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Antinociceptive screening of various 1,2,4-triazole-3-thione derivatives in the hot-plate test in mice

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Abstract

Introduction. Despite the large number of analgesic drugs available currently, pain therapy is still a challenging issue for researchers and clinicians. The search for new drugs that could relieve patients from pain is not only justified, but also highly recommended.

Objective. This study aimed to perform antinociceptive screening of 4 various 1,2,4-triazole-3-thione derivatives (TPB-2, TPB-4, TPF-32 and TPF-38) in the hot-plate test in mice, which is an experimental model allowing the testing of compounds alleviating acute thermal pain.

Materials and method. Experimental verification of the antinociceptive effects of the tested compounds (administered intraperitoneally in a constant dose of 300 mg/kg) was performed in the hot-plate test in mice, by calculating maximum possible antinociceptive effects (MPAE in %) at 4 various pretreatment times (15, 30, 60 and 120 min.).

Results. TPB-2 exerted strong antinociceptive effects with MPAE ranging between 18.54 – 35.43% in the hot-plate test. Similarly, TPF-32 exerted firmly established antinociceptive effects with MPAE ranging from 13.50 – 37.05%. In the case of TPB-4 and TPF-38, both compounds produced slight changes in MPAE in the hot-plate test in mice. These agents can be classified as virtually ineffective in the hot-plate test.

Conclusions. The screening test revealed that TPB-2 and TPF-32 exerted a clear-cut antinociceptive effect in the hot-plate test in mice. If the results from this study were to be translated to clinical settings, both TPB-2 and TPF-32 might be beneficial drugs for pain relief in humans.

Key words

4-triazole-3-thione derivative, hot-plate test, maximum possible antinociceptive effect

INTRODUCTION

Experimental evidence indicates that antiepileptic drugs (AEDs) are a very specific group affecting the central nervous system (CNS). In spite of their anticonvulsant properties, the AEDs also exert antidepressant, antiproliferative and analgesic effects in humans [1–4]. The analgesic effects of AEDs are clearly seen during therapy with tiagabine, gabapentin and pregabalin, because these drugs produce both the anticonvulsant and analgesic effects in patients [5, 6].

At present, the search for novel drugs affecting CNS relies on three main methods. The first focuses on the screening of thousands of newly-synthesized compounds in the hope of finding the most promising and efficacious agent [7–9]. The second method is based on structural transformation and chemical modification of the structure of widely used drugs with firmly established properties with respect to their impact on CNS *in vivo* [10–12]. The changes in chemical structure of currently available drugs are expected to enhance their desired properties. The third method is based on the detection of agents isolated from medicinal plants, which are used by traditional folk medicine to treat some specific illness and diseases [13–15]. All three methods have their opponents and adherents. However, one can also distinguish a fourth method combining all three mentioned-above methods. Thus, isolation of agents from medicinal plants, accompanied by chemical modification of their core structure to enhance their properties, along with preclinical screening of their efficacy, may play a principal role in the search for novel drugs affecting CNS.

Quite recently, a novel group of compounds (i.e., 1,2,4-triazole-3-thione derivatives) has gained attention as potential anticonvulsant drugs in preclinical studies [16–23]. Molecular studies have revealed that agents comprising the 1,2,4-triazole-3-thione structure exerted anticonvulsant effects by affecting $GABA_A$ receptors and blocking sodium channels in neurons [18, 21, 22]. On the other hand, drugs influencing $GABA_A$ receptors and blocking sodium channels possess the antinociceptive properties in both preclinical studies and clinical settings [24–28]. Previously, it has been demonstrated that some 4-substituted derivatives of 5-(4-chlorophenyl)-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione produced the antinociceptive effects in the hot-plate test in mice [29].

Considering the above-mentioned facts, it was of importance to conduct preclinical screening to discover whether or not some other 1,2,4-triazole-3-thione derivatives

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produce antinociceptive properties in mice subjected to the hot-plate test, which is considered a model of acute thermal pain in experimental studies on animals.

