Check for updates

REVIEW

Recent advances in understanding NRF2 as a druggable target: development of pro-electrophilic and non-covalent NRF2 activators to overcome systemic side effects of electrophilic drugs like dimethyl fumarate [version 1; peer review: 2 approved]

Takumi Satoh[1], Stuart Lipton [iD][2-4]

[1]Department of Anti-Aging Food Research, School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan
[2]Neuroscience Translational Center and Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA, USA
[3]Neurodegenerative Disease Center, Scintillon Institute, San Diego, CA, USA
[4]Department of Neurosciences, University of California, School of Medicine, La Jolla, CA, USA

Abstract

Dimethyl fumarate (DMF) is an electrophilic compound previously called BG-12 and marketed under the name Tecfidera®. It was approved in 2013 by the US Food and Drug Administration and the European Medicines Agency for the treatment of relapsing multiple sclerosis. One mechanism of action of DMF is stimulation of the nuclear factor erythroid 2-related factor 2 (NRF2) transcriptional pathway that induces anti-oxidant and anti-inflammatory phase II enzymes to prevent chronic neurodegeneration. However, electrophiles such as DMF also produce severe systemic side effects, in part due to non-specific S-alkylation of cysteine thiols and resulting depletion of glutathione. This mini-review presents the present status and future strategy for NRF2 activators designed to avoid these side effects. Two modes of chemical reaction leading to NRF2 activation are considered here. The first mode is S-alkylation (covalent reaction) of thiols in Kelch-like ECH-associated protein 1 (KEAP1), which interacts with NRF2. The second mechanism involves non-covalent pharmacological inhibition of protein-protein interactions, in particular domain-specific interaction between NRF2 and KEAP1 or other repressor proteins involved in this transcriptional pathway. There have been significant advances in drug development using both of these mechanisms that can potentially avoid the systemic side effects of electrophilic compounds. In the first case concerning covalent reaction with KEAP1, monomethyl fumarate and monoethyl fumarate appear to represent safer derivatives of DMF. In a second

Open Peer Review

Approval Status ✓✓

	1	2
version 1 14 Dec 2017	✓	✓

Faculty Reviews are review articles written by the prestigious Members of Faculty Opinions. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. **Taixing Cui**, University of South Carolina School of Medicine, Columbia, USA

2. **Lu Cai**, Wendy L. Novak Diabetes Care Center, University of Louisville, Louisville, USA Wenzhou Medical University, Zhejiang, China
   Xinyue Hu, The First Hospital of Jilin

approach, pro-electrophilic drugs, such as carnosic acid from the herb *Rosmarinus officinalis*, can be used as a safe pro-drug of an electrophilic compound. Concerning non-covalent activation of NRF2, drugs are being developed that interfere with the direct interaction of KEAP1-NRF2 or inhibit BTB domain and CNC homolog 1 (BACH1), which is a transcriptional repressor of the promoter where NRF2 binds.

University, Changchun, China

Any comments on the article can be found at the end of the article.

Keywords
Nrf2, Dimethyl Fumarate, Electrophilic Drugs, KEAP1

How to cite this article: Satoh T and Lipton S. **Recent advances in understanding NRF2 as a druggable target: development of pro-electrophilic and non-covalent NRF2 activators to overcome systemic side effects of electrophilic drugs like dimethyl fumarate [version 1; peer review: 2 approved]** F1000Research 2017, **6**(F1000 Faculty Rev):2138 https://doi.org/10.12688/f1000research.12111.1

First published: 14 Dec 2017, **6**(F1000 Faculty Rev):2138 https://doi.org/10.12688/f1000research.12111.1

The KEAP1/NRF2 pathway

Neurons are highly sensitive to the balance system between oxidation and reduction, and the disruption of this system can lead to inflammatory reactions contributing to various acute and chronic diseases as well as to the normal aging process[1,2]. Activation of the KEAP1/NRF2/anti-oxidant-response element (ARE) pathway by electrophiles (EPs) can activate this cellular redox defense system against these diseases[2,3]. The NRF2/KEAP1 pathway represents one of the major cellular defense systems against oxidative stress, inflammatory reactions, and exposure to toxic electrophilic compounds[4–7]. NRF2 is a transcription factor that induces various anti-oxidant, anti-inflammatory, and detoxification enzymes[4–7]. Under physiological conditions, KEAP1 protein binds to NRF2 and functions as an adaptor protein for cullin 3 (encoded by *Cul3* in humans) E3 ubiquitin ligase, which polyubiquitinates NRF2. Consequently, NRF2 is ubiquitinated and degraded by the proteasome[4–7]. Hence, the transcriptional activity of NRF2 is potently inhibited under normal conditions[4–7].

KEAP1 contains critical cysteine thiols that react with endogenous and exogenous EPs[6,8–11]. This reaction reduces the ability of KEAP1 to induce ubiquitination and degradation of NRF2[6,8–11]. After EP reaction, NRF2 dissociates from the cytoplasmic complex with KEAP1, enters the nucleus, and accumulates there to drive transcription of its target phase II genes, which encode a coordinated system of anti-oxidant and anti-inflammatory enzymes. These proteins include enzymes that generate the major cellular anti-oxidant, glutathione (GSH)[6,8–11]. Thus, NRF2 activators have been shown to be anti-inflammatory and neuroprotective at least in part via redox regulation[6,8–11].

Additionally, NRF2 activators can potently induce coordinated expression of genes involved in the autophagy system, including p62[12–14]. In turn, p62 protein then activates the NRF2/ARE pathway, representing a positive feedback loop between the NRF2/ARE pathway and autophagy network[12–14]. By simulating autophagy in this fashion, NRF2 activators can potentially remove misfolded proteins and thus suppress several diseases associated with abnormal protein conformation[12–14]. NRF2 activators have also been suggested to be neuroprotective against Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD)[6,8–14] on the basis of results in animal models of these neurodegenerative disorders.

