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Synthesis, Structural Characterization and *In Vitro* Leishmanicide and Antifungal Activities Studies of Schiff Base Compounds

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ABSTRACT

N, N'-bis (2-nitrophenyl methylene)hexane-1,6-diamine, N, N'-bis (4- nitrophenylmethylene)benzene-1,3-diamine, N, N'-bis (p-nitrophenylmethylene) cyclohexane- 1,2-diamine were synthesize and have been characterized by conventional spectrometry methods (NMR, IR and HR-ESI-MS). Antileishmanicide and antifungal screening showed that, N, N'-bis (2-nitrophenyl methylene) hexane- 1,6-diamine, with MIC of 16.40 mg/L and 125 mg /L respectively, is the most active.

Keywords

Schiff base, NMR, IR, MS, Antileishmanicide, Antifungal.

Introduction

Schiff's bases are an important class of organic compounds first reported by Hugo Schiff in 1864 [1,2]. Schiff's bases are condensation products of primary amines with carbonyl compounds. The azomethine group of general formula R1HC = N-R2, where R1 and R2 are alkyl, aryl, cycloalkyl or heterocyclic groups, is the common structural feature of these compounds. Schiff bases have considerable flexible synthetic procedure. The very flexible synthesis procedure of these compounds allows us to prepare a wide variety of them. It should be noted that imine or azomethine groups are present in various naturally occurring and non- natural compounds. The presence of the imine group in these compounds is essential to their biological activities [3-5]. Consequently, Schiff bases present themselves as a promising alternative for the development and provision of new biologically very active molecules. Schiff bases exhibit a wide range of biological activities such as: antibacterial, antifungal, antimalarial, antiproliferative, anti-inflammatory, antiviral, antipyretic, anti-tumor, antioxidant [6-9]. Due to their wide range of industrial applications, Schiff bases are of very significant interest [10,11]. The importance of Schiff base complexes no longer needs to be demonstrated. Its implications in bio-organic chemistry, catalysis and materials science, biomedical applications have been recognized and examined.

Our systematic structural and biological activities research on this kind of compound led us to synthesize many compounds among which the three compounds, which is the subject of this study: N, N'-bis (2-nitrophenyl methylene) hexane- 1,6-diamine (compound **1**), N, N'-bis (p- nitrophenylmethylene)cyclohexane-1,2-

diamine(compound **2**), and N, N'-bis (4- nitrophenylmethylene) benzene-1,3-diamine (compound **3**). The present paper deals with the synthesis, characterization and biological studies of all of the synthesized compounds shown in Figure 1.

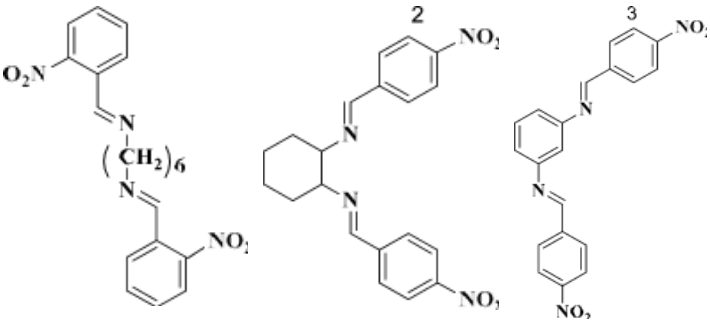

Figure 1: Structures of Schiff bases synthesized.

Material and Methods

Cyclohexane-1,2-diamine, 2-nitro-benzaldehyde, 4-nitro-benzaldehyde, hexane-1,6-diamine and benzene-1,3-diamine were procured from Aldrich and 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 (2-nitrophenylmethylene) hexane-1, 6-diamine: compound 1

2- nitrobenzaldehyde (0.4 mmol) and hexane-1,6-diamine (0.2 mmol) were dissolved in ether (30 mL). At room temperature, the mixture was stirred for six days to give a light brown precipitate. The precipitate obtained was filtered and recrystallized in methanol (Rf: 0.82 in hexane/acetone/acetate diethyl (20; 50; 30).

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

4-nitrobenzaldehyde (0.8 mmol) and cyclohexane-1,2-diamine (0.4 mmol) were dissolved in ether (30 mL). At room temperature, the mixture was stirred for Three days to give a pale yelow precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.75 in hexane/acetone (50;50).

Synthesis of *N, N'*-bis (4-nitrophenylmethylene) benzene-1,3-diamine: compound 3

4-nitrobenzaldehyde (0.8 mmol) and benzene-1,3-diamine (0.4 mmol) were dissolved in ether (30 mL). At room temperature, the mixture was stirred for Three days to give a Red bordeau precipitate. The precipitate obtained was filtered and recrystallized in ethanol (Rf: 0.74 in benzene/acetone (50; 50)

Biological Activity

Synthesized Schiff bases were screened for leishmanicidal and antifungal activity.