MATERIALS AND METHOD

Screening of the antinociceptive effects of four various 1,2,4-triazole-3-thione derivatives was conducted on adult male Swiss mice (weighing 22 – 26 g), maintained under standardized housing and laboratory conditions. Each experimental group in the screening test comprised four randomly selected mice. Experimental procedures involving animals were approved by the Local Ethics Committee and complied with the ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments. Only 64 mice were used in the screening study.

Four various 1,2,4-triazole-3-thione derivatives [5-[(3-chlorophenyl)ethyl]-4-(n-buthyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (TPB-2), 5-[(3-chlorophenyl) ethyl]-4-(n-hexyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (TPB-4), 4-(n-butyl)-5-[(3-fluorophenyl)ethyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (TPF-32), and 5-[(3-fluorophenyl) ethyl]-4-isopropyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (TPF-38)], in one fixed dose of 300 mg/kg, were used. All the tested compounds were suspended in a 1% aqueous solution of Tween 80 (Sigma, Poznań, Poland) and administered intraperitoneally (i.p.) in a volume of 0.01 ml/g of body weight. The compounds were administered at four pretreatment times: 15, 30, 60 and 120 min. before the measurement of the antinociceptive effects in the hot-plate test. These pretreatment times were chosen based upon information about their biological activity from the literature and previous studies by the authors of this study[18].

Hot-plate test. To detect the antinociceptive effects of the tested 1,2,4-triazole-3-thione derivatives with respect to acute thermal nociception, the hot-plate test in mice was used, as described elsewhere [28, 30–33]. The apparatus consisted of an electrically-heated surface and an open Plexiglas tube (17 cm high × 22 cm diameter) to confine the mice to the heated surface (Ugo Basile, Varese, Italy). Each mouse was placed separately on the heated surface (55.0 ± 0.1 °C), and the time period between placement and a shaking, licking, or tucking of the fore- or hind-paws, was recorded by a stopwatch. This period of time (in seconds) was considered a predrug latency response, which served as the control reaction time for each animal.

Subsequently, the animals were administered the 1,2,4-triazole-3-thione derivatives in a fixed-dose of 300 mg/kg. Next, the mice were placed again on the heated surface at four various pretreatment times (i.e., 15, 30, 60 and 120 min. after the 1,2,4-triazole-3-thione derivatives administration). Notably, each animal was challenged with the hot-plate test twice. To prevent thermal injury to animals in the hot-plate test, a maximum cut-off time of 30 seconds was chosen. Mean maximum possible antinociceptive effect (MPAE) values (± S.E.M.) were calculated according to the formula presented elsewhere [34]. Graphical presentation of the results was performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Effects of four various 1,2,4-triazole-3-thione derivatives on the antinociception in the hot-plate test in mice. TPB-2 administered i.p. in a constant dose of 300 mg/kg, at various pretreatment times before the acute thermal pain test, exerted an antinociceptive effect in mice, and the experimentally-derived MPAE ranged from 18.54% – 35.43% (Fig. 1A). The time to peak of the antinociceptive effect for TPB-2 was established at 30 and 60 min. after the drug i.p. administration. Similarly, the experimentally-derived values of MPAE for TPF-32 were between 13.50% and 37.05% (Fig. 1C) and the time to peak-effect was clearly observed at 15 min. after drug administration (Fig. 1C). In the case of TPB-4 and TPF-38, the tested compounds exerted weak antinociceptive effects in the hot-plate test, because their MPAE values ranged from 2.76% – 6.71% (for TPB-4) and 1.42% to 3.23% (for TPF-38), respectively (Fig. 1B, 1D). The time to peak of the anticonvulsant effects for both agents (TPB-4 and TPF-38) was observed at 15 min. after their i.p. administration (Fig.1B,1D).

DISCUSSION

The results obtained in this study confirmed the authors' hypothesis that some 1,2,4-triazole-3-thione derivatives possess the antinociceptive properties in the hot-plate test in mice. Evaluation of MPAE in animals receiving the tested compounds allowed determination not only of the antinociceptive effects of the compounds in *in vivo* model of acute thermal pain, but also the time to peak of the antinociceptive effects in the animals. In this screening test, four 1,2,4-triazole-3-thione derivatives were selected and, by comparing their chemical structure, it was evident that some structural modifications significantly affected and changed the antinociceptive properties of the tested agents, especially, if one compared TPF-32 with TPF-38 (active vs. virtually inactive compound). The most effective compounds exerting strong antinociceptive effects in this study were those containing 4-(n-buthyl)- substituent (TPB-2 and TPF-32).

