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In Vivo Antinociceptive, Muscle Relaxant, Sedative, and Molecular Docking Studies of Peshawaraquinone Isolated from *Fernandoa adenophylla* (Wall. ex G. Don) Steenis

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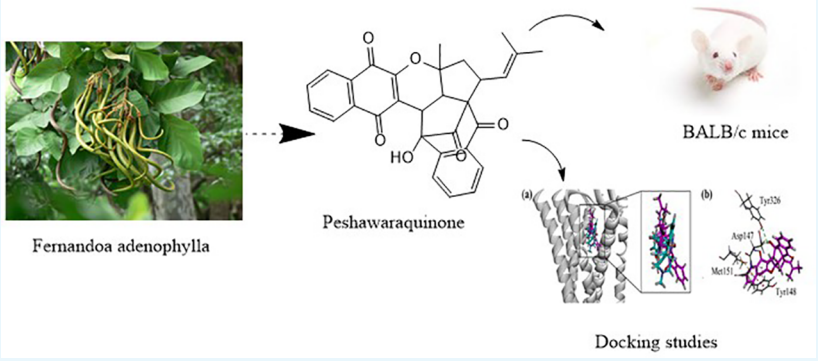

ABSTRACT: *Fernandoa adenophylla* (Wall. ex G. Don) Steenis is traditionally used to cure various diseases and can be included as an ingredient in massage oils, which are supposed to comfort muscular tension and pain. This study was designed to assess the antinociceptive, muscle relaxant, and molecular docking properties of a novel compound, namely, (5a*R*,5a1*R*,6*R*,7a*S*,14b*R*,15*R*)15-hydroxy-7a-methyl-6-(2-methylprop-1-en-1-yl)-7,7a,14b,15-tetrahydro-5*H*-t-5a,15methanobenzo[g]benzo[5,6]azuleno[1,8-*bc*]-chromene-5,9,14,16(5a1*H*,6*H*)- tetraone (peshawaraquinone), isolated from the methanolic extract of *F. adenophylla* in an animal model. The chemical structure of the isolated compound was elucidated using advanced spectroscopic techniques and further confirmed by XRD analysis. Compound **1** was tested against hot plate-induced noxious stimuli at various doses (2.5, 5, 10, and 15 mg/kg i.p.). The muscle relaxation potency of compound **1** was evaluated in the inclined and traction test, while the open-field test was used for the determination of sedative potential. The isolated compound was also subjected to acute toxicity analysis. The compound was then subjected to molecular docking analysis to determine the exact mechanism of action. Compound **1** demonstrated significant ($p < 0.05$) analgesic effect in a dose-dependent manner. A noticeable muscle relaxant effect was observed with the passage of time in both experimental models. The compound **1** showed a significant ($p < 0.05$) sedative effect, and in an acute toxicity study, the compound **1** was devoid of any noxious effects. The docking studies showed preferential affinity for μ-opioid and GABAA receptors. Hence, the prospective antinociceptive and muscle relaxant and sedative properties are probably mediated through these two target interactions.

1. INTRODUCTION

Medicinal plants are the main source of active phytochemicals that can be utilized for the production of modern medicines.[1] Natural product-derived drugs and phytomedicine are becoming popular compared to synthetic drugs because of their low cost and fewer side effects. Synthetic drugs not only have adverse side effects and are more costly but also promote bacterial pathogen resistance.[2] The discovery of crude drugs from medicinal plants is dynamic; therefore, ethnomedicinal studies play a key role in their development.[3] The folkloric usage of medicinal plants plays an important role in the development of the majority of drugs.[4] In this regard, biological and phytochemical screening as well as pharmacological screening assisted the drug industry.[5] Phytochemicals such as 8-hydroxyisodiospyrin, diospyrin, and pistagremic acid isolated from various plant species have been reported for analgesic, anti-inflammatory, and antimicrobial activities.[6−8]

Several plant species with medicinal importance have already been explored; however, there are many unexplored higher plant