During oxidative stress, p62 expression is enhanced via an NRF2-mediated mechanism. The increased p62 can interfere with NRF2/KEAP1 binding and thus results in a positive feedback loop, increasing NRF2 activation[12–14]. The detailed mechanism of p62-KEAP1-NRF2 interaction remains contentious, but some possible scenarios have been proposed[13]. For example, p62 has a STGE motif in its KEAP1-interacting domain and thus p62 may directly bind to KEAP1. The p62 STGE motif may potentially compete with the NRF2 ETGE motif, which is essential for KEAP1-NRF2 interaction[13]. When p62 is upregulated by NRF2 under oxidative stress, p62 then may compete out NRF2 from the KEAP1-NRF2 complex, thus allowing NRF2 to translocate into the nucleus and activate the ARE in the promoter region of phase II genes[13].

However, some NRF2 activators that upregulate p62, such as arsenic, may result in impairment of autophagy, and p62 activation of NRF2 often occurs in the setting of autophagy impairment[13]. Thus, increased p62 can be associated with impairment of autophagy rather than facilitation[12]. Although NRF2 controls the expression of several autophagy-related genes[14], the functional linkage between NRF2 and these putative target autophagy genes under physiological or pathophysiological conditions remains to be determined.

NRF2 manifests both positive and negative attributes with respect to cancer and other diseases[15,16]. On the one hand, NRF2 activators have been proposed for the treatment of various forms of cancer[6,8,9]. In contrast, other recent investigations based on genetic findings suggest that NRF2 activation can promote neoplasia, possibly by enhancing resistance to cancer treatment[15,16]. For example, gain-of-function mutations in NRF2 and loss-of-function mutations in KEAP1 have been encountered in tumors of the digestive tract[15,16]. Further investigation is merited to clarify the biological significance of NRF2 activation in cancer[15,16].

Cysteine-mediated regulation of KEAP1

Among the cysteine thiols of KEAP1 protein, the most characterized reactive thiols are Cys151, Cys273, and Cys288, and they have differential roles in the activation of the KEAP1/NRF2 pathway. The major cysteine residues of KEAP1 that react with EPs are Cys151, Cys273, and Cys288. Each of these cysteine thiols may differentially regulate phase II anti-oxidant gene expression stimulated by the KEAP1/NRF2 transcriptional pathway[17,18].

For example, KEAP1 Cys151 contains the most important thiol for activation of the KEAP1/NRF2 transcriptional pathway[18,19]. Located in the N-terminal BTB domain, Cys151 may be very reactive because of a stretch of basic amino acids in the α5 helical structure[19,20]. One model suggests that covalent modification of Cys151 causes dissociation of the KEAP1/Cullin3 heterodimer, resulting in inhibition of NRF2 ubiquitination[19,20]. Reaction of Cys151 with EPs is thus critical for inhibition of NRF2 degradation mediated by KEAP1-dependent degradation of NRF2[19–21]. In contrast, mutation of KEAP1 Cys151 produces constitutive inhibition of NRF2 under both physiological and pathological conditions in cell-based assays[22,23]. Additionally, ubiquitination and degradation of NRF2 require cysteine residues 273 and 288 of KEAP1. Previous studies of mutations revealed that substitution of Cys273 or Cys288 prevented KEAP1 from repressing NRF2 activity under homeostatic conditions[24–26].

DMF/MMF/MEF
Dimethyl fumarate

Dimethyl fumarate (DMF) is currently approved for clinical use by the US Food and Drug Administration (FDA) and the European Medicines Agency for the treatment of relapsing multiple sclerosis (MS)[27,28]. DMF is an alkylating agent, similar to the classic NRF2 activator sulforaphane, which can non-specifically and covalently modify nucleophilic groups in proteins, including cysteine thiols[29,30]. As a result, serious side effects can occur with this type of drug. For example, a 30% decline in lymphocyte counts has been reported after administration of DMF, which

may predispose to infection[31–34]. DMF has two congeners: mono-methyl fumarate (MMF) and monoethyl fumarate (MEF). Recent research interest has shifted to MEF and MMF with the hope of developing a safer drug than DMF because both of these congeners are less electrophilic than DMF[35–38]. DMF has also been shown to react with other thiol targets, which appear to predominate over KEAP1, at least in T cells[39].

Monoethyl fumarate

DMF and MEF react with disparate KEAP1 thiols, and DMF is more reactive toward a larger number of cysteines[35–39]. MEF appears to solely modulate Cys151 on KEAP1 and manifests sig-nificantly less reaction with other KEAP1 cysteines compared with DMF (Figure 1)[35,36]. On the other hand, DMF induces greater NRF2 protein accumulation than MEF[35,36]. Potentially accounting for some of its side effects, DMF has also been shown to acutely deplete GSH in a concentration-dependent manner[32,34,35,39]. In contrast, MEF maintains GSH levels and, in fact, may produce an increase, possibly due to NRF2 stimulation of GSH synthetic enzymes[35,36]. Thus, MEF may prove to be less toxic than DMF[35,36].

Monomethyl fumarate

A recent study demonstrates similar therapeutic benefits for DMF and its bioactive metabolite MMF in a rat model of PD and brain stroke[37,38]. Despite their similar pharmacological effects *in vivo*, MMF is a less potent NRF2 activator and manifests less toxicity *in vitro*, probably because it manifests orders of magnitude less non-specific alkylating capacity than DMF (Figure 2)[37,38]. The discovery of the therapeutic effects of MMF in an experimental PD model without substantial non-specific alkylating properties compared with DMF suggests that MMF may be a candidate for PD and stroke therapeutics[37,38]. MEF may also potentially be consid-ered as a therapeutic agent since its alkylating capacity is also low

like that of MMF[35–38]. Nonetheless, the lack of specificity of these alkylating NRF2 activators with regard to other protein thiol targets as well as further consideration of their pharmacokinetic and phar-macodynamic properties may limit their ultimate usefulness[37,38].

