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

Neuropathic pain is an obstinate chronic pain state which extremely worsens the quality of life of the suffered population. It is still intended as an intractable disease as existing therapies are not excellent in terms of efficacy and tolerability. Therefore, to search for novel drugs are crucial to acquire satisfactory treatments. The present study investigated the possible antiallodynic, antihyperalgesic and neuroprotective activities of (1,4-bis-(diphenylphosphino) butane) palladium (II)chloride monohydrate-(**1**) in Paclitaxel (PTX)-induced neuropathic pain model (Fig.10). Initially, **1** (5 and 10 mg/kg, b.w) was investigated for antinociceptive activities at behavioral level by performing mechanical and cold allodynia as well as thermal and tail immersion hyperalgesia. RT-PCR was performed to determine the suppressing effect of **1** on mRNA expression level of iNOS, COX-2 and proinflammatory cytokines (TNF-α, IL-1β, IL-6). In addition, antioxidant protein and enzymes (GSH, GST, Catalase), NO, MDA level and muscle activity were also determined. The results demonstrate that once daily dosing of **1** significantly repressed the behavioral pain responses dose dependently. Moreover, the mRNA gene expression of iNOS, COX-2 and inflammatory cytokines were reduced noticeably by **1**. Furthermore, the treatment enhanced the level of antioxidant enzymes and lowered the level of MDA and NO production with no effect on motor activities of rats. These findings suggest the potential of **1** to attenuate neuropathic pain, neuroinflammation and neuroprotective effect. Thereupon, **1** might be a dynamic candidate for the therapeutic management of chronic neuropathic pain.

Introduction

Neuropathic pain occurs due to the damage of peripheral or central nerves [1, 2] and is established as mono-neuropathy, mono-neuropathy multiplex or poly-neuropathy [3]. It is usually particularized by extreme sensitivity to commonly harmless (allodynia) and harmful stimuli (hyperalgesia) [4]. Neuropathic pain is a cureless and painful disorder that can produce prickling, burning sensation and numbness in the body [5, 6]. Its definite cause is oftentimes unidentified [7]. However, it is evident that macrophages are implicated in neuropathic pain models and are hired by chemotactic molecules in injured peripheral nerves [5, 8, 9]. It has been shown by the latest mechanistic studies of neuropathic pain that proinflammatory mediators are exempted by activated glial cells in the spinal cord which in turn, may modify sensitivity and excitability in the nociceptive pathway [10], resulting in neuropathic pain. Moreover, COX-2 (an inducible enzyme) facilitates inflammation and pain [11], peripheral inflammation elevates the expression of COX-2 in the spinal cord [12, 13]. The previous reports have shown that COX-2 expression is stimulated by TNF-α [14, 15] as well as through activation of NF-κB [16]. Stimulation of NF-κB in turn yields several chemokines and pro-inflammatory cytokines [17]. Furthermore, the role of COX-2 [10] and iNOS has also been vigorously associated with the induction and progression of inflammatory and neuropathic pain that can stimulate, or get stimulated by NF-κB [18]. In addition, oxidative stress is also determined as a major pathogenic factor detrimental to peripheral sensory neurons [19] which have substantial involvement in the pathogenesis of neuropathic pain [20].

Neuropathic pain can arise due to trauma, disease and some toxins like chemotherapeutic drugs [21]. Experimental animal models of peripheral nerve injury that yields sensitive behaviors of mechanical allodynia and thermal hyperalgesia have been designed, which reciprocate to some extent, to a clinically related neuropathic pain model [22]. The origination of post-traumatic painful peripheral neuropathic animal models have prominently refined the field of neuropathic pain with an embracive mechanistic perceptive and the potential to discover new anti-neuropathic agents [1]. Although, these models are considerably related to peripheral neuropathies (e.g., causalgia), they are also probably analogous to painful peripheral neuropathies invoked by disease (e.g., diabetes) and toxins (e.g., chemotherapeutics) [1]. Paclitaxel (PTX) is a well-known chemotherapeutic agent that is enormously used for the treatment of breast, ovarian, head, lung, and bladder cancers, and Kaposi's sarcoma as well [23]. However, treatment with PTX may result in peripheral neuropathy which leads the patient non-compliance with the chemotherapy and this adverse-effect can exist for a number of months after the treatment has been terminated [24]. The peripheral neuropathy might then be a reason to reduction of dose or even cessation of chemotherapy, often resistant to conventional analgesics [25]. Numerous research studies reveal that inflammation of spinal cord results in the pathogenesis of chemotherapy-induced peripheral neuropathy, with pro-inflammatory chemokines that are secreted by glial cells to contribute to central sensitization [26, 27]. As a result, illustrating the mechanisms behind PTX-induced neuropathy is important to develop new therapies [23, 25].

