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Pharmacological and computational evaluation of an isoxazolone derivative for mitigating cisplatin-induced neuropathic pain

Abdul Nasir [a,b,1], Abdul Waheed Khan [c,1], Hamid Rafiq Khattak [d], Abdus Samad [e], Sami Ullah [e], Haroon Khan [f], Muhammad Faheem [g,*], Qian Bai [a,b,*]

[a] Department of Anesthesiology, Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[b] Medical Research Center, Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[c] Department of Molecular Science and Technology, Ajou University, Suwon 16499, Korea
[d] Health Department, Government of Khyber Pakhtunkhwa, Pakistan
[e] Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan
[f] Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan
[g] Department of Basic Medical Sciences, Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan

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ABSTRACT

Cisplatin-induced neuropathic pain poses a significant clinical challenge. This study induced neuropathy via cisplatin treatment, measuring pain levels through mechanical threshold and thermal escape latency. Isoxazolone derivatives were administered at doses of 20, 40, and 60 mg/kg to evaluate their effects on allodynia and pregabalin, a known therapeutic agent, was used for comparison. Place preference tests were conducted to gauge responses, while molecular docking and dynamic simulations assessed the binding affinity for relevant receptors. Results showed that cisplatin-induced neuropathy by day four, with reduced mechanical threshold and sustained thermal escape latency for eight days. Isoxazolone derivatives at 20 mg/kg and 40 mg/kg effectively reversed allodynia, while 60 mg/kg showed limited impact. Pregabalin exhibited similar effects, though less significance after day 10. Place preference tests demonstrated positive responses to both isoxazolone derivatives and pregabalin in allodynic rats. Molecular docking analysis highlighted strong binding to receptors, particularly cyclooxygenase-2 (Cox-2), Tumor Necrosis Factor (TNF)-α. Binding free energies indicated potent inhibitory activity of isoxazolone derivatives against Cox-2 and TNF-α. This study underscores the potential of isoxazolone derivatives as therapeutic agents for cisplatin-induced mechanical allodynia, with innovative implication for neuropathic pain treatment. Further research and validation are necessary to confirm their therapeutic efficacy and safety for human clinical use.

1. Introduction

Neuropathic pain is caused by the dysfunction or impairment of the nervous system, caused by a variety of factors, including cancer and its treatments (Fallon, 2013, Kouri et al., 2022, Shkodra and Caraceni, 2022). Notably, chemotherapy medications administered to cancer patients can play a role in the development of neuropathic pain (Maihöfner et al., 2021, Dehnoee et al., 2023). Cancer remains a significant global health concern, ranked the second leading cause of mortality worldwide (Siegel et al., 2023). One of the significant challenges in cancer treatment is the development of chemotherapy-induced peripheral neuropathy (CIPN) (Desforges et al., 2022, Mezzanotte et al., 2022). Notably, taxanes, platinum-based medicines (such as cisplatin), vinca alkaloids, epothilones, and even some novel drugs like lenolidamide and bortezomib have been associated with CIPN (Wolf et al., 2008, Hershman et al., 2014, Nasir et al., 2021, Li et al., 2023). CIPN is characterized by sensory and painful neuropathies, and platinum compounds have been particularly linked to the induction of neuropathy (Hu et al., 2019, Zong et al., 2022). Evidence suggests that rather than direct DNA damage, these compounds disrupt axoplasmic transport and cellular metabolism, culminating in neuronal damage and dysfunction (Lloyd, 2012). The pathogenesis and prognosis of CIPN and other neuropathic

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* Corresponding authors at: Department of Anesthesiology, Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China (Qian Bai).
E-mail addresses: fahimafridi345@gmail.com (M. Faheem), bai_qian@zzu.edu.cn (Q. Bai).
[1] Both authors contributed equally.

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disorders involve various factors, including mitochondrial dysfunction, disruption of calcium signaling pathways, and the generation of reactive oxygen species (ROS) (Jin et al., 2008, Waseem et al., 2018). Additionally, neuro-immune regulatory mechanisms and the activation of inflammatory cascades, along with elevated proinflammatory cytokines, play crucial roles in neuropathic pain development (Uhelski et al., 2021, Mohaghegh et al., 2023). The release of proinflammatory cytokines such as CCL2, IL-6, IL-1, and TNF-α from activated langerhans cells in the skin has been associated with sensory nerve terminal degeneration, further contributing to painful neuropathy (Uhelski et al., 2021, Bai et al., 2023). Current therapies for CIPN, including pregabalin, xaliproden, oxcarbazepine, glutathione, N-acetylcysteine, vitamin E, and glutamine, have shown some efficacy, but the treatment of established CIPN remains challenging (Han et al., 2018). Therefore, novel therapeutic agents with targeted mechanisms of action are needed to effectively alleviate CIPN and improve patients' quality of life.

Isoxazolone derivatives have drawn attention due to their reported analgesic properties and potential as antiandrogenic agents. These compounds have been utilized as herbicides and in the treatment of various diseases (Farahi et al., 2018, Zimecki et al., 2018). Interestingly, a novel isoxazolone derivative, 4-aryl-3-pyrimidinyl, and 4-aryl-3-pyridyl-based, has demonstrated the ability to inhibit the production of TNF-α upon exposure to lipopolysaccharide (Laughlin et al., 2005). Although their antihyperalgesic effects in peripheral neuropathic pain have not been explored, we speculate that they might exhibit activity in the context of neuropathic pain when assessed in a cisplatin-induced neuropathic pain paradigm.

This study aims to explore the potential pharmacological and computational prospects of a specific isoxazolone derivative, 3-methyl-4-benzylidieneisoxazol-5-one (Fig. 1), in alleviating cisplatin-induced neuropathic pain. By employing a range of behavioral testing methods, we will investigate the effect of this compound on test animals. Additionally, based on its TNF-α inhibitory activity, we hypothesize that this isoxazolone derivative holds promise as a potential therapeutic candidate for the treatment of neuropathic pain.

