

# SIMPLE AND EFFICIENT GREEN METHODS INVOLVING MICROWAVES AND MECHANOCHEMISTRY FOR SYNTHESIS OF A SCHIFF LIGAND, 3, 3'-([1,1'-BIPHENYL]-4,4'-DIYLBIS(AZANYLYLIDENE))BIS(INDOLIN-2-ONE) WITH ANTIMICROBIAL ACTIVITY

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Manuscript received: November 2022

## Abstract

An azomethine organic ligand was obtained. The synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) was accomplished by conventional and green protocols. Three methods have been implemented to obtain the compound. The ligand structure was established by NMR spectra, IR spectrum, UV-vis spectrum and elemental analysis. The comparative study of the experimental protocols was performed. The compound has antibacterial and antifungal activity.

## Rezumat

Un ligand organic azometinic a fost obținut. Sinteza 3,3'-([1,1'-bifenil]-4,4'-diilbis(azaniliden))bis(indolin-2-onă) a fost realizată prin protocoale experimentale convenționale și ecologice. Trei metode au fost implementate pentru obținerea compusului. Structura ligandului a fost stabilită prin spectroscopie RMN, IR, UV-vis și analiză elementală. S-a efectuat un studiu comparativ al protocoalelor implementate. Compusul prezintă activitate antibacteriană și antifungică.

**Keywords:** isatin, Schiff base, mechanochemistry, microwaves, structural analysis, antibacterial and antifungal activity

## Introduction

An important class of organic compounds are the Schiff bases. First time synthesis of these compounds was accomplished by Hugo Schiff [1]. Schiff bases also named imines are biologically privileged scaffolds in organic chemistry and important ligands for metal ions in inorganic chemistry [2].

Some of the complexes of Schiff bases with metals of *d*-block as well as with lanthanides metals serve as heterogeneous catalysts for: the oxidation of alkanes, the epoxidation of alkenes and the oxidation of alcohols to carbonyl compounds [3], Suzuki reaction [4], and asymmetry synthesis [5, 6]. Due to the presence of the azomethine functional group many Schiff bases are used as analytical reagents for metal ion detection [7]. Schiff bases exhibits many pharmacological properties. Of these important are antibacterial activity [8, 9], antiviral effect [10, 11], antimalarial action [12], antifungal efficacy [13], anticancer therapy [14], antioxidant potency [15, 16], and anti-inflammatory efficiency [16-18]. The conventional synthesis of Schiff bases is achieved by condensation of carbonyl compounds, aldehydes or ketones, with primary amines. The reaction is reversible, takes place in an organic solvent medium under acidic or basic catalysis during heating [19, 20].

Unconventional methods have also been used to obtain Schiff bases. Of these we can mention microwave irradiation of the chemical medium containing solvent [16, 21] and solvent-free microwave irradiation [22, 23], ultrasound irradiation [24], and mechanochemical reactions [25-27].

We synthesized a Schiff base, 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one), starting from 1*H*-indole-2,3-dione and 1,1'-biphenyl-4,4'-diamine. The compound was obtained by conventional method without catalysts, and unconventional methods without catalysts in paste medium: microwave-assisted synthesis and mechanochemical route.

## Materials and Methods

### Materials

All materials and solvents were obtained from Sigma-Aldrich Chemie GmbH Germany and used without further purification. Ultraviolet-visible spectra were recorded in in  $5 \times 10^{-4}$  mol/L DMF solution using a Cary 50 UV-Vis spectrophotometer manufactured by Varian Inc.. The melting temperature of reagents was determined with a Gallenkamp digital melting point apparatus fabricated by Sanyo Electric Co. The infrared spectra were recorded with the help of an Alpha Bruker Optics spectrometer manufactured by Bruker

