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Original Article

Anti-oxidant and antibacterial activities of novel *N*-sulfonylphthalimide in an ovalbumin-induced inflammation in Wistar rats

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Abstract

Phthalimide and N-substituted phthalimide have a special structure that helps them to be pharmaceutically useful and biologically active. In this study, we investigated the antioxidant, anti-inflammatory and antibacterial effects of a synthetic phthalimide-containing derivative in an experimental asthma model. In vitro determination of antioxidant and chelating activity was carried out by spectrophotometric methods. The in vivo antioxidant activity was carried out in Wistar rats sensitized to ovalbumin in the experimental model of asthma. Our results reveal that that the synthesized N-sulfonylphthalimide molecule has a scavenging capacity against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and a chelating activity on ferrous ions and revealed its protective capacity against altered markers of oxidative stress in the experimental asthma model. All the previous results were confirmed by the result of the histopathological study of the liver. Moreover, neo-synthesized N-sulfonylphthalimide 2 showed antibacterial activity against Gram-positive and Gram-negative bacteria with interesting MIC values. Finally, our study highlights the anti-inflammatory, anti-asthmatic, and antibacterial effects of the N-sulfonylphthalimide molecule, which could potentially be a drug of choice in asthmatic pathology, especially during bacterial superinfections in the respiratory tract.

Keywords: Neo-synthesized N-sulfonylphthalimide; Anti-oxidant; Inflammation; Antibacterial; Asthma experimental model

1. Introduction

In asthma, inflammation plays a crucial role in airway hyperresponsiveness and obstruction. Studies have shown that inflammatory processes involving various immune cells and mediators contribute to the pathogenesis of asthma. Targeting inflammation through interventions such as regulating the NOTCH signaling pathway and inhibiting epithelial-mesenchymal transition may offer potential therapeutic strategies for managing asthma. Understanding the complex inflammatory mechanisms in asthma is essential for developing effective treatments that alleviate symptoms and improve patient outcomes [1,2].

Inflammation in asthma is frequently associated with increased generation of reactive oxygen species (ROS), caused by increased oxidative stress that occurs in the airways of asthmatic patients. This weakens the lungs, making them susceptible to various bacterial superinfections that complicate the state of asthma. The use of drugs that have antioxidant effects has become of interest and has prompted the synthesis of a large number of chemicals that reduce diseases of the respiratory system and at the same time allow the fight against bacteria [1].

Phthalimide and N-substituted phthalimide compounds contain a cyclic imide structure that is known to interact with biological systems effectively. This unique structure allows these compounds to exhibit diverse pharmacological activities, including anti-inflammatory properties by modulating immune responses and antibacterial effects by targeting bacterial growth and survival mechanisms. The presence of functional groups in their structure enables interactions with specific biological targets, making them valuable candidates for drug development and therapeutic interventions in various medical conditions. Further research into the mechanisms of action and specific molecular interactions of these compounds can provide insights

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into their potential applications in pharmacology and medicine [2,3].

Phthalimide and N-substituted phthalimide have a special structure that helps them to be pharmaceutically useful and biologically active, including anti-inflammatory [2] and antibacterial [3] activities. In this study, we investigated the antioxidant, anti-inflammatory, and antibacterial effects of a synthetic phthalimide-containing derivative in an experimental asthma model in ovalbumin-sensitized Wistar rats.

2. Materials and methods

2.1. Chemicals

The N-sulfonylphthalimide 2 (PH) (Figure 1) is easily synthesized in excellent yield (92%) by condensation of phthalic anhydride and sulfonamide derivative 1 of phenylpiperazine under ultrasound irradiation. The structure of the synthesized compound (PH) is unambiguously confirmed by usual spectroscopic methods ^{1}H NMR, ^{13}C NMR, MS, EA and IR [3].

2.2. *In vitro* determination of antioxidant and chelating activity

2.2.1. DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described in a previous study [4]. In succinct terms, the aliquots (50 mL) of various concentrations of the compound tested (PH) were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of free radicals DPPH in percentage (IP %) was calculated in the following way: $IP\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where IP is the inhibition percentage; A blank is the absorbance of the control reaction (containing all reagents except the test sample), and A sample is the absorbance of the test compound. The results are expressed as IC50, the amount of antioxidants necessary to decrease the initial concentration of DPPH by 50%. The lower IC50 values indicate a higher antioxidant activity. Synthetic ascorbic acid was used as positive control (antioxidant).