2. Methods

2.1. Chemicals

Cisplatin and dimethyl sulfoxide (DMSO) were procured from CIPLA Co., Mumbai, India, and Sigma-Aldrich Co., LLC, United States. Pregabalin was sourced from Pfizer Pakistan Ltd, whereas Tween80 was obtained from Sigma Chemicals. The isoxazolone compound was obtained from the Department of Pharmaceutical Chemistry at Riphah International University, Islamabad, Pakistan. The dosage was made using either sterile saline or saline containing DMSO and Tween 80.

2.2. Animal preparation

The Sprague-Dawley male rats, aged 7–8 weeks and weighing 200–220 g, were housed standard cages with a 12-hour light/dark cycle at 23 ± 1 °C and fed with a standard laboratory diet and tap water *ad libitum* in the animal husbandry of the Riphah Institute of Pharmaceutical Sciences, Pakistan. Before the experimentation, the rats were acclimatized for 7 days. All procedures approved by Riphah Institute of Pharmaceutical Sciences (RIPS) ethical committee (Reference No: REC/RIPS/2017/013), which was conducted in accordance with the Institute of Laboratory Animal Resources, Commission on life sciences, university, National research council (1996) laws.

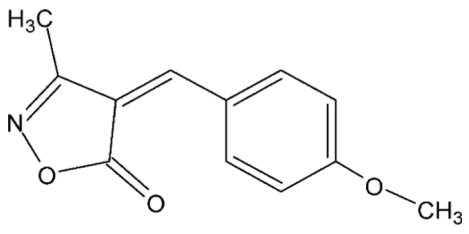

Fig. 1. 3-methyl-4-benzylidieneisoxazol-5-one.

2.3. Cisplatin-induced neuropathic pain and drug treatment

In order to produce chemotherapy-induced peripheral neuropathy (CIPN), a dosage of cisplatin at 3 mg/kg was administered to rats intraperitoneally (ip.) at weekly intervals on days 0, 7, 14, and 21. In order to prevent potential renal damage, a group of rats received a 2 mL injection of sterile saline solution five minutes prior to the administration of cisplatin. Each experiment was comprised of five animals in each group. The presence of mechanical allodynia and mechanical hyperalgesia in both hind paws confirmed the onset of peripheral neuropathy approximately 28 days after the initial administration of cisplatin. Three groups of rats, who had previously been treated with cisplatin, were given oral doses of an isoxazolone derivative at three different dosage levels (20, 40, and 60 mg/kg) once daily for a duration of 14 consecutive days. The treatment started on day 28 and continued until day 42, inclusive. Following the administration of a daily regimen of an isoxazolone derivative, assessments were conducted to evaluate the presence of mechanical allodynia and thermal hyperalgesia (Han et al., 2018).

2.4. Behavioral test

2.4.1. Thermal hyperalgesia

The assessment of thermal latency escape was conducted using the hot plate method, which was set at a temperature of 55 ± 5 °C. The occurrence of paw tissue injury was effectively mitigated by implementing a time limit of 50 s. The assessment of pain sensitivity in rats involved the observation of their responses to painful stimulus, including the speed of their jumping and the occurrence of licking behavior on their forepaws or rear paws. The thermal delay time (sec) of animals was assessed both prior to and subsequent to treatment with or without an isoxazolone derivative (Seto et al., 2017).

2.4.2. Mechanical allodynia

The assessment of mechanical allodynia was conducted via the Von Frey test, employing a series of Von Frey filaments with weights ranging from 4.96 to 6.65 g. The animals were placed in plastic cages on top of a raised wire mesh platform. They were allowed a minimum of 30 min to adapt to the experimental environment before any indications of exploratory behavior were noticed. The up and down method was employed, and the applied force was directed towards the central plantar area of the hind limbs in all of the conducted investigations (Guindon et al., 2014). Pre-experimental testing was conducted to obtain baseline responses. After the paw was removed, the mechanical stimulation was stopped. The pain threshold for paw withdrawal was measured thrice.

2.4.3. Conditioned place preference test

The isoxazolone compounds underwent testing in a conditioned place preference approach utilizing an apparatus consisting of three compartments labeled as A, B, and C. During the adaption phase (days 1 and 2), the rats were allowed unrestricted access to the facility for a duration of 30 min. The researchers monitored the duration of time the rats spent in each cage. The rats were housed in a single shunt compartment for a duration of 30 min on days 3 and 4, specifically in the morning, subsequent to the administration of a vehicle. In this experiment, rats were administered either an isoxazolone derivative or pregabalin, and then placed in a separate compartment for a duration of 30 min. On day five of the experiment, a period of 30 min was allocated for the animals to engage in unrestricted movement in either cage. Time

spent in each section was recorded. To calculate the outcome or demonstrate the effectiveness of the isoxazolone derivative, we subtract the mean time spent in each drug-related compartment on day 5 from the average time spent in corresponding compartments combined (Qu et al., 2011).

2.5. Determination of acute toxicity in an animal model

A group of rats (n = 3) was administered the isoxazolone derivative at a dosage of 100 mg/kg. Following the initial 24-hour period, observations were made regarding the behavior and well-being of the rats, specifically focusing on alterations in locomotion, grooming, feeding patterns, as well as indications of distress and mortality rates (Chinedu et al., 2013).

