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Discovery, Synthesis and Biological Evaluation of Novel SMN Protein Modulators

Jingbo Xiao^{a,*}, Juan J. Marugan^{a,*}, Wei Zheng^a, Steve Titus^a, Noel Southall^a, Jonathan J. Cherry^{b,c}, Matthew Evans^b, Elliot J. Androphy^{b,c}, and Christopher P. Austin^a

^aNIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850

^bDepartment of Medicine, University of Massachusetts Medical School, 364 Plantation Street, LRB 328, Worcester, Massachusetts 01605

Abstract

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder affecting the expression or function of survival motor neuron protein (SMN) due to the homozygous deletion or rare point mutations in the survival motor neuron gene 1 (*SMN1*). The human genome includes a second nearly identical gene called *SMN2* that is retained in SMA. *SMN2* transcripts undergo alternative splicing with reduced levels of SMN. Up-regulation of *SMN2* expression, modification of its splicing, or inhibition of proteolysis of the truncated protein derived from *SMN2* have been discussed as potential therapeutic strategies for SMA. In this manuscript, we detail the discovery of a series of arylpiperidines as novel modulators of SMN protein. Systematic hit-to-lead efforts significantly improved potency and efficacy of the series in the primary and orthogonal assays. Structure property relationships including microsomal stability, cell permeability and *in vivo* pharmacokinetics (PK) studies were also investigated. We anticipate that a lead candidate chosen from this series may serve as a useful probe for exploring the therapeutic benefits of SMN protein up-regulation in SMA animal models, and a starting point for clinical development.

INTRODUCTION

Spinal Muscular Atrophy (SMA), an inherited autosomal neurodegenerative disease, is the leading genetic disorder affecting infant mortality.¹ SMA is a relatively “common” rare disease, affecting approximately 1 in 6000 newborns. Approximately 1 in 40 individuals are genetic carriers.² Clinically, there are four types of SMA (Type I, II, III, and IV). The determination of the type of SMA is based upon the physical milestones achieved. Usually, children with the most severe form of the disease (Type I; Werdnig-Hoffmann disease) die before the age of two years in the absence of supportive respiratory care.³ In fact, SMA is the number one genetic cause of death in children under the age of two and many of those children who make it through infancy are confined to wheelchairs for their entire lives. There is currently no cure or effective therapy for SMA.

Send proofs to: Dr. Juan J. Marugan or Dr. Jingbo Xiao, NIH Chemical Genomics Center, NHGRI, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850, Phone: 301-217-9198, Fax: 301-217-5736, maruganj@mail.nih.gov (Dr. Juan J. Marugan) or xiaoj@mail.nih.gov (Dr. Jingbo Xiao).

^cCurrent address: Department of Dermatology, Indiana University School of Medicine, 980 W. Walnut Street, Indianapolis, Indiana 46202.

SUPPORTING INFORMATION

Assay protocols, experimental procedures and spectroscopic data (¹H-NMR, LCMS and HRMS) are listed for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

SMA is caused by deficiency in survival motor neuron (SMN) protein due to homozygous mutations or deletion of the survival motor neuron 1 telomeric gene (*SMN1*) that is located on chromosome 5q13.⁴ The SMN protein is expressed in all tissues, although there are broad variations regarding levels. Deficiencies in SMN lead to degeneration and dysfunction of alpha motor neurons in the anterior horn of the spinal cord. Studies of the correlation between SMA severity and the amount of SMN protein have shown an inverse relationship.⁵ Humans possess an additional paralogous gene, survival motor neuron 2 (*SMN2*), a centromeric copy gene that differs from the *SMN1* gene at a critical nucleotide at position six in exon 7 (C-T).⁶ While full length 38 kDa SMN protein can be produced from the transcription of both *SMN1* and *SMN2* genes, the small variation in the C-T nucleotide has important consequences for alternative splicing of the *SMN2* transcript. The pre-mRNA transcripts from *SMN1* gene produce full-length mRNA with nine exons encoding full-length of SMN protein, while the C- to T- transition at position six in exon 7 of *SMN2* induces mostly alternative pre-mRNA splicing lacking exon 7, which results in the truncated SMN protein (termed as SMN Δ 7) as its major product. SMN Δ 7 protein has a reduced ability to self-oligomerize, leading to protein instability and rapid degradation.⁷ *SMN2* has been genetically validated as a target for SMA therapy, and there is a striking correlation between SMA type and *SMN2* gene copy numbers.⁸ From the functional point of view, SMN associates with proteins Gemin 2, Gemin 3 and Gemin 4 forming a large complex that plays a role in snRNP assembly, pre-mRNA splicing and transcription.⁹

There are several SMA therapeutic strategies under investigation, including gene therapy,¹⁰ antisense oligonucleotides (ATO)¹¹ and stem cells.¹² However, increasing SMN protein levels by altering expression of the *SMN2* mRNA by small molecules has been proposed as a promising potential therapeutic strategy for SMA. Over the past several years, several classes of small molecules have been identified that increase SMN transcript and/or protein levels in SMA patient-derived cell lines through a variety of mechanisms.¹³ Chang et al. reported that sodium butyrate effectively increased the amount of exon 7-containing SMN protein in SMA lymphoid cell lines by changing the alternative splicing pattern of exon 7 in the *SMN2* gene. *In vivo*, sodium butyrate treatment of SMA-like mice resulted in increased expression of SMN protein in motor neurons of the spinal cord and resulted in significant improvement of SMA clinical symptoms.¹⁴ Sodium 4-phenylbutyrate (PBA), an analogue of sodium butyrate, was also found to increase SMN expression *in vitro*. PBA has a much longer half life time (1.5 hours) in human serum compared with sodium butyrate (6 minutes) which suggested that PBA, also due to its favorable pharmacological properties, could be a candidate for the treatment of SMA.¹⁵ The FDA approved antineoplastic drug hydroxyurea was recently reported as a candidate SMA therapeutic due to its strong safety profile, high pediatric bioavailability, and its known gene up-regulation.¹⁶ Other bioactive agents,¹⁷ such as valproic acid,¹⁸ aclarubicin (aclacinomycin A),¹⁹ tobramycin and amikacin²⁰, Trichostatin A (TSA),²¹ suberoylanilide hydroxamic acid (SAHA),²² EIPA [5-(*N*-ethyl-*N*-isopropyl)-amiloride],²³ LBH589,²⁴ and (*E*)-Resveratrol²⁵ have been shown to increase levels of exon 7-containing SMN transcript and/or overall SMN protein. All of these repurposed agents have significant liabilities that include gastrointestinal bleeding, short half-life in human serum, high toxicity and lack of target specificity; additionally, valproic acid, PBA, and hydroxyurea were not therapeutic in human trials. Lunn et al. identified indoprofen as having an effect on full length *SMN2* expression in a cell-based reporter assay that up-regulated the SMN protein through a cyclooxygenase-independent mechanism, but it was not potent (AC₅₀ >1 μ M) and increased protein expression by only 13% (p-value <0.0139).²⁶ Recently, a tetracycline compound, PTK-SMA1, was found to have the activity to promote *SMN2* exon 7 splicing. However, this compound could not penetrate the blood-brain-barrier or increase SMN protein concentration in the central nervous system.²⁷ Another previous high-throughput screen identified two novel chemical series as HDAC inhibitors that can modulate reporter activity in a cell-based *SMN2* promoter assay.²⁸

Another quinazoline series probe, identified as D156844, targets DcpS, which appears to modulate SMN protein levels by stabilizing the *SMN2* mRNA transcript.²⁹ Oral administration of D156844 significantly increased the mean lifespan of SMN Δ 7 SMA mice by approximately 21–30% when given prior to motor neuron loss.³⁰ After removal of the compound's off-target activity, reducing its toxicity, and improving its potency and brain permeability, it produced the current clinical candidate, D157495.³¹ However, it has not been shown whether this series of molecules acts specifically on *SMN2* mRNA transcript stability or what the safety profile of this molecule might be.³²

We are particularly interested in identifying small molecules that can increase the level of SMN transcript harboring the full length of SMN protein for the therapeutic goal. This may be achieved by modulating splicing, increasing the level of transcript produced, or stabilizing the SMN protein. In order to achieve this goal of looking for molecules able to elevate SMN levels by any of those mechanisms, we developed a new high-throughput screening methodology that allows us to test a large number of compounds in a quantitative manner. In this manuscript, we present some of the results of our screening and optimization campaign.

RESULTS AND DISCUSSION

qHTS Results

Using a luciferase reporter gene assay that combines the promoter and splicing based cassettes in tandem with the major portion of the native *SMN2* cDNA with luciferase, we were able to screen for modulators of all of these mechanisms in a high-throughput manner. Earlier screening assays for SMA only targeted compounds that either stimulate the *SMN2* promoter or decrease exon 7 skipping. In this reporter assay, the *SMN2* promoter drives expression of luciferase reporter that is fused to an SMN splicing reporter (Figure 1). By including our SMN splicing cassette,³³ luciferase is only expressed when exon 7 is included in the mRNA transcript and the full-length SMN protein is generated. This new assay can identify compounds that increase SMN protein levels by three mechanisms: modulating alternative splicing of *SMN2* exon 7, increasing transcription from the *SMN2* promoter, or, due to the presence of the native *SMN2* cDNA, and stabilizing the SMN protein. The assay measures the luminescent signal produced by luciferase, and compounds that increase luminescence are flagged as potential actives.

