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Skin Sensitization Calculation, Topical anti-inflammatory effect and DFT Study of New Indole-Hydrazone

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ABSTRACT

A new indol-hydrazone (**IH**); *N*'-[(*E*)-(5-bromo-1*H*- indol-3-yl) methylidene] pyridine-4-carbohydrazide was selected for theoretical and experimental studies. Molecular structure proprieties were investigated using density functional theory (DFT) *via* B3LYP/6-31G (d,p), skin sensitization prediction was carried out using Pred Skin software program. The obtained results demonstrate the reactivity of **IH** with Energy gap (Δ) of 0.0579 a.u, low sensitizer effect towards human skin with probability of 60 %, and an excellent topical anti-inflammatory effect against xylen-induced ear odema in mice model with inhibition percentages of 81.48%.

Keyword: Hydrazone, skin sensitization, Topical, Anti-inflammatory.

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INTRODUCTION

Indole or benzo [*b*] pyrrole is a planar aromatic heterocyclic compound. It has a bicyclic structure, consisting of a benzene ring fused to a pyrrole ring. It has, an π-electronic system consisting of ten electrons from eight carbon atoms and the non-binding doublet of the nitrogen atom [1]. A considerable number of natural products are derived from this motif. In humans, serotonin or 5-hydroxytryptamine is a potent vasoconstrictor, mainly stored in blood platelets, regulating gastric secretions and intestinal contractions [2]. This substance also acts as a neurotransmitter, involved in the conduction of impulses between nerve cells. In plants, heteroauxine plays a role comparable to serotonin. 5,6-Dihydroxyindole is an essential component of melanin, a brown pigment that stains the skin. Indigo is a compound that is used as a colorant in the textile industry. The indole ring is the most heterocyclic unit in nature and one of the most attractive in the field of organic chemistry concerning the development of new biologically active structures and the discovery of new reactivities [3]. During the 1990s, the discovery of a new class of drugs, the triptans, was a real advance in the management of migraine attacks. For example, Zolmitriptan Zomig® is marketed by Astrazeneca since 1998, Elitriptan (Zophren®), another biologically active product with an indole ring, is marketed as part of the suppression of nausea caused by chemotherapy or radiotherapy in the case of anticancer treatments [4]. In this study, *N*'-[(*E*)-(5-bromo-1*H*- indol-3-yl) methylidene] pyridine-4-carbohydrazide was selected for theoretical and experimental studies. Molecular structure proprieties were investigated using density functional theory (DFT) *via* B3LYP/6-31G (d,p), skin sensitization prediction was carried out using Pred Skin software program and was calculated using P value. In addition, topical anti-inflammatory effect against xylen-induced ear edema was investigated using mice model.

MATERIAL AND METHODS

Quantum chemical calculation

Molecular structure proprieties were investigated using density functional theory (DFT) *via* B3LYP/6-31G (d,p), using the Gaussian 09 program [5], where the following parameters: ionization potential (IP), electron affinities (EA) [6], electronegativity (χ), chemical potential (μ) [7], global hardness (η) [8], global softness (σ), and electrophilicity index (ω) [9] were calculated by using the following equations [10]:

$$IP = - E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$\eta = E_{LUMO} - E_{HOMO} \quad (3)$$

$$\sigma = 1 / \eta \quad (4)$$

$$\chi = -(E_{HOMO} - E_{LUMO}) / 2 \quad (5)$$

$$\mu = (E_{HOMO} - E_{LUMO}) / 2 \quad (6)$$

$$\omega = \mu^2 / 2\eta \quad (7)$$

Skin sensitization prediction

The online software program Pred Skin was used for skin sensitization prediction [11].

Skin sensitization calculation

Chemical-skin sensitizer effect was calculated using the quantum chemical calculation according to the following formulae: Predicted value (P) =15.3 x E_{HOMO} + 5.08

When P is greater than 0.50, the compound is predicted as a sensitizer, otherwise it is predicted as a non-sensitizer [12]. Results compared with Ascorbic acid (**AA**) which is skin whitening Agent [13] with no sensitizer effect.