Optics GmbH.  $^1\text{H-NMR}$  spectra were acquired on Bruker Avance II spectrometer at 300 MHz in  $\text{DMSO-d}_6$ .  $^{13}\text{C-NMR}$  analysis was performed using a Bruker Avance II spectrometer produced by Bruker Optics GmbH at 75 MHz with  $\text{DMSO-d}_6$  as solvent. The chemical shifts ( $\delta$ ) were recorded in parts per million (ppm), compared to the internal standard (TMS) and coupling constants ( $J$ ) in Hz. The following standard abbreviations for signal multiplicity were used for the  $^1\text{H-NMR}$  spectra: s (singlet), sbr (broad singlet), d (doublet), t (triplet), q (quartet), spt (septet), m (multiplet), dd (double doublet), td (triple doublet). The melting point of the product was done by means of Setsys Evolution, Setaram TG-DTG 92-16 thermobalance, Setaram Instrumentation Inc., under an air flow. Elemental analysis was performed with a Carlo Erba model 1106 elemental analyser, Carlo Erba SpA. A digital infrared IR laser thermometer version 900-En-00, Shenzhen Jumaoyuan Science and Technology Co., Ltd, was employed. A Rohnson P-2012 microwaves, Ronson International Limited, was utilized. A BOECO rotary evaporator RVO 400 SD, Germany GmbH was utilized. An Extech EA10 dual input digital thermometer, Extech Instruments Corporation from Nashua, New Hampshire (USA) was employed. A Vilber Lourmat UV lamp ( $\lambda = 254$  nm) and CN6 darkroom for chromatographic plates, Vilber Lourmat Germany GmbH was employed.

*Conventional synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)*

4.32 mmol of benzidine and 2.16 mmol of isatin were dissolved in a 50 mL round bottom flask containing 20 mL of anhydrous methanol. The resulting solution is introduced in a silicone oil bath on a magnetic stirrer hot plate and refluxed for 1.5 h. The initial solution has  $\text{pH}$  of 6.5 and dark red colour. During the reaction, the solution changes its colour to orange. As the reaction product appears, it precipitates at the bottom of the balloon. After cooling to room temperature, the reaction medium is filtered, and the precipitate is washed with its own filtrate, and then washed with hot ethanol. The product is dried in an oven at  $110^\circ\text{C}$  for 4h. Orange crystals of product are obtained. The product is very thermally stable and has a very high melting point  $397^\circ\text{C}$ , determined with the help of a Setsys Evolution, Setaram TG-DTG 92-16 thermobalance. Elemental analysis (calc./found): %C = 76.01 (76.00); %H = 4.07 (4.08%); %N = 12.66 (12.65) The same working protocol was used when other solvents were employed (Table I).

*Microwave synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)*

In a Pyrex 10 mL beaker was introduced 4.32 mmol of benzidine and 2.16 mmol of isatin. A few drops of EtOH were added. The resulting paste was irradiated in a microwave oven ( $\lambda = 12.2$  cm) for

required time (Table III). The resulting heterogeneous medium is mixed with 10 mL of hot ethanol and filtered off. The precipitate was purified by recrystallization from DMF- $\text{H}_2\text{O}$  to give the product.

*Mechanochemical synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)*

In to an agate mortar with pestle were introduced 4.32 mmol of benzidine, 2.16 mmol of isatin, and a few drops of DMF (2 - 3 drops *i.e.* 0.1 - 0.2 mL). The mixture was grounded for required time (Table IV). The resulting medium is mixed with 10 mL of hot ethanol and filtered off. The product was recrystallized from DMF- $\text{H}_2\text{O}$ .

*In vitro antibacterial and antifungal screening*

The compound has been tested as an antibacterial against gram-positive and gram-negative bacteria. The test was performed on two gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and two gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*.

The anti-fungal activity of the compound was evaluated against two pathogenic fungi *Aspergillus flavus* and *Candida albicans*.