Hydroxycarboxylic acid receptor 2 as an alternate target

Other experiments suggest that HCAR2 activation, rather than NRF2 activation, may be partially responsible for the beneficial action of DMF and MMF in PD and MS models[40,41]. HCAR2 is a G protein–coupled receptor whose ligands are hydroxyl-carboxylic acids produced from energy metabolism in order to sense cellular metabolic status[40,41]. HCAR2 is expressed in a number of immune cells and other cell types[40,41]. Emerging evidence suggests that HCAR2 exerts potentially therapeutic anti-inflammatory actions[40,41]. Along these lines, in *Hcar2-/-* mice, the beneficial effect of DMF in a mouse model of MS (autoimmune encephalomyelitis or experimental autoimmune encephalomyelitis) is completely abrogated, consistent with the notion that HCAR2 plays an important role in the effect of DMF[40,41]. Anti-inflammatory effects of DMF in the brain have also been posited to be NRF2-dependent, at least in part[42]. If HCAR2 is indeed a major therapeutic target of DMF in AD, PD, and HD, then the ketone body ß-hydroxybutyrate, a known HCAR2 lig-and, may prove to be a more suitable therapeutic than DMF, MEF, or MMF[43,44]. Hence, additional thiol targets of DMF and related compounds are a major focus of current studies.

Pro-electrophilic drugs as pathologically activated therapeutic drugs
Pro-electrophilic drugs

Redox imbalance (for example, excessive oxidation over reduc-tion) is believed to contribute to a variety of diseases[1]. Prior use of EPs to improve redox balance by activating transcriptional
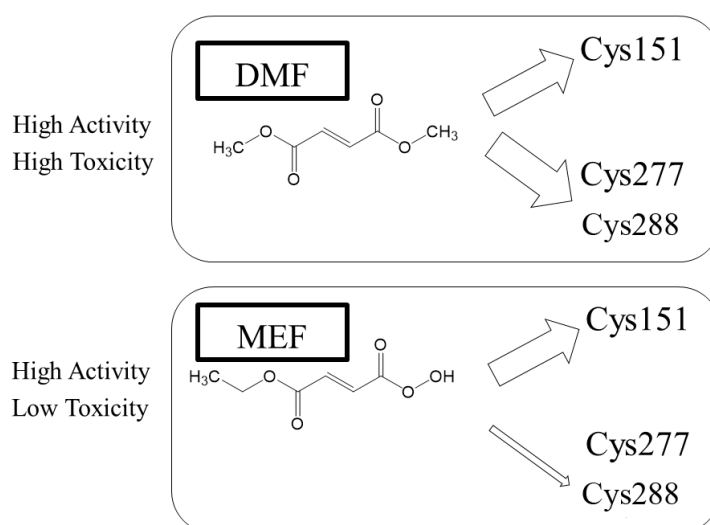
Figure 1. DMF and MEF modulate distinctive repertoires of cysteine thiols on KEAP1. Although DMF reacts with many cysteine residues, including Cys151, Cys273, and Cys288, MEF appears to react preferentially with Cys151. DMF has proven to be more toxic than MEF, although DMF and MEF both activate NRF2, at least *in vitro*[35,36]. DMF, dimethyl fumarate; KEAP1, Kelch-like ECH-associated protein 1; MEF, monoethylfumarate; NRF2, nuclear factor erythroid 2-related factor 2.
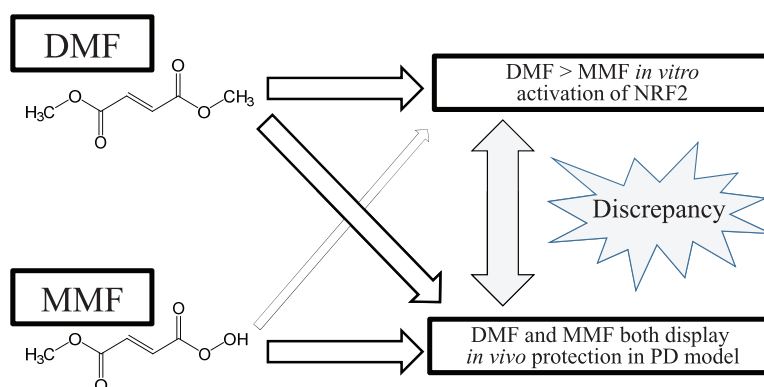
Explanation 1: ADME/T of DMF and of MMF are different.

Explanation 2: HCAR2 or another target mediates protection *in vivo*.

Figure 2. Discrepancy between *in vivo* and *in vitro* actions of DMF and MMF in murine PD models. DMF and MMF show comparable protective action in an *in vivo* rodent model of PD. In contrast, MMF is far less potent than DMF in terms of *in vitro* NRF2 activation[37,38]. There are at least two possible explanations for this discrepancy. One possible interpretation is that DMF and MMF display differential ADME/T (absorption, distribution, metabolism, excretion, toxicity) parameters *in vivo*[37,38]. Another possible explanation is that reaction with HCAR2 or another target mediates the protective effects by DMF[37,38]. DMF, dimethyl fumarate; HCAR2, hydroxycarboxylic acid receptor 2; MMF, monomethylfumarate; NRF2, nuclear factor erythroid 2-related factor 2; PD, Parkinson's disease.

systems against oxidative stress has been met with mixed success, largely because of side effects due to the indiscriminate action of EPs[2]. A newer approach uses pro-drug forms of EPs, known as pro-electrophilic drugs (PEDs), such as carnosic acid (CA), an active ingredient in the herb rosemary (*Rosmarinus officinalis*)[45–50]. Additional compounds of interest include zonarol (ZO) and iso-zonarol (IZ), which are found in seaweed (*Dictyopteris undulata*) (Figure 3)[51,52], as well as related synthetic chemicals[53,54]. Importantly, these PEDs do not react directly with cysteine thiols. However, oxidative stress triggers their conversion from hyd-roquinone to quinone, representing an active EP. The EP then triggers KEAP1/NRF2/ARE transcriptional activity, resulting in the production of anti-oxidant/anti-inflammatory phase II enzymes[45,49].

