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ORIGINAL ARTICLE

Anti-neuropathic pain activity of a cationic palladium (II) dithiocarbamate by suppressing the inflammatory mediators in paclitaxel-induced neuropathic pain model

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Abstract

Background Neuropathic pain is a chronic pain state that negatively impacts the quality of life. Currently, available therapies for the treatment of neuropathic pain often lack efficacy and tolerability. Therefore, the search for novel drugs is crucial to obtain treatments that effectively suppress neuropathic pain.

Objectives The present study was undertaken to investigate the antinociceptive properties of (1,4-bis-(diphenylphosphino) butane) palladium (II) chloride monohydrate (Compound 1) in a paclitaxel (PTX)-induced neuropathic pain model.

Methods Initially, behavioral tests such as mechanical and cold allodynia as well as thermal and tail immersion hyperalgesia were performed to investigate the antinociceptive potential of Compound 1 (5 and 10 mg/kg, b.w). RT-PCR was performed to determine the effect of Compound 1 on the mRNA expression level of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and proinflammatory cytokines such as tumor necrosis factor-alpha (TNF)-α, interleukin (IL)-1β, and IL-6. In addition, antioxidant protein, nitric oxide (NO), and malondialdehyde (MDA) levels were also determined.

Results The results demonstrated that once-daily dosing of Compound 1 significantly suppressed the PTX-induced behavioral pain responses dose-dependently. The mRNA gene expressions of iNOS, COX-2, and inflammatory cytokines were markedly reduced by Compound 1. Furthermore, it enhanced the level of antioxidant enzymes and lowered the level of MDA and NO production.

Conclusion These findings suggest that the antinociceptive potential of Compound 1 in the PTX-induced neuropathic pain model is via suppression of oxidative stress and inflammation. Thus, Compound 1 might be a potential candidate for the therapeutic management of PTX induced neuropathic pain.

Keywords Allodynia · Hyperalgesia · iNOS · COX-2 · Inflammatory mediators · Oxidative stress

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Introduction

Neuropathic pain occurs due to the damage of peripheral or central nerves [1, 2]. It is generally characterized by extreme sensitivity to non-noxious stimuli (allodynia) and noxious stimuli (hyperalgesia). Neuropathic pain arises due to trauma, disease, and chemotherapeutic drugs [2, 3]. Macrophages are a vital component of the immune system and play pivotal roles in the regulation of inflammatory responses [4]. Macrophages are implicated in the pathogenesis of neuropathic pain [5]. Following nerve injury, resident macrophages initiate inflammatory responses and elicit long-lasting neuroinflammation through the recruitment of circulating leukocytes to the site of injury [4]. At the periphery, activated macrophages secrete cytokines/chemokines such as TNF-α (tumor necrosis factor-α) and

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interleukins (IL-1β, IL-6). Cytokines and chemokines secreted by macrophages are potential mediators of pain hypersensitivity in neuropathic pain [5]. Paclitaxel (PTX) is a well-known chemotherapeutic agent that is widely used for the treatment of breast, ovarian, lung, and bladder cancers [6]. However, treatment with PTX may result in peripheral neuropathy that exists for several months even after discontinuation of therapy [7]. Peripheral neuropathy is a major reason for reduction of dose or even cessation of chemotherapy and therefore, results in a great impact on the survival of cancer patients [8].

Numerous studies have revealed that inflammatory responses in the spinal cord result in the development of chemotherapy-induced peripheral neuropathy [9, 10]. It is well recognized that pro-inflammatory mediators are released by activated glial cells in the spinal cord which intensifies the sensitivity and excitability of the neuron [11]. Several lines of evidence have indicated that COX-2 (an inducible enzyme) and iNOS are associated with the induction and progression of neuropathic pain [12]. Peripheral nerve injury elevates the expression of COX-2 in the spinal cord which facilitates inflammation and pain hypersensitivity [13]. In addition, oxidative stress plays a crucial role in the pathogenesis of neuropathic pain [14].

Currently, treatment for neuropathic pain includes NSAIDs, morphine, anti-convulsant, and anti-depressants drug. However, these treatments have been associated with a wide spectrum of adverse effects [2]. The compounds containing structural palladium thiocarbamate have been reported for various anti-inflammatory and analgesic properties [15]. Pronounced pharmacological effects with a better safety profile have been considered by designing and synthesizing palladium-containing complexes [16]. The dithiocarbamates have also been reported for promising activities against oxidative stress and inflammatory cytokine [17]. It has been noted that dithiocarbamates structural analogs exhibit antiarthritic activities via inhibition of NF-κB and COX-2 signaling [18]. The characterization of Compound 1 has recognized it to be an efficient drug moiety. Earlier results have indicated that Compound 1 has suppressed the complete Freund's adjuvant (CFA)-, carrageenan-, histamine-, and serotonin-induced nociception [19]. Thus, bearing in mind the above-mentioned results, we decided to further explore the neuroprotective property of Compound 1 against PTX-induced neuropathic pain model.

