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Original Research Article

Analgesic properties of newly synthesized N-pyrrolyl hydrazide hydrazones

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Abstract

Purpose: *To screen a series of newly synthesized N-pyrrolyl hydrazide hydrazones for analgesic activity via Paw-pressure (PP) test and hot plate test (HPT).*
Methods: *The compounds newly synthesized through the classical Paal-Knor cyclization, N-pyrrolyl hydrazide-hydrazones were administered intraperitoneally at a dose of 20 mg/kg. Paw pressure and hot plate tests were applied to assess the analgesic properties. In addition, stress-induced analgesia with naloxone as a non-selective opioid receptor antagonist was performed.*
Results: *The compound (DI-5g), containing an izatine carbonyl fragment, was the most promising. It presented the highest paw pressure threshold (25 AU at 30th min) by exceeding the analgesic activity of the referent metamizole (23 AU at 30th min). The relative effect from the hot plate test was consistent with the paw pressure results. Opioid receptors were involved in the analgesic activities of N-pyrrolyl hydrazide-hydrazones.*
Conclusion: *The N-pyrrolyl carboxylic acids are synthesized and identified as new compounds and their hydrazide-hydrazone derivatives as promising leads for future design and synthesis of drugs with possibly prolonged analgesic activity.*

Keywords: *Analgesic activity, Pyrrole, Hydrazides, Hydrazones*

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INTRODUCTION

Pain represents a subjective unpleasant sensation usually associated with actual or potential tissue damage. Even without visible morphological changes, the unpleasant sensory and emotional experience of pain impairs quality of life, with expensive socio-economic consequences, understanding the nature and treatment of acute and chronic pain underlay numerous research with multidisciplinary approaches.

Effective treatment of pain fails due to the potential adverse effects of the different analgesics such as gastrointestinal toxicity, hepatotoxicity, and renal insufficiency [1]. This determines the current increased interest in

developing new molecules with identified analgesic effects, combined with decreased toxicity and based on the pyrrole moiety [2].

Pyrrole is a heterocyclic ring template with multiple pharmacophores determining its application as a platform in the generation of a large library of lead molecules. Owing to its pharmacological profile, pyrrole and its analogues have attracted much attention [2]. This fundamental moiety appears in a huge number of chemical and therapeutic agents and natural products. Some of the most traded pyrrole-based pharmaceuticals are Zomepirac, Elopiprazole, Pyrvinium, Chlorfenapyr, Obatoclax, and Atorvastatin [3].

Pyrrole and its derivatives are also constituents in the structure of a variety of natural polymers, dyes, and larger aromatic rings [4,5]. Some of these compounds are major intermediates in the synthesis of biologically important and naturally-occurring bile pigments, coenzymes, and alkaloids [6].

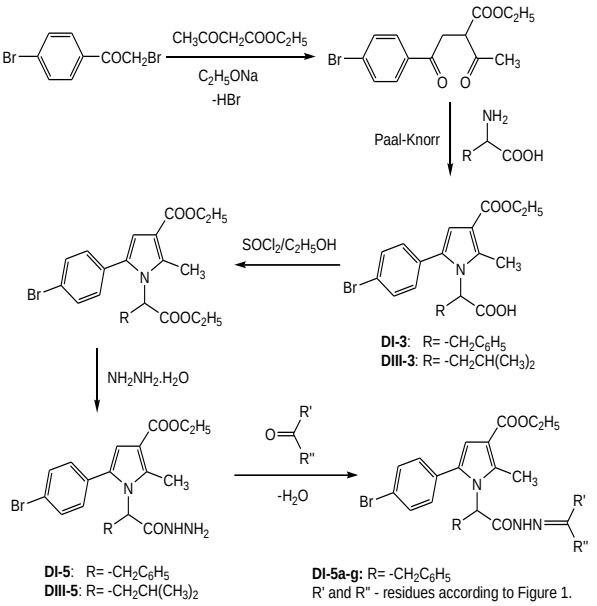
Available literature presents a wide range of various biological effects of pyrrole heterocyclic derivatives, including anti-inflammatory [7], antidepressant [8], antipsychotic [9], antituberculosis [10], antihypertensive [11], anticoagulant [12], antiviral [13], antimicrobial [14], anticonvulsant [15], anticancer [16] and analgesic [17] activities.

