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DRUG CLASSIFICATION AND ACUTE RODENT TOXICITY PREDICTIONS OF BIS-PHENOLIC LIGAND: AS A TOPICAL ANTI-INFLAMMATORY AND ANTIFUNGAL AGENT

Kamel MOKHNACHE1*, Noureddine CHAREF1, Soraya MADOUI1, MOHAMMAD S. MUBARAK 2

1 Laboratory of Applied Biochemistry, University Ferhat Abbas Setif 1,19000, Algeria
2Department of Chemistry, The University of Jordan, Amman 11942, Jordan

Abstract:

The ligand 2,2'-[iminobis(ethane-2,1-diylnitriloeth-1-yl-1-ylidene)]diphenol, was synthesized and characterized by a panel of spectroscopic techniques such as UV, IR, and NMR. In addition, computational methods of the ligand were studied by the prediction of drug classification and acute rodent toxicity using software program such as SuperPred and GUSAR respectively. The obtained results indicate the probability of the ligand to be a topical anti-inflammatory, antifungal and anti-acne, comparable to Ethyl hydroxybenzoate, Salicylic acid, Salicylamide and Potassium salicylate with ATC-Prediction accuracy (%) of 51.67%. In vivo topical anti-inflammatory effect of this Schiff base was examined using the xylene-induced edema method. Results revealed the inhibitory action of the ligand on ear edema with an I% = 44.21%, this result was confirmed with docking study which indicate the high anti-inflammatory effect of the ligand with best docking score of -5.9kcal/mol against Cyclooxygenase-2 Protein (COX-2). In vitro antifungal effect of this Schiff base was examined against *F. oxysporum*, and *A. niger*, the result indicate higher antifungal effect against *F. oxysporum* with diameter of inhibition zone of 23, 20, 13 and 13 mm after 48, 72, 96 and 144h respectively and lower effect against *A. niger* with inhibition zone of 35, 10, 0 and 0 mm after 48, 72, 96 and 144h respectively. We conclude that the ligand can be a safe topical anti-inflammatory and antifungal agent in the future.

Keywords: Schiff base; topical Anti-inflammatory; topical Antifungal; acute toxicity, drug classification

Running title: In Silico and in vivo biological activity of *Bis*-Phenolic Ligand

*Corresponding author: kamelmokhnache@yahoo.com

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Introduction

Drugs discovery has been the major objective of the pharmaceutical researches and, exacting the medicinal chemistry, which is a part of pharmaceutical chemistry (1). New drug discovering process is a very difficult mission, which obligate to pharmaceutical companies to must invest seriously in the discovery of drug that could treat or attenuate a disease (2). Drug design and development are processes that consume a lot of time and resources. There is a growing effort to introduce computational approach in chemical and biological space in order to organize the design and development of drugs and their optimization (3). The computer-aided drug design is a significant means in modern medicinal chemistry (4). The objective of drug design is to find a chemical compound that can fit to a specific cavity on a protein target both geometrically and chemically (5). Computational methods of drug design are based on a postulate that pharmacologically active compounds act by interaction with their macromolecular targets, mainly proteins or nucleic acids. Major factors of such interactions are surfaces of molecules, electrostatic force, hydrophobic interaction and hydrogen bonds formation. These factors are mainly considered during analysis and prediction of interaction of two molecules (6). There is considerable interest in computational models to predict drug safety in drug discovery and development (7).Quantitative structure–activity relationship (QSAR) has a fundamental role in computer-assisted-drug discovery. This method tries to determine a reliable relationship between molecular attributes and biological activity (8). Different QSAR and machine learning methods have different ways of deriving these approximations to provide information about the toxic effect of chemicals (9). In this work, The Schiff base ligand 2,2'-[iminobis(ethane-2,1-diylnitriloeth-1-yl-1-ylidene)]diphenol, was synthesized and characterized by a panel of spectroscopic techniques such as UV, IR, and NMR. In addition, computational methods of the ligand were studied by the prediction of drug classification and acute rodent toxicity using software program such as SuperPred and GUSAR respectively. *In vivo* and *in vitro* topical anti-inflammatory and antifungal effect of this Schiff base were examined.