The new therapeutics' development has been specifically demanding to treat the chronic illness [1]. Mounting evidence determines that currently existing treatment for neuropathic pain, including NSAIDs, morphine, anti-convulsant and anti-depression drugs, has confined efficiency, and is generally followed by untoward side effects [5]. For this reason, the higher efficacious and tolerable drugs are of prior consideration. The compounds containing structural palladium thiocarbamate have been proclaimed for various anti-inflammatory and analgesic properties [28]. The dithiocarbamates have also been reported to reveal promising activities against oxido-nitrosative stress and cytokine suppression [29]. Antiarthritic activities by dithiocarbamates structural analogs have previously been described via mechanism of NF-κB and COX-2 pathways inhibition [30]. Pronounced pharmacological effects with reduced range of side effects have been shown by designing and synthesizing of palladium containing complexes [31] with a better safety profile. Moreover, its characterization has recognized it to be an efficient drug moiety. Earlier results have determined that **1** suppressed the nociception induced by CFA-, carrageenan-, histamine-, and serotonin [17]. Thus, bear in mind the above-mentioned outcomes, we decided to further explore the neuroprotective property of **1** and its effect in a PTX-induced neuropathic pain model.

Materials And Methods

Chemicals and reagents

1 was synthesized according to a procedure as described previously [17]. 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, Gabapentin (GBP), Griess Reagent, 1-Chloro-2,4-dinitrobenzene (CDNB), Dithio-nitrobenzoic acid (DTNB), Hydrogen Peroxide (H_2O_2) and all the other chemicals used were obtained from Sigma (USA) unless otherwise specified. Drugs were dissolved in Dimethyl Sulfoxide (DMSO 2%) and diluted with normal saline up to the final volume.

Animals and experimental design

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

Induction of neuropathic pain and dosing

PTX (4 mg/kg) was injected intraperitoneally (i.p.) in volumes of one ml per kg, four times a week (on days 1, 3, 5, and 7) [32]. PTX dose selection was presumed on a previous report [32]. **1** was administered once daily for 8 days (5 and 10 mg). The doses of **1** was selected based on its previous significant therapeutic activities [17]. 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 final day of experiment (Fig.1).

Animal's models

1 was tested in PTX-induced neuropathic pain animal model. All the animals were allotted in normal, negative (PTX-induced), positive (GBP) and treatment groups (5 and 10 mg/kg of **1**) with 6 animals in each group. Behavioral tests were observed without having the knowledge to which experimental group each rat belonged.

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: **1** 5 mg/kg (once daily for 8 days, i.p)

Group 5: **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 **1** was measured at various intervals (i.e. on day 0, 2, 4, 6 and 8) by using Von Frey (VF) filaments as described previously [17, 33, 34].

Evaluation of cold allodynia (acetone test)

1 (5 and 10 mg/kg) was investigated against PTX-induced cold pain (allodynia) by performing acetone test as described previously [35, 36]. 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 or 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 as a sign of neuropathic pain. Acetone was ejected three times on each hind paw and their average was calculated for each rat.

Evaluation of tail flick latency (hot)

Hot tail immersion assay was performed to evaluate the central anti-nociceptive activity of **1** as described previously [35, 37]. Briefly, rat's tail was immersed into 52 °C warm water for 15s (maximum) and the latency when the rat withdrew the tail was noted. The reaction time was considered as the time taken by a rat to flick its tail from hot water and 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 were calculated as a 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 **1** by dipping the lower half of rat's tail in a beaker filled with cold water (0–4 ^{O}C) [35, 38]. In short, after dipping the tail in 4 °C water, TWL was measured with a 15s cut-off time. The test was studied 3 times at 5 min intervals to avoid tissue damage and its average was calculated. Antihyperalgesic effect was represented by the average of each latency (i.e. the comparatively longer latency was considered the antihyperalgesic effect) [39].

