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Synthesis, Characterization and Antimicrobial Evaluation of Symmetric A-Diimine Schiff Bases Derived from Cis and Trans Racemic Mixture of Cyclohexanediamine

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ABSTRACT

From N,N'-bis(phenylmethylene)cyclohexane-1,2-diamine, substitution of a nitro group on each aromatic ring and its systematic displacement in the positions ortho, meta and para positions allowed to synthesize a homogeneous series of positional isomers. These four symmetric α-diimine Schiff bases derived from cis and trans racemic mixture of cyclohexanediamine have been characterized by conventional spectroscopic methods (NMR, IR and MS). Antimicrobial screening showed that, unlike N, N'-bis (phenylmethylene) cyclohexane-1,2-diamine, the bacterial strain Staphylococcus aureus CIP is sensitive to the other three compounds with MIC values of 93.75µg/ml, 187.5µg/ml and 375µg/ml. The Candida albicans fungal strain shows resistance to all synthesized compounds, but Candida glabrata is sensitive to the non-substituted N, N'-bis(phenylmethylene)cyclohexane-1,2-diamine and ortho substituted compound with a MIC value of 1500µg/ml.

Keywords: Symmetric α-diimine Schiff base; Racemic; Spectral studies; Antibacterial; Antifungal.

Introduction

Schiff bases are condensation products of primary amines with carbonyl compounds**[1-2]**. The common structural feature of these compounds is the azomethine group with the general formula $R_1HC=N-R_2$, where R_1 and R_2 are alkyl, aryl, cycloalkyl, or heterocyclic groups. Schiff bases can be synthesized by considerably flexible procedures. Consequently, a wide variety of these bases can be prepared. Imine or azomethine groups are present in various natural, naturally- derived, and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities **[3-5]**. For this reason, Schiff bases, which are very easy to synthesize, constitute an inexhaustible source of promising versatile pharmacophores for the design and development of new biologically important molecules. Schiff bases are indeed known to exhibit a wide range of biological activities such as: antibacterial, antifungal, antimalarial, antiproliferative, anti-inflammatory, antiviral, antipyretic, anti-tumor, and antioxidant properties **[6-10]**.

As several authors **[11-14]**, our systematic research on Schiff bases allowed us to synthesize many diimines. Gao et al. **[15]** have investigated the actions of *ortho*, *meta* and *para*-NO2 substituted *N,N'*-bis (phenylmethylene)cyclohexane-1,2-diamines in the asymmetric catalytic cyclopropanation of 2,5-dimethyl-2,4-hexadiene with L-menthyl diazoacetate. Moreover, according to Sharma et al.**[16]** antibacterial screening of *N,N'*-bis(phenylmethylene) cyclohexane-1,2-diamine, *N,N'*-bis(*m*-nitrophenylmethylene) cyclohexane-1,2-diamine and *N,N'*-bis(*p*-nitrophenylmethylene)cyclohexane-1,2-diamine Schiff bases were found to be totally inactive on *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. In the other hand, these Schiff bases are also found to have no antifungal activity even at 500µM**[16]**.

In order to test this series of compounds on other patogenic strains and at another initial concentration, we synthesized it with the *ortho* isomer. Inclusion of *ortho* isomer, compared to Sharma et al. Schiff bases series allows us to have a complete sequence of positional isomers. So, the present paper deals with the synthesis, characterization and biological studies of all of the synthesized compounds shown in Figure 1.

Figure1. Condensed molecular structures of the symmetric Schiff bases synthesized with atomic numbering scheme

*Compound **1**: *N,N'*-bis (phenylmethylene) cyclohexane-1,2-diamine
*Compound **2**: *N,N'*-bis (*o*-nitrophenylmethylene) cyclohexane-1,2-diamine
*Compound **3**: *N,N'*-bis (*m*-nitrophenylmethylene) cyclohexane-1,2-diamine
*Compound **4**: *N,N'*-bis (*p*-nitrophenyl methylene) cyclohexane-1,2-diamine

Antibacterial activity of these four compounds was tested at an initial concentration of 1500µM on *Staphylococcus aureus* (CIP) 4.83, *Pseudomonas aeruginosa* (CIP) 103467, *Escherichia coli* (CIP) 54127AF and *Staphylococcus aureus* sensitive to penicillin strains from Pasteur Institute Collection (CIP). The antifungal activity of these molecules was also tested at an initial concentration of 1500µM on the pathogenic strains of *Candida albicans* and *Candida glabata*.