We performed quantitative high-throughput screen (qHTS)³⁴ using this cell-based *SMN2*-luciferase reporter assay to identify compounds that increase expression of the full-length SMN protein. The NIH Molecular Libraries Small Molecule Repository (MLSMR)³⁵ containing 210,386 compounds was screened in the primary assay during the course of this screen campaign (Figure 2). We tested 1,229 1,536-well plates in the primary screen. The signal to background averaged 9 fold and the average Z' for the screen was 0.47 $\pm$ 0.12, excluding 8 plates which failed visual quality control. 6,128 compounds were found to give significant concentration-responses in the primary screen. Compounds were deprioritized for confirmation if they were suspected to interfere with assay readout, or cellular viability using internal SAR data from other related assays of the MLPCN using the same chemical library.³⁶ Recently, it has been brought to our attention that certain classes of luciferase inhibitors can stabilize the luciferase protein and therefore appear as compounds that are able to increase the signal.³⁷ Compounds that act as luciferase inhibitors could score in the *SMN2* assay as false positives due to this phenomenon. Indeed, more than 98% of hits in the primary screen were identified as luciferase inhibitors, which presumably stabilized the *SMN2*-luciferase fusion protein and prevented cellular degradation, although this has not been explicitly proven. Select samples active in the primary screen were obtained in DMSO stock solutions from the MLSMR and/or as powders from compound vendors to confirm

activity in the original assay. A homologous cell line harboring the *SMN1* gene fused to luciferase was used as a counter-screen. The *SMN1* reporter recapitulates the splicing pattern typically seen with the endogenous *SMN1* gene, and predominately includes exon 7. Follow-up compounds that exhibit increased signal in the *SMN1* cell line were flagged as non-specific activators (i.e. transcription or protein stability). Purified luciferase enzyme was used in another counterscreen of the active compounds to filter out the potential false positives. A complete cheminformatics analysis of the follow-up screening data included chemotype clustering, singleton identification, and structural considerations, including physical properties and optimization potential. In addition, potency range, maximum response, and the curve class³⁸ were also considered. Ultimately, this analysis led to selection of 21 candidates for further confirmation. The results of this qHTS screening campaign are detailed in Figure 2.

Ideally, compounds modulating SMN protein production in the *SMN2*-luciferase reporter line would also modulate SMN protein production in primary fibroblasts directly derived from SMA individuals. Therefore, 21 compounds representing the diverse set of chemotypes³⁹ were tested in a SMA-derived patient fibroblast assay for up-regulation of SMN protein production by western blot, in a luciferase-free setting. Ultimately, this campaign led us to focus on two lead series represented by the 5-methoxy-3,6-diphenyl-1,2,4-triazine (**1**) and 1-(4-(4-bromophenyl)thiazol-2-yl)piperidine-4-carboxamide (**2**), which were able to increase the luciferase signal in the *SMN2* reported assay and increase SMN protein production in a dose dependent manner in the western blot (Figure 3). In addition, although we do not know their specific mechanism of action, we noticed that two active compounds **1** and **2** appear to have distinct mechanisms of action. Compound **1** increased the expression of *SMN2* and did not affect the expression of *SMN1*. On other hand, compound **2** increased the expression of *SMN2* but reduced the expression of *SMN1*. This reduction of *SMN1* expression would not be relevant *in vivo*, due to the fact that the *SMN1* gene is not functional at all in SMA patients. The data in Figure 3 also shows the differences in luciferase inhibitory capacity of **1** and **2**. It can be seen that compound **1** was able to both increase the production of SMN protein (western) and inhibit luciferase. So without the creation of another cell line with a different reporter format, such as β -lactamase, GFP or Renilla luciferase, it would be impossible to discriminate between SNM up-regulation and luciferase stabilization effects using our firefly luciferase reporter assay. In addition, the first triazine chemotype was found to have a very narrow SAR. Therefore, we chose compound **2** as the prioritized series for further SAR optimization and biological evaluation, since it possessed a more tractable SAR and drug-like properties. Furthermore, as reported in later section, this series displayed high cell permeability in Caco-2 cell permeability assay,⁴⁰ and it had with no indication of being a glycoprotein (P-gp) substrate.⁴¹

Chemistry

Figure 4 shows the initial SAR strategy designed for the study of compound **2**, including evaluation of the nature and position of the aromatic substituents, the geometry and substituents at the thiazole core, as well as the modification of the piperidine functional group.

We developed convergent synthetic routes toward the original hit compound **2** and its 4-arylthiazolyl piperidine analogues (Scheme 1). Route (a) was designed to prepare analogues with the modification of the phenyl ring. Starting with 2,4-dibromothiazole, selective amination occurred at the 2-position of the thiazole ring to give the key building block 1-(4-bromothiazol-2-yl)piperidine-4-carboxamide.⁴² Then, a mixture of an arylboronic acid or its corresponding pinacol ester and 1-(4-bromothiazol-2-yl)piperidine-4-carboxamide was irradiated under microwaves to induce a Suzuki type of cross-coupling reaction⁴³ using tetrakis(triphenylphosphine)palladium(0) as a catalyst to furnish the final 4-arylthiazolyl

piperidine analogues. Alternatively, the route (b), a Suzuki coupling at the 4-position of the 2-methylthio-4-bromothiazole, followed by MCPBA oxidation of the methylthioyl group to the methylsulfonyl moiety provided another building block 4-aryl-2-(methylsulfonyl)thiazole. However, the displacement of the methylsulfonyl group of the 4-aryl-2-(methylsulfonyl)thiazole with a secondary amine proved to be very difficult. Thus, all of the analogues described in this article were prepared through the amination-Suzuki coupling approach, route (a). This synthetic route was straightforward and provided for rapid SAR studies.

In a similar reaction fashion, if the starting material, 2,4-dibromothiazole is replaced by 2,5-dibromothiazole,⁴⁴ 2,5-dibromothiadiazole,⁴⁵ 5-bromo-2-chloropyrimidine,⁴⁶ 2,4-dichloropyrimidine,⁴⁷ 4,6-dichloropyrimidine,⁴⁸ or 2,4-dichloro-1,3,5-triazine,⁴⁹ the corresponding modifications at the thiazole core template can be accomplished as detailed in Scheme 2. It was also interesting to note that the replacement of thiazole with imidazole or *N*-methylimidazole was unsuccessful under the same reaction conditions. All aminated building blocks underwent the same type of Suzuki coupling procedure to give the desired final analogues.

Similar procedures were utilized to further explore modifications of the piperidine-4-carboxamide moiety (Scheme 3). In the process of typical amination-Suzuki steps, route (a), piperidine-4-carboxamide can be successfully switched to ethyl piperidine-4-carboxylate, piperazine, *tert*-butyl piperidin-4-ylcarbamate, piperidine-4-carbonitrile, 1-(piperidin-4-yl)ethanone as well as 4-(1H-imidazol-2-yl)piperidine, but not piperazine-1-carboxamide. Within our SAR studies, we were interested in examining several related substituted amide derivatives. Saponification of the ethyl ester afforded the appropriate acid, which can be readily coupled with amines to provide the final substituted amide modification analogues. We were also interested in replacing the piperidine with *N*-substituted piperazines. To access these derivatives, the free secondary amine of the piperazine ring can be easily converted into corresponding urea, amides, and sulfonylamides. Finally, a reverse amide bond analogue and a tetrazole analogue were successfully synthesized, as detailed in Scheme 3. Most of the final compounds were purified by preparative scale HPLC with reasonable yields.

SAR Discussion

Tables 1–7 disclose all of the analogues that were generated for this series to derive a SAR. The activity was reported showing two parameters: AC₅₀ (concentration necessary to reach 50% of maximum luciferase signal) and rate of induction (ROI) (efficacy indicated as maximum percentage of luciferase signal with respect to basal levels), which were used to conduct SAR analysis. Both parameters, ROI and AC₅₀ were evaluated and optimized, as the ideal compound should provide maximum SMN protein production with minimal concentration of a given compound.

The hit compound **2** identified from the primary screen was re-synthesized and found to possess an AC₅₀ value of 1.22 μ M with a ROI of 268%. Our early efforts were focused on improving both parameters in order to further explore the SAR for this chemotype. In order to conduct a comprehensive SAR (Table 1) for the aromatic group at the 4-position of the thiazole ring, while exploring the steric and electronic effects of the substituents, the thiazole and piperidine rings were held constant, as shown in Table 1. We first examined the effect of the substituent position at the phenyl ring on potency (AC₅₀) and efficacy (ROI). With the exception of the *ortho*-fluoro substituted analogue **3r** (AC₅₀ = 2.44 μ M, ROI = 274%), all other *ortho*-substituents were either inactive (**3b**, **3e**, **3o**, **4a**, **4c**, **4e**, **4g**, **4m**, and **4p**) or were significant less active (**3u** and **4x**) versus compound **2**. These data indicated the potential necessity of having a co-planar conformation between the two aromatic rings to maintain

good activity. In contrast to *ortho*-substitutions, substituents at *meta* or *para* positions were generally well tolerated. Regarding the electronic nature of the substituents, an electron-donating functional group at the *meta* or *para* position tended to provide better activity and efficacy than the corresponding electron-withdrawing group (**3c**, **3f** vs. **4h**, **4n**, **4q** and **3d** vs. **4i**, **4o**, **4r**). In addition, halo, methyl or trifluoromethyl substitutions at *para* and *meta* position were also tolerated. Several substituents slightly increased or maintained the activity compared to the efficacy of the hit compound **2**, such as the *meta*-methoxy substituted analogue **3c** ($AC_{50} = 1.94 \mu M$, ROI = 357%), *meta*-*N,N*-dimethylamino substituted analogue **3f** ($AC_{50} = 0.97 \mu M$, ROI = 504%), and *para*-methyl substituted analogue **3m** ($AC_{50} = 1.22 \mu M$, ROI = 439%). Regarding hetero-aromatic ring substituents, none of analogues **4s–4u** had a positive impact on either parameter compared to the parent compound **2**. We also studied several polycyclic aromatic ring analogues (**4e**, **4f**, and **4v–4x**). However, most had either no activity or decreased activity and efficacy.