Evaluation of thermal hyperalgesia

In order to evaluate further the analgesic effect of **1** in PTX-induced rats, hot plate test was performed according to the previous procedure performed by Naveed et al. [17, 40]. The once daily dosing of **1** was examined for antihyperalgesic effect on day 0 before any drug administration and then on day 2, 4, 6 and 8 one-hour post treatment.

Evaluation of motor performances

Assessment of **1** treatment on motor performances of rats was evaluated using a rotarod apparatus rotating at a speed up to 40 RPM over 5 min. Before starting the experiment, all the rats were trained for 4 days by placing on a rotating drum with the maximum speed increasing up to 40 RPM as described

previously [41, 42]. All those rats were excluded which did not endure to walk on the rotarod for more than 2 min. 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 day 2, 4, 6 and 8, 1 h after treatment with **1** or GBP [42].

Biochemical assays

RNA extraction and RT-PCR

Total RNA was extracted from L4-6 spinal cord segment of rats according to the manufacturer's protocol using TRI-reagent and purity of total RNA was determined using a UV spectrophotometer. Total RNA was transcribed to cDNA using a cDNA synthesis kit. The expression 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 UV trans-illuminator. The expression level (A.U) was calculated [10, 43-46].

Nitric oxide (NO) determination

The production of NO in blood plasma of rats from all the groups were quantified by Griess reagent assay according to the procedure as described previously by Naveed at al. [17, 47-50].

Determination of antioxidant enzymes

The level of antioxidant enzymes and proteins such as GSH, GST and Catalase was measured following the same methodology as discussed previously [17, 51-53].

Lipid peroxidation assay (LPO)

LPO was estimated through determining the MDA concentration in spinal tissue as per previous method with some modifications [48, 54-56]. Microplate reader was used to measure the absorbance at 535 nm [57].

Pharmacokinetics and toxicokinetic analysis

The *in-silico* analysis was utilized to determine the pharmacokinetic behavior of the studied compounds. The various pharmacokinetics factors that were assessed includes absorption, distribution, metabolism, excretion, volume of distribution, and plasma protein binding. The pharmacokinetic parameters were analyzed using Swiss target prediction and pKCSM online server as reported previously. Similarly, the toxicokinetic analysis was performed to assess the harmful effect of the studied compound against animals, tissue, micro-organism and maximum tolerable dose as reported previously using online computational tools [58].

Statistical analysis

Data expressed as the mean ± SD. ANOVA followed by post hoc Bonferroni correction test was run to define statistical significance between groups at various time points. Statistical analysis of the data was organized using Sigma-plot version 12.5 and were considered significant with a "p" value < 0.05.

Results

Effect of 1 on static mechanical allodynia

Allodynia produced by repeated administration of PTX was considerably inhibited by treatment with **1** and standard GBP in a dose dependent manner. Antiallodynic effect was indicated by increased paw withdrawal threshold (PWT) by treatment with **1** when evaluated at 2^{nd}, 4^{th}, 6^{th}, and 8^{th} day (Fig. 2a). PWT (g) towards the VF filaments was remarkably high in treatment groups as compared to PTX-treated group. All the animals received treatments once daily for 8 days except negative control group and PWT was recorded at basal and on days 2, 4, 6 and 8 post 1 h of drug administration.

Effect of 1 on cold allodynia

Cold allodynia induced by acetone drops in PTX-treated rats was significantly high as compared to the normal control groups, evident from the comparative rise in PWD (s). Opposite to that, this increase in PWD was not significantly different from normal rats showing the attenuation of neuropathic pain by treatment with **1** and standard GBP as shown in (Fig. 2b).

Effect of 1 on tail hot hyperalgesia

The hot tail immersion nociception as indicative of central nociception was evaluated by placing the lower end of rats' tail in hot water to examine the tail withdrawal reflex. Rats treated with PTX exhibited shortened TWL (s) as compared to the normal control. While those treated with **1** was examined to have a pronounced increase in TWL as compared to PTX-induced rats (Fig. 3a).

Effect of 1 on tail cold hyperalgesia

Similar to hot hyperalgesia, the cold hyperalgesic responses were tested by immersing the lower end of tail in cold water. In PTX-treated rats, TWL was significantly reduced than normal animals indicating cold hyperalgesia (Fig. 3b). **1** daily treatment remarkably elevated this reduction in TWL by attenuating the neuropathic pain-induced cold hyperalgesia showing its anti-neuropathic pain potential.

