Expert Opinion on Therapeutic Patents

ISSN: 1354-3776 (Print) 1744-7674 (Online) Journal homepage: https://www.tandfonline.com/loi/ietp20

Recent progress in the development of small molecule Nrf2 activators: a patent review (2017-present)

Haishan Zhou, Yan Wang, Qidong You & Zhengyu Jiang

To cite this article: Haishan Zhou, Yan Wang, Qidong You & Zhengyu Jiang (2020): Recent progress in the development of small molecule Nrf2 activators: a patent review (2017-present), Expert Opinion on Therapeutic Patents, DOI: 10.1080/13543776.2020.1715365

To link to this article: https://doi.org/10.1080/13543776.2020.1715365

Accepted author version posted online: 10 Jan 2020.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Therapeutic Patents*

DOI: 10.1080/13543776.2020.1715365

Recent progress in the development of small molecule Nrf2 activators: a patent review (2017-present)

Haishan Zhou[1,2], Yan Wang[1,2], Qidong You[1,2] and Zhengyu Jiang[1,2]

[1]State Key Laboratory of Natural Medicines, and Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, TongJiaXiang 24, Nanjing 210009, China

[2]Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, TongJiaXiang 24, Nanjing 210009, China

Corresponding authors:

Zhengyu Jiang,

Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, TongJiaXiang 24, Nanjing 210009, China

Email: jiangzhengyucpu@163.com

Qidong You,

Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, TongJiaXiang 24, Nanjing 210009, China

Email: youqd@163.com

Abstract

Introduction: The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is the first line of defense against a plethora of environmental or endogenous deviations in redox metabolism, proteostasis, inflammation, etc. Therefore, pharmacological activation of Nrf2 is a potential therapeutic approach for several diseases related to oxidative stress and inflammation, such as cancer, cardiovascular and neurodegenerative diseases.

Areas covered: The authors first describe the biological function of Nrf2 and the molecular regulatory mechanism of Keap1-Nrf2-ARE ((Kelch-like ECH-Associating protein 1)-Nrf2-(antioxidant response element)). Then they review recent progress of covalent activators and non-covalent Keap1-Nrf2 protein-protein interaction (PPI) inhibitors from patents and publications in 2017-present, consisting of new chemical molecules, structure optimization of reported activators and progress in preclinical or clinical trials.

Expert opinion: Despite significant achievements in the development of Nrf2 activators, the selectivity is the primary consideration. Due to reacting with redox-sensitive cysteines in proteins except for Keap1, electrophilic activators often exhibit off-target effects. For Keap1-Nrf2 PPI inhibitors, how to enhance *in vivo* efficacy and/or penetrate blood-brain barrier (BBB) to reach central nervous system (CNS) is also challenging. Fragment-based drug discovery (FBDD), carboxylic acid bioisosteric replacement and prodrug approach might be used to circumvent this challenge. Moreover, the possibility of cancer risk caused by Nrf2 activation needs to be considered carefully.

Key words: Nrf2 activators, Keap1, ARE, covalent activators, PPI inhibitors

Article highlights

- The Keap1-Nrf2-ARE pathway plays a critical role in the cellular defense system and its dysregulation relates to many diseases.
- The detailed regulatory mechanism of Keap1-Nrf2-ARE signaling is discussed.
- Dimethyl fumarate, Sulforaphane, Bardoxolone methyl and some of their derivatives are undergoing various preclinical or clinical trials. Many other types of covalent Nrf2 activators have also been identified.
- Directly disrupting Keap1-Nrf2 PPI has been an effective strategy to activate Nrf2. Compared with covalent Nrf2 activators, non-covalent Keap1-Nrf2 PPI inhibitors may activate Nrf2 with reduced toxic risk.
- Lots of Keap1-Nrf2 PPI inhibitors have been reported, and some of them have achieved high potency. Selected cases studies in this article show the discovery and development of Keap1-Nrf2 PPI inhibitors.

1. Introduction

The human body is surrounded by endogenous and exogenous electrophilic substances, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Cells will suffer from oxidative stress when continuously exposed to high levels of ROS and RNS. The relentless stresses could destroy cellular nucleic acids, proteins, and lipid membranes, leading to chronic and inflammatory disorders, including cancer, cardiovascular and neurodegenerative diseases [1-5]. To resist such damage, cells have evolved the sophisticated cytoprotective system that can up-regulate cytoprotective factors to maintain body homeostasis [3]. Nrf2 is the chief regulator of this cytoprotective system. More than 100 oxidative stress related genes are regulated by Nrf2, including antioxidant proteins, phase I, II and III detoxification enzymes [5]. Cis-regulatory element sequence ARE (antioxidant response element), the binding target of Nrf2, is existed in their promoter regulatory regions of these cytoprotective genes. The activity of Nrf2 is mainly regulated by Keap1 (Kelch-like ECH-associated protein-1) (Figure 1). Under basal conditions, Nrf2 activity is mainly inhibited by Keap1 via ubiquitination of Nrf2. Upon stress conditions, Keap1 is inactivated, Nrf2 is dissociated from Keap1 and enters the nucleus, which binds to ARE and up-regulates the expression of cytoprotective factors, ultimately protecting the cell [5]. Therefore, Keap1-Nrf2-ARE pathway is a key pathway for cells to resist oxidation and maintain cell homeostasis, and Nrf2 activation agents may be developed as therapeutic drugs for a series of chronic diseases [3].

Human Nrf2 contains 605 amino acids with six highly conserved domains namely Neh1 to Neh6 (Figure 2A) [6-7], and each domain has distinct function. Neh1, Neh3, and Neh6 are situated at the C-terminal half of Nrf2. Neh1 contains a basic leucine zipper motif indispensable for the formation of heterodimer, with the small musculoaponeurotic fibrosarcoma (Maf) protein, or other transcription partners. Neh3 plays a significant role in the transcription activity [8]. Neh6, a serine-rich domain, affects the Keap1-independent negative regulation of Nrf2 [9]. Neh4 and Neh5, locating in the N-terminal half of Nrf2, are transactivation domains that bind to the KIX (kinase-inducible domain interacting) and CH3 (cysteine/histidine-rich domain 3) domains of CBP (CREB (cAMP Responsive Element Binding protein)-binding protein) to induce transactivation [10-12]. The high conserved domain, Neh2, contains two motifs known as DLG and ETGE. These motifs are responsible for the Keap1-Nrf2 PPI that regulate Nrf2 ubiquitination and stability [13-14]. Additionally, Tang et al. also found that the Neh7 domain can interact with retinoic X receptor alpha (RXRα) to inhibit the Nrf2-ARE signaling [15-16].

Keap1, an adaptor section of the Cul3-based ubiquitin E3 ligase responsible for Nrf2 ubiquitination, is a cysteine-rich protein (Figure 2B) [8, 17]. Some of these cysteines (such as

Cys151, Cys273, Cys297, Cys434, and Cys613) are highly sensitive to ROS and electrophilic substances [18-19]. Human Keap1 contains five domains: N-terminal domain (NTD, 1-60); Broad complex, Tramtrack, and Bric-´a-Brac (BTB, 61-178) domain; intervening region (IVR, 179-321); double glycine repeat (DGR, 322-608) and Kelch domain; and C-terminal region (CTR, 609-625) [20-21]. The BTB domain could dimerize with Cullin3 (Cul3) and is critical for the ubiquitination of Nrf2 [22]. The IVR domain contains sensitive cysteines and serves as sensors to oxidative stress [23]. The DGR and CTR, identified as DC domain, regulate the interaction between Keap1 and Nrf2 (Figure 2A).

The molecular mechanism of Nrf2 activity regulation is proposed as three models, the “hinge-and-latch” two points model, the “Cul3-dissociation model”, and the “conformation cycling model”. Several excellent reviews have summarized these cases [11, 24-26]. The “Cul3-dissociation model” proposes that covalent reaction of thiols in sensitive cysteines of BTB domain could disrupt the association between Keap1 and Cul3, and thus suppress ubiquitination of Nrf2, which eventually lead to the stabilization of Nrf2 [27]. In the “hinge-and-latch model” [28], two binding motifs of Neh2, ETGE and DLG, bind to the DC domain of Keap1 with different affinities. The ETGE motif plays a role as the “hinge” to bind to Keap1 dimer with high affinity, while the DLG motif serves as the “latch” with weak activity [29]. Baird et al. proposed a new mode of interaction between Keap1 and Nrf2, the “conformation cycling model” [30]. Under basal conditions, the interaction of Keap1-Nrf2 exists as an alternant mode. Once only ETGE motif binds to the Keap1, it is an “open state”, while both ETGE and DLG motifs bind to the Keap1 homodimer, it is a “closed state”. Besides, the ubiquitination of Nrf2 is proceeding in the “closed state” only, and the “open state” offers further regulatory modes. The “conformation cycling model” provided a new attitude that cysteine modifications of Keap1 could induce an abnormal “closed state” of the Keap1-Nrf2 interaction, while both motifs still bind to Keap1 but ubiquitination stops (Figure 3) [30].

The imbalance of the Keap1-Nrf2-ARE signaling is related to a number of diseases, like Alzheimer’s disease (AD) and Parkinson’s disease (PD), cancer, asthma, atherosclerosis, diabetes, multiple sclerosis (MS), chronic obstructive pulmonary disease (COPD) [26, 31-33]. Development of Nrf2 activators as therapeutic drugs have been regarded as a promising approach. Nowadays, known Nrf2 activators can be commonly divided into two categories based on their functional mechanism: covalent activators through modifying of Keap1 cysteine residues; and non-covalent inhibitors directly disrupting Keap1-Nrf2 PPI [25, 34]. This review summarizes the progress of small molecular Nrf2 activators from patents and publications in 2017-present. Finally, future challenges of developing Nrf2 activators as therapeutic agents are discussed.

2. Covalent activators

Since the Keap1-Nrf2-ARE signaling can be activated by toxic substances, especially electrophiles, a series of Nrf2 activators with electrophilic groups have been found to mimic this activation mode [33]. Many known activators are electrophiles or can be transformed to electrophiles by metabolic pathways [35]. These activators could inhibit the ubiquitination of Nrf2 via reacting with the critical thiols (such as Cys151, Cys273, and Cys288) in Keap1 to induce the expression of Nrf2-regulated cytoprotective factors [36]. Detailed information about these factors has been systematically reviewed elsewhere [11].

2.1. Fumaric acid esters

Fumaric acid esters are a kind of Nrf2 activators belonging to Michael acceptors. Tecfidera (dimethyl fumarate, DMF, **1**, Figure 4), the most successful Nrf2 activator, was approved in 2014 by Biogen for the treatment of patients with relapsing forms of MS [37-40]. The precise mechanism about how DMF plays a part in MS remains unclear, nevertheless, it is commonly believed that its action is compactly relevant with the activation of Nrf2. DMF and the metabolite MMF (Monomethyl fumarate, **2**, Figure 4) activate the Nrf2 pathway by reacting with Cys151 in Keap1 *in vitro* and *in vivo* in both animals and humans [41-42]. Due to non-specifically and covalently modifying nucleophilic groups in proteins [43-44], safety has been a priority for DMF. For instance, one drawback of Tecfidera is leukopenia in patients with low lymphocyte counts after administration of DMF. Therefore, a numerous of efforts are working on improving safety of DMF. As above, DMF could be metabolized *in vivo* to MMF, which inhibited Keap1 through adduct formation at Cys151 [37], several organizations and companies are now exploiting agents with slow and sustained release of MMF that would improve bioavailability and lower side effects. At present, diroximel fumarate **(**BIIB098 (previously ALK8700), **3,** Figure 4) is under phase III trial for MS (NCT03093324, Table 1), which is an oral formulation of an MMF derivative with improved bioavailability and efficacy [45]. XenoPort has identified tepilamide fumarate as MMF prodrug (XP23829, **4**, Figure 4) [46]. Compared with DMF, **4** is much more soluble and penetrable, and has higher oral absorption ratio as well as improved efficacy and reduced GI side effects in preclinical models and is in a phase II clinical trial for plaque psoriasis (NCT02173301) now.

DMF has been used in several clinical trials (Table 1) in relation to its anti-inflammatory properties. Besides, there are ongoing clinical trials (Table 1) related to its anticancer activities in leukemia. Moreover, last 3 years, many dosage forms, components, as well as new adaptations of DMF and its derivatives have been published as patents, some of them are showed in Table 2.

2.2. Sulforaphane

Sulforaphane (SFN, **5**, Figure 5), belonging to isothiocyanates (ITCs), is widely used as electrophilic Nrf2 activator. This agent was initially isolated as the main inducer of Nrf2 downstream enzyme NAD(P)H:quinone oxidoreductase-1 (NQO-1) from extracts of Brassicaceae plants. SFN can react with C151 of Keap1 [47], and has been successfully applied to the treatment of patients with type II diabetes mellitus (T2DM) [48-49]. On account of the BBB permeability [43], SFN can protect against neurodegenerative disorders in numerous preclinical models [50]. For example, in transgenic mouse models of Alzheimer's disease, SFN reduced the amount of amyloid beta (Aβ) and phosphorylated tau proteins as well as their aggregations, and aggregated proteins induced oxidative stress in Alzheimer's disease was also relieved by SFN supplementation [51-52]. SFN has also been reported to prevent or slow the process of normal brain aging and memory problems [53]. At present, more than 20 ongoing clinical trials mainly about neuroprotective effects in various neurologic disease are in process [54]. Considering the instability of SFN, and unmanageable dose control, Evgen Pharma disclosed a pharmaceutical form of SFN, SFX-01 (**6**, Figure 5). **6** is now being studied in clinical trials of subarachnoid haemorrhage (NCT02614742) and metastatic breast cancer (NCT02970682). Otherwise, recent patents have disclosed some dosage forms, compositions, or new indications of SFN and its derivatives since 2017. Patent **MX2017014281** [55] by Alejandro disclosed a composition and method for increasing the bioavailability of sulforaphane and its analogues using ultrasonic approach. Pharmagralabs Inc. reported a method of stabilizing sulforaphane including contacting sulforaphane and a cyclodextrin to form a complex [56].