On the contrary, neither 4-(n-hexyl)- substituent in TPB-4, nor 4-isopropyl- substituent in TPF-38 exerted firmly defined antinociceptive effects in the hot-plate test in mice. It seems that compounds containing 4-(n-buthyl)- structure incorporated into the core of 1,2,4-triazole-3-thione should produce antinociceptive effects. However, this hypothesis should be verified in further experimental studies in various nociceptive models in mice.

The results also confirmed a general hypothesis that an agent possessing the anticonvulsant properties can also produce antinociceptive effects in experimental animals. Previously, it has been documented that tiagabine, gabapentin, pregabalin, and vigabatrin (the second- and third-generation AEDs) exerted antinociceptive properties and prolong the time to the first pain reaction in animals exposed to the heated surface of the hot-plate test [28, 31–33, 35]. It has also been documented that some 4-substituted derivatives of 5-(4-chlorophenyl)-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione, including 2,4-dichlorophenyl- (T-100); 4-chloro-(3-trifluoromethyl)-phenyl- (T-102); 3,4-dichlorophenyl- (T-103); 3-chlorophenyl- (T-104);

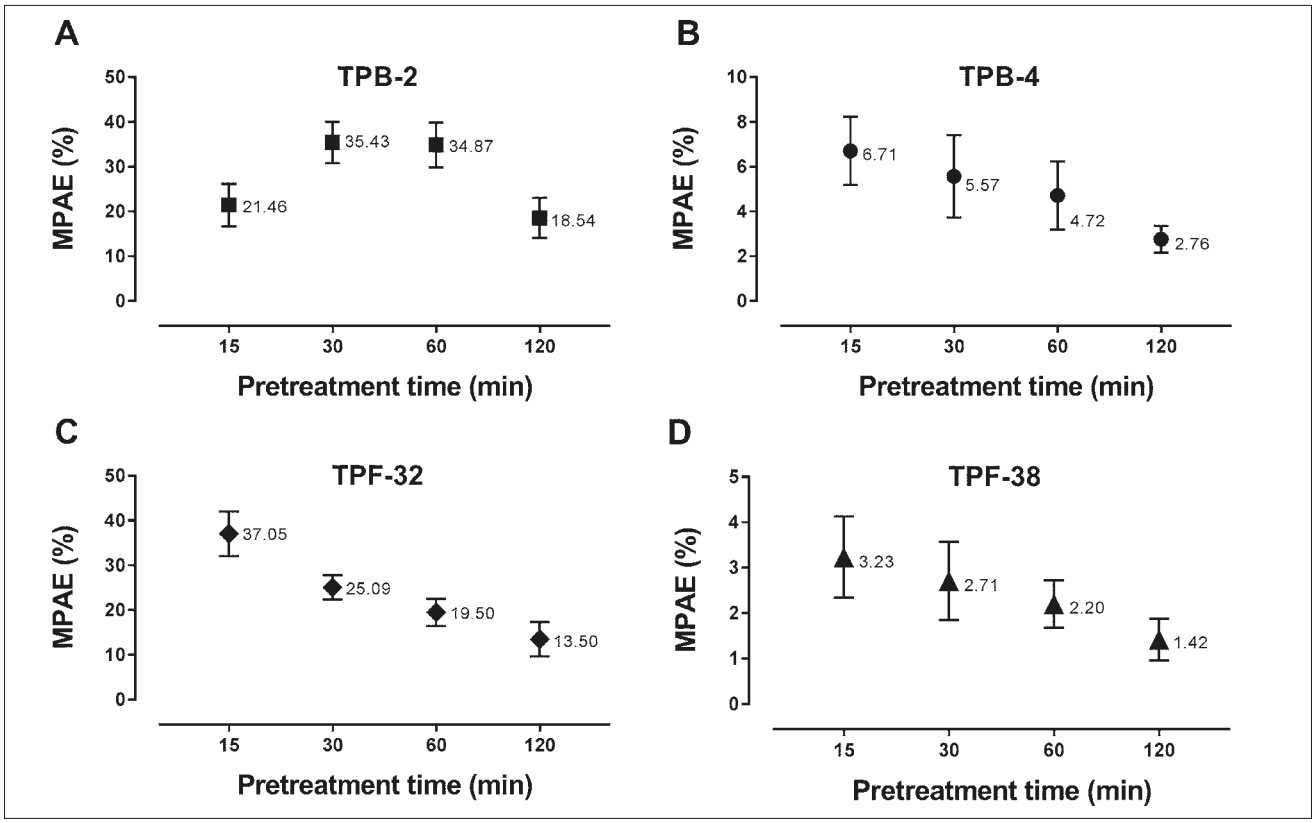

Figure 1A-1D. Effects of various 1,2,4-triazole-3-thione derivatives on antinociception in the hot-plate test in mice.
The antinociceptive effects exerted by four 1,2,4-triazole-3-thione derivatives were transformed to the maximum possible antinociceptive effect (MPAE in % ± S.E.M. as the error bars, n = 4). Four various pretreatment times of the tested compunds (15, 30, 60 and 120 min.) were studied. All the screened compounds (TPB-2, TPB-4, TPF-32 and TPF-38) were administered i.p. at a fixed dose of 300 mg/kg.

and 4-bromophenyl- (T-101), exerted the antinociceptive effects in the hot-plate test in mice [29]. It was also observed that the time to peak of the anticonvulsant effects for the five derivatives was established at 60 min. after their i.p. administration [29]. The hot-plate test is commonly used experimentally, especially, when one can screen various compounds to discover whether or not these compounds exerted antinociception in mice [36, 37].

Limitations of the study. The main limitation in this study was the small number of tested mice in each experimental group. In this screening test, only four mice per group were used, and during evaluation of the antinociceptive effects, four various pretreatment times (15, 30, 60 and 120 min.) were applied. This unique approach, on the one hand, allowed determination of the time to peak of the antinociception, but on the other hand, the S.E.M. values of MPAE were high, and resulted from the range diversity of the four values obtained from the four mice in each group.

