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Research Article

Experimental screening for analgesic and anti-inflammatory effect of novel compounds with a pyrrole heterocycle

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Abstract

The pyrrole heterocycle is found in the chemical structure of numerous drugs with various effects, and it has a reasonably high tolerance and safety profile. The objectives of our study were to assess the analgesic and anti-inflammatory efficacy of six novel compounds with pyrrolic structures. Methods: All trials were carried out on 6-week-old male Wistar rats. Animal pain models using thermal (paw withdrawal, tail-flick) and chemical stimuli (formalin test) were used to examine antinociception. A carrageenan-induced paw edema model was used to assess anti-inflammatory activity. Results: Significant differences between the experimental groups were observed in both early and late phase of the formalin test. Conclusions: The six novel pyrrolic compounds have analgesic action against chemical stimuli in experimental conditions. They do not possess anti-inflammatory activity, or antinociceptive properties against thermal stimuli.

Keywords

Pyrrole, tail-flick, paw withdrawal, formalin model, paw edema

Introduction

Over the preceding two decades, there has been a consistent escalation in analgesic sales, marking them as the most prevalent over-the-counter medication globally (Kristensen et al. 2016). Recent epidemiological studies have unveiled a widespread and uncontrolled use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Fine 2013). A US-based cohort study disclosed that 14.1% of women aged 33–51 consumed aspirin more than once per week, while 42.3% resorted to NSAIDs, and 25.8% opted for acetaminophen (Sasamoto et al. 2021). Similarly, in Germany, there is extensive availability of prescription pain relievers, with approximately 1.9 million individuals, both men and women, using analgesics daily, and an estimated 1.6 million people grappling with painkiller addiction (Leyk et al. 2023). Over the span from 1990 to 2017, a quarter of individuals enduring chronic non-cancer pain turned to opioids for relief (Wertheimer et al. 2021). Despite the notable prevalence of pain-related disorders, effective pain management remains a persistent challenge for healthcare professionals (Polacek et al. 2020).

Numerous analgesics exhibit limited efficacy and can induce severe adverse effects. Consequently, researchers have been prompted to explore novel biological targets and develop alternative approaches to pain management (Liktor-Busa et al. 2021). A variety of medications, such

as nootropics (Piracetam), anti-inflammatory drugs (Indomethacin), statins (Fluvastatin), antipsychotics (Molindone), hypnotics (Zopiclone), antimigraine agents (Sumatriptan), antiparkinsonians (Procyclidine), and drugs utilized in treating erectile dysfunction (Tadalafil), contain a pyrrole ring within their chemical structure. Compounds derived from essential oils containing a pyrrole ring have been employed for their anticancer (5-iodotubercidin) and antibacterial properties (Cefepim, Meropenem) (Wójcicka et al. 2021). Pyrrolic structures serve as promising scaffolds for the development of drugs with anti-inflammatory and analgesic properties (Dannhardt et al. 2000). Notably, the pyrrole ring is present in certain traditional and contemporary NSAIDs, such as ketorolac, tolmetin, and zomepirac. Several studies have underscored the therapeutic potential of this heterocyclic moiety for anti-inflammatory and analgesic effects (Kim et al. 2014; Indumathi et al. 2015). Despite its diverse biological functions, the pyrrole ring exhibits reasonably high tolerance and safety profiles (Rusu et al. 2023; Morales-Salazar et al. 2023). Consequently, the objectives of our study were to assess the analgesic and anti-inflammatory properties of six novel compounds featuring a pyrrolic structure.

Materials and methods

All experimental procedures conducted herein received approval from the Animal Health and Welfare Directorate of the Bulgarian Food Safety Agency, in compliance with Permit No. 255, as endorsed by the Ethics Committee of the Bulgarian Food Safety Agency under Protocol No. 171/08. 10. 2019.

Test substances

Reagents used in the experiments were NaCl 0.9% (Sopharma AD, Sofia, Bulgaria), metamizole sodium amp. 500 mg/mL 2 mL (Sopharma AD, Sofia, Bulgaria), diclofenac sodium amp. 75 mg/3 mL (Hexal AG, Holzkirchen, Germany), lambda-carrageenan (Merck, Darmstadt, Germany), formalin 0.2% (Merck, Darmstadt, Germany). The novel pyrrolic compounds, metamizole, and diclofenac were dissolved in saline and administered via intraperitoneal injection.