2.3. Cyanoenone triterpenoids

Bardoxolone methyl (CDDO-Me, **8**, Figure 6), developed by Reata pharmaceuticals, is another widely known Nrf2 activator. CDDO-Me and its analogues are derivatives of 2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oate (CDDO, bardoxolone, **7**) that resemble the natural product oleanolic acid [57-58]. With α-β unsaturated scaffold, these synthetic and semi-synthetic triterpenoids act as Michael acceptors by interacting with C151 of Keap1 so that obstruct its mutual effects with Cul3, then activate Nrf2 [59]. Many preclinical and clinical trials suggested the usage of these triterpenoids as therapies for diseases related to inflammation. For example, CDDO-Me is proceeded clinical trials to treat advanced chronic kidney disease (CKD) and T2DM [60]. In spite of the beneficial performance in phase II clinical trials, CDDO-Me was halted at phase III due to cardiovascular safety issues [61]. However, many efforts have been made to solve the problem that summarized by other excellent reviews [25, 62-65], and here our main focus is on the latest progress of these triterpenoids in recent 3 years. RTA-408 (Omaveloxlone, **9**), a derivative of

CDDO-Me, is now in phase II clinical trials for Friedreich's ataxia (active, not recruiting) mitochondrial myopathy (completed), ocular inflammation (completed), and pain after ocular surgery (completed) [66]. Recently, a preclinical study evaluated RTA-408 for diabetic wound recovery and pointed Nrf2 upregulation as responsibility for the observed improvement in regenerative capacity [67]. CDDO-Me is now in clinical trials for some particular cases related to CKD, including autosomal dominant polycystic kidney (NCT03366337), focal segmental glomerulosclerosis, Alport syndrome (NCT03019185), immunoglobulin A (IgA) nephropathy, and type 1 diabetes. A phase II clinical trial for CDDO-Me in patients with CKD and T2DM (NCT02316821) has been conducted for its possibility to slow down or impede the recession in glomerular filtration which leads to transplanting or dialysis in patients with Alport syndrome and other rare conditions of CKD. The result showed that treatment of CDDO-Me increased the glomerular filtration rate and exhibited favorable tolerance after exclusion of patients at risk of fluid retention. A phase III clinical trial (NCT03550443) in patients with diabetic kidney disease was initiated by Kyowa Kirin Co., Ltd. Furthermore, CDDO-Me is being tested in patients with kinds of severe progressive diseases leading to heart failure and death, like pulmonary arterial hypertension (PAH) (NCT03068130) and connective tissue disease-associated PAH (CTD-PAH) (NCT02657356).

To reduce the side effects of CDDO-Me, Mou Yi published a patent **CN108440636A** [68], in which disclosed novel CDDO-Me derivatives (**10**). Compared with CDDO-Me, the derivatives exhibited similar activity intensity with lower toxicity. Therefore, the CDDO-Me derivatives can be used as anti-inflammatory agents with higher safety to prevent and treat inflammation-related diseases.

Recently, based on the structure of CDDO-Me, Huang et al. [69-71] designed several hybrid molecules to enhance therapeutic potential of CDDO-Me. To discover new drugs for interference with drug-resistant lung cancer, Huang et al. designed a series of hybrids CDDO and O^2-(2,4-dinitrophenyl) diazeniumdiolate [71]. The best performing compound **12** could produce comparatively high levels of nitric oxide (NO) and ROS in drug-resistant lung cancer A549/Taxol cells which over-express glutathione S-transferase π (GSTπ), and significantly inhibit the cell proliferation superior to the controls. In consideration of the clinical therapeutic efficacy of oral-dosed CDDO-Me and the selective vasodilatory effect caused by inhalation of NO on PAH patients, a new hybrid (CDDO-NO, **13**) from CDDO-Me and NO donor isosorbide 5-mononitrate was designed and synthesized [69]. **13** could liberate CDDO-Me and NO in the lungs of rats after trachea injection, and showed potent inhibitory activity of pulmonary vasodilation and vascular

remodeling, suggesting that it might be a potential agent for PAH intervention. To improve the safety profile of CDDO-Me in diabetic nephropathy (DN), Huang et al. identified a γ-glutamyl transpeptidase (GGT)-based and α-cyano-α, β-unsaturated ketone-modified derivative of CDDO-Me (**11**) [70]. **11** could be specifically cleaved by GGT, which is highly expressed in the kidney, liberate CDDO-Me in situ, and showed anti-DN efficacy compared to that of CDDO-Me with much less toxicity in cells and mice, indicating it a safer agent than CDDO-Me.

2.4. Vinyl Sulfones as Nrf2 activators

A series of vinyl sulfones as Nrf2 activators were reported by Kim et al. in a patent **WO2013165140** in 2013, and the most potent compound **14** exhibited therapeutic potential for PD (Figure 7) [72-73]. Recently, to improve the druglike properties of **14,** Choi et al. disclosed several vinyl sulfone derivatives by introducing nitrogen heterocycles due to the universal application of nitrogen heterocycles [74]. Among them, compound **15** with potent activity showed better *in vitro* absorption, distribution, metabolism, and excretion/toxicity (ADME/Tox) properties compared with **14** [74]. In cell-based assays, **15** exhibited superior effects on Nrf2 activation (**15**: EC_{50} = 346 nM; **14**: EC_{50} = 530 nM). **15** was further verified to up-regulate the expression level of Nrf2-mediated antioxidant enzymes, such as heme oxygenase-1 (HO-1), glutamate-cysteine ligase catalytic (GCLC) and glutamate-cysteine ligase modifier (GCLM), at both mRNA and protein levels. *In vivo*, **15** showed neuroprotective effect in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-challenged mouse model of PD [74]. Meanwhile, Choi et al. [75] altered the vinyl sulfone to vinyl sulfonamide or vinyl sulfonate to enhance activity. The effects on activation of Nrf2 were reduced after introducing of vinyl sulfonamides, while vinyl sulfonate derivatives showed superior activity compared to the vinyl sulfone derivatives. Among them, compound **16** showed favorable Nrf2 activation effects (EC_{50} = 76 nM), induced the expression of Nrf2-mediated antioxidant enzymes and prevented the production of inflammatory factors. Furthermore, **16** showed potent *in vivo* therapeutic efficacy against motor dysfunction in MPTP-induced PD mice and recovered the TH expression and anti-inflammatory responses in the SN and striatum in this mouse model.

2.5. Diterpenoids and sesquiterpenoids as Nrf2 activators

Recently, Li et al. [76] had collected 66 diterpenoids and evaluated the effects of Nrf2 activation via a NAD(P)H: quinone reductase (QR) assay in murine hepatoma Hepa1c1c7 cells. 16 diterpenoids showed Nrf2-dependent protective response. Among them, sphaeropsidin A (SA, **17,** Figure 8) was the most outstanding activator, and exhibited about 5-folds potency than that of SFN. Subsequent cell-based studies showed that SA could activate Nrf2 and its downstream cytoprotective genes (NQO1 and GCLM) via stabilizing Nrf2 in a process involving protein kinases

phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), protein kinase C (PKC), and protein kinase R-like endoplasmic reticulum kinase (PERK). Furthermore, SA prevented human lung epithelial cells against sodium arsenite [As(III)]- and cigarette smoke extract (CSE)-induced oxidative stress and cytotoxicity *in vitro*, and reduced metronidazole (MTZ)-induced oxidative damage in Tg (krt4: NTR-hKikGR)^{cy17} transgenic zebrafish and lipopolysaccharide (LPS)-induced oxidative injury in wild-type AB zebrafish.

Zhou et al. [77] performed a phytochemical investigation of *C. chartophyllum* by a QR screening assay to seek potent Nrf2 activators. Thirty chemical elements of C. *chartophyllum* has been illustrated, and a sesquiterpenoid with α, β-unsaturated ketone group, 3S-(+)-9-oxonerolidol (NLD, **18-1**), and a polyphenol, 3, 3′, 4, 4′-tetrahydroxydiphenyl (THD, **18-2**) were selected for further research. NLD and THD could effectively activate Nrf2 and up-regulate its downstream genes, and as a result protected sodium arsenite [As(III)]-chaleenged human lung epithelial cells.

2.6. Other Nrf2 activators

CXA-10 (10-nitro-9(E)-octadec-9-enoic acid, **19**, Figure 9), an isomer of OA-NO2 (9-Nitro-octadec-9-enoic acid, a nitro-fatty acid), has been confirmed to activate Nrf2 in the model of CKD [78]. At present, several phase I clinical trials for the treatment of this disease and phase II trials for the treatment of pulmonary arterial hypertension and primary focal segmental glomerulosclerosis have been initiated based on CXA-10.

In patent **WO2019122265**, Bartholomeus et al. [79] from Medimmune Limited disclosed a lot of compounds with 6-oxocyclohex-1-ene-1-carbonitrile structure capable of regulating Nrf2 signaling by inhibiting Nrf2 binding to Keap1. EC_{50} values of compounds were evaluated in the HEK-293 cell line-based luciferase assay. Many of them exhibited potent inhibitory effect on Keap1 binding to Nrf2, thus activated the Nrf2 signaling pathway with EC_{50} values of less than 0.1μM. Furthermore, compound **20** was selected to investigate the absolute configuration of compound binding to Keap1. The crystallographic data showed that **20** covalently bound to Cys151 of Keap1 N-BTB, and behaved as *S*-configuration (PDB code is not disclosed) [79].

Patent **CN106265633** by Wang et al. [80] reported the atractylenolide II (**21**) could treat and prevent the diseases associated with Nrf2 pathway. *In vitro*, **21** remarkably activated Nrf2 and increased the expression of its downstream antioxidative enzymes (HO-1 and NQO1), so that protected against LPS-induced inflammation and oxidative stress. Further research showed the cytoprotective effects of **21** were related to a c-Jun N-terminal kinase (JNK)/extracellular signal-regulated kinase (ERK)-Nrf2-ARE-dependent manner. *In vivo*, **21** significantly promoted mRNA and protein levels of Nrf2 and its downstream antioxidative enzymes in

N-Nitroso-N-methylurea-induced rats. **21** showed protective effects against mammary tumorigenesis by reducing tumor incidence, multiplicity and volume, and decreasing inflammation and oxidative stress in rat mammary tissue.

In patent **WO2019104030**, George et al. [81] from Biogen Ma Inc. reported a series of Nrf2 activators containing tetrahydronaphthalene structure. Nuclear Translation assay indicated that compound **22** was the most potent activator with an EC_{50} of less than 0.1 μM. Then **22** was selected for investigating the effects of Nrf2 activation. **22** induced the transcription of Nrf2 target genes in human spinal cord astrocytes, including GCLC, HMOX1, OSGIN1 and NQO1 and increased intracellular glutathione. Furthermore, **22** protected cells from oxidative stress-induced cell death caused by 25 μM sodium arsenite.

3. Non-covalent Keap1−Nrf2 PPI inhibitors

Although numerous achievements have been reached in electrophilic activators of Nrf2, their applications are limited because of their electrophilic nature. To overcome the lack of selectivity, a new class of Nrf2 activators that prevent the interaction of Nrf2 to Keap1 has emerged [27]. By directly disrupting Keap1-Nrf2 interaction, the compounds don't require a covalent group for their mode of action, which may result in a better safety profile [82-83]. The discovery of PPI inhibitors was promoted by the development of screening approaches of Keap1-Nrf2 inhibitors [84] and the availability of the crystal structures of the Kelch domain of Keap1. While the X-ray structure of full-length Keap1 has not been reported, the structure of the Keap1 Kelch domain, which is responsible for the interaction with Nrf2, was determined by several groups using X-ray crystallography [21, 28, 85-87]. The binding cavity of Keap1 has classically been divided into five sub-pockets called P1-P5 (Figure 10A) [88]. The P1 and P2, known as polar sub-pockets, have been shown to play important roles in binding to substrates, represented by the ETGE and DLG motif of Nrf2. Most polar residues (Arg483, Ser508, Ser363, Arg380, Asn382, and Arg415) are located in the P1 and P2 pockets. P4 and P5 mainly contain nonpolar residues, including Tyr525, Gln530, Tyr572, Tyr334, and Phe577. The P3 sub-pocket consists of small polar and nonpolar residues (Gly509, Ala556, Gly571, Ser602, Ser555, and Gly603), which make this pocket sensitive to steric hindrance (Figure 10B). Recently, a hitherto unexplored P6 sub-pocket on the Kelch surface induced by an extended DLG-containing peptide (DLGex) was observed (Figure 10A) [89]. These findings contributed to the understanding of the molecular determinants of Keap1-Nrf2 PPI, which then guided the design of direct PPI inhibitors. Although Lu et al. [90] reported a head-to-tail cyclic peptide inhibitor with high cell potency, the peptide-based inhibitors of Keap1-Nrf2 PPI

generally lack *in vivo* activity, potentially due to the poor bioavailability [91-94]. Here we aim to highlight the recently discovered small-molecule inhibitors (2017-present) and their therapeutic potential, which are organized based on the molecular skeleton.