Antileishmanicide Testing

This test was carried out using the LV9 strain (MHOM/ET/1967/L82) of Leishmania donovani (Pr S. L. Croft, London School of Tropical Medicine and Hygiene, London, England).
Leishmania at the promastigote stage are maintained in culture in 25 ml flasks containing 5 ml of complete medium.

The nutrient medium is composed of: RPMI (10*): 10 mL; decomplemented fetal calf serum: 10 mL; Hepes buffer: 2.5 mL; glutamine 2 mM: 1 mL; gentamicin 40 mg/mL: 0.125 mL; sterile deionized water; qsp 100 mL; pH: adjusted to 7.4 (1M NaoH).

The samples to be evaluated are first dissolved in DMSO, then in RPMI in order to lower the final solvent concentration to less than 2% (v/v) during the tests, such a percentage not affecting the viability of parasites.

The tests are carried out in 96-well flat-bottom plates. The parasites are taken in the exponential growth phase (approximately 72 hours after culturing). For 72 or 96 hour cell cultures, such as promastigotes, the EC50 is always desirable.

In each well, 195 µl of culture medium containing 2.10^5 leishmanias at the promastigote stage are deposited. The plates are then placed in an oven at 27°C for 1 hour. After this incubation, the substances are added at a rate of 5µl per well for 4 hours. Three wells are used for each concentration, and the tests are carried out three times.

The evaluation of antileishmanial activity, expressed in the form of EC50, is carried out by two methods:

- Direct reading under an optical microscope and by comparison with negative controls.

This qualitative test makes it possible to evaluate the vitality of leishmania in each well, based on three criteria: the number of parasites present in each well compared to the controls, the mobility of the parasites and the shape of the cells.

- Quantitative colorimetric assays using MTT (living cell numbering method).It makes it possible to determine cell viability and EC50 *in vitro* (concentration inhibiting 50% of the promastigote culture). The principle of this dosage is based on the reduction of a tetrazolium salt into formazan crystals. MTT is a yellow dye soluble in an aqueous medium, which on contact with living cells, is reduced by enzymes of the mitochondrial respiratory chain (dehydrogenases) into purple MTT-formazan crystals that are insoluble in water.

Antifungal Testing

The reference strains for this test were Aspergillus fumigatus IP 2279.94 from Pasteur Institute of Paris (France). The effective concentration which inhibits 90% of Aspergillus culture was measured by the broth dilution method. Conidia were collected from a culture of A. fumigatus aged 7 days on malt agar at 35°C.

The conidia were purified by centrifugation at 1500 rpm and then washing with PBS buffer containing 0.01% Tween 20. The final concentration in each tube was adjusted to 103 – 5*103 CFU/ml for the strain. The tests were carried out in MOPS buffered RPMI 1640 medium with L-glutamine, but without NaHCO3, containing EtOH at a final concentration of 0.2% (v/v). After shaking, the tubes were incubated at 35°C for 48 h and then assessed for the presence or absence of fungal growth. Amphoterin B is used as a positive control.

Results and Discussion

Three Schiff bases have been synthesized from the condensation of 1,6-diaminohexane with *o*-nitro-benzaldehyde, and cyclohexane-1,2-diamine with *p*-nitro-benzaldehyde, then benzène- 1,3-diamine with *p*-nitro-benzaldehyde.

