Bioorganic & Medicinal Chemistry Letters 22 (2012) 6647–6650

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Substituted pyrazolones require N^2 hydrogen bond donating ability to protect against cytotoxicity from protein aggregation of mutant superoxide dismutase 1

Paul C. Trippier [a], Radhia Benmohammed [b], Donald R. Kirsch [b], Richard B. Silverman [a,c,*]

[a] Department of Chemistry, Northwestern University, Evanston, IL, USA
[b] Cambria Pharmaceuticals, Cambridge, MA, USA
[c] Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, IL, USA

ARTICLE INFO

Article history:
Received 28 June 2012
Revised 14 August 2012
Accepted 28 August 2012
Available online 7 September 2012

Keywords:
Amyotrophic lateral sclerosis
Superoxide dismutase 1
Neuroprotection
Pyrazolone
Pharmacophore

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a debilitating and fatal neurodegenerative disease. Although the cause remains unknown, misfolded protein aggregates are seen in neurons of sporadic ALS patients, and familial ALS mutations, including mutations in superoxide dismutase 1 (SOD1), produce proteins with an increased propensity to misfold and aggregate. A structure activity relationship of a lead scaffold exhibiting neuroprotective activity in a G93A-SOD1 mouse model for ALS has been further investigated in a model PC12 cellular assay. Synthesis of biotinylated probes at the N^1 nitrogen of the pyrazolone ring gave compounds (**5d**–**e**) that retained activity within 10-fold of the proton-bearing lead compound (**5a**) and were equipotent with a sterically less cumbersome N^1-methyl substituted analogue (**5b**). However, when methyl substitution was introduced at N^1 and N^2 of the pyrazolone ring, the compound was inactive (**5c**). These data led us to investigate further the pharmacophoric nature of the pyrazolone unit. A range of N^1 substitutions were tolerated, leading to the identification of an N^1-benzyl substituted pyrazolone (**5m**), equipotent with **5a**. Substitution at N^2 or excision of N^2, however, removed all activity. Therefore, the hydrogen bond donating ability of the N^2–H of the pyrazolone ring appears to be a critical part of the structure, which will influence further analogue synthesis.

Amyotrophic lateral sclerosis (ALS), commonly known as Lou Gehrig's disease, is a progressive and ultimately fatal neurodegenerative disease, with a worldwide prevalence of approximately 2 per 100,000. The disease generally first presents between 40 and 60 years of age; loss of motor neurons controlling voluntary actions results in progressive muscle paralysis and death attributed to respiratory failure, typically within 3–5 years of diagnosis.[1]

Currently, the only approved drug for ALS, riluzole, extends median survival by only 2–3 months.[2] Clearly, there is a need for new therapeutics; however, progress has been impeded because the underlying pathology of the disease remains unknown. There is strong evidence[3] that one pathophysiological mechanism in particular, that of toxic protein misfolding and/or aggregation, may trigger motor neuron dysfunction and loss. Mutations in familial ALS (FALS) patients that promote protein misfolding and aggregation include Cu/Zn superoxide dismutase type 1 (SOD1),[4] UBQLN2,[5] TAR DNA binding protein (TDP-43),[6] fused in sarcoma/translated in liposarcoma (FUS/TLS),[7] and angiogenin (ANG)[8] and, in addition, TDP-43 aggregates are seen in motor neurons of sporadic ALS (SALS) patients.[9]

The clinical phenotype and pathology of SALS, which accounts for 90% of all ALS cases, are indistinguishable from those of the familial form.[10] It has therefore been possible to make significant advances in studying the pathology of ALS through the investigation of the familial form of the disease,[11] which accounts for only approximately 10% of all cases, 20% of which are caused by missense mutations in the gene encoding for the enzyme SOD1. Recent studies[12] have now linked FALS to SALS through common SOD1 containing astrocytes, demonstrating that SOD1 is a viable target for both FALS and SALS, providing further impetus to the identification of compounds active in mutant SOD1 disease models.

We previously described[13] a high throughput screening method for the identification of compounds active in a PC12 cell model in which protein aggregation and cell death depended on the expression of G93A SOD1. One of the chemical scaffolds identified

Abbreviations: ALS, amyotrophic lateral sclerosis; FALS, familial amyotrophic lateral sclerosis; SALS, sporadic amyotrophic lateral sclerosis; SOD1, Cu/Zn superoxide dismutase 1.

* Corresponding author at: Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113, USA. Tel.: +1 847 491 5653; fax: +1 847 491 7713.