The combined efforts of the authors' research groups have led to the development of PEDs that are activated by the very oxida-tive stress that they then serve to counteract. This type of action has been deemed a pathologically activated therapeutic or 'PAT' drug[55,56]—a drug that is active only at the site where it is needed and thus represents a gentle tap or pat compared with more indis-criminant reagents that are reactive throughout the body, such as more conventional EPs[45,49]. Since PEDs are not activated in nor-mal cells, they do not indiscriminately react with other thiols such as GSH; moreover, the cells undergoing oxidative stress in which PEDs are converted to EPs already display depleted levels of GSH; hence, the EP generated from the PED does not encounter the normally high levels of GSH with which to react[45,49]. This type of action may help to minimize the side effects of PEDs while retain-ing beneficial activity[48,49]. Thus, the anti-oxidant NRF2-activating

therapy of PEDs is targeted only to cells 'in need'. Additionally, owing to their stimulation of a transcriptional pathway producing endogenous anti-oxidant enzymes, PEDs exhibit a more sustained and amplified action than standard anti-oxidant compounds[45,48]. Accordingly, our recent neurobehavioral and histological readouts suggest that CA, acting as a PED, and administered orally, transnasally, or parenterally *in vivo*, can be an effec-tive treatment for AD and other neurologic conditions in rodent models[46,47,50].

Inhibitors of protein-protein interaction
KEAP1-NRF2 PPI

NRF2 has a Neh2 domain in its N-terminal regulatory region, which is important for binding to the Kelch-DC domain of the C-terminus of KEAP1[17–20]. Peptides capable of blocking the KEAP1-NRF2 protein-protein interaction (PPI) have been identi-fied and proven to be protective in models of global ischemia[57,58]. Importantly, this non-covalent mechanism of action is com-pletely different from electrophilic NRF2 activators, which react at Cys151 of the N-terminal domain of KEAP1 in a covalent manner[17–20]. Recent structural and functional studies have further illuminated the details of the non-canonical mechanism of NRF2 activation[17–20]. The Kelch-DC domain of KEAP1 binds to NRF2 via either its DLG or ETGE motif; both of these motifs are thought to be the major targets of non-covalent inhibitors of KEAP1-NRF2 PPI[59,60]. In a hinge-and-latch model of this interaction, the ETGE motif has a higher affinity for KEAP1 than the DLG motif, which causes the latter to associate and dissociate from KEAP1 in a dynamic manner, resulting in oscillations between a 'closed' (associated) and 'open' (dissociated) conformation[59,60].
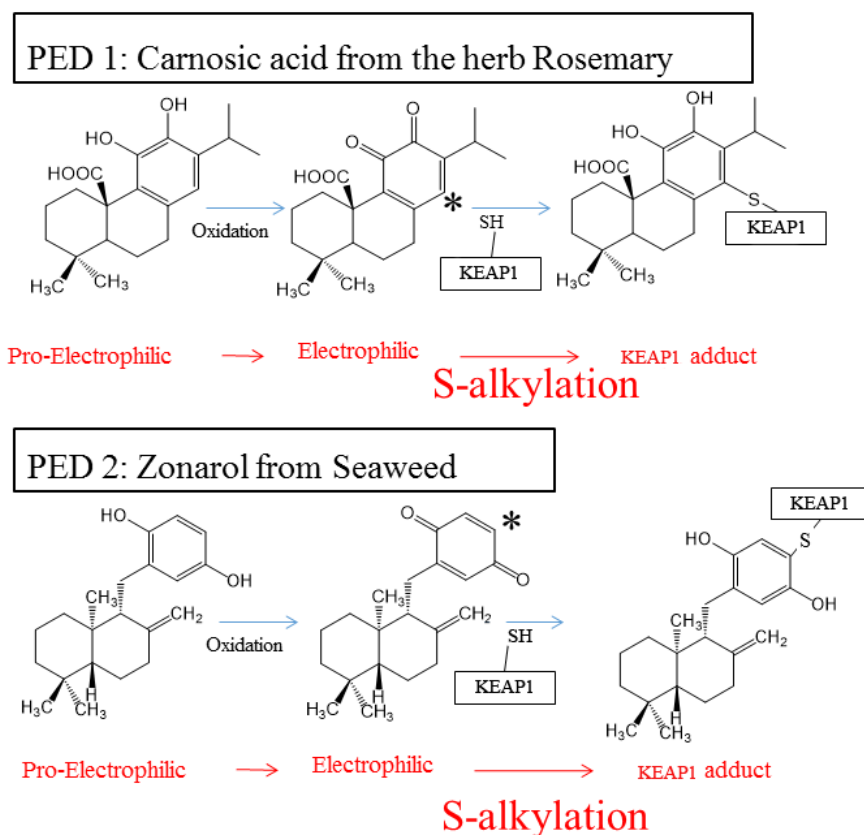
Figure 3. Activation of the KEAP1/NRF2 pathway by PEDs (PED 1, CA; PED 2, ZO). The PED compounds CA (with adjacent or "*ortho-*" position hydroxyl groups)[46,47,49,50] and ZO (with hydroxyl groups located directly across the ring, in the "*para-*" position)[51,52] become oxidized to the electrophilic *quinone* form. CA and ZO quinones undergo nucleophilic attack by a critical KEAP1 cysteine thiol. The reaction forms a KEAP1-CA or KEAP1-ZO adduct. This results in release of NRF2 from KEAP1/NRF2 complexes, accumulation of NRF2 in the nucleus, and subsequent transcriptional activation of phase II enzymes[45,46]. Phase II anti-oxidant and anti-inflammatory enzymes reduce reactive oxygen species and thus improve the resilience of neurons. Importantly, the oxidation of hydroquinone (PED) to quinone (EP) is triggered by oxidative stress, which is then combatted by this transcriptional activity, as described in the text[45,49]. CA, carnosic acid; EP, electrophile; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; PD, Parkinson's disease; PED, pro-electrophilic drug; ZO, zonarol.

