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Research paper

Probing the structural requirements of non-electrophilic naphthalene-based Nrf2 activators

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ABSTRACT

Activation of the transcription factor Nrf2 has been posited to be a promising therapeutic strategy in a number of inflammatory and oxidative stress diseases due to its regulation of detoxifying enzymes. In this work, we have developed a comprehensive structure–activity relationship around a known, naphthalene-based non-electrophilic activator of Nrf2, and we report highly potent non-electrophilic activators of Nrf2. Computational docking analysis of a subset of the compound series demonstrates the importance of water molecule displacement for affinity, and the X-ray structure of di-amide **12e** supports the computational analysis. One of the best compounds, acid **16b**, has an IC_{50} of 61 nM in a fluorescence anisotropy assay and a K_d of 120 nM in a surface plasmon resonance assay. Additionally, we demonstrate that the ethyl ester of **16b** is an efficacious inducer of Nrf2 target genes, exhibiting *ex vivo* efficacy similar to the well-known electrophilic activator, sulforaphane.

1. Introduction

During periods of oxidative or electrophilic stress, one of the body's main defenses is induction of cytoprotective proteins, including detoxification enzymes, such as those that reduce quinones (e.g., NAD(P)H quinone oxidoreductase 1, NQO1) [1], those that degrade heme (heme oxygenase 1, HMOX1) [2], and those involved in glutathione synthesis and transfer (e.g., glutamate-cysteine ligase catalytic subunit, glutamate-cysteine ligase regulatory subunit, glutathione *S*-transferase, GST) [3]. These genes are regulated by the transcription factor, Nrf2 (nuclear factor-erythroid2-related factor 2), which belongs to a cap 'n' collar family of basic leucine zipper transcription factors that comprise seven highly conserved domains (Neh1 to Neh7) [4]. In the absence of electrophilic or oxidative stressors, Nrf2 is negatively regulated by Keap1 (Kelch like ECH associated protein 1), a 69 kDa sensor protein that contains 27 cysteine residues [5]. Keap1 is a tightly-associated dimer that acts as an adaptor protein that simultaneously binds both Nrf2, through Keap1's Kelch domain, and the E3 ubiquitin ligase Cul3 [6], through Keap1's BTB domain. Cul3 poly-ubiquitinates Nrf2, and Nrf2 is subsequently degraded through the ubiquitin/proteasome system [4]. Nrf2 is activated by inhibiting its degradation (see below). Nrf2 is then translocated to the nucleus, where it heterodimerizes with the small MAF (sMAF) transcription factors and binds to antioxidant response elements in the promoter regions of many detoxification genes, such as NQO1, GST, HMOX1 and glutathione peroxidase [7].

As shown in Fig. 1, there are two prevailing mechanisms that

Abbreviations: BTB, Broad complex, Tramtrack and Bric-à-Brac; LE, Ligand efficiency; DMEM, Dubecco's modified Eagle's medium; FBS, fetal bovine serum; HMOX1, heme oxygenase 1; Keap1, Kelch-like ECH-associated protein 1; NQO1, NAD(P)H quinone oxidoreductase 1; MLE, mouse lung alveolar epithelial cell line; Nrf2, nuclear factor (erythroid-derived 2)-like 2; sMAF, small musculoaponeurotic fibrosarcoma oncogene homolog.

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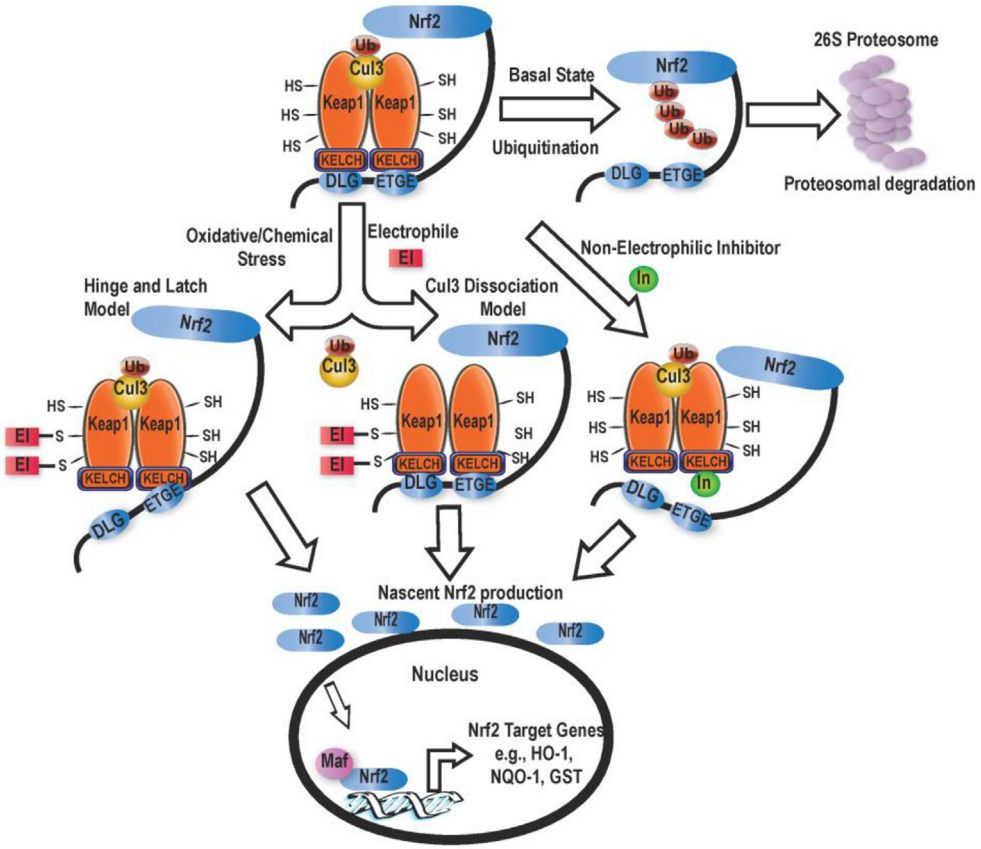