2.6. In silico study

2.6.1. Molecular docking

The molecular docking research of an isoxazolone derivative against targets associated with pain and inflammation was conducted using the dock module of the molecular operating environment. The crystallographic structure of the receptor for all target molecules was obtained from the Protein Data Bank (PDB). Before doing energy minimization, all water molecules were removed. Energy minimization was performed using the MMFF94s force field, with a convergence threshold set at 0.05 gradients (Yeye et al., 2020). The 3-dimensional structural coordinates of the isoxazolone derivative were generated, subjected to energy minimization, and subsequently stored within a newly created MOE database. To achieve the development of the low-energy ligand–protein complex, the flexibility of the ligand atoms was enabled throughout the docking process. The compound was ranked using the GBVI/WSA dG score function (Taha et al., 2016). In order to execute the interaction study of the ligand–protein complex, we utilized Pymol v.1.7.

2.6.2. Molecular dynamic simulation

Based on enhanced interaction and better dock score of isoxazolone derivative against the target receptors, molecular dynamic simulations study was carried out to further explore the mechanisms of interactions and the stability of these compounds at the binding pocket. The protein was parameterized using the FF19SB force field, and all ligands were parameterized using GAFF2 (Samad et al., 2023). After that, the model was solved using a TIP3 water cubic box with a 10 Angstrom box diameter. To balance the system, the LEAP module added counter ions (Na^+ and Cl^-) (Maffucci and Contini, 2013). In order to improve the systems, 500 steepest descent steps and 500 conjugate gradient steps were combined (Dickson et al., 2022). Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method. The temperature was controlled using the Langevin dynamics approach (Mahmood et al., 2023). The SHAKE method was used to restrict the hydrogen atom bonds (Feenstra et al., 1999). At 1 atm pressure, the systems were progressively heated from 0 to 310 Kelvin. Finally, a 200 ns MD simulation conducted in duplicate was carried out on the GPU using PMEMD's CUDA version for all equilibrated complex systems at constant temperature and pressure. Post md simulations analysis was carried out with the use of cpptraj module. Origin ProLab software was employed for data analysis and visualization.

2.6.3. MMGBSA calculations

Calculations of the binding free energy are crucial for understanding the interaction between protein and ligand. The binding free energy between the primary protease and phytochemical inhibitors was computed using the MMPBSA. Py script (Gul et al., 2021). The free energy of each energy term was calculated using the equation below.

$$\Delta G_{bind} = \Delta G_{bind} - [\Delta G_{receptor} + \Delta G_{ligand}]$$

In the equation, G_{bind} stands for total binding free energy, $G_{complex}$ for complex free energy, $G_{receptor}$ for receptor protein free energy, and G_{ligand} for ligand free energy.

2.7. Statistical analysis

The data collected in this study were presented as mean ± SEM and obtained randomly. The data were statistically analyzed with ANOVA with repeated measures. The post hoc Tukey method was employed for pairwise comparisons when ANOVA showed significant differences. Statistically significant was considered when $p < 0.05$. GraphPad Prism 8.0 was used to analyze data.

3. Results

3.1. Behavioral tests

3.1.1. Cisplatin-induced mechanical allodynia

The mechanical threshold showed a decrease by day 4 of the cisplatin treatment in comparison to the baseline of the study ($P < 0.05$). Furthermore, this decrease was even more pronounced by day 8 ($P < 0.001$) and persisted for a duration of two weeks. All of the animals with allodynia displayed symmetrical ambulation, without any motor dysfunction or impairment in positioning or stepping response. The interval of thermal escape latency time exhibited a notable reduction subsequent to a regimen of cisplatin therapy, and this decrease remained persistent for a period of 8 days following exposure to cisplatin (Fig. 2).

3.1.2. Mechanical hyperalgesia

The onset of substantial mechanical hyperalgesia ($P < 0.001$) was seen on the sixth day of cisplatin administration compared to the saline group. This hyperalgesia persisted until the last day of the study. The isoxazolone derivatives demonstrated a significant increase in the paw withdrawal threshold in rats with established allodynia, specifically at doses of 20 mg/kg and 40 mg/kg ($P < 0.001$). This effect was observed approximately 8–14 days after the initiation of cisplatin treatment. However, there was a temporary decline in the paw withdrawal threshold after day 10 of the treatment phase. Notably, the dose of 60 mg/kg did not cause any significant alteration in the paw withdrawal threshold in comparison to lower doses ($P < 0.01$). Pregabalin exhibited a reversal of allodynia; yet, the observed impact was comparatively less pronounced in comparison to the test drugs by day 10 (Fig. 3).

3.1.3. Thermal hyperalgesia

The study observed a statistically significant increase in thermal

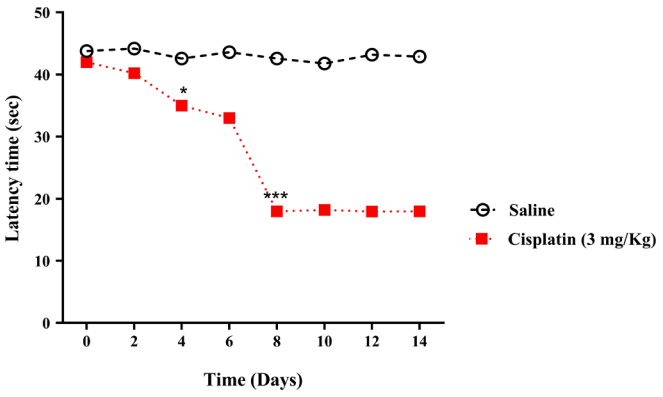

Fig. 2. Graph shows the thermal latency time in the cisplatin-treated group on varying days of cisplatin treatment. Data depicted as ± SEM (n = 5). A one-way ANOVA followed by Tukey's post-hoc test was utilized. ***$p < 0.001$, *$p < 0.05$ shows significancy in difference between the latency time of groups treated with saline and cisplatin.

