



Dihydroquinazolines enhance 20S proteasome activity and induce degradation of α -synuclein, an intrinsically disordered protein associated with neurodegeneration

Taylor J. Fiolek^a, Christina L. Magyar^b, Tyler J. Wall^b, Steven B. Davies^b, Molly V. Campbell^b, Christopher J. Savich^b, Jetze J. Tepe^{a,*}, R. Adam Mosey^{b,*}

^a Department of Chemistry, Michigan State University, East Lansing, MI 48824, United States

^b Department of Chemistry, Lake Superior State University, Sault Sainte Marie, MI 49783, United States

ARTICLE INFO

Keywords:

Proteasome
Activation
Neurodegenerative diseases
Parkinson's disease
Undruggable

ABSTRACT

Aggregates or oligomeric forms of many intrinsically disordered proteins (IDPs), including α -synuclein, are hallmarks of neurodegenerative diseases, like Parkinson's and Alzheimer's disease, and key contributors to their pathogenesis. Due to their disordered nature and therefore lack of defined drug-binding pockets, IDPs are difficult targets for traditional small molecule drug design and are often referred to as "undruggable". The 20S proteasome is the main protease that targets IDPs for degradation and therefore small molecule 20S proteasome enhancement presents a novel therapeutic strategy by which these undruggable IDPs could be targeted. The concept of 20S activation is still relatively new, with few potent activators having been identified thus far. Herein, we synthesized and evaluated a library of dihydroquinazoline analogues and discovered several promising new 20S proteasome activators. Further testing of top hits revealed that they can enhance 20S mediated degradation of α -synuclein, the IDP associated with Parkinson's disease.

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are predicted to become the second-most prevalent cause of death in the next 20 years, making the discovery of disease-modifying therapies an urgent need.^{1,2} Currently there are no such treatments for any neurodegenerative diseases, further exacerbating the problem.^{3–5} Although the pathogenesis of these diseases is unclear, the accumulation and aggregation of intrinsically disordered proteins (IDPs) in affected cells is a common feature among them. IDPs are a class of soluble proteins that generally function as regulatory and signaling proteins and are unique in their ability to interact with numerous binding partners due to their disordered nature.^{6,7} Unfortunately, when these IDPs accumulate within neurons these same features contribute to their aggregation, resulting in harmful signaling events and neurotoxicity.^{8–12} Additionally, IDPs are difficult to target using traditional small molecule drug design because of a lack of defined binding pockets.

The proteasome is a large enzyme complex that is responsible for the proteolytic degradation of misfolded, redundant and damaged proteins within the cell.^{13–17} The proteasome exists in equilibrium between the

26S and 20S forms and both serve important roles in maintaining cellular homeostasis. This equilibrium is dictated by the reversible docking of 19S regulatory units (caps) on the 20S core particle to form the fully assembled 26S proteasome. These caps give the 26S proteasome the ability to recognize and unfold ubiquitinated proteins followed by their proteolytic degradation. The 20S proteasome lacks these 19S caps and constitutes the catalytic core particle of the proteasome. This core particle consists of four stacked concentric rings, two α -rings and two β -rings, each made up of 7 subunits.^{18,19} The top and bottom α -rings act as a gating mechanism to restrict access to the inner catalytic core of the proteasome.^{20,21} This inner core is made up of the two β -rings and contains 6 total catalytic sites with two chymotryptic-like, two trypsin-like and two caspase-like threonine protease activities. The 20S proteasome is, unlike the 26S, unable to recognize ubiquitinated proteins, nor can it unfold and degrade structured proteins.^{18–21} Consequently, the 20S is restricted to the degradation of IDPs and other unfolded proteins. As a result, the 20S plays a critical role in the regulation of free cytosolic levels of IDPs.^{13,17,22}

IDP levels can become dysregulated as we age due to oxidative stress,

* Corresponding authors.

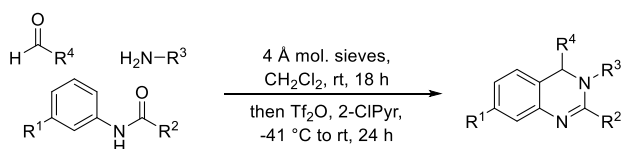
E-mail addresses: tepe@chemistry.msu.edu (J.J. Tepe), rmosey@lssu.edu (R.A. Mosey).

