C_{25}H_{32}N_8O_2S_2$ + H] 541.2162; found 541.2148.

***(R)*-*N*-((1-(4-(Butylamino)-6-(((2-(thiophen-2-yl)thiazol-4-yl)methyl)amino)-1,3,5-triazin-2-yl)pyrrolidin-2-yl)methyl)-3-methoxybenzenesulfonamide and (*R*)-*N*-((1-(4-(Butylamino)-6-(((2-(thiophen-2-yl)thiazol-4-yl)methyl)amino)-1,3,5-triazin-2-yl)pyrrolidin-2-yl)methyl)-3-hydroxybenzenesulfonamide (20i-OMe and 20i).** By following general method B, (*R*)-6-(2-(aminomethyl)pyrrolidin-1-yl)-*N*2-butyl-*N*4-((2-(thiophen-2-yl)-thiazol-4-yl)methyl)-1,3,5-triazine-2,4-diamine TFA salt was first made. To a solution of this intermediate (0.1 g, 0.23 mmol) in THF (10 mL) were added K_2CO_3 (0.09 g, 0.68 mmol) and 3-methoxybenzene-1-sulfonyl chloride (0.07 g, 0.34 mmol). The

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reaction mixture was stirred at 60 °C for 2 h and then concentrated in vacuo. The residue was dissolved in EtOAc (20 mL) and washed with H_2O (10 mL × 3). The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to give *N*-({(2*R*)-1-[4-(butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-3-(methyloxy)benzenesulfonamide (**20i-OMe**) as a crude product. The crude product was dissolved in anhydrous CH_2Cl_2 (10 mL), and the solution was cooled to 0 °C. To this solution, BBr_3 (0.17 g, 0.68 mmol) was added dropwise. The mixture was stirred at 0 °C for 3 h and then was quenched by the addition of ice-cold water (20 mL). Upon cooling, the mixture was extracted with CH_2Cl_2 (10 mL × 3) and the organic phase was combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by preparative HPLC (method F, MeCN/H_2O = 5/3) to afford the title compound (**20i**) (57 mg, 42%). LC−MS (ESI) m/z $[M + 1]^+$ = 601.2, t_R = 1.45 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.2 (1 H), 7.7 (1 H), 7.0−7.6 (8 H), 5.7−6.1 (1 H), 4.5−5.5 (2 H), 4−4.5 (1H), 3.3−3.7 (6 H), 0.8−2.1 (11 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{32}N_8O_3S_3 + H]$ 601.1832; found 601.1809.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-hydroxybenzenesulfonamide (20j).** The title compound was prepared analogously to the synthesis of compound **20i** with additional preparative HPLC purification (method F) in 40% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 601.2, t_R = 1.43 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.8 (1 H), 6.8−7.6 (s or d, 8 H), 5.7−5.9 (1 H), 4.7 (2 H), 4.1−5.0 (3 H), 3.1−3.4 (m, 6 H), 1.8−1.9 (4 H), 1.5−1.6 (2 H), 1.3−1.4 (2 H), 0.9 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{32}N_8O_3S_3 + H]$ 601.1832; found 601.1812.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-chlorobenzenesulfonamide (20a).** The title compound was prepared analogously to the synthesis of compound **20i-OMe** with additional preparative HPLC purification (method F) in 45% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 619.2, t_R = 1.56 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.2 (1 H), 8.0 (1H), 7.0−7.8 (8 H), 5.5−6.0 (1 H), 4.7 (2 H), 4.3 (1 H), 2.8−3.2 (6 H), 1.1−2.1 (8 H), 0.8 (3 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{31}ClN_8O_2S_3 + H]$ 619.1493; found 619.1463.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-3-chlorobenzenesulfonamide (20b).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 48% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 619.2, t_R = 1.54 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.0 (1 H), 8.0−8.3 (d, 1 H), 7.0−7.6 (s or d, 8 H), 6.1 (s, 1 H), 4.7 (2 H), 4.3 (s, 1 H), 2.8−3.6 (m, 6 H), 1.9−2.0 (4 H), 1.5−1.6 (2 H), 1.3−1.4 (2 H), 0.9 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{31}ClN_8O_2S_3 + H]$ 619.1493; found 619.1465.