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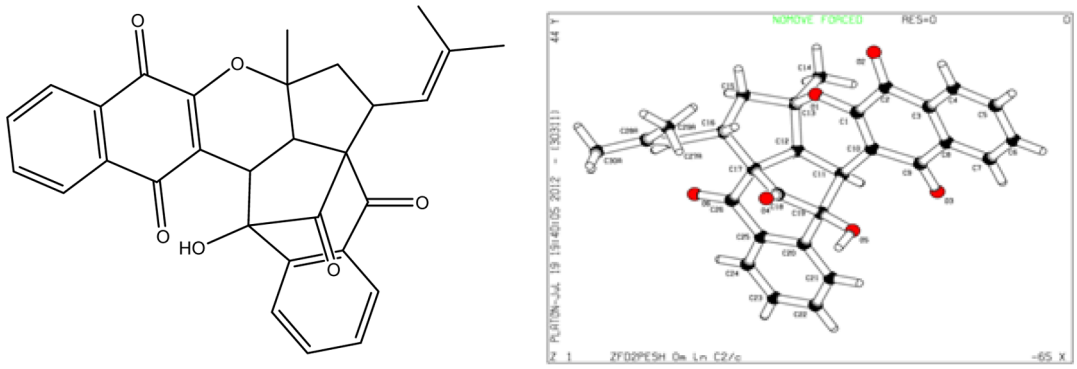

Figure 1. Chemical structure (a) and XRD image (b) of peshawaraquinone (**1**) isolated from the methanolic fraction of *F. adenophylla*.

species. In the search of such species, the present study was conducted, where *Fernandoa adenophylla* was explored scientifically. *F. adenophylla* is a member of the family *Bignoniacea*, which is dispersed in Africa and Southeast Asia.[9,10] This species is also known as Ziron, Dhopa-phali, Mostanphul, Karen wood, and Lotum-poh.[11] *F. adenophylla* has been used in the folkloric system for the treatment of constipation (Bangladesh), haemorrhoids (Bangladesh), and snake bites (India) as well as skin disorders in Thailand.[11] Traditionally, this plant is used by local people for the treatment of premature ejaculation, nocturnal emission, diabetes, amenorrhoea, microbial infections, sepsis, and other skin diseases.[12] The different extracts of *F. adenophylla* have been documented to have various pharmacological effects, including antihypertensive, leishmanicidal antifungal, antibacterial, and anti-TB properties.[13] Plants contain different phytochemicals, such as β-amyrin, lapachol, α-lapchone adenophylone, dehydro-α-lapchone, dehydro-iso-α-lapchone, dilapachone, tecomoquinone-I, and β-sitosterol.[6] All species of *Bignoniacea* contain lapachol in relatively large amounts. Lapachol and its derivatives have been reported to have a wide variety of biological activities, including anti-inflammatory, antimalarial, antiendemic, antiulcer, anticarcinogenic, antiabscess, viricidal, and termiticical activities.[13−15] The chemical constituents including peshawaraquinone, indanone derivatives, lapachol, and α-lapachol have been documented for phosphodiesterase 1 and anti-inflammatory properties. Based on medicinal importance, this study was designed to investigate the antinociceptive effects, muscle coordination, and molecular docking of peshawaraquinone isolated from *F. adenophylla*.

2. MATERIALS AND METHODS

2.1. Plant Material Collection. The root heartwood of *F. adenophylla* was obtained from various regions of the University of Peshawar, Khyber Pakhtunkhwa, Pakistan, in December 2012. The identification of plant specimens was performed by Dr. Muhammad Ilyas University of Swabi KP, Pakistan. The voucher specimen number UOS/Bot761 was kept at the Herbarium University of Swabi, KP, Pakistan.