<https://doi.org/10.1016/j.bmcl.2021.127821>

Received 23 November 2020; Received in revised form 5 January 2021; Accepted 18 January 2021

Available online 27 January 2021

0960-894X/© 2021 Elsevier Ltd. All rights reserved.



Scheme 1. Multicomponent synthesis of dihydroquinazolines.

reduced proteasome activity and changes in IDP production.^{23–29} The result is accumulation and aggregation of IDPs like α -synuclein (α -syn) or amyloid beta ($A\beta$) as seen in PD and AD, respectively.^{10,12,30–38} Due to this role in the regulation of cellular IDP levels, enhancing 20S proteasome-mediated proteolysis has recently emerged as a potential therapeutic strategy for the treatment of neurodegenerative diseases.^{39–44}

Our lab recently reported the discovery of a novel 20S proteasome activator, TCH-165, which induces a conformational change in the α -ring of the 20S proteasome.^{45,46} This leads to an “open-gate” conformation that allows easier access of IDP substrates to the catalytic core of the 20S, thereby enhancing the rate of their degradation. This method for 20S proteasome enhancement shows potential as a new therapeutic strategy by which accumulation and subsequent aggregation of IDPs can be prevented.^{40,45,47} Despite findings like these, there are still relatively few reported small molecule activators of the 20S proteasome⁴⁸ and many still suffer from limitations, such as low potency, off-target effects and poor drug-like properties.^{40,45,49–54} The continued exploration of 20S proteasome activation as a therapeutic method for neurodegenerative diseases will require additional molecular scaffolds to be explored to identify new lead molecules for testing in model systems. As part of an effort to expand upon the imidazoline-mediated allosteric proteasome modulators,^{55–57} we screened a range of structural motifs for new 20S proteasome enhancers. Herein, we report on the structure activity relationship (SAR) and efficacy of a novel class of dihydroquinazoline-based 20S proteasome activators.

The synthesis of dihydroquinazolines is accomplished via our recently reported one-pot multicomponent reaction of amides, amines, and aldehydes (Scheme 1).⁵⁸ This method involves *in situ* imine formation from an amine and an aldehyde in the presence of molecular sieves, followed by tandem assembly of the heterocyclic ring through successive Tf_2O -mediated amide dehydration, imine insertion, and Pictet-Spengler-like cyclization. The multicomponent nature of the method

permits the construction of highly diverse dihydroquinazolines due to the compatibility of a wide range of simple starting materials. A small library of dihydroquinazolines was generated using the multicomponent method to probe the ability of members of this class of compounds to activate the 20S proteasome. Compounds chosen to populate the library differed in their structural features at the 7-, 2-, and 3-positions (R^1 , R^2 , and R^3 , respectively) of the heterocyclic scaffold (Figure 1). Variation at the 7- and 2-positions was accomplished using select amides (e.g. 1–9), while the substituents at the 3-position were introduced using chosen amines (e.g. 10–24). R^1 and R^2 groups introduced from the starting amides provided preliminary structure activity relationship information which was utilized for the construction of the remaining members of the compound library in which the R^3 group was varied. Simple alkyl and alkoxy substituents were explored at R_1 , along with the absence of any additional group at this location (e.g. 1–4), and the investigated R^2 substituents included alkyl and cycloalkyl groups to compare them to the aromatic counterpart (e.g. 5–9 vs 2). A range of R^3 substituents were installed to include aryl and heteroaryl groups (e.g. 10–13), tethered heteroaryl groups (e.g. 14–15), and alkyl groups with varying ring and heteroatom placement (e.g. 16–24).

Screening of this small library of dihydroquinazolines was performed in two stages. In the first stage, each compound was screened at 3 concentrations (3, 10 and 30 μM) to select lead agents.

Secondly, lead agents were further analysed using full concentration responses (6-point titration ranging from 1.25 μM –40 μM) for each of the three proteolytic activities of the 20S proteasome and a combination thereof. The proteolytic activity of the 20S proteasome can be monitored *in vitro* by measuring the cleavage of fluorogenic peptide substrates for the different catalytic sites as an increase in 7-amino-4-methylcoumarin (AMC) fluorescence overtime.^{40,43–45,49,59} A combination of chymotrypsin-like (CT-L, Suc-LLVY-AMC), tryptic-like (T-L, Boc-LRR-AMC) and caspase-like (Casp-L, Z-LLE-AMC) peptide substrates were used in equal amounts to screen compounds 1–24 for overall 20S activity. Pure human 20S proteasome was pre-treated with 3, 10 or 30 μM of one of the analogues or DMSO (vehicle control) for 15 min at 37 °C. To each sample was then added a mixture of the three substrates (13.3 μM each). The release of AMC was monitored as fluorescence overtime for 1 h and the resulting 20S activity changes were determined by comparing to the untreated 20S and calculating the fold-increase in activity for each analogue at a given concentration (Table 1, 30 μM and Table S1, 3 and 10 μM).

