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Research Article

Analgesic and Antioxidant Activities of 4-Phenyl-1,5-benzodiazepin-2-one and Its Long Carbon Chains Derivatives

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The aim of this work is to deepen the pharmacological effect of 4-phenyl-1,5-benzodiazepin-2-one derivatives which have a similar structure to nonionic surfactants: 4-phenyl-1,5-benzodiazepin-2-one is the hydrophilic head, and the carbon chain is hydrophobic tail. The antinociceptive activity of 4-phenyl-1,5-benzodiazepin-2-one derivatives was determined using acetic acid-induced writhing and tail immersion tests. In addition, the in vitro antioxidant activities of the tested derivatives were determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and ferric reducing power assay. A single oral administration of these compounds at the doses of 50 and 100 mg/kg significantly reduced the number of abdominal writhes induced by acetic acid injection. Acute pretreatment with 4-phenyl-1,5-benzodiazepin-2-one derivatives at the dose of 100 mg/kg caused a significant increase in the tail withdrawal latency in the tail immersion test. Additionally, a significant scavenging activity in DPPH and reducing power was observed in testing antioxidant assays. Finally, we carried out a study of the antioxidant activity of these derivatives. The results of this study reveal that these compounds have a low antioxidant activity compared to the BHT. It decreases with the polarity of the molecule. The present study suggests that 4-phenyl-1,5-benzodiazepin-2-one derivatives possess potent antinociceptive and antioxidant effects, which suggest that the tested compounds may be useful in the treatment of pain and oxidation disorders.

1. Introduction

Many heterocyclic organic molecules are synthesized and used in pharmaceutical industries for the preparation of new drugs. It is the case of several benzodiazepines compounds, which are prepared since decades and continue to be marketed specifically for their psychotropic effects on the central nervous system [1–3]. In addition, many researchers suggest the use of various benzodiazepines against cancer [4] and HIV infection [5] and as analgesics [6,7], anti-inflammatories [8], and antioxidant drugs [9].

It is in this context that we prepared derivatives with long carbonaceous chains, 4-phenyl-1,5-benzodiazepin-2-one, having a similar structure to nonionic surfactants and studied their cataleptic hypnotic and sedative activities on the central nervous system. The latter has led to satisfactory results [10]. With an aim of looking further into the exploration of the pharmacological effects of these compounds, we decided to continue, through this work, the study of their action on the nervous system by evaluating their peripheral and central analgesic activities in particular.

In addition, to ensure an antioxidant contribution of the body and given the important role played by these agents

against reactive oxygen species (ROS) and reactive oxygen and nitrogen species (RONS), we will evaluate the antioxidant activity of these derivatives using the DPPH and FRAP methods.

2. Materials and Methods

2.1. Chemistry

2.1.1. Choice of Products to Synthesize. Our objective is to realize a pharmacological study of 4-phenyl-1,5-benzodiazepin-2-one derivatives which have a similar structure to the nonionic surfactants. The (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones were prepared using the following reagent: 1-bromooctane, 1-bromononane, 1-bromodécane, and 1-bromododécane. Indeed, the literature reports on the one hand that the surface-active agents helps in the entrance of active ingredients of drugs in the cells by protecting them from any degradation and on the other hand decreases their toxicity [11].

2.1.2. Synthesis of 4-Phenyl-1,5-benzodiazepin-2-one and Its Derivatives. 4-Phenyl-1,5-benzodiazepin-2-one **3** was prepared by condensation of 1,2-phenylenediamine **1** with ethyl benzoylacetate **2** at xylene reflux for one hour [3]. This compound was subjected to a series of alkylation reactions, under the conditions of phase-transfer catalysis (PTC), with 1-bromo-*N*-(octane, nonane, decane, dodecane) (**4a–d)** to obtain (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** (Scheme 1).