The present study was focused on determination of analgesic properties of newly synthesized N-pyrrolyl hydrazide hydrazones through two methods Paw-pressure test and hot plate test. In addition, an attempt to identify the effects of the tested substances on the opioid receptors was also performed.

EXPERIMENTAL

Chemistry and synthesis of N-pyrrolyl hydrazide-hydrazones

The necessary chemicals and reagents were purchased via Merck (Darmstadt, Germany) and are of synthetic grade. The synthesis was performed according to the procedures earlier described [18,19]. The procedure is based on Paal-Knorr reaction of 1,4-dicarbonyl compound from the interaction of 4-bromoacetophenone and ethyl acetoacetate in sodium ethylate media, as pointed out in reaction 1 of Scheme 1. The next step was the preparation of the target N-pyrrolyl carboxylic acids (**DI-3** and **DIII-3**) using the classical Paal-Knorr cyclization. The desired hydrazides (**DI-5** and **DIII-5**) were synthesized through selective hydrazinolysis of the corresponding ethyl ester. All compounds were synthesized according to the procedure presented in Scheme 1.



Scheme 1: Pathway used in synthesis of the tested compounds

The last synthetic reaction in Scheme 1 describes the formation of the targeted hydrazones, through condensation of the compound obtained in the previous step (hydrazide **DI-5)** with a series of carbonyl derivatives, as presented in Figure 1 to give the target hydrazones (**DI-5a-g)**.

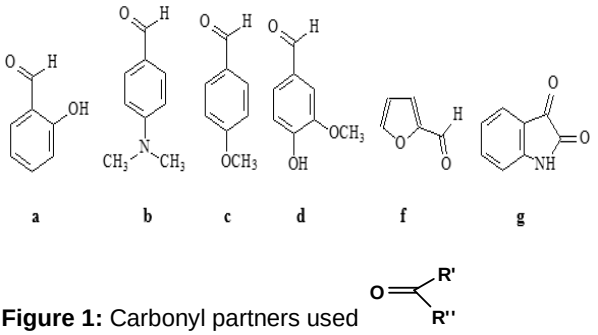


Figure 1: Carbonyl partners used

The structures of the synthesized compounds were determined by spectral methods, including IR, ^{1}H- and ^{13}C-NMR, and MS. The purity of the compounds was elucidated through TLC characteristics, melting points and elemental analyses. The detailed description of the data has been previously reported [18,19].

Animals

The experiments were carried out on male Wistar rats (180 - 200 g) obtained from the National

Breeding Center, Sofia, Bulgaria. The rats were housed individually in polypropylene boxes with free access to fresh drinking water and standard pelleted food suitable for their age. Constant ambient temperature environment (22 **±** 2 ^{o}C), humidity of 72 ± 4 % and a 12/12 light/dark cycle was maintained. Seven days of acclimatization was allowed before the commencement of the study and a veterinary physician regularly monitored the animals' health and Vivarium (certificate of registration of farm No. 0072/01.08.2007) was inspected by the Bulgarian Drug Agency to check the husbandry conditions (№ A-11-1081/03.11.2011). All experiments were carried out between 10:00 a.m. and 1:00 p.m. and according to the requirements of the Bulgarian Food Safety Agency (BFSA) (Ethical clearances no. 187 and No. 190) and the international principles as stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123) [9].

Preparation of drug solutions and administration procedures

All compounds were dissolved in 0.9 % NaCl and were administered intraperitoneally at a dose of 20 mg/kg.

The effects of the newly synthesized N-pyrrolyl carboxylic acids (**DI-3** and **DIII-3**) N-pyrrolyl hydrazides (**DI-5** and **DIII-5**) and N-pyrrolyl hydrazide-hydrazones **DI-5a-g** were compared to a reference. In the current study, as positive reference was used Metamizole (dipyrone)- the broadly known painkiller, applied in therapy as spasm- and fever reliever with additional anti-inflammatory effects.

Assessment of pain perception

Paw-pressure test (PP)

The changes in the mechanical nociceptive threshold of the rats were measured with an analgesiometer (Ugo Basile). Increasing pressure was applied to the hind-paw and the value (in arbitrary units, AU) required to elicit a nociceptive response (a squeak or struggle) was taken as the mechanical nociceptive threshold. A cut-off value (24 AU = 480 g/cm^2) was maintained in order to prevent damage to the paw.