Pyrrolic compounds

1-[1,5-Bis-(4-chloro-phenyl)-2-methyl-1H-pyrrol-3-yl]-ethanone (1a)
1-[5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-2-methyl-1H-pyrrol-3-yl]-ethanone (1b)
1-[5-(4-chloro-phenyl)-1-(3,4-dimethoxy-phenyl)-2-methyl-1H-pyrrol-3-yl]-ethanone (1c)
1,5-Bis-(4-chloro-phenyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (2a)
5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (2b)
5-(4-chloro-phenyl)-1-(3,4-dimethoxy-phenyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (2c)

The structure of the compounds is shown in Fig. 1.

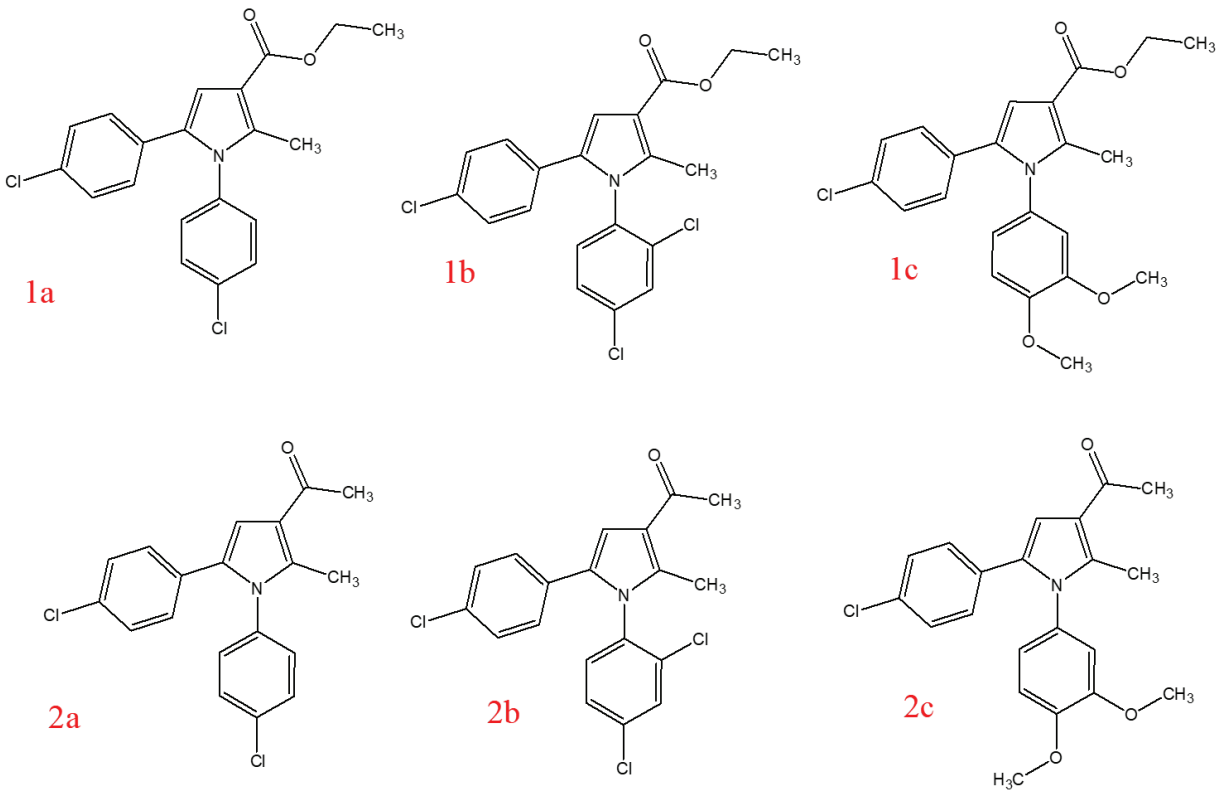

Figure 1. Chemical structure of the six tested pyrrolic compounds.

All commercial chemicals utilized as starting materials and reagents in the synthesis and characterisation of the compounds were obtained from "Merck" (Darmstadt, Germany). The melting points were calculated using a capillary digital melting point apparatus IA 9200 Electrothermal AZ9003MK4, Southend-on-Sea, UK. The IR spectra were recorded with a Carl Zeiss Specord IR-71, Jena, Germany (KBr). The ^{1}H NMR spectra (250 MHz, 20 oC) were recorded using TMS as an internal standard on a Bruker Spectrospin WM250 spectrometer (Faenlanden, Switzerland). TLC properties of the products were tested at ambient temperature on aluminum sheets of silica gel 60 F254, Merck 1.05554 using an appropriate mobile phase. Paal-Knorr pyrrole synthesis was chosen as a practical method for incorporating the selected substituents into the desired pyrroles (Knorr 1884).