These products are obtained according to the following processes:

4-Phenyl-1,5-benzodiazepin-2-one **3**: 0.01 mol of 1,2-phenylenediamine and 0.011 mol of ethyl benzoylacetate are poured into a 250 ml flask containing 50 ml of xylene, and the whole is left to reflux for 1 h. After the reaction and during cooling, a precipitate forms which is filtered and washed with ethanol and dried to obtain a yellowish powder. The product is obtained with a yield of 75% (mp. 196–198°C/EtOH).

(1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** were obtained by reacting, in a 100 ml flask containing 30 ml of tetrahydrofuran, 0.0021 mol of 4-phenyl-1,5-benzodiazepin-2-one, and 0.00315 mol of 1-bromoalkane in the presence of 0.0042 mol of potassium carbonate (weak base) and a pinch of tetrabutylammonium bromide (TBAB). The reaction is stirred at room temperature for 24 hours. The 1-alkyl-4-phenyl-1,5-benzodiazepin-2-one thus prepared is purified by chromatography on silica gel with the hexane/ethyl acetate eluent (80/20) in a yield of 90% for **5a**, 83% for **5b**, 78% for **5c,** and 83% for **5d**.

The spectral data for the identification of these compounds are presented in our previous publication [10].

*2.1.3. Solubility of Products (**5a–d**).* (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a-b)** are soluble in several solvents such as ethanol, methanol, dimethylformamide, tetrahydrofuran, and dimethylsulfoxide (DMSO) among others.

2.2. Evaluation of Analgesic Activity

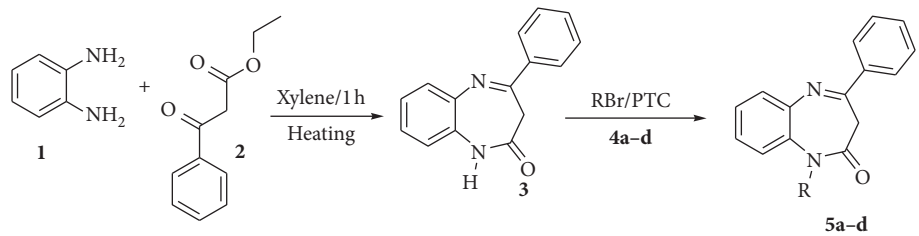
2.2.1. Animals. Mice Swiss adults (20–30 g) and rats Wistar adults were used in the work. The animals were raised in the pharmacology and toxicology laboratory, Faculty of Medicine and Pharmacy of Rabat. All animals were housed in collective cages at controlled temperature (25°C ± 2°C), relative humidity (40 and 70%), and artificially lit rooms on a cycle of 12 h of light/12 h of darkness with free access to water and a standard diet. The animals subjected to the oral administration of the studied molecules or reference drugs were fasting for 16 hours before the experiment. The animals were used in accordance with the Guidelines for the Use of Laboratory Animals [12, 13].

2.2.2. Acetic Acid-Induced Writhing Assay (Peripheral Analgesic Activity). The test is based on the induction of the cramps caused on mice by intraperitoneal injection of the acetic acid and was carried out by using the method of Koster et al. [14]. Female and male mice weighing between 20 and 30 g were divided into twelve groups ($n = 6$). The first group served as a control and received a 10% aqueous solution of arabic gum; the second group was treated with aspirin (Aspegic 100 mg) at the dose of 50 mg/kg; the third group was treated with aspirin (Aspegic 100 mg) at the dose of 100 mg/kg, and the rest of the groups received the different molecules each at 50 and 100 mg/kg, respectively. Cramps were induced by intraperitoneal injection of an acetic acid solution at 1.2% at a rate of 0.15 ml for a mouse of 20 g of body weight, 60 minutes after the administration of the tested product. The mice were placed individually in transparent cages, and the number of cramps was counted during a continuous observation of 10 min as from the 5th minute which follows the injection of acetic acid. The percentage of inhibition of abdominal cramps was calculated according to the formula below:

$$\%\ \text{inhibition} = \left(\frac{1 - \text{Nc}}{\text{Nt}}\right) \times 100, \quad (1)$$

where Nt is the number of cramps of the control group and Nc represents the number of cramps of the batch treated.

