New Terpenyl-Cinnamoyl-Hydrazone Analogues of Cannabidiol with Potent Antinociceptive Effect

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Abstract

Pain is a complex process involving peripheral and central sensitization, neuroinflammation, and altered neurotransmission. Current analgesics often have limited efficacy and undesirable side effects, underscoring the need for safer and more effective options. Cannabidiol (CBD), a non-psychoactive phytocannabinoid from Cannabis sativa, has shown promise as an analgesic through modulation of multiple pathways. In this study, novel terpenyl-cinnamoyl-hydrazone analogs were synthesized and evaluated for antinociceptive potential in preclinical nociception models. The compounds were tested in chemical (formalin-induced licking) and thermal (hot plate) assays in mice. Mechanistic studies employed naloxone (opioid antagonist), atropine (muscarinic antagonist), AM251 (CB1 antagonist), yohimbine (α2-adrenergic antagonist), and ondansetron (5-HT3 antagonist). Most compounds displayed antinociceptive activity, with PQM-274, PQM-291, and PQM-294 showing greater effects than CBD. Naloxone and AM251 reversed the effects of these three compounds. Atropine abolished PQM-291's effect, and ondansetron inhibited PQM-290's activity, whereas yohimbine produced no change. This study reports, for the first time, the antinociceptive properties of terpenyl-cinnamyl-N-acyl-hydrazones with structural features inspired by CBD, suggesting their potential as novel multitarget analgesic candidates.

Introduction

Pain is a multifactorial sensory and emotional experience that serves a protective function in acute contexts, but can become maladaptive when persistent, leading to disability and a reduced quality of life. In 2020, the International Association for the Study of Pain (IASP) updated its definition to emphasize that pain may occur even in the absence of actual tissue damage, adding notes that underscore the cognitive and social dimensions of this phenomenon, with a direct implication for clinical assessment and intervention design[1].

Nociceptive processing involves peripheral transduction, spinothalamic transmission, modulation (both ascending and descending), and cortical perception, stages that are susceptible to adaptive and maladaptive plasticity. In chronic conditions, central sensitization, defined as a state of central nervous system hyperexcitability, has been described as a transdiagnostic mechanism that amplifies nociceptive input and contributes to hyperalgesia and allodynia. Recent reviews have explored its pathophysiological basis, potential biomarkers, and clinical implications, as well as ongoing debates regarding the causal role of this construct in chronic pain[1,2].

Antinociception encompasses endogenous mechanisms and therapeutic strategies capable of reducing nociceptive transmission. In the descending axis, the periaqueductal gray (PAG)–rostroventromedial medulla (RVM) circuit integrates corticolimbic and brainstem signals, modulating the excitability of spinal neurons through on/off populations and inhibitory interneurons. Current evidence details the cellular architecture and conditions under which this system may facilitate or inhibit pain[3].

Descending modulation is mediated by a variety of neurotransmitters and neuromodulators. Among endogenous mediators, the opioid system, comprising μ, δ, and κ receptors and their peptides, plays a central role in antinociception. Recent studies have shown how chronic pain and opioid exposure can remodel this system, and how both neuromodulation and pharmacological interventions target opioid pathways to achieve analgesia, while also highlighting the limitations in efficacy and the risk of adverse effects[4]. Monoamines, particularly serotonin and norepinephrine, also play a pivotal role in pain modulation, with serotonin exerting either inhibitory or facilitatory effects depending on the receptor subtype involved[5]. Moreover, the endocannabinoid system contributes to the fine-tuning of nociceptive processing. In this context, cannabidiol (CBD), a non-psychoactive phytocannabinoid derived from *Cannabis sativa*, has aroused growing scientific interest due to its analgesic and anti-inflammatory potential without inducing the psychotropic effects typical of Δ^9-tetrahydrocannabinol (THC)[6].

CBD exhibits a complex and multifaceted pharmacological profile, interacting with a wide range of molecular targets. Its proposed mechanisms of action include activation of TRPV1 and TRPA1 channels involved in nociception, modulation of 5-HT_1A serotoninergic receptors with both anxiolytic and antinociceptive effects, facilitation of inhibitory neurotransmission via α_3 glycine receptors (GlyRs), and activation of PPAR-γ nuclear receptors, which attenuate the expression of pro-inflammatory mediators such as TNF and IL-1β[7].. Additionally, CBD can indirectly regulate the endocannabinoid system by modulating CB_2 receptors and inhibiting the degradation of endogenous endocannabinoids. It also exhibits antagonistic activity at the σ_1 receptor, which has been associated with both analgesia and neuroprotective effects[8]. Preclinical models have shown that CBD reduces allodynia and hyperalgesia in neuropathic, inflammatory, and chemotherapy-induced pain conditions[8].

However, despite its therapeutical potential, the clinical application of CBD is limited by several factors, including low oral bioavailability, rapid metabolism, and relatively modest potency at its known molecular targets. In view of these limitations, the present work aimed to evaluate a series of novel terpene-cinnamoyl-N-acyl-hydrazone analogues, rationally designed as structural analogues of CBD. In this new molecular architecture (Fig. 1), both terpene and phenyl moieties of CBD were preserved, while a conjugated N-acyl-hydrazone unit was introduced. The inclusion of a Michael-acceptor subunit was expected to function as an auxophoric fragment contributing to antioxidant activity and, at least in part, to retain the antioxidant properties of CBD associated with its two hydroxy groups. Moreover, the more polar N-acyl-hydrazone functionality, containing Hydrogen-bond donor and acceptor groups, could potentially enhance bioavailability.

