Quinoline as TRPV1 Antagonists: A New Approach against Inflammation

Megha P. Ambatkar, Pramod B. Khedekar

Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur - 440 033, Maharashtra, India

ABSTRACT

Inflammation is the first response of the immune system to harmful stimuli such as infection or irritation, consists of a cascade of biochemical events that propagates and matures the inflammatory response. Numbers of anti-inflammatory drugs are available for treatment of acute and chronic inflammation. Many anti-inflammatory drugs cause adverse side effects. The quinoline class of compounds are important for searching the safe and effective anti-inflammatory drugs. These drugs are classified based on the number of substituents present on the quinoline ring or compounds containing a quinoline ring fused to other heterocyclic compounds. Quinolines have the ability to target several causes of inflammation includes transient receptor potential vanilloid 1 receptor. The TRPV1 receptor, first cloned and characterized in 1997, is a non-selective cation channel expressed in primary sensory neurons, and is a key pain sensor and integrator. This review provides the discovery of various quinoline derivatives as transient receptor potential vanilloid 1 (TRPV1) antagonists. Overall, the quinoline moiety will be used as a new template for designing and identifying the novel anti-inflammatory drugs in future.

Keywords: Quinoline, Inflammation, Transient receptor Potential Vanilloid 1, Antagonists.

INTRODUCTION

Inflammation, the first response of the immune system to harmful stimuli such as infection or irritation, consists of a cascade of biochemical events that propagates and matures the inflammatory response. It is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process. However, if uncontrolled, inflammation can lead to a diverse array of acute, chronic and systemic inflammatory disorders.

Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases.

At tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release. Various pathogenic factors, such as infection, tissue injury, or cardiac infarction, can induce inflammation by causing tissue damage. The etiologies of inflammation can be infectious or non-infectious. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses.

Quinolines act as anti-inflammatory agents

Quinoline is a heterocyclic aromatic organic compound containing nitrogen and was first isolated in 1834 by Runge from coal tar and he named it as Leukol. Later in 1842, Gerhardt isolated it by heating the alkaloid cinchonine with alkali and named it as quinoline. These heterocycles have been of considerable interest because a large number of natural products and drugs contain this heterocyclic unit.

Quinolines have attracted particular attention owing to their diverse array of pharmacological properties including the ability to target several causes of inflammation including transient receptor potential vanilloid 1 (TRPV1) antagonists. Several reviews have appeared on the...
anticancer or antitumor, antimarial, and antimicrobial activities of quinolines. This review will focus on targets of inflammation that is transient receptor potential vanilloid 1 followed by the quinoline-based modulators which acts as TRPV1 antagonists.

**Transient Receptor Potential (TRP) Family**

The transient receptor potential (TRP) superfamily is one of the largest families of ion channels and comprises 28 members. Based on amino acid sequence homology, the mammalian members of TRP channel family have been classified into six subfamilies: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin) and TRPA (Ankyrin). The TRPV subfamily consists of a group of four channels, TRPV1, TRPV2, TRPV3 and TRPV4 that are critically involved in nociception and thermosensing, and two additional channels TRPV5 and TRPV6 that appear to be constitutively active and have been proposed to play a role in vitamin D dependant calcium uptake in the kidney and intestine respectively.

**Targeting TRPV1: Vanilloid receptor antagonists**

The TRPV1 receptors, found in the peripheral and central nervous systems, are involved in the transmission and modulation of pain, as well as the integration of diverse painful stimuli. This receptor channel is activated by protons (pH 5, chemical stimuli), heat (>42°C, physical stimuli), endogeneous substances (such as endocannabinoid anandamide, lipoygenase and arachidonic acid metabolites) and also by some natural ligands (such as capsaicin, other vanilloids and resiniferatoxin). This results in Ca\(^{2+}\) influx into the cells through the channel pore, causing cell membrane depolarisation and excitation of primary sensory neurons. It then transmits noxious nerve impulses to the spinal cord and finally delivers the perception of pain. Upon continued stimulation from any external stimuli the activity of neurotransmitters is depleted causing selective damage of the nerves and, thereby, results in desensitisation to further stimuli. As a result, TRPV1 receptors lose sensitivity to painful stimuli. Various TRPV1 agonists were used to treat pains (e.g. capsaicin, resiniferatoxin, etc.). However, several adverse side effects like burning sensation, irritation and neurotoxicity that were associated with this approach shifted the focus toward the discovery of TRPV1 antagonists. These antagonists block the pain signalling pathway with potentially fewer side effects.