Given the improved potency and efficacy observed with the electron-donating substituents, we chose to screen a large number of phenoxy substituents (Table 2). Several additional electron-donating substituents were tolerated including *meta*-isopropoxyphenyl (**5c**, $AC_{50} = 0.77 \mu M$, ROI = 605%). We also examined the 3,5- or 3,4-disubstituted analogues (**5h** and **5i**, $AC_{50} = 7.72 \mu M$ and $12.24 \mu M$, ROI = 45% and 59%, respectively) and 3,4,5-trisubstituted derivative (**5m**, $AC_{50} = 19.4 \mu M$, ROI = 73%). Surprisingly, these modifications resulted in a huge loss of both activity and efficacy. Interestingly, this loss could be regained by bridging two substituents together. Furthermore, the resulting cyclic poly-phenolic-ethers demonstrated a clear trend of improvement in activity in line with ring size ($5 < 6 < 7$, **5j**, **5k**, **5l**, $AC_{50} = 2.44 \mu M$, $0.77 \mu M$, and $0.49 \mu M$, respectively).

With several improved analogues in hand (analogues **5c**, **5k**, and **5l**), we further studied the SAR of the piperidine ring moiety as shown in Table 3. In general, mono or disubstituted amide derivatives **6a–6i** had worse potency and efficacy, with the exception of the phenylamide derivative (**6e**, $AC_{50} = 2.44 \mu M$, ROI = 262%) which was about the same as the hit compound **2**. It is also important to remark that both the carboxylic acid **6k** and the corresponding ethyl ester **6j** derivatives were about 4- and 6-fold less active, respectively, compared to compound **2**, while both showed improved efficacy (**6k**, $AC_{50} = 4.87 \mu M$, ROI = 554% and **6j**, $AC_{50} = 7.72 \mu M$, ROI = 550%, respectively). This result indicated that only the carbonyl functional group might be involved in a potential hydrogen bond interaction. This was further supported by significant loss of potency of the non-carbonyl bearing analogues **6q–6s**. When the amide moiety was replaced by cyano or tetrazole groups, the resulting derivatives (analogues **6l** and **6m**) also showed a loss of potency. Reverse amide **6o** and carbamate **6p** analogues were not tolerated either. Surprisingly, a free amine moiety seemed to be tolerated (**6n**, $AC_{50} = 2.44 \mu M$, ROI = 182%). In contrast to the modification of the amide moiety, the piperidine ring was found to be necessary to maintain potency. Interestingly, a piperazine analogue **6t**, which represents another retro-amide variation, displayed reasonable good activity. In contrast, all of the other piperazine derivatives, **6u–6y**, were essentially inactive.

In general, replacement of the 2,4-disubstituted thiazole ring with a 2,4-disubstituted pyrimidine maintained or improved potency and efficacy (Table 4). Placement of the piperidine-4-carboxamide substituent between the two hetero-aromatic nitrogen atoms and aromatic substituent at the 4-position, increased the potency of the molecule by 2–3 fold (**7d**, $AC_{50} = 0.387 \mu M$, ROI = 387% and **7i**, $AC_{50} = 0.772 \mu M$, ROI = 446%). The aromatic substituent at the 5 position of the pyrimidine was also tolerated as represented by analogue **7b** ($AC_{50} = 0.613 \mu M$, ROI = 236%), although its efficacy was only half in comparison with the above described pyrimidine analogues. The *meta*-isopropoxyphenyl substituted derivative **7g** was found to be the best analogue in this series with about 9-fold increase in

potency (**7g**, $AC_{50} = 0.154 \mu M$, ROI = 298%) versus the hit compound **2**. Derivatives bearing a monofluoro substituent at 5 position of the pyrimidine gave an additional 2–4 fold improvement in potency (**7j** vs. **7i** and **7l** vs. **7n**). Replacement of the pyrimidine for 1,3,5-triazine resulted in analogues with decreased potency (**7i** vs. **7k** and **7h** vs. **7p**). These results indicated that the potency of these compounds would not be improved by the introduction of additional nitrogens in the pyrimidine core ring. An *ortho*-methyl substituted pyrimidine derivative **7o** was found to be totally inactive which further supported an earlier observation for requirement of a co-planar conformation for this portion of the molecule for activity. On the other hand, when the pyrimidine substitution patterns were reversed, with the aromatic substituent between the two hetero-aromatic nitrogen atoms and the piperidine-4-carboxamide substituent at the position 4, the resulting derivatives decreased activity by 8–16 fold (**7i** vs. **7n** and **7j** vs. **7l**). In addition, moving two nitrogen atoms around the pyrimidine also decreased the potency by 5-fold (**7i** vs. **7m** and **7h** vs. **7q**). These findings lead to the hypothesis that the sterically accessible nitrogen atom adjacent to the piperidine group is involved in a hydrogen bond interaction, serving as a hydrogen bond acceptor. Table 5 shows modifications for the thiazole portion of the molecule. Switching the aromatic substituent from the 4 position to 5 position of the thiazole core ring increased the potency by 5-fold and efficacy by 2-fold (**8g**, $AC_{50} = 0.224 \mu M$, ROI = 599%). We speculated that this increment was due to accessibility of the thiazole nitrogen for involvement in hydrogen bond interactions with the putative molecular target. Replacement of a CH with a N atom in the thiazole core resulting in 1,3,4-thiadiazole led to a 3–10 fold loss of potency (**8a** vs. **8b**; **8c** vs. **8d**; **8e** vs. **8f**; and **8g** vs. **8h**). These results are analogous to replacement of the pyrimidine with the triazine and are due to the electronic effect of the core ring.

With the rationale that the SAR of the aromatic substituents from the 2,4-substituted thiazole could be translated to a 2,5-substituted thiazole series, the SAR of the different electron-donating groups on the phenyl moiety was explored (Table 5 and Table 6). Gratifyingly, several electron-donating groups on the phenyl ring indeed produced a synergistic effects on both potency and efficacy (**8a**, $AC_{50} = 0.038 \mu M$, ROI = 370%; **8e**, $AC_{50} = 0.039 \mu M$, ROI = 550%; **8l**, $AC_{50} = 0.077 \mu M$, ROI = 709%; and **8m**, $AC_{50} = 0.031 \mu M$, ROI = 576%). Again, the 3,4-disubstituted derivative **8n** again was less active than the corresponding ring closed analogues **8a** and **8o–8q**. Several cyclic *meta*-*N,N*-disubstituted aminophenyl derivatives, **8r–8t**, were examined and were found to be less active than the parent *meta*-*N,N*-dimethylaminophenyl derivative **8e**.

The modification of the amide moiety in the 2,5-substituted thiazole system was further explored, as shown in Table 7. For these studies, 3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl or *meta*-*N,N*-dimethylaminophenyl were chosen as the two aromatic substituents at the C5 position of thiazole core ring. The results demonstrated that the replacement of amide with an acid had a clear advantage for potency with a 3-fold improvement, but with a slight loss of efficacy (**9a** with best $AC_{50} = 0.012 \mu M$, ROI = 325%). Ethyl ester or phenylamide derivatives were also tolerated (**9b**, $AC_{50} = 0.031 \mu M$, ROI = 320% and **9c**, $AC_{50} = 0.049 \mu M$, ROI = 353%). However, the imidazole and methylketone replacements were not tolerated (**9d**, **9e**, **9f**, and **9g**).

Secondary Validation Assays

With the SAR assessment established for this series, a number of selected analogues featuring desirable potency below 150 nM in the reporter assay were then examined to evaluate the effect on the human SMN protein expression using fibroblasts from SMA patients. We incubated a fibroblast cell line from a type I SMA patient (3813 cell line) with different doses of analogues and assessed the SMN protein level by quantitative western blotting (Figure 5). We observed that analogue **8m** at 37 nM increased the SMN protein

level by 2–3 fold. The SMN protein level was decreased with increasing drug concentration. This result matched with the bell-shaped dose-response curve observed in the *SMN2*-luciferase reporter assay. This could be due to decreased compound solubility at higher concentrations, or it may reflect the intrinsic mechanism of action. Analogue **9a** showed a dose-dependent trend between 37 nM to 1 μ M concentration. Other selected agents (**9c**, **8c** and **8l**) all showed an up-regulation of SMN protein at concentrations ranging between 37 nM and 333 nM. Furthermore, the increase in SMN protein production led us to explore whether the arylpiperidine analogues had effect on the overall number of SMN positive foci or gems in the nucleus. This would corroborate that the increased protein amounts represent functional SMN protein. SMN protein is predominately a cytoplasmic protein, but in the nucleus, SMN localizes to distinct punctuate bodies often referred to as gems (gemini of coiled [Cajal] bodies). There is a direct correlation between the number of gems and total SMN protein production in the cell.⁵⁰ Gem counts are commonly used as another metric to score the amount of total SMN protein expressed on a cell-to-cell basis. The number of nuclei with gems and the number of gems per cell were both significantly reduced in type I SMA cells. Only 3.3% of nuclei have gems in fibroblast cells from SMA type I patients (cell line 3813), while 24.8% of nuclei have gems in fibroblast from a carrier parent (3814 cell line). We treated human type I SMA fibroblasts with increasing concentrations (37 nM – 3000 nM) of arylpiperidine analogues **8a**, **8m**, and **9a** for 3 days, and the number of gems per 100 nuclei were examined (Figure 6). Analogue **8m** treatment yielded more than a 2-fold of increase of the number of the gems at the low 37 nM dose. With increasing concentration, the number of gems was reduced, which agreed with the previous findings on our reporter and westerns experiments. Analogue **9a** also showed an 80% increase of the gem numbers at 37 nM to 1000 nM concentrations, and a slightly decreased at 3000 nM. However, analogue **8a** did not show any activity in this assay.