Results

In a previous work, we reported the synthesis of twenty-six novel CBD-based terpene-cinnamoyl-N-acyl-hydrazone analogues. In that preliminary study, we demonstrated that, following a single oral dose of 10 μmol/kg, all test compounds, except for compounds PQM-375, PQM-378, and PQM-380, produced a significant reduction in mice nociceptive behavior during the neurogenic phase of the formalin test.

Notably, compounds PQM-292, PQM-293, PQM-295, PQM-307, PQM-308, and PQM-309 exhibited particularly potent antinociceptive activity, comparable to morphine, which was used as the reference drug. Regarding the inflammatory phase of the formalin model, PQM-292 stood out for producing the highest antinociceptive effect, almost completely abolishing induced hiperalgesic responses, even when compared with morphine and acetylsalicylic acid (ASA)[9]. Based on these findings, we decided to further evaluate the effects of the new compounds using both chemically induced (formalin-induced licking response) and thermally induced nociception (hot plate model), while also investigating potential mechanisms of action.

• Test Compounds Do Not Exhibit Toxic Effects

To evaluate potential toxic effects of each test compound, analyses of behavioral and hematological parameters in mice were conducted. In this protocol, mice received a single oral dose of 10 µmol/kg of each compound, and their behavior was assessed 24 hours later. No behavior changes or alterations in hematological parameters were observed (data not shown), suggesting that the test compounds do not induce systemic toxicity.

• Test Compounds Can Reduce Chemical and Thermal-Induced Nociception

The intraplantar injection of formalin induces a biphasic nociceptive response, with a first (neurogenic) phase occurring within the first 5 minutes post-injection, resulting in a licking response of 77 ± 15 seconds, and a second (inflammatory) phase occurring between 15 and 30 minutes after injection, with a licking response of 248 ± 44 seconds. Pre-treatment of mice with acetylsalicylic acid (ASA) significantly reduced paw-licking time in both phases, while morphine also produced significant reductions of 71.4% and 43.1% in the first and second phases, respectively. For comparative purposes, as the tested compounds are structural analogues of CBD, we also evaluated the CBD oil. Mice pre-treated with CBD oil (10 µmol/kg) showed a significant reduction in liking behavior by 41.5% and 52.4% in the first and second phases, respectively. Results shown in Fig. 3 indicate that PQM-273, PQM-274, PQM-275, and PQM-276 (Fig. 2) significantly reduced the licking responses in the first phase, with all compounds except PQM-273 exhibiting potent effects at all doses tested. Conversely, PQM-308 and PQM-309 displayed antinociceptive effects only at the highest dose (10 µmol/kg). These compounds also demonstrated significant inhibitory effects in the inflammatory phase. Additionally, PQM-295 (1, 3, and 10 µmol/Kg), PQM-303 (10 µmol/kg), PQM-306 (1 and 3 µmol/kg), PQM-307 (1 µmol/kg, Fig. 2) produced marked reductions in the formalin-induced licking response during the second phase of the assay.

As some tested compounds exhibited significant effects in the neurogenic phase of the formalin-induced paw-licking model, we further evaluated whether these compounds could also exert central antinociceptive effects. Notably, CBD induced a 403% increase in antinociceptive activity, corresponding

to a 3.22-fold increase in the area under the curve (AUC) compared with vehicle-treated animals. Our results showed that PQM-290, PQM-295, and PQM-302 did not produce antinociceptive effects at any of the doses tested. When comparing the groups pre-treated with test compounds to those pre-treated with CBD, compounds PQM-274, PQM-291, and PQM-294 not only exhibited significant antinociceptive effects relative to vehicle-treated animals, but also demonstrated a significantly greater effect than CBD (Fig. 3).

• Evaluation of the mechanism of action

Several CBD-based analogues exhibited significant antinociceptive effects; however, only PQM-274, PQM-291, and PQM-294 demonstrated effects greater than those of CBD (Fig. 4). Based on these results, we further investigated the possible mechanisms of action of these three compounds using pharmacological antagonists targeting different pain-modulating pathways.

Five antagonists were employed: naloxone (an opioid receptor antagonist), atropine (a muscarinic receptor antagonist), yohimbine (an α2-adrenergic receptor antagonist), ondansetron (a 5-HT_3 receptor antagonist), and AM251 (a cannabinoid receptor type 1 antagonist). Each antagonist was administered intraperitoneally 15 minutes prior to oral administration of the respective test compound (PQM), and the antinociceptive response was evaluated as previously described. Both naloxone and AM251 reversed more than 50% of the antinociceptive effect of all three tested compounds, suggesting the involvement of opioid and cannabinoid systems. Notably, atropine completely abolished the antinociceptive effect of PQM-291, while ondansetron exhibited a similar inhibitory effect against PQM-290. In contrast, yohimbine did not affect the antinociceptive activity of PQM-290 and PQM-294 (Fig. 5).

• Compounds PQM-274, PQM-290, and PQM-291 Did Not Affect Spontaneous Activity or Locomotor Performance

The results shown in Fig. 6 indicate that none of the substances, at a dose of 10 μmol/kg, produced any significant effect on the number of falls in the rotarod test at 30 or 180 minutes post-oral administration, suggesting that the compounds did not induce locomotor impairments in mice.

To evaluate a possible effect in spontaneous activity mice pre-treated with compounds were observed in open field apparatus at 30, 90 and 180 minutes post-oral administration. None of compounds tested affect the motor performance and did not affect the spontaneous activity of mice.

Discussion

Pain is a multifaceted phenomenon encompassing sensory-discriminative, affective-motivational, and cognitive dimensions, each subserved by complex and interacting neuronal circuits. Reinforcing that

modulation of nociceptive transmission is not the result of a single receptor or neurotransmitter system, but rather a precise balance among a diversity of them[10].

