Research Article

Antinociceptive Activity of Thymoquinone and its Structural Analogues: A Structure-Activity Relationship Study

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Abstract

Purpose: To investigate the structural features that influence the antinociceptive activity of thymoquinone and their structural analogues.

Methods: The quinones were prepared by an oxidation procedure using molecular oxygen and catalysis with [Co^{III}salen)] from the respective phenols. The antinociceptive activity of para-benzoquinones (10 mg/kg, ip) was evaluated using formalin test in mice. Vehicle (5 % Tween 80) or morphine (10 mg/kg) were used as control group and standard drug, respectively. The amount of time spent licking the injected paw was considered as the nociceptive response.

Results: Among the compounds tested, five para-benzoquinones showed antinociceptive activity. The 2-isopropyl-para-benzoquinone presented the highest potency in first and second phases and produced a near-maximal inhibition (p < 0.001) in the formalin test, similar to morphine (p < 0.001).

Conclusion: Our experimental results show that by appropriate structural modification of para-benzoquinones it may be possible to develop novel analgesic drugs.

Keywords: Quinones, Antinociceptive activity, Analgesic, Central nervous system, Structure-activity relationship, Medicinal plant.

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INTRODUCTION

Although a considerable number of analgesic drugs are available for the treatment of pain, medicinal plants are widely investigated as a rich source of natural components capable of exerting peripheral and central analgesic activity, but with the potential to be devoid of undesirable side effects and addictive properties displayed by conventional analgesic drugs. Furthermore, some chemical constituents of medicinal plants are candidates for analgesic drugs [1,2].

Para-benzoquinones are potentially derivable by oxidation of suitable phenolic compounds. Many of these *para*-benzoquinones have important biochemical functions in electron transport systems for respiration or photosynthesis. Natural products containing the *para*-benzoquinone sub-structural unit are many, for example, vitamins K_1 and K_2, co-enzyme Q (ubiquinone), and also occur in many terpenes [3].

The pharmacological properties attributed to naturally occurring quinones are thus well established. For example, thymoquinone, the principal active constituent of *Nigella sativa* seeds, presents antinociceptive activity [4]. Therefore, these facts led us to compare the antinociceptive activity of six structurally-related *para*-benzoquinones prepared via oxidation reactions.

EXPERIMENTAL

Solvents and reagents were used as received from the manufacturer, or purified, when required, by standard procedures. The six phenols and N,N′-bis(salicylidene) ethylenediaminocobalt(II)[Co(salen)] employed were commercially available products (Aldrich) and used as obtained

General procedure for metal complex-catalyzed oxidation

Phenol (1.0 mmol) was dissolved in dimethylformamide (DMF, 5 mL) and oxygen was bubbled into the reaction mixture for a few minutes by way of a rubber septum and a syringe needle, and then an oxygen atmosphere was maintained with a balloon by filling a toy balloon, and coupling to the same syringe inlet. Co(salen) catalyst (0.0195 g, 0.06mmol) was added, and the reaction mixture stirred at room temperature for 3 h. More of the catalyst (0.0195 g, 0.06 mmol) was added and the reaction mixture stirred for another 3 h at room temperature. The process was repeated once more, for a total addition of 0.18 mmol (0.0585 g) catalyst and a total reaction time of 24 h. Ether (20 mL) was added and the black mixture washed with 0.1 mol·L^{-1} HCl (2 × 10 mL), water and brine. The ethereal solution was dried over anhydrous magnesium sulfate ($MgSO_4$) and the solvent evaporated. The residue was purified by sublimation (or by flash column chromatography when necessary, using as eluent a mixture of 9:1 hexane:ethyl acetate) [5,6].

Nuclear Magnetic Resonance (NMR) and melting point determinations

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 NMR spectrometer at 400 and 100 MHz respectively. Chemical shifts were expressed in ppm downfield from a tetramethylsilane internal standard. Melting point was determined on a Micro Química apparatus (model APF 301) and are uncorrected.

Animals

Male Swiss mice (28 – 34 g) were obtained from the vivarium Prof. Dr. ThomasGeorge of LTF/UFPB, in which they were created. The animals were maintained at constant room temperature (23 ± 1 ^{o}C) and on a 12/12 h light-dark cycle (light from 06:00 to 18:00), with free access to food and water. All behavioral observations were conducted between 08:00 and 12:00 h and the study was approved by the Ethics Committee for the Care and Use of Animals of the Federal University of Paraiba (Comitê de Ética em

Pesquisa Animal do Laboratório de Tecnologia Farmacêutica - CEPA Nº 0202/08) [7].