2.2. 2. Total Antioxidant Activity (TAA) by phosphomolybdenum method

The total antioxidant capacity is based on the reduction of ammonium molybdate (IV) to ammonium molybdate (V) by the sample and the subsequent formation of green phosphate/ Mo (V) compounds with a maximum absorption at 695 nm [4]. A quantity of 0.1 mL of each sample (10 mg/mL) was mixed with the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in 100 mL). The tubes were incubated in boiling water bath at 95°C for 90 min. Then the solution was cooled to room temperature and absorbance was read at 695 nm with spectrophotometer against a blank. The total antioxidant activity results were the ratio between the positive control and the samples under study. The results are expressed in µg positive control (Vitamin C)/µg sample, after the calculation of the following relation: x positive control (µg/mL)/x sample (µg/mL).

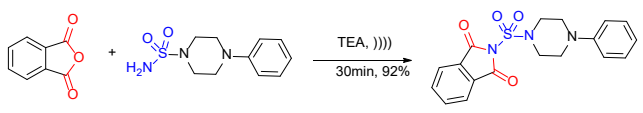

Fig. 1. Synthesis of N-sulfonylphthalimide derivative of phenylpiperazine.

2.2.3. ABTS assay

2,2'-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS solution (7 mM) with ammonium persulfate (2.45 mM). The mixture was allowed to stand in dark at room temperature for 12-16 h before use. For the study of PH samples, the ABTS solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. In brief, one milliliter of diluted ABTS solutions was mixed with 10 µL of sample or Trolox standards and the percentage inhibition was calculated and TEAC (Trolox equivalent antioxidant capacity) values were determined from the decreases in absorbance from the Trolox standard curve [4].

2.2.4. Ferrous ion-chelating ability assay

The ferrous ion-chelating ability was determined according to the method of Decker and Welch [5] with minor modifications. One hundred microliters of each sample stock solution (5 mg/mL) were mixed with 135 µL of distilled water and 5 µL of 2 mM $FeCl_2$ in a microplate. The reaction was initiated by the addition of 10 µL of 5 mM ferrozine. The solutions were well mixed and allowed to stand for 10 min at room temperature. After incubation, the absorbance was measured at 562 nm. Distilled water (100 µL) instead of sample solution was used as a control. Distilled water (10 µL) instead of ferrozine solution was used as a blank; Ethylenediaminetetraacetic acid disodium salt; EDTA-Na_2 was used as reference standard. The ferrous ion-chelating ability was calculated as follows:

Ferrous ion- chelating ability (%) = [(A0-(A1-A2))]/A0×100. where A0 is the absorbance of the control, A1 is the absorbance of the sample or standard and A2 is the absorbance of the blank.

2.3. *In vivo* antioxidant activity

2.3.1. Animals

Thirty-two male rats (Albino Wistar), weighing approximately 100-150 g at the age of 6-8 weeks, were provided from Algiers Pasteur Institute. All animals were housed in cages that were cleaned every two days and kept in regular settings of 25°C and a cycle of 12h light and 12h dark, with free access to food and water. Before beginning the experiments, the rats were given a 15-day adaptation period. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by our Ethics Committee of our DGRSDT institution (under number's project PRFU: D01N01UN230120200008).

2.3.2. Experimental groups design

Experimental rats were distributed in 4 groups of nine (n=9) males receiving the following treatments: Rats of group 1 were untreated and served as control (C). Animals of group 2 were orally given a dose of N-sulfonylphthalimide (PH) of 24–96 µmol/kg, according to Neves et al [6]. While rats of group 3 were sensitized by ovalbumin (OVA). Animals of group 4 were sensitized and treated by N-sulfonylphthalimide (OVA + PH).

