International Journal of Drug Development and Research

Saqib and Baig, Int J Drug Dev & Res 2016, 8:3

Mini Review

Inhibitors of Toll-Like Receptor 4 (TLR4) – Homodimerization: Nipping in the Bud

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Received July 20, 2016; **Accepted** August 23, 2016; **Published** August 29, 2016

Abstract

Toll-like receptors (TLRs) play a key role in sensing microbial components and hence in eliciting innate immune responses. Lipopolysaccharide (LPS)-induced dimerization of TLR4 is a prerequisite for the activation of downstream signaling cascade including nuclear factor-kappa B (NF-κB). Hence, the receptor dimerization is a primary regulatory event in the activation of TLR- signaling which could be targeted to inhibit the inflammatory pathway. Many small organic molecules of both natural and synthetic origin have been discovered in the past few years. In this brief review, we throw light on a common structural motif of an α,β-unsaturated carbonyl group utilized by various natural and synthetic inhibitors of TLR-4 signaling. These inhibitors disrupt the disulphide bonds present between the cysteine residues at the extracellular domain of TLR4 which eventually inhibits its dimerization. This brief review focusses on how the present understanding about the mode of inhibition of these few but important inhibitors could be well utilized for further drug discovery and development.

Keywords: TLR4; Inflammation; LPS; NF-κB; Phytochemicals

Introduction

TLRs were originally discovered based on homology to the *Drosophila melanogaster* Toll protein [1], and hence derived their name from it [2]. Structurally, TLRs are integral glycoproteins characterized by an extracellular ligand-binding domain containing leucine-rich repeat (LRR) motifs and a cytoplasmic signaling Toll/interleukin-1 (IL-1) receptor homology (TIR) domain [3]. TLR4 has been identified as the receptor for lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, which causes septic shock through intensive systemic inflammatory responses. It was demonstrated that ligand-induced homotypic oligomerization is required for LPS-induced activation of TLR4 signaling pathways [4]. Dimerization of TLR4 leads to the recruitment of one or more TIR domain containing adapter molecules, such as myeloid differentiation protein-88 (MyD88) or TLR domain containing adapter inducing interferon-β (TRIF) [5]. Hence, the receptor dimerization in response to a LPS insult is considered to be one of the first means of regulation in activating TLR-mediated downstream signaling pathways. Plant secondary metabolism provides an endless source of chemically diverse bioactive and pharmacologically active compounds. Interestingly, many herbs used in traditional Chinese and Indian medicine seem to be quite rich in molecules that interfere with TLR4 dimerization hence promoting its inactivation thus further inhibiting the signaling pathway [6,7]. These include curcumin [8], shogaol [9], isoliquiritigenin [10], cinnamaldehyde [11] etc. A detailed review on plant based TLR4 modulators has been nicely described by Peri and Calabrese [12]. After understanding the potency of these natural compounds many chemists tried to develop synthetic compounds which could mimic the mode of action of these natural inhibitors. This resulted in the introduction of a fumaryl pyrrolidinone, (E)-isopropyl 4-oxo-4-(2-oxopyrrolidin-1-yl)-2- butenoate (IPOP) [13]. IPOP is a potent synthetic compound known for its TLR4-dimerization inhibitory activity. It has been suggested that phytochemicals or synthetic compounds with the structural motif of α,β-unsaturated carbonyl group undergoes Michael addition by interacting with cysteine residues in TLR4 leading to inhibition of TLR4 dimerization. The Figure 1 clearly shows the redundancy of α,β-unsaturated carbonyl group in all TLR4 inhibitors. We discuss below the major TLR4 inhibitors, which have been previously as well as recently used as anti-inflammatory agents by specifically inhibiting TLR4 dimerization and signaling.