All bacteria were grown on Mueller–Hinton agar plates at  $37^\circ\text{C}$  for 24 h and fungi were grown on Sabouroud's dextrose agar plates at  $26^\circ\text{C}$  for 48 h. Ciprofloxacin has been used as antibacterial drug reference and clotrimazole as antifungal reference drug. To choose the solvent for the study of the antibiotic activity of the compound, the solubility data from Table II are usefully. Dimethylformamide (DMF, Table II, entry 10) is a very good solvent for 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) and for the drugs used as reference.

The compound was dissolved in DMF solutions with a concentration of 0.1%. The disk diffusion assay - Kirby Bauer method [22] was used. The diameter of the inhibition zones was measured. The relative percentage inhibition is determined using the following equation [23]:

$$P = \frac{(A_1 - A_2)}{A_3 - A_2} \times 100\%$$

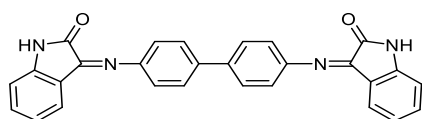
where: P, relative percentage inhibition of the test sample;  $A_1$  [ $\text{mm}^2$ ], surface of inhibition of the tested compound;  $A_2$  [ $\text{mm}^2$ ], surface of inhibition of the solvent,  $A_3$  [ $\text{mm}^2$ ], surface of inhibition of the standard drug. The surfaces were calculated using the circle area formula,  $\pi r^2$ , where  $r$  is radius of inhibition zone.

## Results and Discussion

The reaction between the carbonyl compounds and the primary amines by which the imine results is generally carried out by refluxing the mixture of reactants. The reaction is reversible. Mechanistically, this reaction takes place in two steps. First, nucleophilic addition of amino function to the carbonyl group occurs and a carbinolamine intermediate is formed. Second, the

carbinolamine loses a water molecule by an E<sub>2</sub> elimination reaction and forms the Schiff base. The second stage is the rate-limiting step of the reaction mechanism. The carbinolamine intermediate loses water through the acid or base catalysed pathways [2, 19]. The water removal is important because the reaction is reversible. In order to accomplish this, the use of a Dean Stark apparatus or the use of a molecular sieve and dehydrating agents are useful.

The new Schiff base ligand, 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one), results from the reaction of isatin with benzidine and has the structure depicted in Figure 1.



**Figure 1.**

Structure of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)

#### Conventional synthesis

In the conventional approach, the synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) occurs in homogeneous medium. Both reagents, benzidine and isatin are dissolved in anhydrous methanol. The solution is heated to reflux temperature.

The synthesis of the product was carried out under different experimental conditions. The molar ratio of the reactants follows the stoichiometry of the reaction, isatine:benzidine = 2:1. The synthesis of the Schiff base was carried out in different solvents involving the reflux of the solvent: water, lower alcohols, halogenated compounds and aromatic hydrocarbons. The best yield (90%) has been obtained when methyl alcohol is used as solvent for the reaction medium and 1.5 hours reaction time at reflux temperature. (Table I). The synthesis of the product does not require any catalyst.

**Table I**

Synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) by conventional method refluxing in solvent

| Entry | Solvent   | Molar ratio |           | Time [h] | Yield [%] |
|-------|---|-------------|-----------|----------|-----------|
|       |   | Isatin      | Benzidine |          |           |
| 1     | H <sub>2</sub> O                                | 2           | 1         | 3        | 0         |
| 2     | CH <sub>3</sub> OH                              | 2           | 1         | 1.5      | 90        |
| 3     | C <sub>2</sub> H <sub>5</sub> OH                | 2           | 1         | 2        | 81        |
| 4     | <i>n</i> -C <sub>3</sub> H <sub>7</sub> OH      | 2           | 1         | 3        | 65        |
| 5     | <i>i</i> -C <sub>3</sub> H <sub>7</sub> OH      | 2           | 1         | 3        | 57        |
| 6     | CCl <sub>4</sub>                                | 2           | 1         | 4        | 34        |
| 7     | C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub>   | 2           | 1         | 4.5      | 40        |
| 8     | C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub> * | 2           | 1         | 4.5      | 70        |
| 9     | CCl <sub>4</sub> *                              | 2           | 1         | 4        | 48        |
| 10    | Isooctane                                       | 2           | 1         | 3        | 0         |