Materials and methods

Chemicals and reagents

The DNA Extraction Kit (Novel Genomic DNA Mini Kit), TRI-Reagent (Bioshop, Canada), cDNA Synthesis Kit (ABM, Canada), PCR Primers (Macrogen Korea), PCR Master Mix (Thermo Fisher Scientific US). PTX, Compound 1, gabapentin (GBP), Griess Reagent, 1-chloro-2,4-dinitrobenzene (CDNB), dithio-nitrobenzoic acid (DTNB), hydrogen peroxide (H2O2), and all the other chemicals used were obtained from Sigma (USA). Drugs were dissolved in dimethyl sulfoxide (DMSO 2%) and diluted with normal saline up to the final volume.

Synthesis of compound 1

Compound 1 (1,4-bis-(diphenylphosphino) butane) palladium (II)chloride monohydrate) was synthesized according to the literature procedure [19]. Briefly, it was prepared by simultaneous and dropwise addition of 1,4-bis-(diphenylphosphino) butane (0.426 g, 1 mmol) in acetone (40 mL) and sodium 4-benzylpiperidine-1-carbodithioate (0.273 g, 1 mmol) in methanol (25 mL) to a methanolic suspension of palladium (II) chloride (0.177 g, 1 mmol). The reaction mixture was refluxed for 6 h with constant stirring. It was then filtered, and the filtrate was rotary evaporated. The product was soluble in acetone, chloroform, methanol, and dimethylsulfoxide.

Animals and experimental design

Sprague–Dawley female rats (200–250 g) were used in the present study. The animals were obtained from the NIH Islamabad, Pakistan. All the rats were accommodated in standard environmental conditions (22 ± 1 ºC, 52 ± 3% humidity) and a light/dark period of 12-h with unrestricted access to food and water.

Induction of neuropathic pain and dosing

PTX (4 mg/kg) was injected intraperitoneally (i.p.) in volumes of 1 ml per kg, 4 times a week (on days 1, 3, 5, and 7) [20]. PTX dose selection was selected based on a previous report [20]. Compound 1 (5 and 10 mg) was administered once daily for 8 days. The doses of Compound 1 were selected based on its previously reported therapeutic activities in animal models of arthritis [19]. Previously, in the first set of experiments, different doses of Compound 1 were screened to select an optimum and effective dose. Compound 1 was tested at a dose level of 0.1, 1, and 10 mg/kg. It exhibited optimum anti-inflammatory and analgesic effects at the doses of 5 and 10 mg/kg of body weight in mice [19]. The schedule of behavioral experimentations was on day 0 i.e., before first drug administration and then on every other day of PTX administration i.e., on days 2, 4, 6, and 8 after 1 h of treatment. Samples were collected after all the rats were sacrificed from each group on the final day of the experiment (Fig. 1).

Animal's models

Compound 1 was tested in a PTX-induced neuropathic pain model. All the animals were allotted in normal, negative (PTX-induced), positive (GBP) and treatment groups (5 and 10 mg/kg of Compound 1) with 6 animals in each group. Behavioral testing was carried out by an experimenter blinded to the treatment groups.

PTX-induced model

Rats were distributed into five groups with six rats per group:

Group 1: Normal control.
Group 2: PTX-induced (4 mg/kg, i.p).
Group 3: GBP 75 mg/kg (once daily for 8 days, i.p).
Group 4: Compound 1 (5 mg/kg, once daily for 8 days, i.p).
Group 5: Compound 1 (10 mg/kg, once daily for 8 days, i.p).

Behavioral experiments

Evaluation of static mechanical allodynia in PTX-induced rats

The anti-allodynic effect of Compound 1 was measured at various intervals (i.e. on days 0, 2, 4, 6, and 8) using Von Frey (VF) filaments as described previously [21–23].

Evaluation of cold allodynia (acetone test)

Compound 1 (5 and 10 mg/kg) was investigated against PTX-induced cold pain (allodynia) by performing an acetone test as described previously [24, 25]. In short, rats were habituated for 15 min in a transparent plastic box with a wire mesh floor. After that, a drop (0.05 mL) of acetone was put on the rat's plantar skin surface using a syringe. The behaviors of rats (i.e. time taken by a rat while flinching, licking, withdrawing, and biting of hind paw) after the acetone spray was examined within 15 s and measured as paw withdrawal duration (PWD). A comparative increase in PWD (s) is considered to be a sign of neuropathic pain. Acetone was ejected three times on each hind paw and their average PWD was calculated for each rat.