In the present work, we demonstrated that several compounds among a series of 26 novel synthetic Terpenyl-cinnamoyl-N-acyl-hydrazones designed as new structural analogs of cannabidiol demonstrated significant effects in chemical (formalin-induced licking response) or thermal (hot plate) models of nociception.

The formalin-induced paw-licking test is a well-established model for assessing both acute and tonic phases of pain and provides valuable insights into peripheral and central nociceptive mechanisms. Following subcutaneous injection of formalin into the hind paw, the animal exhibits a biphasic nociceptive behavior characterized by an early neurogenic phase (0–10 min), resulting from direct activation of C-fiber afferents, followed by a tonic inflammatory phase (15–60 min) associated with peripheral inflammation and central sensitization within the spinal cord dorsal horn[11]. The first phase reflects the direct depolarization of primary nociceptive neurons through activation of TRPA1 and TRPV1 channels, as well as the release of substance P and glutamate, while the second phase involves the release of inflammatory mediators such as prostaglandins, bradykinin, histamine, and cytokines, which sustain nociceptor sensitization[12]. This biphasic pattern makes the formalin test a useful tool for differentiating centrally acting analgesic agents, which tend to inhibit both phases, from peripherally acting drugs, which primarily attenuate the second phase[13]. Moreover, formalin injection leads to prolonged neuronal hyperexcitability, resembling aspects of chronic pain and central sensitization, thus serving as a predictive model for screening novel analgesic compounds targeting glutamatergic, serotonergic, opioid, and endocannabinoid systems[14,15]. The reproducibility, behavioral quantification, and translational relevance of this model make it one of the most widely used paradigms for studying the mechanisms underlying persistent pain and testing new therapeutic strategies.

A novel compound may attenuate this early nociceptive response through several mechanistic pathways, such as the direct blockade or desensitization of nociceptive ion channels, prevention of C-fiber depolarization, modulation of peripheral neurotransmitter release leading to reduced excitatory signaling to spinal neurons, activation of inhibitory receptors on primary afferents, including presynaptic opioid (μ, δ, κ), cannabinoid (CB1), or α2-adrenergic receptors, which decrease nociceptive firing, as well as antioxidant or anti-inflammatory actions that limit local sensitization mediated by reactive oxygen species or early mediator release[15]. Our data suggests that PQM-273, PQM-274, PQM-275, and PQM-276 exert significant effects in both phases of the chemical nociception model. Moreover, most of these compounds also inhibited the second phase of the formalin test. Therefore, any of these mechanisms or a combination thereof, could account for the observed reduction in nociceptive behavior during the neurogenic phase of the formalin model.

As several compounds exhibited antinociceptive effects in the first phase of the formalin-induced licking behavior, we further assessed their potential central antinociceptive activity using the hot plate test. This model is widely employed to evaluate the central antinociceptive properties of novel analgesic

substances. It primarly reflects the activation of Aδ and C-fibers and the involvement of both supraspinal and spinal nociceptive pathways, including modulation of descending inhibitory systems such as opioidergic and serotonergic circuits. In contrast to inflammatory pain assays, the hot plate test measures acute thermal nociception, providing a screening tool for drugs acting at the central level (e.g., opioids, cannabinoids, and centrally acting adjuvants)[16]. Hence, the hot plate test is ideal for evaluating acute, centrally mediated analgesia, whereas the formalin model is superior for investigating chronic and inflammatory pain mechanisms and for distinguishing between central and peripheral sites of drug action. The combined use of these models offers a comprehensive pharmacological profile of novel analgesic compounds[17].

Pharmacological inhibition using selective antagonists demonstrated distinct roles of the opioid, cholinergic, adrenergic, serotonergic, and endocannabinoid systems in modulating the antinociceptive responses induced by PQM-274, PQM-291, and PQM-294. Our data confirmed the involvement of endogenous opioid pathways, as naloxone (a non-selective opioid receptor antagonist) reversed the antinociceptive effect of all three compounds. We also demonstrated that atropine, a non-selective muscarinic acetylcholine receptor antagonist, partially reversed the antinociceptive effect of PQM-274 and completely abolished the effect of PQM-291. Atropine has been shown to produce complex effects on pain modulation. In experiments using hot-plate, tail flick, and writhing tests, very low doses of atropine (1-100 µg/kg) induced antinociception, whereas high doses (~ 5 mg/kg) resulted in hyperalgesia[18]. When administered intrathecally, atropine significantly attenuates morphine-induced analgesia in radiant heat stimulation, suggesting the involvement of spinal cholinergic pathways in mediating opioid analgesia[19]. Moreover, atropine blocks the antinociceptive effects of nonsteroidal anti-inflammatory drugs (NSAIDs) in tail-flick tests, both after intraperitoneal and intrathecal administration, indicating that even in acute thermal pain without overt inflammation, intrinsic muscarinic cholinergic facilitation is required for full antinociceptive expression[18].

We also observed that yohimbine, an α2-adrenergic receptor antagonist, partially reversed (by approximately 30%) the antinociceptive effect of PQM-291, suggesting the involvement of the adrenergic system in the antinociceptive activity of this compound. This finding supports the hypothesis that noradrenergic descending inhibition contributes significantly to pain suppression. Although recent research has focused more on adrenergic agonists or novel receptor modulators, these results are consistent with the literature asserting that α2-receptor activation limits excitatory neurotransmission within dorsal horn circuits.