Material and Methods

Benzaldehyde, 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, and *cis* and *trans* racemic mixture of cyclohexane-1,2-diamine were procured from Sigma-Aldrich and were used without further purification. All organic solvents were purchased from Merck and dried before use. Melting points were determined in capillary tube using an MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus and are uncorrected. IR spectra in the range 400-4000 cm^{-1} were obtained on a Bruker-Vector FTIR spectrophotometer, with samples investigated as thin film from CDCl3 solution. The ^{1}H NMR spectra were recorded on a Bruker-Avance-300 spectrometer, operating at 300 MHz. The mass spectra were recorded on a TOF LCT Premier (WATERS) Spectrometer coupled to an HPLC Alliance 2695 chain.

*Synthesis of *N,N'*-bis(phenylmethylene)cyclohexane-1,2-diamine

Benzaldehyde (0.4mmol) and cyclohexane-1,2-diamine (0.2mmol) were dissolved in diethylether (30ml). At room temperature, the mixture was stirred for 3 day to give a brown precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.81 in hexane/acetone (50:50), yield: 90%, mp: 103^{o}C).

*Synthesis of *N,N'*-bis(2-nitrophenylmethylene)cyclohexane-1,2-diamine

2-Nitrobenzaldehyde (0.4mmol) and cyclohexane-1,2-diamine (0.2mmol) were dissolved in diethylether (30ml). At room temperature, the mixture was stirred for 4 day to give a brown precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.77 in hexane/acetone (50:50), yield: 79%, mp 108^{o}C).

**N,N'*-bis(3-nitrophenylmethylene)cyclohexane-1,2-diamine

3-Nitrobenzaldehyde (0.4mmol) and cyclohexane-1,2-diamine (0.2mmol) were dissolved in diethylether (30 ml). At room temperature, the mixture was stirred for 3 day to give a pale yelow precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.73 in hexane/acetone (50:50), yield: 80%, mp 77^{o}C).

We have recently reported the crystal structure of this compound [17].The single crystal X-ray diffraction characterization showed that this molecule, which crystallizes in non-standard I2/a monoclinic space group is an enantiopure *trans*(1*R*, 2*R*)*N*, *N'*-bis(3-nitrophenylmethylene)cyclohexane-1,2-diamine, in contrast to the racemic *cis* and *trans* cyclohexane α-diamine mixture used for the synthesis.

***Synthesis of *N,N'*-bis(4-nitrophenylmethylene)cyclohexane-1,2-diamine**

4-Nitrobenzaldehyde (0.8mmol) and cyclohexane-1,2-diamine (0.4mmol) were dissolved in diethylether (30ml). At room temperature, the mixture was stirred for Three day to give a pale yelow precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.75 in hexane/acetone (50:50), yield: 83%, mp 111^{o}C).

Biological Activity

***Antibacterial Assays**

The bacterial cultures: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (CIP) 4.83, *Pseudomonas aeruginosa (*CIP) 103467, *Escherichia coli* (CIP) 54127AF and *Staphylococcus aureus* sensitive to penicillin were obtained from Pasteur Institute Collection (CIP) and also provided by the National Laboratory of Public Health of Côte D'Ivoire. The bacterial cultures were incubated at 37 °C for 18 hours by inoculation into nutrient agar. Schiff bases were stored dry at room temperature and were dissolved in dimethylsulfoxide (DMSO) at concentrations of 1500 µg/mL followed by dilution to 250µg/mL. Antibacterial activities of each compound were evaluated by the agar disc-diffusion method. Mueller Hinton Agar Media (15 cm^3) kept at 45^{o}C was poured in the Petri dishes and allowed to solidify. Poured Petri plates (9 cm) were incubated with 50µL of normal saline solution of the above culture media (10^5-10^6 bacteria per ml). Discs injected with prepared Schiff bases (50µL) were applied on the solid agar medium by pressing tightly. The Petri plates were placed at 37^{o}C for 18 hours. At the end of period the inhibition zones formed on media were measured with a zone reader.

***Antifungal Assays**

Pathogenic strains of *Candida albicans* and *Candida glabata* were obtained from National Laboratory of Public Health and the Microbiology Laboratory of Swiss Centre of Scentific Research of Côte D'Ivoire. Schiff bases were stored dry at room temperature and dissolved at 1500 µg/mL in dimethylsulfoxide (DMSO). Antifungal activities of each compound were evaluated by the agar disc-diffusion method. Sabouraud agar media (15 cm^3) kept at 45^{o}C was poured in the Petri-dishes and allowed to solidify. Sterile, filter paper discs of 10mm diameter were impregnated with prepared Schiff bases (50µL) and were placed on to the media, seeded with fungus. The plates were then incubated at 37^{o}C for 1-3 days. At the end of period the inhibition zones formed on media were measured with a zone reader in millimeters.