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-3,4-dimethylbenzenesulfonamide (20c).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 46% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 613.2, t_R = 1.55 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.4 (1 H), 8.2 (1 H), 7.0−7.6 (5 H), 5.8 (s, 1 H), 4.7 (2 H), 4.0−4.4 (s, 1 H), 2.8−3.6 (m, 6 H), 2.3 (6 H),1.9−2.0 (4 H), 1.5−1.6 (2 H), 1.3−1.4 (2 H), 0.9 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{28}H_{36}N_8O_2S_3 + H]$ 613.2196; found 613.2186.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-biphenylsulfonamide (20d).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 37% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 661.2, t_R = 1.62 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.1 (s, 1 H), 8.1−8.3 (d, 1 H), 7.0−8.0 (s or d, 13 H), 6.0 (d, 1 H), 4.7 (m, 2 H), 4.1−4.3 (m, 1 H), 2.8−3.6 (m, 6 H), 1.8−2.6 (t, 4 H), 1.4−1.6 (m, 3 H), 1.2−1.4 (m, 3 H), 0.9 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{32}H_{36}N_8O_2S_3 + H]$ 661.2196; found 661.2169.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}-amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-(methylsulfonyl)benzenesulfonamide (20e).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 47% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 663.2, t_R = 1.45 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.2 (1 H), 8.2 (d, 1 H), 7.8−8.0 (s or d, 4 H), 7.0−7.5 (s or d, 4 H), 5.6 (s, 1 H), 4.6−5.0 (d, 2 H), 4.3 (s, 1 H), 3.2−3.6 (m, 4 H), 3.0 (s, 3 H), 2.8−3.0 (m, 2 H), 1.3−2.1 (m, 8 H), 0.8 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{34}N_8O_4S_4 + H]$ 663.1659; found 663.1641.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-cyanobenzenesulfonamide (20f).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 39% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 610.2, t_R = 1.49 min (method D). ^1H NMR ($CDCl_3$, 300 MHz) δ 9.0 (1 H), 8.0−8.3 (d, 1 H), 7.0−8.0 (s or d, 8 H), 6.6 (1 H), 4.7 (2 H), 4.0−4.3 (s, 1 H), 2.8−3.6 (m, 6 H), 1.8−2.5 (4 H), 1.5−1.6 (2 H), 1.3−1.4 (2 H), 0.8 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{31}N_9O_2S_3 + H]$ 610.1836; found 610.1814.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-3-cyanobenzenesulfonamide (20g).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 48% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 610.2, t_R = 1.49 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.9 (1 H), 8.3 (1 H), 7.0−8.2 (8 H), 6.0−6.5 (s, 1 H), 4.7 (2 H), 4.0−4.5 (s, 1 H), 2.8−3.6 (m, 6 H), 1.9−2.1 (4 H), 1.5−1.6 (2 H), 1.3−1.4 (2 H), 0.9 (t, 3 H). HRMS $(M + H)^+$ calcd for $[C_{27}H_{32}N_9O_2S_3 + H]$ 610.1841; found 610.1842.

***N*-({(2*R*)-1-[4-(Butylamino)-6-({[2-(2-thienyl)-1,3-thiazol-4-yl]methyl}amino)-1,3,5-triazin-2-yl]-2-pyrrolidinyl}methyl)-4-fluorobenzenesulfonamide (20h).** The title compound was prepared analogously to the synthesis of **20i-OMe** with additional preparative HPLC purification (method F) in 41% yield. LC−MS (ESI) m/z $[M + 1]^+$ = 603.2, t_R = 1.51 min (method D); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.1 (1 H), 8.1−8.3 (1 H), 7.0−8.0 (8 H), 6.1−6.3 (1 H), 4.7 (2 H), 4.1−4.4 (1 H), 2.8−3.7 (m, 6 H), 1.8−2.0 (3 H), 1.3−1.5 (3 H), 1.2−1.4 (3 H), 0.85 (3 H). HRMS $(M + H)^+$ calcd for $[C_{26}H_{31}FN_8O_2S_3 + H]$ 603.1789; found 603.1779.