E-mail addresses: Agman@chem.northwestern.edu, r-silverman@northwestern.edu (R.B. Silverman).

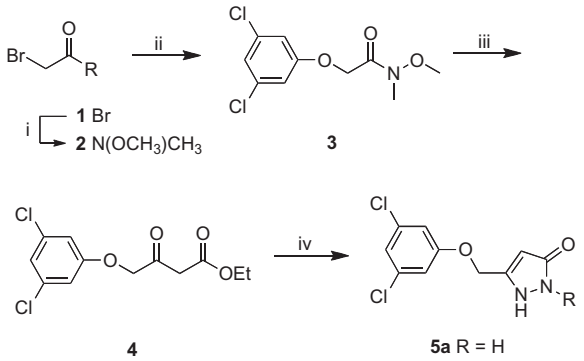
0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.114

in this screen was the arylsulfanylpyrazolones.[14] Although ineffective as a potential therapeutic because of the metabolic instability of the sulfanyl linker, this scaffold has served as a valuable lead for detailed structure–activity relationship (SAR) studies,[15] resulting in **5a** (Scheme 1, Table 1), which has an EC_{50} = 0.067 μM in our G93A-SOD1 cell assay and is active in a mutant SOD1 mouse model of ALS. Methylation of **5a** to **5b** (Table 1) or conjugation with biotin (**5d**) gave compounds with similar EC_{50} values, but dimethylation to **5c** gave an inactive compound. This intriguing result led us to investigate the cause for the inactivity of **5c** relative to much more sterically-demanding active compounds, such as **5b** and **5d**. Here, we describe the SAR profile of the pyrazolone ring; substitution, excision, and replacement of one or both of the two nitrogen atoms is described, and the pharmacophoric nature of the N^2-position is demonstrated.

The synthetic route delineated in Scheme 1 was modified in step iv to include the addition of suitably substituted hydrazine reagents, providing access to a wide range of N^1-substituted pyrazolone analogues (Table 1). Phenyl, heteroaryl, alkyl, and acyl-substituted pyrazolones were synthesized and their activities determined.

Alkyl substitution at the N^1-position of the pyrazolone ring is tolerated, albeit with an approximately 10-fold reduction in cell activity compared to **5a**. All of the N^1-substituted compounds were active except for **5f**, **5j**, **5k**, and **5o**. Steric hindrance, apparently, plays little or no role in the activity of the compounds synthesized; compounds with methyl (**5b**, EC_{50} = 0.67 μM), biotin (**5d**, EC_{50} = 0.56 μM) and tetraethyleneglycol-linked biotin (**5e**, EC_{50} = 0.67 μM) all display the same activity over a substituent length from 1 to 24 atoms including, in the case of biotin compounds **5d** and **5e**, the presence of a bulky tetrahydro-1*H*-thieno(3,4-*d*)imidazol-2(3*H*)-one ring.

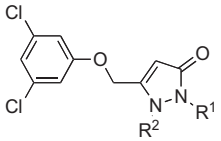
The inactivity of **5f** is intriguing because it is small and not bulky. As an explanation for this lack of activity, we considered the possibility that the compound exists in a bicyclic structure with the hydroxyl hydrogen bonded to the N^2 position, thereby forming a six-membered intramolecular hydrogen-bonded ring and blocking the hydrogen bond donating ability of the N^2 position. To support this hypothesis, the hydroxypropyl-substituted compound (**5g**) was synthesized from known 3-hydrazinylpropan-1-ol.[16] The corresponding hydrogen bonding in **5g** would lead to an unfavorable seven-membered ring, which therefore would not be expected to form, allowing the hydrogen bond donating ability of the N^2 position to be unobstructed. In agreement with this hypothesis, **5g** was active (EC_{50} = 0.34 μM). The stability of the proposed six-membered hydrogen bonded ring has been

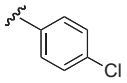
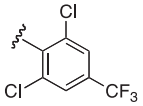
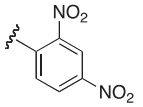
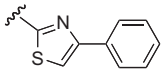
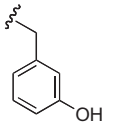
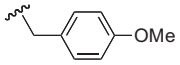
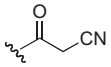
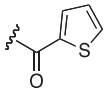
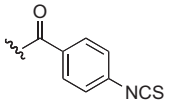
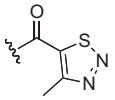
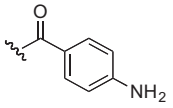
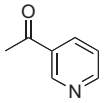


Reactants and conditions: i) TEA, (Me)N(OMe).HCl, DCM (65%); ii) 3,5-dichlorophenol, NaOEt, EtOH, heat (47%); iii) LiHMDS, EtOAc, THF,-78 °C (56%); iv) NH_2NHR, EtOH, RT (28%).