2.2.3. Tail Immersion Test (Activity Analgesic Power Station). The test was performed according to the method described by Sewell and Spencer [15]. Female and male rats (160–180 g) were used. Rats were divided into seven groups ($n = 6$). The first group served as a control and received a 10% aqueous solution of arabic gum; the second group was treated with morphine at a dose of 1 mg/kg, and the rest of the groups received orally the different molecules at the dose of 100 mg/kg. We performed this work at the dose 100 mg/kg because it is at this dose that we recorded better results for peripheral analgesic activity. 4 cm of tail of the animal was immersed in hot water (50°C). The time passed between the immersion of the tail, and the deflection of the tail was recorded at 30 min,



Scheme 1

60 min, and 120 min after the treatment by morphine or of 4-phenyl-1,5-benzodiazepin-2-one derivatives by using a digital stop watch. A time of cut of 10 s was maintained to avoid the tissue lesions of the tail in the rodents.

2.3. Evaluation of the Antioxidant Activity. The antioxidant effect of 4-phenyl-1,5-benzodiazepin-2-one and its derivatives was performed according to the DPPH test and the FRAP test described below and the readings using the 6705 UV/Vis Spectrophotometer.

2.3.1. DPPH Protocol. The ability of our 4-phenyl-1,5-benzodiazepin-2-ones derivatives to trap the free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was estimated according to the method described by Sahin et al. [16]. In this test, the 4-phenyl-1,5-benzodiazepin-2-ones derivatives reduce the radical DPPH whose color is violet in a yellowish compound, the diphenylpicrylhydrazine, whose intensity of the color is inversely proportional to the reducing capacity of the antioxidant agents. Dilutions of each molecule are carried out using dimethylsulfoxide (DMSO), and the study is carried out according to the following protocol: 50 μL of various concentrations of the samples of the products studied are added to 2 ml of a methanolic solution of DPPH with 60 μM at a rate of 0.0023%. The reaction mixture is well-stirred and homogenized and then incubated for 20 min at room temperature in complete darkness. After this incubation, the absorbance fading relative to the negative control containing only the DPPH solution is measured at the wavelength of 517 nm. For every sample, the experience was repeated three times in the same experimental conditions. The percent inhibition of DPPH (%I) is calculated by the following formula [17]:

$$\%I = \left(\frac{1 - A_e}{A_b}\right) \times 100, \quad (2)$$

where A_b represents the absorbance of the negative control and A_e is the absorbance of the sample.

The concentration of a molecule responsible for 50% inhibition of the DPPH (IC_{50}) is determined using the graph representing the percent inhibition as a function of the concentrations. The BHT is used as a reference in this work.

2.3.2. FRAP Protocol. The test is based on the reduction of Fe^{3+} present in the potassium ferricyanide complex $K_3Fe(CN)_6$ to Fe^{2+}. The reducing capacity of 4-phenyl-1,5-benzodiazepin-2-one and derivatives is studied according to the method described by Oyaizu [18]. Initially, 0.2 ml of each compound was mixed with various studied concentrations, 2.5 ml of a buffer solution phosphates (0.2 M, pH = 6.6), and 2.5 ml of ferricyanide of potassium $K_3Fe(CN)_6$ to 1%. The whole is incubated in the hot bath at 50°C for 20 min. During this time of incubation, the reaction of reduction of Fe^{3+} to Fe^{2+} of each sample proceeds perfectly. Then, after 20 min of incubation (50°C), the reaction is stopped by adding of 2.5 ml of 10% trichloroacetic acid. The different samples are then centrifuged at 3000 rpm for 10 minutes.

Finally, 2.5 mL of the solutions obtained are mixed with 2.5 and 0.5 mL of 0.1% $FeCl_3$. Absorbances are measured at 700 nm. The concentration of the sample providing 0.5 optical density (IC_{50}) is calculated by plotting the curve showing the absorbance relative to the concentration of the sample. BHT is used as a reference compound.