KEAP1-NRF2 PPI inhibitors

Non-electrophilic NRF2 activators have been proposed as therapeutic agents for chronic neurodegeneration and inflammation because of their potentially lower incidence of side effects compared with EPs (Figure 4)[59,60]. Using peptide displacement for high-throughput screening, small molecules have been identified that interfere with KEAP1-NRF2 binding[57–60]. Accordingly, KEAP1-NRF2 PPI inhibitors are being studied as NRF2 activators in several disease models[6,61,62]. Taking advantage of this molecular mechanism of action should allow chemists to optimize such agents for the development of non-covalent NRF2 activators[63–67]. To date, many studies of KEAP1-NRF2 PPI inhibitors have focused on the KEAP1-NRF2 ETGE motif[59,60]. However, the affinity of this binding reaction is very high and difficult to inhibit[59,60]. In contrast, as alluded to above, the KEAP1-NRF2 DLG interaction is weaker and has rapid association and dissociation rates[59,60]. Thus, inhibition of binding at the KEAP1-NRF2 DLG may represent an improved approach to further develop effective KEAP1-NRF2 PPI inhibitors[59,60]. Another possible target is the p62 STGE motif, which can compete with the NRF2 ETGE motif for binding to KEAP1[12–14].

BTB and CNC homology 1 inhibitors

Yet another mechanism for ARE-mediated gene regulation involves BACH1, which functions as an inhibitor of NRF2-mediated transcription by binding to small musculoaponeurotic fibrosarcoma proteins (sMAFs) and occupying ARE promoter elements[68–71]. As shown in Figure 4, the basic concept of BACH1 inhibition is competition between BACH1 and NRF2 for dimer formation with sMAFs on ARE-containing promoters[68–71]. In essence, BACH1 inhibitors serve to inhibit the action of an inhibitor, resulting in NRF2 activation. sMAFs are leucine zipper–type transcription factors containing basic regions[72,73]. The basic region of sMAF family members contributes to the distinct DNA-binding mode of this class of proteins[72,73]. sMAFs form homodimers as well as heterodimers with NRF2 or BACH1[72,73]. Because NRF2 and BACH1 cannot bind to DNA as monomers, sMAFs are indispensable partners in order to bind to ARE-containing promotors. In contrast, sMAF
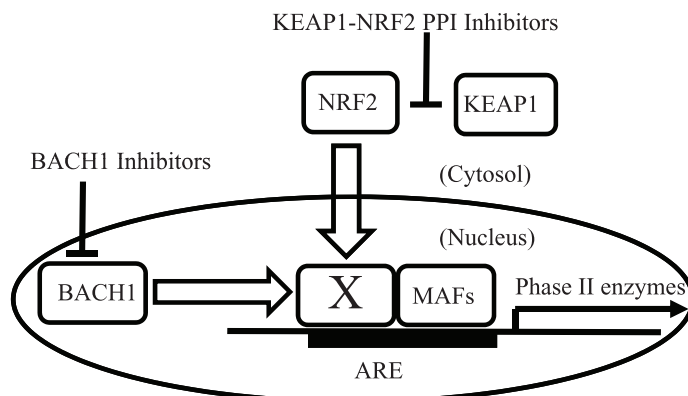
Figure 4. Two types of PPI inhibitors can activate NRF2. NRF2-KEAP1 PPI inhibitors directly inhibit binding of NRF2 and KEAP1 proteins and result in NRF2 release, translocation into the nucleus, and activation of phase II gene transcription[59,60]. Under physiological conditions, BACH1 constitutively inhibits NRF2-mediated transcriptional activity[68–71]. BACH1 inhibitors bind to BACH1[68–71]. Thus, BACH1 inhibitors can activate transcription of NRF2-dependent phase II genes[74]. In this figure, the "X" designates a partner of sMAFs[72,73]. X and sMAFs can form homo- or hetero-dimers and bind to ARE elements[72,73]. When "X" is a sMAF or BACH1, phase II enzymes are not induced; in contrast, when "X" is NRF2, phase II enzymes are induced[72,73]. ARE, anti-oxidant-response element; BACH1, BTB and CNC homology 1; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; PPI, protein-protein interaction; sMAF, small musculoaponeurotic fibrosarcoma protein.

homodimers basically act as transcriptional repressors[72,73]. Additionally, binding of heme to BACH1 will displace this repressor, allowing it to be degraded[68–71]. As expected, BACH1 gene knockout results in activation of the KEAP1/NRF2 pathway and protection in various disease models[68–71]. Hence, the development of drugs that bind BACH1 could also contribute to activation of NRF2-dependent phase II enzymes and prove therapeutic in the future[70,74].

Summary

In conclusion, new forms of both covalent and non-covalent NRF2 activators have recently shown promise as protectants from neurologic diseases; they may also be beneficial for other cell types affected in systemic diseases, including type 2 diabetes mellitus and possibly even normal aging. The new compounds offer hope of efficacy without indiscriminately reacting with protein thiols, which contribute to the multiple side effects observed with the older EP-like drugs, including curcumin and DMF. Recently, excitement has been generated over the possibility of developing non-covalent NRF2 activators. However, the pathologically targeted covalent-reacting PED, CA, appears on the 'generally regarded as safe' (GRAS) list approved by the FDA and has been consumed in large quantities by humans for over two thousand years without incident. It is not yet clear whether the newer non-covalent NRF2 activators will be as well tolerated by humans and avoid systemic toxicity. Considerable further discovery, optimization, and clinical testing will be needed to bring these new drugs to market for neurological as well as systemic diseases.

Abbreviations

AD, Alzheimer's disease; ARE, anti-oxidant-response element; BACH1, BTB and CNC homology 1; CA, carnosic acid; DMF, dimethyl fumarate; EP, electrophile; FDA, US Food and Drug Administration; GSH, glutathione; HCAR2, hydroxycarboxylic acid receptor 2; HD, Huntington's disease; KEAP1, Kelch-like ECH-associated protein 1; MEF, monoethylfumarate; MMF, monomethylfumarate; MS, multiple sclerosis; NRF2, nuclear factor erythroid 2-related factor 2; PD, Parkinson's disease; PED, pro-electrophilic drug; PPI, protein-protein interaction; sMAF, small musculoaponeurotic fibrosarcoma protein.