Animals

All experiments were performed on 6-week-old male Wistar rats weighing approx. 200 g, randomly divided into 21 parallel experimental groups, each consisting of 8 animals (Table 1). The rats were housed in standard laboratory conditions: temperature-controlled environment, light/dark cycle of 12/12 h, access to food and water ad libitum.

Experimental models

Tail flick

An infrared heat source (Ugo Basile, Italy) was positioned beneath the tail of the experimental animal, approximately 3 cm from its distal end, with tail withdrawal time automatically recorded in seconds. To prevent tissue damage, the radiation intensity was regulated to 80 mW/cm^2, and the maximum heating duration was limited to 15 seconds. Each animal underwent testing after the first, second, and third hours following treatment with the experimental substances. The extension of reaction time relative to saline-treated rats served as the criterion for evaluating analgesic efficacy.

Paw withdrawal

The experimental animal was housed in a separate Plexiglas enclosure with freedom to move. Following an acclimatization period, an infrared heat source (Ugo Basile, Italy) was positioned directly beneath one of the rat's hind paws, and the paw withdrawal time in seconds was automatically recorded. To prevent significant paw injury, the intensity of the infrared radiation was set at 50 mW/cm^2, with a maximum heating duration of 30 seconds. To prevent inadvertent signaling to the animal, a specialized filter was employed to block the visible light spectrum. Each animal underwent testing after the first, second, and third hours after treatment with the experimental compounds. Criteria for determining analgesic activity included a delay in reaction time compared to animals in the control group.

Formalin model

One hour following the administration of the test substances, an intradermal injection of 0.2% formalin (200µl) was administered into one of the rear feet of the animals. The time spent licking the injected paw was measured in seconds during two distinct phases: the initial ten minutes post-injection (1st phase) and the period between the 20th

Table 1. Experimental animal groups.

Experimental group	Tested substance	Number of animals
1 – negative control	Saline solution	8
2 – positive control for pain models	Metamizole 200 mg/kg b.w.	8
3 – positive control for inflammation model	Diclofenac 25 mg/kg b.w.	8
4	Compound 1a 10 mg/kg b.w.	8
5	Compound 1a 20 mg/kg b.w.	8
6	Compound 1a 40 mg/kg b.w.	8
7	Compound 1b 10 mg/kg b.w.	8
8	Compound 1b 20 mg/kg b.w.	8
9	Compound 1b 40 mg/kg b.w.	8
10	Compound 1c 10 mg/kg b.w.	8
11	Compound 1c 20 mg/kg b.w.	8
12	Compound 1c 40 mg/kg b.w.	8
13	Compound 2a 10 mg/kg b.w.	8
14	Compound 2a 20 mg/kg b.w.	8
15	Compound 2a 40 mg/kg b.w.	8
16	Compound 2b 10 mg/kg b.w.	8
17	Compound 2b 20 mg/kg b.w.	8
18	Compound 2b 40 mg/kg b.w.	8
19	Compound 2c 10 mg/kg b.w.	8
20	Compound 2c 20 mg/kg b.w.	8
21	Compound 2c 40 mg/kg b.w.	8

Note: mg/kg b.w., milligrams per kilogram body weight.

and 30th minute post-injection (2nd phase). A reduction in paw licking time, relative to the control group, served as a criterion for assessing analgesic efficacy.

Carrageenan-induced paw edema

The volume of the right hind paw of all animals was measured before treatment. Then, 0.1 ml of a 1% carrageenan solution in 0.9% sodium chloride was injected into each animal's right hind paw to elicit edema. Immediately after the carrageenan injection, the animals were injected intraperitoneally with saline or the tested substances, respectively. The volume of fluid displaced from the right hind paw of the rat was measured using a plethysmometer (Ugo Basile, Italy) at the second, third, and fourth hour post-carrageenan treatment. The percentage of paw edema was calculated using the following formula:

$$\text{Paw edema (\%)} = [(Vt - Vo)/Vo] \times 100$$

Vo – mean paw volume before treatment, Vt – mean paw volume at the respective hour.