2.3.3. Sensitization and airway challenge

OVA and OVA/PH groups were sensitized with ovalbumin (grade II, ref. A5253-250 G, Sigma Aldrich), according to the methods of Moura et al. [7] and Yang et al. [8]. They were actively sensitized by intraperitoneal injections of ovalbumin (1 mg/mL) with alum (1 mg/mL in saline) as an adjuvant on days 0 and 14. On days 21, 22 and 23, the rats were challenged, for 30 min, with inhalation of either OVA (5 mg/mL) via a nebulizer (OMRON, NE-C29-E) coupled to a plastic box. C and PH groups received injections and were challenged with saline only.

2.3.4. Preparation of Liver Homogenates

Liver was quickly removed, washed in 0.9 % NaCl solution and weighed after the careful removal of the surrounding connective tissues, and then, a quantity of 1 g was homogenized in 2 mL of phosphate buffer solution (PBS: Tris 50 mM, NaCl 150 mM, pH 7.4) at 1:2 (w/v), in ice-cold condition. Homogenates were centrifuged at 3,000×g for 35 min at 4°C; the supernatants were divided into aliquots and then stored at −20°C [9].

2.3.5. Estimation of Lipid Peroxidation (LPO) Levels

The detection of malondialdehyde (MDA) resulting from the degradation of polyunsaturated fatty acids with 3 or 4 double bonds peroxidized by a colorimetric reaction with thiobarbituric acid (TBA), is a highly sensitive method for determining lipid peroxidation *in vitro*. This assay is performed according to the method by Esterbauer et al. [10] in liver tissue. Supernatant (375 µL) was homogenized by sonication with PBS (150 µL), TCA-BHT (375 µL) (trichloroacetic acid- butyl hydroxytoluene) in order to precipitate proteins, and then centrifuged (1000 g, 10 min, and 4 °C). After that, 400 µL of supernatant was mixed with 80 µL of HCl (0.6 M) and TBA (320 µL) dissolved in Tris, and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm.

2.3.6. Estimation of reduced glutathione (GSH) Levels

Glutathione level is determined in the liver tissue according to the method of Weckbercker and Cory [11]. The principle of this assay is based on measuring the optical absorbance of the 2-nitro-5-mercapturic resulting from the reduction of 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB) by groups SH of glutathione. To this end, deproteinization is made to keep only SH groups specific to glutathione. Briefly, liver supernatant (0.8 mL) was added to 0.25% sulphosalycylic acid (0.3 mL) and tubes were centrifuged at 2500 g for 15 min. The supernatant (0.5 mL) was mixed with 0.025 mL of 0.01M DTNB and 1 mL phosphate buffer (0.1 M, pH 7.4). Finally, absorbance at 412nm was recorded. Total GSH content was expressed as n mol GSH/mg protein.

2.3.7. Measurement of glutathione peroxidase (GPx) activity

The enzymatic activity of GPx was measured by the method of Flohe and Günzler [12]. This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH). The latter is transformed into glutathione disulphide (GSSG) under the influence of GPx. Supernatant obtained after centrifuging liver homogenate at 1500 g for 10 min followed by 10,000 g for 30 min at 4°C was used for GPx assay. Reaction mixture (1 mL) was prepared which contained 0.3mL of phosphate buffer (0.1 M, pH 7.4), 0.2mL of GSH (2 mM), 0.1mL of sodium azide (10 mM), 0.1mL of H_2O_2 (1mM) and 0.3 mL of liver supernatant. After incubation at 37°C for 15 min, reaction was terminated by addition of 0.5mL 5% TCA. Tubes were centrifuged at 1500 g for 5 min and the supernatant was collected. 0.2 mL of phosphate buffer (0.1 M, pH 7.4) and 0.7 mL of DTNB (0.4 mg/mL) were added to 0.1 mL of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

2.3.8. Measurement of catalase (CAT) activity

CAT activity was measured at 240nm using a UV/visible by the variation of the consecutive optical density at the dismutation of hydrogen peroxide (H_2O_2) [13]. The reaction mixture consists of 780 µL phosphate buffer (pH 7.5), 200 µL of hydrogen peroxide (500mM) and 20 µL supernatant in a final volume of 1 mL. Absorbance was recorded at 240 nm every 15s for 1 min. The enzyme activity was calculated by using an extinction coefficient of 0.043mM cm^{-1}.