3.1. Small molecule inhibitors containing a 1,4-diaminonaphthalene core

The compounds with 1,4-diaminonaphthalene skeleton discovered by Biogen via a high-throughput screening (compound **23**, Figure 11A) [95], and then have been developed by our group [96-97] and Moore's group [98]. These bis-carboxylic acid compounds (**24** and **25**, Figure 11A) could directly disrupt Keap1-Nrf2 PPI with excellent potency, and up-regulated the expression level of Nrf2-mediated proteins and genes. The action modes of this chemotype have been identified by Keap1 co-structure with inhibitors, such as compound **23** (PDB code: 4IQK, Figure 11B**)** and **25** (PDB code: 4XMB, Figure 11C**)**. By analyzing these co-structures, the polar interactions formed by arginines (Arg380, Arg415 and Arg483) and inhibitors are critical for the tight binding of small molecules to Keap1. Inspired by information of these co-structures, many achievements have recently been reported by different research groups.

To investigate the therapeutic potential of **CPUY192018** (compound **26**, Figure 12), a high potent Keap1-Nrf2 PPI inhibitor previously developed by our group, a follow-up study was conducted and reported recently [99]. Treatment of **26** in lipopolysaccharide (LPS)-stimulated human proximal tubular epithelial HK-2 cells showed cytoprotective effects through up-regulating the Nrf2-mediated antioxidant pathway and alleviated the LPS-stimulated inflammatory response via preventing the ROS-mediated activation of the NF-κB pathway. Moreover, **26** treatment equilibrated renal oxidative stress and decreased inflammatory responses by activating Nrf2 in the LPS-challenged mouse model of chronic renal inflammation. This study proved the potential of small molecule inhibitors of Keap1-Nrf2 PPI in the treatment of renal diseases related to oxidative stress (Figure 12). Hui et al. reported that **CPUY192018** activated Nrf2-regualted cytoprotective system in human retinal endothelial cells and ameliorated retinal ischemia-reperfusion injury [100].

In patent **CN108101821** published in 2018 by our group, several potent Keap1 inhibitors with amino acids substituents were designed [101-102]. By analyzing some potent inhibitors reported before, some natural α-amino acid fragments might occupy the P1 or P2 polar pockets of Keap1 kelch domain. Based on the previously discovered potent inhibitor **24** [96], different α-amino acid groups were incorporated into the diaminonaphthalene core and led to a series of small molecules with novel chemical skeletons. By means of structure-activity relationship (SAR) study, the Pro and Phe were identified as two preferential groups, and derivative **27** with Pro substituent was the most potent inhibitor (fluorescence polarization (FP) assay: IC_{50} = 43 nM) (Figure 13). This effective

Keap1 inhibitor showed potent Keap1 binding affinity (isothermal scanning calorimetry (ITC) assay: K_d = 53.7 nM; biolayer interferometry (BLI) assay: K_D = 28.5 nM) both *in vitro* and in cells, and subsequent cellular assay proved potent Nrf2 activation effects, and further study showed that **27** exhibited cytoprotective effects against acetaminophen (APAP) induced hepatic damage in both normal hepatic L02 cells and mouse liver by activating the Nrf2-ARE pathway.

To investigate safer Nrf2 activators, Moore et al. replaced the naphthalene scaffold in Keap1-Nrf2 inhibitors with non-naphthalene and heterocyclic scaffolds [103]. Many research results have shown that 1,4-diaminonaphthalene core may not be a good choice for development of drug-like candidates, owing to metabolic instability [104-108], carcinogenic and mutagenic properties [109-110]. In consideration of these defects, a series of Keap1-Nrf2 inhibitors containing non-naphthalene and heterocyclic scaffolds were designed based on the compound **24**. The results indicated that the 1,4-substituted isoquinoline scaffold derivative (**28**) showed similar potency (FP assay: IC_{50} = 60 nM), high aqueous solubility, and metabolic stability to the 1,4-substituted naphthalene (**24**). Moreover, a mini-Ames assay showed that **28** has a better mutagenic profile than **24** (as shown in Figure 14). This study provided a favor chemical skeleton for novel Keap1-Nrf2 PPI inhibitors.

To identify specific inhibitor(s) of the interaction between phosphorylated p62 (*p*-p62) and Keap1, Komatsu et al. [111] discovered compound **29** (Figure 15A), similar to **23**, through a FP-based high-throughput screening (HTS) of the Drug Discovery Initiative Library. **29** could disrupt the interaction between Keap1 and *p*-p62 through binding to Keap1 as Nrf2 does (Figure 15B). Subsequently, with a SAR investigation, the inhibitory activity of **29** was confirmed based on FP assay (Keap1-*p*-p62: IC_{50} = 1.5 μM, Keap1-Nrf2: IC_{50} = 6.2 μM) [112]. Moreover, X-ray crystal structural analysis of the complex between Keap1-DC and **29** revealed a same binding mode as **23** (PDB code: 4ZY3, Figure 15B) [111]. In 2017, Mashino et al. [113] disclosed a new Keap1-Nrf2 PPI inhibitor (compound **30**, Figure 15C) containing benzoindole core and indole-3-hydroxamic acid moiety by the FP-based HTS (IC_{50} of 0.20 μM), and then cellular effect was confirmed by evaluating the mRNA expression levels of NQO1. **30** exhibited more metabolically stable in human liver microsomes than that of **23**. Subsequently, a series of N-substituted hydrazide derivatives were synthesized and showed similar inhibitory activity and metabolic stability as **30**. Interestingly, a latest article by Tran et al. [114] claimed that compound **30** showed a robust Keap1-Nrf2 inhibition (K_i = 0.0021 μM) by a FP assay [114]. Then stabilization activity by TSA (thermal shift assay), binding activity by SPR (surface plasmon resonance) assay, cell activity by NQO1 induction assay have showed **30** is a superior Keap1-Nrf2 PPI inhibitor (Figure 15C).

In patent **WO2013067036** Hu et al. [115] disclosed a small molecule, **31** with naphthalene ring as the Keap1-Nrf2 inhibitor through FP-based HTS of the MLPCN library (Figure 16). In 2018, Zhuang et al. [116] reported a subsequent study. By fragment growing method, nearly 20 novel compounds with hydronaphthoquinones were designed and synthesized as new Nrf2 activators. Among them, **32** and **33** exhibited excellent inhibitory activity in disturbing the interaction of Keap1-Nrf2, as detected by fluorescent anisotropy assay (**32**: K_D = 1.14 μM; **33**: K_D = 8.52 μM) and confirmed by surface plasmon resonance (SPR) (**32**: K_D = 0.45 μM; **33**: K_D = 5.17 μM) and differential scanning fluorimetry (DSF) assays (**32**: ΔT_m = 2.5 ^oC; **33**: ΔT_m = 1.5 ^oC) (Figure 16). They showed protective effects on the H9c2 cardiac cells against LPS-challenged damage in a dose-dependent manner. They up-regulated the Nrf2-mediated genes (HO-1, NQO-1) while decreased the level of ROS and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Subsequently, the *in vivo* cardioprotective effect was confirmed using LPS-induced acute death mouse model with pre-treatment and post-treatment of compounds. Both **32** and **33** prolonged the survival of mice, while inhibited the pro-inflammatory cytokines. Although containing a pan-assay-interference-compounds (PAINS) fragment hydronaphthoquinone [117], authors demonstrated that the inhibitory activity of Keap1-Nrf2 PPI of these compounds by performing several biological experiments.

3.2. Small molecule inhibitors based on a 3-phenylpropanoic acid core

Astex and GlaxoSmithKline disclosed a series of monoacidic inhibitors of Keap1-Nrf2 PPI by an X-ray crystallography fragment-based screening method [118-119]. The representative compound **39** (Figure 17) was low-nanomolar inhibitor that enhanced the expression level of NQO1 in cells. In the chronic obstructive pulmonary disease (COPD) model, after intravenous infusion, **39** reduced lung inflammation in rats and significantly suppressed ozone-induced increases of leukocytes and restore glutathione (GSH) levels. Many patents have been published which revealed many other derivatives and their applications as Nrf2 activators [120-121]. Compounds from these patents have exhibited protective or therapeutic effects in certain pulmonary disease models of oxidative stress. Recently, the detailed process of discovering of **39** has been disclosed by Davies et al. (Figure 17) [122]. As described previously, a crystallographic screen of approximately 330 fragments identified three active fragments that occupied three separate hot-spots in Keap1, which referred as the “acid”, “planar acceptor”, and “sulfonamide” pockets. Due to its multiple synthetically accessible exit vectors for hit elaboration and probing the site integrally, the fragment 4-chlorophenyl propionic acid **34** fitting the “acid” pocket, was chosen as a “anchor” fragment, and a fragment growing approach was employed via introducing functionality simulating the modes of

interaction between Keap1 hot-spots and fragments. FP and ITC assays were used to examine the potency of compounds. Inspired by the X-ray structure of the Kelch domain with **34**, fragment **35** could occupy the "planar acceptor" pocket through growing out of the benzylic position of **34** and form the stacking and hydrogen-bonding interactions with Gln 530 and Ser 555 in this subsite via introduction of functionality. Through SAR in the "planar acceptor" pocket, a methyl benzotriazole could form stacking and polar interactions with the amino acid residues in this pocket, and this effort obtained an intermediate with enhanced binding activity (**37**, IC_{50} = 61 μM). Introducing the sulfonamide moiety **36** into **34** provided the interaction with Ser525 in "sulfonamide" binding pocket and led to a more active hit (**38**, IC_{50} = 0.27 μM). Following optimizations of **38** led to a highly potent Keap1-Nrf2 PPI inhibitor **39** (FP assay: IC_{50} =15 nM, ITC assay: K_d = 1.3 nM), including replacement of the *p*-chloro-phenyl with methyl, introduction of the methoxy group at the benzotriazole 7-position and cyclization of the phenyl sulfonamide to form a fused 7-membered benzoxathiazepine [122].

3.3. Small molecule activators based on a 1-phenylpyrazole core

In 2017, Astex Therapeutics Ltd. and GlaxoSmithKline Intellectual Property Ltd. disclosed two patents about novel Keap1-Nrf2 PPI inhibitors with 1-phenylpyrazole ring. In patent **WO2017060854** [123], approximately 65 compounds with the phenylpyrazole core were present. *In vitro* target experiments showed that **40** is the most active inhibitor (FP assay: IC_{50} =10-100 nM, time-resolved FRET (TR-FRET) assay: IC_{50} < 10 nM, Figure 18). In patent **WO2017060855** [124], about 25 compounds containing a cycloalkylarylpyrazole ring were presented, and **41** was the most potent inhibitor with good Keap1-Nrf2 inhibitory activity (FP assay: IC_{50} =10-100 nM, TR-FRET assay: IC_{50} =10-100 nM) and remarkable effect in cells (EC_{50} = 79 nM) (Figure 18).

3.4. Small molecule activators based on a five-membered heterocyclic core

In 2015, Xu et al. [125] published a range of Nrf2 activators containing 1,2,4-oxadiazole core. After SAR study, compound **42** was selected for further biological evaluation as the most potent derivative (Figure 18). The results showed **42** could up-regulate the expression levels of Nrf2 and Nrf2-regulated enzymes such as NQO1 and HO-1 *in vitro* and significantly suppress inflammation in LPS-induced mouse model *in vivo* [125]. In 2018, a follow-up research about compound **42** was disclosed by Xu et al. [126]. To investigate the therapeutic potential of Nrf2 activators, **42** was chosen for further research. The results showed that Nrf2-ARE activity was induced by **42** through activating of the ERK1/2 phosphorylation. The effects of **42** on the nuclear translocation of Nrf2 protein were based on the phosphorylation of Nrf2 Ser40 by *p*-ERK. To further explore this chemotype of Nrf2 activators, Xu et al. [127] gained compound **43** by screening the Specs

database virtually based on hit substructure search. **43** was more potent than hit compound **42**, which could induce Nrf2 translocation into nuclear and upregulate Nrf2-regualted genes, and then showed cytoprotective effects on H_2O_2-induced cellular injury [127]. In 2018, a subsequent study was published [128], in which they disclosed a novel Nrf2 activator **44** through structure optimization of **43**. **44** showed appropriate physicochemical properties and protected hepatocyte L02□cells from APAP-induced cytotoxicity *in vitro* by activating the Nrf2-dependent signaling. Noticeably, **44** exhibited therapeutic potential in APAP-induced mouse model of acute liver injury via activating Nrf2 and its downstream protective proteins and decreasing the liver injury markers [128]. **44** was selected as a molecular tool for investigating the therapeutic effects on neurodegenerative diseases, and the positive outcomes further proposed **44** as a potential candidate for neurodegenerative diseases such as PD [129]. However, these compounds did not directly disrupt Keap1-Nrf2 PPI, and the precise mechanism by which they regulated the Nrf2-ARE pathway has not yet been elucidated.

4. Conclusion

The Keap1-Nrf2-ARE pathway has become a promising target to regulate numerous diseases associated with oxidative stress and inflammation. Over the past 3 years, lots of Nrf2 activators have been identified and some are under preclinical or clinical developments, including electrophilic and non-covalent compounds. Particularly, the small molecule Keap1-Nrf2 PPI inhibitors exhibit therapeutic potential in multiple animal models. In order to bring these agents into clinical application, numerous efforts need to be carried out.

5. Expert opinion

For the moment, a lot of Nrf2 activators with diverse chemotypes are at clinical studies, such as DMF, SFN, CDDO-Me and their derivatives as mentioned above. It is worth noting that the development of Keap1-Nrf2 PPI inhibitors has achieved remarkable progress. Although the potency of various types of Nrf2 activators have been confirmed both *in vitro* and *in vivo*, several certain problems are urgently needed to be solved.