Topical anti-inflammatory effect

Experimental animals

Experiments were performed using mice, weighing 25-30 g. They were obtained from Pasteur institut (Algeria) and housed in plastic cages under normal laboratory conditions (12 h light / dark cycle, 23 ± 2 °C) for an acclimatization period of 7 days prior to the experiments. All the animals were given food and water *ad libitum*.

Xylene-induced ear edema in mice

Adult albino female's mice (25-30g) were randomised into different groups of 6 mice each were used for the experiment. The investigated compounds (0.5 mg/ear edema), were topically applied to various groups. Inflammation was induced in mice by topical application of 30 µl of xylene and 30 µl of different synthetic product at the inner surface of the right ear. The thickness of the ear is measured before and half hour after the induction of inflammation by a digital caliper. The difference in thickness before and after the application of xylene is calculated [14].

RESULTS AND DISCUSSION

Quantum chemical calculation

Geometry optimization

Geometry optimization of Isoniazid **INH** and its synthesized hydrazone analog **IH** was performed in DFT/B3LYP/6-31G^{++}(d,p) level calculations. The optimized structure is shown in **Fig. 1**.

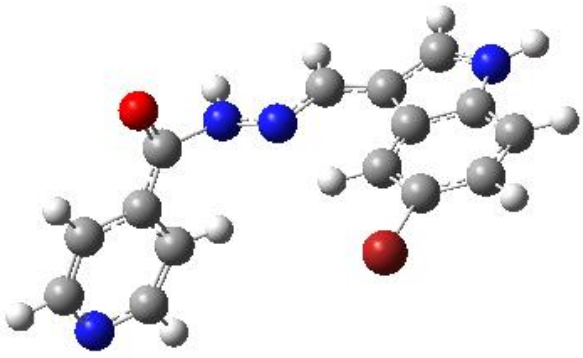

Fig. 1. Optimized structure of IH

The analysis of the wave function indicates that the electron absorption corresponds to the transition between the ground state and the excited state, the electron donor distribution in the busiest molecular orbital (HOMO) and the electron acceptor in the least occupied molecular orbital (LUMO). **Table 1** and **Figure 2** illustrate the molecular energy, LUMO represents the ability to gain an electron, so the HOMO represents an ability to lose an electron. The energy of the HOMO is directly proportional to the ionization potential and the energy of the LUMO is directly proportional to the electronic affinity.

The difference in orbital energy between the HOMO and LUMO is called the HOMO-LUMO gap. The high HOMO energy corresponds to a molecule more reactive with electrophiles in reactions, low energy LUMO is reactive with nucleophiles.

According to the theory of molecular orbitals, a high HOMO energy of one reagent molecule and a low LUMO energy of another reagent are advantageous for the reaction between the two molecules, because the electron transfers are easier from the HOMO of a LUMO reagent on the other in the orbital interaction. The HOMO, LUMO and the energy difference (HOMO-LUMO) of the monomer in the DFT with the 6-31G base (d, p) were calculated. The HOMO-LUMO energy gap reveals that the difference in energy reflects the chemical activity of the molecule.