TRPV1 has been shown to be important in both nociception (the perception of pain) and the inflammatory responses. It is considered to be a highly validated target for pain, and both agonists and antagonists of TRPV1 are being evaluated as potential analgesics in clinical trials. Simply, its antagonists block the function of TRPV1 and relieve pain behaviours caused by inflammation, osteoarthritis and cancer in rodent models. Its agonists, such as capsaicin, cause desensitization of TRPV1 channels that relieves pain behaviours in preclinical species. The initial drug discovery research targeting TRPV1 was mainly followed by the quinolines derivative such as capsaicin, resiniferatoxin and capsazepine, three important pharmacophore regions are marked.

Nonclassical TRPV1 ligands have a carbonyl group, which is either present as a part of heterocyclic ring or is unrecognizable. The preclinical studies and clinical trials showed that the side effects such as hyperthermia and impaired noxious heat sensation (burn risk) are the main obstacles for developing TRPV1 antagonists. There has been a need to distinguish the excitatory effects and the analgesic effects of TRPV1, and it has been actively studied whether these side effects are on-target, that is, unavoidable, or can be mitigated by chemical modifications. Based on these efforts, second generation of TRPV1 antagonists that do not induce hyperthermia are under development, and several compound series show promising results for overcoming these side effects.

This review focuses on the development of quinoline as TRPV1 antagonists with an emphasis on recent research in this field.

**Quinoline as TRPV1 Antagonist**

Many monosubstituted quinolines have been reported as TRPV1 antagonists. Compounds 1 and 2 were prepared by acylation of quinoline with 4-(trifluoromethylthio)phenyl isocyanate. The activities of compounds were measured by a Ca\(^{2+}\) influx assay using human TRPV1 receptor. The screening of compounds with single nitrogen containing bicyclic cores for their antagonistic activity at human TRPV1 revealed that the quinoline derivative 2 (IC\(_{50} = 420\) nM) exhibited superior potency to the regioisomer quinoline 1 (IC\(_{50} = 1500\) nM).
N-Aryl cinnamide 3 (AMG9810), identified independently through the screening studies at Amgen, was also found to be a highly selective TRPV1 receptor antagonist when profiled against more than 80 different drug targets. Compound 3 blocks the capsaicin-induced uptake of Ca\(^{2+}\) in TRPV1 expressing CHO cells with an IC\(_{50}\) value of 17 nM. Compound 3 was found to block TRPV1 activation by capsaicin as well as other agonists such as acids and heat.\(^{15}\) SAR studies using 3 as a lead compound led to a series of N-aryl cinnamide, the most potent of which were the quinolines 4a and 4b with IC\(_{50}\) values of 1.9 and 0.42 nM, respectively. These compounds exhibit good oral bioavailability in rats with F\(_{oral} = 39\)% and 17\%, respectively, compared to negligible oral bioavailability (F\(_{oral} = 3\)%) observed for the lead 3.\(^{26}\)

SAR studies around HTS hit 5 led to the identification of the N-quinolin-7-yl-6-phenylnicotinamide 6 which was identified as a potent TRPV1 antagonist with activity in an in vivo model of inflammatory pain.\(^{37}\)