\*Refluxing under Dean–Stark apparatus

Solubility of reagents, isatin and benzidine, and the reaction product, Schiff base, 3,3'-([1,1'-biphenyl]-

4,4'-diylbis(azanylylidene))bis(indolin-2-one), has been studied in several solvents. It is observed that the reagents and product are completely insoluble in water (Table II, Entry 1) and for this reason the synthesis cannot be accomplished in aqueous medium (Table I, Entry 1). For the same reasons, product synthesis cannot be performed in isooctane, 0% yield (Table I, Entry 10). The alcoholic solvents dissolve the two reagents, benzidine and isatin, and precipitate the final product. They are good candidates for the conventional synthesis of our product (Table II, Entries 2-9).

Methyl alcohol is a polar solvent having a moment dipole of 2.87 D and dielectric constant of 32.7, which allows it to solve very well the both reactants. Next, the Schiff base, which is insoluble in methanol, will precipitate out of the reaction medium, and the reaction as a whole becomes irreversible. The synthesis of the product accomplished in methanol gains a good yield (Table I, entry 2).

We have also tried to remove the water from the reaction medium to increase efficiency. But, the use of a Dean–Stark distilling trap for removing water from the reaction medium in the case of solvents forming azeotrope, toluene (azeotropic boiling point 77°C) and carbon tetrachloride (azeotropic boiling point 65°C), does not considerably increase the yield (Table I, Entries 8, 9).

Finally, we wanted to perform the synthesis of the Schiff base ligand also in acetic acid medium at refluxing temperature. This organic acid is a polar protic solvent and a Brønsted acid. However, the heterocyclic structure of the isatin molecule does not resist at refluxing temperature of the reaction medium (118°C) to the attack of acetic acid and is destroyed. In these conditions, the synthesis of Schiff base cannot be carried out.

#### Synthesis under microwave irradiation

Electromagnetic microwaves are an unconventional alternative for activating chemical reactions, either in the presence of the solvent or in its absence. Recently, some Schiff bases have been prepared using microwaves in the presence of the organic solvent [16, 21] or in the absence of the solvent [22, 23]. Green chemistry or sustainable chemistry is the field which is oriented towards obtaining chemical products by ecological methods, which minimize the use and generation of materials dangerous for the environment. The activation of organic reactions using electromagnetic microwaves follows this line [28-30]. At the time, we showed that the activation of the chemical reaction using microwaves is due to the formation of hot spots in the reaction medium [28-33]. Starting from our previous experience [29, 30], we set out to obtain the product, 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one), through a green method involving microwaves. The results obtained in the synthesis of the Schiff base under microwaves are shown in Table III.