Evaluation of thermal hyperalgesia

To evaluate further the analgesic effect of Compound 1 in PTX-induced rats, a hot plate test was performed according to the previously reported protocols [26–28]. The once-daily dosing of Compound 1 was examined for antihyperalgesic effect on days 0, 2, 4, 6, and 8.

Evaluation of tail-flick latency (hot)

A hot tail immersion assay was performed to evaluate the central anti-nociceptive activity of Compound 1 as described previously [29, 30]. Briefly, the rat's tail was immersed into 52 °C water for 15 s (maximum), and the latency when the rat withdrew the tail was noted. The time taken by a rat to flick its tail from hot water was recorded as tail withdrawal latency (TWL). Each rat was exposed to three trials with a break of 5 min in between. The average of 3 trials was calculated as latency for each rat.

Evaluation of tail-flick latency (cold)

The cold-water tail-flick assay was performed to evaluate the cold hyperalgesia and antihyperalgesic effect of Compound 1 by dipping the lower half of the rat's tail into a beaker filled with cold water (0–4 °C) [29, 31]. After dipping the tail, the TWL was measured with a 15 s cut-off time. The test was studied 3 times at 5 min intervals to avoid tissue damage and the average was calculated. The antihyperalgesic effect was represented by the average of each latency (i.e. the comparatively longer latency was considered the antihyperalgesic effect) [32].

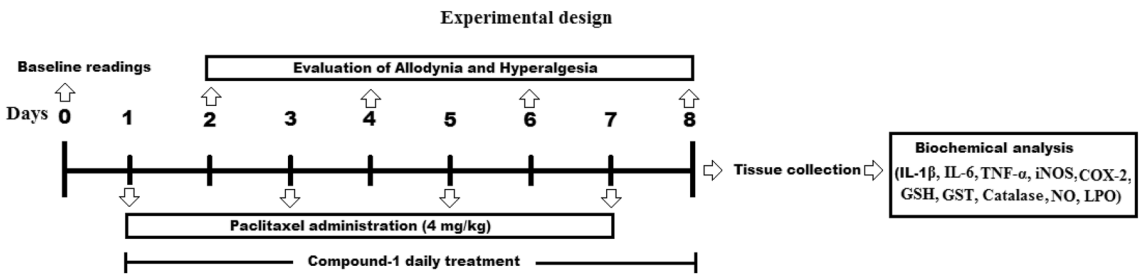

Fig. 1 A schematic representation of overall study plan

Evaluation of motor performances

Assessment of Compound 1 treatment on motor performances of rats was evaluated using a rotarod apparatus, which was rotating at a speed up to 40 rpm for 5 min. Before starting the experiment, all the rats were trained for 4 days by placing them on a rotating drum with a maximum speed of 40 rpm as described previously [33, 34]. The rats which did not endure walking on the rotarod for more than 2 min were excluded. After that baseline trials were performed before any drug administration to start the experiment. Each rat underwent 3 trials. The latency of time to fall and the falling frequency over a 5-min period were measured. The same trials were repeated on days 2, 4, 6, and 8, 1 h after treatment with Compound 1 or GBP.

Table 1 The sequences of PCR primers

Primer	Sequence
IL-1β	F- 5'-TGATGACGACCTGCTAGTGTG-3°
	R- 5'- TCCATTGAGGTGGAGAGCTT-3°
IL-6	F- 5'-GGAGTTTGTGAAGAACAACT 3°
	R- 5'- CTAGGGTTTCAGTATTGCTC-3°
TNF-α	F- 5'- ATGAGCACAGAAAGCATGATC-3'
	R- 5'-TACAGGCTTGTCACTCGAATT-3'
iNOS	F- 5'-CACCACCCTCCTTGTTCAAC-3'
	R; 5'-CAATCCACAACTCGCTCCAA-3'
COX-2	F- 5′-TGTATGCTACCATCTGGCTTCGG-3’
	R- 5’-GTTTGGAACAGTCGCTCGTCATC-3’
β-actin	F- 5′- CGTTGACATCCGTAAAGACCTC-3';
	R- 5'-TAGGAGCCA GGGCAGTAATCT-3'

Biochemical assays

RNA extraction and RT-PCR

Total RNA was extracted from the L4-6 spinal cord segment of rats according to the manufacturer's protocol using the TRI-reagent and the purity of the total RNA was determined using a UV spectrophotometer. Total RNA was transcribed to cDNA using a cDNA synthesis kit. The expressions of targeted genes like TNF-α, IL-1β, IL-6, iNOS, and COX-2 were determined (Table. 1). β-actin was incorporated as the housekeeping gene. Amplified products were isolated by the use of 1.5% agarose gel electrophoresis and visualized through a UV trans-illuminator. The expression level (A.U) was calculated [11, 35–38].