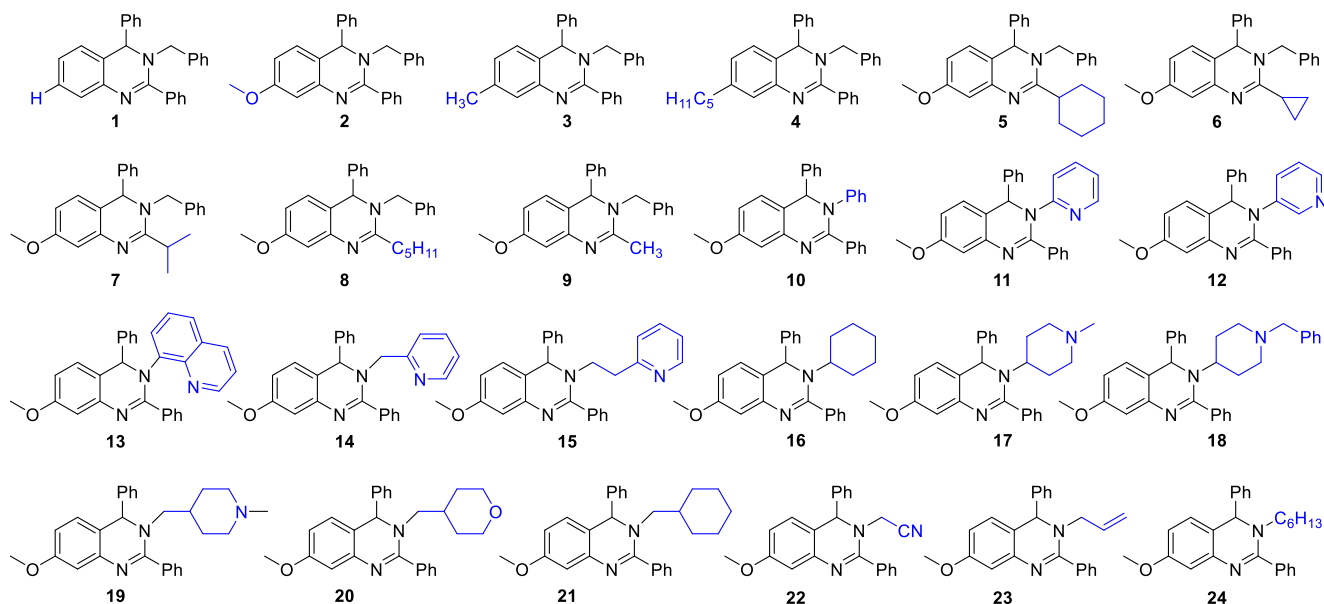


Fig. 1. Structures of dihydroquinazoline analogues.

Table 1Compounds 1–24 ranked by fold enhancement of 20S activity at 30 μ M.

Compound	Fold increase over vehicle (30 μ M)
10	9.5
21	8.2
3	8.1
2	7.9
18	7
11	6.9
16	6.7
5	6.5
12	5.9
13	5.3
1	4
8	2.8
15	2.8
4	2.8
6	2.7
22	2.7
7	2.3
23	2.3
14	2
9	1.8
24	1.6
20	1.6
19	1.2
17	1

The data collected from this screen (Table 1 and see Table S1 for 3 and 10 μ M results) shows a few insightful trends in the SAR of the dihydroquinazolines. Small changes in substitution at the 7-position appear to have a significant effect on activity of the dihydroquinazolines. Compound 1, which displays a 4-fold (i.e. 400%) increase over background 20S activity, lacks a substituent at the 7-position but is otherwise identical to compounds 2 (7.9-fold increase) and 3 (8.1-fold

increase). This small change results in a reduction in 20S activity from 8-fold enhancement down to a 4-fold enhancement at 30 μ M. Similarly, the addition of a longer alkyl chain on compound 4 resulted in a steep drop in 20S activity to 2.8-fold at 30 μ M. Changes at the 2-position show similar effects to that of the 7-position, where most substitutions other than a phenyl group (compounds 5–9) caused marked decreases in 20S activity. All have <3-fold activation at 30 μ M, apart from compound 5 (6.5-fold increase).