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MATERIAL AND METHODS

Materials

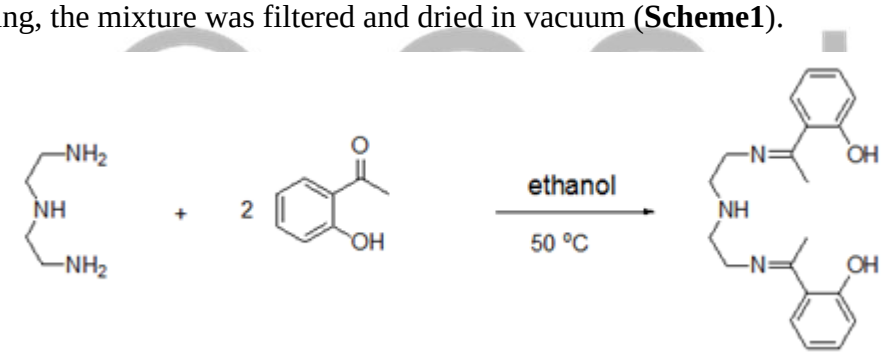
1-(2-hydroxyphenyl)ethan-1-one (Aldrich), Bis (2-aminoethyl)amine (Sigma–Aldrich). Reactions were monitored by thin layer chromatography (TLC) using silica.

Computer software program

SuperPred (10) web server was employed to calculate drug properties and to predict drug classification of the ligand. GUSAR: General Unrestricted Structure-Activity Relationships, web server was used for various activity/ property endpoints and the prediction of different toxicity (11): **GUSAR eco-toxicity** http://www.pharmaexpert.ru/GUSAR/AcuToxPredict/.

Synthesis of the Schiff base ligand, 2,2'-[iminobis(ethane-2,1-diylnitriloeth-1-yl-1-ylidene)]diphenol

Ethanolic solution of Bis(2-aminoethyl)amine (0.516 g, 5mmol) was added to 1-(2-hydroxyphenyl)ethan-1-one (1.36 g, 10 mmol). The mixture was refluxed for 3 h at 55 °C. After cooling, the mixture was filtered and dried in vacuum (**Scheme1**).



Scheme 1. Synthesis of Schiff base ligand.

Yield 98%. M.p.: 93-94 oC. UV–Vis (EtOH) λmax (nm) 321, 389. IR (KBr, cm^{-1}): νmax: 3453 (br, OH), 1616 (C=N). 1H-NMR (500 MHz, DMSO-*d6*) δ(ppm): 2.33 (s, 3H, CH3), 2.88 (t, *J* = 13.0 Hz, 2H), 3.61 (t, *J* = 12.6 Hz, 2H), 6.69 (dd, *J* = 24.9, 16.8 Hz, 2H), 7.21 (t, *J* = 14.9 Hz, 1H), 7.57 (d, *J* = 16.2 Hz, 1H,), 16.49 (s, 1H, OH). 13C-NMR (75 MHz, DMSO-*d6*) δ (ppm): 14.8(CH3), 49.3 (CH2), 49.9 (CH2), 116.7 (CH), 118.8 (CH), 119.2 (C), 129.2 (CH), 132.8 (CH), 164.9 (C), 173.2 (C).

Topical anti-inflammatory effect

The anti-inflammatory effect was evaluated using xylene-induced acute ear edema in adult female *Swiss albino* mice (25-30 g). The animals obtained from 'Institut Pasteur d'Algérie', were treated by topical application of xylene on the inner surface of the right ear, and

immediately treated (20 µL/ear) on the outer surfaces with the ligand (22 mg/mL in acetone). Dexamethasone (0.05 mg/ear) was used as positive control. After 1 h, the ear edema was evaluated using a digital micrometer (12). The inhibition of inflammation was calculated using the formula: $\text{Inhibition}(\%) = 100\,\text{x}\,(\text{Vc} - \text{Vt}) \times \text{Vc}$, where Vc = mean edema in control and Vt = mean edema in the group treated with dexamethasone or the ligand (13).