Affinity Selection. Selections were done using streptavidin matrix tips (Phynexus) and chemically biotinylated ADAMTS-5 (262−624) protein. Three rounds of selections were performed. Each tip in each round of selection had 10 μg of protein loaded onto it. Tips were prepared as follows: tips were washed three times with blocking buffer (5% casein in 1× selection buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 0.05% Brij35, 2 mM β-mercaptoethanol (BME), and 1.0 mg/mL sheared salmon sperm DNA (sssDNA, Ambion))) and stored for 12 h at 4 °C in the same buffer. Tips were rinsed two times with blocking buffer. A final buffer exchange was performed with 1.0 mg/mL sssDNA in 1× selection buffer, and the tips were stored in 80 mL of the sssDNA in 1× selection buffer for 12 h at 4 °C.

For selection round 1, 10 mg of ADAMTS-5 protein was immobilized on a previously prepared streptavidin matrix tip and the tip was washed four times with selection buffer. An amount of 5 nmol of DEL-A was diluted in 60 mL of 1× selection buffer and passed over the streptavidin matrix tip for 1 h at room temperature. The tip was washed 8 times with 1× selection buffer and two times with DNA free 1× selection buffer. Binders were eluted by passing heated (80 °C) DNA free 1× selection buffer over it for 12 min. The eluted material was passed over a fresh streptavidin matrix tip for 10 min at room temperature to remove any denatured protein (postclear). The postclear step was repeated a second time to the round 1 selection output (1 μL of the round 1 selection output was retained to be used for qPCR in order to monitor the output from this round of selection). The remaining round 1 selection output was brought to 60 μL by adding the necessary volume of sssDNA and selection buffer.

For selection round 2, the above selection procedure was repeated with fresh protein, a previously prepared fresh tip (washed an additional four times with selection buffer before use), and the round 1 selection output. At the end of round 2, the selection output was

postcleared two times as previously described (5 μL of the round 2 selection output was retained to be used for qPCR in order to monitor the output from this round of selection). The remaining round 2 selection output was brought to 60 μL by adding the necessary volume of sssDNA and selection buffer.

For selection round 3, the selection procedure was repeated with fresh protein, a previously prepared fresh tip (washed an additional four times with selection buffer before use), and the round 2 selection output. The postclear steps were not repeated at the end of round 3. Quantitative PCR was run with the outputs from each round of selection to assess selection yields for each step. The round 3 output was sequenced using Roche/454 technology (454 contract sequencing). The no target control selection was performed in the same fashion but without the protein input.

ADAMTS-5 Assay. The biological activity of the ADAMTS-5 compounds was measured using an assay described by Zhang et al.[22] WAAG-3R substrate was purchased from Anaspec (catalog no. 60431-1). Truncated ADAMTS-5 (262−624) was expressed with a C-term affinity tag in the Chinese hamster ovary cell line, purified from conditioned medium via affinity tag, released from matrix by proteolytic cleavage, and further resolved by size exclusion chromatography. Enzyme and substrate concentrations were 60 nM and 25 μM, respectively. The 1× buffer consists of 50 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$, 0.1% CHAPS, and 5% glycerol. Assay was run in 384-well black plates (Greiner catalog no. 781209). Compounds were suspended in 100% DMSO at 10 mM and diluted serially (final compound concentration 1% DMSO and 1× assay buffer) and preincubated with the enzyme for 15 min. Substrate was added, and the plate was read at every 30 s for 1 h at 37 °C. Excitation and emission wavelengths were 340 and 420 nm, respectively. The enzyme rate in the presence of inhibitor was compared to the rate with 1% DMSO alone to determine percent inhibition at a particular inhibitor concentration. Compound IC_{50} values were determined using a sigmoidal dose−response curve fitting routine (Graphpad Prism).