Mechanism of Action

Compounds were initially characterized using RT-PCR to measure RNA expression levels and exon 7 inclusion within our reporter cell line (not endogenous SMN). Some analogues (**2**, **3f**, and **8e**) showed a slight increase in total SMN-luciferase transcripts, but there was almost no increase in exon 7 mRNA (data not shown). Therefore, the mechanism of action of our series is likely post-transcriptional, in contrast to the HDAC and DcpS inhibitors previously identified. Perhaps this mechanism includes increasing the stability of the SMN protein and/or affecting its degradation, but it is distinct from D156844, a deCode compound,^{29a} which had no activity in the present screening assay. So far, no other SMN modulator has been described as having this type of activity, and therefore it will be interesting for the scientific community to have a better understanding of its properties, mechanism and potential pharmaceutical application. The SMN Δ 7 protein, the primary product from *SMN2*, is functional in the context of low levels of exon-7 included SMN protein, but it is proteolytically unstable. It will be interesting to explore whether the present series has an impact on the stability of the SMN Δ 7 derived truncated SMN protein, thereby establishing a new paradigm for the treatment of SMA.

ADME Properties and Pharmacokinetics

In addition, several of the most potent analogues were profiled for mouse liver microsomal stability and cell permeability in Caco-2 cells (Table 8). For the microsomal stability tests, we decided to evaluate the amount of parent compound remaining following incubation at three different time periods: 10, 15 and 60 minutes. This allowed us to both calculate both intrinsic clearance and model half lives. The original hit compound **2** was found to have a relatively short half life (12 minutes). Analogues **8c**, **8m**, and **9c** all had a very short half lives < 10 minutes. Analogues **8b**, **8l**, and **9a** had half lives of more than 30 minutes. In the bidirectional Caco-2 cell permeability assay, analogues **7g**, **8b**, and **9a** were found to exhibit

high permeability, while analogues **8b** and **9a** had efflux ratios of 2.4 and 1.3 respectively, and analogue **8l** had a promising efflux ratio of 0.05. The cell permeability of analogue **8m** was not great, though the efflux ratio was acceptable (0.125).

In consideration of a balance of potency, efficacy, and ADME properties of all current analogues, we selected analogues **8l** and **9a** for full mouse *in vivo* pharmacokinetic (PK) studies to evaluate the central nervous system (CNS) penetration of this series (Table 9). The plasma and brain concentration of analogues **8l** and **9a** in male Swiss Albino mice after a single oral gavage administration at a dose of 30 mg/kg were measured. As shown in Table 9, the plasma to brain ratio of analogues **8l** and **9a** in male Swiss Albino mice was found to be 0.29 and 0.028, respectively. Both analogues **8l** and **9a** possessed a reasonable long half life in brain ($t_{1/2}$ = 5.44 h and 13.69 h), as well as in plasma ($t_{1/2}$ = 5.78 h and 11.18 h). The concentration of analogue **8l** in brain reached at 1.28 $\mu\text{mol/kg}$ (C_{max}) in only 5 minutes, and a concentration above its AC_{50} of 0.077 μM was maintained for more than 4 hours. Despite its high plasma protein binding (94.9%) for analogue **9a**, its concentration in brain reached 1.23 $\mu\text{mol/kg}$ (C_{max}) in 15 minutes and a concentration above AC_{50} concentration of 0.012 μM for over 24 hours. In addition, no behavioral disturbance was observed in animals administered with both tested compounds throughout the whole study period. Oral administration with good brain exposure will be preferred for demonstrating *in vivo* activity, as many of the current animal models require chronic administration of the drug.

CONCLUSION

In summary, after several rounds of medicinal chemistry SAR studies, we were able to improve the potency of the hit compound by ~ 100-fold from μM to low double digit nM in our cell-based *SMN2*-luciferase reporter assay, we were also able to increase the rate of SMN protein production 3–7 fold. From this study, analogues **8a** (AC_{50} = 0.038 μM , ROI = 370%), **8e** (AC_{50} = 0.039 μM , ROI = 550%), **8l** (AC_{50} = 0.077 μM , ROI = 709%), **8m** (AC_{50} = 0.031 μM , ROI = 576%), and **9a** (AC_{50} = 0.012 μM , ROI = 325%) were identified as novel SMN protein modulators. In the field, it is generally believed that an up-regulation of SMN production of at least 20% above patient basal levels will be necessary to have a therapeutic impact. Our best molecules clearly surpass that mark, *in vitro*, increasing levels of SMN protein between 100 to 300% at low nanomolar concentrations when they were tested in our primary reporter assay, as well as in relevant orthogonal assays such as western blot and gems assays with patient fibroblasts. In consideration of all the aspects including ADME properties, analogues **8l** and **9a** possessed the best combination of potency, efficacy, mouse liver microsomal stability and cell permeability of all the analogues presented in this study. Moreover, analogues **8l** and **9a** showed good oral absorption and CNS penetration upon oral gavage administration at a reasonable dose of 30 mg/kg providing levels of exposure above their AC_{50} for more than 4 hours in brain, with no sign of toxicity or behavior disturbance in animals.

EXPERIMENTAL SECTION

Biology Reagents

All cell culture reagents were purchased from Invitrogen. The white solid bottom 1,536 well, tissue culture treated plates came from Greiner. The OneGlo luciferase detection reagent was from Promega. The luciferase enzyme and buffers came from Sigma Aldrich. The 4f11 and 4B7 SMN antibodies were provided to EJA as a generous gift from Christian Lorson (University of Missouri). Goat anti-mouse HRP-conjugated secondary and α -tubulin antibodies (DM1 α) were supplied by Sigma.

Luciferase Reporter Assay

HEK-293 cells stably expressing the *SMN1* or *SMN2* reporter construct were maintained and passaged in complete DMEM media containing phenol red, glutamax, sodium pyruvate, 10 % FCS, 1x pen/strep, and 200 μ g/mL hygromycin. Cells were assayed in the same media lacking only hygromycin. For the HTS, cells were plated at a density of 2,000 cells/well in 5 μ L of media and were allowed to adhere overnight at 37 °C in 90% humidity with 5% CO₂. After incubation, cells received 23 nL of compound library (in 100% DMSO) by pintool addition (Kalypsys). The final concentration of DMSO was 0.46%. The screen was conducted in a 7-point qHTS titration with a final concentration of 50 μ M to 25 nM. The positive control compound used was sodium butyrate (4.5 mM final concentration) and the negative control compound was DMSO. Plates were incubated in the presence of compounds for 30 to 36 hours in the humidified incubator. After incubation, cells were treated with 3 μ L of OneGlo luciferase detection reagent and incubated at room temperature for 5 to 15 minutes. Luminescence was detected on a Viewlux CCD based instrument (Perkin Elmer) with settings of 60 second integration and high speed 2x binning. Data were normalized against sodium butyrate (100%) and DMSO (0%) treatments.

Firefly Luciferase Enzyme Assay

The firefly luciferase enzyme was assayed at 5 nM in buffer consisting of 50 mM Tris-acetate pH 7.6, 10 mM Mg acetate 0.05% BSA, 0.01% Tween. Enzyme (2.5 μ L/well) was dispensed in a white solid bottom 1,536 well plate and compounds (23 nL) were added using a pintool. Plates were incubated at room temperature for 5 minutes followed by the addition of 2.5 μ L of OneGlo luciferase detection reagent. Luminescence was detected in the Viewlux with a 1 second integration time, no binning and medium camera sensitivity.

SMN Protein Detection

For detection of SMN protein in patient fibroblasts, 8,000 cells per cm² were plated 24 hours prior to drug addition. Fresh media and compound were added every 24 hours. After 72 hours of incubation, cells were harvested, washed with cold PBS, and lysed as above. We have determined that 10 μ g total protein per lane is within the linear range for immunoblot detection of SMN and α -tubulin. Western blots were probed for SMN with the 4f11 mouse monoclonal antibody and α -tubulin. Quantification of protein was performed with Fujifilm LAS-4000 Multifunctional Imaging System. The signal intensity was measured for each band on an immunoblot, normalized to the loading control, and the fold increase was determined in relation to the appropriate DMSO treated control.