Scheme 1. Synthesis of lead compound **5a** via Weinreb amide intermediate **3**.

Table 1
SAR studies of substitution of the pyrazolone ring



Compound	R^1	R^2	EC_{50} (μM)
a	H	H	0.067
b	Me	H	0.67
c	Me	Me	>32[a]
d	Biotin	H	0.56
e	Peg4–Biotin	H	0.67
f	CH_2CH_2OH	H	>32
g	$CH_2CH_2CH_2OH$	H	0.34
h	Ph	H	1.03
i		H	1.73
j		H	>32
k		H	>32
l		H	1.00
m	Bn	H	0.13
n		H	0.36
o		H	>32
p	CHO	H	0.49
q	Ac	H	0.27
r		H	0.42
s		H	0.31
t		H	0.33
u		H	1.71
v		H	0.93
w		H	1.32

[a] >32 μM indicated EC_{50} not reached at the highest concentration used. All values are reported as the mean average of three determinations. Average Z′ factor value = 0.5.

estimated to be 20 kJ/mol[17] (4.8 kcal/mol) and 29 kJ/mol (6.9 kcal/mol),[18] which could account for the large difference in

activity between **5f** and **5g**. Explanations relating to solubility, cell permeability, or bioavailability are unlikely, given the one methylene difference from that of **5g**.

An alternative possibility regarding the importance of hydrogen bonding for activity is that the tautomeric enol form of the pyrazolone is the active form, and it is the enol hydroxyl that is essential for activity. If that were the case, there are two tautomeric forms, one that retains the N^1–H and one that retains the N^2–H (both still having the enol OH) (Fig. 1). If the OH were the important H-bond donor, the activity would not be affected if the compound were methylated at N^1 or N^2. In a related series of compounds from our group,[19] the N^1-methylated analogue was active, but the N^2-methylated analogue was not active. If the enol OH were relevant, both of those methylated compounds would have been active.

An increase in electron-withdrawing ability should have an important effect on activity because of its decrease in the pK_a of N^2–H and corresponding promotion of hydrogen bond donation ability. Suprisingly, phenyl substitution at N^1 attenuated the activity of the pyrazolone (**5h**, EC_{50} = 1.03 μM), allowing further characterization of the relatively expansive pocket environment the N^1 substituents inhabit. A further decrease in activity of analogue **5i** and total loss of activity with N^1-phenyl analogues **5j** and **5k**, containing multiple strong electron-withdrawing substituents was observed. An explanation for the reduced activity seen with **5h** and **5i** may be the steric bulk of the introduced phenyl ring, which impedes the N^2 position and prevents hydrogen bond donation. The lack of activity of **5j** and **5k** may be attributed to steric crowding by the ortho substituents, which completely block the hydrogen bond donating ability at N^2, further supporting our assertion that this position represents an important pharmacophore of the pyrazolone moiety. These results support the importance of N^2–H in its activity, presumably some hydrogen-bonding interaction with a target that is essential for activity. The N^1-phenylthiazole substituted analogue (**5l**) is equipotent (EC_{50} = 1.00 μM) with phenyl substituted pyrazolone **5h**, illustrating that five-membered aromatics and bulky bicyclics are tolerated at this position.

The moderate activity displayed by phenyl substitution, coupled with the relatively large steric pocket that N^1 substituents inhabit, suggested the synthesis of a benzyl-substituted pyrazolone, providing steric relief from the phenyl moiety by the methylene spacer. Compound **5m** was the most potent of the substituted pyrazolones yet synthesized, having an EC_{50} = 0.13 μM, essentially equivalent to that of **5a** (EC_{50} = 0.067 μM) within the reproducibility of the assay.[13] Substitution around the benzyl ring attenuates bioactivity; *meta*-hydroxybenzyl analogue **5n** exhibits an almost three-fold reduction in potency (EC_{50} = 0.36 μM). Introduction of a *para*-methoxyl substituent (**5o**) renders the compound inactive. It is not clear why the *para*-methoxyl functionality exerts a deactivating effect.