**Table II**

Solubility of reactants and product in different solvents

| Entry | Solvent                          | Solubility |        |        |        |             |       | $\mu$<br>[34] | $\epsilon$<br>[34] |
|-------|----------------------------------|------------|--------|--------|--------|-------------|-------|---------------|--------------------|
|       |                                  | Benzidine  |        | Isatin |        | Schiff base |       |               |                    |
|       |                                  | RT         | BS     | RT     | BS     | RT          | BS    |               |                    |
| 1     | H <sub>2</sub> O                 | insol      | spsol  | insol  | vpsol  | insol       | insol | 1.87          | 78                 |
| 2     | MeOH                             | vsol       | vsol   | vsol   | vsol   | insol       | vpsol | 2.87          | 32.7               |
| 3     | EtOH                             | sol        | solsol | sol    | solsol | insol       | insol | 1.66          | 24.55              |
| 4     | <i>n</i> -PrOH                   | sol        | solsol | sol    | solsol | insol       | insol | 3.09          | 20.33              |
| 5     | <i>i</i> -PrOH                   | sol        | vsol   | sol    | vsol   | insol       | insol | 1.66          | 19.92              |
| 6     | <i>n</i> -BuOH                   | msol       | sol    | spsol  | msol   | insol       | insol | 1.66          | 19.5               |
| 7     | <i>i</i> -BuOH                   | sol        | solsol | spsol  | sol    | insol       | insol | 1.64          | 18.8               |
| 8     | <i>t</i> -BuOH                   | msol       | solsol | spsol  | sol    | insol       | insol | 1.31          | 10.9               |
| 9     | Allyl alcohol                    | sol        | solsol | sol    | solsol | spsol       | sol   | 1.52          | 20.3               |
| 10    | DMF                              | vsol       | vsol   | vsol   | vsol   | vsol        | vsol  | 3.86          | 36.71              |
| 11    | DMSO                             | vsol       | vsol   | vsol   | vsol   | vsol        | vsol  | 4.1           | 46.68              |
| 12    | 1,4-Dioxane                      | sol        | solsol | sol    | solsol | spsol       | vpsol | 0.24          | 2.25               |
| 13    | THF                              | vsol       | vsol   | vsol   | vsol   | insol       | insol | 1.75          | 7.58               |
| 14    | PhNO <sub>2</sub>                | vsol       | vsol   | vsol   | vsol   | sol         | vsol  | 4.02          | 34.82              |
| 15    | Me <sub>2</sub> CO               | vsol       | vsol   | vsol   | vsol   | insol       | insol | 2.69          | 20.7               |
| 16    | AcOH                             | vsol       | vsol   | vsol   | vsol   | insol       | spsol | 1.68          | 6.15               |
| 17    | MeCOEt                           | vsol       | vsol   | vsol   | vsol   | insol       | insol | 2.78          | 18.5               |
| 18    | CHCl <sub>3</sub>                | insol      | msol   | insol  | msol   | insol       | vpsol | 1.15          | 4.81               |
| 19    | CCl <sub>4</sub>                 | msol       | sol    | vpsol  | msol   | insol       | vpsol | 0             | 2.24               |
| 20    | C <sub>6</sub> H <sub>6</sub>    | vsol       | vsol   | vpsol  | msol   | insol       | insol | 0             | 2.27               |
| 21    | C <sub>6</sub> H <sub>5</sub> Me | vsol       | vsol   | vpsol  | msol   | insol       | insol | 0.43          | 2.38               |
| 22    | Heptane                          | insol      | insol  | insol  | insol  | insol       | insol | 0             | 1.92               |
| 23    | Isooctane                        | insol      | insol  | insol  | insol  | insol       | insol | 0             | 1.94               |

RT-room temperature; BS – boiling solvent; sol – soluble; slsol - slightly soluble; vsol - very soluble; msol - moderately soluble; spsol - sparingly soluble; vpsol - very sparingly soluble; insol – insoluble;  $\mu$  - dipole moment of solvent[D];  $\epsilon$  - dielectric constant of solvent

The synthesis occurs in a solvent-free heterogeneous medium without catalyst. The optimal synthesis time is 4 minutes at a microwave power of 600 W. Further increase in irradiation time does not improve the reaction yield.

**Table III**

Synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) under microwaves at 600W

| Entry | Molar ratio |           | Time [min] | Yield [%] |
|-------|-------------|-----------|------------|-----------|
|       | Isatin      | Benzidine |            |           |
| 1     | 2           | 1         | 2          | 35        |
| 2     | 2           | 1         | 4          | 75        |
| 3     | 2           | 1         | 6          | 94        |
| 4     | 2           | 1         | 8          | 94        |

#### Mechanochemical synthesis

The solid state sometimes offers good conditions for carrying out chemical reactions [35, 36]. Chemical reactions can occur between reactants in solid state and through mechanical action on substances. Mechanochemistry is the science of the interface between mechanic and chemistry. The mechanical energy is transferred to the reaction medium by friction and milling [25, 26].