Chemistry General Methods

All solvents were of anhydrous quality, purchased from Aldrich Chemical Co., and used as received. Commercially available starting materials and reagents were purchased from Aldrich, TCI and Acros, and were used as received. Analytical thin layer chromatography (TLC) was performed with Sigma Aldrich TLC plates (5 $\times$ 20 cm, 60 Å, 250 μ m). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KPSil pre-packed cartridges and using the Biotage SP-1 automated chromatography system. ¹H NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (CDCl₃ 7.27 ppm, DMSO-*d*₆ 2.49 ppm, for ¹H NMR). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, quin = quintet, br = broad, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to an Agilent Technologies 1200 series HPLC. The HPLC retention

time were recorded through standard gradient 4% to 100% acetonitrile (0.05% TFA) over 7 minutes using Luna C₁₈ (3 μ m, 3 mm $\times$ 75 mm) column with a flow rate of 0.800 mL/min. High resolution mass spectral data were collected in-house using an Agilent 6210 time-of-flight mass spectrometer coupled to an Agilent Technologies 1200 series HPLC system. If needed, products were purified via a Waters preparative HPLC equipped with a Phenomenex Luna or Gemini C₁₈ reverse phase (5 μ m, 30 mm $\times$ 75 mm) column having a flow rate of 45 mL/min. The mobile phase was a mixture of acetonitrile (0.1% TFA) and H₂O (0.1% TFA) for acidic condition and a mixture of acetonitrile and H₂O (0.1% NH₄OH) for basic condition. Samples were analyzed for purity on an Agilent 1200 series LC/MS equipped with a Luna C₁₈ reverse phase (3 μ m, 3 mm $\times$ 75 mm) column having a flow rate of 0.800 mL/min over a 7.0 minutes gradient and a run time of 8.5 minutes. Purity of final compounds was determined to be > 95%, using a 3 μ L injection with quantitation by AUC at 220 and 254 nm (Agilent diode array detector).

General protocol A

A mixture of bromo- or chloro-substituted building block (0.100 mmol), boronic acid or pinacol ester (0.200 mmol) and tetrakis(triphenylphosphine)palladium (5.00 μ mol) in DMF (1.50 mL) or CH₃CN (1.50 mL) and 2.0 M Na₂CO₃ aqueous solution (0.50 mL) was heated in a microwave at 100 °C for 30 minutes. The reaction mixture was cooled to room temperature, treated with a small portion of Si-THIOL to get rid of palladium. The mixture was filtered through a frit to give a light yellow solution. The crude material was purified by preparative HPLC under acidic or basic condition to give the final product.

General protocol B

A mixture of carboxylic acid (0.082 mmol), EDC (0.082 mmol), HOBt (0.082 mmol), DMAP (0.082 mmol) and amine (0.163 mmol) in DMF (1.50 mL) was heated in a microwave at 100 °C for 1.5 hours. The crude material was purified by preparative HPLC to give the final product.

General protocol C

A solution of 4-(4-bromophenyl)-2-(piperazin-1-yl)thiazole (0.093 mmol), Et₃N (0.139 mmol) in CH₂Cl₂ (2.00 mL) was treated at 0 °C with carboxylic chloride or sulfonyl chloride (0.111 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 1 hour, and concentrated *in vacuo*. The crude material was purified by preparative HPLC to give the final product.

1-(4-(4-Bromophenyl)thiazol-2-yl)piperidine-4-carboxamide (2)

A general protocol for amination: A mixture of 2,4-dibromothiazole (2.06 g, 8.48 mmol), piperidine-4-carboxamide (1.30 g, 10.1 mmol) and Et₃N (2.50 mL) in ethanol (5.00 mL) was heated in a microwave at 100 °C for 1 hour. The reaction mixture was cooled to room temperature, diluted with water, and extracted with a mixture of MeOH and CH₂Cl₂. The organic layer was separated, dried with Na₂SO₄, and concentrated as a light brown solid. The crude mixture was purified by Biotage with 0–10% of MeOH in CH₂Cl₂ with 1% Et₃N to give 2.08 g (85%) of 1-(4-bromothiazol-2-yl)piperidine-4-carboxamide as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.31 (br. s., 1 H), 6.85 (s, 1 H), 6.82 (br. s., 1 H), 3.77 – 3.90 (m, 2 H), 3.03 (td, *J*=12.5, 2.8 Hz, 2 H), 2.29 – 2.39 (m, 1 H), 1.78 (dd, *J*=13.5, 3.1 Hz, 2 H), 1.50 – 1.63 (m, 2 H); LCMS RT = 4.13 min, *m/z* 289.9 [M+H⁺]. The title compound was prepared according to the general protocol A: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.83–7.79 (m, 2 H), 7.59–7.55 (m, 2 H), 7.33 (s, 1 H), 7.32 (br. s., 1 H), 6.82 (br. s., 1 H), 3.96 (br. d., *J*=12.8 Hz, 2 H), 3.06 (td, *J*=12.6, 2.9 Hz, 2 H), 2.36 (tt, *J*=11.6, 3.8 Hz, 1 H), 1.82 (dd, *J*=13.0, 2.6 Hz, 2 H), 1.61 (qd, *J*=12.4, 4.0 Hz, 2 H); LCMS RT =

5.16 min, m/z 366.0 $[M+H^+]$; HRMS (ESI) m/z calcd for $C_{15}H_{17}^{79}BrN_3OS$ $[M+H^+]$ 366.0276, found 366.0272.

1-(4-(4-Bromophenyl)thiazol-2-yl)-*N*-methylpiperidine-4-carboxamide (6a)

The compound was prepared according to the general protocol B as a TFA salt: 1H NMR (400 MHz, $CDCl_3$) δ ppm 7.60 – 7.71 (m, 2 H), 7.47 – 7.57 (m, 2 H), 6.72 (s, 1 H), 5.78 (br. s., 1 H), 4.15 (dt, $J=13.3, 3.5$ Hz, 2 H), 3.10 – 3.30 (m, 2 H), 2.84 (d, $J=5.1$ Hz, 3 H), 2.41 (tt, $J=11.1, 3.8$ Hz, 1 H), 1.95 – 2.05 (m, 2 H), 1.81 – 1.95 (m, 2 H); ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm –75.97 (s); LCMS RT = 5.34 min, m/z 380.0 $[M+H^+]$; HRMS (ESI) m/z calcd for $C_{16}H_{19}^{79}BrN_3OS$ $[M+H^+]$ 380.0432, found 380.0425.

1-(4-(4-Bromophenyl)thiazol-2-yl)piperidine-4-carboxylic acid (6k)

A solution of ethyl 1-(4-(4-bromophenyl)thiazol-2-yl)piperidine-4-carboxylate (3.34 g, 8.45 mmol) in THF (24.0 mL) and H_2O (8.00 mL) was treated at room temperature with LiOH (1.01 g, 42.2 mmol). The reaction mixture was stirred at room temperature for 24 hours, diluted with 100 mL of CH_2Cl_2 and washed with 2.0 N HCl (25.0 mL). The organic layer was separated, dried with Na_2SO_4 , and concentrated to give a yellow oil. The crude product was purified by Biotage with 0–15% of MeOH in CH_2Cl_2 to give 2.79 g (90%) of product as a white solid. A small amount of sample was re-purified by preparative HPLC under acidic condition to give the product as a TFA salt: 1H NMR (400 MHz, $DMSO-d_6$) δ ppm 7.74 – 7.88 (m, 2 H), 7.50 – 7.64 (m, 2 H), 7.33 (s, 1 H), 3.90 (dt, $J=12.7, 3.5$ Hz, 2 H), 3.04 – 3.23 (m, 2 H), 2.51 – 2.59 (m, 1 H), 1.94 (dd, $J=13.1, 3.7$ Hz, 2 H), 1.51 – 1.75 (m, 2 H); ^{19}F NMR (376 MHz, $DMSO-d_6$) δ ppm –74.87 (s); LCMS RT = 5.84 min, m/z 367.0 $[M+H^+]$; HRMS (ESI) m/z calcd for $C_{15}H_{16}^{79}BrN_2O_2S$ $[M+H^+]$ 367.0116, found 367.0103.

2-(4-(1H-tetrazol-5-yl)piperidin-1-yl)-4-(4-bromophenyl)thiazole (6m)

A solution of 1-(4-(4-bromophenyl)thiazol-2-yl)piperidine-4-carbonitrile (59.5 mg, 0.171 mmol) in water (1.00 mL) and 1,4-dioxane (0.58 mL) was treated at room temperature with sodium azide (33.3 mg, 0.513 mmol) and zinc bromide (57.7 mg, 0.256 mmol). The pH value of the solution was adjusted to about 7 with 1 N NaOH (~ 6 drops). The reaction mixture was heated in oil bath at 120 °C for 60 hours. Another aliquot of reagents was added to the reaction solution and the mixture was re-heated at 120 °C for additional 60 hours. The reaction mixture was cooled to room temperature, diluted with EtOAc, and washed with H_2O . The organic layer was separated, dried with Na_2SO_4 , and concentrated *in vacuo*. The crude product was purified by preparative HPLC under basic condition to give 15.0 mg (22%) of product: 1H NMR (400 MHz, $DMSO-d_6$) δ ppm 7.76 – 7.87 (m, 2 H), 7.53 – 7.62 (m, 2 H), 7.33 (s, 1 H), 3.97 (dt, $J=13.2, 3.7$ Hz, 2 H), 3.20 – 3.30 (m, 2 H), 3.14 (tt, $J=11.1, 3.6$ Hz, 1 H), 2.04 (dd, $J=13.3, 3.1$ Hz, 2 H), 1.71 – 1.87 (m, 2 H); LCMS RT = 5.62 min, m/z 391.0 $[M+H^+]$; HRMS (ESI) m/z calcd for $C_{15}H_{16}^{79}BrN_6S$ $[M+H^+]$ 391.0341, found 391.0338.