2.3.9. Measurement of superoxide dismutase (SOD) activity

The dosage of SOD activity was carried out by the NBT test, which is a photoreduction method riboflavin complex/methionine, which generates superoxide anions [14]. The oxidation of NBT by the superoxide anion O_2 is used due to the presence of SOD detection base. In an aerobic environment, riboflavin blend, methionine and NBT give a bluish coloration. The presence of SOD inhibits the oxidation of NBT. Briefly, 5 µL of the supernatant was combined with 1mL of EDTA/methionine (0.3 mM), 1890mL phosphate buffer (pH 7.8), 85 µL of 2.6mM NBT; 22 µL of riboflavin (0.26mM) were added as the last and the light was switched. The reaction changes in absorbance at 560 nm were recorded after 20 min.

2.3.10 Histopathological Examination

Liver was dissected and immediately fixed in formol solution to 10% for 24 h, processed by using a graded ethanol series, and then embedded in paraffin (increased to 56/58°C). The paraffin sections were cut into 5µm thick slices using a microtome (Leica RM2125RT), followed by staining with hematoxylin and eosin (Leica ST4040) and a mounting EUKITT. Finally, the sections were observed and analyzed under an optical microscope (Leica DM LB 2) and then photographed.

2.4. Antibacterial Activity Assay

Susceptibility testing of selected strains of Gram-positive and Gram-negative bacteria against the neo-synthesized N-sulfonylphthalimide was performed using the diffusion method according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) [15].

Briefly, for each strain, a bacterial suspension corresponding to 0.5 Mac Farland was prepared. Solution of the compound in Dimethyl sulfoxide (DMSO) was prepared to obtain semi-log dilutions of half-to-half. In Petri dishes, 2 mL of each concentration are added to 18 mL of Muller-Hinton Agar and left to solidify. Each Petri dish was seeded by spotting the strains. The incubation was done at 37°C for 18-24h. The clinical strains were obtained from

the laboratory of microbiology of the University Hospital Center of Dr. Dorban in Annaba (Algeria). The typed Strains were provided by Laboratory of Microbiology, Faculty of Pharmacy, University of Annaba (Algeria). As a negative control, DMSO was used. For each strain growth, different concentrations of N-sulfonylphthalimide were appreciated and the Minimal Inhibitory Concentration (MIC) values were determined.

2.5. Statistical analysis

All data are expressed as mean ± SD for nine rats of each group, with biological replicates included to ensure robustness of the findings. These calculations were performed using Microsoft Excel (2010). Significant differences between the group's means were determined by the Student's t-test. The statistical signification of difference was taken as $p<0.05$.

3. Results

3.1. Determination of *in vitro* antioxidant activities

In order to estimate antioxidants capacities of N-sulfonyl phthalimide (PH) sample we used DPPH, TAA, ABTS and Ferrous ion-chelating ability assay. Concerning DPPH assay we found that PH sample has antioxidant activity better than BHT. In ABTS test, PH was also found to be the most potent followed by Trolox. Whereas, in TAA, Vit C has the most capacity antioxidant compared with PH sample the same with ferrous ion chelating ability assay (FICAA) shows that EDTA has the most ability to chelate ions followed by PH sample. The results are presented in Table 1.

3.2. Effects of treatments on lipid peroxidation and non-enzymatic antioxidant (GSH)

In OVA-treated group, results (Figure 2) showed a highly significant increase in MDA level ($p<0.001$) and a highly significant decrease in the liver GSH contents ($p<0.01$). Whilst, the co-administration of PH with OVA has improved the GSH content ($p<0.001$) and showed a decrease in liver MDA level ($p<0.001$) compared to the OVA-treated group.

3.3. Effects of treatments on antioxidant enzymes

Antioxidant enzyme activities of the hepatic tissues are shown in Figure 3. Thus, SOD, CAT, and GPx activities decreased significantly in OVA-treated group ($p<0.001$, $p<0.05$ and $p<0.05$) respectively, compared to the control. In addition, a significant recovery of SOD, CAT, and GPx ($p<0.001$, $p<0.05$, and $p<0.05$) respectively, was observed in response to the presence of PH.