Hot plate test (HP)

The latency of response to pain was measured from the moment an animal was placed on a metal plate heated to 55 ± 0.5 ^{o}C to the first signs of pain (paw licking, jumping) [20]. A cut-off time of 30 s was marked.

Statistical analysis

The results were evaluated through one-way analysis of variance ANOVA followed by students *t*-test comparison. Values are presented as mean ± SEM. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Based on the structural characteristics of the newly synthesized pyrrole-based molecules, two groups were formed. **Group I** contain the initial N-pyrrolyl carboxylic acids **DI-3** and **DIII-3** and the corresponding N-pyrrolyl hydrazides **DI-5** and **DIII-5**. **Group II** formed by the derived N-pyrrolyl hydrazones **DI-5a-g.** In addition, the compounds forming Group **II** were separated depending on the type of the carbonyl partner to Subgroup 1 - containing benzaldehyde and substituted benzaldehyde fragments (**DI-5a-d**) and Subgroup 2 containing selected heterocyclic fragments (**DI-5f-g**).

Analgesic effects of compounds from Group I

The results from evaluation of analgesic effect of N-pyrrolyl hydrazide-hydrazones through the paw pressure (PP) test (A) and hot plate test (HPT) (B) are pointed in Figure 2.

Figure 2 A shows that compound **DIII-5** produced progressively increasing analgesia which maximal value (on the 40th min of the experiment) reached approximately 73 % of the estimated metamizole analgesia (EMA). The results indicate that compound **DI-3** also showed increasing analgesia arising from the 30th min, and reaching approximately 73 % of EMA with maximal values on the 50th min.

On the other hand, the N-pyrrolyl carboxylic acid **DIII-3** showing maximal analgesia of approximately 73 % of EMA on the 20th min, and hydrazide **DI-5** showing maximal analgesia of approximately 49 % of EMA on the 30th min, may be considered as short lasting analgesics.

In addition, a hot plate test (HPT) was applied as second test for estimation of analgesic effect. The results for the compounds from **Group I** are presented on Figure 2 B. The results from both tests are consistent.

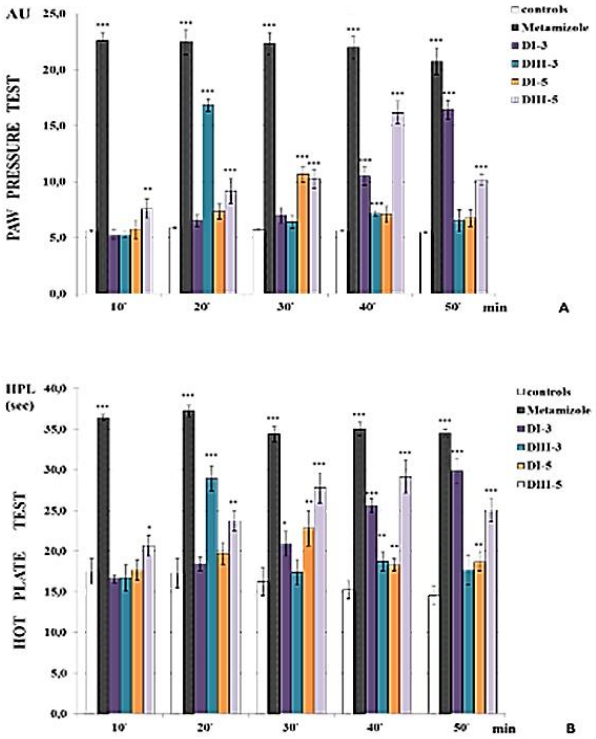


Figure 2: Analgesic activity of newly synthesized N-pyrrolyl carboxylic acids (**DI-3** and **DIII-3**) and N-pyrrolyl hydrazides (**DI-5** and **DIII-5**), estimated by (A) PP test: Paw pressure thresholds are presented in arbitrary units (AU) as mean ± SEM, compared to controls ***p < 0.001; **p < 0.01, and (B). HP test: Hot plate latencies (HPL) are presented in seconds (sec) as mean ± SEM, ***p < 0.001; **p < 0.01; *p < 0.05 compared to controls

Analgesic effects of compounds from Group II

The results of the PP test of the derivatives in Subgroup 1 of **Group II** compounds are presented in Figure 3. The results indicated that compound **DI-5c** showed the highest analgesic activity of approximately 93 % of estimated metamizole analgesia (EMA) in the 10th min and 77 % of EMA in the 20th min. **DI-5a** and **DI-5b** led to analgesia (compared to controls) during the time of the experiment, with **DI-5a** producing the higher (and constant) value approximately 62 % of EMA. The graphical representation of the effectivity of **DI-5d** follows a biphasic-shaped curve with peaks on the 10th (approximately 52 % of EMA) and on the 40th (approximately 41 % of EMA) min of the experiment.