For electrophilic activators, selectivity is the most noticeable consideration. Some redox-sensitive cysteines of Keap1 are essential for the interaction between electrophilic compounds and such ubiquitin ligase substrate adaptor [59]. A potential defect of these activators is that they might show effect on some proteins with highly reactive cysteines besides Keap1, consequently causing unexpected effects [130-131]. For example, the CDDO-Me showed

cardiotoxic side effects [61]. Nowadays, with more and more covalent agents are reported, some new strategies should be applied to the development of electrophilic Nrf2 activators with target selectivity.

For Keap1-Nrf2 inhibitors, one critical challenge is how to selectively target a particular organ *in vivo* [25]. CNS diseases, especially AD and PD, have always been global problem. It is gratifying that the therapeutic potency of Nrf2 activation in neurological disorders has been confirmed, and development of Keap1-Nrf2 inhibitors targeting brain tissues might serve as an alternative strategy for the treatment of CNS disorders [132-134]. Nevertheless, mainly due to the polar binding pocket of the Keap1 binding domain, most of Keap1-Nrf2 PPI inhibitors contain carboxyl or polar groups that usually lead to poor BBB penetrability [135]. Because the carboxyl group is a common substituent in most potent Keap1-Nrf2 inhibitors, an effective approach to circumvent this issue is bioisosteric replacement. For example, after replacing carboxylic acid groups with tetrazole groups, the highest potency inhibitor of Keap1 exhibited better physicochemical properties and penetrability than **26** [136]. With derivatization of carboxylic acid groups, the prodrug strategy might be an effective method for targeting CNS disorders. Other alternative approaches include reducing the amounts of polar substituents and increasing hydrophobic interactions.

Noticeably, the double roles of Nrf2 in the development and progression of cancer has been expounded [137-139]. Tumor cells may benefit from the activation of Nrf2, such as escaping from cancer therapies. Although the risk of cancer from Nrf2 activators has not been eliminated, it's worth noting that a phase III clinical trial of DMF showed no difference in cancer incidence between placebo and DMF treatment groups [140].

Funding

This study was supported by Projects 81773581, 81773639, and 81930100 of the National Natural Science Foundation of China, the Natural Science Foundation of Jiangsu Province of China (no. BK20160746), National Science & Technology Major Project “Key New Drug Creation and Manufacturing Program”, China (no. 2018ZX09711002 and 2017ZX09302003), the Priority Academic Program Development of Jiangsu Higher Education Institutions, CPU2018GY02 of Double First Class Innovation Team of China Pharmaceutical University, Program for Outstanding Scientific and Technological Innovation Team of Jiangsu Higher Education, the “Qing Lan” Project of Jiangsu Province, and the Young Elite Scientists Sponsorship Program by CAST.

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as:

** of interest*

*** of considerable interest*

1. Finkel THolbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239-47.

2. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004;3:205-14.

3. Kensler TW, Wakabayash N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacool Toxicol 2007;47:89-116.

4. Benz CCYau C. Ageing, oxidative stress and cancer: paradigms in parallax. Nat Rev Cancer 2008;8:875-79.

5. Hu R, Saw CL, Yu R, et al. Regulation of NF-E2-Related Factor 2 Signaling for Cancer Chemoprevention: Antioxidant Coupled with Antiinflammatory. Antioxid Redox Sign 2010;13:1679-98.

6. Cho HY, Reddy SP, Kleeberger SR. Nrf2 defends the lung from oxidative stress. Antioxid Redox Sign 2006;8:76-87.

7. Lyakhovich VV, Vavilin VA, Zenkov NK, et al. Active defense under oxidative stress. The antioxidant responsive element. Biochemistry-Moscow 2006;71:962-74.

8. Itoh K, Wakabayashi N, Katoh Y, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 1999;13:76-86.

9. Nioi P, Nguyen T, Sherratt PJ, et al. The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. Mol Cell Biol 2005;25:10895-906.

10. Katoh Y, Itoh K, Yoshida E, et al. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes Cells 2001;6:857-68.

11. Lu MC, Ji JA, Jiang ZY, et al. The Keap1-Nrf2-ARE Pathway As a Potential Preventive and Therapeutic Target: An Update. Med Res Rev 2016;36:924-63.

**** This review detailedly describes the biological functions of Keap1-Nrf2-ARE pathway and its modulators.**

12. Chowdhry S, Zhang Y, McMahon M, et al. Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene 2013;32:3765-81.

13. McMahon M, Thomas N, Itoh K, et al. Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. J Biol Chem 2004;279:31556-67.

14. Itoh K, Chiba T, Takahashi S, et al. An Nrf2 small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313-22.

15. Wang H, Liu K, Geng M, et al. RXRalpha inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2. Cancer Res 2013;73:3097-108.

16. Namani A, Li Y, Wang XJ, et al. Modulation of NRF2 signaling pathway by nuclear receptors: Implications for cancer. BBA-Mol Cell Res 2014;1843:1875-85.

17. Wakabayashi N, Itoh K, Wakabayashi J, et al. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat Genet 2003;35:238-45.

18. Yamamoto T, Suzuki T, Kobayashi A, et al. Physiological significance of reactive cysteine residues of keap1 in determining Nrf2 activity. Mol Cell Biol 2008;28:2758-70.

19. Li WKong A-N. Molecular Mechanisms of Nrf2-Mediated Antioxidant Response. Mol Carcinog 2009;48:91-104.

20. Itoh K, Mimura J, Yamamoto M. Discovery of the Negative Regulator of Nrf2, Keap1: A Historical Overview. Antioxid Redox Sign 2010;13:1665-78.

21. Lo S-C, Li X, Henzl MT, et al. Structure of the Keap1 : Nrf2 interface provides mechanistic insight into Nrf2 signaling. EMBO J 2006;25:3605-17.

22. Zhang DD, Lo SC, Cross JV, et al. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol 2004;24:10941-53.

23. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, et al. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci USA 2002;99:11908-13.

24. Pallesen JS, Tran KT, Bach A. Non-covalent Small-Molecule Kelch-like ECH-Associated Protein 1 - Nuclear Factor Erythroid 2-Related Factor 2 (Keap1-Nrf2) Inhibitors and Their Potential for Targeting CNS Diseases. J Med Chem 2018;61:8088-103.

**** This Perspective describes current non-covalent small-molecule Keap1-Nrf2 inhibitors' potential as CNS tool compounds by analyzing physicochemical properties, including CNS multiparameter optimization (MPO) scoring algorithms and several strategies for identifying CNS-targeting Keap1 inhibitors.**

25. 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;59:10837-58.

**** This paper is a comprehensive review of the progress in the discovery and development of Keap1-Nrf2 PPI inhibitors.**

26. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. Med Res Rev 2012;32:687-726.

27. Richardson BG, Jain AD, Speltz TE, et al. Non-electrophilic modulators of the canonical Keap1/Nrf2 pathway. Bioorg Med Chem Lett 2015;25:2261-68.

28. Tong KI, Padmanabhan B, Kobayashi A, et al. Different electrostatic Potentials define ETGE and DLG motifs as hinge and latch in oxidative stress response. Mol Cell Biol 2007;27:7511-21.

29. Tong KI, Katoh Y, Kusunoki H, et al. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. Mol Cell Biol 2006;26:2887-900.

30. Baird L, Swift S, Lleres D, et al. Monitoring Keap1-Nrf2 interactions in single live cells. Biotechnol Adv 2014;32:1133-44.

31. Kensler TW, Wakabayashi N. Nrf2: friend or foe for chemoprevention? Carcinogenesis 2010;31:90-99.

32. Copple IM. The Keap1-Nrf2 cell defense pathway--a promising therapeutic target? Adv Pharmacol (San Diego, Calif.) 2012;63:43-79.

33. Hur W, Gray NS. Small molecule modulators of antioxidant response pathway. Curr Opin Chem Biol 2011;15:162-73.

34. Wells G. Peptide and small molecule inhibitors of the Keap1-Nrf2 protein-protein interaction. Biochem Soc Trans 2015;43:674-79.

35. Hong F, Freeman ML, Liebler DC. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. Chem Res Toxicol 2005;18:1917-26.

36. Sporn MBLiby KT. NRF2 and cancer: the good, the bad and the importance of context. Nat Rev Cancer 2012;12:564-71.

37. Linker RA, Lee D-H, Ryan S, et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 2011;134:678-92.

38. Gold R, Kappos L, Arnold DL, et al. Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis. N Engl J Med 2012;367:1098-107.

39. Gold R. Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis (vol 367, pg 1098, 2012). N Engl J Med 2012;367:2362-62.

40. Fox RJ, Miller DH, Phillips JT, et al. Placebo-Controlled Phase 3 Study of Oral BG-12 or Glatiramer in Multiple Sclerosis. N Engl J Med 2012;367:1087-97.
41. Prosperini LPontecorvo S. Dimethyl fumarate in the management of multiple sclerosis: appropriate patient selection and special considerations. Ther Clin Risk Manag 2016;12:339-50.
42. Jing X, Shi H, Zhang C, et al. Dimethyl fumarate attenuates 6-OHDA-induced neurotoxicity in SH-SY5Y cells and in animal model of Parkinson's disease by enhancing Nrf2 activity. Neuroscience 2015;286:131-40.
43. Lastres-Becker I, Garcia-Yague AJ, Scannevin RH, et al. Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. Antioxid Redox Sign 2016;25:61-77.
44. 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;16:13885-907.
45. Palte MJ, Wehr A, Tawa M, et al. Improving the Gastrointestinal Tolerability of Fumaric Acid Esters: Early Findings on Gastrointestinal Events with Diroximel Fumarate in Patients with Relapsing-Remitting Multiple Sclerosis from the Phase 3, Open-Label EVOLVE-MS-1 Study. Adv Ther 2019;
46. Mrowietz U, Morrison PJ, Suhrkamp I, et al. The Pharmacokinetics of Fumaric Acid Esters Reveal Their In Vivo Effects. Trends Pharmacol Sci 2018;39:1-12.
47. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 2003;23:8137-51.
48. Axelsson AS, Tubbs E, Mecham B, et al. Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. Sci Transl Med 2017;9:
49. Bahadoran Z, Mirmiran P, Hosseinpanah F, et al. Broccoli sprouts powder could improve serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients: A randomized double-blind placebo-controlled clinical trial. Diabetes Res Clin Pract 2012;96:348-54.
50. Holmstrom KM, Kostov RV, Dinkova-Kostova AT. The multifaceted role of Nrf2 in mitochondrial function. Curr Opin Pharmacol 2016;1:80-91.
51. Lee S, Choi B-R, Kim J, et al. Sulforaphane Upregulates the Heat Shock Protein Co-Chaperone CHIP and Clears Amyloid-beta and Tau in a Mouse Model of Alzheimer's Disease. Mol Nutr Food Res 2018;62:
52. Hou T-T, Yang H-Y, Wang W, et al. Sulforaphane Inhibits the Generation of Amyloid-beta

Oligomer and Promotes Spatial Learning and Memory in Alzheimer's Disease (PS1V97L) Transgenic Mice. J Alzheimers Dis 2018;62:1803-13.

53. Sunkaria A, Bhardwaj S, Yadav A, et al. Sulforaphane attenuates postnatal proteasome inhibition and improves spatial learning in adult mice. J Nutr Biochem 2018;51:69-79.

54. Klomparens EADing Y. The neuroprotective mechanisms and effects of sulforaphane. Brain circulation 2019;5:74-83.

55. Alejandro B. Sulforaphane Compositions and Method for Increasing Bioavailability by Ultrasonic Techniques. MX20170014281 (2019).

56. Frisbee A, Newsome P, Baudet M, et al. Stabilized Sulforaphane. HUE039549T2 (2019).

57. Honda T, Rounds BV, Bore L, et al. Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages. J Med Chem 2000;43:4233-46.

58. Sporn MB, Liby KT, Yore MM, et al. New Synthetic Triterpenoids: Potent Agents for Prevention and Treatment of Tissue Injury Caused by Inflammatory and Oxidative Stress. J Nat Prod 2011;74:537-45.

59. Cleasby A, Yon J, Day PJ, et al. Structure of the BTB Domain of Keap1 and Its Interaction with the Triterpenoid Antagonist CDDO. PLoS One 2014;9:

60. Pergola PE, Raskin P, Toto RD, et al. Bardoxolone Methyl and Kidney Function in CKD with Type 2 Diabetes. N Engl J Med 2011;365:327-36.

61. Zhang DD. Bardoxolone Brings Nrf2-Based Therapies to Light. Antioxid Redox Sign 2013;19:517-18.

62. Sun H, Zhu J, Lin H, et al. Recent progress in the development of small molecule Nrf2 modulators: a patent review (2012-2016). Expert Opin Ther Pat 2017;27:763-85.

63. Robledinos-Anton N, Fernandez-Gines R, Manda G, et al. Activators and Inhibitors of NRF2: A Review of Their Potential for Clinical Development. Oxid Med Cell Longev 2019;2019: 9372182.

64. Cuadrado A, Rojo AI, Wells G, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov 2019;18:295-317.

**** Ref 64 is an excellent review for the therapeutic potential of Nrf2 and Keap 1 partnership.**

65. Cuadrado A, Manda G, Hassan A, et al. Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine Approach. Pharmacol Rev 2018;70:348-83.

66. Lynch DR, Farmer J, Hauser L, et al. Safety, pharmacodynamics, and potential benefit of omaveloxolone in Friedreich ataxia. Ann Clin Trans Neurol 2019;6:15-26.

