Bioorganic & Medicinal Chemistry Letters 27 (2017) 5144–5148

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimization of a series of heterocycles as survival motor neuron gene transcription enhancers

Sungwoon Choi [a], Alyssa N. Calder [a], Eliza H. Miller [a], Kierstyn P. Anderson [a], Dawid K. Fiejtek [a], Anne Rietz [b], Hongxia Li [b], Jonathan J. Cherry [b], Kevin M. Quist [b], Xuechao Xing [a], Marcie A. Glicksman [a], Gregory D. Cuny [a], Christian L. Lorson [c], Elliot A. Androphy [b], Kevin J. Hodgetts [a,*]

[a] Laboratory for Drug Discovery in Neurodegeneration, Brigham and Women's Hospital and Harvard Medical School, 65 Landsdowne Street, Cambridge, MA, USA
[b] Department of Dermatology, Indiana University School of Medicine, Indianapolis, IN, USA
[c] Department of Veterinary Pathobiology, Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

ARTICLE INFO

Article history:
Received 2 September 2017
Revised 22 October 2017
Accepted 24 October 2017
Available online 26 October 2017

Keywords:
Spinal muscular atrophy
Survival motor neuron

ABSTRACT

Spinal muscular atrophy (SMA) is a neurodegenerative disorder that results from mutations in the *SMN1* gene, leading to survival motor neuron (SMN) protein deficiency. One therapeutic strategy for SMA is to identify compounds that enhance the expression of the *SMN2* gene, which normally only is a minor contributor to functional SMN protein production, but which is unaffected in SMA. A recent high-throughput screening campaign identified a 3,4-dihydro-4-phenyl-2(1H)-quinolinone derivative (**2**) that increases the expression of *SMN2* by 2-fold with an EC_{50} = 8.3 μM. A structure-activity relationship (SAR) study revealed that the array of tolerated substituents, on either the benzo portion of the quinolinone or the 4-phenyl, was very narrow. However, the lactam ring of the quinolinone was more amenable to modifications. For example, the quinazolinone (**9a**) and the benzoxazepin-2(3H)-one (**19**) demonstrated improved potency and efficacy for increase in *SMN2* expression as compared to **2**.

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by progressive muscle wasting, loss of motor function and premature death in the most severe cases.[1–3] Two genes, *SMN1* and *SMN2*, produce survival motor neuron (SMN) protein,[4–6] which is ubiquitously expressed with the highest levels in the spinal cord[7] and functions in the assembly of spliceosomal small nuclear ribonucleoproteins.[8] The majority of SMN protein normally is produced from the *SMN1* gene, while the almost identical *SMN2* gene only produces approximately 10% of functional protein.[9,10]

SMA results from mutations within exon 7 of *SMN1*, leading to SMN protein deficiency.[11] The clinical severity of this disease is, therefore, indirectly proportional to the copy number of *SMN2* genes, the sole source of protein in these patients.[12,13] Although the threshold level of SMN protein necessary to maintain healthy motor neurons is not known, it has been estimated that increasing the amount of functional protein by only 2- or 3-fold may be clinically significant.[14] Thus, the *SMN2* gene has become a therapeutic target, in which multiple strategies (*i.e.*, small molecules, antisense oligonucleotide) work to increase the transcription of *SMN2*, increase the inclusion of exon 7 *SMN2*, stabilize the full-length exon-7 included *SMN2* mRNA, or stabilize the SMN protein.[15–17] As SMA is the leading heritable cause of infant mortality, it is critical to build upon the one recently FDA-approved treatment, Nusinersen, an antisense oligonucleotide[18] and to provide a variety of treatment options with different modes of action. Several repurposed drugs, such as riluzole, phenylbutyrate, valproic acid, albuterol and hydroxyurea, have advanced into clinical trials, but none has elicited convincing improvement in muscle function or survival in SMA. Currently, there are several small molecules in Phase I to Phase III clinical trials for SMA, including compounds that increased exon 7 inclusion of SMN2.[19]

Previously, we reported an *SMN2*-lucifrase reporter assay for identifying compounds that increase SMN expression from the *SMN2* gene and its use in high-throughput screening.[20] Using the reporter assay, we discovered two hit compounds, **LDN-75654** (**1**) and **LDN-76070** (**2**) (Fig. 1), that increase expression of *SMN2* by 2-fold, but that have different mechanisms of action than the small molecules already in clinical trials to treat SMA. Compound **1** increases the stability of SMN protein, whereas compound **2** acts in a transcriptional manner.[21]