Antifungal activity

In vitro antifungal activity was carried out using the standardized disc-agar diffusion method (14). Antifungal effect of the ligand (100 mg/mL) was evaluated against *F. oxysporum*, and *A. niger* 2AC 936. The fungal cultures were sub cultured in potato dextrose agar medium. Econazole was used as reference antifungal drug. After incubation at 27 ºC (15), the inhibition zone was measured after 48, 72, 96 and 144h.

RESULTS

The results displayed in (**Table1**) showed the similarity of the ligand to Ethyl hydroxybenzoate, Salicylic acid, Salicylamide and Potassium Salicylate with ATC-Prediction accuracy (%) of 51.67%. Ethyl hydroxybenzoate; (**D01AE**: Antifungals for Topical Use). Salicylic acid (**D01AE**: Other Antifungals for Topical Use, **D02AF**: Salicylic Acid Preparations, **D10AX**: Other Anti-acne Preparations for Topical Use, **D11AF**: Wart and Anti-corn Preparations, **S01BC**: Antiinflammatory Agents, Non-steroids). Salicylamide and Potassium salicylate (**N02BA**: Salicylic Acid and Derivatives).

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Table 1. Drug classification of the Schiff base ligand

	Schiff base ligand	**Most similar compound**			
Compound Names	2,2'-Iminobisethylenebis(nitriloethylidyne)bisphenol	Ethyl hydroxybenzoate	Salicylic acid	Salicylamide	Potassium salicylate
ATC-Prediction accuracy (%)	-	**51.67**	**51.67**	**51.67**	**51.67**
Tanimoto	-	**0.38**	**0.37**	**0.36**	**0.35**
Drug Classification code	-	**(D01A)**	**(D01AE) (D02AF) (D10AX) (D11AF) (S01BC)**	**(N02BA)**	**(N02BA)**
Drug proprieties					
Formula	C20H25N3O2	C9H10O3	C7H6O3	C7H7NO2	C7H5KO3
Molweight	339.431	166.174	138.121	137.136	176.211
xlogP	3.397	1.569	1.09	1.191	0
Heavy Atoms	25	12	10	10	11
Rotatable Bonds	8	3	1	1	1
H-bond Donors	3	0	1	2	1
H-bond Acceptors	5	3	3	2	3
Bonds	26	12	10	10	15
Rings	2	1	1	1	1
Polar Surface Area	77.210	46.53	57.53	63.32	60.36
Total Charge	0	0	0	0	0
SMILES	N(CC/N=C(/C)\c1ccccc1O)CC/N=C(/C)\c1c(cccc1)O	O(C(=O)c1c(O)cccc1)CC	c1ccc(c(c1)C(=O)O)O	c1ccc(c(c1)C(=O)N)O	[K+].Oc1c(cccc1)C(=O)[O-]

Abbreviation

ATC: Anatomical Therapeutic Chemical. D01AE: Other Antifungals for Topical Use. D02AF: Salicylic Acid Preparations. D10AX: Other Anti-acne Preparations for Topical Use. D11AF: Wart and Anti-corn Preparations. S01BC: Antiinflammatory Agents, Non-steroids. N02BA: Salicylic Acid and Derivatives