In the laboratory, mechanochemistry is powered by grinding by hands or mill. Manual grinding is done in a mortar and pestle. The mechanical grinding is

accomplished in a mixer, a shaker or a planetary mill with a frequency of 5-60 Hz [27].

We developed a solvent-less mechanochemical method for synthesis of Schiff-base, 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one). The reaction occurs into an agate mortar with pestle. The grinding of the two reagents, isatin and benzidine happens for 45 minutes to get the Schiff base (Table IV). To trigger the reaction a few drops of DMF were added to the reaction medium.

**Table IV**

Mechanochemical synthesis of 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)

| Entry | Molar ratio |           | Time [min] | Yield [%] |
|-------|-------------|-----------|------------|-----------|
|       | Isatin      | Benzidine |            |           |
| 1     | 2           | 1         | 15         | 25        |
| 2     | 2           | 1         | 30         | 37        |
| 3     | 2           | 1         | 45         | 50        |
| 4     | 2           | 1         | 60         | 53        |

The three methods of synthesis of Schiff base ligand 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) are summarized Table V. Synthesis under microwave provides the lower reaction time and also the greatest yield. From the Table V, it is clearly that the microwave activation is the simple way to access to the Schiff base.

**Table V**

The compare of three methods of Schiff base ligand

| Method | Reaction condition          | Time   | Yield [%] |
|--------|-----------------------------|--------|-----------|
| 1      | Conventional (reflux, MeOH) | 1.5 h  | 90        |
| 2      | Microwaves                  | 6 min  | 94        |
| 3      | Mechanochemistry            | 45 min | 50        |

**FT-IR spectrum**

The IR spectrum of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) reveals the stretching vibration characteristic for band of Schiff base at 1613 cm<sup>-1</sup>. This is a very strong absorption of  $\nu(\text{C}=\text{N})$  azomethine group and demonstrates the formation of the desired reaction product.

**Table VI**

The electronic spectrum of the ligand 3,3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)

| Compound  | $\lambda_{\text{max}}$<br>nm (cm <sup>-1</sup> ) | Transition              | $\epsilon_{\text{max}}$ |
|---|--|-------------------------|-------------------------|
| C <sub>28</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub> | 294 (34013)                                      | $\pi \rightarrow \pi^*$ | 6855                    |
|   | 440 (22727)                                      | $n \rightarrow \pi^*$   | 2446                    |
|   | 694 (14409)                                      | $\pi \rightarrow \pi^*$ | 23                      |

A second band with a very strong intensity appears at 1738 cm<sup>-1</sup> and is assigned to  $\nu(\text{C}=\text{O})$  stretching frequency. The broad bands between 3121 and 3236 cm<sup>-1</sup> is assigned to symmetric and asymmetric stretching valence vibrations of NH. This  $\nu(\text{NH})$  stretching vibration displays a medium intensity. The stretching vibration of  $\nu(\text{C}-\text{N})$  displays at 1285 cm<sup>-1</sup> and has strong light infrared absorption, and  $\nu(\text{C}=\text{C})$  vibration occurs at 1462 and also exhibits a strong infrared

light absorption. Below 900 cm<sup>-1</sup> are found the vibrations of the benzenoid ring [37].

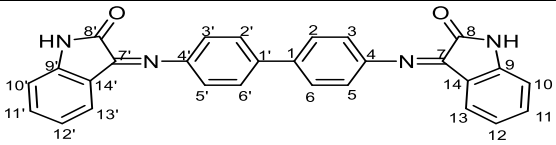
The most important absorption bands provided by the IR spectrum of this Schiff base and their intensities are the following: 577(s), 703(m), 729(s), 746(s), 992(m), 1093 (s), 1285 (vs), 1462 (vs), 1487 (vs), 1613(vs), 1651 (m), 1722 (s), 1738 (vs), 2796 (vw), 2880 (vw), 3121 (w), 3169 (m), 3236 (m). (s = strong, m = medium, w = weak, vs = very strong, vw = very weak).