Curcumin

Curcumin (Figure 2a) is a naturally occurring yellow pigment derived from the plant *Curcuma longa*. Before being known to disrupt TLR4 dimerization, it was known to inhibit the LPS mediated activation of NFkB via inhibition of IkB kinase β (IKKβ) which phosphorylates IkBα leading to its ubiquitinylation and degradation [14,15]. Previously, Fang *et.al* has shown that curcumin binds to the catalytically active cysteine residue of thioredoxin reductase and inhibits its activity [16]. In a similar manner the thiol (–SH) group containing cysteine residue in the activation loop of IKKβ reacts with the α,β-unsaturated carbonyl group of curcumin thereby inhibiting the kinase activity of IKKβ [17]. Further, it was postulated that molecules with the structural motif of α,β-unsaturated carbonyl group can react with biological nucleophiles such as sulfhydryl group by a Michael addition [18,19]. It could also be noted that curcumin is considered to be a very strong inhibitor of many signaling molecules present in the TLR4 pathway, possibly due to the presence of more than one α,β-unsaturated carbonyl group in its structure (Figure 2a).

Isoliquiritigenin

Isoliquiritigenin is a flavonoid with a simple chalcone core of 4,2,4-trihydroxychalcone (Figure 2b). It is a major compound derived from licorice root. From many years licorice (*Glycyrrhiza uralensis*) has been used as a flavoring agent in foods and beverages as well as a traditional natural medicine for treating several diseases like gastric ulcers, sore throats, coughs, bronchitis, arthritis, adrenal insufficiency, and allergies and many other inflammatory diseases [20-26]. Isoliquiritigenin is shown to inhibit LPS-induced TLR4 dimerization resulting in inhibition of NF-κB and interferon regulatory factor 3 (IRF3) activation [10]. Similar to curcumin, Isoliquiritigenin also consists of the α,β-unsaturated carbonyl group conferring the Michael addition. This group disrupts the dimerization of TLR4. TLRs have several cysteine residues in extracellular and cytoplasmic domains that may be involved in disulfide bond formation for dimerization of TLRs [27]. Therefore, TLR4 receptor cysteine residues have been suggested as the potential targets for ILG, which is an α,β-unsaturated carbonyl substituted chalcone.