2.2. Extraction, Fractionation, and Isolation. The collected root heartwood (5 kg) of *F. adenophylla* was kept in the shade until dried. The dried plant sample was ground and then subjected to cold extraction with methanol (20 L) for 2 weeks. The obtained methanolic extract (112.89 g) was subjected to successive fractionation using polar and nonpolar organic solvents in the order of increasing polarity as per standard procedures.[16,17] Upon fractionation and then successive concentration on a rotary evaporator, different fractions, including hexane (9.76 g), chloroform (27.32 g), ethyl acetate (21.11 g), and methanolic fractions (25.98 g), were obtained. Based on comparative TLC, the methanolic fraction (25.98 g) was subjected to silica gel chromatographic analysis using chloroform and methanol in various proportions, which afforded 114 fractions. These collected subfractions were compiled through TLC, which yielded seven subfractions. The subfractions were subjected to a normal-phase, repeated chromatographic technique using chloroform and methanol as a solvent system (7:3), which yielded crystalline compound **1** (90 mg; 99.87% pure). The purity of compound **1** was confirmed by TLC. The chemical structure of compound **1** (Figure 1) was identified by comparing the spectroscopic data with those of previously reported compounds.[9]

2.3. Animals. BALB/c mice of both sexes having weight 18−22 g (21 weeks old) were used for *in vivo* pharmacological evaluations as per standard methods.[18] Animals were fed standard laboratory food and water *ad libitum*. Before the experimental procedure, animals were acclimated under laboratory conditions (12/12 h dark and light and a temperature of 25 °C). All animals were examined physically for any health issues, and only healthy, that is, physically active, mice were included in the study. All the experimental procedures were presented and thoroughly approved by the ethical committee of the Department of Pharmacy UOS (Phrm323) University of Swabi KPK, Pakistan. The same animals were used throughout experimental procedures including acute toxicities.

2.4. Antinociceptive Activity. The hot-plate test was used for the evaluation of the tested compound following our published protocol.[19,20] Animals were categorized into different groups ($n = 6$), that is, the positive control, negative control, and test groups. A pretest was performed for the experimental animals on a hot plate (Harvard apparatus) maintained at 55 ± 1 °C. All animals that demonstrated a latency time greater than 15 s were excluded from the study. The positive group was treated with tramadol (10 mg/kg, i.p.), and the negative control group was injected with normal saline (10 mL/kg, i.p.). The tested groups (four) were administered peshawaraquinone 1 (2.5, 5, 10, and 15 mg/kg, PO). Thirty minutes after the above-mentioned administration, the animals were placed on the hot plate maintained at 55 ± 1 °C, and the latency time (time for which the mouse remained on the hot plate (55 ± 0.1 °C) without licking or flicking of the hind limb or jumping) was recorded in seconds. To prevent tissue harm, a cut-off time of 30 s was used for each animal. The latency time was noted for all groups at regular intervals, that is, after 30, 60, 90, and 120 min.

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Table 1. Antinociceptive Effect of Compound 1 Isolated from *F. adenophylla*[a]

		time (minutes)			
group	dose mg/kg	30	60	90	120
normal saline	10 mL	9.20 ± 0.08	9.20 ± 0.09	9.21 ± 0.07	9.18 ± 0.12
tramadol	10	24.34 ± 0.08**	26.09 ± 0.100***	25.76 ± 0.12***	25.68 ± 0.44***
peshawaraquinone	2.5	13.80 ± 0.52*	14.84 ± 0.88*	14.83 ± 0.65*	14.66 ± 1.65*
	5	15.70 ± 0.89**	18.65 ± 0.93**	18.98 ± 0.92**	17.67 ± 1.17**
	10	17.71 ± 0.56**	19.68 ± 0.91**	19.40 ± 0.79**	18.99 ± 1.21**
	15	22.76 ± 0.45***	22.90 ± 1.00***	21.97 ± 1.32***	21.99 ± 1.40***

[a]Data are presented as the mean ± SEM of animal tolerance to thermal stimuli in seconds. The level of significance was determined by ANOVA followed by Dunnet's test.