Effect of 1 on thermal hyperalgesia

Treatment with **1** extensively lowered the hyperalgesic responses (paw licking, jumping etc.) dose dependently in PTX-treated rats. At second, fourth, sixth and eighth day of experiment, the behavioral evaluation of thermal hyperalgesia was performed which was remarkably attenuated as denoted by increased paw withdrawal latency (PWL) in treatment groups. During the entire period, the inhibiting effect of **1** on hyperalgesia was significant (Fig. 4).

Effect of 1 on motor activity of animals

Muscle coordination and general body weakness were assessed through rotarod test before any drug administration and then every alternate day of PTX administration, one hour after treatment. As compared to normal control rats, no abnormal effect was observed on muscle activity and coordination after the treatment with **1 (**Fig. 5**)**. However, within treatment groups' GBP exhibited significant motor deficit as compared to normal and PTX-induced group indicated from low rotarod latency time (sec) (Fig. 4).

Effect of 1 on inflammatory cytokines

To further investigate and confirm the behavioral anti-neuropathic pain activities of **1**, the mRNA level of inflammatory cytokines in spinal cord tissues were analyzed by RT-PCR at the end of experiment. A significant induction in the expression of these cytokines were observed in PTX-treated rat's tissue as shown in (Fig. 5a). However, treatment with **1** significantly lowered the expression of these cytokines as compared to negative control group (Fig. 5 b, c, d).

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

Further to determine the inhibitory effect of **1** on key mediators of inflammatory pain (NO and PGE2), we evaluated the mRNA expression level of iNOS and COX-2 in spinal cord of PTX-induced rats via RT-PCR respectively. There was an up-regulation of iNOS and COX-2 mRNA expression after neuropathic pain induction which was significantly inhibited by treatment with **1** (Fig. 5 e, f).

Effect of 1 on antioxidant protein and enzymes

The level of antioxidant protein and enzymes were significantly declined due to oxidative stress induced by PTX treatment as compared to normal and treatment groups. However, this oxidative stress was prevented and reversed after a significant enhancement in the level of these antioxidant protein and enzymes were seen in the rats receiving treatment as shown in (Table. 2).

Effect of 1 on nitric oxide (NO) production

To determine whether the iNOS regulates the production of pro-inflammatory mediator and a marker of inflammation and pain (NO), we investigated NO in blood plasma of PTX-induced rats by performing Griess reagent assay (Table. 2). Treatment with **1** remarkably inhibited the PTX-induced nitrite production, thereupon this correlates with the reduction in the corresponding mRNA expression (Table. 2).

Effect of 1 on LPO

It was observed from the result that PTX administration elevated the MDA level to a significant level (Table. 2). **1** treatment showed marked decline 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). 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 showed a good safety profile as shown in Table 3.

Discussion

PTX therapy, may result in severe peripheral neuropathy, which is commonly used as an anticancer drug for the treatment of solid tumors. As a result, there is a reduction in adherence to chemotherapy and this adverse effect may persist up to certain months even after discontinuation of therapy [24]. Neuropathic pain is the consequence of peripheral nerve injury; it is often constant and the current therapies are unable to treat it properly because of provoking obscure mechanism of neuronal injury [21]. It is important to find out the PTX-induced mechanism of pain for the discovery of novel therapies [23]. Numerous studies have shown the role of inflammatory cytokines, NO, COX-2 and oxidative stress in the initiation and maintenance of chronic neuropathic pain [59-61]. Therefore, reagents interfering with these mediators possibly be the potential candidates to treat neuropathic pain. Previous findings have demonstrated that **1** exhibited remarkable anti-inflammatory and analgesic activities by suppressing the inflammatory mediator's production. Therefore, keeping in view the previous promising therapeutic activities, **1** was further evaluated as anti-neuropathic pain agent in the present study.

PTX significantly induced allodynia and hyperalgesia by increasing rat's plantar sensitivity to VF filaments, hot and cold stimulus by performing various behavioral nociceptive tests. The neuropathic pain produced by PTX administration was consistent as per previous reports [62] and measured in the form of various pain parameters such as decreasing PWT, PWL, TWL and increase in PWD [35]. However, these parameters of nociception were significantly changed by daily treatment with **1** and GBP. Results from all the behavioral responses demonstrated the significant antiallodynic and antihyperalgesic activities of **1** as that of standard used. These behavioral antinociceptive activities were further confirmed by investigating the key mediators of these processes such as cytokines, iNOS, COX-2 and oxidative stress in experimental animal tissues.