When ondansetron, a 5-HT3 receptor antagonist, was administered, a complete reversal of the PQM-274 antinociceptive effect and a partial reduction of those of PQM-291 and PQM-294 were observed. The participation of the serotonergic system in pain modulation is complex. Recent studies have demonstrated that 5-HT_3 receptors play a significant modulatory role in both neuropathic and pain facilitation states. For instance, in a rat model of brachial plexus avulsion, 5-HT_3A receptor expression was upregulated in the spinal dorsal horn, on both neurons and microglia, along with markers of central

sensitization (c-Fos, GFAP, IL-1β, TNF-α). Intrathecal administration of the 5-HT_3 antagonist ondansetron reduced mechanical and cold hypersensitivity and attenuated those molecular changes[20]. Similarly, in a rat model of complex regional pain syndrome (CRPS), expression of 5-HT_3 receptors increased, and treatment with the antagonist ramosetron produced analgesia in paw withdrawal tests, correlating with reduced inflammatory mediators such as substance P and IL-6[21]. Together, these findings support that 5-HT_3 receptors facilitate pain transmission in persistent and pathological states and that their antagonists possess therapeutic potential in reducing hyperalgesia, allodynia, and central sensitization. The fact that the antagonist completely reversed the PQM-274-induced antinociception suggests that this compound may interfere with a key serotonergic pain-modulatory pathway.

We further demonstrated that AM251, a CB1 receptor antagonist, reversed more than 50% of the antinociceptive effects of all three compounds. Recent evidence underscores the central importance of CB_1 receptors in modulating various pain states, particularly neuropathic and chronic pain. Mice with conditional deletion of CB_1 in primary sensory neurons (CB_1cKO) develop persistent hyperalgesia; transcriptomic analyses of their dorsal root ganglia reveal altered expression of genes involved in immune, inflammatory, and neuronal conduction pathways, indicating that CB_1 normally acts as an endogenous analgesic buffer[22]. In inflammatory pain models, the peripherally selective CB_1-preferring agonist CB-13 reduces mechanical allodynia and thermal hyperalgesia, likely by preventing TRPV1 sensitization and neuronal hyperexcitability induced by inflammatory mediators, although repeated dosing leads to tolerance and central side effects[23].

The “cannabinoid tetrad” is another important tool for characterizing CB1 receptor agonists. This term refers to the four hallmark effects of systemic administration of cannabinoid receptor agonists: analgesia, hypolocomotion, hypothermia, and catalepsy. Our data suggest that the PQM compounds may reduce nociceptive signal transduction by modulating CB1 or opioid receptor activity. Importantly, despite producing antinociception, these compounds did not induce cross-tolerance, as neither hypolocomotion nor catalepsy was observed.

Because pain modulation involves multiple signaling pathways that interact in a complex manner, confirming and identifying the precise molecular targets of the tested compounds solely through vivo models remains challenging. Another limitation concerns the ability of certain drugs to cross the blood–brain barrier, which complicates the determination of their exact site of action, whether peripheral or central. One possible approach to overcome this issue would be the use of compounds that do not penetrate the CNS, such as naloxone methiodide or methylnaltrexone. However, access to these agents is restricted by federal regulations intended to prevent uncontrolled distribution. Consequently, the results presented here should be interpreted as indicative rather than definitive evidence of the potential mechanisms involved. To elucidate the precise mechanism of action and receptor targets, additional in vivo experiments with selective ligands, as well as in vitro receptor-binding assays, will be required. At present, such studies are limited by the poor solubility of the tested compounds. Ongoing efforts are therefore directed toward improving solubility, which will enable the implementation of more detailed in vitro investigations.

Collectively, our findings reveal that a subset of the novel terpenyl-cinnamoyl-N-acyl-hydrazone derivatives, particularly PQM-273, PQM-274, PQM-275, and PQM-276, exert robust antinociceptive effects, implicating a multifaceted modulation of pain pathways. Pharmacological antagonism highlights the critical involvement of endogenous opioid, cholinergic, adrenergic, serotonergic, and/or endocannabinoid systems, with PQM-274, PQM-291, and PQM-294 demonstrating notable reliance on CB1 and 5-HT_3 receptor-mediated mechanisms. Importantly, these compounds effectively attenuate the neurogenic and inflammatory phases of the formalin-induced licking response without eliciting locomotor deficits or catalepsy, suggesting selective engagement of central and peripheral antinociceptive pathways without overt adverse effects.

Methods

• Animals

All protocols used Swiss Webster mice (male, 20–25 g, 8–10 weeks) donated by the Instituto Vital Brazil (Niterói, Rio de Janeiro, Brazil). All methods were carried out in accordance with relevant guidelines and regulations. National Council for the Control of Animal Experimentation (CONCEA/Brazil), the Biomedical Science Institute/UFRJ, and Ethical Committee for Animal Research approved the protocols used (numbers 151/19 and 96/23). all methods are reported in accordance with ARRIVE guidelines. The animals were maintained under standard conditions (a room with a 12 h light–dark cycle at 22 ± 2°C, 60% to 80% humidity, and with food and water provided ad libitum).

• Drugs and Treatments

Acetylsalicylic acid (ASA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Morphine sulfate was kindly provided by Cristália (São Paulo, Brazil) and formalin was purchased from Isofar (Rio de Janeiro, Brazil). The tested compounds were dissolved in DMSO to generate a stock solution (100 µmol/mL). From this stock solution, intermediate solutions were prepared and administered by oral gavage at doses varying from 1 to 10 µmol/kg, in a final volume of 0.1 mL of water. The tested compounds, as well as all drugs, were diluted just before use.

• Formalin-Induced Licking Behavior

Formalin (2.5%, 50 µL v/v) was injected into the dorsal surface of the left hind paw of mice. The time in which animals remained licking the formalin-injected paw was recorded according to Hunskaar & Hole[24] with modifications done by Matheus et al.[25]. The response was divided into two phases: the first one (neurogenic phase) occurs in the first 5 min post-formalin injection and the second one (inflammatory phase) occurs between 15- and 30-minutes post-formalin injection.