Table 2. Muscle Relaxant Effect of Compound 1 Isolated from *F. adenophylla*[a]

		inclined plane test (%) time in minutes			traction test (%) time in minutes		
group	dose mg/kg	30	60	90	30	60	90
distilled water	10 mL						
diazepam	1	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00	100 ± 00
peshawaraquinone	2.5	18.30 ± 1.88	24.57 ± 1.76	23.58 ± 1.78	19.28 ± 1.57	24.66 ± 2.12	23.98 ± 2.88
	5	25.45 ± 2.00	34.87 ± 2.54	35.80 ± 2.60	26.65 ± 2.19	34.12 ± 2.40	35.58 ± 2.88
	10	32.87 ± 1.93	43.98 ± 2.00	44.12 ± 2.01	33.40 ± 2.44	45.12 ± 2.64	44.55 ± 2.90
	15	43.76 ± 2.21	51.56 ± 2.76	52.12 ± 2.88	44.20 ± 2.12	52.96 ± 2.22	51.90 ± 2.40

[a]Data are presented as the mean ± SEM of the percent effect.

2.5. Muscle Relaxant Activity. The muscle coordination effect of peshawaraquinone **1** (2.5, 5, 10, and 15 mg/kg, PO) was evaluated through traction tests and inclined plane tests using specific wires for hanging and special wood inclined planes, respectively. The activity was performed exactly following our published procedures.[21] Animals were classified into positive control (diazepam 0.5 mg/kg, IP), negative control (normal saline 10 mL/kg, ip), and test groups (n = 6). Thirty minutes after these administrations, each animal was tested for muscle relaxant effects in both models. In the case of the traction experimental model, each animal was allowed to hang by a wire on their hind leg, and the duration of holding the wire by claws was noted in seconds. Hanging for less than 5 s means the muscle relaxant potential and vice versa. Regarding the inclined plane 30 min after administration, animals were allowed to slide on the inclined plane. Resistance to sliding was considered for no muscle coordination, and ease of sliding was considered for an animal with relaxed muscles.

2.6. Open-Field Test. This experimental system was used for the evaluation of sedative potential. Animals were classified as described above. The apparatus used for this activity comprised an area of white wood (150 cm diameter) surrounded by stainless-steel walls and was divided into 19 squares by black lines. The open-field apparatus was placed inside a light- and sound-attenuated room.[20,21] Thirty minutes after treatment, each animal was allowed to move from the center of the box. The maximum number lines crossed by the animal were considered no sedation. Animal was considered to be sedated with hindered movement. The number of lines crossed by each animal was used to find sedative effects in statistical calculations. The doses of diazepam, normal saline, and compound **1** were the same as in previous experiments.

2.7. Acute Toxicity. Peshawaraquinone 1 was evaluated for acute toxicity effects, and animals were classified into a saline-treated group (n = 6) and peshawaraquinone **1**-treated group. Peshawaraquinone 1 was administered at doses of 10, 25, 50, 100, and 200 mg/kg (P.O). After 30 min of these treatments, each animal was monitored carefully for any gross effect for 8 h. The mortality rate was observed for 24 h.[22]

2.8. Statistical Analysis. The results obtained from biological studies are displayed as the mean ± standard deviation to identify the level of significant difference (p < 0.05 or 0.01) for each group of experimental mice. One-way analysis of variance (ANOVA) was performed by Dunnett's multiple comparison test.

2.9. Docking Studies. The docking experiments were performed using the molecular operating environment (MOE) docking program version 2016.08.[23] The three-dimensional (3D) structures of the enzymes with their cocrystallized ligands were downloaded from the Protein Data Bank. The 3D crystal structure of the μ-opioid receptor has a native ligand (PDB id = 5C1M). The GABAnergic homopentamer from humans was obtained from the Protein Data Bank (PDB id = 4COF). The docking algorithm was authenticated by the redocking of native ligands. The computed root mean square deviation (rmsd) between the experimental and redocked poses was found within the threshold limit (<2 Å). The 3D structure of the compound was built in an MOE using a builder module. Energy minimization of the ligand, preparation of structures of the downloaded enzymes, and active site identification were carried out according to a previously reported procedure.[24−26] The assessment of the docking results and investigation of their surface with graphical representations were carried out using MOE and a discovery studio visualizer.[27]