**NMR spectra**

As shown in Table II, the ligand is insoluble in many organic solvents and therefore it was a problem to find the suitable solvent for NMR spectra recording. Fortunately, this ligand dissolves very well at room temperature in dimethylsulfoxide (DMSO, Table II, entry 11) and therefore DMSO-d<sub>6</sub> was used to record <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectra. The absorption signals of the carbon atoms of the compound are recorded at 75 MHz using the *J*-modulated spin-echo experiment. Through spin-echo <sup>13</sup>C-NMR spectroscopy, the peaks of the secondary and quaternary carbon atoms are located above the baseline, and the peaks of the primary and tertiary carbon atoms are located below the baseline of the spectrum [29, 30, 32, 37]. Our compound contains twenty-eight carbon atoms in the molecule. The molecule of the Schiff base is symmetrical having twelve carbon nucleus different magnetically. Of these, two carbon atoms are secondary and ten carbon atoms are tertiary. The twelve magnetically different <sup>13</sup>C nucleus appear as twelve distinct peaks: ten peaks below the baseline and two peaks above the spectrum baseline (Table VII). The peak of the solvent DMSO-d<sub>6</sub> displays at 39.5 ppm.

**Table VII**

<sup>13</sup>C-NMR chemical shifts ( $\delta$ , ppm) of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)

| Compound          |  |  |  |                                   |                                   |                                   |                                   |                                     |                                     |                                     |                                     |                                     |
|-------------------|--|--|--|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Carbon atoms      | C <sub>1</sub> ,<br>C <sub>1'</sub>  | C <sub>2</sub> , C <sub>6</sub><br>C <sub>2'</sub> , C <sub>6'</sub> | C <sub>3</sub> , C <sub>5</sub><br>C <sub>3'</sub> , C <sub>5'</sub> | C <sub>4</sub><br>C <sub>4'</sub> | C <sub>7</sub><br>C <sub>7'</sub> | C <sub>8</sub><br>C <sub>8'</sub> | C <sub>9</sub><br>C <sub>9'</sub> | C <sub>10</sub><br>C <sub>10'</sub> | C <sub>11</sub><br>C <sub>11'</sub> | C <sub>12</sub><br>C <sub>12'</sub> | C <sub>13</sub><br>C <sub>13'</sub> | C <sub>14</sub><br>C <sub>14'</sub> |
| ( $\delta$ , ppm) | 126.8  | 121.7  | 118.3  | 134.3                             | 148.3                             | 115.7                             | 127.4                             | 114.2                               | 126.1                               | 120                                 | 125.2                               | 111.4                               |

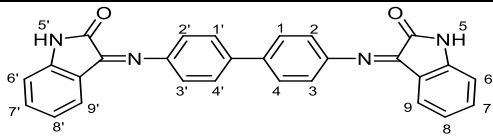
The <sup>1</sup>H-NMR spectrum of Schiff base has been analysed. The peaks of the solvent DMSO are assigned at 2.55 ppm (quintet) and 3.3 ppm. Pure dimethylsulfoxide always contains a small amount of water due to its strong affinity for water molecules. A small part of it, by isotope exchange with the solvent, becomes semi-deuterated water HOD. Therefore, in the <sup>1</sup>H-NMR spectrum, besides the residual signal of dimethylsulfoxide, it is also the singlet peak of H<sub>2</sub>O and the triplet due to HOD, 1:1:1. This signal is observed at 3.3 ppm.

The <sup>1</sup>H-NMR chemical shift range 0 - 13.5 ppm and expanded in the chemical shift region 6.4 - 8 ppm. The

absorption peaks of protons are integrated. As can be seen the integral of the signal of the aromatic protons,  $\delta = 6.58 - 7.92$  ppm, is  $I_1 = 7.95$ , and the integral of the absorption signal of the proton of the region  $\delta = 10.90 - 11.00$  is  $I_2 = 1$ . The ratio of the two integrals is identical to the ratio of the number of protons in the molecule (two protons in NH and sixteen protons on the four benzene rings):  $I_1:I_2 = 7.95:1 \approx 16:2$ . Thus, the structure of the Schiff base is again demonstrated by the number of hydrogen atoms that the ligand molecule contains. The absorption signal that appears at  $\delta = 10.90 - 11.00$  ppm is due to the proton from the NH function.