Substitutions at the 3-position showed more flexibility to changes than either the 7- or 2-positions, while still having a significant effect on the relative 20S activities of the analogues. Many of the most potent analogues, like compounds 10, 3, 2 and 5 (9.5, 8.1, 7.9 and 6.5-fold increase of 20S activity, respectively), contain a phenyl or benzyl functionality at the 3-position. Other similarly sized and shaped substituents like cyclohexane (compound 16 (6.7-fold)) and pyridine compounds 11 (6.9-fold) and 12 (5.9-fold)) also provided some of the most potent analogues. Interestingly, larger substituents at the 3-position as seen in compounds 18 (7-fold) and 13 (5.3-fold) also yielded potent analogues, suggesting that additional functionalities may be incorporated here for further optimization if necessary. The substitution of the phenyl or benzyl groups for some other heterocycles such as *N*-methyl piperidine (compounds 17 (1-fold) and 19 (1.2-fold)), tetrahydropyran (compound 20 (1.6-fold)) or even a pyridine linked by a methylene group in compound 14 (2-fold) lead to significant decreases in 20S activity. This suggests that placement of heteroatoms at the 3-position may disrupt hydrophobic interactions in that region. The difference in activity shown between 17 and 18 could be caused by a disruption of hydrophobic interactions with the addition of the piperidine nitrogen, which could then be replaced by new interactions made by the phenyl group in 18. The addition of non-cyclic substituents at the 3-position (compounds 22, 23 and 24) resulted in very little 20S activity