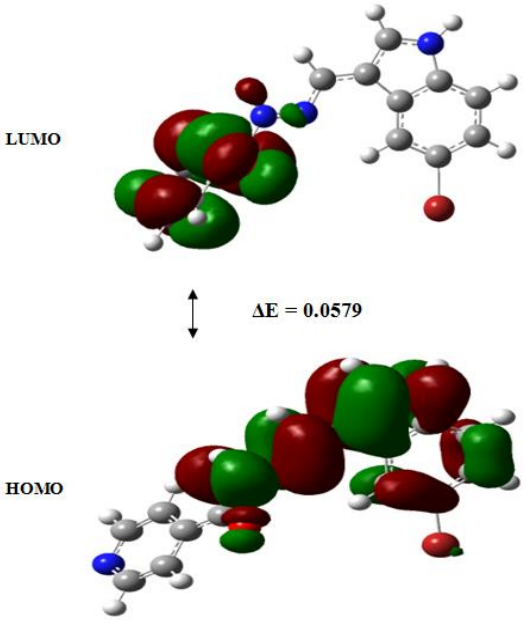

Fig 2. Frontiers orbital of the hydrazone

Table 1. Calculated global scalar properties of **IH**

B3LYP/6-31G (d,p)		
Molecular energy (a.u)	**INH**	**Hyd1**
E(B3LYP)	–472.2814	–3444.13
E_{LUMO}	–0.1045	–0.1228
E_{HOMO}	–0.2174	–0.1804
Energy gap (Δ)	0.1129	0.0579
Ionization potential (I)	0.2174	0.1804
Electron affinity (A)	0.1045	0.1228
Global hardness (η)	0.05645	0.0288
Global softness (S)	0.02822	0.0144
Chemical potential (µ)	–0.3219	–0.1516
Electophilicity (ω)	0.9177	0.3975
Electronegativity (χ)	0.1609	0.1516

Skin sensitization calculation

In first time, skin sensitization was predicted by P value calculation using E_{HOMO} (Hartree), results shown in **Table 2** show sensitizer effect of **IH** comparable with **INH** and sensitizer effect comparable with **AA** with P values of 23198, 1.75378 and 0.4301 respectively.

Table 2. P value and predicted sensitization effect of **INH, Hyd** and **AA**

Compound	E_{HOMO}	P values	Skin sensitization
INH	-0.2174	1.75378	Sensitizer
IH	-0.1804	2.3198	Sensitizer
AA	-8.27	0.4301	Non-Sensitizer

Skin sensitization prediction

Results displayed in **Figures 3**, and **4** explain the reasons of precedent results in **Table 2**, which indicate the atoms or the fragments contributed in the sensitizing effect, green atom or fragment represent an increase in skin sensitization potential; whereas, pink fragments represent a decrease in skin sensitization potential, and gray fragments do not contribute to skin sensitization potential.

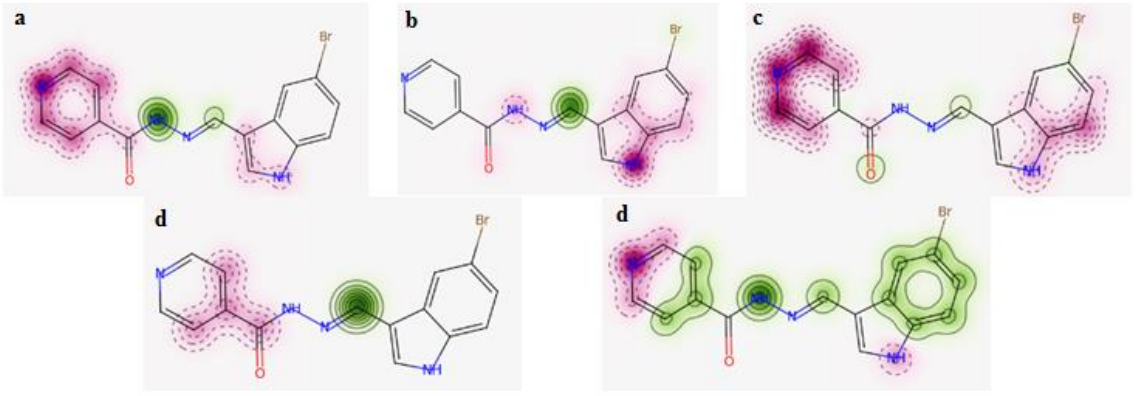

Fig. 3. Effect of **IH** on a. Human skin sensitization, b. Murine local lymph node assay (LLNA), c. Direct peptide reactivity assay (DPRA), d. Human cell line activation test (h-CLAT), e. KeratinoSens™.