3. Results

3.1. Acetic Acid-Induced Writhing Assay. After a careful study, the results obtained vary according to the dose studied. Thus, at the therapeutic dose of 50 mg/kg, 4-phenyl-1,5-benzodiazepin-2-one **3** has a lower peripheral analgesic activity than aspirin with a cramping number of 29.20 ± 2.38 and 23.25 ± 1.75, respectively. On the other hand, the derivatives (1-octyl, 1-nony)-4-phenyl-1,5-benzodiazepin-2-ones (**5a-b)** have a higher activity than aspirin at this same dose with a mean number of cramps respective of 19.2 ± 2.58 and 19.8 ± 1.92. The derivatives (1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5c-d)** have a lower activity than aspirin with a mean number of cramps, respectively, 25 ± 1.41 and 28 ± 2.44.

In addition, at the therapeutic dose of 100 mg/kg, the results obtained show that the aspirin used is more active than all the products studied. Indeed, the average number of cramps of each product becomes at this dose 10.25 ± 1.70 for **5a**, 12.60 ± 2.86 for **5b**, 15.25 ± 8.65 for **5c,** and 18.20 ± 2.44 for **5d** against 9.60 ± 1.50 for aspirin (Table 1).

Compound **3** was studied by P. M. Kanyonga et al. [7] at the dose of 100 mg/kg. They obtained an average number of cramps of 26.00 ± 2.0, 20 minutes after acetic acid administration.

3.2. Tail Immersion Test. The results obtained confirm the analgesic effect observed in the acetic acid-induced writhing test and show that all the products studied have a central analgesic activity comparable to morphine at dose of 1 mg/kg (Table 2).

Table 1: Effect of 4-phenyl-1,5-benzodiazepin-2-one **3** and (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** on acetic acid-induced cramps in mice.

Products	Doses (mg/kg)	Number of cramps in 10 (and 20 for 3) min	Percentage of inhibition (%)
3	50	29.2 ± 2.38	26.07 ± 6.04
5a	50	19.2 ± 2.58	51.39 ± 6.55
5b	50	19.8 ± 1.92	49.87 ± 4.86
5c	50	25 ± 1.41	36.70 ± 3.58
5d	50	28 ± 2.44	29.11 ± 6.20
Aspirin	50	23.25 ± 1.75	41.13 ± 4.43
3	100	26 ± 2.00	34.17
5a	100	10.33 ± 2.08	73.84 ± 5.27
5b	100	12.60 ± 2.88	68.10 ± 7.29
5c	100	15.25 ± 2.75	61.39 ± 6.97
5d	100	18.20 ± 1.92	53.92 ± 4.86
Aspirin	100	9.60 ± 1.50	75.69 ± 3.79
Control	—	39.50 ± 2.60	

Table 2: Effect of 4-phenyl-1,5-benzodiazepin-2-one **3** and (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** on nociceptive responses in the tail immersion test.

Products	Dose (mg/kg)	Reaction time (s)		
		30 min	60 min	120 min
3	100	6.78 ± 1.78	5.29 ± 1.26	5.01 ± 1.56
5a	100	5.29 ± 2.57	4.55 ± 1.27	5.86 ± 1.32
5b	100	5.37 ± 0.99	4.27 ± 1.69	4.15 ± 1.28
5c	100	5.03 ± 1.78	5.41 ± 2.52	5.46 ± 1.23
5d	100	4.37 ± 1.43	7.42 ± 1.85	4.72 ± 0.83
Morphine	1	7.20 ± 0.83	7.12 ± 0.75	6.58 ± 0.30
Control	—	2.19 ± 1.14	1.85 ± 0.84	1.62 ± 0.59