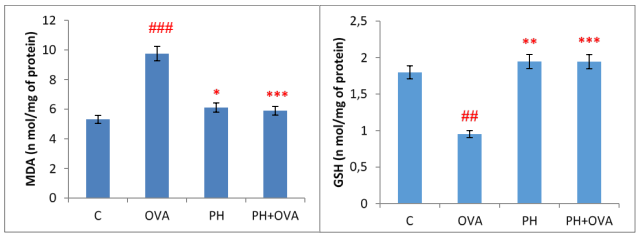

Fig. 2. Effect of PH on non-enzymatic antioxidant (GSH) and lipid peroxidation (MDA). Values are given as mean ± SD. Significant difference: all treated groups compared to the control one (## $p<0.01$ and ### $p<0.001$). All treated groups compared to the OVA treated one (*$p<0.05$, **$p<0.01$, and ***$p<0.001$).

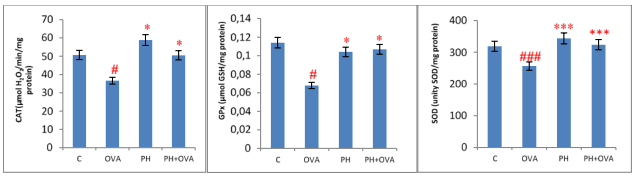

Fig. 3. Effect of PH on enzymatic antioxidant (GPx, CAT, and SOD). Values are given as mean ± SD. Significant difference: all treated groups compared to the control one (# $p<0.05$ and ### $p<0.001$). All treated groups compared to the OVA treated one (*$p<0.05$ and ***$p < 0.001$).

3.4. Histopathological Results

Microscopic observation of rat liver control shows a regular histological structure and normal hepatocyte cell morphology (Figure 4-A). In contrast, OVA-treated group had histological alterations such as dilatation of sinusoidal space (red arrows), infiltration of inflammatory cells (yellow circle) and cell necrosis (double green arrow direction) (Figure 4-B). However, the OVA –PH group showed a reduction of sinusoidal space (blue arrows), decrease in inflammatory cells (green circle) (Figure 4-C). No histological changes in the liver of PH group compared to the control group were found (Figure 4-D).

3.5. Antibacterial Activity

The compound (PH) has shown antibacterial effects in 98% of the tested concentrations (Table 2). The MIC modal of 3.51µg/mL was determined against Gram-negative bacteria except *E. coli ESBL* producer and *Acinetobacter baumannii* 13 Colistine resistant with, respectively, 28.125µg/mL and 14.06 µg/mL values. In case of Gram-positive bacteria, the MIC values recorded were 3.51µg/

Table 1. Antioxidant *in vitro* activities of N-sulfonyl phthalimide.

Samples	Antioxidant activity			
	DPPH IC_{50} (µg/mL)	TAA (mg/g)	ABTS (µg/mL)	FICAA (µg/mL)
N-sulfonyl phthalimide	73.07 ± 1.00	03.45 ± 0.40	64.59 ± 0.50	43.79 ± 0.10
Vit C	43.15 ± 1.03	00.21 ± 0.10	-	-
BHT	302.00 ± 1.00	-	-	-
Trolox	-	-	74.54 ± 0.40	-
EDTA	-	-	-	23.52 ± 0.09