1-(4-(4-Bromophenyl)thiazol-2-yl)piperidin-4-amine (6n)

A solution of *tert*-butyl 1-(4-(4-bromophenyl)thiazol-2-yl)piperidin-4-ylcarbamate (78.7 mg, 0.180 mmol) in CH_2Cl_2 (2.00 mL) was treated at 0 °C with TFA (1.00 mL). The reaction mixture was stirred at 0 °C for 30 minutes and at room temperature for additional 10 minutes. The solvents were removed *in vacuo*. The crude product was filtered through a short cartridge column to remove TFA to give 43.4 mg (72%) of product as a white solid. Small portion of sample was re-purified by preparative HPLC under acidic condition to give the final product as a TFA salt: 1H NMR (400 MHz, $DMSO-d_6$) δ ppm 7.93 (br. s., 2 H), 7.72 – 7.85 (m, 2 H), 7.50 – 7.65 (m, 2 H), 7.37 (s, 1 H), 4.00 (d, 2 H), 3.25 – 3.35 (m, 1 H), 3.14 (td, $J=12.8, 2.6$ Hz, 2 H), 1.99 (dd, $J=12.6, 3.0$ Hz, 2 H), 1.57 (qd, $J=12.4, 4.4$ Hz, 2

H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm -73.54 (s); LCMS RT = 4.66 min, m/z 338.0 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{14}\text{H}_{17}^{79}\text{BrN}_3\text{S}$ [$\text{M}+\text{H}^+$] 338.0327, found 338.0323.

1-(4-(4-(4-Bromophenyl)thiazol-2-yl)piperazin-1-yl)ethanone (6t)

The compound was prepared according to the general protocol C as a TFA salt: ^1H NMR (400 MHz, CDCl_3) δ ppm 7.64 – 7.74 (m, 2 H), 7.47 – 7.55 (m, 2 H), 6.82 (s, 1 H), 3.77 – 3.92 (m, 2 H), 3.66 (br. s., 4 H), 3.48 – 3.58 (m, 2 H), 2.20 (s, 3 H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm -76.04 (s); LCMS RT = 5.89 min, m/z 366.0 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{15}\text{H}_{17}^{79}\text{BrN}_3\text{OS}$ [$\text{M}+\text{H}^+$] 366.0276, found 366.0274.

4-(4-(4-Bromophenyl)thiazol-2-yl)piperazine-1-carboxamide (6u)

A solution of 4-(4-bromophenyl)-2-(piperazin-1-yl)thiazole (40.0 mg, 0.123 mmol) in H_2O (2.00 mL) was treated at room temperature with KOCN (20.0 mg, 0.247 mmol). The reaction mixture was stirred at room temperature overnight and extracted with EtOAc. The organic layer was separated, dried with Na_2SO_4 , and concentrated *in vacuo*. The crude mixture was purified by preparative HPLC under basic condition to give 14.2 mg (31%) of product: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 7.82 (d, $J=8.4$ Hz, 2 H), 7.57 (d, $J=8.4$ Hz, 2 H), 7.37 (s, 1 H), 6.11 (br. s., 2 H), 3.37 – 3.57 (m, 8 H); LCMS RT = 5.33 min, m/z 367.0 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{14}\text{H}_{16}^{79}\text{BrN}_4\text{OS}$ [$\text{M}+\text{H}^+$] 367.0228, found 367.0215.

1-(4-(3-Isopropoxyphenyl)pyrimidin-2-yl)piperidine-4-carboxamide (7g)

The compound was prepared according to the general protocol A as a TFA salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 8.41 (d, $J=5.1$ Hz, 1 H), 7.66 (ddd, $J=8.1, 1.3, 1.0$ Hz, 1 H), 7.57 – 7.63 (m, 1 H), 7.41 (t, $J=7.9$ Hz, 1 H), 7.29 (br. s., 1 H), 7.19 (d, $J=5.3$ Hz, 1 H), 7.03 – 7.13 (m, 1 H), 6.78 (br. s., 1 H), 4.55 – 4.84 (m, 3 H), 2.88 – 3.10 (m, 2 H), 2.41 (tt, $J=11.5, 3.8$ Hz, 1 H), 1.80 (dd, $J=12.7, 2.9$ Hz, 2 H), 1.50 (qd, $J=12.4, 3.9$ Hz, 2 H), 1.30 (d, $J=6.1$ Hz, 6 H); ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ ppm -74.96 (s); LCMS RT = 4.45 min, m/z 341.2 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{25}\text{N}_4\text{O}_2$ [$\text{M}+\text{H}^+$] 341.1978, found 341.1983.

1-(4-(3,4-Dihydro-2H-benzo[b][1,4]dioxepin-7-yl)pyrimidin-2-yl)piperidine-4-carboxamide (7h)

The compound was prepared according to the general protocol A: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 8.36 (d, $J=5.1$ Hz, 1 H), 7.62 – 7.80 (m, 2 H), 7.29 (br. s., 1 H), 7.01 – 7.14 (m, 2 H), 6.78 (br. s., 1 H), 4.63 – 4.84 (m, 2 H), 4.10 – 4.26 (m, 4 H), 2.93 (td, $J=12.7, 2.5$ Hz, 2 H), 2.40 (tt, $J=11.5, 3.7$ Hz, 1 H), 2.14 (quin, $J=5.6$ Hz, 2 H), 1.79 (dd, $J=12.9, 2.7$ Hz, 2 H), 1.49 (qd, $J=12.4, 4.3$ Hz, 2 H); LCMS RT = 3.84 min, m/z 355.2 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{23}\text{N}_4\text{O}_3$ [$\text{M}+\text{H}^+$] 355.1770, found 355.1770.

1-(5-(3,4-Dihydro-2H-benzo[b][1,4]dioxepin-7-yl)thiazol-2-yl)piperidine-4-carboxamide (8a)

The compound was prepared according to the general protocol A: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 7.47 (s, 1 H), 7.31 (br. s., 1 H), 7.07 (d, $J=2.3$ Hz, 1 H), 6.97 – 7.04 (m, 1 H), 6.89 – 6.96 (m, 1 H), 6.82 (br. s., 1 H), 4.12 (dt, $J=9.3, 5.5$ Hz, 4 H), 3.89 (dt, $J=12.8, 3.3$ Hz, 2 H), 3.04 (td, $J=12.5, 2.9$ Hz, 2 H), 2.36 (tt, $J=11.5, 3.7$ Hz, 1 H), 2.02 – 2.15 (m, 2 H), 1.79 (dd, $J=13.2, 2.8$ Hz, 2 H), 1.50 – 1.68 (m, 2 H); LCMS RT = 3.74 min, m/z 360.1 [$\text{M}+\text{H}^+$]; HRMS (ESI) m/z calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_3\text{S}$ [$\text{M}+\text{H}^+$] 360.1382, found 360.1379.

1-(5-(3,4-Dihydro-2H-benzo[b][1,4]dioxepin-7-yl)-1,3,4-thiadiazol-2-yl)piperidine-4-carboxamide (8b)

The compound was prepared according to the general protocol A as a TFA salt: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 7.23 – 7.52 (m, 3 H), 6.95 – 7.11 (m, 1 H), 6.84 (br. s., 1 H),

4.19 (t, $J=5.6$ Hz, 4 H), 3.88 (dt, $J=12.8, 3.5$ Hz, 2 H), 3.19 (td, $J=12.4, 2.9$ Hz, 2 H), 2.35 (tt, $J=11.4, 4.0$ Hz, 1 H), 2.14 (dt, $J=11.2, 5.6$ Hz, 2 H), 1.82 (dd, $J=13.5, 2.9$ Hz, 2 H), 1.65 (qd, $J=12.4, 3.9$ Hz, 2 H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm -74.79 (s); LCMS RT = 4.21 min, m/z 361.1 $[\text{M}+\text{H}^+]$; HRMS (ESI) m/z calcd for $\text{C}_{17}\text{H}_{21}\text{N}_4\text{O}_3\text{S}$ $[\text{M}+\text{H}^+]$ 361.1334, found 361.1332.

1-(5-*p*-Tolylthiazol-2-yl)piperidine-4-carboxamide (8c)

The compound was prepared according to the general protocol A as a TFA salt: ^1H NMR (400 MHz, DMSO- d_6) δ ppm 7.58 (s, 1 H), 7.34 – 7.45 (m, 2 H), 7.32 (br. s., 1 H), 7.18 (d, $J=8.0$ Hz, 2 H), 6.84 (br. s., 1 H), 3.91 (dt, $J=12.9, 3.2$ Hz, 2 H), 3.12 (td, $J=12.6, 2.8$ Hz, 2 H), 2.38 (tt, $J=11.4, 3.8$ Hz, 1 H), 2.29 (s, 3 H), 1.82 (dd, $J=13.2, 2.8$ Hz, 2 H), 1.48 – 1.72 (m, 3 H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm -74.86 (s); LCMS RT = 3.91 min, m/z 302.1 $[\text{M}+\text{H}^+]$; HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{20}\text{NOS}$ $[\text{M}+\text{H}^+]$ 302.1327, found 302.1328.