• Thermal Nociception Model (Hot-Plate)

The reaction time (licked fore and hind paws) that mice remained on a hot plate (Insight Equipment, Brazil) set at 55 ± 1°C was recorded as previously described by Eddy and Leimbach[26] at several intervals of 30 min post-oral administration of morphine, vehicle or test compounds (1, 3, or 10 μmol/kg). Baseline was calculated by the mean of two reaction time measurements at 60 and 30 minutes before oral administration[25]. Area under the curve (AUC) graphs were calculated from time–course graphs. The following formula, which is based on the trapezoid rule, was used to calculate the AUC: AUC = 30 × IB [(min 30) + (min 60) +… + (min 180)]/2, where IB is the increase from the baseline (in %).

• Analysis of the Mechanisms of Action

One of the following treatments was given i.p. 30 min before PQMs (10 μmol/kg, p.o.): naloxone (opioid receptor antagonist, 3 μmol/kg), atropine (muscarinic receptor antagonist, 3.5 μmol/kg), AM251 (cannabinoid CB1 receptor antagonist, 2 μmol/kg), yohimbine (an α2-adrenergic antagonist, 3.5 μmol/kg) or ondansetron (5-HT3 serotoninergic receptor antagonist, 3 μmol/kg). The antinociceptive effect was evaluated in the hot plate test as described above.

• Analysis of the Spontaneous Activity and Locomotor Performance

To evaluate these effects, mice (n = 7 per group) were previously treated with vehicle and test compounds (10 μmol/kg). The open field method was used to evaluate the spontaneous activity of mice[27]. After oral administration of the tested substances, mice were individually placed in a box, in which the floor had marked squares (5 × 5 cm). Over a period of five minutes, the number of squares by which each mouse crossed was counted. The locomotor performance, from 0.5 up to 3.5 h after administration was evaluated using the rotarod apparatus. The number of falls from the apparatus was recorded.

• In Vivo Toxicity Test

For different groups of animals, 10 μmol/kg of each test compound was administered orally. After 24 h, mice were sacrificed with an overdose of ketamine/xylazine solution (150 and 50 mg/kg, respectively), and a blood sample was collected in a heparinized tube. The femur was removed, and the bone marrow from each femur was washed with 1 mL of saline solution (0.9% NaCl) with heparin. Both bone marrow and blood samples were subjected to complete blood count and cell count using an automatic cell counter (PocH-100iV Diff, Sysmex, Kobe, Japan).

• Statistical Analysis

Each experimental group consisted of 6 to 10 mice, and the results are expressed as the mean ± S.D. The area under the curve (AUC) was calculated using Prism Software 8.02 (GraphPad Software, La Jolla, CA, USA). Significant differences between the groups were established using Tukey test for multiple comparisons after analysis of variance (ANOVA) testing. p values less than 0.05 were considered significant.

Declarations

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Author contributions statement

PDF, CVJ conceptualization, formal analysis, data curation, funding acquisition, project administration, resources, supervision, writing – original draft and review and editing, JPBP, RMC, MLS, ACPL, GRRF Investigation, Methodology

Additional information

Competing interests: The authors declare no competing interests.

Data availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Figures

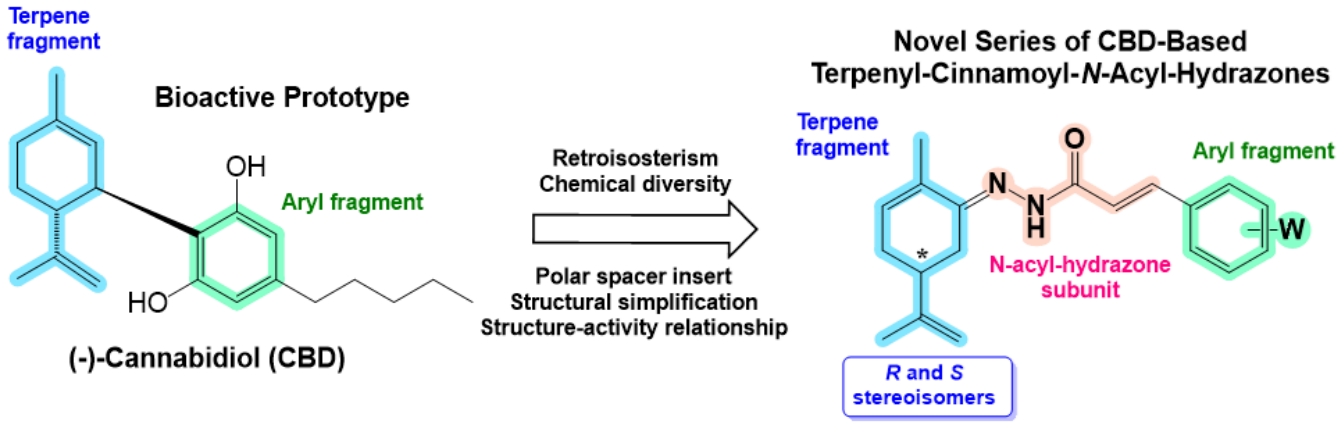

Figure 1

Rational design of a novel series of terpene-cinnamoyl-N-acyl-hydrazone analogues based on the structure of cannabidiol