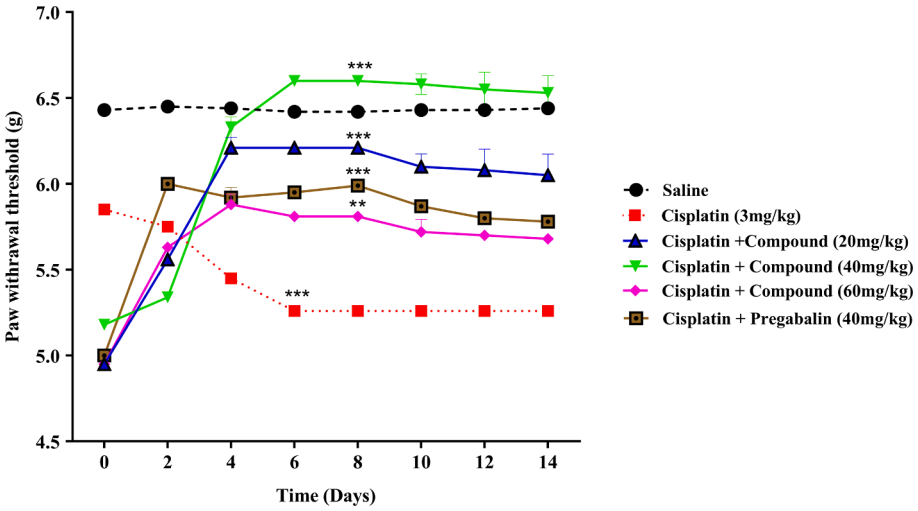

Fig. 3. Graph shows the paw withdrawal thresholds of control group, cisplatin-treated group, isoxazolone-derivative (20, 40, and 60 mg/kg doses), and pregabalin (40 mg/kg) treated group against cisplatin-induced peripheral neuropathy. Data is given as mean SEM (n = 5). A one-way ANOVA and Tukey's post hoc test were used for analysis. Paw withdrawal threshold comparisons between the isoxazolone derivative and pregabalin treated groups and the cisplatin group were statistically significant (***'$P < 0.001$ and **'$P < 0.01$).

hyperalgesia ($P < 0.001$) on day 4 after cisplatin administration compared to the saline group that was persisted until the last day of the study. In contrast to the observed changes in the paw withdrawal threshold, the thermal escape latency time did not exhibit significant alterations following the initiation of the treatment phase. However, it is worth noting that the test compound administered at a dosage of 40 mg/kg induced a significant delay in latency time ($P < 0.001$). At 20 mg/Kg, compound effect was non-significant while at 60 mg/Kg, it caused a little delay in the latency time ($P < 0.05$). The pregabalin at 40 mg/Kg, also showed a significant delay in latency time ($P < 0.001$). Nevertheless, this delay was not enduring, as evidenced by a subsequent decline in latency time after day 6 of the treatment phase. which was - then persisted in the later stages of the study (Fig. 4).

3.1.4. Conditioned place preference

There was no discernible variation in the duration required for preconditioning between the saline and pregabalin groups, as well as the groups connected with the isoxazolone derivative chambers. The study initially implemented a two-day adaptation phase protocol, succeeded by a two-day treatment pairing protocol, and subsequently followed by a one-day test plan. In accordance with the experimental protocol, the administration of pregabalin at a dosage of 40 mg/kg resulted in the development of place preference in rats exhibiting allodynia, as opposed to rats treated with saline. The results of the drug coupling pattern experiment conducted over a two-day period revealed a significant place preference in the hyperalgesic state when pregabalin was administered. Conversely, the group treated with saline did not exhibit any place preference, indicating a lack of positive reinforcement in the untreated rats and their indifference towards the compartment associated with saline. At a dosage of 40 mg/kg, an isoxazolone derivative exhibited a significant preference for the compartment linked with the drug. However, 20 mg/Kg and 60 mg/kg, no significant place preference was detected (Fig. 5).

3.2. Acute toxicity

There was no evidence of any significant changes in locomotion, grooming, and feeding patterns of the animals that were treated with high doses of test drugs. Also, no mortality and morbidity indicators were found in any animal.

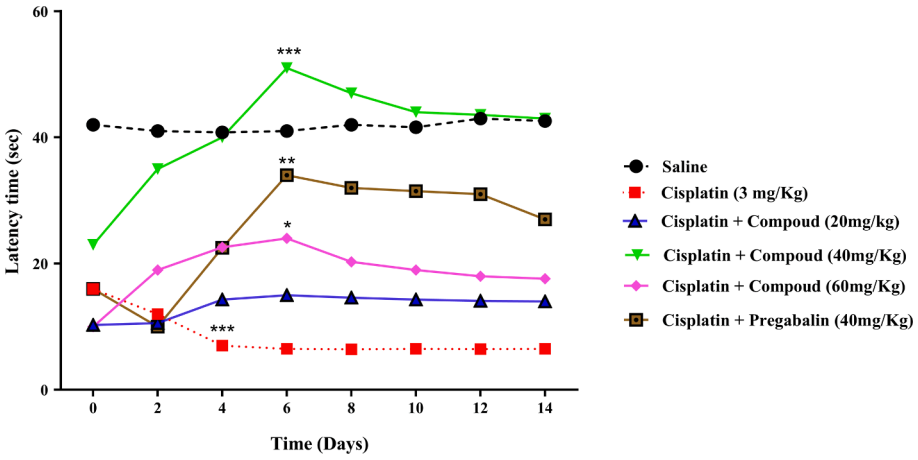

Fig. 4. Graph shows the thermal latency time of control group, cisplatin-treated group, isoxazolone-derivative (20, 40, and 60 mg/kg doses), and pregabalin (40 mg/kg) treated group against cisplatin-induced peripheral neuropathy. Data is given as mean SEM (n = 5). A one-way ANOVA and Tukey's post hoc test were used for analysis. The latency time comparisons between the isoxazolone derivative and pregabalin treated groups and the cisplatin group were statistically significant (***'$P < 0.001$, **'$P < 0.01$ and *'$P < 0.05$).