Nitric oxide (NO) determination

The production of NO in the spinal tissue was quantified using the Griess reagent assay according to the previously reported protocols [39–41].

Determination of antioxidant enzymes

The levels of antioxidants such as GSH, GST, and catalase were measured according to the previously reported method [42, 43].

Lipid peroxidation assay (LPO)

LPO was estimated by measuring the MDA concentration in spinal tissue according to the previously reported method [44–46]. A microplate reader was used to measure the absorbance at 535 nm.

Pharmacokinetics and toxicokinetic analysis

An *in-silico* analysis was performed to determine the pharmacokinetic behavior of Compound 1. The various pharmacokinetics factors that were assessed include absorption, distribution, metabolism, excretion, the volume of distribution, and plasma protein binding. The pharmacokinetic parameters were analyzed using Swiss target prediction and pK-CSM online server as reported previously [47]. Similarly, the toxicokinetic analysis was performed to assess the harmful effect of Compound 1 against animals, tissue, and micro-organisms, and the maximum tolerated dose was established using online computational tools [47].

Statistical analysis

Data expressed as the mean ± SD. Two-way ANOVA was applied followed by Bonferroni's post hoc test for the assessment of statistical significance amongst various treated groups. Statistical analysis of the data was performed using Sigma-plot version 12.5. p" value < 0.05 was considered to be statistically significant.

Results

Effect of Compound 1 on static mechanical allodynia

Allodynia produced by administration of PTX was considerably ($p < 0.001$) inhibited by treatment with Compound 1 and GBP in a dose-dependent manner. The antiallodynic effect was indicated by increased paw withdrawal threshold (PWT) by treatment with Compound 1 when evaluated on

the 2nd, 4th, 6th, and 8th day. The PWT (g) was remarkably high in the treatment groups as compared to the PTX group (Fig. 2a).

Effect of Compound 1 on cold allodynia

Cold allodynia in PTX-treated rats was significantly ($p < 0.001$) high as compared to the normal control groups as evident from the comparative rise in PWD (s). However, this increase in PWD was reduced by treatment with Compound 1 and GBP (Fig. 2b).

Effect of Compound 1 on thermal hyperalgesia

Treatment with Compound 1 markedly ($p < 0.001$) lowered the hyperalgesic responses (paw licking and jumping) dose-dependently in PTX-treated rats as denoted by increased paw withdrawal latency (PWL) in treatment groups (Fig. 2c).

Effect of Compound 1 on tail hot hyperalgesia

The hot tail immersion nociception is indicative of central nociception. Rats treated with PTX exhibited shortened TWL (s) as compared to the normal control. However, Compound 1 treatment markedly ($p < 0.001$) increased TWL (Fig. 2d).

Effect of Compound 1 on tail cold hyperalgesia

In PTX-treated rats, TWL was significantly reduced than normal animals indicating cold hyperalgesia. Compound 1 daily treatment remarkably ($p < 0.001$) elevated this reduction in TWL showing its anti-neuropathic pain potential (Fig. 2e).

Effect of Compound 1 on motor activity

Motor coordination was assessed through a rotarod test. No abnormal effect was observed on motor activity after the treatment with Compound 1. However, GBP exhibited a significant motor deficit indicated from low rotarod latency time (sec) (Fig. 2f).

Effect of Compound 1 on inflammatory cytokines

The mRNA level of inflammatory cytokines in spinal cord tissues was analyzed using RT-PCR. A significant ($p < 0.001$) induction in the expression of these cytokines was observed in the PTX-treated group. However, treatment with Compound 1 significantly ($p < 0.001$) lowered the expression of these cytokines as compared to the negative control group (Fig. 3b-d).