3.3. Antioxidant Activity. According to the results of our study (Table 3), it should be noted that each studied product has a capacity to neutralize free radicals and a good reducing power of Fe^{3+}. Indeed, the concentration required to reduce the 50% of DPPH radical (**IC_{50}**) is 7.83 ± 1.33 for **3**, 12.19 ± 0.00 for **5a**, 17.03 ± 2.16 for **5b**, 18.05 ± 0.00 for **5c,** and 40.12 ± 4.76 for **5d** (Table 3). On the contrary, the concentration required to reduce 50% of Fe^{3+} is 3.27 ± 0.27 for **5a**, 7.87 ± 0.19 for **5b**, 8.80 ± 0.04 for **5c,** and 9.57 ± 0.31 for **5d** (Table 3), compared with the reference molecule (BHT).

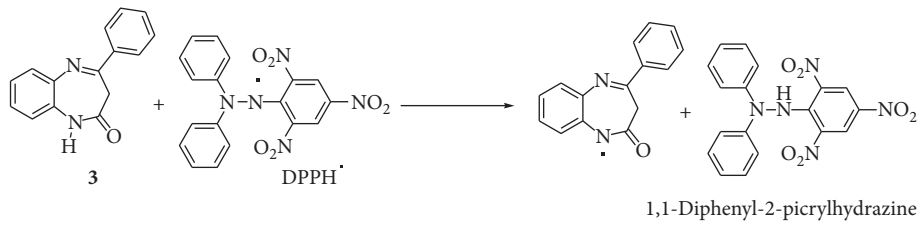
Table 3: DPPH and FRAP test results.

Tests	Products	IC_{50} (mg/mL)
DPPH	**3**	7.83 ± 1.33
	5a	12.19 ± 0.00
	5b	17.03 ± 2.16
	5c	18.05 ± 0.00
	5d	40.12 ± 4.76
FRAP	**3**	—
	5a	3.27 ± 0.27
	5b	7.87 ± 0.19
	5c	8.80 ± 0.04
	5d	9.57 ± 0.31
Reference	BHT	$7.02 \times 10^{-3} \pm 0.02$

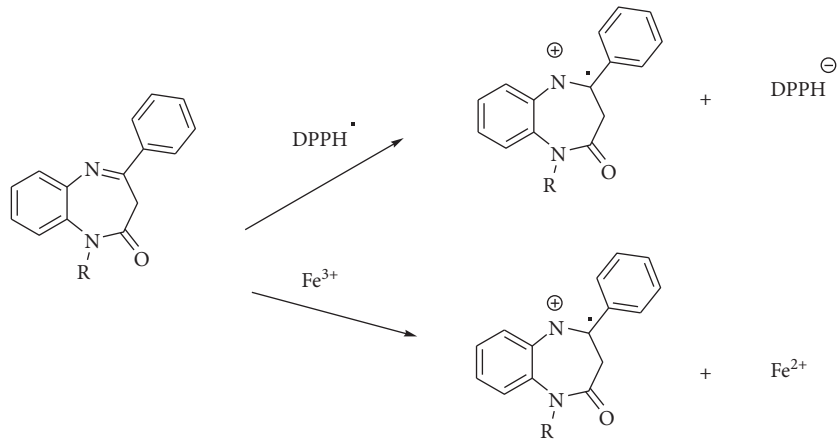
BHT is butylated hydroxytoluene.