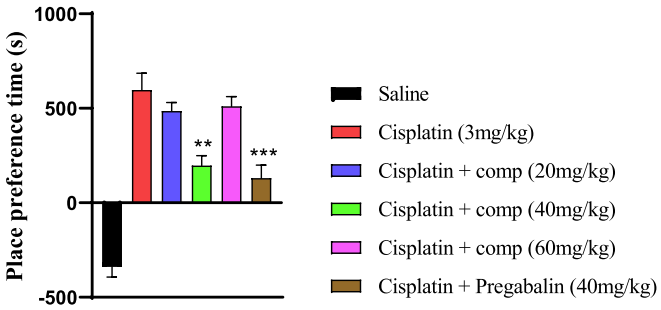

Fig. 5. Graph shows the effect of isoxazolone derivative on cisplatin-induced hyperalgesia by performing condition place preference test. Data is given as mean SEM (n = 5). A one-way ANOVA and Tukey's post hoc test were used for analysis. ***'$P < 0.001$, and **'$P < 0.01$ exhibit the significant difference in condition place preference between the tested compounds and saline.

3.3. *In silico study*

3.3.1. *Molecular docking analysis*

The isoxazolone derivative docking studies against all the target receptors showed a wide range of potential interactions with the binding site residues (Fig. 6**A-I**). The interaction details and docking score are enlisted in Table 1. Among all the target receptor, isoxazolone derivative showed better binding affinity for Cox-2, forming three hydrogen bond acceptor interactions and one H-pi with the key binding site residues (His386, Trp387, Leu390, Leu391), having docking score of −6.68 kcal/mol. Followed by TNF-α which has the docking score of −6.25 kcal/mol, mediates three non-covalent interactions with isoxazolone derivative, the Ser60 of chain-A form hydrogen bond donor interaction while Ser60 of chain-B was found in the hydrogen bond donor interactions, and ring of Tyr119 mediates H-pi. Similarly, Mu-opioid, IL-6, Nicotinic α7, Delta-opioid, and NMDA form two hydrogen bonds with their key binding residues with the docking score of −5.92, −5.74, −5.69, −5.50, and −5.25 Respectively. NFkB and TLR4 share a single non bonded contact. Overall docking analysis demonstrates the significant inhibitory activity of isoxazolone derivative against all receptors.

3.3.2. *Dynamic stability and residual flexibility of COX-2 and TNF-α upon binding of isoxazolone derivative*

To determine structural stability, dynamic stability assessment is essential. Root means square deviation (RMSD) was used to evaluate the stability of each system. The pharmacological potential of a chemical is estimated based on the dynamic stability of a ligand-bound system. A smaller deviation experienced by a system during its period of simulation indicates a stable complex formation and vice versa. We also estimated RMSD for each complex since it is crucial to comprehend how such complexes behave. Since understanding how these complexes behave is essential, we also computed RMSD for each complex as shown in Fig. 7A-B. We analyzed the thermodynamic stability index for isoxazolone derivative bound with COX-2, the mean RMSD value was found to be 2.14 Å, with a standard deviation of 0.34 Å. A minor divergent from its mean position was observed at 60 and 190 ns as shown in Fig. 7A. For TNF-α in complex with isoxazolone derivative, the averaged RMSD value was found to be 2.46 Å, initially a slight raised in the RMSD curved was observed up to 10 ns, afterward the system was converged up to 100 ns, minor fluctuation and raised was experienced up to 160 ns followed by the convergence of the system with a standard deviations of 0.21 Å. Overall, our RMSD data show that upon binding of isoxazolone derivative with COX-2 and TNF-α contributed to the stability of System.

Residue flexibility offers vital details on molecular patterns of interaction, inter-residue communication, and inhibitory potential.