Acyl substitution appears to be well tolerated. Pyrazolones **5p**–**5t** have comparable EC_{50} values, and **5u**–**5w** are comparable but about one-third less potent. To aid in future target identification studies of these compounds phenylisothiocyanate **5t** was prepared. It was anticipated that this compound would act as a covalent linker, forming a bond with a suitably disposed residue within the target of action active site for use in subsequent affinity chromatography experiments. This was among the most potent of the acyl-substituted pyrazolones. Appendage of a biotin or affinity gel moiety to **5t** will determine if this approach will be successful in a protein pull-down experiment.

To further establish the pharmacophoric nature of the N^2 hydrogen bond donor group, a series of pyrazolone ring analogues was synthesized, which replaced N^2 and other positions around the pyrazolone ring with heteroatoms and/or methylene groups. Lactone **9** was obtained from the nucleophilic coupling of known bromo-substituted lactone **8**[20] and 3,5-dichlorophenol (Scheme 2) in analogy with compounds **5a**–**w**. The lactone, lacking the hydrogen bond donor ability of the pyrazolone nitrogen at N^2, was inactive in the assay, providing further evidence for the importance of the N^2 position for bioactivity.

Replacement of the pyrazolone ring with a cyclopentanone ring (**13**) was achieved through an analogous coupling of known iodide **12**[21] with 3,5-dichlorophenol (Scheme 3) to produce a compound devoid of both nitrogen atoms of the pyrazolone ring and of biological activity.

The isoxazol-3(2*H*)-one analogue (**17**) was synthesized (Scheme 4) using a modified procedure.[22] The lithium acetylide of propargyl bromide was treated with ethyl chloroformate to yield bromotetrolate **15**; addition of an aqueous methanolic solution of hydroxylamine and careful control of pH afforded bromomethylisoxazole **16**. Subsequent addition of 3,5-dichlorophenol provided **17**, which, lacking the crucial N^2 hydrogen bond donating group, displayed no activity.

Another ring system lacking the N^2 hydrogen bond donor is illustrated by benzyl protected unsaturated pyrolidinone **22** (Scheme 5). This was obtained by reduction of commercially available pyrrolidinone ester **18** to form alcohol **19**, followed by mesylation and nucleophilic addition of a bromide ion to furnish bromide **20** in good yield (42%) relative to the alternative Appel reaction (PBr_3, pyridine; 2% yield); addition of 3,5-dichlorophenol to **20** provided pyrrolidinone **21**. Subsequent addition of phenylselenyl bromide to the prepared enolate and selenoxide elimination provided unsaturated pyrrolidinone **22**, identical to the most potent analogue (**5m**) except lacking the crucial N^2–H. Again, both **21** and **22**, possessing no N^2 hydrogen bond donor, had no activity (EC_{50} = >32 μM).

In conclusion, we have shown that the N^2 pyrazolone position is essential for the cellular activity of this class of compounds. While other factors such as cell membrane penetration may play a role in the activity patterns of some of these compounds, a sufficient degree of evidence has been accumulated to support the hypothesis that the hydrogen bond donating N^2–H group is pharmacophoric in these compounds. SAR data have provided further information on the interactions of **5a** and its analogues. Steric considerations are of little importance around the pyrazolone N^1 area, suggesting a large open pocket or corridor within the target structure. Placement of a benzyl group β to the pyrazolone N^1 (**5m**) enhances its

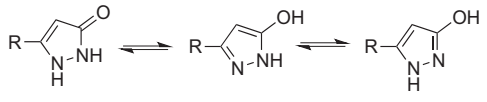
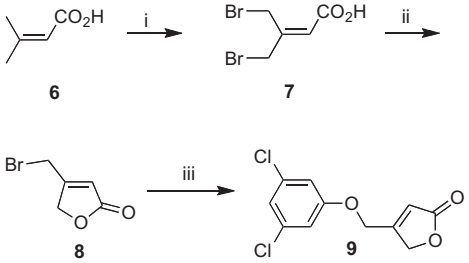
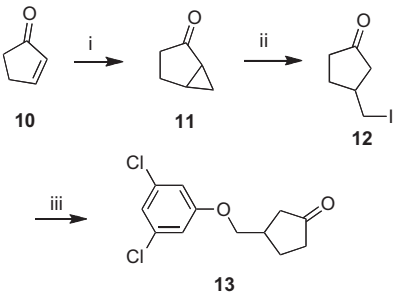


Figure 1. Tautomeric forms of the pyrazolones.