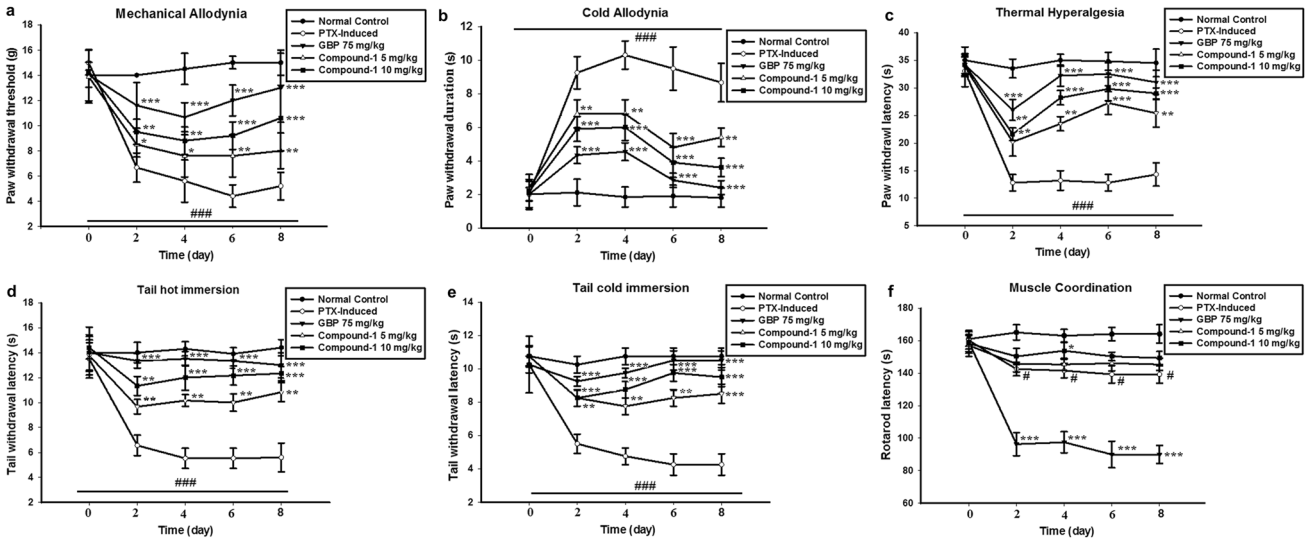

Fig. 2 Dose-dependent effect of Compound 1 in doses of 5 and 10 mg/kg in PTX-induced rats. Compound 1 pretreatment inhibited PTX-induced (**a**) mechanical allodynia (**b**) cold allodynia (**c**) thermal hyperalgesia (**d**) tail thermal hyperalgesia (**e**) tail cold hyperalgesia and (**f**) motor activity. The data is displayed as the mean (n = 6) ± SD. ANOVA followed by a post hoc Bonferroni test was applied for comparing statistical differences between groups. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$ represent a statistically significant difference from the PTX-induced group. (###) indicates comparison to the normal control group

Effect of Compound 1 on iNOS and COX-2 mRNA expression levels

To further determine the inhibitory effect of Compound 1 on inflammatory mediators, we evaluated the mRNA expression level of iNOS and COX-2 in the spinal cord. There was an up-regulation of iNOS and COX-2 mRNA expression after neuropathic pain induction which was significantly ($p < 0.001$) inhibited by treatment with Compound 1 (Fig. 3e, f).

Effect of Compound 1 on antioxidants

The levels of antioxidants were significantly ($p < 0.001$) reduced in the PTX-treated group. However, Compound 1 increased the level of these antioxidant proteins as shown in (Table. 2).

Effect of Compound 1 on nitric oxide (NO) production

The NO production was significantly ($p < 0.001$) increased in the PTX-treated group. Treatment with Compound 1 remarkably ($p < 0.001$) inhibited the PTX-induced NO production (Table. 2).

Effect of Compound 1 on LPO

PTX administration significantly ($p < 0.001$) elevated the MDA level. Compound 1 treatment showed a marked ($p < 0.001$) decrease in MDA level as compared PTX-treated group (Table. 2).

Pharmacokinetics and toxicokinetic analysis

The pharmacokinetic analysis of the compounds showed variable pharmacokinetic properties using online pKCSM software (http://biosig.unimelb.edu.au/pkcsm/prediction).