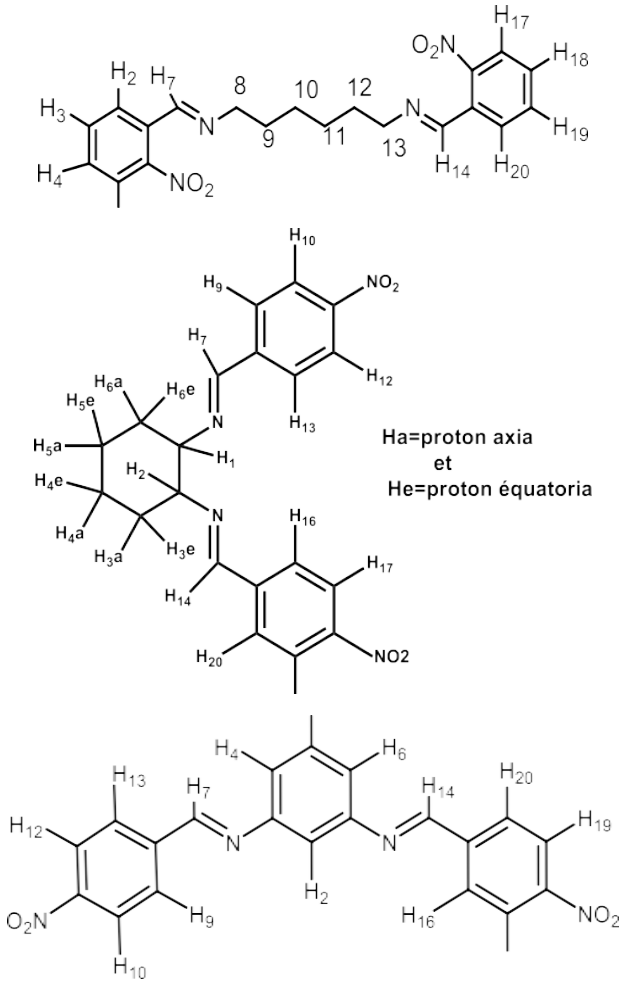

Figure 1: Condensed molecular structures with atomic numbering scheme.

Compound 1: **N, N'-bis (2-nitrophenylmethylene) hexane-1,6-diamine**
Compound 2: ***N, N'*-bis (*p*-nitrophenylmethylene) cyclohexane-1,2-diamine**
Compound 3: ***N, N'*-bis (4-nitrophenylmethylene) benzene-1,3-diamine**

^{1}H NMR and ^{13}C NMR Spectroscopy

Synthesis of N, N'-bis (2-nitrophenylmethylene) hexane-1,6-diamine (Compound 1)

yield: 98,19 %, mp: 65,6°C. 1H-NMR (CDCl3): δ (ppm) = 8.56 (*s*, 2H, CH), 1.38-3.61 (*m*, 6H, CH), 7.75-8.10 (*m*, 8H, CH*arom*). 13C-NMR (CDCl3): δ (ppm) = 24.46, 28.01, 59.05, 121.67, 130.87, 129.08, 146.29, 154.10. Anal calcd for $C_{20}H_{22}N_4O_4$.

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

yield: 83%, mp: 110,6°C. 1H-NMR (CDCl3): δ (ppm) = 8.46 (s, 2H, CH), 3.51 (*q*, 2H, CH), 7.85-8.15 (*d,* 8H, CH*arom*), 1.50-1.78(*m*, 4Ha, CH), 1.63-2.05(*m*, 4He, CH). 13C-NMR (CDCl3): δ (ppm) = 24.22, 30.89, 74.01, 124.25, 130.47, 141.60, 148.89, 158.57, Anal calcd for $C_{20}H_{20}N_4O_4$.

Synthesis of: *N, N'*-bis (4-nitrophenylmethylene) benzene-1,3-diamine (Compound 3)

yield: 80,25 %, mp: 185,6°C. 1H-NMR (CDCl3): δ (ppm) = 8.90 (s, 2H, CH), 7.30-7.53 (*dd*, 3H, CH*arom*), 8.25 (m, 4H, CH*arom*), 7.35 (*t*, 1H, CH*arom*), 8.38 (*d*, 4H, CH*arom*). 13C- NMR (CDCl3): δ (ppm) = 120.13, 124.00, 129.73, 129.47, 141.40, 148.93, 151.63, 159.67. Anal calcd for $C_{20}H_{14}N_4O_4$

The resonance of protons had been assigned on the basis of their integration and multiplicity pattern [11].

Mass spectra (SM) and infrared spectrum (IR)

Mass spectrum (ESI-MS) and the infrared spectrum of the synthesized compounds are given in Table 1.

Table 1: Mass spectral and infrared data.

Compound	Molar mass (g/mol)	Mass spectrum $[M+H]^+$ (g/mol)	infrared spectrum: (Cm^{-1}) (υC=N)
1	**382**	383,1726	1633
2	**380**	381,1595	1642
3	**374**	375.1095	1660

**MS study*

The mass spectra (HR-ESI-MS) of compounds 1,2,3 showed peaks corresponding respectively to the molecular ions at m/z 383.1726 $[M + H]^+$, 381.1595 $[M + H]^+$, 375.1091 $[M + H]^+$, that allow to propose respectively, $C_{20}H_{22}N_4O_4$, $C_{20}H_{20}N_4O_4$, $C_{20}H_{14}N_4O_4$ has empirical formula for these compounds.

**IR study*

The IR spectra show characteristic bands at 1633cm^{-1} for compound **1**, 1642 cm^{-1} for compound **2**, 1660 cm^{-1} for compound **3,** indicating the presence of two azomethine functions (C=N) in each compound.