1-(5-(3-Isopropoxyphenyl)thiazol-2-yl)piperidine-4-carboxamide (8l)

The compound was prepared according to the general protocol A as a TFA salt: ^1H NMR (400 MHz, DMSO- d_6) δ ppm 7.65 (s, 1 H), 7.32 (br. s., 1 H), 7.24 (t, $J=8.0$ Hz, 1 H), 6.93 – 7.03 (m, 2 H), 6.83 (br. s., 1 H), 6.76 – 6.81 (m, 1 H), 4.55 – 4.75 (m, 1 H), 3.91 (dt, $J=12.8, 3.4$ Hz, 2 H), 3.11 (td, $J=12.5, 2.9$ Hz, 2 H), 2.37 (tt, $J=11.4, 3.7$ Hz, 1 H), 1.81 (dd, $J=13.3, 2.9$ Hz, 2 H), 1.52 – 1.68 (m, 2 H), 1.26 (d, $J=6.1$ Hz, 6 H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm -74.91 (s); LCMS RT = 4.30 min, m/z 346.1 $[\text{M}+\text{H}^+]$; HRMS (ESI) m/z calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}^+]$ 346.1589, found 346.1593.

1-(5-(4-(Methylthio)phenyl)thiazol-2-yl)piperidine-4-carboxamide (8m)

The compound was prepared according to the general protocol A as a TFA salt: ^1H NMR (400 MHz, DMSO- d_6) δ ppm 7.58 (s, 1 H), 7.37 – 7.45 (m, 2 H), 7.32 (br. s., 1 H), 7.21 – 7.29 (m, 2 H), 6.83 (br. s., 1 H), 3.91 (dt, $J=12.9, 3.0$ Hz, 2 H), 3.09 (td, $J=12.5, 2.8$ Hz, 2 H), 2.48 (s, 3 H), 2.37 (tt, $J=11.6, 4.0$ Hz, 1 H), 1.81 (dd, $J=13.5, 3.5$ Hz, 2 H), 1.54 – 1.67 (m, 2 H); ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm -74.56 (s); LCMS RT = 4.07 min, m/z 361.1 $[\text{M}+\text{H}^+]$; HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{20}\text{N}_3\text{OS}_2$ $[\text{M}+\text{H}^+]$ 334.1048, found 334.1048.

1-(5-(3,4-Dihydro-2H-benzo[b][1,4]dioxepin-7-yl)thiazol-2-yl)piperidine-4-carboxylic acid (9a)

A solution of ethyl 1-(5-(3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl)thiazol-2-yl)piperidine-4-carboxylate (284 mg, 0.72 mmol) in THF (6.00 mL) and H_2O (2.00 mL) was treated at room temperature with LiOH (86.0 mg, 3.60 mmol). The reaction mixture was stirred at room temperature for 24 hours, diluted with 100 mL CH_2Cl_2 , and washed with 2 N HCl (25.0 mL). The organic layer was separated, dried with Na_2SO_4 , and concentrated as a yellow oil. The crude product was purified by Biotage with 0–15% of MeOH in CH_2Cl_2 to give 233 mg (90%) of product as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ ppm 12.32 (br. s., 1 H), 7.48 (s, 1 H), 7.07 (d, $J=2.2$ Hz, 1 H), 6.97 – 7.03 (m, 1 H), 6.89 – 6.96 (m, 1 H), 4.12 (dt, $J=9.2, 5.5$ Hz, 4 H), 3.83 (ddd, $J=13.2, 3.7, 3.5$ Hz, 2 H), 2.98 – 3.20 (m, 2 H), 2.51 – 2.57 (m, 1 H), 2.04 – 2.17 (m, 2 H), 1.87 – 1.98 (m, 2 H), 1.49 – 1.69 (m, 2 H); LCMS RT = 4.18 min, m/z 361.1 $[\text{M}+\text{H}^+]$; HRMS (ESI) m/z calcd for $\text{C}_{18}\text{H}_{21}\text{N}_2\text{O}_4\text{S}$ $[\text{M}+\text{H}^+]$ 361.1222, found 361.1221.

1-(5-(3,4-Dihydro-2H-benzo[b][1,4]dioxepin-7-yl)thiazol-2-yl)-*N*-phenylpiperidine-4-carboxamide (9c)

The compound was prepared according to the general protocol A: ^1H NMR (400 MHz, DMSO- d_6) δ ppm 9.95 (s, 1 H), 7.60 (dd, $J=8.7, 1.1$ Hz, 2 H), 7.49 (s, 1 H), 7.24 – 7.33 (m,

2 H), 6.97 – 7.11 (m, 3 H), 6.92 – 6.97 (m, 1 H), 4.13 (dt, $J=9.4, 5.5$ Hz, 4 H), 3.90 – 4.03 (m, 2 H), 3.10 (td, $J=12.6, 2.7$ Hz, 2 H), 2.62 (tt, $J=11.5, 3.7$ Hz, 1 H), 2.10 (quin, $J=5.4$ Hz, 2 H), 1.90 (dd, $J=13.0, 2.8$ Hz, 2 H), 1.71 (qd, $J=12.4, 4.3$ Hz, 2 H); LCMS RT = 4.94 min, m/z 436.2 $[M+H]^+$; HRMS (ESI) m/z calcd for $C_{24}H_{26}N_3O_3S$ $[M+H]^+$ 436.1695, found 436.1699.

Supplementary Material

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Abbreviations

SMA	spinal muscular atrophy
SMN	survival motor neuron
<i>SMN1</i>	survival motor neuron gene 1
<i>SMN2</i>	survival motor neuron gene 2
qHTS	quantitative high-throughput screen
ROI	rate of induction
NIH	National Institutes of Health
RNA	ribonucleic acid
snRNP	small nuclear ribonucleoproteins
mRNA	messenger RNA
SAR	structure-activity relationships
SPR	structure property relationships
PK	pharmacokinetics
PBA	sodium 4-phenylbutyrate
FDA	U.S. Food and Drug Administration
cDNA	complementary DNA
DMSO	dimethyl sulfoxide
MLSMR	Molecular Libraries-Small Molecule Repository
GFP	green fluorescent protein
P-gp	P-glycoprotein
MCPBA	<i>meta</i> -chloroperoxybenzoic acid
HPLC	high-performance liquid chromatography
RT-PCR	real-time polymerase chain reaction
HDAC	histone deacetylases
DcpS	decapping enzyme scavenger
SMNΔ7	delete exon 7 SMN protein
ADME	absorption, distribution, metabolism, and excretion
CNS	central nervous system

HRP	horseradish peroxidase
HEK-293	human embryonic kidney 293
DMEM	Dulbecco's modified eagle medium
FCS	fetal calf serum
CCD	charge-coupled device
TLC	thin layer chromatography
DMF	dimethylformamide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	hydroxybenzotriazole
DMAP	4-dimethylaminopyridine
TEA	triethylamine
TFA	trifluoroacetic acid

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Figure 1.
SMN2-luciferase reporter assay.

Figure 2.
Overview of screening and hits selection process.

Figure 3.

A. Chemical structures of **1** and **2** from qHTS. B. Activity of compounds **1** and **2** in *SMN1* (cyan) and *SMN2* (blue) reporter assays, indicating the rate of SMN induction (ROI) at different concentrations, and luciferase inhibition assay (black). C. Quantification by western blot of SMN levels after treatment with compounds **1** and **2** at different concentrations, as indicated.

Figure 4.
SAR strategy.

Figure 5.
Quantification by western blot of SMN levels after treatment with drug compounds at different concentrations, as indicated.

Figure 6.
Number of gems per 100 nuclei after treatment with drug compounds at different concentrations, as indicated.

Scheme 1.

Synthesis of the hit compound **2** and 4-arylthiazolyl piperidine analogues.^a

^a Conditions and reagents: (i) triethylamine, μ W, 100 °C; (ii) arylboronic acids or pinacol esters, 2.0 N Na_2CO_3 , 5% $Pd(PPh_3)_4$, μ W, 100 °C; (iii) a. 4-bromophenylboronic acid, 2.0 N Na_2CO_3 , 5% $Pd(PPh_3)_4$, μ W, 100 °C; b. MCPBA; (iv) triethylamine, μ W, 100 °C.

Scheme 2.

Modifications at the thiazole core template.a

^a Conditions and reagents: (i) piperidine-4-carboxamide, triethylamine, μW , 100 or 120 °C; (ii) arylboronic acids or pinacol esters, 2.0 N Na_2CO_3 , 5% $\text{Pd(PPh}_3)_4$, μW , 100 °C; (iii) piperidine-4-carboxamide, *N,N*-diisopropylethylamine, 0 °C.

Scheme 3.

Synthesis of the piperidine modification analogues.^a

^a Conditions and reagents: (i) triethylamine, μ W; (ii) arylboronic acids or pinacol esters, 2.0 N Na_2CO_3 , 5% $\text{Pd}(\text{PPh}_3)_4$, μ W, 100 °C; (iii) LiOH , THF, H_2O ; (iv) EDC, DMAP, μ W, 100 °C; (v) KOCN , H_2O , or R_5COCl , triethylamine, or $\text{R}_6\text{SO}_2\text{Cl}$, triethylamine; (vi) trifluoroacetic acid; (vii) acetyl chloride, triethylamine; (viii) NaN_3 , ZnBr_2 , NaOH , dioxane, H_2O , 120 °C.

Table 1

aromatic substituent.