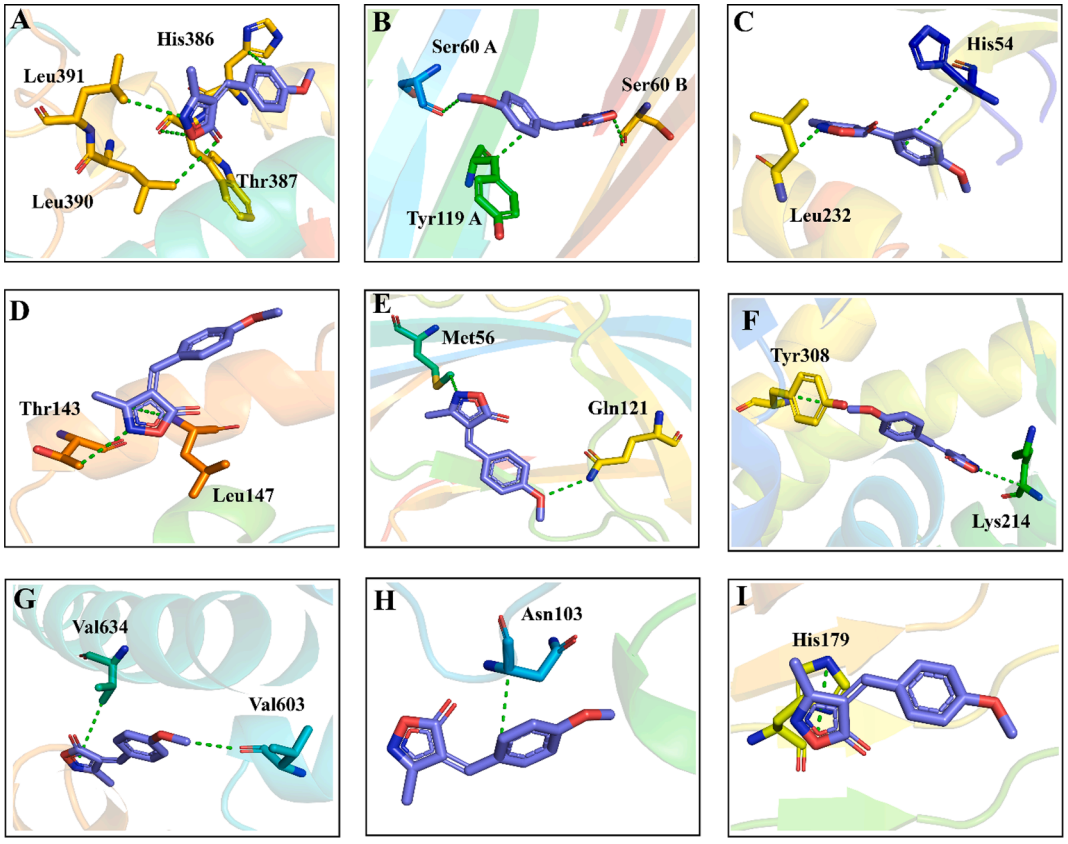

Fig. 6. Represent the 3D interactions pattern of all target receptors with isoxazolone derivative. (A) indicates COX-2, (B) for TNF-α (C) for Mu-opioid, (D) for IL-6 (E) for Nicotinic α7 (F) for Delta-opioid (G) for NMDA (H) for NFKB, and (I) for TLR4 in complex with isoxazolone derivative.

Table 1
Represent the interactions details, PDB IDs and docking scores for all the receptors.

Target protein	PDB IDs	Compound	Interaction type	Interacting residues	Distance	Energy (Kcal/mol)	Docking score
COX-ll	5F1A	isoxazolone derivative	H-acceptor	Leu391	3.49	−0.3	−6.68
			H-acceptor	Leu390	3.70	−0.5	
			H-acceptor	Trp387	3.64	−0.4	
			H-pi	His386	4.03	−0.2	
TNF-α	2AZ5	isoxazolone derivative	H-donor	Ser60	3.41	−0.1	−6.25
			H-acceptor	Ser60	3.67	−0.3	
			pi-H	Tyr119	3.49	−0.6	
Mu-opioid	5C1M	isoxazolone derivative	H-acceptor	Leu232	3.83	−0.2	−5.92
			pi-H	His54	4.53	−0.4	
IL-6	1P9M	isoxazolone derivative	H-acceptor	Thr143	3.70	−0.1	−5.74
			pi-H	Leu147	4.32	−0.4	
Nicotinic α7	3SQ9	isoxazolone derivative	H-acceptor	Gln121	4.18	−0.2	−5.69
			pi-H	Met56	4.03	−0.5	
Delta-opioid	4EJ4	isoxazolone derivative	H-acceptor	Lys214	3.62	−0.2	−5.50
			H-pi	Tyr308	3.79	−0.5	
NMDA	5UN1	isoxazolone derivative	H-donor	Val603	3.25	−0.5	−5.25
			pi-H	Val634	4.07	−0.4	
NFkB	1NFK	isoxazolone derivative	pi-H	Asn103	3.93	−0.7	−4.92
TLR4	2Z65	isoxazolone derivative	pi-pi	His179	3.34	−0.0	−4.83

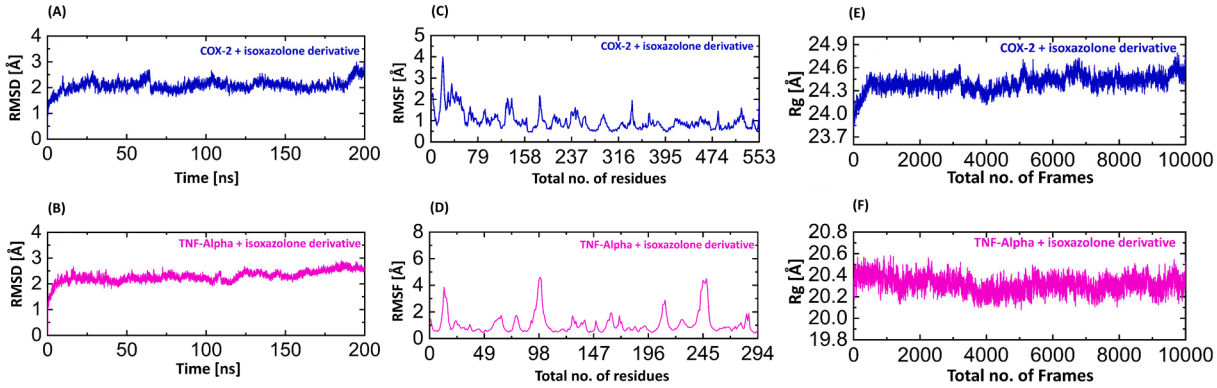

Fig. 7. RMSD, RMSF and Rg plots of isoxazolone derivative bound to COX-2 and TNF-α.

Fig. 7C-D represents the flexibility of COX-2 bound to isoxazolone derivative (blue line), indicating that binding residues have lesser fluctuation, while the loop region has relative high fluctuations i.e., 25–30, 85–95 and 176–185. For TNF-α the most fluctuating regions were found to 15–24, 95–110 and 240–245. While binding interface has slightly reduced RMSF values indicating a stable complex formation.

The radius of gyration is helpful for evaluating the protein's compactness and folding. Less compactness (more unfolded) is indicated by higher Rg values, while greater structural compactness is indicated by lower Rg values. The Rg value of the COX-2 in complex with isoxazolone derivative was found to be 17.8A during the 200 ns MD simulation as shown in Fig. 7E-F. While for TNF-α bound to isoxazolone derivative has Average Rg value of 20.3 Å. Both systems have significantly converged Rg curved which further validates the finding of our previous analysis.