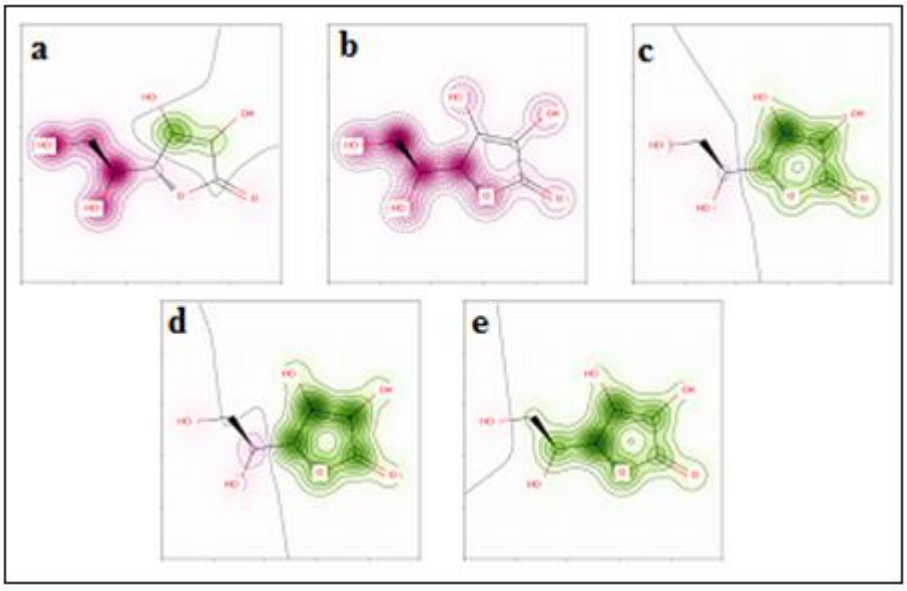

Fig. 4. Effect of **AA** on **a.** Human skin sensitization**, b.** Murine local lymph node assay (LLNA)**, c.** Direct peptide reactivity assay (DPRA)**, d.** Human cell line activation test (h-CLAT)**, e.** KeratinoSens™.

Topical anti-inflammatory

Inflammation is a complex biological response to harmful stimuli mainly mediated by two enzymes: cyclooxygenase and lipoxygenase that generate prostaglandins and leukotrienes respectively [15]. The clinical signs of this process are: The clinical signs of this process are heat, redness, swelling and pain, and impaired functioning of the affected organ may occur. At the tissue level, the inflammatory response is characterized by increased vascular permeability, increased protein denaturation and cell membrane alteration [16]. Topical anti-inflammatory effect against xylen-induced ear edema was investigated using mice model. The ability of **IH** to inhibit inflammation induced by xylene was estimated. The obtained results show

that **IH** has an inhibitory effect against topical inflammation. This effect is significantly ($p \leq 0.001$) lower than that of the positive control indomethacin (**IND**) which expresses a percentage inhibition of 87.65 ± 0.13%. However, the inhibition percentages obtained with **IH** is 81.48 ± 0.32%. Whereas, **INH** exhibited similar effect to **IND** with inhibition percentage of 87.65±1.45 % (**Figure 5**).

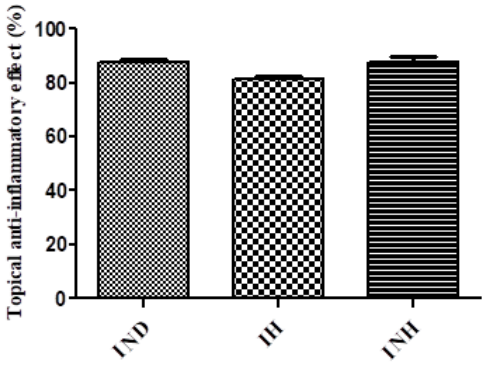

Figure 5. Topical anti-inflammatory effect of IH, INH and IND

CONCLUSION

A new hydrazone **IH**, derived from the isoniazid (anti-tubercular drug) was investigated for their skin sensitization and anti-inflammatory effects. The predicted results indicate the sensitizer effect of this hydrazone. On the other hand, results revealed an excellent topical anti-inflammatory effect of **IH**.

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