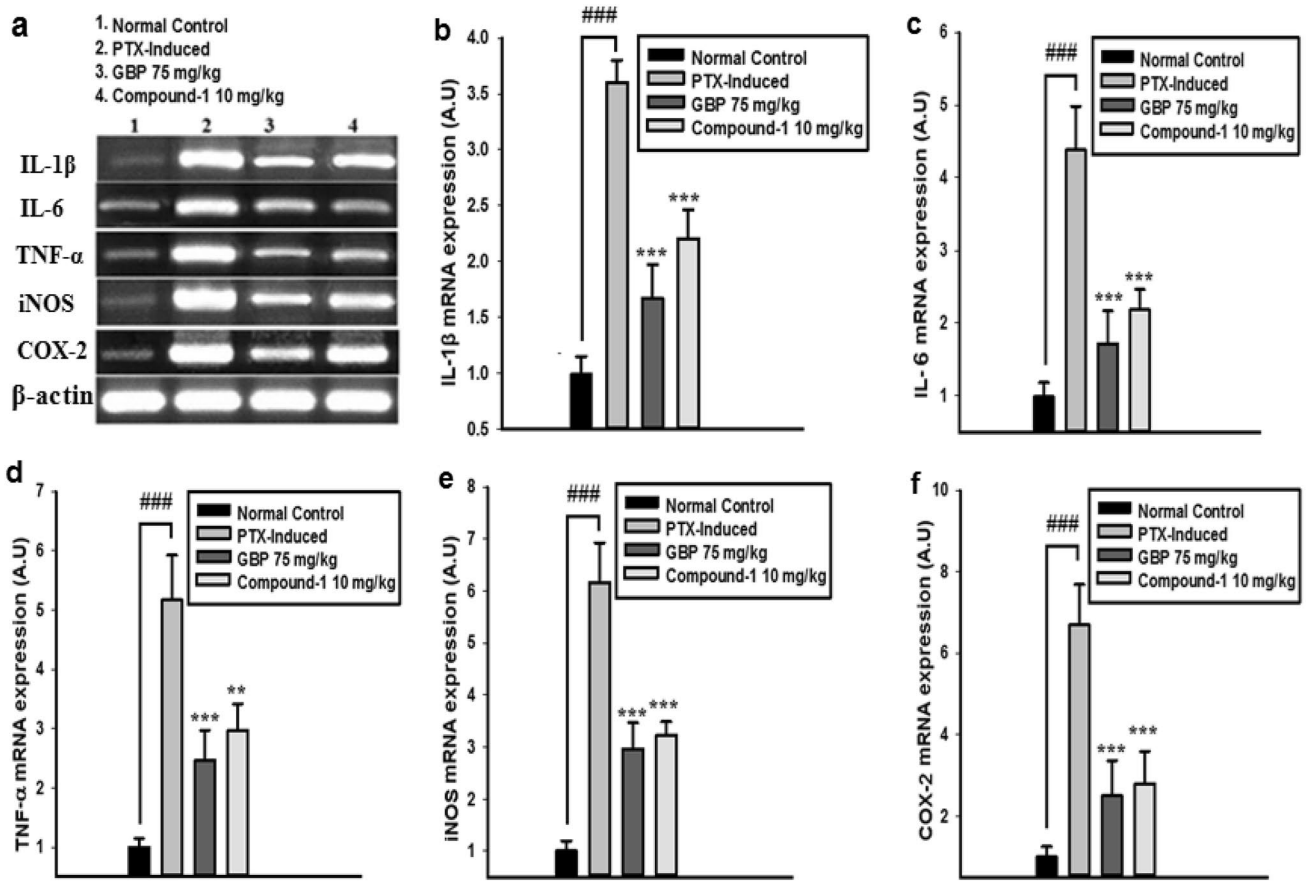

Fig. 3 **a** Effect of Compound 1 treatment on mRNA expression level of (**b**) IL-1β (**c**) IL-6 (**d**) TNF-α (**e**) iNOS and (**f**) COX-2. The results are shown in a relatively arbitrary unit (A.U). The data is displayed as the mean (n = 6) ± SD. ANOVA followed by a post hoc Bonferroni test was applied for comparing statistical differences between groups. $*p \leq 0.05$, $**p \leq 0.01$, and $***p \leq 0.001$ represent a statistically significant difference from the PTX-induced group. (###) indicates comparison to the normal control group

Table 2 Effect of Compound 1 on antioxidants, LPO and NO production in PTX-induced rat's spinal tissue

Parameters	Normal control	PTX	GBP (75 mg/kg)	Compound 1 (10 mg/kg)
GSH concentration (μM)	14.47 ± 0.39	3.45 ± 0.28$^{###}$	11.42 ± 0.39***	10.90 ± 0.36***
GST concentration (μM)	38.92 ± 1.27	12.43 ± 1.38$^{###}$	36.70 ± 1.14***	36.25 ± 1.66***
Catalase activity (unit/μL)	11.6 ± 0.17	3.88 ± 0.09$^{###}$	11.16 ± 0.09***	11.03 ± 0.14***
MDA level (%)	10.68 ± 0.30	100 ± 2.86$^{###}$	16.89 ± 1.68***	18.01 ± 0.68***
NO production (μM)	7.61 ± 0.51	56.06 ± 6.56$^{###}$	11.29 ± 1.90***	11.64 ± 1.34***