4. Discussion

After the work on the psychotropic effects of 4-phenyl-1,5-benzodiazepin-2-one **3** and (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d**), in which we have shown that these products have a sedative activity of interest at the dose of 200 mg/kg, comparable to the Bromazepam at the dose of 20 mg/kg, we decided to deepen the exploration of the pharmacological effects of these derivatives in order to enhance their value. For this, we began by studying their analgesic activity since it is exerted on the nervous system, the main place of action of benzodiazepines. The *in vivo* peripheral analgesic activity that we performed at the dose of 50 mg/kg shows that the molecules (1-octyl, 1-nonyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a-b)** are more active than aspirin since their percentages of cramping inhibition are, respectively, 51.39 ± 6.55 and 49.87 ± 4.86, while aspirin is 41.13 ± 4.43 (Table 1). 4-Phenyl-1,5-benzodiazepin-2-one **3** and (1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5c-d)** derivatives appear to be less active than Aspirin with respective inhibition percentages of 26.07 ± 6.04, 36.70 ± 3.58 and 29.11 ± 6.20 at this same dose. We also find that the addition of an alkyl chain to the compound **3** increases its activity. When we increase the dose of the products studied to 100 mg/kg, the result becomes much better. Indeed, all derivatives (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** possess interesting peripheral analgesic activity comparable to the aspirin at the same dose (Table 1). In addition, they are more active than their parent molecule **3**. We also note that the activity of **5a–d** chemicals decreases as the alkyl chain increases. These results confirm our previous work [10], in which we have shown that the addition of an alkyl chain to molecule **3** increases its pharmacological activity, and that this activity decreases as the alkyl chain increases. All results at 50 and 100 mg/kg confirm that some benzodiazepines can be used as analgesics. This is the case of tetrazepam, which is marketed at the therapeutic dose of 50 mg/kg for its effect against painful muscle contractures [19,20]. The results of the central analgesic activity show that 4-phenyl-1,5-benzodiazepin-2-one **3** and (1-octyl, 1-nonyl, 1-decyl,



Scheme 2



Scheme 3

1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d)** have slightly higher activity than morphine at a dose of 1 mg/kg (Table 2). These results corroborate the work of G. Roma et al. [8], which showed that 4-phenyl-1,5-benzodiazepin-2-ones derivatives have an interesting analgesic activity. Other researchers like Gaudy et al. [21] and Ostermann [22] also suggest the use of benzodiazepines in some types of painful infections.

Variation of analgesic activity in (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones (**5a–d**). we showed that the analgesic activity of (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones is greater than the analgesic activity of the 4-phenyl-1,5-benzodiazepin-2-one, which meets our goal of increasing the activity of this molecule. But we find that this activity decreases as the alkyl chain increases. This is in agreement with the action of analgesics. Indeed, the pharmacological action of the analgesic molecules results in their attachment to the opioid receptors of the central nervous system. However, to easily reach these receptors, the drugs must cross the blood-brain barrier. This crossing is easily done if these drugs are small. This explains in a remarkable way the results obtained in this work. Indeed, small molecules have greater analgesic effects compared to larger ones. This observation could also justify the absence of long-chain benzodiazepines on the world market [23].

We continued to valorize our molecules by evaluating their antioxidant activity using the DPPH and FRAP tests. These tests permit to study their capacity to reduce the free radicals and the metal ions, respectively. The results obtained in vitro with the DPPH test show that all the products studied reduce very slightly the free radical DPPH compared to BHT (Table 3). In addition, this activity decreases as the alkyl chain increases. This observation confirms the work of G. C. Neochoritis et al. [9], which showed that benzodiazepines reduce the radical DPPH. In addition, using the FRAP test, we observe that the products (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones reduce slightly Fe^{3+} in Fe^{2+} (Table 3). For solubility problems with the phosphate buffer used, we were unable to study the ability of 4-phenyl-1,5-benzodiazepin-2-one to reduce Fe^{3+}.

Variation of the antioxidant activity. we observe that the antioxidant activity decreases when the alkyl chain of the molecule increases. This observation permits to say that this activity is linked to the polarity of the molecules studied. Thus, a compound will be more active as its polarity will be high. This observation also explains why BHT (very polar) is a good antioxidant. Moreover, the antioxidant activity of molecule **3** can be explained by the presence of the –NH group that it contains. Indeed, it can easily give a proton to the radical DPBH to stabilize it (Scheme 2).

However, the activity of its derivatives (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones, which do not contain a labile proton, can be explained by the presence, among others, of the imine link they contain. Indeed, they can transfer an electron to the free radical or to the metal ion. In this case, the stabilization of the radical will be done with a negative charge through a process that we have not identified (Scheme 3).