References

F F1000 recommended

1. Coyle JT, Puttfarcken P: **Oxidative stress, glutamate, and neurodegenerative disorders.** *Science.* 1993; **262**(5134): 689–95.
   PubMed Abstract | **Publisher Full Text**

2. **F** Satoh T, Lipton SA: **Redox regulation of neuronal survival mediated by electrophilic compounds.** *Trends Neurosci.* 2007; **30**(1): 37–45.
   PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

3. Satoh T, Okamoto S, Cui J, *et al.*: **Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic [correction of electrophillic] phase II inducers.** *Proc Natl Acad Sci U S A.* 2006; **103**(3): 768–73.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

4. **F** Tebay LE, Robertson H, Durant ST, *et al.*: **Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease.** *Free Radic Biol Med.* 2015; **88**(Pt B): 108–46.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

5. Joshi G, Johnson JA: **The Nrf2-ARE pathway: a valuable therapeutic target for the treatment of neurodegenerative diseases.** *Recent Pat CNS Drug Discov.* 2012; **7**(3): 218–29.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

6. Magesh S, Chen Y, Hu L: **Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents.** *Med Res Rev.* 2012; **32**(4): 687–726.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

7. **F** Deshmukh P, Unni S, Krishnappa G, *et al.*: **The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases.** *Biophys Rev.* 2017; **9**(1): 41–56.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

8. Poillet-Perez L, Despouy G, Delage-Mourroux R, *et al.*: **Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy.** *Redox Biol.* 2015; **4**: 184–92.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Copple IM, Dinkova-Kostova AT, Kensler TW, *et al.*: **NRF2 as an Emerging Therapeutic Target.** *Oxid Med Cell Longev.* 2017; **2017**: 8165458.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

10. **F** Huang Y, Li W, Su ZY, *et al.*: **The complexity of the Nrf2 pathway: beyond the antioxidant response.** *J Nutr Biochem.* 2015; **26**(12): 1401–13.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

11. Senger DR, Li D, Jaminet SC, *et al.*: **Activation of the Nrf2 Cell Defense Pathway by Ancient Foods: Disease Prevention by Important Molecules and Microbes Lost from the Modern Western Diet.** *PLoS One.* 2016; **11**(2): e0148042.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

12. **F** Liu WJ, Ye L, Huang WF, *et al.*: **p62 links the autophagy pathway and the ubiqutin-proteasome system upon ubiquitinated protein degradation.** *Cell Mol Biol Lett.* 2016; **21**: 29.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

13. **F** Jiang T, Harder B, Rojo de la Vega M, *et al.*: **p62 links autophagy and Nrf2 signaling.** *Free Radic Biol Med.* 2015; **88**(Pt B): 199–204.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

14. **F** Pajares M, Cuadrado A, Rojo AI: **Modulation of proteostasis by transcription factor NRF2 and impact in neurodegenerative diseases.** *Redox Biol.* 2017; **11**: 543–53.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

15. Sporn MB, Liby KT: **NRF2 and cancer: the good, the bad and the importance of context.** *Nat Rev Cancer.* 2012; **12**(8): 564–71.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

16. **F** Menegon S, Columbano A, Giordano S: **The Dual Roles of NRF2 in Cancer.** *Trends Mol Med.* 2016; **22**(7): 578–93.
    PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

17. **F** Dinkova-Kostova AT, Kostov RV, Canning P: **Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants.** *Arch Biochem Biophys.* 2017; **617**: 84–93.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

18. **F** Saito R, Suzuki T, Hiramoto K, *et al.*: **Characterizations of Three Major Cysteine Sensors of Keap1 in Stress Response.** *Mol Cell Biol.* 2015; **36**(2): 271–84.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

19. Sekhar KR, Rachakonda G, Freeman ML: **Cysteine-based regulation of the CUL3 adaptor protein Keap1.** *Toxicol Appl Pharmacol.* 2010; **244**(1): 21–6.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

20. Zhang DD, Lo S, Cross JV, *et al.*: **Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex.** *Mol Cell Biol.* 2004; **24**(24): 10941–53.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

21. Dunlap T, Piyankarage SC, Wijewickrama GT, *et al.*: **Quinone-induced activation of Keap1/Nrf2 signaling by aspirin prodrugs masquerading as nitric oxide.** *Chem Res Toxicol.* 2012; **25**(12): 2725–36.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

22. Antelmann H, Helmann JD: **Thiol-based redox switches and gene regulation.** *Antioxid Redox Signal.* 2011; **14**(6): 1049–63.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

23. Shen T, Jiang T, Long M, *et al.*: **A Curcumin Derivative That Inhibits Vinyl Carbamate-Induced Lung Carcinogenesis via Activation of the Nrf2 Protective Response.** *Antioxid Redox Signal.* 2015; **23**(8): 651–64.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

24. Takaya K, Suzuki T, Motohashi H, *et al.*: **Validation of the multiple sensor mechanism of the Keap1-Nrf2 system.** *Free Radic Biol Med.* 2012; **53**(4): 817–27.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

25. He X, Ma Q: **Critical cysteine residues of Kelch-like ECH-associated protein 1 in arsenic sensing and suppression of nuclear factor erythroid 2-related factor 2.** *J Pharmacol Exp Ther.* 2010; **332**(1): 66–75.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

26. Yamamoto T, Suzuki T, Kobayashi A, *et al.*: **Physiological significance of reactive cysteine residues of Keap1 in determining Nrf2 activity.** *Mol Cell Biol.* 2008; **28**(8): 2758–70.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

27. Prosperini L, Pontecorvo S: **Dimethyl fumarate in the management of multiple sclerosis: appropriate patient selection and special considerations.** *Ther Clin Risk Manag.* 2016; **12**: 339–50.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

28. Smith MD, Martin KA, Calabresi PA, *et al.*: **Dimethyl fumarate alters B-cell memory and cytokine production in MS patients.** *Ann Clin Transl Neurol.* 2017; **4**(5): 351–5.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