The data is presented as the mean (n = 6) ± SD

(###) denotes comparison to normal control group

***p denotes comparison to PTX-induced group

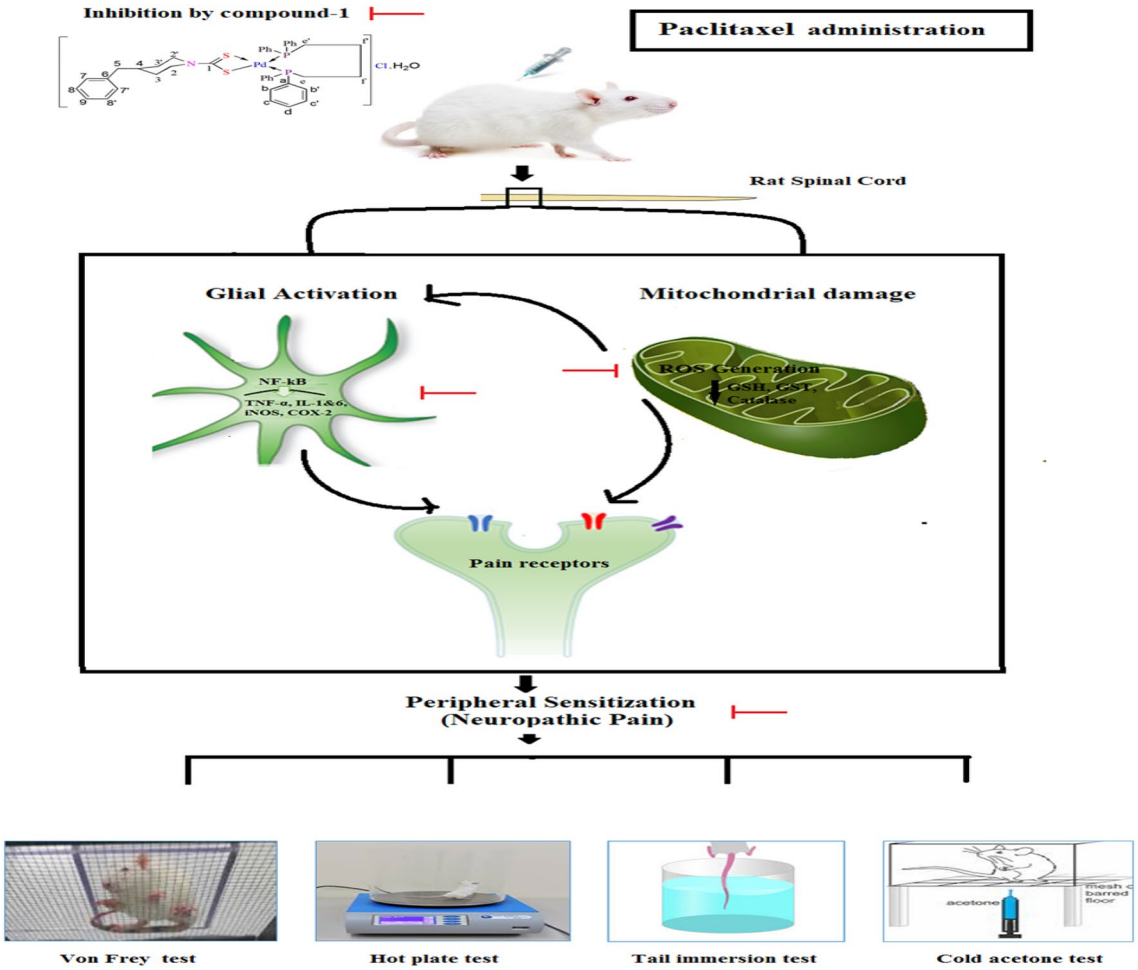

Fig. 4 Graphical abstract representing anti-neuropathic pain activity of Compound 1 in PTX-induced neuropathic pain model

The various properties that were predicted include drug-like properties, physicochemical properties, lipophilicity, water solubility, and pharmacokinetic properties. Similarly, the toxicokinetic analysis of the Compound 1 showed a good safety profile as shown in Table 3.