**** Refs 65 and 66 present the attempt to address the role of Nrf2 in human chronic disease**

67. Rabbani PS, Ellison T, Waqas B, et al. Targeted Nrf2 activation therapy with RTA 408 enhances regenerative capacity of diabetic wounds. Diabetes Res Clin Pract 2018;139:11-23.

68. Mou Y. CDDO-Me derivative, preparation method and medical application. CN108440636A (2018).

69. Cheng Y, Gong Y, Qian S, et al. Identification of a Novel Hybridization from Isosorbide 5-Mononitrate and Bardoxolone Methyl with Dual Activities of Pulmonary Vasodilation and Vascular Remodeling Inhibition on Pulmonary Arterial Hypertension Rats. J Med Chem 2018;61:1474-82.

70. Huang Z, Mou Y, Xu X, et al. Novel Derivative of Bardoxolone Methyl Improves Safety for the Treatment of Diabetic Nephropathy. J Med Chem 2017;60:8847-57.

71. Kang F, Ai Y, Zhang Y, et al. Design and synthesis of new hybrids from 2-cyano-3,12-dioxooleana-9-dien-28-oic acid and O-2-(2,4-dinitrophenyl) diazeniumdiolate for intervention of drug-resistant lung cancer. Eur J Med Chem 2018;149:269-80.

72. Woo SY, Kim JH, Moon MK, et al. Discovery of Vinyl Sulfones as a Novel Class of Neuroprotective Agents toward Parkinson's Disease Therapy. J Med Chem 2014;57:1473-87.

73. Kim D, Park K, Park W, et al. Benzyl derivative compound containing activated vinyl group capable of being used for preventing and treating neurological disorders through nitric oxide generation inhibition and nrf2 activation, and pharmaceutical composition thereof. WO2013165140 (2013).

74. Choi JW, Kim S, Park JH, et al. Optimization of Vinyl Sulfone Derivatives as Potent Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Activators for Parkinson's Disease Therapy. J Med Chem 2019;62:811-30.

75. Choi JW, Shin SJ, Kim HJ, et al. Antioxidant, Anti-inflammatory, and Neuroprotective Effects of Novel Vinyl Sulfonate Compounds as Nrf2 Activator. ACS Med Chem Lett 2019;10:1061-67.

76. Li A-L, Shen T, Wan T, et al. Novel diterpenoid-type activators of the Keap1/Nrf2/ARE signaling pathway and their regulation of redox homeostasis. Free Radical Biol Med 2019;141:21-33.

77. Zhou M-X, Li G-H, Sun B, et al. Identification of novel Nrf2 activators from Cinnamomum chartophyllum HW Li and their potential application of preventing oxidative insults in human lung epithelial cells. Redox Bio 2018;14:154-63.

78. Arbeeny CM, Ling H, Smith MM, et al. CXA-10, a Nitrated Fatty Acid, Is Renoprotective in Deoxycorticosterone Acetate-Salt Nephropathys. J Pharmacol Exp Ther 2019;369:503-+.

79. Bartholomeus J, Burli R, Jarvis R, et al. Small Molecule Modulators of the BTB Domain of Keap1. WO2019122265 (2019).

80. Wang L, Wang YR, Chen X, et al. Nrf2 activator compound, drugs and new application of atractylenolide II. CN106265633 (2017).
81. George CA, Edward L, Stuart LB. Tetrahydronaphthalene Derivatives Useful as Nrf2 Activators. WO2019104030 (2019).
82. 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 Pharmaceutica Sinica B 2015;5:285-99.
83. Zhuang C, Miao Z, Sheng C, et al. Updated Research and Applications of Small Molecule Inhibitors of Keap1-Nrf2 Protein-Protein Interaction: a Review. Curr Med Chem 2014;21:1861-70.
84. Leung CH, Zhang JT, Yang GJ, et al. Emerging Screening Approaches in the Development of Nrf2-Keap1 Protein-Protein Interaction Inhibitors. Int J Mol Sci 2019;20:4445
85. Hoerer S, Reinert D, Ostmann K, et al. Crystal-contact engineering to obtain a crystal form of the Kelch domain of human Keap1 suitable for ligand-soaking experiments. Acta Crystallogr F 2013;69:592-96.
86. Padmanabhan B, Tong KI, Ohta T, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell 2006;21:689-700.
87. Beamer LJ, Li XC, Bottoms CA, et al. Conserved solvent and side-chain interactions in the 1.35 angstrom structure of the Kelch domain of Keap1. Acta Crystallogr D 2005;61:1335-42.
88. Jiang ZY, Xu LL, Lu MC, et al. Investigation of the intermolecular recognition mechanism between the E3 ubiquitin ligase Keap1 and substrate based on multiple substrates analysis. J Comput Aided Mol Des 2014;28:1233-45.
89. Fukutomi T, Takagi K, Mizushima T, et al. Kinetic, Thermodynamic, and Structural Characterizations of the Association between Nrf2-DLGex Degron and Keap1. Mol Cell Biol 2014;34:832-46.
90. Lu MC, Jiao Q, Liu T, et al. Discovery of a head-to-tail cyclic peptide as the Keapl-Nrf2 protein-protein interaction inhibitor with high cell potency. Eur J Med Chem 2018;143:1578-89.
91. Lu MC, Yuan ZW, Jiang YL, et al. A systematic molecular dynamics approach to the study of peptide Keap1-Nrf2 protein-protein interaction inhibitors and its application to p62 peptides. Mol Biosyst 2016;12:1378-87.
92. Hancock R, Schaap M, Pfister H, et al. Peptide inhibitors of the Keap1-Nrf2 protein-protein interaction with improved binding and cellular activity. Org Biomol Chem 2013;11:3553-57.
93. Inoyama D, Chen Y, Huang X, et al. Optimization of Fluorescently Labeled Nrf2 Peptide Probes and the Development of a Fluorescence Polarization Assay for the Discovery of Inhibitors of

Keap1-Nrf2 Interaction. J Biomol Screen 2012;17:435-47.

94. Hancock R, Bertrand HC, Tsujita T, et al. Peptide inhibitors of the Keap1-Nrf2 protein-protein interaction. Free Radical Biol Med 2012;52:444-51.

95. Marcotte D, Zeng W, Hus J-C, et al. Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism. Biorg Med Chem 2013;21:4011-19.

96. Jiang ZY, Lu MC, Xu LL, et al. Discovery of potent Keap1-Nrf2 protein-protein interaction inhibitor based on molecular binding determinants analysis. J Med Chem 2014;57:2736-45.

97. Jiang ZY, Xu LL, Lu MC, et al. Structure-Activity and Structure-Property Relationship and Exploratory in Vivo Evaluation of the Nanomolar Keap1-Nrf2 Protein-Protein Interaction Inhibitor. J Med Chem 2015;58:6410-21.

98. Jain AD, Potteti H, Richardson BG, et al. Probing the structural requirements of non-electrophilic naphthalene-based Nrf2 activators. Eur J Med Chem 2015;103:252-68.

99. Lu MC, Zhao J, Liu YT, et al. CPUY192018, a potent inhibitor of the Keap1-Nrf2 protein-protein interaction, alleviates renal inflammation in mice by restricting oxidative stress and NF-kappaB activation. Redox Biol 2019;26:101266.

100. Hui Q, Karlstetter M, Xu Z, et al. Inhibition of the Keap1-Nrf2 protein-protein interaction protects retinal cells and ameliorates retinal ischemia-reperfusion injury. Free Radic Biol Med 2019;

101. Lu MC, Zhang X, Wu F, et al. Discovery of a Potent Kelch-Like ECH-Associated Protein 1-Nuclear Factor Erythroid 2-Related Factor 2 (Keap1-Nrf2) Protein-Protein Interaction Inhibitor with Natural Proline Structure as a Cytoprotective Agent against Acetaminophen-Induced Hepatotoxicity. J Med Chem 2019;62:6796-813.

102. You QD, Jiang ZY, Tan SJ, et al. Naphthyl sulfamide amino acid derivative, preparation method and medical application thereof. CN108101821 (2018).

103. Richardson BG, Jain AD, Potteti HR, et al. Replacement of a Naphthalene Scaffold in Kelch-like ECH-Associated Protein 1 (KEAP1)/Nuclear Factor (Erythroid-derived 2)-like 2 (NRF2) Inhibitors. J Med Chem 2018;61:8029-47.

104. Winkel AF, Engel CK, Margerie D, et al. Characterization of RA839, a Noncovalent Small Molecule Binder to Keap1 and Selective Activator of Nrf2 Signaling. J Biol Chem 2015;290:28446-55.

105. Schwarz LR, Mezger M, Hesse S. Effect of decreased glucuronidation and sulfation on covalent binding of naphthalene in isolated rat hepatocytes. Toxicology 1980;17:119-22.

106. Buckpitt A, Boland B, Isbell M, et al. Naphthalene-induced respiratory tract toxicity: Metabolic mechanisms of toxicity. Drug Metab Rev 2002;34:791-820.

107. Tsuruda LS, Lame MW, Jones AD. Formation of epoxide and quinone protein adducts in B6C3F1 mice treated with naphthalene, sulfate conjugate of 1,4-dihydroxynaphthalene and 1,4-naphthoquinone. Arch Toxicol 1995;69:362-67.

108. Buckpitt AR, Warren DL. Evidence for hepatic formation, export and covalent binding of reactive naphthalene metabolites in extrahepatic tissues in vivo. J Pharmacol Exp Ther 1983;225:8-16.

109. Hsu KH, Su BH, Tu YS, et al. Mutagenicity in a Molecule: Identification of Core Structural Features of Mutagenicity Using a Scaffold Analysis. PLoS One 2016;11:

110. Bulbulyan MA, Figgs LW, Zahm SH, et al. Cancer incidence and mortality among beta-naphthylamine and benzidine dye workers in Moscow. Int J Epidemiol 1995;24:266-75.

111. Saito T, Ichimura Y, Taguchi K, et al. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. Nat Commun 2016;7:12030.

112. Yasuda D, Nakajima M, Yuasa A, et al. Synthesis of Keap1-phosphorylated p62 and Keap1-Nrf2 protein-protein interaction inhibitors and their inhibitory activity. Bioorg Med Chem Lett 2016;26:5956-59.

113. Yasuda D, Yuasa A, Obata R, et al. Discovery of benzo g indoles as a novel class of non-covalent Keap1-Nrf2 protein-protein interaction inhibitor. Bioorg Med Chem Lett 2017;27:5006-09.

114. Tran KT, Pallesen JS, Solbak SMO, et al. A Comparative Assessment Study of Known Small-Molecule Keap1-Nrf2 Protein-Protein Interaction Inhibitors: Chemical Synthesis, Binding Properties, and Cellular Activity. J Med Chem 2019;62:8028-52.

115. Hu L, Magesh S, Chen L, et al. Direct inhibitors of Keap1-Nrf2 interaction as antioxidant inflammation modulators. WO2013067036 (2018).

116. Meng N, Tang H, Zhang H, et al. Fragment-growing guided design of Keap1-Nrf2 protein-protein interaction inhibitors for targeting myocarditis. Free Radical Biol Med 2018;117:228-37.

117. Dahlin JL, Inglese J, Walters MA. Mitigating risk in academic preclinical drug discovery. Nat Rev Drug Discov 2015;14:279-94.

118. Davies TG, Wixted WE, Coyle JE, et al. Monoacidic Inhibitors of the Kelch-like ECH-Associated Protein 1: Nuclear Factor Erythroid 2-Related Factor 2 (KEAP1:NRF2) Protein Protein Interaction with High Cell Potency Identified by Fragment-Based Discovery. J Med Chem 2016;59:3991-4006.

119. Boehm JC, Davies TG, Woolford AJ-A, et al. Preparation of sulfonylamino phenyl propanoic acid derivatives as Nrf2 regulators. WO2015092713 (2015).
120. Boehm JC, Davies TG, Woolford AJ-A, et al. Nrf2 Regulators. US2019002454 (2019).
121. Matthews JM. Nrf2 Compounds. WO2018109646 (2018).
122. Heightman TD, Callahan JF, Chiarparin E, et al. Structure-Activity and Structure-Conformation Relationships of Aryl Propionic Acid Inhibitors of the Kelch-like ECH-Associated Protein 1/Nuclear Factor Erythroid 2-Related Factor 2 (KEAP1/NRF2) Protein-Protein Interaction. J Med Chem 2019;62:4683-702.
123. Callahan JF, Kerns JJ, Li T, et al. Biaryl Pyrazoles as Nrf2 Regulators. WO2017060854 (2017).
124. Callahan JF, Kerns JJ, Li T, et al. Arylcyclohexyl Pyrazoles as Nrf2 Regulators. WO2017060855 (2017).
125. Xu LL, Zhu JF, Xu XL, et al. Discovery and Modification of in Vivo Active Nrf2 Activators with 1,2,4-Oxadiazole Core: Hits Identification and Structure-Activity Relationship Study. J Med Chem 2015;58:5419-36.
126. Xu LL, Liu T, Wang L, et al. 3-(1H-Benzo d imidazol-6-yl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (DDO7232), a Novel Potent Nrf2/ARE Inducer, Ameliorates DSS-Induced Murine Colitis and Protects NCM460 Cells against Oxidative Stress via ERK1/2 Phosphorylation. Oxid Med Cell Longev 2018;
127. Xu LL, Zhang X, Jiang ZY, et al. Molecular similarity guided optimization of novel Nrf2 activators with 1,2,4-oxadiazole core. Biorg Med Chem 2016;24:3540-47.
128. Xu LL, Wu YF, Wang L, et al. Structure-activity and structure-property relationships of novel Nrf2 activators with a 1,2,4-oxadiazole core and their therapeutic effects on acetaminophen (APAP)-induced acute liver injury. Eur J Med Chem 2018;157:1376-94.
129. Xu LL, Wu YF, Yan F, et al. 5-(3,4-Difluorophenyl)-3-(6-methylpyridin-3-yl)-1,2,4-oxadiazole (DDO-7263), a novel Nrf2 activator targeting brain tissue, protects against MPTP-induced subacute Parkinson's disease in mice by inhibiting the NLRP3 inflammasome and protects PC12 cells against oxidative stress. Free Radical Biol Med 2019;134:288-303.
130. Zheng S, Laxmi YRS, David E, et al. Synthesis, Chemical Reactivity as Michael Acceptors, and Biological Potency of Monocyclic Cyanoenones, Novel and Highly Potent Anti-inflammatory and Cytoprotective Agents. J Med Chem 2012;55:4837-46.
131. Bradshaw JM, McFarland JM, Paavilainen VO, et al. Prolonged and tunable residence time using reversible covalent kinase inhibitors. Nat Chem Biol 2015;11:525-+.