Reduced swelling of the paw in comparison to the control group is a criterion for anti-inflammatory activity.

Statistical analysis

Statistical evaluation was executed with IBM SPSS 26.0 software, employing One-Way ANOVA, Tukey, and Games–Howell post hoc depending on Levene's test for equal variances and Welch and Brown-Forsythe's robust tests of equality of means. Normality of distribution was assessed using the Shapiro-Wilk test. Results are presented as arithmetic mean and standard error of the mean (mean ± SEM). A p-value of ≤ 0.05 was considered statistically significant.

Results

Synthesis of the target compounds

All pyrrolic compounds were synthesized according to the procedure presented in Fig. 2. The design, detailed synthesis, and characterization of the compounds by spectroscopic analysis and thin-layer chromatography are described by Vladimirova and Bijev (2015) in "Synthesis of new derivatives of pyrrole tuberculostatics providing structural diversity".

Evaluation of the analgesic and anti-inflammatory activity of the new compounds

In both the tail-flick and paw withdrawal models, ANOVA testing revealed significant differences between groups (p<0.001 for each of the three tested hours). However, further post hoc analysis demonstrated that this significance was primarily attributable to the positive control metamizole. The reference analgesic exhibited significant analgesic effect in both tests, thereby validating the models. Nevertheless, none of the experimental compounds managed to significantly decrease withdrawal latency.

Significant differences between the experimental groups were observed in the formalin model (ANOVA, p=0.008 for the 1st phase; p<0.001 for the 2nd phase). Metamizole-treated rodents spent significantly less time licking and biting their paws. During the initial phase, the compounds that significantly reduced paw-licking time were as follows: 1c at 20 and 40 mg/kg b.w., 2b at 10, 20, and 40 mg/kg b.w., and 2c at 20 and 40 mg/kg b.w. Paw-licking time during the second phase was significantly affected by

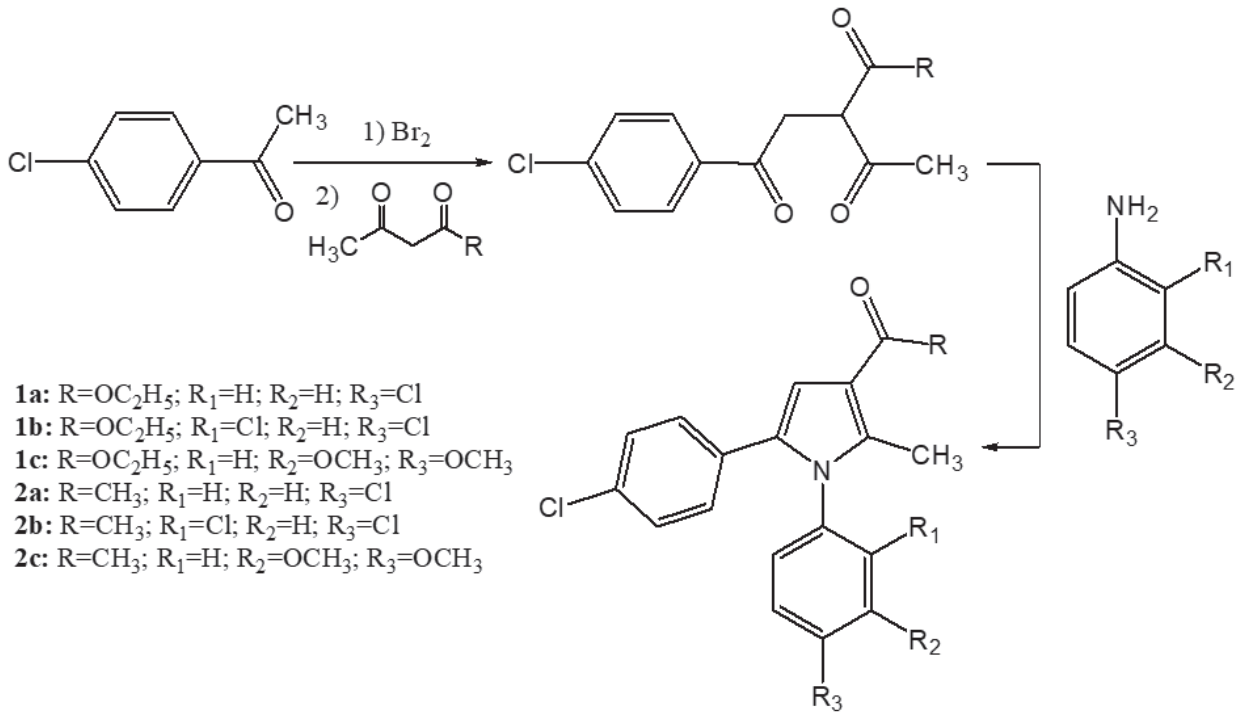

Figure 2. Pathway used in the synthesis of the tested compounds.