This compound possessed excellent potency at human and rat TRPV1 receptors and a favourable in vitro DMPK profile. However, the low intrinsic clearance observed in liver microsomes [Cl\(_i\) 3.1 (human), 2.2 (rat), 3.9 (guinea pig) mL/min/g tissue] did not translate into acceptable in vivo stability in the rat (Cl\(_b\) 33 mL/min/kg, T\(_{1/2}\) 0.36 h at 1 mg/kg iv). Furthermore, compound 6 showed only modest activity in the guinea pig model of Freund's Complete Adjuvant (FCA)-induced inflammatory pain and was less efficacious than expected based on its high level of in vitro potency. Further optimisation of this lead series targeting, in particular, an improved in vivo profile. This work has resulted in the identification of a compound which showed excellent levels of activity in the guinea pig FCA model on oral dosing. Initial efforts focused on modification of the terminal phenyl ring of 6. Substituted analogues 7 and 8 were prepared from methyl 6-chloronicotinate and the appropriate boronic acid using Suzuki–Miyaura coupling conditions\(^{38}\) followed by hydrolysis and standard EDCI-mediated amide coupling. Alternatively, compounds 9 – 12 were prepared directly from chloro-nicotinamide.\(^{37}\)
As illustrated in Table 1,[17] the introduction of a fluoro-substituent into the 2- or 3-positions, 7 and 8, was well tolerated giving compounds with potency similar to that of the unsubstituted parent 6. The 4-fluoro substituted compound 9 showed ~3-fold lower potency at TRPV1 with pKb 8.2 but following determination of in vitro ADME properties it was found that this compound possessed the best overall in vitro profile in this series with an acceptable P450 inhibition profile (IC50 > 10 μM at five major human isoforms), and low intrinsic clearance in human and rat liver microsomes [Clint 1.7 (human), 1.1 (rat) mL/min/g tissue]. However, in guinea pig microsomes, 9 proved to be a high-clearance compound (Clint 42 mL/min/g). Replacement of 4-F with 4-Cl, 4-CF3 or 4-CN 10-12 was detrimental to potency and complete replacement of the terminal phenyl ring with smaller groups such as cyano 13 or chloro 14 also resulted in reduced levels of TRPV1 antagonist activity.

Table 1: Antagonist activity of nicotinamide derivatives 6 and 7-14 at human TRPV1 expressed in a 1321N1 cell line[40] against capsaicin in FLIPR (Fluorescence Imaging Plate Reader) assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>hTRPV1=pKb</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Ph</td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>7</td>
<td>2-FPh</td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>8</td>
<td>3-FPh</td>
<td></td>
<td></td>
<td>8.6</td>
</tr>
<tr>
<td>9</td>
<td>4-FPh</td>
<td></td>
<td></td>
<td>8.2</td>
</tr>
<tr>
<td>10</td>
<td>4-CF3Ph</td>
<td></td>
<td></td>
<td>7.3</td>
</tr>
<tr>
<td>11</td>
<td>4-CF3Ph</td>
<td></td>
<td></td>
<td>&lt;6.6</td>
</tr>
<tr>
<td>12</td>
<td>4-CNPh</td>
<td></td>
<td></td>
<td>&lt;6.7</td>
</tr>
<tr>
<td>13</td>
<td>4-CN</td>
<td></td>
<td></td>
<td>&lt;6.7</td>
</tr>
<tr>
<td>14</td>
<td>4-Cl</td>
<td></td>
<td></td>
<td>&lt;6.7</td>
</tr>
</tbody>
</table>

* Standard error of mean (SEM) for all pKb values ≤ 0.1

To investigate the effect of altering the conformation around the biaryl amide linker, the 2- and 4-methylnicotinamide analogues 15, 16 and 17 were prepared.

Interestingly, it was found that the presence of a methyl in the 2-position was beneficial to TRPV1 antagonist potency, compounds 15 and 16, whereas the 4-methylnicotinamide 17 was much less active (Table 2).[17]

The reduced potency seen with 17 may be due to the 4-methyl group causing a conformational change resulting in the pyridyl nitrogen in 17 occupying an alternative and less favourable position in the active site when compared to the 2-methyl analogue 15 and unsubstituted analogue 6. The 2-methylnicotinamide 16 was tested in a guinea pig model of FCA induced inflammatory pain and showed 42% reversal of hyperalgesia at a dose of 5 mg/kg ip. Although this represented a significant improvement over the activity of 6 in the same in vivo model (32% reversal at 30 mg/kg sc).