Formalin test

The animals received an injection of 20 µl of 2.5 % formalin (0.92 % formaldehyde diluted in normal saline) in the subplantar area of the right hind paw. The duration of paw licking was measured in the periods 1 - 5 min (first phase) and 15 - 30 min (second phase) after the formalin injection. The amount of time spent licking the injected paw was considered as the nociceptive response. Animals were divided into eight groups (*n* = 8) and subjected to intraperitoneal (ip) administration of *para*-benzoquinones **1-6** (10 mg/kg, ip), vehicle (5 % Tween 80) or morphine (10 mg/kg), respectively, 30 min prior to the injection of formalin [8,9].

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA), followed by Tukey's multiple comparison test. A probability level of 0.05 was regarded as significant..

RESULTS

The results in respect of the prepared quinones are shown in the Scheme 1 and Table 1. while antinociceptive activity data are presented in Figures 1 and 2.

1 (30%)   **2** (54%)   **3** (34%)

4 (95%)   **5** (90%)   **6** (93%)
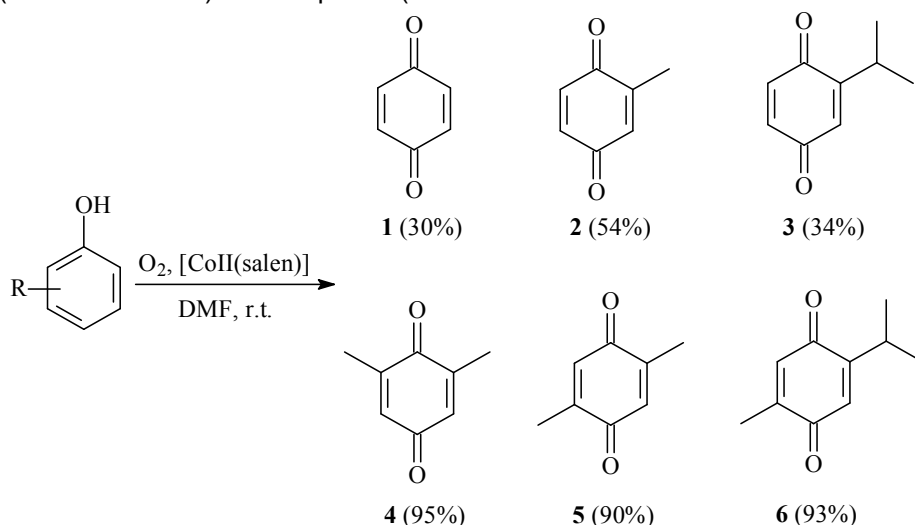
Scheme 1: Oxidation of mono-phenols to *para*-benzoquinones

DISCUSSION

Previous studies showed that *Nigella sativa* seeds oil and its major component, thymoquinone, have significant antinociceptive activity [4]. Assessment of this pharmacological activity with regard to *para*-benzoquinones using formalin test in mice was investigated in the present work. This test is a model of acute and tonic pain, being considered a more valid model for clinical pain than tests based on mechanical or thermal stimulation [8].

The first phase results from direct chemical stimulation of the nociceptive afferent fibers, mainly C fibers, and the release of substance P [14], and may be inhibited by centrally acting analgesics such as morphine. The second phase is a consequence of the action