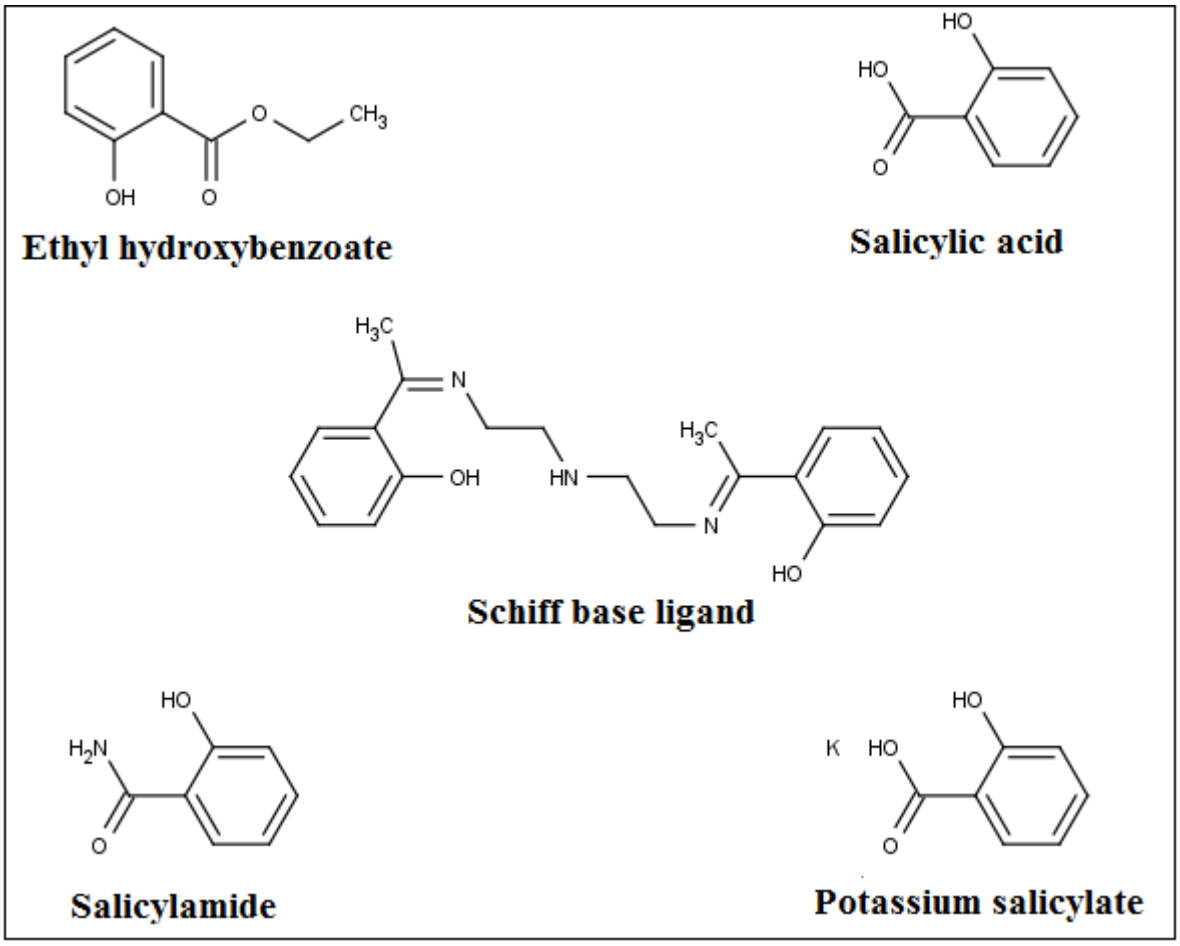

Figure 1. Most similar drug of the ligand

Topical anti-inflammatory effect

The anti-inflammatory activity of the ligand was evaluated in vivo using the xylene-induced edema method. Results revealed the inhibitory action of the ligand on ear edema with an I% = 44.21%. Meanwhile, dexamethasone, as a control, showed a maximum inhibition at 50.40%. Results represented in (**Table 2**) and **(Figure 2)** show the anti-inflammatory effect of the ligand with best docking score of -5.9kcal/mol against Cyclooxygenase-2 Protein (COX-2).