3. RESULTS

3.1. Antinociceptive Effect. The compound **1** demonstrated a dose- and duration-dependent central antinociceptive effect, as demonstrated in Table 1. Peshawaraquinone **1** significantly increased the pain threshold level and mitigated pain sensation in mice. This effect was seen in the first 30 min postadministration and remained significant (p < 0.001) for 120 min of the experimental duration.

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3.2. Muscle Relaxant Effect. The muscle coordination effect of compound **1** in both experiments is presented in Table 2. The muscle relaxant effect was significant at the tested dose of 15 mg/kg in both experiments. The percentage of effect at the higher dose was 43.76% at the early period, which was improved up to 52.12% after 90 min postadministration (inclined plane). In the case of the traction test, the same pattern of muscle coordination effect was observed.

3.3. Sedative Effect. Compound **1** significantly hindered ($p < 0.001$) the movement of animals in the special box, which reflects its sedative potential (Table 3). The tested compound

Table 3. Effect of Compound 1 Isolated from *F. adenophylla* in the Open-Field Test (Locomotive Activity)[a]

sample	dose mg/kg	no of lines crossed
control	5 mL	129.24 ± 3.91
diazepam	0.5	9.00 ± 0.55
peshawaraquinone	2.5	79.66 ± 0.97**
	5	70.41 ± 1.24***
	10	61.95 ± 2.63***
	15	52.01 ± 3.26***

[a]Data are presented as the mean ± SEM of the number of lines crossed by animals. The level of significance was determined by ANOVA followed by Dunnet's test.

demonstrated significant ($p < 0.01$) effect. In comparison with the negative control, the compound **1** restricted the movement of animals, indicating the sedative effect.

3.4. Acute Toxicity. During the acute toxicity study, no mortality was observed at 200 mg/kg.

3.5. Molecular Docking Studies. We performed molecular docking studies to explore the mechanism of analgesic activity. Based on the mechanism of action of tramadol, a standard drug used, we first performed docking studies on the μ-opioid receptor (μOR). For this purpose, we obtained the 3D crystal structure of the μ-opioid receptor with the native ligand BU72 (PDB id = 5C1M). The redocking process was carried out to assess the docking reliability. After docking validation, the standard drug used in the current study, that is, tramadol, was docked into the active site of 5C1M. Moreover, we also analyzed the binding orientation and binding energy of the irreversible morphinan antagonist β-funaltrexamine (β-FNA) (PDB-entry 4DKL). The 3D interaction plots of the three studied drugs are shown in Figure 2. The native ligand (BU72) with a binding energy value of −7.7408 kcal/mol interacts with His54 and Asp147 *via* hydrogen-bond interactions. Cys217 forms π-interactions with the phenyl ring (Figure 2a). Tramadol (−6.6084 kcal/mol) formed one hydrogen-bond interaction with Asp147. Tyr326 formed a π-alkyl interaction (Figure 2b). The computed binding energy of the antagonist β-FNA was −8.6277 kcal/mol. It formed two hydrogen-bond interactions with Asp147 and Lys303. Met151 formed π-sulfur interactions. The ligand enzyme complex was stabilized by a $\pi-\sigma$ interaction with Val300 (Figure 2c).