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.22	268	3p		387	100	4i		inactive	inactive
7.72	165	3q		2.44	386	4j		3.87	356

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁
inactive	inactive	3r		2.44	274	4k
12.24	130

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.94	357	3s		9.72	64	4l		12.24	51
7.72	205	3t		3.87	288	4m		inactive	inactive

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive	3u		7.72	57	4n		3.87	20
0.97	50	3v		12.24	53	4o		15.41	254

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
30.74	64	3w		2.44	381	4p		inactive	inactive
6.13	100	4a		inactive	inactive	4q		inactive	inactive

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive	4b	<p>Chemical structure of a brominated benzene ring, specifically 1-bromo-4-(4-cyanophenyl)benzene.</p>	30.75	57	4r	<p>Chemical structure of a 4-cyanophenyl group, consisting of a benzene ring with a cyano group (-CN) at the para position.</p>	9.72	49

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁
inactive	inactive	4c	 <chem>c1ccc(cc1)-c2ccccc2</chem>	inactive	inactive	4s	 <chem>Cc1cc(s1)C</chem>
inactive	inactive	4c	 <chem>c1ccc(cc1)-c2ccccc2</chem>	7.72	51		

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive	4d		24.42	58	4t		inactive	inactive

AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)	#	R_1
30.74	187	4e		inactive	inactive	4u	
				inactive	inactive

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.22	439	4f		7.72	162	4v		7.72	534
3.87	245	4g		inactive	inactive	4w		15.41	100

AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)
inactive	inactive	4h		inactive	inactive	4x		30.74	369

Table 2

ing a phenoxy substituent.

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.22	268	5c		0.77	605	5j		2.44	359
7.72	167	5d		3.87	178	5k		0.77	459

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive	5e		3.07	54	5l		0.49	443

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.94	357	5f		4.87	240	5m		19.40	73
7.72	209	5g		Inactive	Inactive	5n		12.24	401

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
3.07	297	5h		7.72	45	50		15.41	589
3.87	212	5i		12.24	59				

Table 3

ns of the primary amide moiety and piperidine ring.

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
1.22	268	6i		inactive	inactive	6q		19.40	272
7.72	201	6j		7.72	550	6r		inactive	inactive

AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)
30.75	43	6k	 <chem>BrC1=CC=C(C=C1)c2nc(s2)C(=O)O</chem>	4.87	554	6s	 <chem>BrC1=CC=C(C=C1)C2=CC(=O)N(C2)R1</chem>	24.42	-97
30.75	77	6l	 <chem>BrC1=CC=C(C=C1)c2nc(s2)C#N</chem>	30.74	78	6t	 <chem>BrC1=CC=C(C=C1)C2=CC(=O)N(C2)R1</chem>	3.07	234

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive	6m		12.24	67	6u		12.24	89
2.44	182	6n		2.44	182	6v		inactive	inactive
inactive	inactive	6o		15.41	-57	6w		inactive	inactive
6.13	32	6p		inactive	inactive	6x		inactive	inactive

AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
inactive	inactive					6y		inactive	inactive

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Table 4

SAR of analogues with modifications of thiazole core.

#	Structure	AC ₅₀ (μM)	ROI (%)	#	Structure	AC ₅₀ (μM)	ROI (%)
7a		inactive	inactive	7j		0.387	373
7b		0.613	236	7k		1.22	200
7c		inactive	inactive	7l		3.07	141
7d		0.387	387	7m		3.87	81

#	Structure	AC ₅₀ (μM)	ROI (%)	#	Structure	AC ₅₀ (μM)	ROI (%)
7e		0.387	444	7n		12.24	306
7f		1.22	364	7o		inactive	inactive
	0.154	298	7p		2.44	186
	1.54	234	7q		7.72	211
	0.772	446				

Table 5

SAR of analogues having 2,5-substituted thiazole and thiadiazole cores.

#	Structure	AC ₅₀ (μM)	ROI (%)
8a		0.038	370
8b		0.122	257
8c		0.077	609
8d		0.244	54
8e		0.039	550
8f		0.387	448
8g		0.244	599

#	Structure	AC ₅₀ (μM)	ROI (%)
8h	<chem>N#CN1C(S1)=C(c2ccc(Br)cc2)N3CCCCC3C(=O)N</chem>	inactive	inactive

Table 6

SAR of 2,5-substituted thiazole analogues containing an aromatic substituent.

#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
8i		inactive	inactive	8n		9.72	209
8j		0.244	599	8o		0.049	590
		0.154	498	8p		0.097	424

#	R ₁	AC ₅₀ (μM)	ROI (%)	#	R ₁	AC ₅₀ (μM)	ROI (%)
8k		2.44	601	8a		0.038	370
8c		0.077	609	8q		0.194	598
8l		0.077	709	8r		0.387	685

#	R_1	AC_{50} (μM)	ROI (%)	#	R_1	AC_{50} (μM)	ROI (%)
8e		0.039	550	8s		1.54	565
8m		0.031	576	8t		2.44	275

Table 7

SAR of 2,5-substituted thiazole analogues with modifications of the primary amide.

#	Structure	AC ₅₀ (μM)	ROI (%)
8a		0.038	370
9a		0.012	325
9b		0.031	320
9c		0.049	353
9d		2.44	129
9e		inactive	inactive

#	Structure	AC ₅₀ (μM)	ROI (%)
8e		0.039	550
9f		7.72	188
9g		3.07	224

Table 8

Selected ADME properties assessment for chosen lead compounds.^a

#	SMN2 AC ₅₀ (μM)	ROI (%)	Luciferase inh. IC ₅₀ (μM)	Mouse liver microsome T _{1/2} (minute)	Caco-2 Permeability mean P _{app} A → B (10 ⁻⁶ cm s ⁻¹)	Caco-2 Permeability mean P _{app} B → A (10 ⁻⁶ cm s ⁻¹)
2	1.22	268	inactive	12	30.7	12.1
6k	4.87	554	1.60	ND	32.6	12.0
7g	0.154	298	10	18	40.1	17.3
8a	0.038	370	3.2	30	13.1	9.9
8b	0.122	257	3.2	>60	21.2	51.2
8c	0.077	609	6.4	7	ND	ND
8l	0.077	709	0.80	30	6.1	0.3
8m	0.031	576	40	2	1.6	0.2
9a	0.012	325	0.005	>60	33.8	44.1
9c	0.049	353	4.0	<15	ND	ND

^aMicrosomal stability analysis was performed by Cerep and are based upon duplicate incubations of test reagent with mouse liver microsomes at 37 °C as described (www.cerep.com). LC/MS/MS is utilized to quantitate remaining test compound. Test compounds were applied at standard concentrations (1 μM). Caco-2 permeability analysis was performed by Cerep as described (www.cerep.com). Data are expressed in P_{app} (apparent permeability) in 10⁻⁶ cm s⁻¹. Colchicine and Ranitidine were used as a low permeability controls (Colchicine: mean A→B < 0.1 and mean B→A = 7.9; Ranitidine mean A→B = 0.2 and mean B→A = 2.8), Propranolol was used as a high permeability control (mean A→B = 41.0 and mean B→A = 39.4) and Labetalol was used as a P-gp substrate control (mean A→B = 7.3 and mean B→A = 36.6). Test compounds were applied at standard concentrations (10 μM).

Table 9

Pharmacokinetic data for analogues **8l** and **9a** following oral gavage administration dosing to mice.

Oral administration of 30 mg/kg of 8l in male Swiss Albino mice					
Mean plasma concentration			Mean brain concentration		
Sampling time (hr)	Mean (ng/mL)	Mean (μM)	Mean (ng/g)	Mean (μmol/kg)	
0	0	0	0	0	
0.083	1286	3.72	444	1.28	
0.25	416	1.2	109	0.31	
0.5	416	1.2	138	0.4	
1	439	1.27	207	0.6	
2	206	0.6	85	0.23	
4	0.98	0.0028	29	0.083	
8	8.49	0.025	0	0	
12	6.71	0.019	0	0	
24	20.64	0.06	7.78	0.023	

PK parameters	Unit	Estimate	PK parameters	Unit	Estimate
T _{max}	hr	0.083	T _{max}	hr	0.083
C _{max}	μM	3.72	C _{max}	μmol/kg	1.28
T _{1/2}	hr	5.78	T _{1/2}	hr	5.44
AUC _{plasma} (AUC _{last})	hr*ng/mL	1857	AUC _{plasma} (AUC _{last})	hr*ng/mL	546
AUC _{INF}	hr*ng/mL	1429	AUC _{INF}	hr*ng/mL	607
AUC _{brain} /AUC _{plasma}	%	29			

Oral administration of 30 mg/kg of **9a** in male Swiss Albino mice

Mean plasma concentration				Mean brain concentration			
Sampling time (hr)	Mean (ng/mL)	Mean (μM)	Free concentration (μM) ^d	Mean (ng/g)	Mean (μmol/kg)		
0	0	0	0	0	0		
0.083	15990	44.4	2.26	173	0.48		
0.25	15038	41.7	2.13	442	1.23		
0.5	14822	41.1	2.1	409	1.14		
1	7575	21	1.07	214	0.59		
2	5990	16.6	0.85	157	0.43		
4	3890	10.8	0.55	129	0.36		
8	4537	12.6	0.64	114	0.32		
12	1645	4.57	0.23	47	0.13		
24	1570	4.36	0.22	48	0.13		

PK parameters	Unit	Estimate	PK parameters	Unit	Estimate
T _{max}	hr	0.083	T _{max}	hr	0.25
C _{max}	μM	44.4	C _{max}	μmol/kg	1.23
T _{1/2}	hr	11.18	T _{1/2}	hr	13.69
AUC _{plasma} (AUC _{last})	hr ⁸ ng/mL	77768	AUC _{plasma} (AUC _{last})	hr ⁸ ng/mL	2167
AUC _{INF}	hr ⁸ ng/mL	103094	AUC _{INF}	hr ⁸ ng/mL	3115
AUC _{brain} /AUC _{plasma}	%	2.8			

^aBased on 94.9% of plasma protein binding for **9a**