* Corresponding author.
E-mail address: khodgetts@bwh.harvard.edu (K.J. Hodgetts).

https://doi.org/10.1016/j.bmcl.2017.10.066
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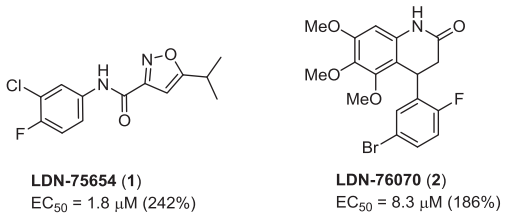


Fig. 1. Two SMN mRNA expression enhancers identified following high-throughput screening.

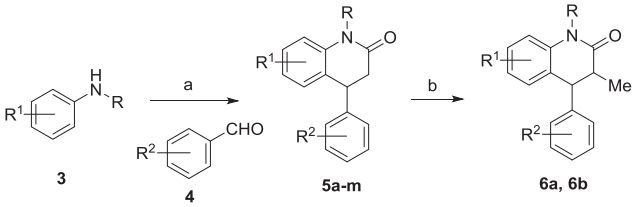
We recently reported the structure-activity relationship (SAR) of analogs of the 5-isopropylisoxazole-3-carboxamide **1**.[22] In this paper, we report preliminary SAR of the 3,4-dihydro-4-phenyl-2(1H)-quinolinone **2** for increasing SMN expression.

The 3,4-dihydro-4-phenyl-2(1H)-quinolinone derivatives were prepared according to the procedure outlined in Scheme 1. A three-component coupling of an aniline (**3**), aromatic aldehyde (**4**) and Meldrum's acid in refluxing ethanol generated the quinolinones **5a–m** in good yield.[23] These compounds were transformed further into the methyl substituted derivatives **6a–b**, which were isolated as mixture of *cis*- and *trans*-isomers.

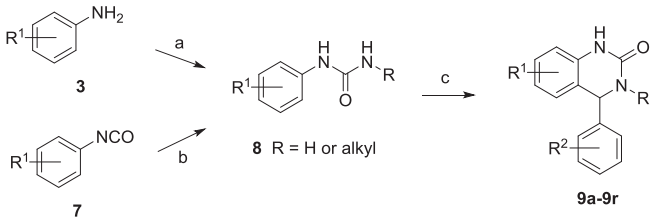
A series of 3,4-dihydro-quinazolinone derivatives **9a–9l** was prepared according to the procedures outlined in Scheme 2.[24] Anilines **3** were treated with potassium isocyanate to give the ureas **8** (*e.g.*, R = H). Alternatively, phenyl isocyanates **7** were treated with primary amines to yield substituted ureas **8** (*e.g.*, R = alkyl). The ureas then were treated with catalytic methane sulfonic acid in refluxing toluene, with azeotropic removal of water, to produce the 3,4-dihydro-quinazolinones **9a–9r**.

The synthesis of the 2-quinazolinone **13** and the seven-membered 1,4-benzodiazepin-2-one **14** is outlined in Scheme 3. The pivolate-protected 3,4,5-trimethoxylaniline **10** was lithiated with butyllithium in THF at -78 °C, and the resulting anion was quenched with methyl 5-chloro-2-fluorobenzoate to give the ketone **11** in modest yield. The pivolate group was cleaved with refluxing sulfuric acid and gave the versatile intermediate **12**. For example, the ketone **12** was treated with trichloroacetyl chloride, and the product, upon treatment with ammonium acetate, cyclized to **13**. On the other hand, the ketone **12** also was treated with glycine ethyl ester, with azeotropic removal of water, and gave the 1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one **14**.