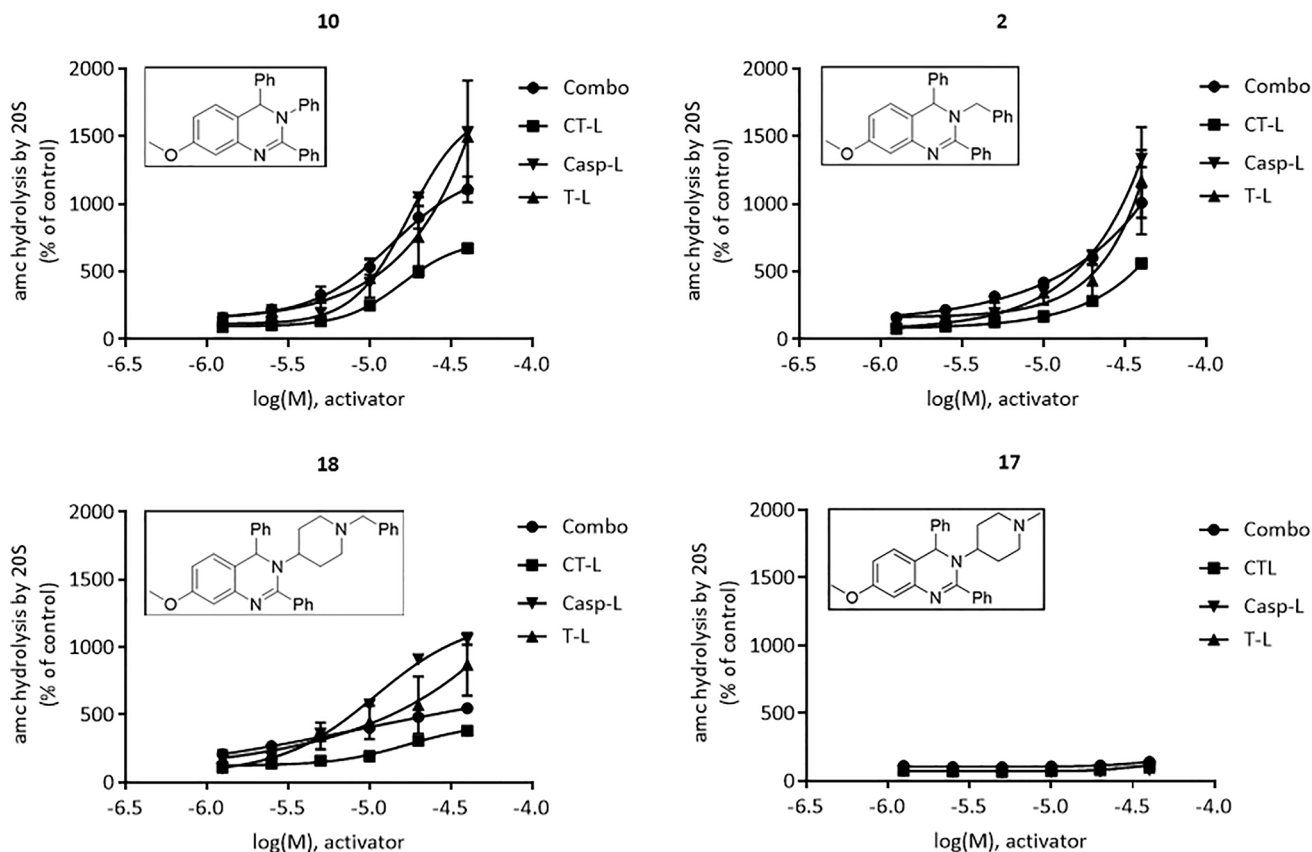


Fig. 2. Concentration response (0–40 μ M) from fluorogenic peptide digestions with compounds 2, 10, 17 and 18. Error bars denote standard deviation. These data were collected in triplicate.

Table 2

Detailed analysis of 20S activation by select dihydroquinazoline analogues.

Compound	Combo		CT-L		Casp-L		T-L	
	AC ₂₀₀ (μM)	Max Fold	AC ₂₀₀ (μM)	Max Fold	AC ₂₀₀ (μM)	Max Fold	AC ₂₀₀ (μM)	Max Fold
10	2.3	11.1	8.1	6.7	5.8	15.3	2.5	15.0
2	2.0	10.1	12.9	5.6	5.6	13.4	5.0	11.7
18	1.3	5.5	10.5	3.8	2.8	10.6	1.7	8.7
17	N/A	1.4	N/A	1.1	N/A	0.8	N/A	1.0

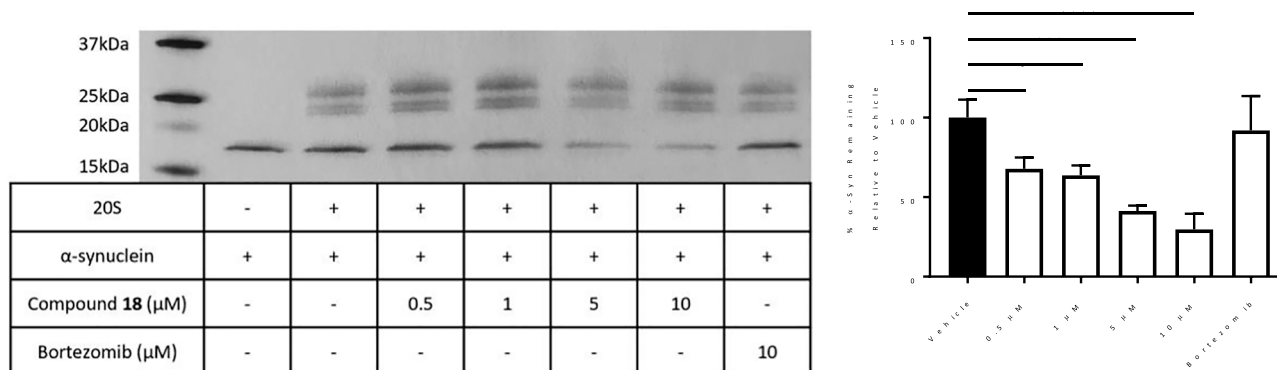


Fig. 3. *In vitro* digestion of purified α -synuclein with human 20S proteasome and enhancement by compound **18**. Representative silver stain of these α -synuclein digestions and quantification of three trials (see Fig. S1 for other replicates). 20S proteasome subunits were used as a loading control in the quantifications (right). These data were collected in triplicate ($n = 3$). Error bars denote standard deviation. Ordinary one-way ANOVA statistical analysis was used to determine statistical significance (ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

(2.7, 2.3 and 1.6-fold increase in 20S activity, respectively) in all cases suggesting that larger hydrophobic groups at the 3-position are likely required for 20S activity.

