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Chloro-oxime derivatives as novel small molecule chaperone amplifiers

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ABSTRACT

Chloro-oxime derivatives were investigated as novel small molecule chaperone amplifiers. Lead optimization led to the discovery of compounds that displayed potent HSF1 activation activity, significant cytoprotection in MG-132 stress, ER stress and PolyQ stress cell models (EC_{50} < 10 μM).

Neurodegenerative disease is a condition in which cells of the brain and spinal cord become dysfunctional and ultimately die, leading to disabilities in memory, movement, sensory processing and decision making. At present there are few effective pharmaceuticals for the wide range of neurodegenerative diseases and none of them can provide a cure. Therefore there are significant unmet medical needs to find better therapies and approaches to treat neurodegenerative diseases.

In recent years, substantial experimental evidence has accumulated indicating that protein misfolding may be a universal underlying mechanism in the pathogenesis of several neurodegenerative diseases including sporadic and familial amyotrophic lateral sclerosis (ALS and FALS), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and related polyglutamine (polyQ) expansion diseases as well as transmissible spongiform encephalopathy (TSE) diseases.[1–5] Heat shock proteins (HSPs) are molecular chaperones whose expressions are induced as part of the cellular stress response. HSPs recognize and either repair or destroy misfolded proteins.

Increased expression of many HSPs is regulated at least in part by the activation of heat shock transcription factor-1 (HSF1).[6–9] Up-regulation of heat shock proteins (HSPs) via small molecules has shown great therapeutic promise in a number of disease areas where the probable common cause is the accumulation of misfolded proteins.[10–12] For example, Arimoclomol is now in phase II/III clinical trial in ALS patients,[11] and Celastrol is in clinical trial for treating rheumatoid arthritis.[12] Several therapeutically active small molecules that increase cellular HSP levels have been reported (e.g., celastrol, radicicol, galdanamycin, 17-AAG). These compounds seem to mediate their effect on HSPs by indirect activation of HSF1.[13] Other compounds, exemplified by bimoclomol arimoclomol, and iroxanadine do not activate HSF1 or induce HSP expression in normal unstressed cells, but only amplify the already activated chaperone response in stressed or diseased cells that accumulate misfolded proteins.[10] Such a unique molecular mechanism that specifically targets stressed cells potentially offers greater safety than compounds that induce the chaperone response in all cells indiscriminately.[10] Our interest is to discover more potent small molecule chaperone amplifiers to effectively treat neurodegenerative diseases and other diseases of protein misfolding.

Previous investigation revealed that the transcriptional activity of HSF1 in cultured cells correlated well with the formation of intranuclear HSF1 granules.[14,15] Hence we developed a quantitative high-throughput assay for HSF1 transcriptional activity using

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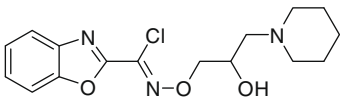
Table 1
The percentage of HSF1 granule formation (average) with or without heat shock stress



1

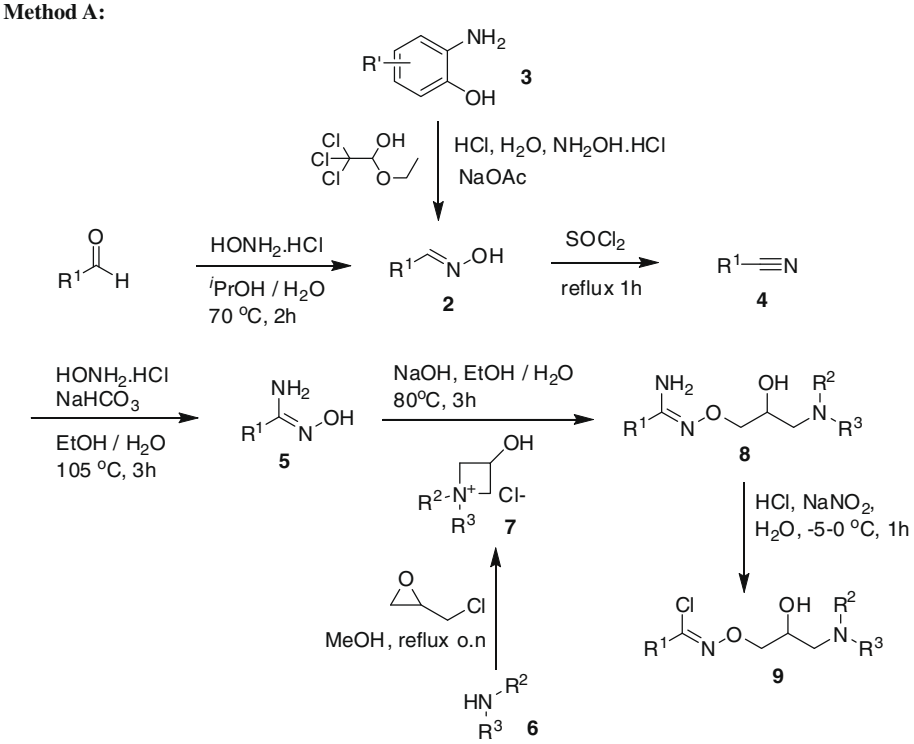
% of granule formation (standard deviation)	DMSO	Compound **1**
No heat shock stress (37 °C)	5.6 (±0.7)	6.7 (±0.91)
Heat shock stress (41 °C)	7.3 (±1.2)	58.0 (±4.0)