The results of the second test (HPT) (not shown) were consistent with the results presented in Figure 3, with **DI-5c** showing highest analgesic properties. The results from the PP test of the derivatives included in Subgroup 2 of **Group II** are presented in Figure 4, with **DI-5g** performing higher analgesic activity compared to metamizole on the 20th min, and even higher on the 30th min. Both **DI-5g** and Metamizole showed the highest analgesic activity amplitudes on the 30th min after application, slowly decreasing (but not disappearing) after that period.

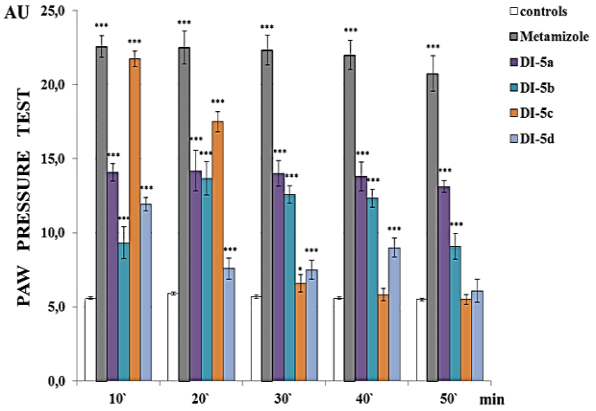


Figure 3: Comparison of analgesic properties of newly synthesized N-pyrrolyl hydrazones containing substituted aromatic fragment (**DI-5a-d**), estimated by PP test. Paw pressure thresholds are presented in arbitrary units (AU) as mean ± SEM. ***p < 0.001; *p < 0.05 vs controls

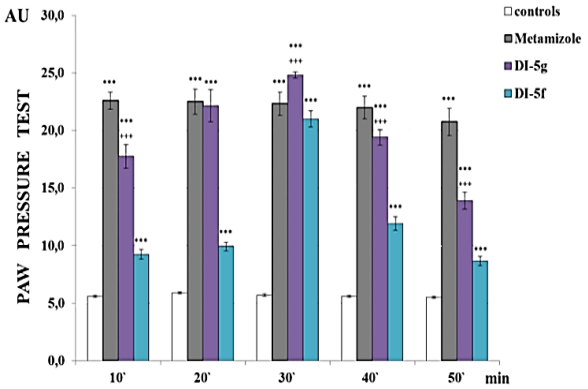


Figure 4: Analgesic activity of newly synthesized N-pyrrolyl hydrazones containing heterocyclic fragments (**DI-5f-g**), estimated via PP test. Paw pressure thresholds are presented in arbitrary units (AU) as mean ± SEM. ***P < 0.001 vs. control; ^{+++}p < 0.001 vs. metamizole

The results from HP test were concordant with the performance determined through the PP test confirming that from the discussed Subgroup 2 of **Group II**, **DI-5g** is the most promising derivative.

Stress-induced analgesia (SIA)

Stress-induced analgesia (SIA) depends on two components – opioid and non-opioid. For the most promising derivatives, the involvement of the opioid receptors was estimated via pretreatment with the non-selective opioid receptor antagonist; Naloxone (1 mg/kg, i.p.).