132. Mazzuferi M, Kumar G, van Eyll J, et al. Nrf2 Defense Pathway: Experimental Evidence for Its Protective Role in Epilepsy. Ann Neurol 2013;74:560-68.
133. Gao B, Doan A, Hybertson BM. The clinical potential of influencing Nrf2 signaling in degenerative and immunological disorders. Clinical pharmacology : advances and applications 2014;6:19-34.
134. Sivandzade F, Prasad S, Bhalerao A, et al. NRF2 and NF-B interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. Redox Biol 2019;21:101059.
135. Sheng C, Dong G, Miao Z, et al. State-of-the-art strategies for targeting protein-protein interactions by small-molecule inhibitors. Chem Soc Rev 2015;44:8238-59.
136. Lu MC, Tan SJ, Ji JA, et al. Polar Recognition Group Study of Keap1-Nrf2 Protein-Protein Interaction Inhibitors. ACS Med Chem Lett 2016;7:835-40.
137. Jiang ZY, Lu MC, You QD. Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Inhibition: An Emerging Strategy in Cancer Therapy. J Med Chem 2019;62:3840-56.
138. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the Hallmarks of Cancer. Cancer Cell 2018;34:21-43.
139. Menegon S, Columbano A, Giordano S. The Dual Roles of NRF2 in Cancer. Trends Mol Med 2016;22:578-93.
140. Pakpoor J, Disanto G, Altmann DR, et al. No evidence for higher risk of cancer in patients with multiple sclerosis taking cladribine. Neurol Neuroimmunol Neuroinflamm 2015;2:158.

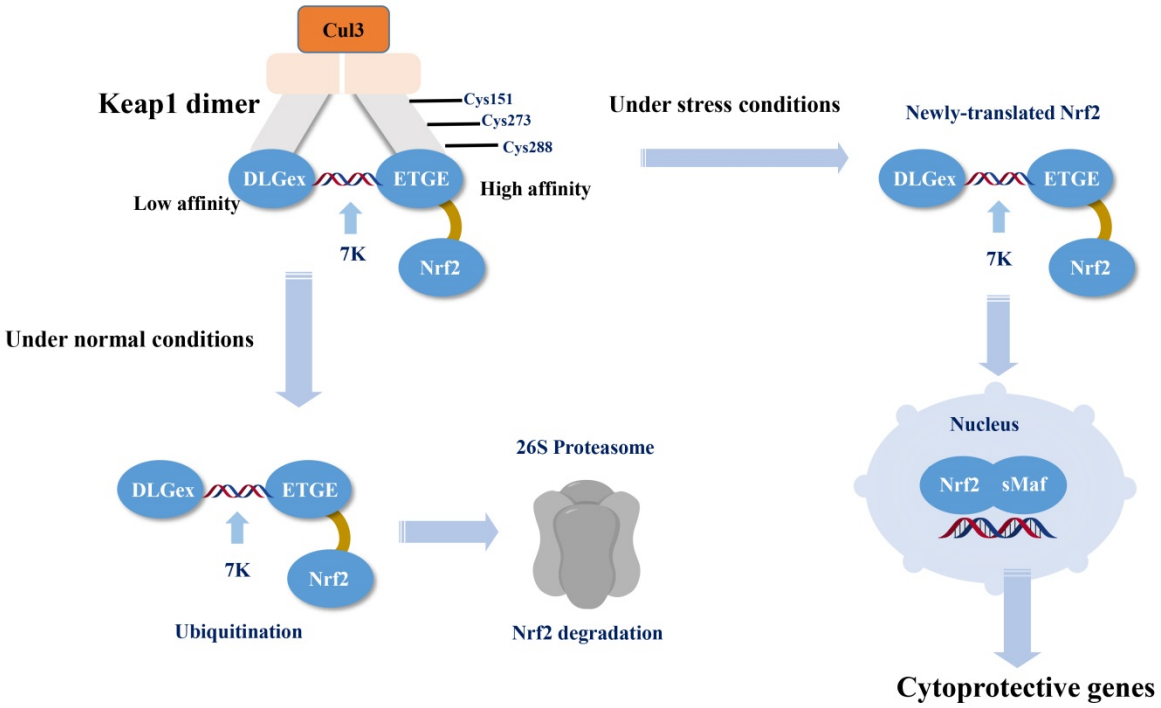


Figure 1. Schematic diagram for the regulation of Nrf2 activity.

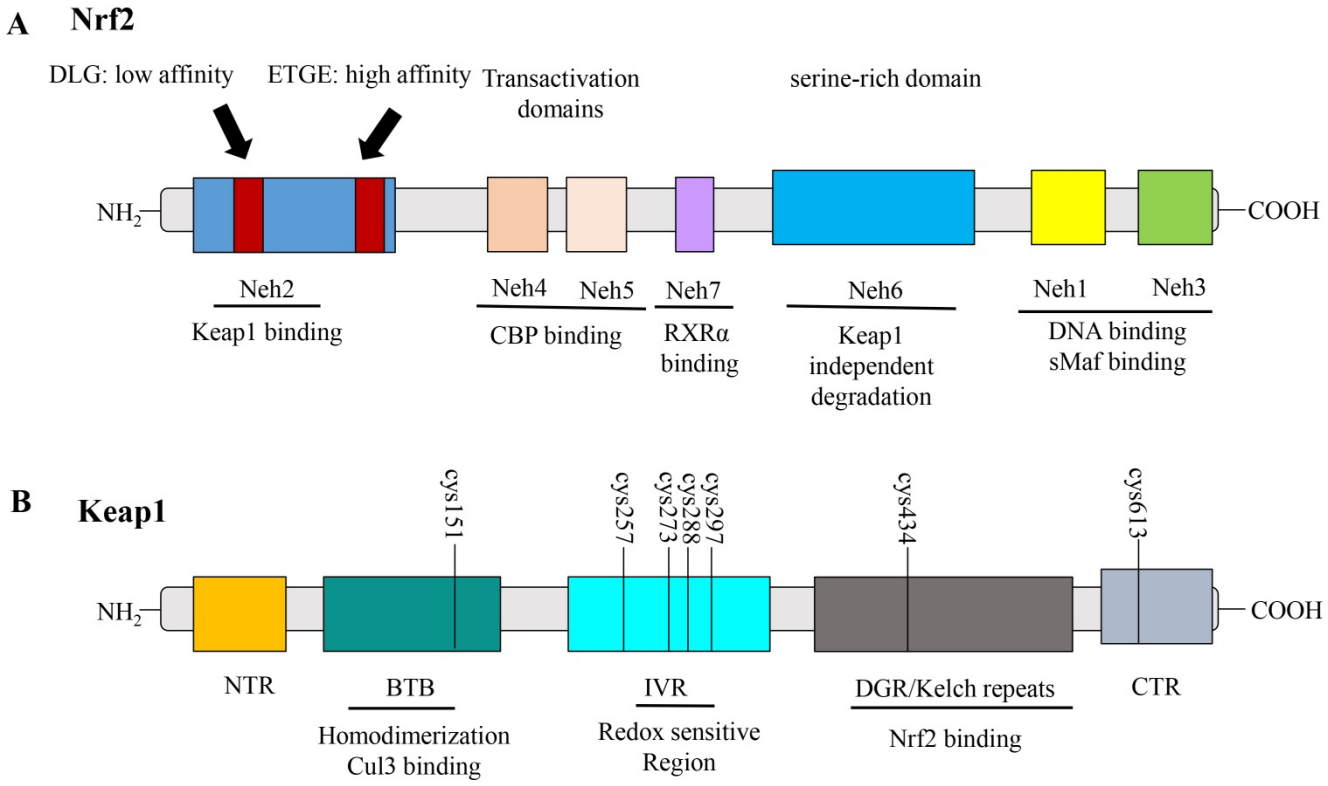


Figure 2. Domain structures of Nrf2 and Keap1.

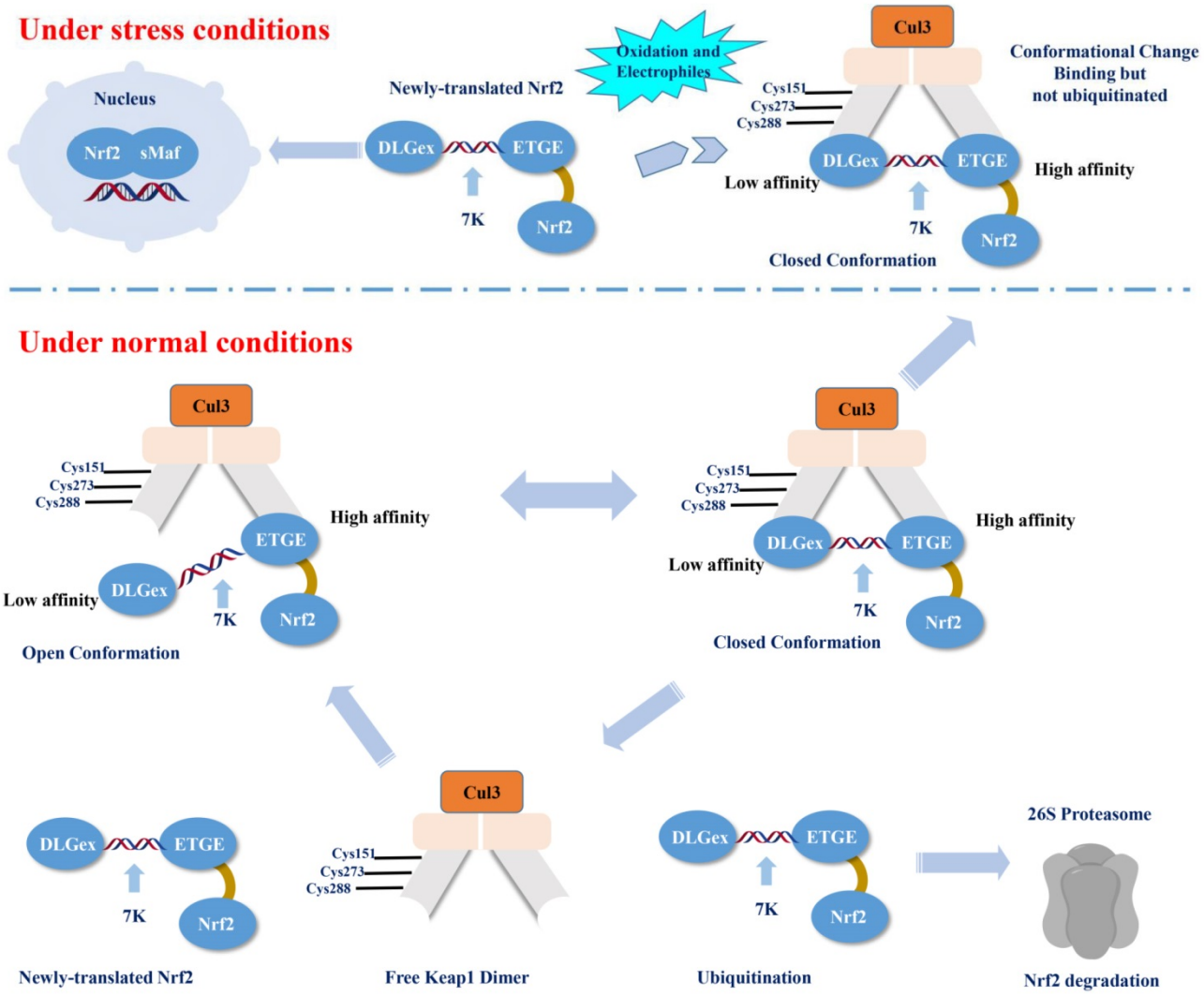


Figure 3. Conformation cycling model of Keap1-Nrf2 regulation.

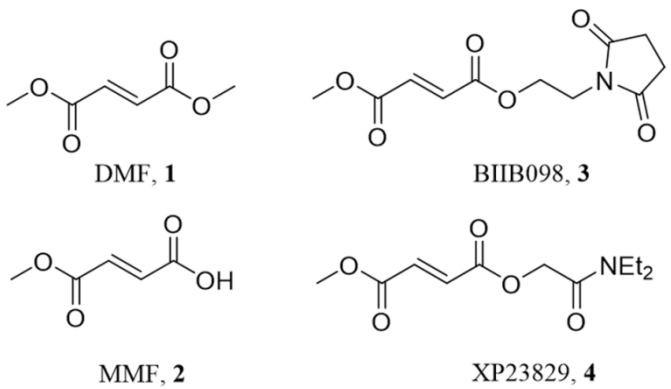


Figure 4. The structures of DMF and its analogs.