The chemical mediators secreted all over the inflammatory process sensitizes the nociceptors that produce inflammatory pain resulting in hyperalgesia and allodynia [17]. Inflammatory cytokines have a considerable role in nerves damage [1] which are implicated in the sensitization of the nociceptive pathway [9], especially in painful neuropathy. PTX has been revealed to induce proinflammatory cytokines expression such as IL-1 and TNF-α etc. in rat's spinal cord [63]. In the present study, PTX treatment considerably raised the expression of inflammatory cytokines in spinal tissues which were observed to be remarkably reduced in **1** treated group (Fig. 6). Similarly, NO and PGE2 (Prostaglandin E2) production are induced by iNOS and COX-2 which enhanced the performance of nociceptive stimuli resulting in inflammatory pain [64]. Further, NO and PGE2 possess an imperative role in the activation of macrophages that are linked with both acute and chronic inflammation [65, 66]. Therefore, the iNOS and COX-2 expression levels were also evaluated in PTX-induced rats' spinal tissues. The present study showed that **1** significantly suppressed iNOS and COX-2 expressions as well as NO production.

Moreover, reactive oxygen species (ROS) are accelerated by distorted stimulation of phagocytes and neutrophils [51, 67]. It is well known that oxidative stress plays a primary part in chronic inflammation and pain [68]. In the present study, PTX repeated administration significantly lowered the level of endogenous antioxidants i.e., GSH, GST and catalase, respectively. The data obtained reveals that **1** exhibited protective role in PTX-induced oxidative stress model by enhancing the level of antioxidants in spinal tissues while decreasing the MDA level. Furthermore, **1** chronic use did not show any deteriorating effect on muscle strength. Likewise, the pharmacokinetic analysis using pK-CSM software showed that **1** exhibit high GIT absorption, BBB permeability, and interaction with the cytochrome p450 system, and partial risk of toxicity.

Conclusion

In summary, **1** was evaluated for neuroprotective activity in a well-known classical PTX-induced neuropathic pain model. The study revealed that the compound possesses significant antiallodynic and antihyperalgesic activities at the behavioral level which was further supported by *in vitro* assays. Therapeutic effect of **1** appears to be due to inhibiting the induction of proinflammatory cytokines, iNOS, COX-2 as well as attenuating the oxidative stress. The pharmacokinetic analysis showed that 1 exhibit good ADME properties. More investigation is still needed to simplify the comprehensive mechanism. We suggest from the present study that **1** could be developed for the management of chronic pain and might be a useful candidate for the treatment of chronic neuropathic pain.

Declarations

Funding

None

Conflicts of interest

The authors have no conflict of interest.

Ethics 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 to participate

N/A

Consent for publication

N/A

Availability of data and material

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

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. MN, AK, BS, AUK, and SK analyzed the data and drafted the manuscript. SK supervised the project. All authors read and approved the final manuscript.

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Tables

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'

Table. 2: Effect of Compound-**1** on antioxidant enzymes, LPO and NO production in PTX-induced rat's spinal tissue

Parameters	Normal Control	PTX	GBP (75mg/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.

Table. 3. Pharmacokinetic and toxicokinetic analysis of the **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 Numeric (log PS)	VDss log L/kg	CYP 2D6 3A4 1A2 2C19 2C9 2D6 3A4 Substrate Inhibitor Categorical (Yes/No)	Numeric (log ml/min/
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