The results of our work reveal the pharmacological importance of benzodiazepines and justify the fact that many

researchers continue to synthesize new benzodiazepines that may have biological activity [24, 25, 26].

5. Conclusion

In conclusion, the work we have done shows that 4-phenyl-1,5-benzodiazepin-2-one and (1-octyl, 1-nonyl, 1-decyl, 1-dodecyl)-4-phenyl-1,5-benzodiazepin-2-ones have a good peripheral analgesic activity in mice at the dose of 100 mg/kg, compared with aspirin at the same dose. In addition, at the dose of 100 mg/kg, they have slightly higher central analgesic activity than morphine used at the dose of 1 mg/kg. Analgesic activity of the products studied decreased when the alkyl chain increased because this effect is more important when the molecules of small sizes are fixed to the opioid receptors present on the central nervous system. Furthermore, these compounds have low antioxidative activity compared with the BHT. It decreases with the polarity of the molecule.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] L. H. Sternbach, "The benzodiazepine story," *Journal of Medicinal Chemistry*, vol. 22, no. 1, pp. 1–7, 1979.

[2] L. O. Randall, "Progress in drug research," in *Psychopharmacological Agents*, M. Gordon, Ed., vol. 3, p. 175, Academic Press, New York, NY, USA, 1974.

[3] P. M. Kanyonga, M. Y. A. Faouzi, A. Zellou, M. E. Essassi, and Y. Cherrah, "Evaluation de l'activité anxiolytique de la 4-phényl-1,5-benzodiazépin-2-one," *International Journal of Biological and Chemical Sciences*, vol. 3, no. 4, pp. 646–652, 2009.

[4] A. Kamal, N. Shankaraiah, S. Prabhakar et al., "Solid-phase synthesis of new pyrrolobenzodiazepine-chalcone conjugates: DNA-binding affinity and anticancer activity," *Bioorganic and Medicinal Chemistry Letters*, vol. 18, no. 7, pp. 2434–2439, 2008.

[5] M. Dibraccio, G. C. Grossi, G. Roma, L. Vargiu, M. Mura, and M. Marongiu, "1,5-Benzodiazepines. Part XII. Synthesis and biological evaluation of tricyclic and tetracyclic 1,5-benzodiazepine derivatives as nevirapine analogues∗1," *European Journal of Medicinal Chemistry*, vol. 36, no. 11-12, pp. 935–949, 2001.

[6] A. Sandhar and R. Singh, "Rapid and efficient synthesis of 2,3-dihydro-1*H*-1,5-Benzodiazepines catalyzed by chloroacetic acid screened among various aliphatic acids under solvent free conditions," *Chemical Science Transactions*, vol. 2, no. 1, pp. 176–180, 2013.

[7] P. M. Kanyonga, A. Zellou, M. Essassi, and Y. Cherrah, ""Synthèse et évaluation de l'activité pharmacologique de la 4-phényl-1,5-benzodiazépin-2-one et ses dérivés," *International Journal of Biological and Chemical Sciences*, vol. 4, no. 1, pp. 19–24, 2010.

[8] G. Roma, G. Grossi, M. Di Braccio, M. Ghia, and F. Mattioli, "1,5-Benzodiazepines IX. A new route to substituted 4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amines with analgesic and/or anti-inflammatory activities," *European Journal of Medicinal Chemistry*, vol. 26, no. 5, pp. 489–496, 1991.

[9] C. G. Neochoritis, C. A. Tsoleridis, J. Stephanidou-Stephanatou, C. A. Kontogiorgis, and D. J. Hadjipavlou-Litina, "1,5-Benzoxazepines vs 1,5-benzodiazepines. One-pot microwave-assisted synthesis and evaluation for antioxidant activity and lipid peroxidation inhibition," *Journal of Medicinal Chemistry*, vol. 53, no. 23, pp. 8409–8420, 2010.