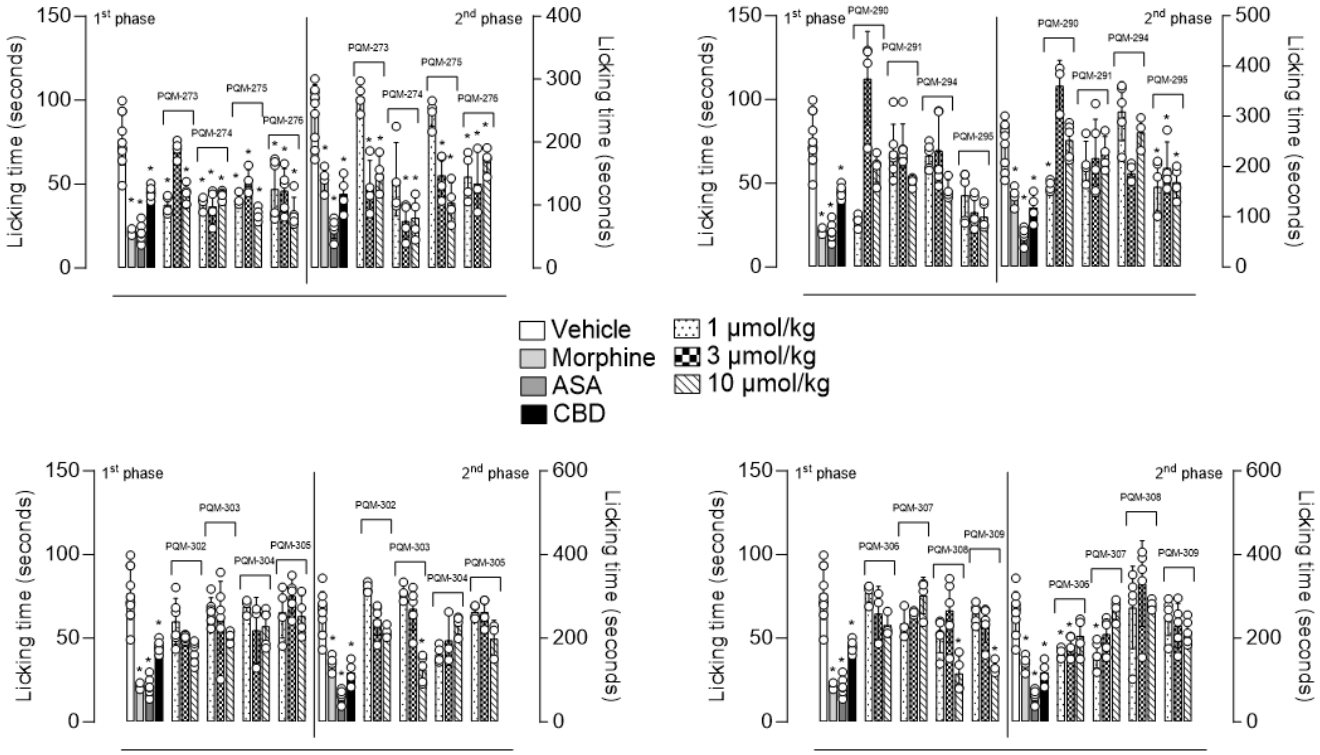

Figure 2

Effects of the CBD-based terpene-cinnamoyl-N-acyl-hydrazone analogues in an inflammatory pain model (the formalin- induced licking response). Mice were orally treated with each of the substances in three different doses, acetylsalicylic acid (ASA, 1,100 µmol/kg), morphine (10 µmol/kg), CBD (10 µmol/kg), test compounds (1, 3, 10 µmol/kg), or vehicle. One hour later the nociceptive response was induced by intraplantar injection of formalin (2.5%) in right hind paw. Results are expressed as mean ± standard

deviation (n= 5-8). The statistical analyses were done using GraphPad Prism 8.02 (San Diego, CA, USA) software with analyses of variance (ANOVA) followed by Tukey post-test with $p < 0.01$ (*) when comparing treated groups with vehicle-treated group.

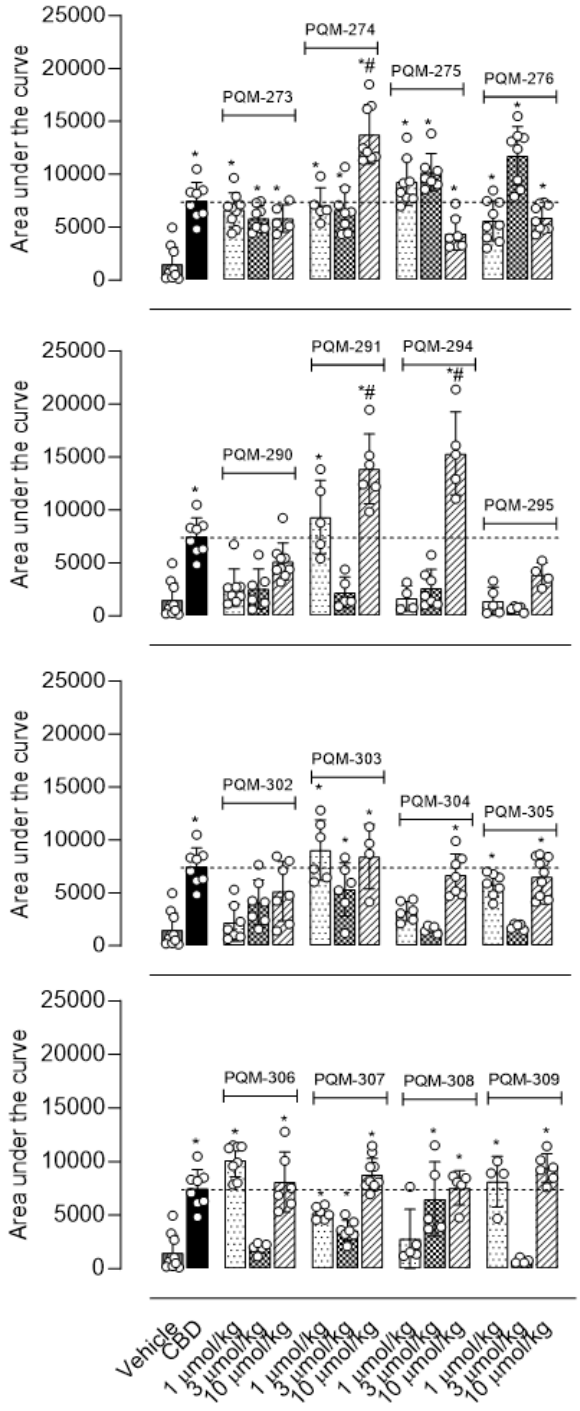

Figure 3

Antinociceptive effect of the CBD-based terpene-cinnamoyl-N-acyl-hydrazone analogues in thermal-induced pain model (hot plate). Mice were orally treated with each of the PQMs in three different doses