Table 1: Characterization data of prepared compounds

Compound	Physical and spectral data
1	Yield: 30%; Mp ^{o}C: 110-114; Lit. Mp ^{o}C: 113-115 [10]; CAS 106-51-4; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 6.79 (4H, s); ^{13}C NMR (CDCl$_3$/TMS) δ: 136.6, 187.2
2	Yield: 54%; Mp ^{o}C: 65–68; Lit. Mp ^{o}C: 67-70 [11]; CAS 553-97-9; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 1.90 (3H,s), 6.62 (1H, s), 6.75 (1H, d, *J*=10.1 Hz), 6.77 (1H, d, *J*=10.1 Hz); ^{13}C NMR (CDCl$_3$/TMS) δ: 15.8, 133.3, 136.4, 136.5, 145.9, 187.5, 187.7
3	Yield: 34%; Mp ^{o}C: 57-60; Lit. Mp ^{o}C: 54-58 [10]; CAS 15232-10-7; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 1.14 (6H, d, *J*=6.8 Hz), 3.05 (1H, dhept, *J*=6.8; 1.1 Hz), 6.55 (1H, d, *J*=1.1 Hz), 6.73 (1H, d, *J*=2.5 Hz), 6.74 (1H, d, *J*=2.5 Hz); ^{13}C NMR (CDCl$_3$/TMS) δ: 16.5, 26.2, 130.3, 135.9, 137.0, 154.9, 187.1, 188.1
4	Yield: 95%; Mp ^{o}C: 69–72; Lit. Mp ^{o}C: 71–73 [12]; CAS 527-61-7; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 2.10 (6H, d, *J*=1.0 Hz), 6.56 (2H, q, *J*=1.0 Hz); ^{13}C NMR (CDCl$_3$/TMS) δ: 15.9, 133.3, 145.7, 187.1
5	Yield: 90%; Mp ^{o}C: 121-123; Lit. Mp ^{o}C: 124–125 [13]; CAS 137-18-8; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 2.04 (6H, d, *J*=1,6Hz), 6.60 (2H, q, *J*=1,6Hz); ^{13}C NMR (CDCl$_3$/TMS) δ: 15.6, 133.3, 145.7, 188.7
6	Yield: 93%; Mp ^{o}C: 47–48; Lit. Mp ^{o}C: 45–47 [5]; CAS 490-91-5; ^{1}H NMR (CDCl$_3$/TMS) δ, *J*(Hz): 1.13 (6H, d, *J*=6.8Hz), 2.04 (3H, d, *J*=1.6Hz), 3.03 (1H, d hept, *J*=1.2 and 6.8Hz), 6.52 (1H, d, *J*=1.2Hz), 6.59 (1H, q, *J*=1.6Hz); ^{13}C NMR (CDCl$_3$/TMS) δ: 15.3, 21.4, 26.5, 130.3, 133.8, 145.1, 154.9, 187.4, 188.5
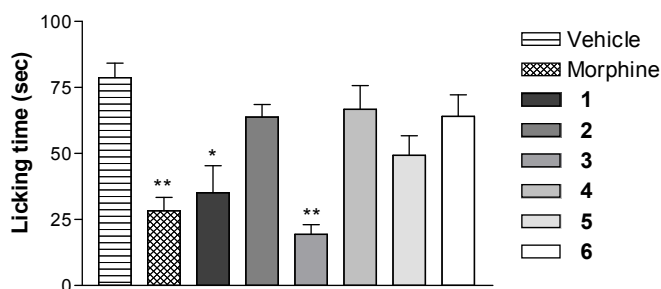
Fig 1: Effect of *para*-benzoquinones 1-6 (10 mg/kg, ip) and morphine (10 mg/kg, ip) on licking time (formalin test) during the First Phase in mice. The values represent mean ± S.E.M. (*n* = 8); *$p < 0.01$, **$p < 0.001$ significantly different from control (vehicle)
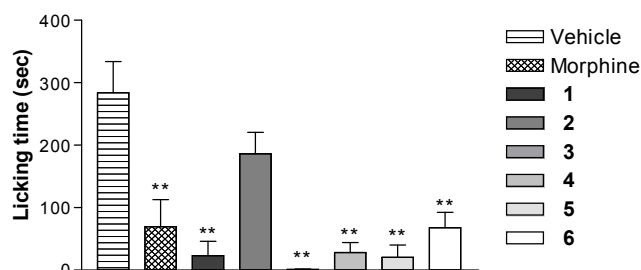
Fig 2: Effect of *para*-benzoquinones **1-6** (10 mg/kg, ip) and morphine (10 mg/kg, ip) on licking time (formalin test) during the Second Phase in mice. The values represent mean ± S.E.M. (*n* = 8); *$p < 0.01$, **$p < 0.001$ significantly different from control (vehicle)

of inflammatory mediators released locally, such as prostaglandins, serotonin, histamine, and bradykinin [15,16] and also from enhanced synaptic transmission in the spinal cord [17].

Among the compounds tested, five *para*-benzoquinones were found to have antinociceptive activity (Figure 2). The 2-isopropyl-*para*-benzoquinone (3) (isopropyl group at carbon 2), (p < 0.001), was the compound that presented the highest potency (Figure 1) and has effect in both phases. These results show that the mode of action of 3 is via central and peripheral pathways. In fact, this benzoquinone produced a near-maximal inhibition on the formalin test (p < 0.001), similar to morphine (p < 0.001) on second phase. The unsubstituted *para*-benzoquinone (1) (no alkyl groups) exhibited a similar effect to 3, but was less potent in the first phase (p < 0.01). The other quinones were inactive in this phase. These data demonstrate that the absence of alkyl groups or the presence of a bulky alkyl group in the structure of *para*-benzoquinones results in psychoactive compounds with antinociceptive activity. Interestingly, only compound 2 was inactive in the second phase. The other *para*-benzoquinones showed significant antinociceptive effect (p < 0.001). Therefore, *para*-benzoquinones 4, 5, and 6 are compounds that act peripherally, at a dose of 10 mg/kg.

CONCLUSION

An attempt has been made in this study to understand the structural relationship between *para*-benzoquinones and their analgesic-like effects. The results suggest that by appropriate structural modification of *para*-benzoquinones, it may be possible to develop novel analgesic drugs

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