Table 2. Docking scores of the ligand and Salicylic acid against Cyclooxygenase-2 Protein (COX-2)

Compound	**Docking scores (kcal/mol)**				**Best score (kcal/mol)**
	1	2	3	4	
Ligand	-5.9	-5.9	-5.6	-5.5	-5.9
Salicylic acid	-4.7	-4.6	-4.5	-4.4	-4.7

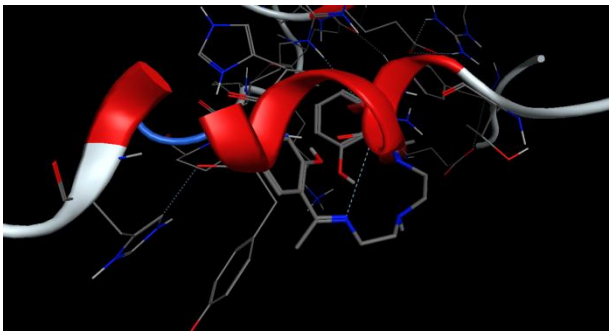

Figure 2. Schiff base ligand docked with Cyclooxygenase-2 (COX-2)

Antifungal activity

In this study and in order to prepare topical antifungal agent, we evaluate the antifungal activity of the synthesized ligand against A. *niger* and F. *oxysporum*, the inhibition zone was measured after 48, 72, 96 and 144h. The results presented in (**Figure 2**), showed higher antifungal activity of the ligand before 72h. But after 72h, the results showed lesser or no activity. The result also indicate higher antifungal effect against *F. oxysporum* with diameter of inhibition zone of 23, 20, 13 and 13 mm after 48, 72, 96 and 144h respectively and lower effect against *A. niger* with inhibition zone of 35, 10, 0 and 0 mm after 48, 72, 96 and 144h respectively.

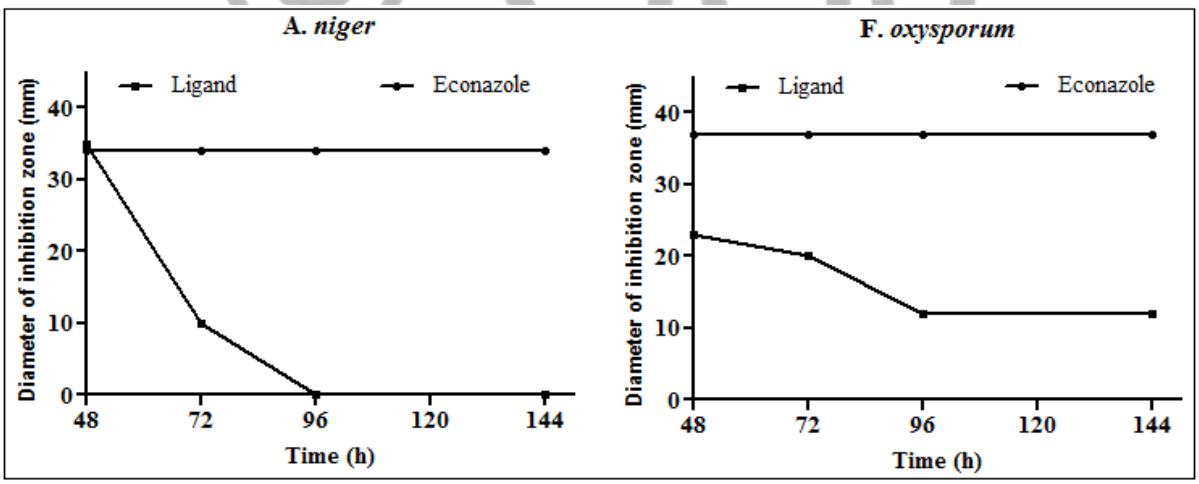

Figure 3. Evolution of inhibition zone the ligand and its metal complexes against fungi after 48, 72, 96 and 144 h.

Acute rodent toxicity prediction

The development and the progress in theoretical chemistry led to developed numerous *in silico* models for chemicals toxicity prediction. In this study, LD50 values and the predicted toxicity class of the Schiff base ligand were predicted by GUSAR software in rodent with different administration route (intraperitoneal, intravenous, oral and subcutaneous). The

results in **Table 3**, showed low toxicity in subcutaneous administration with LD50 value of 1273.00 mg/kg, which classified in the class 5. The results for the other administration route classified the ligand in the class 4, with LD50 values of 47.910, 267.50 and 1431.00 mg/kg for intravenous, intrapereteneal and oral administration respectively.