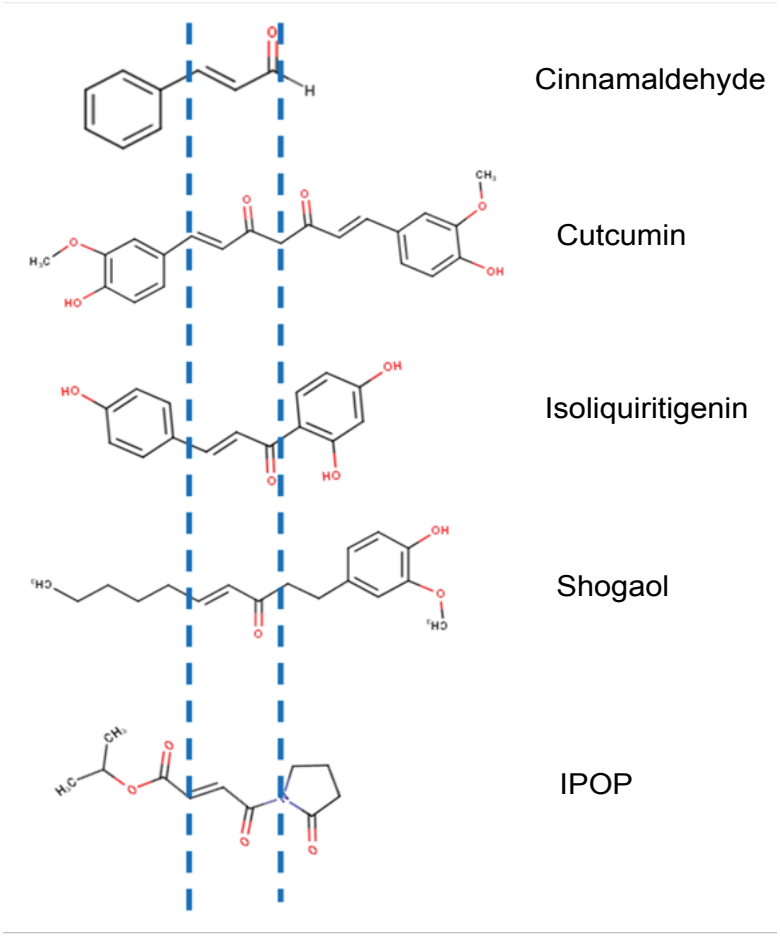

Figure 1: Chemical structures of TLR4 dimerization inhibitors. The α,β-unsaturated carbonyl groups responsible for disrupting bonds between cysteine residues in TLR4 are demarcated in dashed blue lines.

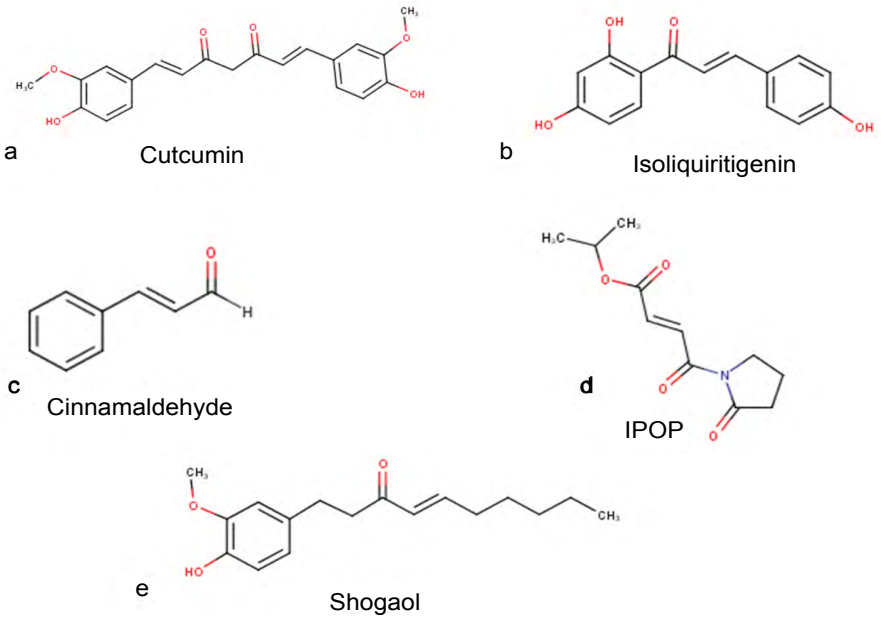

Figure 2: Chemical structures of TLR4 dimerization inhibitors.

Cinnamaldehyde

Cinnamaldehyde (3-phenyl-2-propenal) is the major constituent of the essential oil obtained from the bark of cinnamon isolated from Cinnamomum trees. It is a spice compound in cinnamon and has been widely used as a major ingredient of foodstuffs such as chewing gum, ice cream, candy and beverages as well as in perfumes, fungicides and flavoring agents. Cinnamaldehyde has been previously known to have various biological activities including anti-inflammatory and anti-bacterial properties [28,29]. However, when it was understood that cinnamaldehyde too carries an α,β-unsaturated carbonyl derivative with a mono-substituted benzene ring (Figure 2c), it was further investigated to have anti-inflammatory properties through TLR4 inhibition leading to suppression of LPS-induced NF-κB activation (11). Hence, similar mode of inhibition on TLR4 oligomerization induced by LPS was observed for cinnamaldehyde as was for isoliquiritigenin and curcumin. Further, cinnamaldehyde also inhibited ligand-independent NF-κB activation induced by constitutively active TLR4 or wild-type TLR4 (11). Although cinnamaldehyde is quite a small molecule but contains the essential α,β-unsaturated carbonyl core which binds to the –SH groups of cysteine residues and further disrupts the oligomerization process of TLR4 receptor and hence further signaling.