Discussion

PTX is an effective chemotherapeutic drug indicated in the treatment of breast, ovarian, lung, and bladder cancers. However, PTX-induced peripheral neuropathy is a

Table 3 Pharmacokinetic and toxicokinetic analysis of the Compound 1

Name	Absorption				Distribution			Metabolism	Excretion
	Water solubility (log mol/l)	Caco2 Cell	Intestinal absorption	P-gp Substrate Categorical (Yes/No)	BBB permeability (logBB)	Fraction Unbound Numeric (Fu)	CNS VDss Numeric (log PS) log L/kg	CYP 2D6 3A4 1A2 2C19 2C9 2D6 3A4 Substrate Inhibitor Categorical (Yes/No)	Numeric (log ml/min/kg)
Compound 1	− 5.34	1.009	94.37	Yes	1.445	0.300	− 0.056 − 0.753	No Yes No No No No No	− 1.109
Molecular properties	Molecular weight		Logp		Rotatable bonds		Acceptors	Donors	Surface area
Compound 1	785.328		8.918		6		2	0	290.311
Toxicity	Ames toxicity	Max dose	hERG I inhibitor	hERG II inhibitor	Oral toxicity acute (LD50)	Oral chronic toxicity	Hepatotoxicity	Skin sensitivity T. Pyriformis toxicity	Minnow toxicity
Compound 1	No	0.45	No	Yes	3.263	− 0.367	No	No 0.285	3.039

major adverse effect and causes premature termination of cancer therapy. This adverse effect may persist up to several months even after discontinuation of therapy [7]. Current therapies have been associated with a wide spectrum of adverse effects that limit their satisfactory clinical use. It is important to identify novel drugs that effectively suppress neuropathic pain without attenuating their anticancer effects [2]. Numerous studies have shown the role of inflammatory cytokines, NO, COX-2, and oxidative stress in the initiation and maintenance of neuropathic pain [48–50]. Therefore, the drug that inhibits the release of inflammatory mediators might be the potential candidate to treat neuropathic pain. Previous findings have demonstrated that Compound 1 exhibited remarkable anti-inflammatory and analgesic activities by suppressing the inflammatory mediator's production. Therefore, keeping in view the previous promising therapeutic activities, Compound 1 was further evaluated as an anti-neuropathic pain agent in the present study.

PTX significantly induced allodynia and hyperalgesia by increasing the rat's plantar sensitivity to VF filaments, hot, and cold stimuli. The neuropathic pain produced by PTX administration was measured in the form of various pain parameters such as decreasing PWT, PWL, TWL, and increase in PWD [29]. However, these parameters of nociception were significantly improved by daily treatment with Compound 1 and GBP. Results from all the behavioral responses demonstrated the significant antiallodynic and antihyperalgesic activities of Compound 1. Furthermore, Compound 1 did not show any deteriorating effect on motor activity.

Inflammatory cytokines play a crucial role in neuropathic pain by increased hypersensitivity [1] and lead to excessive nociceptive transmission in the spinal cord [51]. The chemical mediators secreted all over the inflammatory process sensitizes the nociceptors that produce pain hypersensitivity resulting in hyperalgesia and allodynia [19]. PTX has been revealed to induce the expression of proinflammatory cytokines, such as IL-1 and TNF-α, in the rat spinal cord [52]. In the present study, PTX treatment considerably increased the expression of inflammatory cytokines in spinal tissues. These inflammatory cytokines were remarkably reduced by Compound 1. Similarly, the iNOS and COX-2 expression levels were also measured. The present study showed that Compound 1significantly suppressed iNOS and COX-2 expressions as well as NO production.

It is well known that oxidative stress plays a vital role in neuropathic pain [2]. In the present study, PTX administration significantly lowered the level of endogenous antioxidants i.e., GSH, GST, and catalase, respectively. The results demonstrated that Compound 1 exhibited a protective role against the PTX-induced oxidative stress by enhancing the level of antioxidants in spinal tissues while decreasing the MDA level. Additionally, the pharmacokinetic analysis using pK-CSM software showed that Compound 1 exhibits high GIT absorption, BBB permeability, and interaction with the cytochrome p450 system, and partial risk of toxicity.

Conclusion

In summary, the present study revealed that the compound possesses significant antiallodynic and antihyperalgesic activities in the well-known PTX-induced neuropathic pain model. Compound 1 markedly suppressed the levels of proinflammatory cytokines, iNOS, COX-2, and oxidative stress (Fig. 4). The pharmacokinetic analysis showed that Compound 1 exhibits good ADME properties. We suggest from the present study that Compound 1 might be a useful candidate for the treatment of neuropathic pain. Additional research is needed to confirm these results.

Authors' contributions MN, RU designed and performed research including behavioral and biochemical assays. SZK and ZR synthesized the compound. AK helped in behavioral and biochemical assays. AK and MN write the original manuscript. MN, AK, BS, AUK, and SK analyzed the data. SK supervised the project. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval "QAU guidelines for animal's care" Islamabad were followed for the overall experiments involving animals. QAU, Islamabad Bioethical Committee (Approval No: BEC-FBS-QAU 2017–59) approved the study. Maximum care was assured to minimize harm to animals.

Consent for publication N/A

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