After analyzing the results in Table 1, three of the most promising analogues were selected for further studies into their 20S activity. Compounds **10**, **2** and **18** were selected to be carried forward based on their fold increase (Table S1, fold 20S activity increase > 200% at 3.0 μM) and the highest Max-Fold activities (Table S1, at high dose of 30 μM). Compound **17** was also carried forward to use as a negative control since it had no discernible activity towards the 20S. These compounds were then tested to obtain a full concentration–response (Fig. 2) of their activities towards the 20S proteasome using each of the three substrates for the three catalytic sites individually and the combination of the three substrates. This was done to ensure that each of the selected compounds activate the 20S proteasome at all three catalytic sites, which is critical for effective IDP degradation, as these proteins are likely to contain multiple cleavage sites for each. Previously identified 20S proteasome activators that were only able to activate a single catalytic site showed poor enhancement of IDP degradation *in vitro* when compared to those that activated all three catalytic sites.⁴⁰

Using the data in Figure 2, the concentration at which 20S activity was doubled (AC₂₀₀) was calculated for each compound using each substrate and the combination of the three substrates (Table 2). Because of variations in the maximum fold enhancement between 20S enhancers, AC₂₀₀ values allow for easy comparisons to be made between activators. It was found that each of the active compounds (**10**, **2** and **18**) achieved both high maximum fold increases (>500%) in 20S activity and doubled 20S activity in the combination at low μM concentrations.

Although the three compounds showed near equipotent activities, compound **18** was selected to move forward with due to its lowest overall AC₂₀₀ values (Table 2: combo, AC₂₀₀ 1.3 μM) and the individual activities of the catalytic sites of the 20S. The efficacy of compound **18** was tested by observing its ability to enhance 20S-mediated degradation of α -synuclein, the IDP associated with the development of Parkinson's Disease. Briefly, the 20S proteasome was incubated with compound **18**,

followed by addition of pure human α -synuclein. This mixture was then incubated for 4 h at 37 °C. The digestions were analyzed using silver stain and quantified (Fig. 3 and Fig. S1) to determine the ability of compound **18** to enhance IDP degradation by the 20S. It was found that compound **18** effectively enhanced the rate of degradation of α -synuclein by the 20S *in vitro* in a concentration dependant manner. As a control, we used the proteasome inhibitor, bortezomib, which prevented α -synuclein degradation, confirming that the clearance of α -synuclein in Fig. 3 is a proteasome mediated event. This shows that the prior peptide substrate-based results are likely to translate to the degradation of full IDPs and that dihydroquinazolines represent a promising new lead from which potent 20S activators that enhance IDP degradation can be developed.

In conclusion, this study demonstrates that dihydroquinazolines represent a promising scaffold from which potent 20S activators can be developed. Additionally, recently developed synthetic methods allow for access to a broad scope of dihydroquinazoline analogues, allowing for exploration of a variety of different substituents and substitution patterns. Among the analogues tested, we found several active compounds and a few of the most potent 20S activators identified to date. Further optimization and testing of dihydroquinazoline analogues may yield even more potent and drug-like leads, which can assist in the exploration of 20S activation as a novel therapeutic method.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully appreciate assistance from Thomas Dexheimer at the Assay Development and Drug Repurposing Core Facility at Michigan State University. The authors gratefully acknowledge financial support for this work from the National.

Institutes of Aging 1R01 AG066223-01A1 (JJT), National Institute of General Medical Sciences, T32GM092715 (TF) of the National Institutes of Health, and the National Science Foundation (MRI award 1626523).

Appendix A. Supplementary data

Supplemental information for this paper includes full compound characterization, detailed experimental procedures, and reproducibility of experiments. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmcl.2021.127821>.