Table 3. Acute Rodent Toxicity and toxicity Classification of the Schiff base ligand

Administration route	LD50 log10(mmol/kg)	LD50 (mg/kg)	Predicted toxicity class
Intraperitoneal (IP)	-0.103	267.500	4
Intravenous (IV)	-0.850	47.910	4
Oral administration	0.625	1431.00	4
Subcutaneous (SC)	0.574	1273.00	5

DISCUSSIONS

Anatomical Therapeutic Chemical (ATC) system was recognized by World Health Organization (WHO) for drug classification, which classified the drugs from their anatomical, therapeutic, pharmacological and chemical properties, in five classes (anatomical group, therapeutic subgroup, pharmacological subgroup, chemical subgroup and chemical substance) (16). In this study, for classified the Schiff base ligand according to (ATC) system, we employed SuperPred web server. For Ethyl hydroxybenzoate was used as additive agent in cosmetics, food products, and pharmaceutical formulations (17). From these predicted results, the synthesized ligand has topical proprieties of antifungal, anti-acne and non steroid anti-inflammatory agent, which can use in pharmaceutical preparations.

Molecular descriptors of drugs; Ethyl hydroxybenzoate, Salicylic acid, Salicylamide, Potassium salicylate and the synthesized ligand indicate that ligand properties are consistent with Lipinski's drug discovery rule (18). In addition, structure similarity of the precedent drugs with the ligand was represented in (**Figure1**), which indicate; the presence of phenol group in all compound structure, ethylic group and amide function in Ethyl hydroxybenzoate and Salicylamide respectively. These results explain the similarity in biological effect (19).

Topical anti-inflammatory effect

Inflammation is a physical response to microbe infections, physical or chemical agents induced cell damage and pain, which characterized by perturbation in physiological functions. Medications by steroidal and non steroidal anti-inflammatory drugs are mainly employed in the world for reduced or attenuated inflammation. Oral administration of anti-inflammatory

drugs can induce several adverse gastrointestinal effects, which obligate to researchers to prepare new drugs with low undesirable effects or to find other drug-formulations. In this study, the obtained results indicate potential topical anti-inflammatory effect of the ligand *in silico* using drug classification models and docking studies with Cyclooxygenase-2 Protein (COX-2) and *in vivo* using the xylene-induced edema method, which confirmed that the ligand as a topical anti-inflammatory without the passage to oral administration and its gastrointestinal complications.

Antifungal activity

Organism protection against environmental invective is the most important function of the skin (20), (21). Infection of skin by fungus is one of the frequently problem in dermatology (22). Topical antifungals are an important adjuvant in treatment of dermatophytosis (23). Currently, topical formulation is the major preparation in antifungal drug formulation. Preparation of new and safe topical antifungal drug is the major objective of researchers. In this study, the obtained results completed the precedent theoretical results represented in (**Table1**) and confirmed the possibility of the ligand to be an efficacy topical antifungal agent in new pharmaceutical formulations.

Acute rodent toxicity prediction

Currently, *in silico* new computational methods facilitate the early stages of drug discovery processes of researchers from pharmaceutical industry, by the prediction of chemical toxicity before the synthesis in a short time, with low expenses and without the use of animals. In literature, more than 30 % of drug candidates failing in clinical trials because of undetected toxic effects (24). In this investigation and before the synthesis, the ligand was predicted for their toxicity by GUSAR software with different administration route (intraperitoneal, intravenous, oral and subcutaneous), the obtained results showed that subcutaneous which can defined as “under skin” was the best administration route. These predictions, confirmed the safety of the ligand when used as a topical drug “on the skin”.

CONCLUSIONS

The ligand 2,2'-[iminobis(ethane-2,1-diylnitriloeth-1-yl-1-ylidene)]diphenol was synthesized, characterized and was subjected to computational studies, which we predicted their drug classification and their acute toxicity. The topical anti-inflammatory and antifungal properties are the major effects of the ligand were evaluated which displayed significant topical anti-inflammatory activity. In addition, lower acute toxicity of the ligand was evaluated**.** Results obtained in our studies indicate that the ligand can at least be a potential topical anti-inflammatory agent.

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