Finally, we docked our isolated compound peshawaraquinone (**1**) into the binding site of 5C1M. Its superimposed binding pose with native morphinan is shown in Figure 3a. The 3D interaction plot showed two hydrogen-bond interactions with Asp147 and Tyr326 *via* hydroxy groups. Tyr148 forms a bifurcated $\pi-\pi$ interaction with the naphthalene-1,4-dione ring. Another bifurcated $\pi-\pi$ interaction was observed between naphthalene-1,4-dione and Trp318 (Figure 3b). Met151

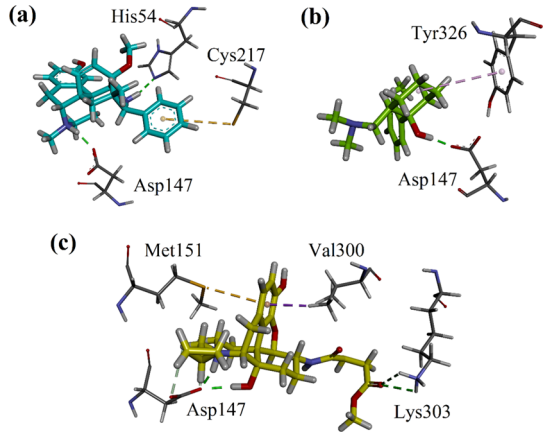

Figure 2. Close-up 3D interaction plot of (a) native ligand BU72 and (b) tramadol into the binding site of the μ-opioid receptor 5C1M. (c) Binding interactions of the μOR antagonist (from PDB id = 4DKL).

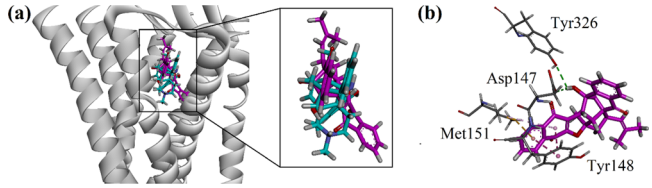

Figure 3. (a) Ribbon model of the superimposed binding poses of isolated compound (pink) on the native drug (yellow) into the binding site of 5C1M; (b) close-up 3D interaction plot of the isolated compound into the binding site of 5C1M.

displayed π-sulfur interactions. The computed binding energy for the isolated compound was −8.8430 kcal/mol.

Subsequently, the muscle relaxant effect of the isolated compound was explored using docking simulations. The docking simulations were carried out on GABAnergic receptors. The GABAnergic homopentamer from humans was obtained from the Protein Data Bank (PDB id = 4COF). The receptor was in complex with an agonist benzamidine located in each extracellular domain (ECD).

The docking simulation of the standard drug diazepam and isolated compound was performed. The lowest energy 3D interaction plots of the docked compounds are shown in Figure 4. The nitrogen atom of the seven-membered diazepam ring

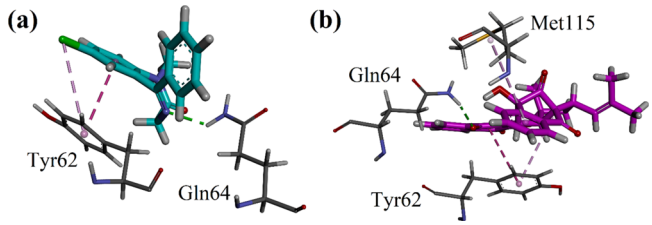

Figure 4. Close-up 3D interaction plot of diazepam and (b) isolated compound into the binding site of the GABA receptor (PDB id = 4COF).

formed hydrogen-bond interactions with Gln64. The phenyl ring forms $\pi-\pi$ interactions with Tyr62 (Figure 4a). Our isolated compound peshawaraquinone formed hydrogen-bond interactions with Gln64. Similarly, naphthalene-1,4-dione formed $\pi-\pi$ interactions with Tyr62 (Figure 4a). Met115 formed π-alkyl interactions. The computed binding energy

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values for diazepam and the isolated compound were −5.6005 kcal/mol and −5.9300 kcal/mol, respectively.

4. DISCUSSION

In Pakistan, alternative medicines from *F. adenophylla* are locally used for topical muscle relaxation and pain killer formulations. The local community uses topical oil with *F. adenophylla* extract as one of the ingredients to relieve muscular tension and pain. This folkloric practice encouraged us to test the currently isolated molecule such as peshawaraquinone **1**, for pain reliving, muscle coordination, and sedative effects. The testing of chemical constituents against the folklore of medicinal plants is a good push toward novel drug discovery. Even these biological experiments provide a sound background to the extract.