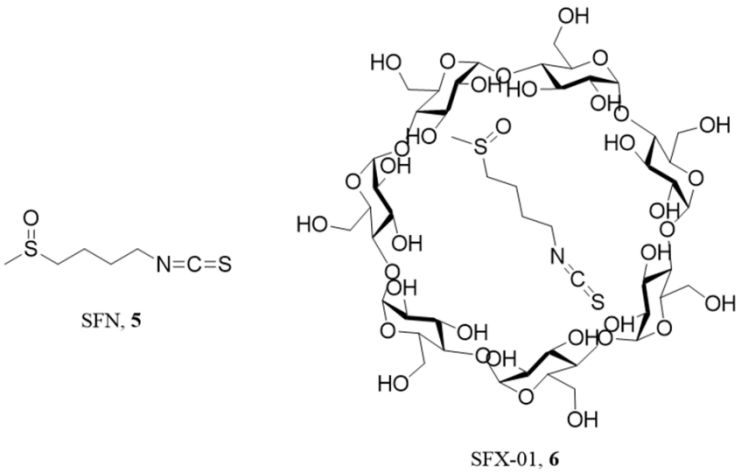


Figure 5. The structures of SFN and its analogs.

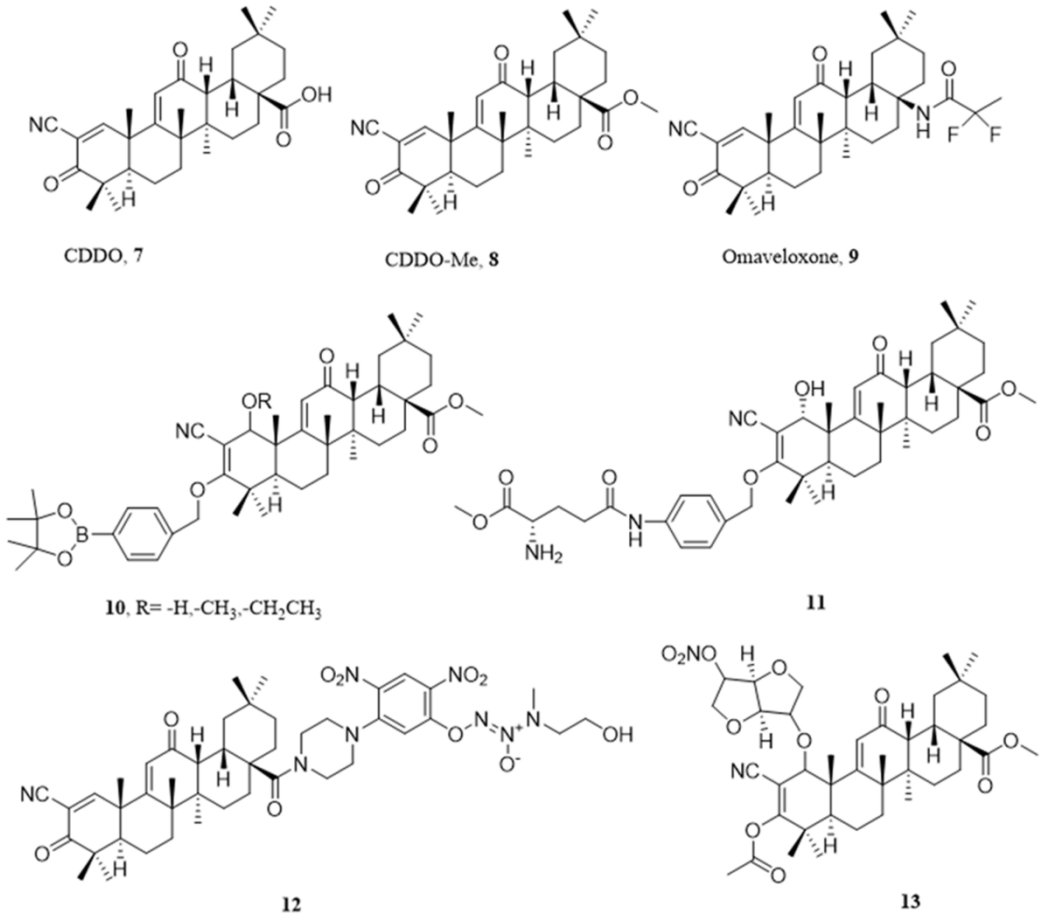


Figure 6. The structures of CDDO-Me and its derivatives.

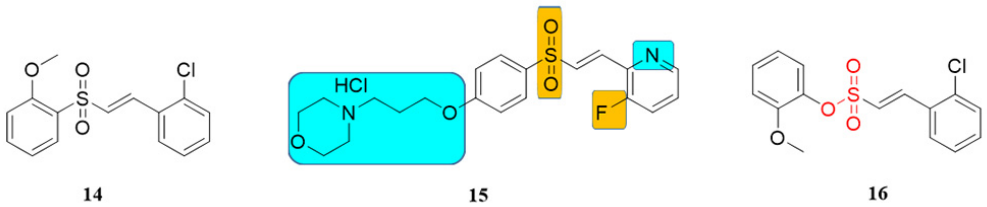


Figure 7. The structures of vinyl sulfones.

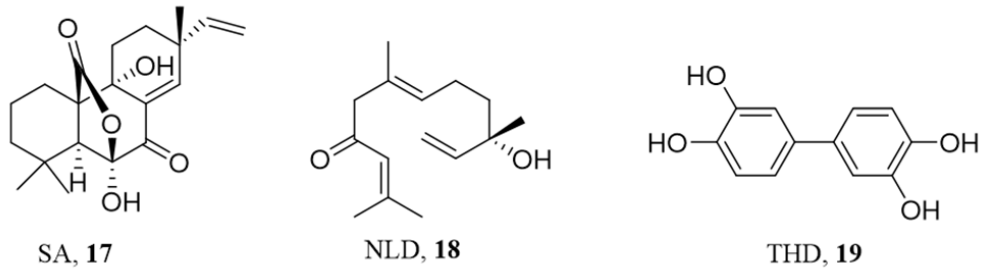


Figure 8. The structures of diterpenoid **17** and sesquiterpenoids **18-1** and **18-2**.

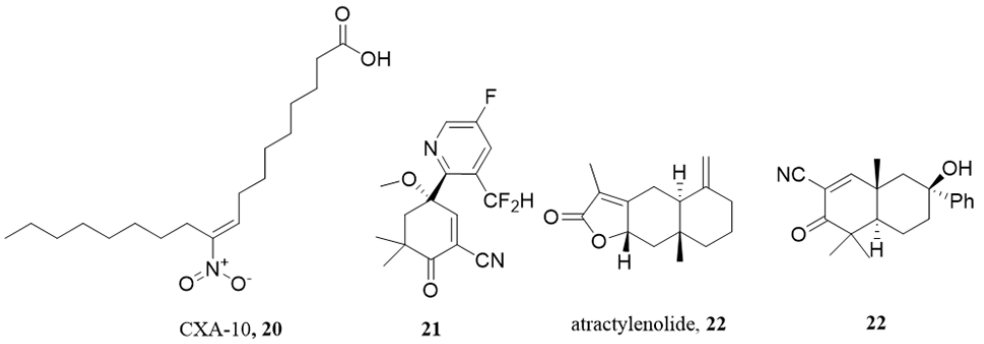


Figure 9. The structures of compounds **20-22**.

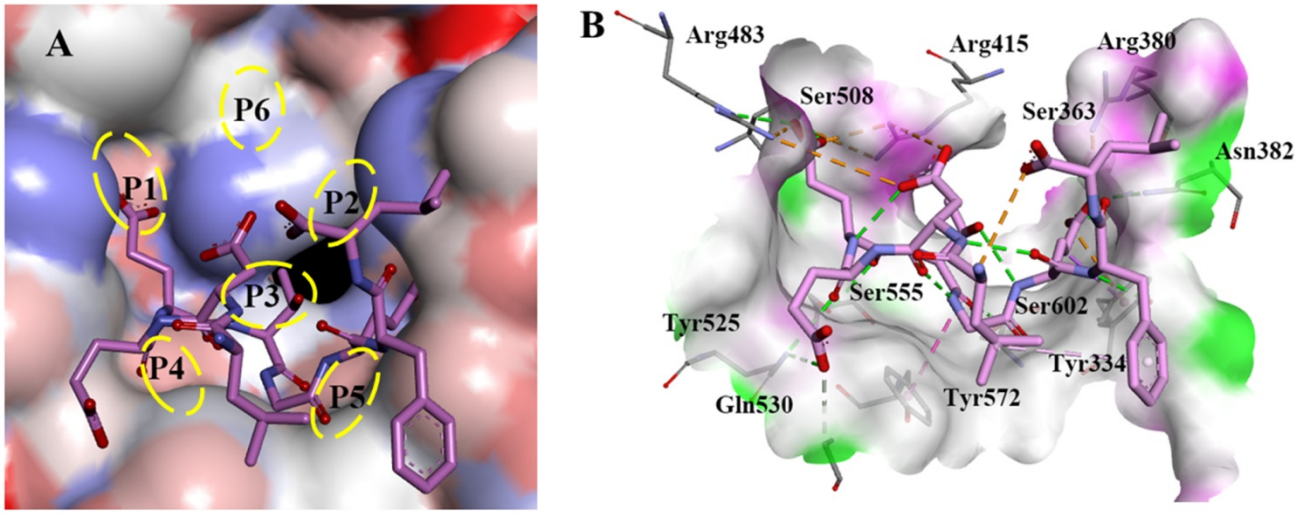


Figure 10. Sub-pockets of the Keap1 binding cavity. (A) The binding cavity based on Keap1-Nrf2 ETGE complex (PDB code: 1X2R); (B) Binding surface and key polar residue interactions of the Keap1-Nrf2 ETGE motif (PDB code: 1X2R).

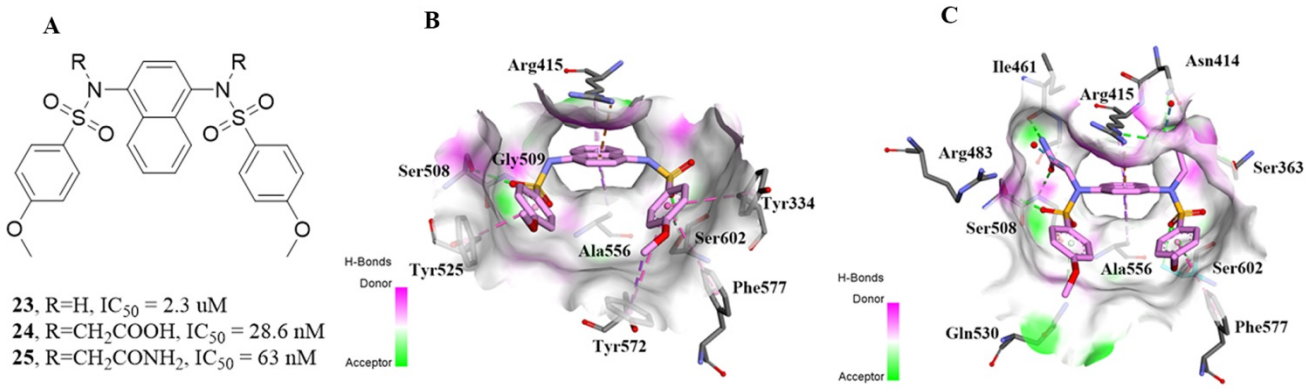


Figure 11. The structures of naphthalene sulfonamide class of the Keap1-Nrf2 PPI inhibitors **23-25** and binding mode of **23** and **25**. (A) The structures of **23-25**; (B) The binding mode of **23** (PDB code 4IQK); (C) The binding mode of **25** (PDB code 4XMB). Hydrogen bonds are represented by green dashed lines, and electrostatic interactions are represented by yellow dashed lines. The carbon atoms of small molecules and Keap1 residues are colored purple and gray, respectively. Key water molecules are represented by red dots.

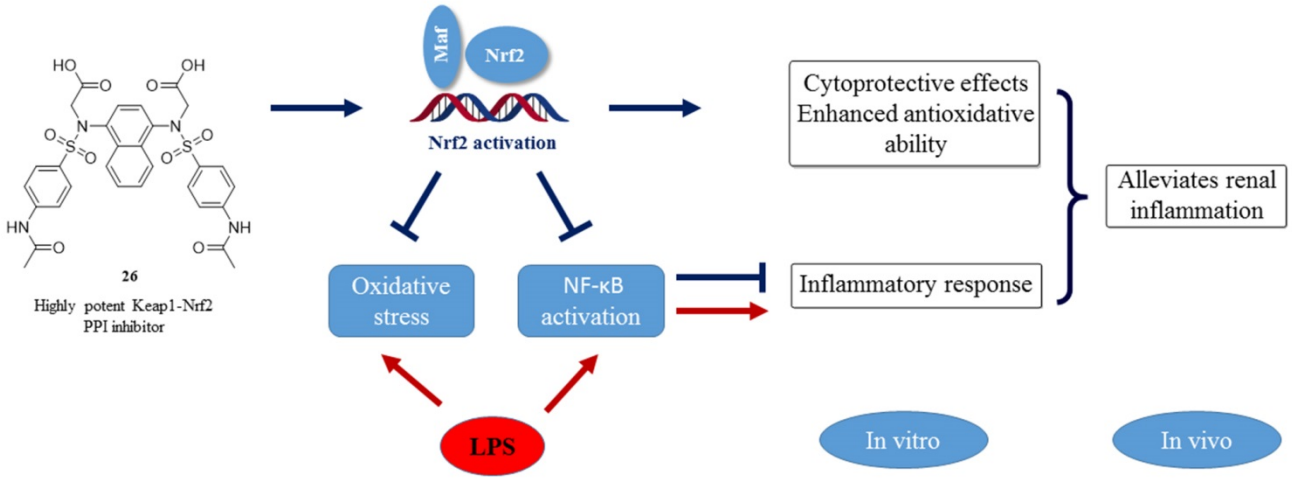
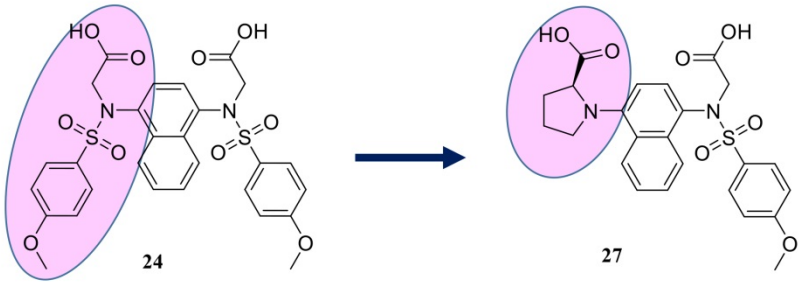


Figure 12. The structures and therapeutic potential in inflammatory kidney disorders of **26**.