The screening test was conducted in a specific manner because the same animals were tested twice, i.e., before administration of the 1,2,4-triazole-3-thione derivatives (pre-test), and at the respective pretreatment times after i.p. injection of the tested compounds (post-test). This experimental paradigm eliminated the control (naïve) animals in order not to expose the animals to unnecessary pain and suffering, which is in agreement with the 3R rules (Replacement, Reduction, Refinement) when conducting experiments on animals [38].

CONCLUSIONS

In conclusion, TPB-2 and TPF-32 produced the antinociceptive effects in mice with the peak of the antinociceptive effects established at 30 min. and 15 min. after drug administration, respectively. Although the antinociceptive effects of TPB-4 and TPF-38 were observed in the hot-plate test, their antinociceptive strength (power) was insufficient to classify them as antinociceptive agents. This was the reason that both TPB-4 and TPF-38 were considered as virtually inactive in the hot-plate test in mice. The screening of various novel compounds in the hot-plate test allowed the selection of the most active compounds offering antinociception in experimental animals. If the results from this screening test were to be translated to clinical settings, TPB-2 and TPF-32 might be favourable for pain relief in patients.

REFERENCES

1. Cooper TE, Wiffen PJ, Heathcote LC, Clinch J, Howard R, Krane E, et al. Antiepileptic drugs for chronic non-cancer pain in children and adolescents. Cochrane Database Syst Rev. 2017;8:Cd012536.
2. Chen B, Choi H, Hirsch LJ, Katz A, Legge A, Buchsbaum R, et al. Psychiatric and behavioral side effects of antiepileptic drugs in adults with epilepsy. Epilepsy Behav. 2017; 76: 24–31.
3. Abaza MS, Bahman AM, Al-Attiyah RJ. Valproic acid, an anti-epileptic drug and a histone deacetylase inhibitor, in combination with proteasome inhibitors exerts antiproliferative, pro-apoptotic and chemosensitizing effects in human colorectal cancer cells: underlying molecular mechanisms. Int J Mol Med. 2014; 34: 513–532.
4. Rizzo A, Donzelli S, Girgenti V, Sacconi A, Vasco C, Salmaggi A, et al. In vitro antineoplastic effects of brivaracetam and lacosamide on human glioma cells. J Exp Clin Cancer Res. 2017; 36: 76.