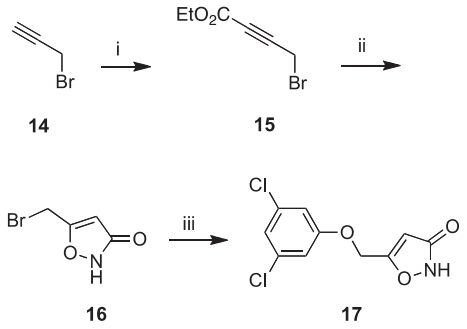
Reagents and Conditions: i) NBS, CCl_4, Benzoyl peroxide, reflux (98%); ii) 5% NaOH (61%); iii) NaOEt, EtOH, reflux (14%)

Scheme 2. Synthesis of lactone analogue **9**.



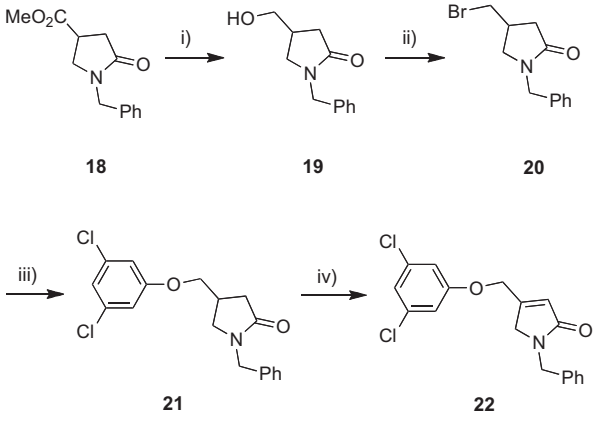
Reagents and Conditions: i) Me_3SOI, DMSO, NaH (33%); ii) Me_3SiCl, NaI, MeCN, RT (39%); iii) 3,5-dichlorophenol, NaOEt, EtOH, reflux (27%)

Scheme 3. Synthesis of cyclopentanone **13**.



Reagents and Conditions: i) a, ^{n}BuLi, THF, Et_2O, -40 °C; b, Ethyl chloroformate (11%); ii) NH_2OH, MeOH, -35 °C - RT (47%); iii) 3,5-dichlorophenol, NaOEt, EtOH, 70 °C (20%)

Scheme 4. Synthesis of 3-bromo-4,5-dihydroisoxazole analogue **17**.



Reagents and Conditions: i) $LiBH_4$, THF, RT (99%); ii) PBr_3, Py, DCM (2%) or MsCl, NEt_3 then LiBr (42%); iii) 3,5-dichlorophenol, NaOEt, DMF, reflux (24%); iv) a) LDA, PhSeBr, THF, -78°C, b) H_2O_2, H_2O, DCM (27%).

Scheme 5. Synthesis of protected pyrolidinone analogue **22**.

potency, suggesting the presence, within the target, of a hydrophobic pocket, possibly containing aromatic residues. The importance of the N^2–H group to participate in hydrogen bond donating interactions with the biological target serves to promote this scaffold as both a lead compound for further therapeutic investigation and as a probe compound to potentially identify the biological target for these compounds.

Acknowledgments

We thank the National Institutes of Health (Grant 1R43NS057849), the ALS Association (TREAT program), and the Department of Defense (Grant AL093052) for funding.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.114.