Leishmanicidal activities

In vitro leishmanicidal activities of the different compounds were evaluated using the LV9 strains of *L. donovani*. The activity values, in EC_{50} (effective concentration causing 50% inhibition), are expressed in mg/L, and are recorded in table 2.

The results indicate that, these compounds show significant activity against *L. donovani*. We note that compound **1** having its nitro group in the ortho position on the benzene nucleus with the central structure of the linear hexane chain, is the most active with EC_{50} (mg/L) of 16.40. Compound **2**, having as intermediate structure a hexanic ring and its nitro group in the para position on the benzene ring, appears less active with an EC_{50} of 55 mg/L. Compound **3**, having a benzene ring as its intermediate structure, and its nitro group in the para position on its external benzene rings, is the least active with EC_{50} >125 mg/L.

Antifungal activity

All compounds show antifungal activity against Aspergilus fumigatus as shown in Table 3, as well as amphotericin B. here again, compound 1 presents the best antifungal activity with EC_{90} of 125 mg/L. Compounds 2 and 3 are the least active with an EC_{90}>250 mg/L.

Table 2: Measurement of inhibition diameters and Value of minimum inhibition concentration (MIC) for antibacterial activity.

Strain tested	*L. donovani*
Compounds	**Value of MIC (µg /mL)**
1	**16.40**
2	**55**
3	**>125**
Witnesses	
Pent	2.6

Values are averages of three repetitions; ***Pent****: Pentamidine*

Table 3: Measurement of Value of minimum inhibition concentration (MIC) for antifungal activity.

Strain tested	*Aspergilus fumigatus*
Compounds	**Value of MIC CE90(mg /L)**
1	**125**
2	**>250**
3	**>250**
Witness	
Ampho B	**0.500**

Ampho B: *Amphotherine B*

Conclusion

Schiff bases of compounds 1, 2, 3, were synthesized and characterized by analytical and spectral techniques. Compound 1 which contain nitro group in the ortho position on the benzene nucleus with the central structure of the linear hexane chain, is the most active on LV9 strains of L. donovani and on Aspergilus fumigatus. Compounds 2 and 3 having nitro groups in the para position on their different benzene rings, with central structures different from compound 1, showed less activity on the strains tested. The structure of compound 1 is therefore seens to be critical for obtaining important activity on the strain tested.

References

1. Arulmurugan S, Kavitha PH, Venkatraman RP. Biological activities of Schiff base and its omplexes: a review. Rasayan J Chem. 2010; 3: 385-410.
2. Schiff H. Mitteilungen aus dem universitats laboratorium in Pisa: Eineneue reihe organischer basen. Justus Liebigs Ann Chem. 1864; 131:118-119.
3. Bringmann G, Dreyer M, Faber JH, et al. Ancistrotanzanine C and related 5,1'- and 7,3'-coupled naphthylisoquinoline alkaloids from Ancistrocladus tanzaniensis. J Nat Prod. 2004; 67: 743-748.
4. Salimon J, Salih N, Ibraheem H, at al. Synthesis of 2-N-salicylidene5-(substituted)-1,3, 4-thiadiazole as potential antimicrobial agents. Asian J Chem. 2010; 22: 5289-5296
5. Guo Z, Xing R, Liu S, et al. Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. Carbohydr Res. 2007; 342: 1329-1332.
6. Kangah NJB, Kodjo CG, Ouattara ZA, et al. Synthesis, Characterization and Biological Evaluation of New Series of Schiff Bases Derived from Hexamethylenediamine as Potential Antibacterial and Antifungal Agents, IRA-International Journal of Applied Sciences. 2017; 07: 69-74.
7. Kangah NJB, Kodjo CG, Kablan ALC, et al. Synthesis, Characterization and Antimicrobial Evaluation of Symmetric A-Diimine Schiff Bases Derived from Cis and Trans Racemic Mixture of Cyclohexanediamine, IRA-International Journal of Applied Sciences. 2017; 06: 23-30.
8. Sari N, Arslan S, Logoglu E, et al. Antibacterial activities of some new amino acid Schiff bases. Journal of the Institute of Science and Technology, Gazi Uni. 2003; 16: 283.
9. Verma M, Pandeya SN, Singh KN,et al. Anticonvulsant activity of Schiff bases of isatin derivatives. Acta Pharm. 2004; 54: 49-56.
10. Li Y, Yang ZS, Zhang H, et al. Artemisinin derivatives bearing Mannich base group: synthesis and antimalarial activity. Bioorg Med Chem. 2003: 11: 4363-4368.
11. Silverstein RM, Kiemle DJ, Webster FX. Identification Spectrométrique de composés Organiques De Boeck Supérieur, 3ième edition. collection chimie (in)organique. 2016; 175.