the following compounds: 1b at 10, 20 and 40 mg/kg b.w.; 1c at 20 and 40 mg/kg b.w.; 2a at 20 and 40 mg/kg b.w.; 2b at 10, 20 and 40 mg/kg b.w.; and 2c at 20 and 40 mg/kg b.w.. Detailed results are presented in Table 2.

Table 2. Comparison of the time spent licking/biting paw (in seconds) in formalin test between the control group and the groups treated with metamizole and pyrrolic compounds.

Group	Phase	Mean ± SEM	*p*
Control	1st	72.13 ± 3.73	–
	2nd	62.13 ± 4.76	
Metamizole	1st	15.88 ± 4.48	<0.001*&
	2nd	20.75 ± 4.45	0.002*&
1a 10 mg/kg	1st	33.75 ± 11.44	0.314
	2nd	39.88 ± 21.02	0.999
1a 20 mg/kg	1st	46.00 ± 14.64	0.929
	2nd	39.50 ± 10.29	0.849
1a 40 mg/kg	1st	43.38 ± 15.81	0.917
	2nd	45.75 ± 13.94	0.999
1b 10 mg/kg	1st	35.00 ± 10.75	0.287
	2nd	23.38 ± 7.26	0.044*&
1b 20 mg/kg	1st	33.38 ± 10.01	0.186
	2nd	19.63 ± 5.66	0.005*&
1b 40 mg/kg	1st	44.63 ± 9.30	0.487
	2nd	18.00 ± 6.42	0.008*&
1c 10 mg/kg	1st	38.50 ± 10.04	0.325
	2nd	26.88 ± 9.31	0.234
1c 20 mg/kg	1st	22.00 ± 8.78	0.023*&
	2nd	19.75 ± 6.09	0.008*&
1c 40 mg/kg	1st	26.50 ± 8.29	0.029*&
	2nd	6.50 ± 2.84	<0.001*&
2a 10 mg/kg	1st	42.75 ± 10.03	0.487
	2nd	23.88 ± 7.60	0.062
2a 20 mg/kg	1st	44.50 ± 5.49	0.069
	2nd	10.00 ± 5.74	0.001*&
2a 40 mg/kg	1st	36.50 ± 9.94	0.255
	2nd	4.13 ± 2.41	<0.001*&
2b 10 mg/kg	1st	38.38 ± 2.67	0.001*&
	2nd	21.63 ± 6.41	0.015*&
2b 20 mg/kg	1st	29.50 ± 4.56	0.001*&
	2nd	16.50 ± 5.83	0.003*&
2b 40 mg/kg	1st	22.25 ± 6.09	0.001*&
	2nd	18.88 ± 3.64	0.001*&
2c 10 mg/kg	1st	29.25 ± 10.59	0.151
	2nd	25.13 ± 8.69	0.142
2c 20 mg/kg	1st	20.50 ± 5.67	0.001*&
	2nd	12.00 ± 4.41	<0.001*&
2c 40 mg/kg	1st	15.25 ± 5.43	<0.001*&
	2nd	19.13 ± 6.82	0.014*&

Note: * $p < 0.05$ compared to control; & Games-Howell post hoc test.

ANOVA revealed significant differences between groups ($p<0.001$ for each of the three tested hours) in the carrageenan-induced paw edema. Similar to the findings from the pain models involving thermal stimuli, subsequent post hoc analysis demonstrated that this significance was primarily attributable to the positive control diclofenac. The reference anti-inflammatory drug demonstrated a significant anti-inflammatory effect, thereby validating the model. However, none of the experimental compounds were able to significantly reduce paw edema.

Discussion

Our study indicates that the six new pyrrolic compounds described above possess analgesic activity against chemical stimuli in experimental settings. However, they do not exhibit anti-inflammatory activity or antinociceptive properties against thermal stimuli.