29. **F** Wang Q, Chuikov S, Taitano S, *et al.*: **Dimethyl Fumarate Protects Neural Stem/Progenitor Cells and Neurons from Oxidative Damage through Nrf2-ERK1/2 MAPK Pathway.** *Int J Mol Sci.* 2015; **16**(6): 13885–907.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

30. **F** Lastres-Becker I, García-Yagüe AJ, Scannevin RH, *et al.*: **Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease.** *Antioxid Redox Signal.* 2016; **25**(2): 61–77.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

31. **F** Chaves C, Ganguly R, Ceresia C, *et al.*: **Lymphocyte subtypes in relapsing-remitting multiple sclerosis patients treated with dimethyl fumarate.** *Mult Scler J Exp Transl Clin.* 2017; **3**(2): 2055217317702933.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

32. **F** Michell-Robinson MA, Moore CS, Healy LM, *et al.*: **Effects of fumarates on circulating and CNS myeloid cells in multiple sclerosis.** *Ann Clin Transl Neurol.* 2016; **3**(1): 27–41.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

33. **F** Brennan MS, Matos MF, Richter KE, *et al.*: **The NRF2 transcriptional target, *OSGIN1*, contributes to monomethyl fumarate-mediated cytoprotection in human astrocytes.** *Sci Rep.* 2017; **7**: 42054.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

34. **F** Lin R, Cai J, Kostuk EW, *et al.*: **Fumarate modulates the immune/inflammatory response and rescues nerve cells and neurological function after stroke in rats.** *J Neuroinflammation.* 2016; **13**(1): 269.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

35. **F** Brennan MS, Matos MF, Li B, *et al.*: **Dimethyl fumarate and monoethyl fumarate exhibit differential effects on KEAP1, NRF2 activation, and glutathione depletion *in vitro*.** *PLoS One.* 2015; **10**(3): e0120254.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

36. **Fumaderm Summary of Product Characteristics.** Biogen Idec. 2009.

37. **F** Ahuja M, Ammal Kaidery N, Yang L, *et al.*: **Distinct Nrf2 Signaling Mechanisms of Fumaric Acid Esters and Their Role in Neuroprotection against 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Experimental Parkinson's-Like Disease.** *J Neurosci.* 2016; **36**(23): 6332–51.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

38. **F** Yao Y, Miao W, Liu Z, *et al.*: **Dimethyl Fumarate and Monomethyl Fumarate Promote Post-Ischemic Recovery in Mice.** *Transl Stroke Res.* 2016; **7**(6): 535–47.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

39. **F** Blewett MM, Xie J, Zaro BW, *et al.*: **Chemical proteomic map of dimethyl fumarate-sensitive cysteines in primary human T cells.** *Sci Signal.* 2016; **9**(445): rs10.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

40. **F** Offermanns S, Schwaninger M: **Nutritional or pharmacological activation of HCA(2) ameliorates neuroinflammation.** *Trends Mol Med.* 2015; **21**(4): 245–55.
    PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

41. Chen H, Assmann JC, Krenz A, *et al.*: **Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE.** *J Clin Invest.* 2014;

124(5): 2188–92.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

42. Schulze-Topphoff U, Varrin-Doyer M, Pekarek K, *et al.*: **Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2.** *Proc Natl Acad Sci U S A.* 2016; **113**(17): 4777–82.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

43. Newman JC, Verdin E: **Ketone bodies as signaling metabolites.** *Trends Endocrinol Metab.* 2014; **25**(1): 42–52.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

44. Newman JC, Verdin E: **β-hydroxybutyrate: much more than a metabolite.** *Diabetes Res Clin Pract.* 2014; **106**(2): 173–81.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

45. Satoh T, McKercher SR, Lipton SA: **Nrf2/ARE-mediated antioxidant actions of pro-electrophilic drugs.** *Free Radic Biol Med.* 2013; **65**: 645–57.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

46. Nagar S, Noveral SM, Trudler D, *et al.*: **MEF2D haploinsufficiency downregulates the NRF2 pathway and renders photoreceptors susceptible to light-induced oxidative stress.** *Proc Natl Acad Sci U S A.* 2017; **114**(20): E4048–E4056.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

47. Lipton SA, Rezaie T, Nutter A, *et al.*: **Therapeutic advantage of pro-electrophilic drugs to activate the Nrf2/ARE pathway in Alzheimer's disease models.** *Cell Death Dis.* 2016; **7**(12): e2499.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

48. Satoh T, Stalder R, McKercher SR, *et al.*: **Nrf2 and HSF-1 Pathway Activation via Hydroquinone-Based Proelectrophilic Small Molecules is Regulated by Electrochemical Oxidation Potential.** *ASN Neuro.* 2015; **7**(4): pii: 1759091415593294.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

49. Satoh T, Kosaka K, Itoh K, *et al.*: **Carnosic acid, a *catechol-type* electrophilic compound, protects neurons both *in vitro* and *in vivo* through activation of the Keap1/Nrf2 pathway via *S*-alkylation of targeted cysteines on Keap1.** *J Neurochem.* 2008; **104**(4): 1116–31.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

50. Rezaie T, McKercher SR, Kosaka K, *et al.*: **Protective effect of carnosic acid, a pro-electrophilic compound, in models of oxidative stress and light-induced retinal degeneration.** *Invest Ophthalmol Vis Sci.* 2012; **53**(12): 7847–54.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

51. Shimizu H, Koyama T, Yamada S, *et al.*: **Zonarol, a sesquiterpene from the brown algae *Dictyopteris undulata*, provides neuroprotection by activating the Nrf2/ARE pathway.** *Biochem Biophys Res Commun.* 2015; **457**(4): 718–22.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

52. Yamada S, Koyama T, Noguchi H, *et al.*: **Marine hydroquinone zonarol prevents inflammation and apoptosis in dextran sulfate sodium-induced mice ulcerative colitis.** *PLoS One.* 2014; **9**(11): e113509.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

53. Satoh T, Rezaie T, Seki M, *et al.*: **Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat-shock proteins and Nrf2-activated phase 2 antioxidant response enzymes.** *J Neurochem.* 2011; **119**(3): 569–78.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