5. Derry S, Bell RF, Straube S, Wiffen PJ, Aldington D, Moore RA. Pregabalin for neuropathic pain in adults. Cochrane Database Syst Rev. 2019; 1: Cd007076.
6. Todorov AA, Kolchev CB, Todorov AB. Tiagabine and gabapentin for the management of chronic pain. Clin J Pain. 2005; 21: 358–361.
7. Abdel Salam OI, Al-Omar MA, Khalifa NM, Amr Ael G, Abdallah MM. Analgesic and anticonvulsant activities of some newly synthesized trisubstituted pyridine derivatives. Z Naturforsch C. 2013; 68: 264–268.
8. Al-Omar MA, Amr Ael G, Al-Salahi RA. Anti-inflammatory, analgesic, anticonvulsant and antiparkinsonian activities of some pyridine derivatives using 2,6-disubstituted isonicotinic acid hydrazides. Archiv der Pharmazie. 2010; 343: 648–656.
9. Said SA, Amr Ael G, Sabry NM, Abdalla MM. Analgesic, anticonvulsant and anti-inflammatory activities of some synthesized benzodiazipine, triazolopyrimidine and bis-imide derivatives. Eur J Med Chem. 2009; 44: 4787–4792.
10. Brenneman DE, Smith GR, Zhang Y, Du Y, Kondaveeti SK, Zdilla MJ, et al. Small molecule anticonvulsant agents with potent in vitro neuroprotection. J Mol Neurosci. 2012; 47: 368–79.
11. King AM, De Ryck M, Kaminski R, Valade A, Stables JP, Kohn H. Defining the structural parameters that confer anticonvulsant activity by the site-by-site modification of (R)-N'-benzyl 2-amino-3-methylbutanamide. Journal of medicinal chemistry. 2011; 54: 6432–6442.
12. Sysak A, Obminska-Mrukowicz B. Isoxazole ring as a useful scaffold in a search for new therapeutic agents. Eur J Med Chem. 2017; 137: 292–309.
13. Mandegary A, Sharififar F, Abdar M. Anticonvulsant effect of the essential oil and methanolic extracts of Zataria multiflora Boiss. Cent Nerv Syst Agents Med Chem. 2013; 13: 93–7.
14. Ya'u J, Yaro AH, Malami S, Musa MA, Abubakar A, Yahaya SM, et al. Anticonvulsant activity of aqueous fraction of Carissa edulis root bark. Pharmaceut Biol. 2015; 53: 1329–1338.
15. Goel RK, Gawande D, Lagunin A, Randhawa P, Mishra A, Poroikov V. Revealing medicinal plants that are useful for the comprehensive management of epilepsy and associated comorbidities through in silico mining of their phytochemical diversity. Planta Med. 2015; 81: 495–506.
16. Flieger J, Kowalska A, Pizon M, Plech T, Luszczki J. Comparison of mouse plasma and brain tissue homogenate sample pretreatment methods prior to high-performance liquid chromatography for a new 1,2,4-triazole derivative with anticonvulsant activity. J Sep Sci. 2015; 38: 2149–2157.
17. Flieger J, Pizon M, Plech T, Luszczki JJ. Analysis of new potential anticonvulsant compounds in mice brain tissue by SPE/HPLC/DAD. J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 909: 26–33.
18. Kapron B, Luszczki J, Paneth A, Wujec M, Siwek A, Karcz T, et al. Molecular mechanism of action and safety of 5-(3-chlorophenyl)-4-hexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione – a novel anticonvulsant drug candidate. Int J Med Sci. 2017; 14: 741–749.
19. Luszczki JJ, Plech T, Wujec M. Influence of 5-(3-chlorophenyl)-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione on the anticonvulsant action of 4 classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Pharmacol Rep. 2012; 64: 970–978.
20. Luszczki JJ, Plech T, Wujec M. Effect of 4-(4-bromophenyl)-5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione on the anticonvulsant action of different classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Eur J Pharmacol. 2012; 690: 99–106.