IPOP

After the discovery showing curcumin, cinnamaldehyde, 6-shogaol, and isoliquiritigenin inhibiting TLR4 dimerization due to the presence of an α,β-unsaturated carbonyl group, it was clearly realized that more such molecules are needed. Hence, molecules like IPOP (13) with an α,β-unsaturated carbonyl group structural motif were designed which could interact with biological nucleophiles such as sulfhydryl group of cysteine by Michael addition as previously discussed. Hence, cysteine residues have been implicated as potential targets for IPOP as well. Considering the similar structure of IPOP with other natural compounds, it is conceivable that IPOP interacts with the cysteine residue in TLR4 leading to the inhibition of TLR4 dimerization. The structure of chemically synthesized IPOP (Figure 2d) though is lengthy as compared to cinnamaldehyde, however its mode of action is exactly similar to its natural contemporary.

[6]-Shogaol

Shogaols are an active constituent of ginger (*Zingiber officinale*). Although ginger is known to be composed of various other constituents, including diarylheptanoids, paradols, gingerols [30], however 6-shogaol is one of its major active component [31,32]. A large number of studies have demonstrated that 6-shogaol has a strong ability to inhibit inflammation [33-40]. It has been also reported to be the most potent anti-inflammatory and anti-oxidant component in ginger [41]. It has been lately reported that 6-shogaol also inhibits LPS-induced dimerization of TLR4, resulting in the inhibition of NF-κB activation and the expression of cyclooxygenase-2. Furthermore, due to the presence of the signature α,β-unsaturated carbonyl group in its structure, (Figure 2e) it was shown that 6-shogaol can directly inhibit TLR-mediated signaling pathways at the receptor level by inhibition of the TLR4 dimerization through the well-studied reaction with the thiol group of cysteine residues in the TLR4 domain. As could be observed from Figure 2e, the structure of 6-shogaol is somewhat similar to curcumin, which itself describes how both of these structurally similar natural compounds have been naturally evolved for inhibiting the TLR4 pathway at the receptor level.

Conclusions

The dimerization of TLR4 was shown to be a prerequisite for the ligand-induced receptor activation. TLRs are Type I transmembrane receptors, consisting of leucine-rich repeats (LRRs) and cysteine-rich region in the extracellular domain and TIR region in the cytoplasmic domain. TLRs have several cysteine residues in both cytoplasmic and extracellular domains, which may form disulfide bonds for the dimerization of the receptors. Since receptor dimerization is required to activate downstream inflammatory signaling pathways, inhibitors targeting this point of signal propagation would be the ideal staring points. It has been clearly shown in many studies that phytochemicals or synthetic compounds with the structural motif of α,β-unsaturated carbonyl group, conferring Michael addition may interact with cysteine residues in TLR4 leading to inhibition of TLR4 dimerization. Therefore, TLR4 receptor cysteine residues have been suggested as the potential targets for many randomly discovered phytochemicals as well as synthesized chemicals as they contain the α,β-unsaturated carbonyl group to react with thiol group of cysteine.

Hence, TLR4 activity can be regulated by certain phytochemicals such as cinnamaldehyde, isoliquiritigenin, shogaol, curcumin, etc. as well as synthetic chemicals like IPOP via the modulation of receptor oligomerization leading to decreased inflammatory gene expression. This notion provides a new paradigm in identifying specific molecular targets of anti-inflammatory agents. Furthermore, this commentary suggests and encourages that more such inhibitors having novelty and potency along with the required structural motif should be designed as a preventive and therapeutic strategy against many chronic diseases.

Acknowledgements

This work was supported by the Department of Biotechnology sponsored Ramalingaswami Fellowship (BT/ HRD/35/02/2006) to MSB and the Science and Engineering Research Board (SERB) Young Scientists Start-Up Research Grant under grant number YSS/2015/001279 to US. The authors also gratefully acknowledge the Indian Institute of Technology Indore for providing facilities and other support.

Competing Interest

None of the authors have any competing interests in the manuscript.

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