An efficient synthesis of 2-amino-oxazolo[4,5-c]quinoline TRPV1 antagonists is described via a thiourea formation/carbodiimide cyclization sequence. Synthetic route optimization eliminates intermediate isolations and facilitates the rapid preparation of a series of novel pentacyclic TRPV1 antagonists 18-24. From this series, compound 22 was identified as a potent and selective TRPV1 antagonists with hTRPV1 IC50 = 5 nM and > 100-fold selectivity versus other TRP channels.[41]
Many monosubstituted quinolines have been reported as TRPV1 antagonists. One such report identified quinoline derivatives 25 and 26 possessing oral bioavailability in rats (F = 39% and 17%, respectively) as promising agents in the capsaicin (CAP) mediated functional assay ([IC\textsubscript{50} = 1.9 and 0.42 nM, respectively] and the pH-mediated assay ([IC\textsubscript{50} = 1.3 and 1.0 nM, respectively]). The maximum plasma concentrations for 25 and 26 were C\textsubscript{max} = 540 ng/ml and 320 ng/ml at 5 mg/kg p.o., respectively.\textsuperscript{26}

![Image of compound 25](image1.png)

![Image of compound 26](image2.png)

However, it was inefficacious at preventing thermal hyperalgesia generated by complete Freund's adjuvant in rats.\textsuperscript{32} Therefore, to improve the potency and pharmacokinetic (PK) properties of 27, the 8-oxoquinoline derivative 28 [rTRPV1 (CAP) IC\textsubscript{50} = 15 nM] was designed with an encouraging microsomal stability.\textsuperscript{43} Compound 28 was cleared at the rate of 44 and 135 ml/min/mg in rat and human, respectively, compared with 111 and 250 ml/min/mg for compound 27.

![Image of compound 27](image3.png)

![Image of compound 28](image4.png)

A quinoline carboxamide derivative 29 with N-methyl substitution showed moderate activities against hTRPV1 (pkb = 6.5) with capsaicin as the agonist.\textsuperscript{44} Optimisation of 29 led to the development of compound 30, with a carboxamide at the 7-position and no substitution on the quinoline nitrogen. Compound 30 exhibited good levels of in vitro metabolic stability (CL\textsubscript{i} < 5 ml/min/g liver) in human, rat, guinea pig and dog liver microsomes and also P450 inhibition with IC\textsubscript{50} values >18 \mu M at five major human isoforms (1A2, 2C9, 2C19, 2D6 and 3A4). It also showed excellent potency against hTRPV1 [pIC\textsubscript{50} (acid) = 8.1] and rTRPV1 receptors [pIC\textsubscript{50} (acid) = 7.6].\textsuperscript{44} Overall, quinolines with carboxamide substitution and 8-oxo-substituted quinolines showed promising TRPV1 antagonism.\textsuperscript{1}

![Image of compound 29](image5.png)

![Image of compound 30](image6.png)

Trisubstituted tetrahydroquinoline urea 31 has been reported as a TRPV1 antagonist in human TRPV1 calcium influx assay and an inhibitor of CYP3A4 enzyme [hTRPV1 IC\textsubscript{50} = 7 nM and 47% CYP3A4 inhibition at 10 \mu M].\textsuperscript{45} Another compound, 32 with 5,5 diphenyl pentadienamide moiety at the 4- position was also reported as a TRPV1 antagonist.\textsuperscript{46} The (R)-enantiomer of 32 [hTRPV1 IC\textsubscript{50} = 0.14 and rTRPV1 IC\textsubscript{50} = 0.35 nM] was more potent than the (S)-32 in the capsaicin-based assay and showed good PK profile in rats, dogs and monkeys. The (R)-32 was effective at preventing mechanical allodynia in rats in a dose dependent manner and reversed thermal hyperalgesia in a model of neuropathic pain induced by sciatic nerve injury.\textsuperscript{46}

![Image of compound 31](image7.png)

![Image of compound 32](image8.png)
CONCLUSION
From all above the information, it is cleared that the heterocyclic moiety quinolines have importance in searching the safe and effective next generation anti-inflammatory drugs. This is explained by the discovery of various quinoline derivatives as TRPV1 receptor antagonists and the activities of these derivatives depend on the nature and position of substituents present on the quinoline ring or compounds containing a quinoline ring fused to other heterocyclic compounds. Overall, the quinoline moiety can be used as a new template for designing and identifying the novel anti-inflammatory drugs to treat the diseases associated with inflammation in future.

REFERENCES