Of course, our research has certain limitations. Despite consultation with a statistician, the sample size of animals used remains relatively small. The pyrrolic compounds have only been tested on rats, and the sensitivity of other animal species to these compounds may vary. Although we endeavored to minimize human intervention in our experimental techniques by employing tail-flick and plantar tests with automatic measurement, some aspects still involve manual recording, such as the seconds spent biting/licking their paw in the formalin test. Additionally, in the tail-flick test, the animal is held stationary by the handler. Our dose selection is likewise limited. While based on acute toxicity studies, the utilization of only three increasing dosages makes observing dose-dependency of the effect with certainty problematic; higher doses may potentially demonstrate a more pronounced pharmacological effect.

Pain perception and nociceptive reflexes are intricately linked processes. Nociceptors are responsive to various types of noxious stimuli (e.g., thermal, chemical, mechanical), eliciting withdrawal reflexes that serve as proxies for human pain experiences in animal models (Negus et al. 2006). Despite their inherent limitations and the the challenges in translating fundamental scientific data into effective analgesic therapies, nociception experimental models remain invaluable pharmacological tools in preclinical research for evaluating the potential analgesic efficacy of novel drugs (Mogil et al. 2010; Szandruk-Bender et al. 2020).

The plantar test stands out as a sensitive procedure that relies on supraspinal processes to evoke behavioral reactions. This test employs a localized heat source, applying thermal stimulus exclusively to the paw of the test animal (Gregory et al. 2013). Additionally, the plantar test offers the advantage of automatically detecting latency time. Since there is no specific cue prompting a conditioned response, animals cannot learn to influence the test, thereby enhancing the accuracy of the results (Le Bars et al. 2001). Brito et al. (2017) found that intraperitoneal administration of metamizole at 270 mg/kg b.w. increased paw withdrawal time in the plantar test in a mouse model of cancer. Similarly, El-Awdan et al. (2015) observed an increase in the time taken by rats to respond to thermal stimulation in the plantar test following meloxicam administration.

The "tail flick" test also utilizes a thermal stimulus. This test is considered precise as it is electronically assessed, ensuring consistency in reaction times across different animals (Gregory et al. 2013). The involvement of spinal and supraspinal systems in the tail-flick response depends on the intensity of the heat stimulus. When the tail-flick apparatus elicits a latency response within 4–5 seconds, the tail-flick reflex engages higher brain regions, partic-

ularly the spinobulbospinal loop. The latency period in the tail-flick test is directly proportional to the central analgesic effect of administered drugs (Hutchinson et al. 2004). However, the tail flick test has limitations; the site of stimulation on the tail significantly affects the response, with the distal region being the most sensitive (Yoburn et al. 1984), and minor differences in the heated area can lead to changes in latency. Dogrul et al. (2007) found that topical application, but not systemic administration, of NSAIDs induced antinociception in rats in the tail flick test. Carlsson et al. (1986) reported that both intraperitoneal and intrathecal injection of metamizole in rats resulted in a dose-dependent prolongation of tail withdrawal time. In experiments involving the tail-flick test, Szandruk-Bender et al. (2020) discovered that pyrrolo[3,4-d] pyridazinone derivatives exhibit dose-dependent antinociceptive effects.

In both the plantar and tail-flick tests, the six pyrrolic compounds subject of this study did not exhibit any antinociceptive properties. This lack of efficacy could potentially be attributed to a peripheral mode of action. Hence, we further investigated their effects using a formalin model. The formalin test serves as a suitable model for exploring the potential involvement of peripheral and spinal pathways in modulating pain transmission. Intraplantar administration of formalin induces biphasic pain responses. The two phases are caused by alterations of varying nature and magnitude, allowing for insights into distinct mechanisms underlying antinociception. The initial phase, lasting approximately 10 minutes, is triggered by direct stimulation of peripheral nociceptors. Following a ten-minute break period, the second phase ensues, driven by the release of local endogenous proinflammatory mediators, leading to peripheral inflammatory processes and subsequent sensitization of nociceptive spinal neurons (Meunier et al. 1998). Given its incorporation of neurogenic, inflammatory, and supraspinal nociception pathways, the formalin test is a particularly valuable model. Furthermore, when compared to other experimental models, the spontaneous nociceptive response in freely moving, unrestrained animals with a relativley prolonged duration closely resembles clinical pain experiences in this test. It is hypothesized that prolonged afferent input to the spinal cord following formalin injection induces functional alterations in the dorsal horn (Yin et al. 2016). Notably, medications limiting COX activity are known to be less effective in attenuating nociceptive behavior during the neurogenic phase of the formalin test when compared to opioids (Bastos et al. 2006).