ADAMTS-4 Assay. The biological activity of the ADAMTS-4 compounds was measured using an assay described by Zhang et al.[22] ADAMTS-4, similar to that previously described in Wayne et al.,[32] and substrate concentrations were 48 nM and 25 μM respectively. The 1× buffer consists of 50 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$, 0.1% CHAPS, and 5% glycerol. Assay was run in 384-well black plates (Greiner catalog no. 781209). Compounds were suspended in 100% DMSO at 10 mM and diluted serially (final compound concentration 1% DMSO and 1× assay buffer) and preincubated with the enzyme for 15 min. The substrate (WAAG-3R) was added, and the plate was read every 30 s for 1 h at 37 °C. Excitation and emission wavelengths were 340 and 420 nm, correspondingly. IC_{50} values were determined as described above.

TACE Assay. The assay was run with TACE enzyme (R&D 930-ADB) and TACE substrate (R&D ES003). The assay was run in 1× TACE buffer: 25 mM Tris (pH 8.0), 2.5 μM $ZnCl_2$, and 0.1% Brij-35. TACE enzyme and substrate concentrations were 20 nM and 100 μM, respectively. Compounds were diluted serially and preincubated with the enzyme for 15 min. Substrate was added, and the plate was read every 30 s for 30 min. Excitation and emission wavelengths were 355 and 405 nm, correspondingly. IC_{50} values were determined as described above.

MMP-13 Assay. MMP-13 enzyme was purchased from R&D Systems (R&D 511-MM). The assay was run in 50 mM HEPES (pH 7.5), 150 mM NaCl, 10 mM $CaCl_2$, 1 μM $ZnAc_2$, and 600 μM CHAPS. Substrate was custom synthesized 5-FAM-TPGPLGL[Dap-(DNP)]ARRK(5-TAMRA)-amide. Compounds were diluted serially in DMSO (final 1%) and added to the plate (Greiner catalog no. 784076). Assay buffer, substrate (final 1 μM), and MMP-13 enzyme (final 480 pM) were added sequentially to the plate and incubated at room temperature for 45 min. The reaction was stopped by adding EDTA (final 10 mM). The assay plates were read at 485 nm excitation/530 nm emission with a 505 nm dichroic filter. IC_{50} values were determined as described above.

ADAMTS-1 Assay. ADAMTS-1 was purchased from R&D Systems (no. 2197-AD). The assay was run in 50 mM HEPES (pH 7.5), 150 mM NaCl, 1 μM ZnAc, 10 mM $CaCl_2$, and 600 μM CHAPS. Substrate FAM (5-carbosyfluorescein)-AE*LQGRPISIAK-TAMRA(5(6)-carbosytetramethylrhodamine) was custom synthesized by Enzyme Systems Products, Inc. Compounds were diluted serially in DMSO (final 1%) and added to the plate (Greiner no. 784076). Assay buffer, substrate (final 2.5 μM), and ADAMTS-1 enzyme (final 20 nM) were added to the plates and incubated at room temperature for 2 h. The reaction was stopped by adding EDTA (final 10 mM). The assay plates were read at 485 nm excitation and 530 nm emission with a 505 nm dichroic filter. IC_{50} values were determined as described above.