Figures

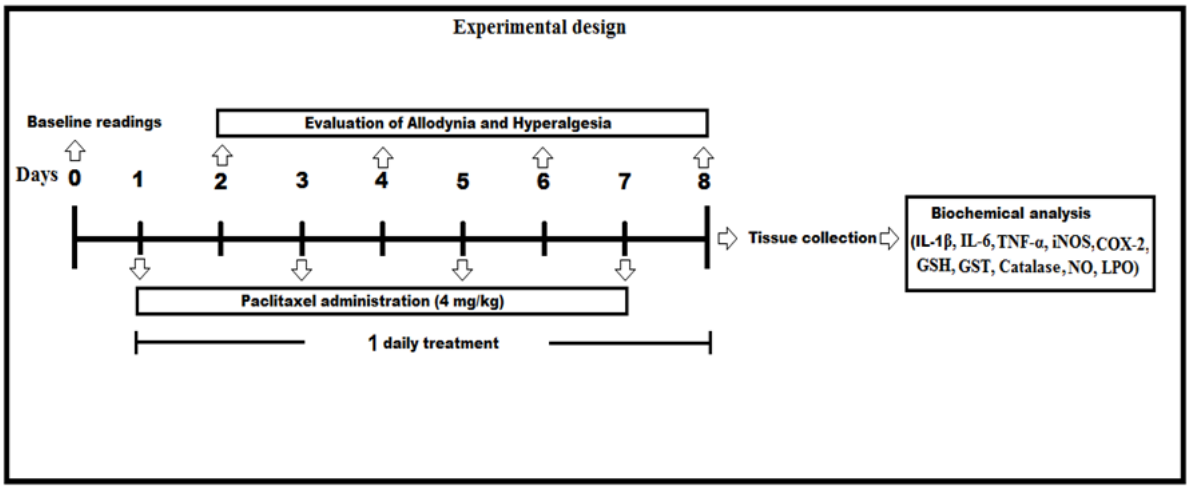

Figure 1

A schematic representation of overall study plan

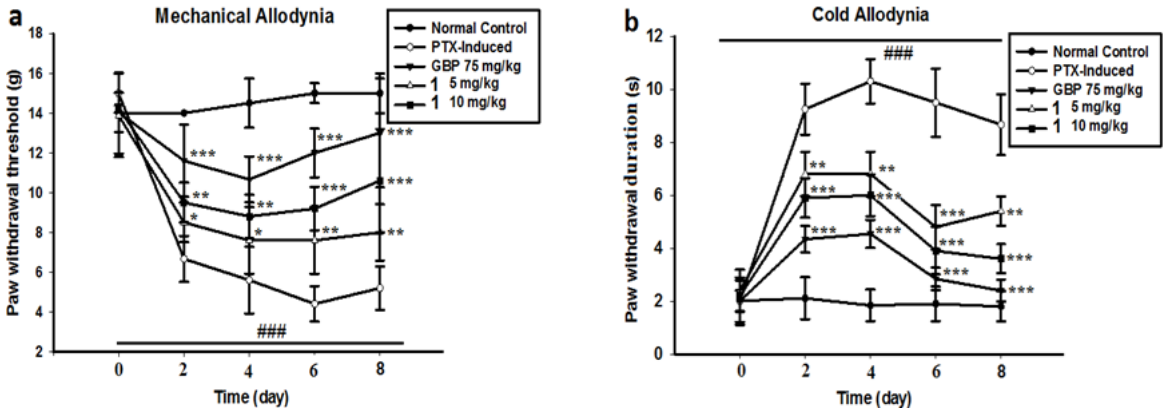

Figure 2

Dose-dependent effect of Compound-1 in doses of 5 and 10mg/kg in PTX-induced rats. Compound-1 pretretment inhibited (a) mechanical allodynia and (b) cold allodynia. The paw withdrawl responses were recorded pre-and post PTX injection at 0, 2, 4, 6 and 8th day one hour after treatment as discussed in Materials and Methods. The data is displayed as the mean (n=6) ± SD. ANOVA followed by post hoc bonferroni test was applied for comparing statistical differences between groups. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represents a statistically significant difference from PTX-induced group. (###) indicates comparison to normal control group