[10] T. Nguema Ongone, R. Achour, M. El Ghoul et al., "Synthesis and pharmacological valorization of derivatives of 4-phenyl-1,5-benzodiazepin-2-one," *Advances in Pharmacological Sciences*, vol. 2018, Article ID 6042602, 8 pages, 2018.

[11] A. Boudier, P. Castagnos, E. Soussan et al., "Polyvalent cationic vesicles: exploring the drug delivery mechanisms," *International Journal of Pharmaceutics*, vol. 403, no. 1-2, pp. 230–236, 2011.

[12] NAP, *Guidelines for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Academy Press, Washington, DC, USA, 1996.

[13] E. D. Olfert, B. M. Cross, and A. A. McWilliam, *Guide to the Care Use of Experimental Animals*, Canadian Council on Animal Care, vol. 1, 2nd edition, 1993.

[14] R. Koster, M. Anderson, and E. J. De Beer, "Acetic acid-induced analgesic screening," *Federation Proceeding*, vol. 18, p. 412, 1959.

[15] R. Sewell and P. S. Spencer, "Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats," *Neuropharmacology*, vol. 15, no. 11, pp. 683–688, 1976.

[16] F. Sahin, R. Cakmakci, and F. S. Kantar, "Sugar beet and barley yields in relation to inoculation with N-fixing and phosphate solubilizing bacteria," *Plant and Soil*, vol. 265, no. 1-2, pp. 123–129, 2004.

[17] F. Sharififar, M. H. Moshafi, S. H. Mansouri, M. Khodashenas, and M. Khoshnoodi, "In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss," *Food Control*, vol. 18, no. 7, pp. 800–805, 2007.

[18] M. Oyaizu, "Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine," *The Japanese Journal of Nutrition and Dietetics*, vol. 44, no. 6, pp. 307–315, 1986.

[19] J. Simiand, P. E. Keane, K. Biziere, and P. Soubrie, "Comparative study in mice of tetrazepam and other centrally active skeletal muscle relaxants," *Archives Internationales de Pharmacodynamie et de Thérapie*, vol. 297, pp. 272–285, 1989.

[20] P. E. Keane, J. Simiand, M. Morre, and K. Biziere, "Tetrazepam: a benzodiazepine which dissociates sedation from other benzodiazepine activities. I. Psychopharmacological profile in rodents," *Journal of Pharmacology and Experimental Therapeutics*, vol. 245, no. 2, pp. 692–698, 1988.

[21] J. F. Gaudy and D. Arreto, *Manuel d'Analgésie en Odontostomatologie*, Masson, Paris, France, 2nd edition, 2005.

[22] P. Queneau and G. Ostermann, *Le Médecin, le Malade et la Douleur*, Masson, Paris, France, 2004.

[23] M. V. Sokolovskaya, S. E. Lyubimov, I. S. Mikhel, K. P. Birin, and V. A. Davankov, "Asymmetric Ir-catalyzed hydrogenation of 1,3-dihydro-2H-1,5-benzodiazepin-2-ones using phosphoramidites," *Russian Chemical Bulletin*, vol. 67, no. 2, pp. 260–264, 2018.

[24] E. Chetraru, A. Ameline, L. Gheddar, J.-S. Raul, and P. Kintz, "Les " designer benzodiazepines » : qu'en sait-on aujourd'hui ?," *Toxicologie Analytique et Clinique*, vol. 30, no. 1, pp. 5–18, 2018.

[25] S. Chander, C.-R. Tang, H. M. Al-Maqtari et al., "Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro- 2H -1,5-benzodiazepin-2-one derivatives," *Bioorganic Chemistry*, vol. 72, pp. 74–79, 2017.

[26] H. Salah, E. A. Ahmed, and M. M. Hassan, "Ring rearrangements and reactivity of 3-((4-oxo-4 H -chromen-3-yl) methylene)-4-phenyl-1 H -[1,5]benzodiazepin-2(3 H)-one toward some nucleophiles," *Arabian Journal of Chemistry*, vol. 10, no. 4, pp. 548–555, 2017.