high content image-based HSF1 granule formation in heat-shocked HeLa cells in order to screen for potential amplifiers of HSF1.[16] We began our screening efforts with an in-house directed library containing compounds structurally similar to arimoclomol, bimoclomol, and iroxanadine. We discovered that chloro-oxime **1** showed potent HSF1 amplification activity (Table 1). Treatment with **1** resulted in a significant increase in the percentage of cells with active HSF1 granules following mild heat shock compared to cells treated with **1** without heat shock (58% vs 7.3%, respectively). However, there is no noticeable change in granule formation when cells were treated with **1** at non-stress conditions (6.7% vs 5.6%), suggesting that **1** by itself is not a stressor. Thus, **1** is a bona fide amplifier of HSF1 activity, not a de novo activator of HSF1. In addition, **1** also increased HSP70 expression under identical heat shock condition (41 °C with 2 h recovery time) with maximum percentage activation of 51% (EC_{50} = 32.9 μM) indicating that compound **1** is a HSF1/HSP70 co-inducer.[17] We focused on lead optimization of this chemical series aiming to improve activity for chaperone amplification that would provide cytoprotection in neurodegenerative disease cell models for potential use as targeted chaperone therapies.

The synthesis of the chloro-oxime derivatives was fairly straightforward and there are a number of synthetic methods (**Methods A** and **B**, Schemes 1 and 2)[18] available to prepare the compounds shown in Tables 2–4.

Oximes **2** were synthesized by the reaction of commercially available aldehydes with hydroxylamine under heating conditions in >90% yield. Alternatively oximes **2** could be provided by a one step synthesis directly from *ortho*-hydroxyanilines **3** by treatment with 2,2,2-trichloro-1-ethoxyethanol in good yields.[19] Oximes **2** were then treated with thionyl chloride to form nitriles **4** which were then converted to hydroxyimidamides **5** by treating with hydroxylamine in good yield. Compounds **5** were then converted to the key intermediates **8** in moderate yields by treating with quaternary azetidinium salts **7** in the presence of a base under heating. Azetidinium salts **7** were synthesized by reacting epichlorohydrin with secondary amines **6** under heating in moderate yields.[20] The key intermediates **8** were then treated with $NaNO_2$ and HCl to provide the desired chloro-oxime derivatives **9** in low to good yields. Alternatively in **Method B** (Scheme 2), hydroxyimidamides **5** were directly treated with epichlorohydrin to form compounds **10** in moderate to good yields, which were then treated with either amines **6** or methyl sulfonamide to form key intermediates **8** or **11** in ⩾80% yields. Intermediates **8** and **11** were then converted to the desired products **9** and **12** respectively in the same way as that described in **Method A**. Methods **A** and **B** diverge at compounds **5**. For most substrates, both methods worked equally well. However, in some cases reactions of amino-oximes **5** with **7** in **Method A** gave very low yields while **Method B** resulted in fairly good yields. On the other hand, **Method A** is more convergent.

Our lead optimization focused on the modification of the left side (R^1), right side (R^2 and R^3) of the molecules **9** and the replacement of the Cl moiety in the chloro-oxime core to study the structure and activity relationship (SAR). We used the high content image-based HSF1 granule formation in HeLa cells to measure the HSF1 activation activity.[21] Additionally, we developed the MG-132 cell stress model in SK-N-SH neuroblastoma cells to systematically measure the cytoprotection of the analogs in this series.[22] MG-132 is a potent and selective 26S proteasome inhibi-



Scheme 1. Synthetic method A for chloro-oxime derivative preparation.