Results and Discussion

Mass Spectra (MS) and IR

The mass spectra and the infrared spectra of the synthesized compounds are given in Table 1

Table 1: Mass spectrum and selected infrared data

Compound	molar mass (g/mol)	Mass spectrum $[M+H]^+$ (g/mol)	infrared spectrum: (Cm^{-1}) ($\upsilon_{C=N}$)	(υ_{C-H})
1	290	291.2062	1639	2941-2950
2	380	381.1522	1637	2964-2973
3	380	381.1560	1642	2929-2847
4	380	381.1595	1643	2939-2852

MS study

The mass spectra (HR-ESI-MS) of the title compounds show peaks corresponding to the molecular ions at *m/z* 381.1560 $[M + H]^+$; 381.1560 $[M + H]^+$; 381.1522 $[M + H]^+$, corresponding to $C_{20}H_{20}N_4O_4$ for compounds **2, 3** and **4**. For compound **1** the peak at *m/z* 291.2062 $[M + H]^+$, corresponds to the molecular formula $C_{20}H_{22}N_2$.

IR study

The IR spectra show characteristic bands at 1639 cm^{-1} for compound **1**, 1637 cm^{-1} for compound **2**, 1642 cm^{-1} for compound **3** and 1643 cm^{-1} for compound **4**. These bands correspond to the elongation vibration of the two azomethine vibrators C=N present in each molecule structure. Thus, the fact of obtaining only one vibration band $\upsilon_{C=N}$ for the two C=N bonds attests that the molecules studied are symmetric. The absence of N-H vibrator bands around 3500cm^{-1} in the spectra confirms the absence of an amine group in the synthesized products. The multi-bands located between 2941 cm^{-1} and 2847 cm^{-1} indicated in Table **1**, correspond to υ_{C-H} elongation vibrations of cyclohexane and aromatic fragments.

^{1}H NMR Spectroscopy

^{1}H NMR spectral data in deturated CDCL3 solution of the synthesized compounds are given in Table **2**.

Table 2: ^{1}H NMR data^{a-c} of compounds with general formula R1N=CHR2

Compound	Molecular formula	N=C<u>H</u> (s)	$C_6\underline{H}_{4(5)}$ (m)	-C<u>H</u>$_2$- (m)	-C<u>H</u>- (m)
1	$C_{20}H_{22}N_2$	8.70 ;(2H)	7.83-7.52;(10H)	2.19-1.56;(8H)	3.60-3.55;(2H)
2	$C_{20}H_{20}N_4O_4$	8.72 ;(2H)	8.09-7.59;(8H)	2.19-1.56;(8H)	3.66-3.58;(2H)
3	$C_{20}H_{20}N_4O_4$	8.45 ;(2H)	8.28-7.90;(8H)	2.16-1.59;(8H)	3.50-3.47;(2H)
4	$C_{20}H_{20}N_4O_4$	8.46 ;(2H)	8.15-7.85;(8H)	2.05-1.50;(8H)	3.69-3.52;(2H)

a Multiplicity is given as s = singlet, m = multi-signals
b Chemical shits in ppm ; c Integration: number of protons in brackets

The resonance of protons has been assigned on the basis of their integration and multiplicity patterns [18]. The ^{1}H NMR spectra exhibit signals at 8.70 ppm, 8.72 pp,; 8.45ppm and 8.46 ppm for compounds **1**, **2**, **3** and **4**, respectively, attributed to the iminic C<u>H</u>=N- protons**.** The multi-signals within the 8.28-7.52 ppm range are assigned to the aromatic protons of both rings**.** The cyclohexane protons exhibit multi-signals within the 1.50-3.69 ppm range attributed to -C<u>H</u>$_2$- and -C<u>H</u>- protons. The ^{1}H-NMR spectral data of the Schiff bases synthesized are in accord with the proposed structures.

