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# Anticonvulsant activity of thymoquinone and its structural analogues

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**Abstract:** It has been widely reported that the crude oil of *Nigella sativa* L., Ranunculaceae, seeds and its major chemical component thymoquinone present anticonvulsant activity. These facts led us to verify the pharmacological potential of five structurally related *para*-benzoquinones on the pentylenotetrazol-induced seizures model, and establish the structural characteristics that influence the anticonvulsant activity of thymoquinone. The unsubstituted *para*-benzoquinone was the compound that exhibited the highest potency, while 2-methyl-*p*-benzoquinone was inactive. It was found that the presence of alkyl groups attached to the ring influence the pharmacological activity of the *para*-benzoquinones. In addition, the number, position, and size of these groups change the anticonvulsant potency of the compounds.

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## Introduction

Diseases of the central nervous system are amongst the most common ailments afflicting mankind. Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures (Engel, 1995) and up to 5% of the world population develops epilepsy in their lifetime (Sander & Shorvon, 1996). There appear to be many causes for epilepsy, but epileptic seizures are characterized by simultaneous firing of cortical neurons. Therapeutic use of antiepileptic drugs has focused on lowering $Na^+$, $K^+$, or $Ca^{2+}$ flux in neurons, inhibiting glutamate (Glu) neurotransmission, or promoting γ-aminobutyric acid (GABA) activity at $Cl^-$ channels (Clement et al., 2004). Several studies have shown the anticonvulsant activity of natural products (Almeida et al., 2003). A significant number of herbal medication and dietary supplements are used for treating patients with neurological or psychiatric complaints. Some of these products may be anticonvulsant and thus of possible benefit in patients with epilepsy. There are more studies that report anti-seizure activity of a non-allopathic preparation with animal models of epilepsy (Tyagi & Delanty, 2003). Evidence for the pharmacological effect of these components on experimental tests has been provided (De Sousa et al., 2006; 2007; De Almeida et al., 2008).

Quinones are ubiquitous in nature and constitute an important class of naturally occurring compounds found in plants, fungi, and bacteria. Current human exposure to quinones occurs via the diet as well as clinically. Benzoquinones are potentially derivable by oxidation of suitable phenolic compounds. Many of these benzoquinones have important biochemical functions in electron transport systems for respiration or photosynthesis. Natural products frequently occur containing the benzoquinone sub-structural unit within the global structure, as can be exemplified by vitamins $K_1$ and $K_2$, co-enzyme Q (ubiquinone), and in many terpenes (Dewick 2001). The pharmacological properties attributed to naturally occurring quinones are thus well established. For example, thymoquinone, the principal active constituent of *Nigella sativa* seeds, presents anticonvulsant activity in the petit mal epilepsy (Hosseinzadeh & Parvardeh, 2004). *N. sativa* has been traditionally used as a natural remedy for a number of illnesses and conditions such as diabetes, inflammation, bronchitis, fever, and influenza (Ali & Blunden, 2003). Mechanism of action studies have shown that this quinone exerts its anticonvulsant activity through the stimulation of opioid receptors in the central nervous system (Hosseinzadeh et al., 2005). These facts led us to verify the pharmacological potential of

five structurally related *para*-benzoquinones on the pentylenotetrazol (PTZ)-induced seizures model, and establish the structural characteristics that influence the anticonvulsant activity of thymoquinone.

## Materials and Methods

### *General*

$^{1}H$ and $^{13}C$ NMR spectra were obtained on a Bruker DRX-400 spectrometer at 400 and 100 MHz respectively. Chemical shifts are quoted in ppm downfield from a tetramethylsilane internal standard. Melting points were determined on a Micro Química model APF 301 apparatus and are uncorrected. Solvents and reagents were used directly from the manufacturer, or purified when required by standard procedures. The six phenols and [$Co^{II}$(salen)] are commercially available products (Aldrich) and used as obtained.

### *General procedure for the metal complex catalyzed oxidations*

The phenol (1.0 mmol), was dissolved in DMF (5 mL), and oxygen was bubbled into the reaction mixture for a few minutes, and then an oxygen atmosphere was maintained with a balloon. The $Co^{II}$(salen) catalyst (6% mol) was added, and the reaction mixture stirred at room temperature for 3 h. Further catalyst (6% mol) 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 18% mol catalyst, and a total reaction time of 24 h. Ether (20 mL) was added and the black mixture washed with 0.1 $molL^{-1}$ HCl (2× 10 mL), water and brine. The ethereal solution was dried over anhydrous $MgSO_4$ and the solvent evaporated. The residue was usually purified by sublimation (or by flash column chromatography when necessary, using as eluent a mixture of 9:1 hexane:ethyl acetate) (Dockal et al. 1985; Uliana et al. 2008), and the corresponding *para*-benzoquinones 1-4 were obtained with 30-95% yield (Table 1).