Pain is considered as a classic sign of the inflammation. A number of chemical mediators are released during the inflammatory process. These mediators cause nociceptive sensitization. Medicinal plants are the main source of active phytochemicals that can be utilized for the production of modern medicines.[1] Natural product-derived drugs and phytomedicine are becoming popular compared to synthetic drugs because of their low cost and fewer side effects. de Oliveira *et al.* studied the antinociceptive properties of Bergenin, a C-glucoside of 4-O-methylgallic acid that occurs naturally in several plant genera. The study showed that bergenin has consistent antinociceptive and anti-inflammatory properties.[28] Martins *et al.* isolated (−)-4′-methylepigallocattechin from Maytenus rigida Mart. which demonstrated the antinociceptive activity in mice.[29] Rodrigues *et al.* investigated the antinociceptive effect of three alkaloids (dihydro-piplartine, piplartine, and 3,4,5-trimethoxydihydrocinnamic acid) and crude extracts from the fruits of *Piper tuberculatum* J_{ACQ} (Piperaceae).[30] Similarly, essential oils and their monoterpenes/sesquiterpenes possess analgesic properties. Lenardão *et al.* compiled antinociceptive of about 63 essential oils and their constituents.[31]

Naphthoquinones are structurally diverse secondary metabolites found in bacteria, fungi, and higher plants and exhibited several biological activities including anti-inflammatory and antinociceptive activity. Soares *et al.* for the first time explored the anti-inflammatory and antinociceptive activity of five naphthoquinones isolated from tubers of *Sinningia reitzii*.[32] Considering the key role of natural products especially naphthoquinones in nociception, we planned the study to evaluate peshawaraquinone **1** for its central analgesic, muscle relaxant, and sedative effects. Compound **1** belongs to the naphthoquinone class of chemical constituents, which have been reported to have various biological activities, such as anticancer, sedative, analgesic, and antimicrobial activities. The structure of peshawaraquinone **1** bears minor resemblance with benzodiazepines which are significant sedative hypnotic, muscle relaxant, and adjuvant analgesics.[19] The biological actions of benzodiazepines are attributed to their strong interactions with GABA channels.[33] Once the GABA channels are occupied at allosteric sites, the chloride channel opens, and more chloride influx occurs, which shifts the membrane potential toward being more electronegative, and the cell becomes inhibited. Once the membrane potential becomes more electronegative, the calcium channels remain closed, and no calcium influx occurs. These intracellular cascades hinder the release of neurotransmitters and thus mitigate the interactions of neurotransmitters with postsynoptic receptors. This attenuated situation keeps the muscles relaxed and induces sedation, decreasing pain sensation. The significant analgesic potential of peshawaraquinone **1** might be due to the interaction with GABA channels or with opioid receptors. If peshawaraquinone **1** is considered an agonist for opioid receptors, it might be a muscle relaxant.[34] Opioid receptors are known as G protein-coupled receptors (GPCRs). Once an agonist binds with these inhibitory heptahelical receptors, intracellular signaling inhibits protein kinase A and stimulates the potassium channel to stay open, and more potassium efflux occurs, which causes the membrane potential to become more electronegative. Again, calcium channels are not opening, and no calcium is available for neurotransmitter exocytosis. Once the exocytosis of neurotransmitters (especially substance p) diminishes, the pain threshold level is set to convert algesia into analgesia. These cascades show potential muscle relaxation effects and probably sedative effects as well.