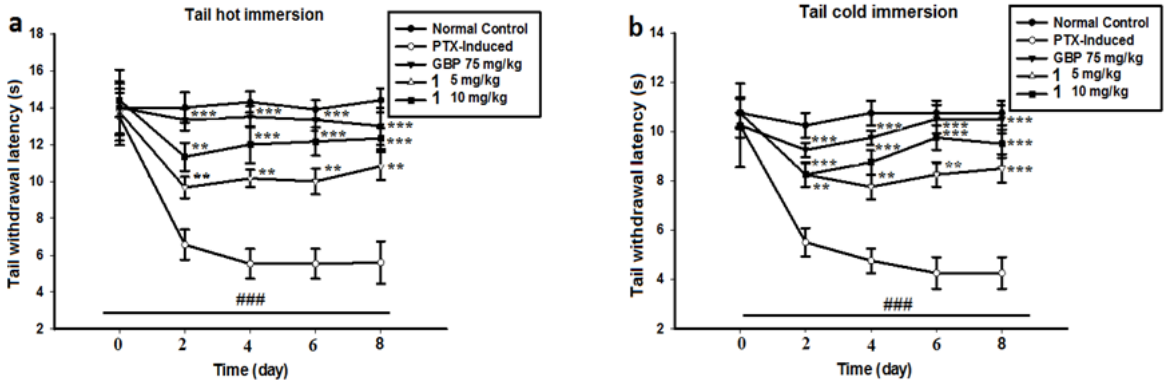

Figure 3

Treatment with Compound-1 inhibited (a) PTX-induced tail thermal hyperalgesia and (b) PTX-induced tail cold hyperalgesia. The data is displayed as the mean (n=6) ± SD. ANOVA followed by post hoc bonferroni test was applied for comparing statistical differences between groups. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represents a statistically significant difference from PTX-induced group. (###) indicates comparison to normal control group

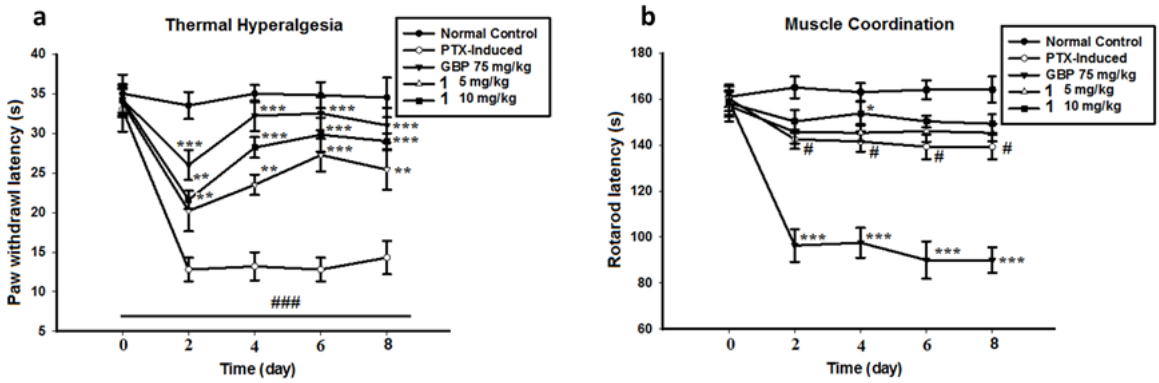

Figure 4

Inhibitory effect of Compound-1 treatment on thermal hyperalgesia in PTX-induced rats (a). Effect of Compound-1 on motor activity of rats by using rotarod (b). The data is displayed as the mean (n=6) ± SD. ANOVA followed by post hoc bonferroni test was applied for comparing statistical differences between groups. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represents a statistically significant difference from PTX-induced group. (###) indicates comparison to normal control group