3.3.3. MMGBSA analysis

The MD simulation trajectory protein–ligand complexes were used to compute the energy parameters to evaluate the energetics of COX-2 and TNF-α to the ligands. Using the MM-GBSA approach, the binding free energies of each system were determined. The calculated average binding free energies and particular energetic contribution elements of the last 500 frames are shown in Table 2. For COX-2 the total binding (ΔG) was found to be −45.37 kcal/mol. while the van der Waals and electrostatic energies was observed to be −48.39 and −0.21 kcal/mol indicating the higher binding affinity for isoxazolone derivative. Next, we analyze the binding energies of TNF-α in complex with isoxazolone derivative, for this type of system the total binding energy was found to be −29.11 kcal/mol. Our findings have demonstrated that the isoxazolone derivative potent inhibitors of COX-2 and TNF-α.

4. Discussion

Chemotherapy-induced peripheral neuropathy (CINP) is a noteworthy neurological complication resulting from the administration of cisplatin, a widely used platinum-based medication employed in the management of lung, ovarian, and testicular cancers. This condition is often accompanied by the distressing symptom of neuropathic pain, which can significantly impact patients' overall well-being and quality of life (Kerckhove et al., 2017).

Table 2
The MMPBSA binding free energy (kcal/mol) calculation for isoxazolone derivative in complex with Cox-2 and TNF-α.

S.no	Protein	Compound	ΔE_{vdW}	ΔE_{ele}	ΔE_{GB}	ΔE_{Surf}	ΔG_{total}
1	COX-2	isoxazolone derivative	−48.4 ± 0.3	−0.2 ± 0.8	8.8 ± 0.2	−5.6 ± 0.1	−45.4 ± 0.1
2	TNFα	isoxazolone derivative	−36.5 ± 0.6	−23.3 ± 0.7	34.4 ± 0.3	−3.7 ± 0.1	−29.1 ± 0.1

vdW = van der Waals energy, ele = electrostatic energy, Surf = surface areas energy, GB = the electrostatic contribution to the solvation-free energy calculated by GB.

The results of the study showed that cisplatin treatment in animals led to the development of mechanical allodynia and increased sensitivity to thermal stimuli, which are consistent with neuropathic pain. These effects became more pronounced with time and persisted for an extended period. Importantly, despite the heightened sensitivity to mechanical and thermal stimuli, the animals maintained normal ambulation, indicating that their motor functions were not impaired.

According to the findings of current research, giving cisplatin over a period of two weeks causes considerable hyperalgesia, which can be efficiently treated with pregabalin as well as isoxazolone derivatives. The effectiveness of these was found to be highest at a dose of 40 mg/Kg. The antiallodynic action of isoxazolone derivatives at the dose of 40 mg/Kg is particularly remarkable. This effect highlights the positive reinforcement potential of the compound, which results in conditioned location preference in rats that have been treated with cisplatin.

Cisplatin-induced neuropathy is a source of considerable distress for patients and frequently serves as a dose-limiting adverse effect that affects their ability to engage in routine daily activities. Patients commonly exhibit heightened tactile sensitivity, abnormal sensations such as tingling, and unpleasant sensations in the afflicted regions, especially the limbs (Garcia et al., 2008). The study found that significant mechanical hyperalgesia was observed on the sixth day of cisplatin administration when compared to a saline group ($P < 0.001$). The study evaluated the impact of isoxazolone derivatives on rats with established allodynia in Fig. 3. The isoxazolone derivatives demonstrated a significant increase in the paw withdrawal threshold in these rats, and reduced the heightened sensitivity to mechanical stimuli, alleviating hyperalgesia. This effect was particularly prominent when the derivatives were administered at doses of 20 mg/kg and 40 mg/kg ($P < 0.05$). It became noticeable approximately 8–14 days after the initiation of cisplatin treatment, indicating a delayed but effective response to the treatment. However, there was a temporary decline in the paw withdrawal threshold after day 10 of the treatment phase, suggesting that the efficacy of the derivatives diminished after this point. Notably, the highest dose of 60 mg/kg did not produce any significant alteration in the paw withdrawal threshold ($P < 0.05$). Pregabalin, a standard treatment for neuropathic pain, also reverses allodynia, though its efficacy is comparatively less pronounced when compared to the isoxazolone derivatives by day 10 as observed in previous studies (Kremer et al., 2016, Iseppon et al., 2023). These findings suggest that isoxazolone derivatives may offer a more potent and sustained relief from cisplatin-induced hyperalgesia compared to pregabalin.

Several previous studies have showed the effect of isoxazole derivatives in neuropathic pain(hi Cho et al., 2015, Alam et al., 2023). The study observed a statistically significant increase in thermal hyperalgesia on day 4 after cisplatin administration compared to a saline group ($P < 0.001$) as shown in Fig. 4. This indicates a rapid development of increased sensitivity to heat in the cisplatin-treated rats. Importantly, this heightened thermal sensitivity persisted until the last day of the study, suggesting that cisplatin-induced thermal hyperalgesia is a long-lasting effect. A test compound administered at a dosage of 40 mg/kg induced a significant delay in thermal escape latency time ($P < 0.001$). This suggests that this specific compound had a notable effect in reducing thermal hyperalgesia by delaying the rats' response to thermal stimuli. At a dosage of 20 mg/kg, the test compound did not have a significant impact on thermal escape latency time. At a higher dosage of 60 mg/kg, the compound caused a slight delay in the latency time ($P < 0.05$), but it was less pronounced than the effect observed with the 40 mg/kg dosage. Pregabalin, administered at 40 mg/kg, also showed a significant delay in thermal escape latency time ($P < 0.001$). However, overall, the delay was not enduring, as there was a subsequent decline in latency time after day 6 of the treatment phase, which persisted in the later stages of the study.