References

- Gammon K. Neurodegenerative disease: brain windfall. *Nature*. 2014;515:299–300.
- Gitler AD, Dhillon P, Shorter J. *DMM Dis Model Mech*. 2017;10:499–502.
- Dang CV, Reddy EP, Shokat KM, Soucek L. Drugging the ‘undruggable’ cancer targets. *Nat Rev Cancer*. 2017;17:502–508.
- Lazo JS, Sharlow ER. Drugging Undruggable Molecular Cancer Targets. *Annu Rev Pharmacol Toxicol*. 2016;56:23–40.
- Hu G, Wu Z, Wang K, Uversky VN, Kurgan L. Untapped Potential of Disordered Proteins in Current Druggable Human Proteome. *Curr Drug Targets*. 2016;17:1198–1205.
- Dunker AK, Lawson JD, Brown CJ, et al. Intrinsically disordered protein. *J Mol Graph Model*. 2001;19:26–59.
- DeForte S, Uversky V, DeForte S, Uversky VN. Order, Disorder, and Everything in Between. *Molecules*. 2016;21:1090.
- Babu MM, van der Lee R, de Groot NS, Sponser J. *Curr Opin Struct Biol*. 2011;21:432–440.
- R. Berrocal, V. Vasquez, S. R. KRS, B. S. Gadad, R. KS, *Mol Neurobiol* 2015, 51, 1417–1431.
- Korsak M, Kozyreva T. Springer. *Cham*. 2015:401–421.
- Uversky VN, Na I, Landa KS, Schenck RO. Highly disordered proteins in prostate cancer. *Curr Protein Pept Sci*. 2017;18:453–481.
- Uversky VN, Oldfield CJ, Dunker AK. Intrinsically Disordered Proteins in Human Diseases: Introducing the D² Concept. *Annu Rev Biophys*. 2008;37:215–246.
- Ben-Nissan G, Sharon M. Regulating the 20S proteasome ubiquitin-independent degradation pathway. *Biomolecules*. 2014;4:862–884.
- Jung T, Höhn A, Grune T. The proteasome and the degradation of oxidized proteins: Part III—Redox regulation of the proteasomal system. *Redox Biol*. 2014;2:388–394.
- Höhn TJA, Grune T. *Redox Biol*. 2014;2:99–104.
- Tanaka K, Mizushima T, Saeki Y. *Biol Chem*. 2012;393:217–234.
- Kumar Deshmukh F, Yaffe D, Olshina M, Ben-Nissan G, Sharon M. The Contribution of the 20S Proteasome to Proteostasis. *Biomolecules*. 2019;9:190.
- Budenholzer L, Cheng CL, Li Y, Hochstrasser M. *J Mol Biol*. 2017;429:3500–3524.
- Groll M, Ditzel L, Löwe J, et al. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature*. 1997;386:463–471.
- Finley D, Chen X, Walters KJ, Gates. Channels, and Switches: Elements of the Proteasome Machine. *Trends Biochem Sci*. 2016;41:77–93.
- Groll M, Bajorek M, Köhler A, et al. A gated channel into the proteasome core particle. *Nat Struct Biol*. 2000;7:1062–1067.
- Erales J, Coffino P. Ubiquitin-independent proteasomal degradation. *Biochim Biophys. Acta - Mol. Cell Res*. 2014;1843:216–221.
- Ding Q, Cecarini V, Keller JN. Interplay between protein synthesis and degradation in the CNS: physiological and pathological implications. *Trends Neurosci*. 2007;30:31–36.
- Ding Q, Dimayuga E, Keller JN. Proteasome Regulation of Oxidative Stress in Aging and Age-Related Diseases of the CNS. *Antioxid Redox Signal*. 2006;8:163–172.
- Chondrogianni N, Sakellari M, Lefaki M, Papaevgeniou N, Gonos ES. Proteasome activation delays aging in vitro and in vivo. *Free Radic Biol Med*. 2014;71:303–320.
- Chondrogianni N, Georgila K, Kourtis N, Tavernarakis N, Gonos Efstathios S. Enhanced proteasome degradation extends *Caenorhabditis elegans* lifespan and alleviates aggregation-related pathologies. *Free Radic Biol Med*. 2014;75:518.
- Bulteau A-L, Szewda LI, Friguet B. Age-Dependent Declines in Proteasome Activity in the Heart. *Arch Biochem Biophys*. 2002;397:298–304.
- E. Gonos, in *Advances in experimental medicine and biology*, 2015, vol. 821, pp. 7–7.
- Keller JN, Gee J, Ding Q. The proteasome in brain aging. *Ageing Res. Rev*. 2002;1:279–293.
- Longhena F, Spano P, Bellucci A. Springer. *Cham*. 2017:85–110.
- Uversky VN. Wrecked regulation of intrinsically disordered proteins in diseases: pathogenicity of deregulated regulators. *Front Mol Biosci*. 2014;1:6.
- Uversky VN. Targeting intrinsically disordered proteins in neurodegenerative and protein dysfunction diseases: another illustration of the D(2) concept. *Expert Rev Proteomics*. 2010;7:543–564.