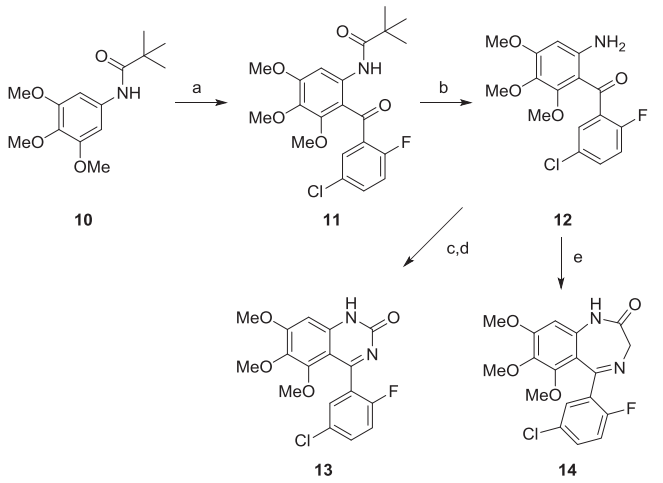
Several oxygen-containing heterocycles (*e.g.*, **16**, **19–21**) also were prepared from the ketone **12** (Schemes 4 and 5). Ketone **12** was reduced to the racemic alcohol **15** with sodium borohydride in ethanol. Treatment of **15** with carbonyl diimidazole (CDI) directly gave the oxazinone **16** in good yield. Acylation of **15** with 2-bromoacetyl chloride or 2-bromopropionyl chloride gave the amides **17** and **18**, respectively. Treatment of **17** with two equivalents of sodium hydride gave the 7-membered benzoxazepin-2(3H)-one **19** in moderate yield.[25] Similarly, cyclization of **18** gave the 3-methyl-benzoxazepin-2(3H)-one **20** as a mixture of diastereoisomers. Finally, treatment of **19** with methyl iodide gave the *N*-methyl amide **21**.



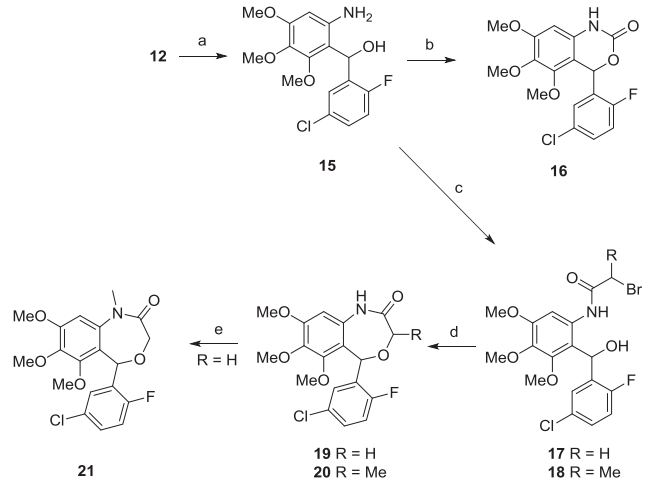
Scheme 1. (a) Meldrum's acid, EtOH, 85 °C, 16 h (55–78%); (b) BuLi, THF, 0 °C then MeI (48%).



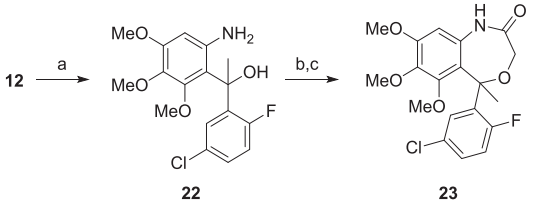
Scheme 2. (a) KNCO, AcOH, RT; (84–97%); (b) RNH_2, THF, 0 °C to RT (63–89%); (c) R^2PhCHO, cat. $MeSO_3H$, toluene, reflux (31–81%).



Scheme 3. (a) BuLi, THF −78 °C then methyl 5-chloro-2-fluorobenzoate (15%); (b) H_2SO_4, EtOH, reflux (57%); (c) Cl_3CCOCl, DCM, Et_3N, RT; (d) NH_4OAc, DMSO, 80 °C (41% over 2-steps); (e) $HCl•NH_2CH_2CO_2Et$, Py, Dean-Stark trap, reflux, 24 h (38%).



Scheme 4. (a) $NaBH_4$, EtOH, RT (82%); (b) CDI, THF, RT (76%); (c) BrCHRC(O)Cl, Et_2O, Et_3N, 0 °C to RT (62–73%); (d) NaH, THF, RT then reflux (43–72%); (e) MeI, Cs_2CO_3, THF, RT (81%).



Scheme 5. (a) MeMgBr, THF, 0 °C (67%); (b) $BrCH_2C(O)Cl$, Et_2O, Et_3N, 0 °C to RT; (c) NaH, THF, RT then reflux (54% over 2-steps).