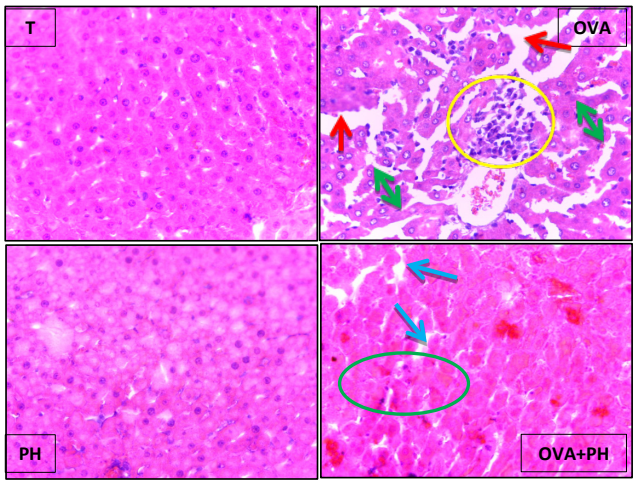

Fig. 4. Histological sections of the liver in rats of different Experimental groups (X40). (T) normal histological structure, (OVA) dilatation of sinusoidal space (red arrows), infiltration of inflammatory cells (yellow circle) and cells necrosis (double green arrow direct ion), (PH) normal histological structure. (OVA+PH) reduction of sinusoidal space (blue arrows) and decrease in inflammatory cells (green circle).

Table 2. MICs values of N-sulfonylphthalimide against tested bacteria.

Strains	MIC (µg/mL) values
Gram-negative bacteria :	
Klebsiella pneumoniae KPC-	3.51
Klebsiella pneumoniae KPC+	3.51
Klebsiella pneumoniae 2	3.51
Escherichia coli ATCC 25922	3.51
E.coli ESBL producer	28.125
Pseudomonas aeruginosae ATCC27853	3.51
Pseudomonas NDM1	3.51
Acinetobacter baumannii 13 ColR	14.06
Ac. baumannii 5 ImpR 5	3.51
Gram-positive bacteria :	
Staphylococcus aureus ATCC 29213	3.51
St. aureus Cipro R	7.031
Enterococcus feacalis	14.06

mL against *Staphylococcus aureus* ATCC 29213, the value of 7.03 µg/mL was recorded with *Staphylococcus aureus* Ciprofloxacin resistant and 14.06 µg/mL with *Enterococcus feacalis*.

4. Discussion

Phthalimide and N-substituted phthalimides are a crucial class of compounds because they possess important biological activities [16]. In addition, in general, the compounds containing the sulfonamide group are interesting derivatives that have polyvalent and potential biological activities (such as anti-inflammatory, anti-tumor, etc.). So, in order to confirm the antioxidant effect of a new N-sulfonylphthalimide and its anti-asthma therapeutic effect, we used an allergic asthma model in rats. Indeed, oxidative stress plays an important role in the pathophysiology of asthma in which damage to oxidative stress biomarkers is an established consequence of inflammation [17] [18].

Our results show that synthesized N-sulfonylphthalimide molecule has scavenging ability against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and chelating activity on ferrous ions, according to Karthik et al [19] the scavenging ability against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) in which the IC50 values of the higher radical inhibition activity it was 15.40 µg/mL comparing to the Vit C (16.94 µg/mL). In addition, this synthesized phthalimide was capable of chelating Fe^{2+} ions whose found that N-sulfonylphthalimide has the best value (14.12 µg/mL) comparing to standards (EDTA 13.88 µg/mL) . Fe^{2+} ions initiate free radicals through the Fenton and Haber-Weiss reaction. This reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases [20]. Also, our molecule has a total antioxidant capacity so some correlations are evident between the antioxidant activity measured and the type of spacer linking the aromatic ring to the carboxylic acid group or the type of substituent on ortho-position to the hydroxyl group responsible for the activity [21].

To reinforce the precedent results we exposed rats to allergen by inhalation-induced inflammatory airway response and treated them with N-sulfonylphthalimide molecule, the results were compared firstly with the control group (C) and secondly with the OVA group (OVA-sensitized rats). Our data show evaluation of certain oxidative stress biomarkers, especially in liver. The diminution level of GSH which could be explained by an adaptive response to oxidative stress, and the elevated level of MDA in the OVA-challenged group could be linked to the peroxidation damages of biological membranes, caused by an increased reactive Fe^{2+} and/or inactivation of enzymes involved in antioxidant defense and it's confirmed by the decreasing of GPx, SOD, and CAT activities. This result is in agreement with those of Chekchaki et al [22] and Khaldi et al [23], which showed in their experimental work oxidative inactivation of SOD, catalase, and the decrease in MDA formation in OVA Treated asthmatic rats.