Antibacterial activity

The results of the antibacterial screening of compounds **2**, **3,** and **4** at a concentration of 1500µg/ml against *Staphylococcus aureus* (CIP) 4.83 and *Staphylococcus aureus sensitive to penicillin* are shown in Table **3**. The inhibition zones were measured in mm and results are shown in Table **3**. The inhibition zones diameters were between 9 and 18 mm. The results indicate that, this compounds show significant activity against *Staphylococcus aureus* (CIP) 4.83 than *Staphylococcus aureus* sensitive *to penicillin*, while they were found to be inactive against *Escherichia coli* (CIP) 54127AF, *Pseudomonas aeruginosa* (CIP) 103467, *Staphylococcus aureus* ATTC 25923. We note that compound **1** without a nitro group on the benzene rings is inactive on all the strains.

Table 3: Mean diameters (mm) of the inhibition zones and Value of Minimum Inhibitory Concentration (MIC) values for antibacterial activity

Mean diameters of the inhibition zones (mm)											**Value of MIC (µg/mL)**	
Strains tested	***Pseu a*** CIP		***Sta a*** CIP		***Sta a sens***		***E. coli*** CIP		***Sta a*** ATTC			
Concentrations (µg/mL) C1=1500 : C2=250 : C3=25											***Sta a*** CIP	***Sta* a sens**
Compounds	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2		
1	0	0	0	0	0	0	0	0	0	0	-	-
2	0	0	12	9	11	0	0	0	0	0	93.75	>1500
3	0	0	18	11	10	0	0	0	0	0	375	>1500
4	0	0	18	10	11	0	0	0	0	0	187.5	>1500
Witnesses	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3		
Gen	23	10	29	23	32	23	12	0	19	10	0.78	12.5
Tetra	0	0	36	29	27	19	29	17	24	15	0.0976	12.5

Values are averages of three repetitions; ***Gen****: Gentamicin;* ***Tetra****: Tetracycline;* ***Sta a sens****: Staphylococcus aureus sensitive to penicillin;* ***Sta a****: Staphylococcus aureus ;* ***Pseu a*** *: Pseudomonas aeruginosa ;* ***E. coli :*** *Escherichia coli*

On the strain *Staphylococcus aureus* CIP, the compound **2** in which the nitro group is substituted in *ortho* position is the most active with a MIC of 93.75 µg/mL. On the strain *Staphylococcus aureus* sensitive to compounds **2**, **3** and **4** have inhibition concentrations greater than 1500µg/mL. Gentamicin and tetracycline showed activities (10-36 mm) on all strains with MIC values of 0.78 and 0.0976 µg/mL respectively on Staphylococcus aureus (CIP) 4.83.

Antifungal activity

All the compounds including amphotericin B show no antifungal activity against *C. albicans* with the exception of nystatin as shown in Table **4**. However, on *C. glabrata*, compounds **1** and **2** show activities with inhibition diameters between 10 mm and 11mm, but compounds **3** and **4** as well as amphotericin B and nystatin remain insensitive. Thus, on the other hand, on *C. glabrata*, the compound **1** is active with a MIC greater than 1500 µg/mL and as before the *ortho*-substituted compound **2** is the most active with a MIC of 1500 µg /mL.

Conclusion

We can therefore conclude herein that in contrast to Sharma et al. study, *ortho*, *meta* and *para*-NO2 substituted *N*, *N'*-bis(phenylmethylene)cyclohexane-1,2-diamines are activities on the bacterial strains *Staphylococcus aureus* (PIC) 4.83 and *Staphylococcus aureus* sensitive to penicillin as evidenced by the MIC values of 93.75µg/ml, 187.5µg/ml and 375µg/ml respectively. In the other hand, antifungal assays have shown that *ortho*-NO2 substituted compound is the one among the three positional isomers that is active with a MIC of 1500 µg/mL. Consequently, this *ortho* position seems to be a privileged position for important biological activity of this pharmacophore. We will soon design and synthesize new series taking into account this result.

Table 4: Mean diameters (mm) of the inhibition zones and Value of Minimum Inhibitory Concentration (MIC) values for antifungal activity

Mean diameters of the inhibition zones (mm)			**MIC Value (µg/mL)**	**MIC Value (µg/mL)**
Strains tested	*C. albicans*	*C. glabrata*	*C. albicans*	*C. glabrata*
Compounds	Concentrations (µg/mL) C1=1500 : C3=25			
	C1	C1		
1	0	10	-	**>1500**
2	0	11	-	**1500**
3	0	0	-	-
4	0	0	-	-
Witnesses	**C3**	**C3**		
Ampho B	0	0	-	-
Nyst	11	0	**50**	-

Ampho B: *Amphotericin B;* ***Nyst****: Nystatin*

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