(1, 3, 10 µmol/kg), CBD (10 µmol/kg), or vehicle. The results are presented as the mean ± SD (n=5–10) of the area under the curve (AUC). Statistical significance was calculated using GraphPad Prism 8.02 software (San Diego, CA, USA) using analyses of variance (ANOVA) followed by Tukey post-test with $p < 0.01$ when comparing CBD- or PQM-treated groups with vehicle-treated group and # $p < 0.01$ when comparing PQM-treated mice with CBD-treated animals.

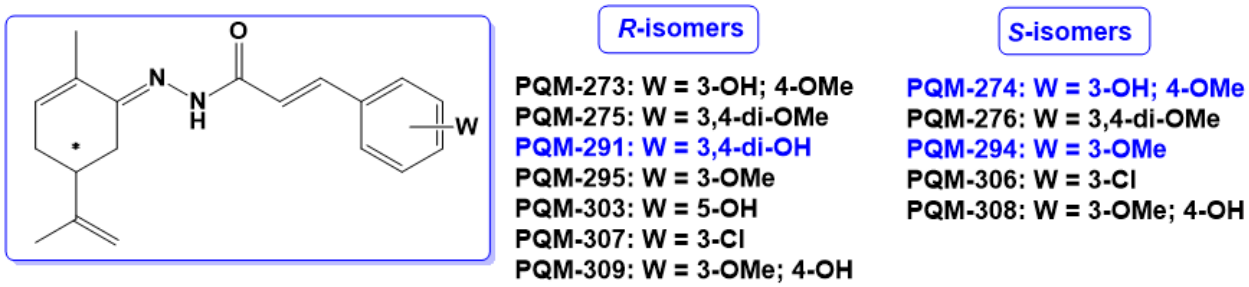

Figure 4

Chemical structure of the most active compounds in the hot plate assay when compared to CBD-treated animals.