21. Plech T, Kapron B, Luszczki JJ, Paneth A, Siwek A, Kolaczkowski M, et al. Studies on the anticonvulsant activity of 4-alkyl-1,2,4-triazole-3-thiones and their effect on GABAergic system. Eur J Med Chem. 2014; 86: 690–699.
22. Plech T, Kapron B, Luszczki JJ, Wujec M, Paneth A, Siwek A, et al. Studies on the anticonvulsant activity and influence on GABA-ergic neurotransmission of 1,2,4-triazole-3-thione-based compounds. Molecules. 2014; 19: 11279–11299.
23. Plech T, Luszczki JJ, Wujec M, Flieger J, Pizon M. Synthesis, characterization and preliminary anticonvulsant evaluation of some 4-alkyl-1,2,4-triazoles. Eur J Med Chem. 2013; 60: 208–215.
24. Azmi S, ElHadd KT, Nelson A, Chapman A, Bowling FL, Perumbalath A, et al. Pregabalin in the management of painful diabetic neuropathy: a narrative review. Diabetes Ther. 2019; 10: 35–56.
25. Chincholkar M. Analgesic mechanisms of gabapentinoids and effects in experimental pain models: a narrative review. Br J Anaesth. 2018; 120: 1315–1334.
26. Smith MD, Woodhead JH, Handy LJ, Pruess TH, Vanegas F, Grussendorf E, et al. Preclinical comparison of mechanistically different antiseizure, antinociceptive, and/or antidepressant drugs in a battery of rodent models of nociceptive and neuropathic pain. Neurochem Res. 2017; 42: 1995–2010.
27. Luszczki JJ, Kolacz A, Czuczwar M, Przesmycki K, Czuczwar SJ. Synergistic interaction of gabapentin with tiagabine in the formalin test in mice: an isobolographic analysis. Eur J Pain. 2009; 13: 665–672.
28. Luszczki JJ, Kolacz A, Wojda E, Czuczwar M, Przesmycki K, Czuczwar SJ. Synergistic interaction of gabapentin with tiagabine in the hot-plate test in mice: an isobolographic analysis. Pharmacol Rep. 2009; 61: 459–467.
29. Listos J, Talarek S, Orzelska J, Fidecka S, Wujec M, Plech T. The antinociceptive effect of 4-substituted derivatives of 5-(4-chlorophenyl)-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thion e in mice. Naunyn Schmiedebergs Arch Pharmacol. 2014; 387: 367–375.
30. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther. 1953; 107: 385–393.
31. Luszczki JJ. Dose-response relationship analysis of pregabalin doses and their antinociceptive effects in hot-plate test in mice. Pharmacol Rep. 2010; 62: 942–948.
32. Luszczki JJ, Florek-Luszczki M. Synergistic interaction of pregabalin with the synthetic cannabinoid WIN 55,212-2 mesylate in the hot-plate test in mice: an isobolographic analysis. Pharmacol Rep. 2012; 64: 723–732.
33. Luszczki JJ, Czuczwar SJ. Dose-response relationship analysis of vigabatrin doses and their antinociceptive effects in the hot-plate test in mice. Pharmacol Rep. 2008; 60: 409–414.
34. Schmauss C, Yaksh TL. In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther. 1984; 228: 1–12.
35. Luszczki JJ, Czuczwar SJ. Isobolographic characterization of interactions between vigabatrin and tiagabine in two experimental models of epilepsy. Prog Neuropsychopharmacol Biol Psychiatry. 2007; 31: 529–538.
36. Bannon AW, Malmberg AB. Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Curr Protoc Neurosci. 2007; Chapter 8: Unit 8.9. https://doi.org/10.1002/0471142301.ns0809s41.
37. Dhouibi R, Moalla D, Ksouda K, Ben Salem M, Hammami S, Sahnoun Z, et al. Screening of analgesic activity of Tunisian Urtica dioica and analysis of its major bioactive compounds by GCMS. Arch Physiol Biochem. 2018; 124: 335–343.
38. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 2010; 160: 1577–1579.