Computational studies have led to the developing of new drugs especially analgesics. Docking simulations predict the binding orientation of a compound of therapeutic interest into the active site of the target and also helps in understanding the biological activity. Moreover, the binding orientations and interactions also help in structure−activity relationship (SAR) studies. Computational docking simulations demonstrated that the isolated compound (**1**) has shown favorable binding orientations and binding energy values against the μ-opioid and GABAnergic receptors. The μ-opioid receptors (μORs) are membrane proteins and members of the opioid neuromodulatory system. These receptors are the therapeutic targets of opioid drugs for the treatment of moderate to severe pain. However, the binding pattern of μOR-targeting drugs (antagonist *vs* agonist) is not unclear. There are some hypotheses about the substitution pattern at the nitrogen atom on these receptors. It is assumed that bulky groups such as allyl or cyclopropylmethyl are associated with antagonist activity. The N-methyl group is considered to show agonist properties.[34,35] However, there are a number of examples displaying agonist activity with bulky groups on nitrogen atoms.[36,37]

Describing μOR interactions helps us to identify the responses and initiate the selective activation of signaling pathways which may be exploited to develop novel and potent anti-inflammatory agents/analgesics. The solved crystal structures of μOR-bound antagonists and agonists provided a detailed analysis of the ligand−receptor-binding pattern. The first three-dimensional structure of μOR in complex with the irreversible antagonist β-FNA was published in 2012 (PDB ID = 4DKL). The antagonist displayed hydrogen-bond interactions with Asp147 and Tyr148. It was also involved in hydrophobic interactions with Met151, Tro293, Ile296, and Val300.[38,39] Later, insights into the binding pattern of the agonist BU72 into the binding site of μORs were published.[40] The agonist BU72 showed hydrogen-bond interactions with Asp147, Tyr148 (water-mediated), and His54.

It is interesting to note that SAR studies revealed that a slight change in the structure of compound can convert an agonist to antagonist. We performed docking analysis by comparing the binding poses of the compound under study with an antagonist- and agonist-binding pattern. The detailed binding-pose analysis and binding-energy calculations of our isolated compound **1** allowed us to gain further insights into its mode of action. Structurally, compound **1** is a structurally diverse compound with fused rings. The binding energies for agonist, tramadol, antagonist, and compound **1** are −7.7408, 6.6084, −8.6277, and

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−8.8430 kcal/mol, respectively. This shows that our compound binds more tightly than the other studied compounds. As far as the binding interaction is concerned, it forms two hydrogen-bond interactions with Asp147 and Tyr326. Hydrophobic interactions were also observed with Tyr148 and Met 151. However, detailed experimental data related to the mechanism of action of structurally diverse ligands are required.

The possible mechanism behind these pharmacological actions might be interaction with GABA or opioids, as discussed above. However, to find safe, effective, and nonaddictive molecules, peshawaraquinone **1** must be subjected to structure−activity relationship (SAR) analysis and a detailed mechanistic study.

5. CONCLUSIONS

In the current study, peshawaraquinone 1 isolated from *F. adenophylla* demonstrated significant analgesic, sedative, and muscle relaxant effects. The traditional use of *F. adenophylla* is mainly in massage oils, which are supposed to comfort muscular tension and pain. The traditional usage of this plant was strongly augmented by peshawaraquinone 1. Thus, our findings provide scientific rationale for the folkloric usage of *F. adenophylla* for the treatment of various diseases. Thus, peshawaraquinone 1 is a novel candidate for additional detailed study to ascertain its clinical use. A detailed comparison of the binding orientations and binding energy values computed through docking was carried out among agonists, antagonists, and the isolated compound. The interactions of peshawaraquinone 1 with pharmacological targets suggest that the compound may act as a novel nociceptive and inflammatory pain alleviator, as supported by *in vivo* studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05720.

Spectroscopic data associated to this paper have been provided as supplementary file (PDF)

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Notes

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■ ABBREVIATIONS

F. adenophylla, *Fernandoa adenophylla*; TLC, thin layer chromatography; MOE, molecular operating environment (MOE); PDB, Protein Data Bank; rmsd, root-mean-square deviation; 3D, three-dimensional; ECD, extracellular domain

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