Chemotherapeutic agents such as cisplatin have the potential to elicit alterations in both thermal and mechanical threshold assays. The effects of the test compounds in a standardized neuropathic pain model were revealed by CPP testing (Qu et al., 2011). This study explored the effects of isoxazolone derivatives, and pregabalin on the development of place preference in rats with allodynia, a condition characterized by heightened sensitivity to pain. At a dosage of 40 mg/kg, both test compound and pregabalin resulted in the development of place preference in rats exhibiting allodynia as shown in Fig: 5. This suggests that similar to pregabalin, the isoxazolone derivative at this specific dosage had positive reinforcing effects on rats with allodynia like previously shown by Asaoka et al., (Asaoka et al., 2018). However, at 20 mg/Kg, and 60 mg/kg, no prominent place preference was detected. This indicates that these doses of the isoxazolone derivative did not have the same reinforcing effects and did not create a significant preference for the drug-associated compartment.

This approach provides evidence that isoxazolone derivatives specifically affect discomfort, without any intrinsic reinforcing qualities. The results of our study provide valuable insights into the impact of isoxazolone derivatives and pregabalin on CPP formation. The effectiveness of the test compound and pregabalin in different models of nerve damage, place preference, and cisplatin-induced hyperalgesia corresponds to its influence on the Von Frey threshold as previously used (Han et al., 2018). The robust location preference was established through the dose-dependent reversal of tactile allodynia, with non-allodynic animals serving as important control subjects.

In acute toxicity test, after the administration of high dose, the animals were observed for 24 h for different adverse effects of the test compound. There was not any morbidity and mortality, or any significant changes in grooming and feeding pattern except slight changes in the locomotion and behavior of the rats.

Previous studies have revealed the role of TNF-α and COX-2 in the mechanism of neuropathic pain and their inhibition by different isoxazole derivatives have shown analgesic activities (Razzaq et al., 2022, Alam et al., 2023). In this study, the isoxazolone derivative, having similar properties to isoxazole, was subjected to molecular docking research in order to evaluate its interactions with various receptors. The results revealed that the derivative displayed notable affinity for Cox-2, as evidenced by the formation of hydrogen bonds with crucial residues. Furthermore, it demonstrated inhibitory effects on many receptors, including TNF-α, Mu-opioid, IL-6, Nicotinic α7, Delta-opioid, and NMDA. The stability investigation provided evidence of stable complex formation between COX-2 and TNF-α complexes. The MMGBSA research revealed that the isoxazolone derivative had significant inhibitory action against both COX-2 and TNF-α. Several compounds have identified to prevent cisplatin-induced neuropathic pain like Berberine at 60–120 mg/Kg OD for 2 weeks, and then every other for next 2 weeks in mice via Inhibition of NF-κB and p-ERK overexpression (Meng et al., 2022), Rapamycin at 3 mg/Kg every other day for 12 days in mice via Inhibition of caspase-3, and downregulation of $p21^{Cip1}$, p53, and PUMA proteins expression (Alotaibi et al., 2022), gabapentin at 75 mg/Kg OD i.p., and its topical gel 10 % w/w three times a day on hind paws for 28 days via increasing GABA level in rats (Shahid et al., 2019). Like the isoxazole derivatives, our test compound showed to inhibit the main pain causing factors i.e., COX-2, and TNF as shown in docking and simulation techniques. Which increases the importance of these novel derivatives to be studies in detail for chemotherapy induced neuropathic pain.

In summary, the results of our study indicate that the isoxazolone derivative exhibits promise as a therapeutic intervention that specifically targets the COX-2 and TNF-α receptors, with the aim of alleviating neuropathic pain generated by cisplatin. In future, nanoparticles approach may be applied for getting better results of isoxazolone derivative like recently used palladium, and iron nanoparticles green synthesis techniques (Bai et al., 2021, Shahriari et al., 2022). Nanoparticles have been found to possess the ability to boost the solubility and stability of phytochemicals, facilitate their absorption, shield them from premature destruction within the physiological environment, and extend their circulation duration.

5. Conclusions

The study emphasizes the noteworthy involvement of the inflammatory cytokine in the development and advancement of chemotherapy-induced neuropathy. We confirm that cisplatin administration triggers mechanical allodynia in rats. Our isoxazolone derivative, especially at 40 mg/kg, demonstrates a significant antiallodynic effect and promising potential as an effective analgesic. Docking analysis reveals strong binding affinity to COX-2 and TNF-α receptors, highlighting its multi-target potential. Dynamic stability and residue flexibility tests confirmed complex stability. The MMGBSA study indicates potent inhibitory activity against both COX-2 and TNF-α. Our finding suggests the isoxazolone derivative potential as a therapeutic agent for selectively targeting COX-2 and TNF-α receptors in cisplatin-induced neuropathic pain management. Further research and validation studies are necessary for potential clinical applications.

Ethics approval

The research study was approved by the Research and Ethical Committee at Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, and reference number is REC/RIPS/2017/013.

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Availability of data and materials

Data in this study is available from the corresponding author upon request.

CRediT authorship contribution statement

Abdul Nasir: Writing – review & editing, Writing – original draft, Conceptualization, Methodology, Investigation. **Abdul Waheed Khan:** Writing – review & editing, Writing – original draft, Conceptualization, Methodology, Investigation. **Hamid Rafiq Khattak:** Writing – original draft. **Abdus Samad:** Conceptualization, Methodology, Investigation. **Sami Ullah:** Writing – review & editing. **Haroon Khan:** . **Muhammad Faheem:** Writing – review & editing, Writing – original draft. **Qian Bai:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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