### *Animals*

Male Swiss mice (28-34 g) were obtained from our research animal facility. The animals were maintained at constant room temperature (23+1 °C) and on a 12/12 h light-dark cycle (light from 6 a.m. to 6 p.m.), with free access to food and water. All behavioral observations were conducted between 8:00 and 12:00 h and approved by the Institution's Ethics Committee for the Care and Use of Animals.

### *PTZ-induced convulsions*

Mice were divided into eight groups (n=8). The control and positive control groups received 5% Tween 80 or diazepam (DZP) (4 mg/kg, *i.p.*), respectively. The remaining groups received an injection of the benzoquinones at dose of 80 mg/kg, *i.p.* Thirty minutes after drug administration, the mice were treated with PTZ (*i.p.*) at a dose of 60 mg/kg and observed for at least 15 min to detect the occurrence of the first episode of forelimb clonus (Swinyard et al., 1989).

### *Statistical Analysis*

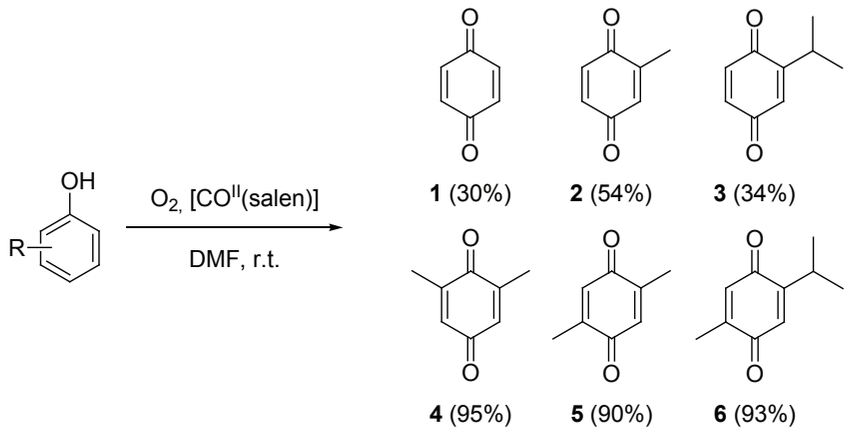
The statistical analysis was performed using analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A probability level of 0.05 was regarded as significant.

## Results and Discussion

*Nigella sativa* L., Ranunculaceae, seeds have been used for thousands of years as a protective and curative remedy for numerous disorders. It has been demonstrated that the crude oil prepared from the seeds, and its major chemical component thymoquinone, exhibit anticonvulsant activity (Ilhan et al., 2005; Hosseinzadeh & Parvardeh, 2004; Hosseinzadeh et al., 2005). Beneficial interaction of thymoquinone and sodium valproate in experimental models of epilepsy was demonstrated. This benzoquinone combined with sodium valproate increases its antiepileptic response (Raza et al., 2006). Considering the importance of benzoquinones as candidates for anticonvulsant drugs, we report in this comparative study, the findings from the assessment of the anticonvulsant activity of thymoquinone and their structural analogues using PTZ-induced seizures model.

The quinones were synthesized using as starting materials a non-substituted phenol, three di-alkyl-substituted phenols, and two mono-alkyl-substituted phenols with standard $O_2$ and [$Co^{II}$(salen)]. Table 1 and Scheme 1 shows the products of the oxidation of the phenols against the metal-ligand catalyst. The *para*-benzoquinone products are all known compounds, and identification was performed by comparison of NMR spectral data, as well as melting point determinations.

Among the compounds tested, five *para*-benzoquinones were found to have anticonvulsant action (Figure 1). The unsubstituted *para*-benzoquinone **1** (no alkyl groups) ($p<0.001$), was the compound that presented the highest potency. This result shows that the absence of alkyl groups increase the anticonvulsant activity of *para*-benzoquinones. In fact, there was a significant decrease of pharmacological activity in the other para-benzoquinones with regard to compound **1**. Comparing 2-methyl-*p*-benzoquinone **2** (methyl