All the previous results were confirmed by the result of the histopathological study of liver. Hence an infiltration of inflammatory cells in the OVA-Challenged group was observed and dilatation in sinusoids and cell necrosis, due certainly to immune reaction and possibly to the formation of reactive radicals and to subsequent lipid peroxidation. As a result, the accumulated hydroperoxides in this organ could cause cytotoxicity associated with membrane phospholipids peroxidation, the basis of liver damage. A similar concept was found in the literature by Khaldi et al [23] demonstrating the alterations in liver histoarchitecture in asthma rat model.

Furthermore, our study provides evidence of the anti-inflammatory and anti-asthmatic effect of the N-sulfonylphthalimide molecule. In fact, phthalimides possess a structural feature –CO-N(R)-CO- and an imide ring this framework plays an immense role which helps them to be biologically active and pharmaceutically useful [24]. As the results of Lima et al [25] confirm the role of the phthalimide ring in the anti-inflammatory activity. As part of an

ongoing effort to develop new thalidomide analogues as anti-inflammatory lead candidates, Machado et al [2] describe the synthesis and anti-inflammatory activity of novel N-phenyl-phthalimide functionalized derivatives. The target compounds were assayed in an acute lung inflammatory model and all compounds were able to inhibit TNF-alpha production and subsequent neutrophil recruitment in the LPS-acute lung inflammatory model [2]. Indeed, the administration of N-sulfonylphthalimide provided significant protection to the altered markers of oxidative stress, since the histological observation of rat liver tissues, of the OVA/PH group, showed a decrease in the influx of inflammatory cells to the as well as in the signs of inflammation and reduce the sinusoidal dilatation. Additionally, N-sulfonylphthalimides can inhibit enzymes like cyclooxygenase (COX), involved in the inflammatory pathway, thereby reducing the production of pro-inflammatory prostaglandins. This mechanism supports the observed reduction in inflammation and corroborates findings from other studies on similar compounds.

The combination of pharmacophores is widely used to improve the activity of the derivatives compared to the starting drug [26] [27] [28]. Phthalimide derivatives are mostly studied for their antimycobacterial activity [29]. Paraiso and Alea [30] found one of the fourth new compounds, showed an appreciable antimycobacterial activity. In this study, the neo-synthesized N-sulfonylphthalimide 2 showed an antibacterial activity against Gram-positive and Gram-negative bacteria with interesting MICs values. It's not always the case for phthalimide derivatives previously tested in anterior studies in which a weak inhibitory activity against the tested bacteria was determined [3]. Previous studies have demonstrated that phthalimide derivatives exhibit significant anti-inflammatory and antimicrobial activities [1, 2]. However, the specific mechanisms by which N-sulfonylphthalimides exert these effects remain underexplored. However, proposed mechanisms for the antibacterial activity of phthalimides include inhibition of protein synthesis by binding to bacterial ribosomes, inhibition of cell wall synthesis by targeting essential enzymes, disruption of bacterial cell membrane integrity, and inhibition of key bacterial enzymes like topoisomerases. Finally, this novel compound has the potential to be considered a new core drug for the treatment of multidrug-resistant bacteria.

5. Conclusion

The treatment of rats sensitized to ovalbumin with N-sulfonylphthalimide against asthma and inflammation, was proven to be effective in reduction of inflammatory markers in blood and liver and scavenging of the generated free radicals through the stimulation of antioxidant enzymes activities. According to these results, it's possible to develop new anti-asthma compounds based on the synthesis of N-sulfonylphthalimide. The positive antibacterial activity of the N-sulfonylphthalimide should be undertaken to develop a potent antibacterial substance although further studies are needed on a wider range of microorganisms including Fungi and with lower concentrations of N-sulfonylphthalimide.

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Author contribution

Silini Ilhem, Rouibah Zineb, and Ahmida Meryem performed the in vitro and in vivo experiments, analyzed the data, and wrote the article. **Djahoudi Abdelghani and Bouraoui Houda** conceived and performed the microbiological experiments. **Nedjai Sabrina, Grib Ismahene and Berredjem Malika** provided chemicals and microbiological samples. **Boumendjel Amel and Messarah Mahfoud** contributed to the study conception and design and manuscript correction.

Interest conflict

Authors reported no conflict of interest.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

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