Rat PK Study. Characterization of the pharmacokinetics of **15f**, **13g**, and **13e** (Table 6) was done after cassette administration of single intravenous (iv) and oral (po) doses in male Sprague−Dawley rats. The iv infusion dose was administered to fed rats through the femoral vein at 4 mL/kg over 30 min (0.8−0.9 mg compound/kg). Formulation was a filtered solution in 5% DMSO and 20% HPbCD (hydroxypropyl-β-cyclodextrin, also known as Cavitron) in water, pH 3−5, and sampling was done at 0, 5, 15, 30, 32, 35, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 1440 min. The po dose was administered by gavage at 16 mL/kg (2.0 mg of **15f**/kg, 0.5 mg of **13g**/kg, and 0.6 mg of **13e**/kg) to fasted rats (fed 4 h postdose). Formulation was a filtered solution in 5% DMSO and 6% HPbCD in water, pH 3−5, and sampling was done at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 1440 min. Blood was collected from the jugular vein, and plasma was analyzed by LC/MS/MS.

Human OA Cartilage Explant Assay. Tissue was obtained from a 68 year old human osteoarthritis patient within 24 h of undergoing total knee replacement (NDRI, Philadelphia, PA) under IRB approval and in compliance with UK Human Tissue Act 2004 guidelines. Cartilage tissue was processed from the bone into uniform 3 mm diameter disks using a scalpel and leather punch in a sterile hood. Disks were randomized and placed into 96-well tissue culture plates in DMEM supplemented with 0.5% FBS, Penn/Strep and L-glutamine. Following a 3-day equilibration to culture, the medium was removed and fresh medium containing compound and control treatments and recombinant human IL-1β and oncostatin M was added to the wells in replicates of seven per treatment group. IL-1β and oncostatin M were added to the wells in a stepwise fashion (0.5 ng/mL each on days 0−7, 5.0 ng/mL each on days 7−14, and 50 ng/mL each on days 14−18). Each change of cytokine levels required a complete medium change, and fresh medium including treatments at consistent concentration was made (days 7 and 14). Conditioned medium time points were sampled throughout the study and tested for ^{374}ARGS neoepitope and GAG release.

^{374}ARGS Neoepitope Release Detection. Levels of ^{374}ARGS were quantified using a sandwich ELISA format as described previously.[29] Briefly, assay plates (Perkin-Elmer) were coated [10 μg/mL] overnight at 4 °C with a monoclonal antihuman aggrecan capture antibody specific to the keratin sulfate domains (Invitrogen, AHP0012). Plates were washed in 1× DELFIA wash buffer (Perkin-Elmer) and an equal volume (25 μL) of 1× DELFIA assay buffer and cartilage explant samples were added to the wells according to the experimental plate layout. Plates were incubated for 2 h at 37 °C, washed, and incubated with a biotinylated ^{374}ARGS neoepitope specific detection antibody (OA-1, 50 μL/well at 1ug/mL) for 1 h at room temperature and washed. Released ^{374}ARGS neoepitope was detected using streptavidin−europium (50 μL of 100 ng/mL for 30 min at room temperature) and DELFIA enhancement solution (200 μL/well for 3−5 min on a shaker) followed by europium signal acquisition (320 nm excitation, 615 nm emission) on an Envision plate reader.

Glycosaminoglycan Release Detection. Release of glycosaminoglycan (GAG) from cartilage was quantified in the tissue culture medium using the 1,9-dimethylmethylene Blue (DMMB) dye binding procedure as described previously.[28]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis of the DNA encoded library, the ADAMTS-5 inhibition experiment with various concentrations of $ZnCl_2$, the substrate−inhibitor competition experiment, the solubility and artificial membrane permeability assays, and rat liver microsomal stability assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ELT, encoded library technology; OA, osteoarthritis; siRNA, small interfering RNA; DEL, DNA encoded library; AOP, 15-amino-4,7,10,13-tetraoxapentadecanoic acid; BB, building block; Nvoc, *o*-nitroveratryloxycarbonyl; NTC, no-target control; TACE, tumor necrosis factor α-converting enzyme; MMP, matrix metalloproteinase; GAG, glycosaminoglycan; IL-1β, interleukin-1β; OSM, oncostatin M; PK, pharmacokinetic; DIPEA, diisopropylethylamine

■ ADDITIONAL NOTE

The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents. All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

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