**Scheme 1.** Oxidation of mono-phenols to *para*-benzoquinones.

**Table 1.** Characterization data of prepared compounds

| Compound | Physical and spectral data |
|---|---|
| *p*-Benzoquinone (**1**) | Yield: 30%; Mp 110-114 °C; Lit. Mp: 113-115 °C (Acros Organics, 2007/2008); CAS 106-51-4 °C; $^1$H NMR ($CDCl_3$/TMS) δ 6.79 (4H, s); $^{13}$C NMR ($CDCl_3$/TMS): δ 136.6, 187.2. |
| 2-Methyl-*p*-benzoquinone (**2**) | Yield: 54%; Mp 65-68 °C; Lit. Mp 67-70 °C (Saladino et al., 2002); CAS 553-97-9; $^1$H NMR ($CDCl_3$/TMS): δ 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. |
| 2-Isopropyl-*p*-benzoquinone (**3**) | Yield: 34%; Mp 57-60 °C; Lit. Mp: 54-58 °C (Acros Organics, 2007/2008); CAS 15232-10-7; $^1$H NMR ($CDCl_3$/TMS): δ 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. |
| 2,6-Dimethyl-*p*-benzoquinone (**4**) | Yield: 95%; Mp 69-72 °C; Lit. Mp 71-73 °C (Barton et al., 1988); CAS 527-61-7; $^1$H NMR ($CDCl_3$/TMS): δ 2.10 (6H, q, *J*=1.0 Hz), 6.56 (2H, d, *J*=1.0 Hz); $^{13}$C NMR ($CDCl_3$/TMS): δ 15.9, 133.3, 145.7, 187.1. |
| 2,5-Dimethyl-*p*-benzoquinone (**5**) | Yield: 90%; Mp 121-123 °C; Lit. 124-125 °C (Adam et al., 1994); 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. |
| Thymoquinone **(6)** | Yield: 93%; Mp 47-48 °C; Lit. 45-47 °C (Dockal et al., 1985); CAS 490-91-5; $^1$H NMR ($CDCl_3$/TMS): δ 1.13 (6H, d, *J*=6.8Hz), 2.04 (3H, d, *J*=1.6Hz), 3.03 (1H, d hept, *J*=1.2 e 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. |

group at carbon 2) and compound **1**, it is shown that the presence of a methyl group induces the loss of anticonvulsant activity. Interestingly, 2-isopropyl-*p*-benzoquinone **3** was bioactive ($p$<0.01). This result suggests that bulky groups are important to preserve the pharmacological activity in monosubstituted para-benzoquinones. Similarly, anticonvulsant activity was maintained in both the dimethyl-*p*-benzoquinones **4** (methyl groups at carbons 2 and 6), and **5** (methyl groups at carbons 2 and 5). These results show that *para*-benzoquinones with two methyl groups attached to the ring are also bioactive. However, the comparison between the pharmacological effects of compounds **4** ($p$<0.01) and **5** ($p$<0.05) shows that changing the position of these groups in the ring may alter the anticonvulsant potency. Replacing the methyl group at carbon 2 by an isopropyl group as in thymoquinone **6** ($p$<0.01), increases this activity, compared to compound 5. This effect confirms that the presence of bulky alkyl groups attached to the ring enhances the anticonvulsant activity.

Most of the *para*-benzoquinones investigated in this study present pharmacological activity close to that of diazepam ($p$<0.001), a standard anticonvulsant drug. PTZ is the prototype agent in the class of systemic convulsants, and is used as a screening test for anticonvulsant activity. The mechanism of action of PTZ is only partially understood. At a synaptic level PTZ appears to interact with the (GABA receptor-benzodiazepine-chloride ionophore) complex, decreasing the potency of inhibition and leading to seizures (Fisher, 1989). The enhancement of

neural inhibition by GABA is a common therapeutic strategy for treating CNS diseases such as sleep disturbances, muscle spasms and seizure disorders (Chebib & Johnston, 2000). Generally, compounds with anticonvulsant activity in petit mal epilepsy are effective in the PTZ-induced seizure model (Vida, 1995).

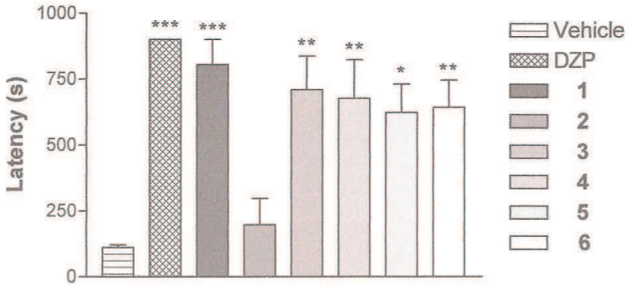



**Figure 1.** Effect of para-benzoquinones (80 mg/kg) on the latency of the first post-injection convulsion induced by pentylenetetrazol. The bars indicate mean±S.E.M. (n=8). Statistically significant differences *$p$<0.05, **$p$<0.01, ***$p$<0.001 with respect to control according to one-way ANOVA, followed by Dunnett's test.

However, using Structure-Activity Relationships knowledge, we identified structural characteristics which may contribute to the understanding of the anticonvulsant activity of quinones and their derivatives. It was found that the presence of alkyl groups attached to the ring influence the pharmacological activity of the *para*-benzoquinones. In addition, the number, position, and size of these groups change the potency of the compounds. Factors such as lipophilicity may be related to these anticonvulsant potency differences. Our experimental results also suggest that by appropriate structural modification of *para*-benzoquinones it may be possible to develop novel anticonvulsant drugs potentially suitable to control seizures.

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