The cellular response to tissue damage triggers heightened sensitivity, facilitating the influx of activated cells such as macrophages, lymphocytes, and mast cells. Inflammatory mediators such as bradykinin, ATP, purines, and oxidative stress products are released from nociceptor membranes. Evidence suggests that oxidative and nitrosative stress-induced mitochondrial dysfunction contributes to peripheral and central sensitization. Owing to the abundance of phospholipids and axonal mitochondria, mammalian nerves are particularly vulnerable to free radicals, both oxygen (ROS) and nitrogen (RNS). Animal studies on antioxidant supplementation indicate that hydroxyl radicals (OH), superoxide (O_2□), and nitric oxide (NO) may play a role in peripheral sensitization through detrimental effects on lipids and nucleic acids, protein carbonylation, and subsequent involvement of organelles and antioxidant enzymes (Carrasco et al. 2018). Moreover, ROS have been identified as signaling molecules in various cellular processes, including proliferation, survival, and antioxidant gene regulation (Gupta et al. 2014). The inflammatory response is further exacerbated by an increase in free radicals. Conversely, antioxidant supplementation in animal models has been shown to mitigate peripheral sensitization induced by ROS (Jiménez-Cabrera et al. 2021). The anti-radical properties of any drug may thus play an essential role in reducing inflammatory nociception (Szandruk-Bender et al. 2020).

Celecoxib and two of its derivatives were found to exert a considerable analgesic effect in a formalin test conducted in rats by Lu et al. (2015). In both the initial amd secondary stages of the formalin test, intraperitoneally administered metamizole demonstrated dose-dependent antinociception in mice (Beirith et al. 1998). Hunskaar and Hole (1987) discovered that morphine, codeine, nefopam, and orphenadrine, as centrally acting analgesics, displayed antinociception in both phases of the formalin test in mouse trials. Glucocorticosteroids like dexamethasone and hydrocortisone, along with indomethacin and naproxen, inhibited paw licking only in the second phase, whereas aspirin and paracetamol displayed antinociceptive effects in both phases. In our previous investigations of novel pyrrolic compounds, 2-[3-acetyl-5-(4-chlorophenyl)-2-methyl-pyrrol-1-yl]-3-(1H-indol-3-yl)-propionic acid demonstrated antinociceptive activity in both phases of the formalin test (Zlatanova et al. 2019). In formalin experiments, Szandruk-Bender et al. (2020) illustrated that pyrrolo[3,4-d]pyridazinone derivatives produce dose-dependent antinociceptive action. Arylated 5-hydroxy-pyrrol-2-ones also demonstrated a pronounced antinociceptive effect in both phases of the formalin test (Lattmann et al. 2017). Compounds 1c, 2b and 2c exhibited antinociceptive properties in both phases of the formalin model, which aligns with the hypothesized peripheral action of the experimental substances. Given that the aforementioned compounds, along with 1b and 2a, demonstrated antinociceptive effects in the late phase of the formalin test, it is plausible to hypothesize that they might also possess anti-inflammatory properties.

The inflammation induced by carrageenan is acute, non-immune, extensively studied, and easily reproducible. Carrageenan-induced edema in rats and acute exudative inflammation in humans have similar vascular and cellular responses. This inflammation has two distinct phases: vascular and cellular (Kostadinov et al. 2014). Bradykinin, serotonin, histamine, and many proinflammatory cytokines (TNF-α, IL-1β) are involved in vasodilation and early extravasation. Prostanoids and kinins play crucial