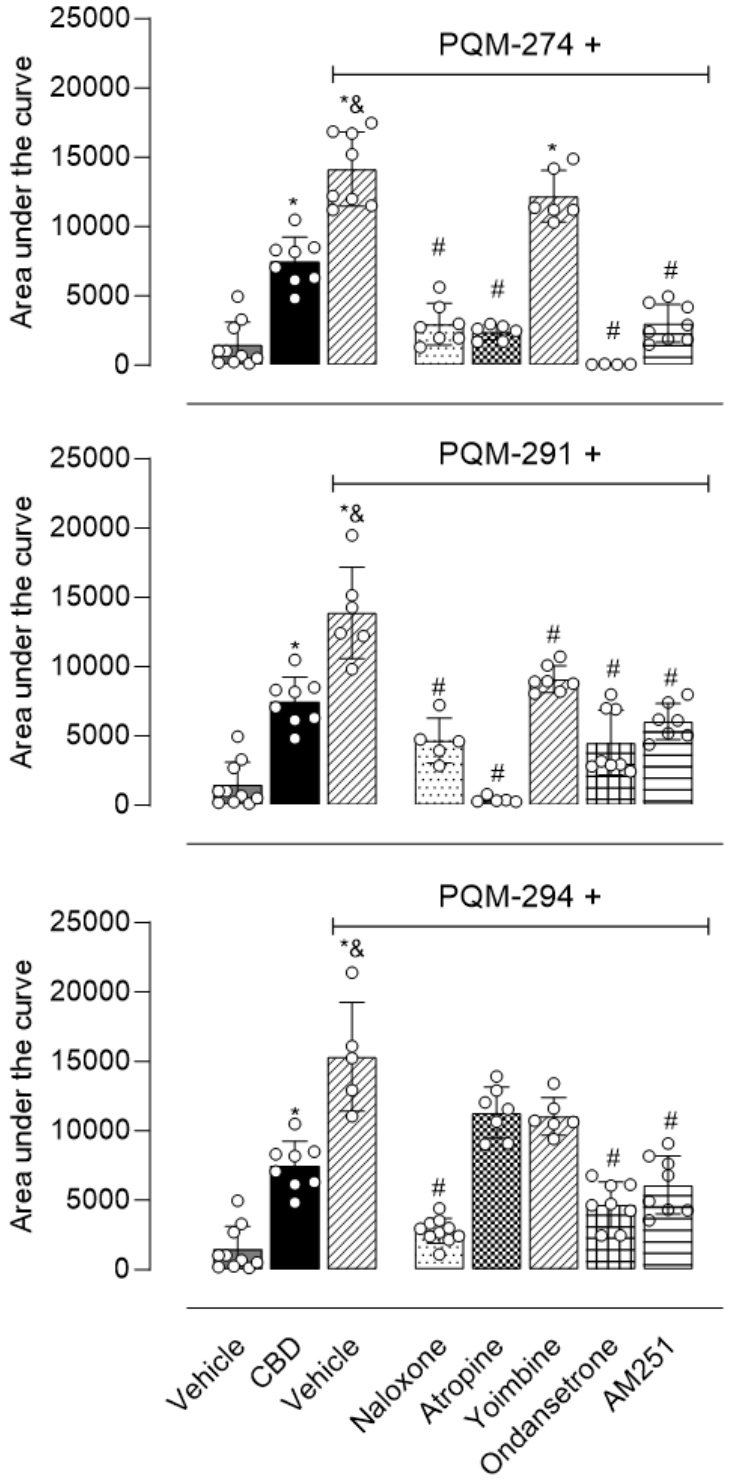

Figure 5

Effects of different antagonists on the antinociceptive activity of CBD-based terpene-cinnamoyl-*N*-acyl-hydrazone analogues evaluated in the hot plate model. Naloxone (3 μmol/kg, i.p.), ondansetron (3 μmol/kg, i.p.), atropine (3.5 μmol/kg, i.p.), Yohimbine (5 μmol/kg), or AM251 (2 μmol/kg, i.p.), were administered 30 min prior to oral administration of test compounds (10 μmol/kg). Data are expressed as mean ± SD (n= 5-10). One-way ANOVA followed by Tukey's post hoc test was used to calculate statistical

significance. *p < 0.05, when comparing CBD- or PQM-treated mice to the vehicle-treated group, and $^{\&}$p < 0.05, when comparing PQM-treated mice with CBD-treated animals; $^{\#}$p < 0.05, when comparing antagonists pretreated mice with PQM-treated groups.

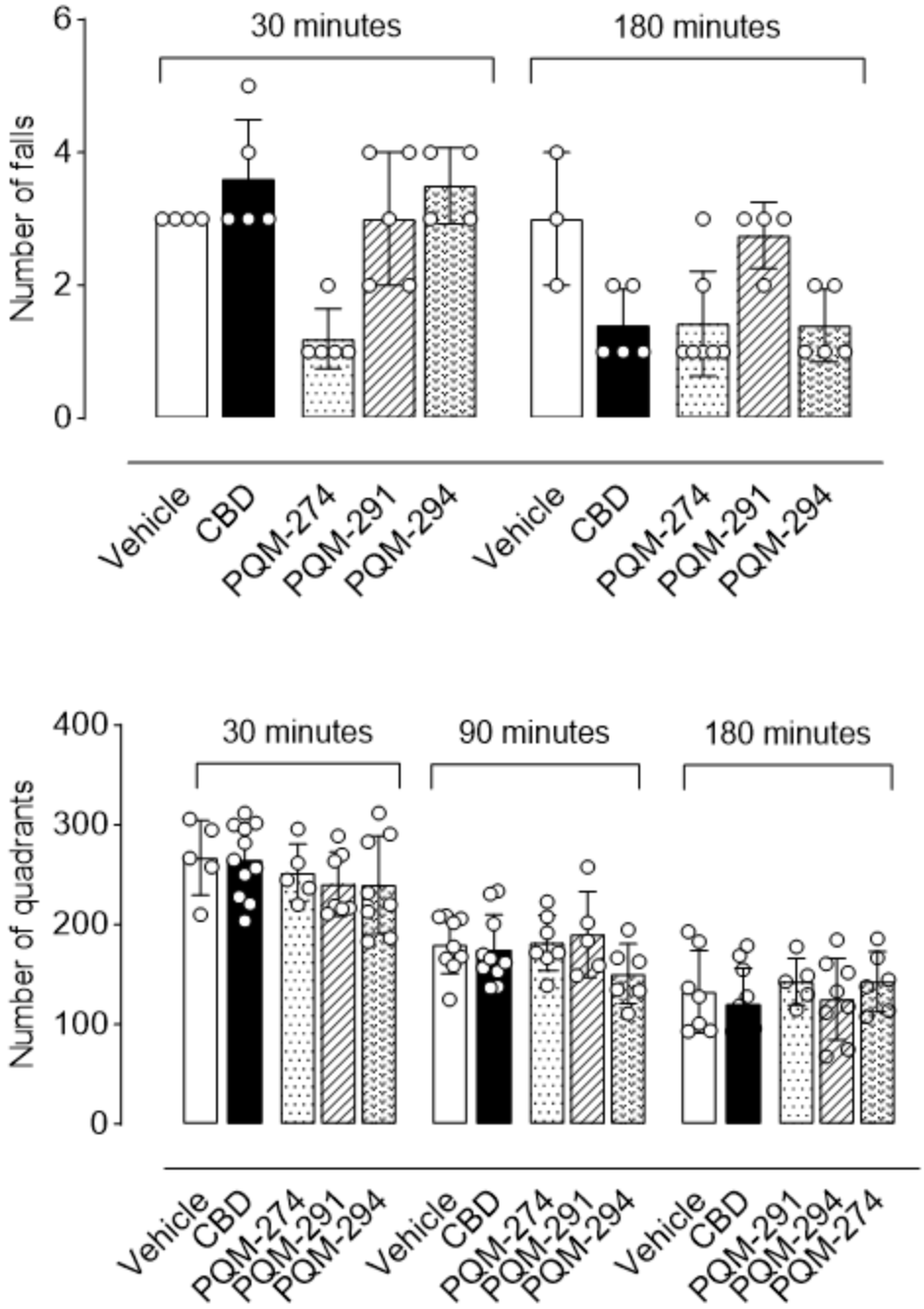

Figure 6

Effects of compounds in the rotarod and open field apparatus. Mice received oral administration of PQMs (at 10 µmol/kg dose). Mice were evaluated in the rotarod apparatus at 30 or 180 minutes, and in the open field at 30, 90 and 180 minutes after oral administration. Data are expressed as mean ± SD (n= 4-11). One-way ANOVA followed by Tukey's post hoc test was used to calculate statistical significance.