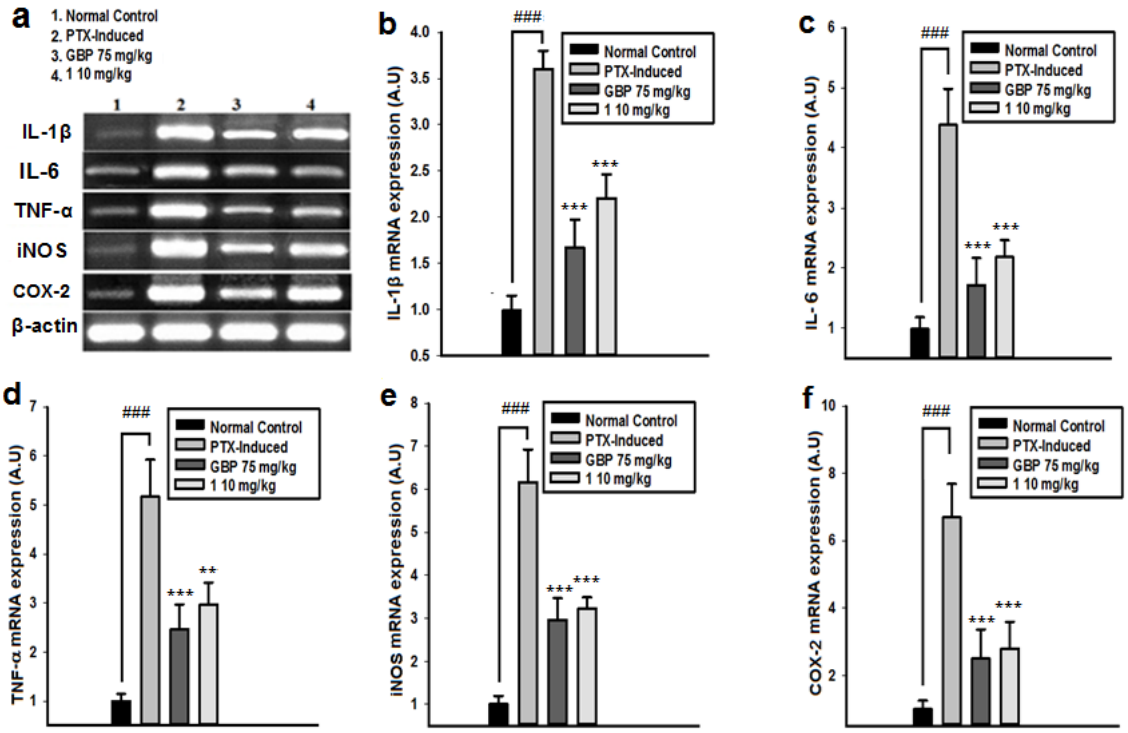

Figure 5

(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 relative arbitrary unit (A.U). The data is displayed as the mean (n=6) ± SD. ANOVA followed by post hoc bonferroni test was applied for comparing statistical differences between groups. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represents a statistically significant difference from PTX-induced group. (###) indicates comparison to normal control group

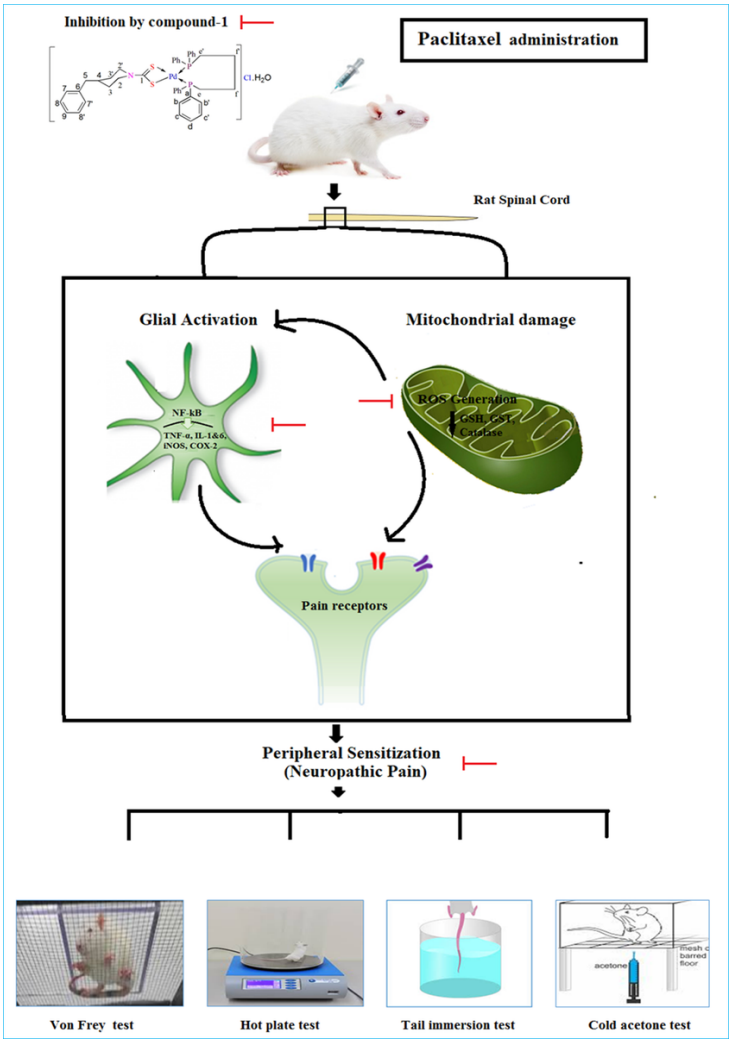

Figure 6

Graphical abstract representing anti-neuropathic pain activity of 1 in PTX-induced neuropathic pain model