Fig. 1. A model of Nrf2 activation by both electrophilic and non-electrophilic inhibition of Keap1-Nrf2 complex.

explain how Nrf2 degradation is inhibited: 1) the Cul3 dissociation model [8,9] and 2) the hinge and latch model [10]. In the Cul3 dissociation model, Keap1 recognizes and binds Nrf2 through the ETGE and DLG motifs of the Neh2 region of Nrf2. Keap1 brings Nrf2 into close proximity with a ubiquitin-conjugating enzyme (Cul3), which then transfers ubiquitin to target lysine residues on Nrf2. If electrophilic inhibitors bind to Keap1 so that Cul3 is dissociated from Keap1, Nrf2 is not degraded, but it is also not released from Keap1 [8,9,11]. Newly formed Nrf2 is then available for regulating expression of cytoprotective enzymes. The alternative hinge and latch model is governed by modifications affecting Nrf2 [10,12–14]. In this model, upon electrophilic modification of key cysteine residues in the IVR region of Keap1, the protein undergoes a slight conformational modification and releases the DLG motif, while maintaining binding to the ETGE motif [12,13]. Once the DLG motif is released, Nrf2 swings out from its ideal position, becoming inaccessible for ubiquitination [13]. With this change, Nrf2 does not get degraded, and nascent Nrf2 production gives rise to Nrf2 accumulation and activation. A recent structural study [15] lends support for the Cul3-dissociation model, but there is still an active dialogue in the literature as to which model dominates in Nrf2 activation or whether both are operative [8–10,16–18].

Given its central importance in regulating cytoprotective enzymes, Nrf2 activation has been proposed as a promising pharmacotherapeutic strategy in a number of inflammatory and oxidative stress disorders, including chronic kidney disease [19], multiple sclerosis [20], pulmonary fibrosis [21], cancer chemoprevention [22], and chronic obstructive pulmonary disorder [23]. As alluded to above, electrophilic Nrf2 activators are known (Chart 1). Two of the more well-known electrophilic Nrf2 activators are sulforaphane (**1**), an isothiocyanate derived from cruciferous vegetables like broccoli; and dimethyl fumarate, a new therapeutic for multiple sclerosis (i.e., Tecfidera® (**2**); see Chart 1). The therapeutic benefit of each of these electrophilic molecules is thought to arise, in part, from Nrf2 activation. These compounds covalently react with the sensor cysteines of Keap1, particularly Cys 151 [24–28], which results in Nrf2 activation (see above). These covalent activators are obviously efficacious, but, because they are electrophiles, they may not be selective for Keap1. This point is exemplified by a proteomics study done with the oleanic triterpenoid bardoxolone imidazole (**3**) (See Chart 1) [29]. That study found that this potent electrophilic activator of Nrf2 interacts with at least 577 different proteins in whole cells [29]. It acts as a Michael acceptor for reactive Cys residues on Keap1 *en route* to activating Nrf2. A related compound, bardoxolone methyl (**4**), proceeded as far as a phase III clinical trial in patients with type 2 diabetes and chronic kidney disease before adverse cardiovascular events derailed its development [30]. Although their cause is unknown, these adverse events may be attributable to off-target toxicity.

Recently, there has been great interest in developing reversible covalent drugs to activate Nrf2 [31,32]; however, we and others have taken a different approach and have begun to develop non-covalent compounds that might be more selective Nrf2 activators [31,33–37]. Non-covalent compounds may serve two important functions: first, as tool compounds that can help to disentangle the rather complicated pharmacology of Nrf2 activation, and, second, as lead compounds for eventual therapeutic development.

In developing non-covalent activators of Nrf2, a sensible approach is to inhibit the interaction of Nrf2 with its negative regulator, Keap1. In this case, an inhibitor would occupy the site on