54. Satoh T, Saitoh S, Hosaka M, *et al.*: **Simple *ortho-* and *para-*hydroquinones as compounds neuroprotective against oxidative stress in a manner associated with specific transcriptional activation.** *Biochem Biophys Res Commun.* 2009; **379**(2): 537–41.
PubMed Abstract | **Publisher Full Text**

55. Lipton SA: **Pathologically activated therapeutics for neuroprotection.** *Nat Rev Neurosci.* 2007; **8**(10): 803–8.
PubMed Abstract | **Publisher Full Text**

56. Lipton SA: **Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation.** *Curr Drug Targets.* 2007; **8**(5): 621–32.
PubMed Abstract | **Publisher Full Text**

57. Steel R, Cowan J, Payerne E, *et al.*: **Anti-inflammatory Effect of a Cell-Penetrating Peptide Targeting the Nrf2/Keap1 Interaction.** *ACS Med Chem Lett.* 2012; **3**(5): 407–10.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

58. [F] Tu J, Zhang X, Zhu Y, *et al.*: **Cell-Permeable Peptide Targeting the Nrf2-Keap1 Interaction: A Potential Novel Therapy for Global Cerebral Ischemia.** *J Neurosci.* 2015; **35**(44): 14727–39.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

59. [F] Gazaryan IG, Thomas B: **The status of Nrf2-based therapeutics: current perspectives and future prospects.** *Neural Regen Res.* 2016; **11**(11): 1708–11.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

60. [F] Jiang ZY, Lu MC, You QD: **Discovery and Development of Kelch-like ECH-Associated Protein 1. Nuclear Factor Erythroid 2-Related Factor 2 (KEAP1: NRF2) Protein-Protein Interaction Inhibitors: Achievements, Challenges, and Future Directions.** *J Med Chem.* 2016; **59**(24): 10837–58.
PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

61. Wang L, Lewis T, Zhang YL, *et al.*: **The identification and characterization of non-reactive inhibitor of Keap1-Nrf2 interaction through HTS using a fluorescence polarization assay.** Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): Nat Cent Biotech Inform (US); 2010–2012.
Reference Source

62. [F] Abed DA, Goldstein M, Albanyan H, *et al.*: **Discovery of direct inhibitors of Keap1-Nrf2 protein-protein interaction as potential therapeutic and preventive agents.** *Acta Pharm Sin B.* 2015; **5**(4): 285–99.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

63. [F] Hu L, Magesh S, Chen L, *et al.*: **Discovery of a small-molecule inhibitor and cellular probe of Keap1-Nrf2 protein-protein interaction.** *Bioorg Med Chem Lett.* 2013; **23**(10): 3039–43.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

64. Marcotte D, Zeng W, Hus JC, *et al.*: **Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism.** *Bioorg Med Chem.* 2013; **21**(14): 4011–9.
PubMed Abstract | **Publisher Full Text**

65. [F] Quinti L, Casale M, Moniot S, *et al.*: **SIRT2- and NRF2-Targeting Thiazole-Containing Compound with Therapeutic Activity in Huntington's Disease Models.** *Cell Chem Biol.* 2016; **23**(7): 849–61.
PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

66. [F] Kerr F, Sofola-Adesakin O, Ivanov DK, *et al.*: **Direct Keap1-Nrf2 disruption as a potential therapeutic target for Alzheimer's disease.** *PLoS Genet.* 2017; **13**(3): e1006593.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

67. [F] Lu MC, Ji JA, Jiang YL, *et al.*: **An inhibitor of the Keap1-Nrf2 protein-protein interaction protects NCM460 colonic cells and alleviates experimental colitis.** *Sci Rep.* 2016; **6**: 26585.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

68. [F] Zhou Y, Wu H, Zhao M, *et al.*: **The Bach Family of Transcription Factors: A Comprehensive Review.** *Clin Rev Allergy Immunol.* 2016; **50**(3): 345–56.
PubMed Abstract | **Publisher Full Text** | **F1000 Recommendation**

69. So AY, Garcia-Flores Y, Minisandram A, *et al.*: **Regulation of APC development, immune response, and autoimmunity by Bach1/HO-1 pathway in mice.** *Blood.* 2012; **120**(12): 2428–37.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

70. Attucks OC, Jasmer KJ, Hannink M, *et al.*: **Induction of heme oxygenase I (HMOX1) by HPP-4382: a novel modulator of Bach1 activity.** *PLoS One.* 2014; **9**(7): e101044.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

71. Ohta R, Tanaka N, Nakanishi K, *et al.*: **Heme oxygenase-1 modulates degeneration of the intervertebral disc after puncture in Bach 1 deficient mice.** *Eur Spine J.* 2012; **21**(9): 1748–57.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

72. [F] Katsuoka F, Yamamoto M: **Small Maf proteins (MafF, MafG, MafK): History, structure and function.** *Gene.* 2016; **586**(2): 197–205.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

73. [F] Tsuchiya M, Misaka R, Nitta K, *et al.*: **Transcriptional factors, Mafs and their biological roles.** *World J Diabetes.* 2015; **6**(1): 175–83.
PubMed Abstract | **Publisher Full Text** | **Free Full Text** | **F1000 Recommendation**

74. Davudian S, Mansoori B, Shajari N, *et al.*: **BACH1, the master regulator gene: A novel candidate target for cancer therapy.** *Gene.* 2016; **588**(1): 30–7.
PubMed Abstract | **Publisher Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of Faculty Opinions. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

1. **Lu Cai**
   [1] Department of Pediatrics, Pediatric Research Institute, Wendy L. Novak Diabetes Care Center, University of Louisville, Louisville, USA
   [2] Chinese-American Research Institute for Diabetic Complications, School of Pharmaceutical Science, Wenzhou Medical University, Zhejiang, China
   Xinyue Hu
   Center of Cardiovascular Diseases, The First Hospital of Jilin University, Changchun, China
   Competing Interests: No competing interests were disclosed.

2. **Taixing Cui**
   Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, Columbia, USA
   Competing Interests: No competing interests were disclosed.