Table VIII

<sup>1</sup>H-NMR chemical shifts ( $\delta$ , ppm) of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)

| Compound          |  |  |                                  |                                  |                                  |                                  |   |
|-------------------|--|--|----------------------------------|----------------------------------|----------------------------------|----------------------------------|---|
|                   | Protons  | H <sub>1</sub> , H <sub>1'</sub> ,<br>H <sub>4</sub> , H <sub>4'</sub> | H <sub>6</sub> , H <sub>6'</sub> | H <sub>7</sub> , H <sub>7'</sub> | H <sub>8</sub> , H <sub>8'</sub> | H <sub>9</sub> , H <sub>9'</sub> | H <sub>2</sub> , H <sub>2'</sub> ,<br>H <sub>3</sub> , H <sub>3'</sub> ,<br>H <sub>4'</sub> |
| ( $\delta$ , ppm) | 6.58 - 6.82  | 6.89 - 6.95  | 7.01 - 7.13                      | 7.14 - 7.20                      | 7.36 - 7.51                      | 7.63 - 7.92                      | 10.90 - 11.00   |
| <i>J</i> (Hz)     | 9; 6   | 9; 6, 3  | 9; 6                             | 9; 6;3                           | 6; 3;                            | 9; 6                             | -   |

The sixteen aromatic protons form a very well represented massif. This massif includes four proton nuclei with nonequivalent magnetic nuclei AA'BB'. Such a spin system can be solved by computing. However, manual analysis can be performed if the system has sufficient number of detectable peaks [37]. We performed the <sup>1</sup>H-NMR analysis of the Schiff base and the results are reported in the Table VIII. Nuclear magnetic resonance coupling constants

show <sup>3</sup>*J* = 9 Hz for vicinal protons coupled in *cis*, <sup>4</sup>*J* = 6 Hz for spin-spin couplings through four covalent bonds and <sup>5</sup>*J* = 3 Hz for spin-spin coupling constants at large distances.

*Antimicrobial activity of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one)*

The Antimicrobial activity of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) is presented in Table IX.

Table IX

Antimicrobial activity of 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one).

| Relative percentage inhibition, P [%] |                               |                        |                              |                           |                         |
|---------------------------------------|-------------------------------|------------------------|------------------------------|---------------------------|-------------------------|
| Gram-positive bacteria                |                               | Gram-negative bacteria |                              | Fungi                     |                         |
| <i>Escherichia coli</i>               | <i>Pseudomonas aeruginosa</i> | <i>Bacillus cereus</i> | <i>Staphylococcus aureus</i> | <i>Aspergillus flavus</i> | <i>Candida albicans</i> |
| 16                                    | 32                            | 25                     | 15                           | 12                        | 10                      |

The Schiff ligand 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) exhibits antibacterial and antifungal activity. However, the antifungal activity is weaker than the antibacterial one. The most intense effect was in the case of gram-positive bacteria *Pseudomonas aeruginosa*.

## Conclusions

An efficient one pot synthesis of the Schiff ligand 3'-([1,1'-biphenyl]-4,4'-diylbis(azanylylidene))bis(indolin-2-one) has been obtained. Three methods were developed: a conventional synthesis and two green synthesis - microwaves assisted synthesis, and mechanochemical synthesis. Using microwaves the synthesis occurs very quickly in heterogeneous medium and no catalyst is needed. The structure of the new ligand was confirmed by structural analysis. All the recorded spectra (IR, UV-vis, <sup>1</sup>H- and <sup>13</sup>C-NMR) were interpreted. Compound displayed mild antibacterial and antifungal activity against the tested microorganisms.

## Conflict of interest

The authors declare no conflict of interest.

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