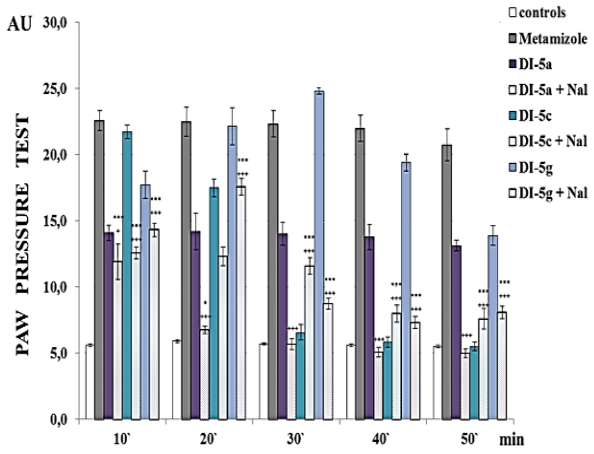


Figure 5: Analgesic property of **DI–5a**, **DI-5c**, and **DI-5g** after Naloxone pretreatment, estimated via PP test. Paw pressure thresholds are presented in arbitrary units (AU) as mean ± SEM, with ***p < 0.001, *p < 0.05 vs controls and ^{+++}p < 0.001, ^{+}p < 0.05 vs metamizole

The most significant analgesic effects throughout the experiment with highest threshold of 25 AU occurred for compound **DI-5g** at 30th min, slowly decreasing but not disappearing by the end of the evaluation.

DISCUSSION

The chemical structure of newly synthesized compounds is determined by the presence of two essential functional groups in the alkyl spacer between the carboxyl group at compounds **DI-3**, and additional phenyl radical in the spacer and **DIII-3** with branched alkyl chain in the spacer. These discrepancies in addition determine the same structural differences in the corresponding hydrazides (**DI-5** and **DIII-5)** and the related hydrazones, identified as **DI-5a-g**. These differences in structure of the compounds are also responsible for the probable analgesic effects they exhibit and the influence of aromatic moiety on them. Thus, N-pyrrolyl carboxylic acids, hydrazides, and hydrazide-hydrazones obtained were subjected to PP and HP tests to evaluate their analgesic properties [20].

The estimation of analgesic effect of compounds included in **Group I** showed that N-pyrrolyl carboxylic acids and their hydrazides exhibited different degrees and duration of analgesia compared to metamizole, used as a control. The results indicated that the duration of effect is closely related to the availability of carboxylic group and the presence of an additional aromatic ring in the spacer between the free carboxylic group and the pyrrole ring [18,19]. It was observed that derivatives containing branched alkyl chains in spacers show faster and shorter analgesic effect with **DIII-3** manifesting higher activity at 20th min while the compounds with an additional phenyl in spacer showed prolonged effect with **DI-3** expressing its highest effect at the 50th min. It is worth mentioning that the introduction of a hydrazine group does not change the analgesic properties significantly.

The corresponding N-pyrrolyl hydrazones containing substituted aromatic fragments (**DI-5a-d**) also led to different degrees and duration of analgesia when compared to metamizole. The presence of a –OCH_3 group at the 4th position in the carbonyl fragment (**DI-5c**) produced the highest analgesic effect, compared to that of metamizole at 10th min of application, which decreased at 30th min after which it was kept relatively constant. The removal of this substituent (**DI-5a**) or its replacement (**DI-5b** and **DI-5d**) resulted in a decrease in analgesic effect.

On the other hand, the introduction of salicylic aldehyde fragment in the carbonyl part of the structure produced a relatively constant analgesic effect throughout the experiment. The assessment of the analgesic properties of Subgroup 2 compounds (**DI-5f** and **DI-5g**, from **Group II)**, revealed comparable analgesic effect from both representatives, and metamizole. Subgroup 2 compounds are relatively better analgesics than Subgroup 1, with izatine containing **DI-5g** exhibiting the most promising analgesic properties.

In order to identify the reason for analgesic effect of the N-pyrrolyl hydrazide-hydrazones, the compounds were subjected to a stress-induced analgesic activity evaluation using Naloxone as a non-selective opioid receptor antagonist to induce a decreased analgesic effect. The application of Naloxone led to a decrease in the paw pressure threshold on the 10th min of the experiment to a total decline at the end. The results indicated that the type of carbonyl fragment is a determining factor for the performance of an opioid effect, with compound **DI-5g** appearing as the most promising analgesic with activity towards opioid receptors.

CONCLUSION

Newly synthesized pyrrole-based carboxylic acids, hydrazides and hydrazones demonstrates promising analgesic effects with hydrazone **DI-5g** (containing izatine as a carbonyl fragment) possessing the highest analgesic property. A possible mechanism for their analgesic activities is the effect of the tested molecules on the opioid receptors. Pyrrole-based carboxylic acids and

their derivatives are promising lead compounds for the future design and synthesis of drugs with possibly prolonged analgesic activity.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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