References and notes

1. Rothstein, J. D. *Anal. Neurol.* **2009**, *65*, S3.
2. Bensimon, G.; Lacomblez, L.; Meininger, V. N. *Engl. J. Med.* **1994**, *330*, 585.
3. Brown, R. H., Jr.; Robberecht, W. *Semin. Neurol.* **2001**, *21*, 131.
4. Kerman, A.; Liu, H. N.; Croul, S.; Bilbao, J.; Rogaeva, E.; Zinman, L.; Robertson, J.; Chakrabartty, A. *Acta Neuropathol.* **2010**, *119*, 335.
5. Deng, H. X.; Chen, W.; Hong, S. T.; Boycott, K. M.; Gorrie, G. H.; Siddique, N.; Yang, Y.; Fecto, F.; Shi, Y.; Zhai, H.; Jiang, H.; Hirano, M.; Rampersaud, E.; Jansen, G. H.; Donkervoort, S.; Bigio, E. H.; Brooks, B. R.; Ajroud, K.; Sufit, R. L.; Haines, J. L.; Mugnaini, E.; Pericak-Vance, M. A.; Siddique, T. *Nature* **2011**, *477*, 211.
6. Sreedharan, J.; Blair, I. P.; Tripathi, V. B.; Hu, X.; Vance, C.; Rogelj, B.; Ackerley, S.; Durnall, J. C.; Williams, K. L.; Buratti, E.; Baralle, F.; de Belleroche, J.; Mitchell, J. D.; Leigh, P. N.; Al-Chalabi, A.; Miller, C. C.; Nicholson, G.; Shaw, C. E. *Science* **2008**, *319*, 1668.
7. Kwiatkowski, T. J., Jr.; Bosco, D. A.; Leclerc, A. L.; Tamrazian, E.; Vanderburg, C. R.; Russ, C.; Davis, A.; Gilchrist, J.; Kasarskis, E. J.; Munsat, T.; Valdmanis, P.; Rouleau, G. A.; Hosler, B. A.; Cortelli, P.; de Jong, P. J.; Yoshinaga, Y.; Haines, J. L.; Pericak-Vance, M. A.; Yan, J.; Ticozzi, N.; Siddique, T.; McKenna-Yasek, D.; Sapp, P. C.; Horvitz, H. R.; Landers, J. E.; Brown, R. H., Jr. *Science* **2009**, *323*, 1205.
8. Greenway, M. J.; Andersen, P. M.; Russ, C.; Ennis, S.; Cashman, S.; Donaghy, C.; Patterson, V.; Swingler, R.; Kieran, D.; Prehn, J.; Morrison, K. E.; Green, A.; Acharya, K. R.; Brown, R. H., Jr.; Hardiman, O. *Nat. Genet.* **2006**, *38*, 411.
9. Chen-Plotkin, A. S.; Lee, V. M.-Y.; Trojanowski, J. Q. *Nat. Rev. Neurol.* **2010**, *6*, 211.
10. Bruijn, L. I.; Houseweart, M. K.; Kato, S.; Anderson, K. L.; Anderson, S. D.; Ohama, E.; Reaume, A. G.; Scott, R. W.; Cleveland, D. W. *Science* **1851**, *1998*, 281.
11. Pasinelli, P.; Brown, R. H., Jr. *Nat. Rev. Neurol.* **2006**, *7*, 710.
12. Haidet-Phillips, A. M.; Hester, M. E.; Miranda, C. J.; Meyer, K.; Braun, L.; Frakes, A.; Song, S.; Likhite, S.; Murtha, M. J.; Foust, K. D.; Rao, M.; Eagle, A.; Kammesheidt, A.; Christensen, A.; Mendell, J. R.; Burghes, A. H.; Kaspar, B. K. *Nat. Biotechnol.* **2011**, *29*, 824.
13. Benmohamed, R.; Arvanites, A. C.; Kim, J.; Ferrante, R. J.; Silverman, R. B.; Morimoto, R. I.; Kirsch, D. R. *Amyotroph Lateral Scler.* **2011**, *12*, 87.
14. Chen, T.; Benmohamed, R.; Arvanites, A. C.; Ralay Ranaivo, H.; Morimoto, R. I.; Ferrante, R. J.; Watterson, D. M.; Kirsch, D. R.; Silverman, R. B. *Bioorg. Med. Chem.* **2011**, *19*, 613.
15. Chen, T.; Benmohamed, R.; Kim, J.; Smith, K.; Amante, D.; Morimoto, R. I.; Kirsch, D. R.; Ferrante, R. J.; Silverman, R. B. *J. Med. Chem.* **2012**, *55*, 515.
16. Muehlebach, M.; Boeger, M.; Cederbaum, F.; Cornes, D.; Friedmann, A. A.; Glock, J.; Niderman, T.; Stoller, A.; Wagner, T. *Bioorg. Med. Chem.* **2009**, *17*, 4241.
17. Porath, B.; Rademacher, P.; Boese, R.; Blaser, D. Z. *Naturforsch.* **2002**, *57*, 365.
18. Ferguson, L. N. *The modern structural theory of organic chemistry*; Prentice-Hall: Englewood Cliffs, NJ, 1963. p. 128.
19. Zhang, Y.; Kirsch, D.R.; Silverman, R.B. Manuscript in preparation.
20. Liu, G. Z.; Xu, H. W.; Chen, G. W.; Wang, P.; Wang, Y. N.; Liu, H. M.; Yu, D. Q. *Bioorg. Med. Chem.* **2010**, *18*, 1626.
21. Dieter, R. K.; Pounds, S. *J. Org. Chem.* **1982**, *47*, 3174.
22. Orth, R.; Bottcher, T.; Sieber, S. A. *Chem. Commun.* **2010**, *46*, 8475.