- V. N. Uversky, *Synuclein Misfolding and Neurodegenerative Diseases*, 2008, vol. 9.
- Levine ZA, Larini L, LaPointe NE, Feinstein SC, Shea J-E. Regulation and aggregation of intrinsically disordered peptides. *Proc Natl Acad Sci USA*. 2015;112:2758–2763.
- Raychaudhuri S, Majumder P, Sarkar S, Giri K, Mukhopadhyay D, Bhattacharyya NP. Huntingtin interacting protein HYPK is intrinsically unstructured. *Proteins Struct Funct Bioinforma*. 2007;71:1686–1698.
- Uversky VN, Oldfield CJ, Midic U, et al. Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. *BMC Genomics*. 2009;10(Suppl 1):S7.
- Uversky VN. in *Protein Folding and Misfolding: Neurodegenerative Diseases*. Netherlands, Dordrecht: Springer; 2009:21–75.
- Midic U, Oldfield CJ, Dunker AK, Obradovic Z, Uversky VN. Protein disorder in the human diseaseome: unfoldomics of human genetic diseases. *BMC Genomics*. 2009;10: S12.
- Njomen E, Tepe JJ. Proteasome Activation as a New Therapeutic Approach to Target Proteotoxic Disorders. *J Med Chem*. 2019;62:6469–6481.
- Jones CL, Njomen E, Sjögren B, Dexheimer TS, Tepe JJ. Small Molecule Enhancement of 20S Proteasome Activity Targets Intrinsically Disordered Proteins. *ACS Chem Biol*. 2017;12:2240–2247.
- Opoku-Nsiah KA, Gestwicki JE. *Transl Res*. 2018;198:48–57.
- Jones CL, Tepe JJ. *Molecules*. 2019:24.
- Coleman RA, Trader DJ. Methods to Discover and Evaluate Proteasome Small Molecule Stimulators. *Molecules*. 2019;24:2341.
- Zerfas BL, Coleman RA, Salazar-Chaparro AF, Macatangay NJ, Trader DJ. Fluorescent Probes with Unnatural Amino Acids to Monitor Proteasome Activity in Real-Time. *ACS Chem Biol*. 2020;15:2588–2596.
- Njomen E, Osmulski PA, Jones CL, Gaczynska M, Tepe JJ. Small Molecule Modulation of Proteasome Assembly. *Biochemistry*. 2018;57:4214–4224.
- Njomen E, Tepe JJ. Regulation of Autophagic Flux by the 20S Proteasome. *Cell Chem Biol*. 2019;26(1283–1294), e5.
- E. Njomen, T.A. Lansdell, A. Vanecek, et al. Enhancing c-MYC degradation via 20S proteasome activation in-duces in vivo anti-tumor efficacy, *bioRxiv*, 2020, (08), 2020, pp. 24.265470.
- Coleman RA, Muli CS, Zhao Y, Bhardwaj A, Newhouse TR, Trader DJ. Analysis of chain length, substitution patterns, and unsaturation of AM-404 derivatives as 20S proteasome stimulators. *Bioorg Med Chem Lett*. 2019;29:420–423.
- Trader DJ, Simanski S, Dickson P, Kodadek T. Establishment of a suite of assays that support the discovery of proteasome stimulators. *Biochim Biophys Acta - Gen Subj*. 2017;1861:892–899.
- Lee B-H, Lee MJ, Park S, et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature*. 2010;467:179–184.
- Leestemaker Y, de Jong A, Witting KF, et al. Proteasome Activation by Small Molecules. *Cell Chem Biol*. 2017;24(725–736), e7.
- Myeku N, Clelland CL, Emrani S, et al. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. *Nat Med*. 2016;22:46–53.
- Krahn JH, Kaschani F, Kaiser M. *Cell Chem Biol*. 2017;24:653–655.
- Trippier PC, Zhao KT, Fox SG, et al. Proteasome activation is a mechanism for pyrazolone small molecules displaying therapeutic potential in amyotrophic lateral sclerosis. *ACS Chem Neurosci*. 2014;5:823–829.
- Lansdell TA, Hurchla MA, Xiang J, et al. Noncompetitive modulation of the proteasome by imidazoline scaffolds overcomes bortezomib resistance and delays MM tumor growth in vivo. *ACS Chem Biol*. 2013;8:578–587.
- Kahlon DK, Lansdell TA, Fisk JS, Tepe JJ. Structural-activity relationship study of highly-functionalized imidazolines as potent inhibitors of nuclear transcription factor- κ B mediated IL-6 production. *Bioorganic Med Chem*. 2009;17:3093–3103.
- Azevedo LM, Lansdell TA, Ludwig JR, et al. Inhibition of the Human Proteasome by Imidazoline Scaffolds. *J Med Chem*. 2013;56:5974–5978.
- Magyar CL, Wall TJ, Davies SB, et al. Triflic anhydride mediated synthesis of 3,4-dihydroquinazolines: A three-component one-pot tandem procedure. *Org Biomol Chem*. 2019;17:7995–8000.
- Gaczynska M, Osmulski PA. *Methods Enzymol*. 2005;398:425–438.