roles in the cellular phase, which typically occurs around 4 hours after intraplantar administration carrageenan (Meshram et al. 2016). Eicosanoids stimulate neutrophil chemotaxis and the synthesis of elastase, collagenase, and other compounds, leading to the breakdown of structural proteins into peptides. Consequently, vascular permeability and hydrostatic pressure rise, causing edema and neutrophil migration to the injured tissue (Fachini-Queiroz et al. 2012). COX-2 is essential for cytokine synthesis and the release of prostanoid mediators. The inhibition of carrageenan-induced inflammation serves as an indicator of anti-inflammatory drug activity in human inflammatory diseases, with NSAID doses in this model correlating well with their effective dose in patients (Morris, 2004). Unlike indomethacin, selective COX-2 inhibitors such as celecoxib and rofecoxib exhibit weak anti-inflammatory action after a single dosage but significantly reduce carrageenan edema in experimental settings (Pinheiro and Calixto 2002). However, Francischi et al. (2002) found limited anti-edematogenic efficacy of selective COX-2 inhibitors in the carrageenan model of inflammation. Maddila et al. (2016) discovered considerable anti-inflammatory action in a carrageenan inflammatory model in an experimental study of 1,3,4-thiadiazoles with a pyrrole nucleus. Mohamed et al. (2010) tested pyrrole derivatives for anti-inflammatory activity and found results comparable to ibuprofen, used as a positive control. In comparable tests of novel pyrrole compounds, Lessigiarska et al. (2005) and Bijev et al. (2006) discovered that these compounds significantly decreased paw edema, exhibiting anti-inflammatory effects even greater than indomethacin. Biava et al. (2008) discovered that orally administered 1,5-diapyrrole derivatives exhibited strong anti-inflammatory effect in a carrageenan model of inflammation, with complete remission of edema observed one hour after administration. Previous studies by our team revealed similar findings regarding novel pyrrolic compounds. For instance, 2-[3-Acetyl-5-(4-chlorophenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid exhibited a significant anti-inflammatory effect, close to that of diclofenac, particularly following long-term treatment (Zlatanova et al. 2018). However, in the current study's inflammatory model, none of the tested drugs demonstrated antiphlogistic efficacy.

The release of oxygen-free radicals is a major contributor to the development of acute inflammation (El-Shitany and Eid 2019). Oxidative stress can induce inflammation by activating several transcription factors, leading to variable gene expression (Ahmed et al. 2020). ROS and RNS are highly reactive free radicals that interact with biological macromolecules, resulting in DNA, protein, and lipid damage. Oxidants activate the redox-sensitive NF-kB signaling pathway, which promotes the expression of cell adhesion receptors, chemokines, and proinflammatory cytokines, all contributing to free radical generation and the perpetuation of inflammation (Matsuyama et al. 2021). Mehrzadi et al. (2021) demonstrated that Zingerone, a bioactive molecule derived from ginger root, reduced paw edema in carrageenan-injected rats. Zingerone boosted both enzymatic and nonenzymatic antioxidants in the paw, while also reducing levels of malondialdehyde (MDA), NO, COX-2, PGE2, TNF-α, and IL-1. These findings suggest that Zingerone may offer clinical benefits in managing inflammation due to its antioxidant and anti-inflammatory properties.

Based on the outcomes observed in the formalin model, we hypothesize that our novel pyrrolic compounds exert peripheral antinociceptive effects and influence the inflammatory phase due to their antioxidant properties. In our previous studies, these six compounds were evaluated for their antimycobacterial activity, corresponding drug-like properties, and potential toxicity risks. The preliminary *in vitro* tests registered encouraging anti-*Mycobacterium tuberculosis* activity (Georgieva et al. 2017). The involvement of oxidative stress in infection remains poorly understood. Patients with tuberculosis (TB) often exhibit elevated levels of oxidative stress markers and depletion of antioxidants such as vitamin C, vitamin E, and glutathione (Vijayamalini and Manoharan 2004). A TB guinea pig model investigation revealed the presence of oxidative stress markers alongside increased oxidant-mediated lung and spleen lesions. N-acetyl cysteine (NAC) supplementation in *M. tuberculosis*-infected guinea pigs partly restored serum total antioxidant capacity, leading to reduced lung and spleen bacterial levels and decreased lesion necrosis (Matsuyama et al. 2021). We hypothesize that the potential antioxidant properties of the compounds were also responsible for their antimycobacterial effects. Therefore, our next research endeavors will focus on elucidating the alleged antioxidant activity of the compounds and exploring additional models based on peripheral antinociceptive processes.

Conclusions

The six novel pyrrolic compounds demonstrate analgesic effects against chemical stimuli in experimental conditions, impacting both peripheral and inflammatory nociceptive responses. However, they do not exhibit anti-inflammatory activity or antinociceptive properties against thermal stimuli after short-term administration. We postulate that these compounds possess antioxidant properties. Therefore, our upcoming research endeavors will concentrate on investigating the alleged antioxidant activity of the compounds, as well as exploring further models based on peripheral antinociceptive processes.

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