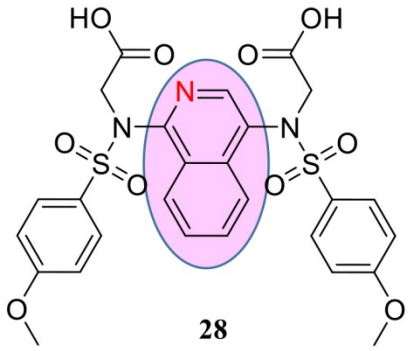
FP assay: IC_{50} = 43 nM

ITC assay: K_d = 53.7 nM

BLI assay: KD = 28.5 nM

In vivo efficacy on APAP-induced Acute Live Injury

Figure 13. The development strategy of **27**.



High potency

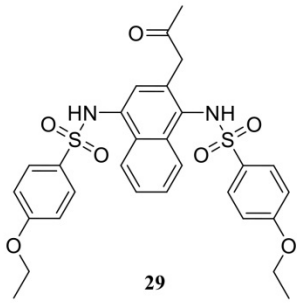
High solubility

Cellular activity

Metabolic stability

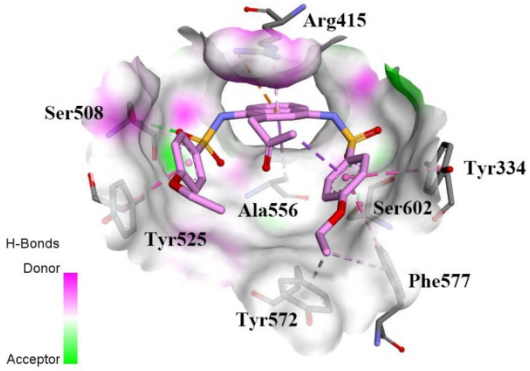
Decreased mutagenicity

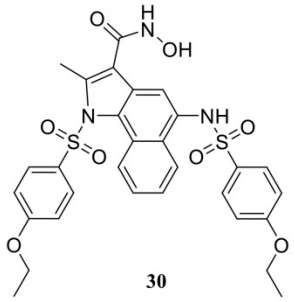
Figure 14. The superior properties of **28**.



IC_{50}= 1.5 μM Keap1-*p*-p62 FP Assay

IC_{50}= 6.2 μM Keap1-Nrf2 FP Assay





IC_{50}= 0.20 μM FP Assay by Mashino et al.

K_i = 0.0021 FP Assay

ΔTm-max = 28 ℃ TSA Assay

*K*d = 0.026 μM SPR Assay by Tran et al.

Figure 15. The structures of **29** and **30** and binding mode of **29**. (A) The structures of **29**; (B) The binding mode of **29** (PDB code 4ZY3); (C) The structures of **30**. Hydrogen bonds are represented by green dashed

lines, and electrostatic interactions are represented by yellow dashed lines. The carbon atoms of small molecules and Keap1 residues are colored purple and gray, respectively. Key water molecules are represented by red dots.

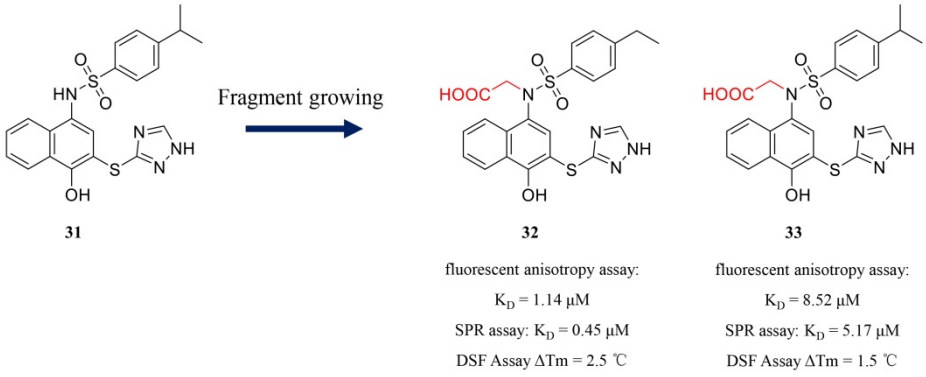


Figure 16. The development strategy of **32** and **33**.

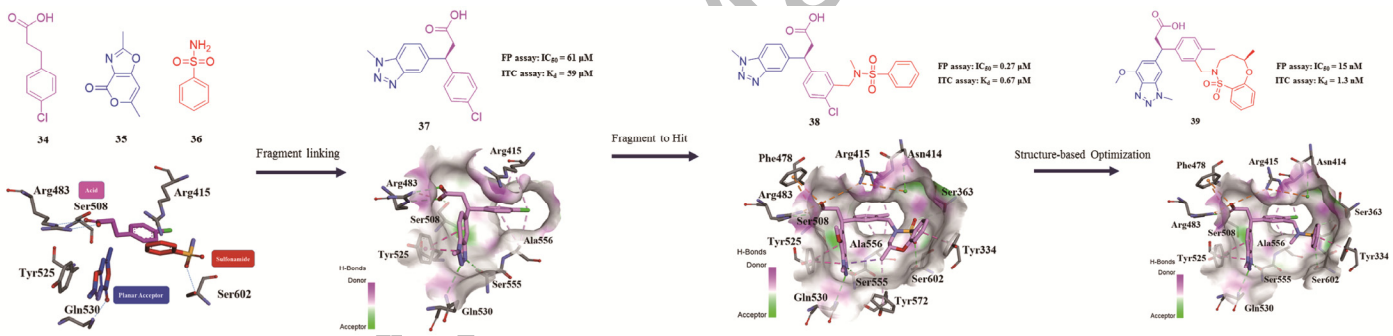
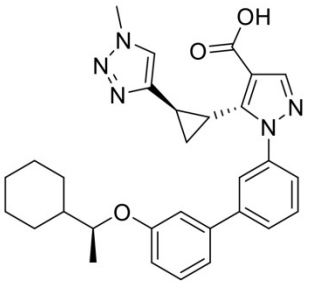


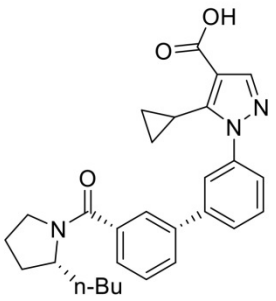
Figure 17. Fragment-based and structure-based discovery of compound **39**. Three distinct fragments were identified through a crystallographic screen of fragments library (Fragment **34**: PDB code 5FNQ; Fragment **35**: PDB code 5FZJ and Fragment **36**: PDB code 5FZN). The first hit in fragment to hit process, **37**, was designed based on the binding mode of fragment 1 and 2 (PDB code: 5FNR). The structure of fragment **36** was integrated into **37**, resulting in a good hit, **38** (PDB code: 5FNT). Further structure-based optimization gave the nanomole inhibitor, **39** with a quite ingenious seven-member heterocyclic structure (PDB code: 5FNU).



40

FP assay: IC_{50} = 10-100 nM

TR-FRET assay : IC_{50} < 10 nM



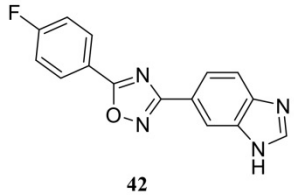
41

FP assay: IC_{50} = 10-100 nM

TR-FRET assay : IC_{50} = 10-100 nM

BEAS-2B NQO1 MTT assay: EC_{50} = 79 nM

Figure 18. The structures of **40** and **41**.

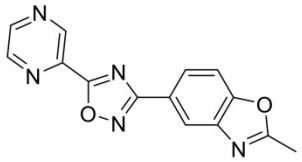


42

Potent Nrf2 Activator

In vivo efficacy on DSS-induced Murine Colitis and LPS-challenged Mouse Model

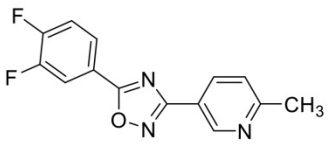
Hit-based Virtual Screening



43

In Vitro Efficacy on HCT116, LO2, NCM460, PC12 cells

Structure-based Optimization



44

Therapeutic Effects on APAP-induced Acute Liver Injury and MPTP-induced Parkinson's Disease in Mice

Figure 19. The structures of **42**, **43** and **44**.

Table 1. Use of dimethyl fumarate in clinical trials.

Sponsor/Collaborators	Official title	Clinical trials (Status)	NCT Number
Biogen/ University Hospital Muenster	Investigation of the Effect of Dimethyl Fumarate on T Cells in Patients with Relapsing Remitting Multiple Sclerosis	Phase IV (Completed)	NCT02461069
Biogen Inc.	Dimethyl Fumarate (DMF) Observational Study	Recruiting	NCT02047097
XenoPort Inc.	A Study to Assess the Efficacy and Safety of XP23829 in Subjects with Moderate-to-Severe Chronic Plaque-Type Psoriasis	Phase II (Completed)	NCT02173301
Michael Choi/ The Leukemia and Lymphoma Society/ University of California, San Diego	Dimethyl fumarate (DMF) in Relapsed/Refractory CLL/SLL	Phase I	NCT02784834
Robert Lafyatis/ Biogen/ University of Pittsburgh	Dimethyl Fumarate (DMF) in Systemic Sclerosis-Associated Pulmonary Arterial Hypertension	Recruiting	NCT02981082
Multiple Sclerosis Center of Northeastern New	Investigating Indirect Mechanism of Neuroprotection of Tecfidera® (Dimethyl Fumarate) in RRMS and	Phase IV (Active, not recruiting)	NCT03092544

York/ Icahn School of Medicine at Mount Sinai/ Biogen	Progressive Patients		
LEO Pharma	A Trial Comparing the Efficacy of Subcutaneous Injections of Brodalumab to Oral Administrations of Fumaric Acid Esters in Adults with Moderate to Severe Plaque Psoriasis	Phase IV (Completed)	NCT03331835
Alkermes Inc	A Tolerability Study of ALKS 8700 in Subjects with Relapsing Remitting Multiple Sclerosis (RRMS) EVOLVE-MS-2	Phase III (Active, not recruiting)	NCT03093324

Table 2. Patent applications or patents about the indications of DMF published during 2017-present.

Pub./Pat. no.	Pub./Pat. date	Title	Applicants	Targeted diseases
US2019247485A1	15 August 2019	Dimethyl Fumarate and Vaccination Regimens	Biogen Inc.	a method of treating or preventing a disease or disorder (e.g., MS) Combination of drug comprising DMF and vaccines
US2019201369A1	14 July	Pharmaceutical	FWP IP	pharmaceutical composition

	2019	Composition Containing Dimethyl Fumarate for Administration at a low Daily Dose	APS	for oral use in treating hyperproliferative, inflammatory or autoimmune disorders by administering a low daily dosage dimethyl fumarate
US2019175510A1	13 June 2019	Pharmaceutical Matrix Formulations Comprising Dimethyl Fumarate	Biogen Inc.	methods of using novel pharmaceutical compositions of dimethyl fumarate for treating multiple sclerosis
JP2019059732A	18 April 2019	Pharmaceutical Composition Containing Dimethyl Fumarate	Biogen Inc.	neurodegenerative diseases including multiple sclerosis
US2019091146A1	28 March 2019	Controlled Release Dosage Form for Once Daily Administration of Dimethyl Fumarate	Biogen Inc.	multiple sclerosis
WO2018234584A1	27 December 2018	Pharmaceutical Composition Comprising Dimethyl Fumarate	Almirall Sa.	inflammatory autoimmune diseases or disorders
US2018050031A1	22	Treatment of Multiple	Teva	multiple sclerosis or presenting

	February 2018	Sclerosis with Combination of Laquinimod and Dimethyl Fumarate	Pharma.	a clinically isolated syndrome
WO2017145036A1	31 August 2017	Pharmaceutical Compositions Comprising Dimethyl Fumarate	Kannusamy Saravanan. Das Samir. Nalla Priyanka. Meenakshis underam Sivakumaran.	multiple sclerosis
CN106814144A	9 June 2017	Determination and analysis method of dimethyl sulfate content in dimethyl fumarate	Shanghai Syncore Tech Inc.	hyperproliferative, inflammatory or autoimmune disorders