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Dedicated to the memory of
Professor Victor-Emanuel Sahini (1927–2017)

SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF A NEW BIS-AZOMETHINE WITH TRIMETHYLSILYL TERMINAL GROUPS

Angelica VLAD,[a] Maria CAZACU,[a] Nicoleta VORNICU,[b] Sergiu SHOVA[a] and Mirela-Fernanda ZALTARIOV[a,*]

[a]"Petru Poni" Institute of Macromolecular Chemistry, Aleea Gr. Ghica Voda 41A, 700487 Iaşi, Roumania
[b]Metropolitan Center of Research T.A.B.O.R, The Metropolitanate of Moldavia and Bukovina, Iaşi, Roumania

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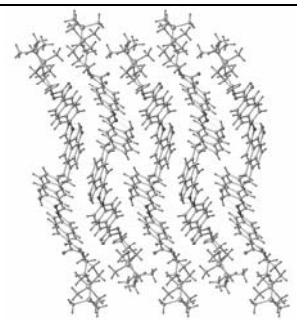
A Schiff base was synthesized by the reaction of 5,5'-methylene-bis-salicylaldehyde and *p*-aminobenzoate-propyl-trimethylsilyl. Results of spectroscopic (FTIR, ^{1}H NMR, ^{13}C NMR, 2D NMR spectra, ESI–MS) and elemental analyses for the resulting compound were in a very good concordance with the structure determined by single-crystal X-ray diffraction. The antimicrobial activity of this Schiff base containing highly hydrophobic tails was studied *in vitro* by the disk diffusion assay against some fungi and bacteria and the results displayed a better inhibition of fungi than the standard Caspofungin, making it suitable for further development as potential antifungal agents.



INTRODUCTION

Azomethines or Schiff bases represent an important class of organic compounds with applicability in a wide range of fields.[1-7] The relatively easy way of achieving these compounds and the presence of the lone electron pair in a sp^2 hybridized orbitals of the nitrogen atom of the azomethine group[8] give them a remarkable ability to coordinate different metals ions from *s, p, d* or *f* blocks. Therefore, Schiff base-type compounds represent one of the most used ligands in the coordination chemistry field, for their preparation being used a wide variety of carbonyl and amine precursors.[6,7] Recent concerns in this area are directed toward getting new homo- and heteronuclear complexes through diversification of the azomethine ligands for testing their potential application in the fields of high interest.[9-13]

In this respect, the design of molecular structures with targeted properties (optical, magnetic or electrical) based on Schiff bases having different donor atoms (starting with the *Salen*-type N_2O_2 up to polydentate N_xO_y structures) is an innovative direction regarding their applicability in a range of fields such as medicine (for the obtaining of active principles),[2] biology (as antibacterial, antifungal or antitumoral agents),[3] analytical chemistry (optical, electrochemical and chromatographic sensors)[4,5] and materials science

* Corresponding author: zaltariov.mirela@icmpp.ro

(asymmetric catalysts) or as useful substrates for the production of new optical and organic conducting polymers (emitting diode OLED, PLED). These compounds have been found to possess a significant biological activity and a diversity of valuable useful applications. A number of Schiff bases such as rhodopsin or those derived from dihydroxyacetone phosphate are well-known to be involved in metabolic pathways of biologically molecules (amino acids, carbohydrates or lipids).[1] Thus, the diversified azomethine structures still remain a promising source for the rational design of novel molecules.

Hence, in this work we have focused our attention on the preparation of a Schiff base by using an original aldehyde-amine pair, *i.e.,* 5,5'-methylene-bis-salicylaldehyde and *para*-aminobenzoate-propyl-trimethylsilyl, that are not commercially available. Co-existence of azomethine and trimethylsilyl groups in product creates prerequisites for a high biocidal activity through an adequate hydrophilic-hydrophobic balance.

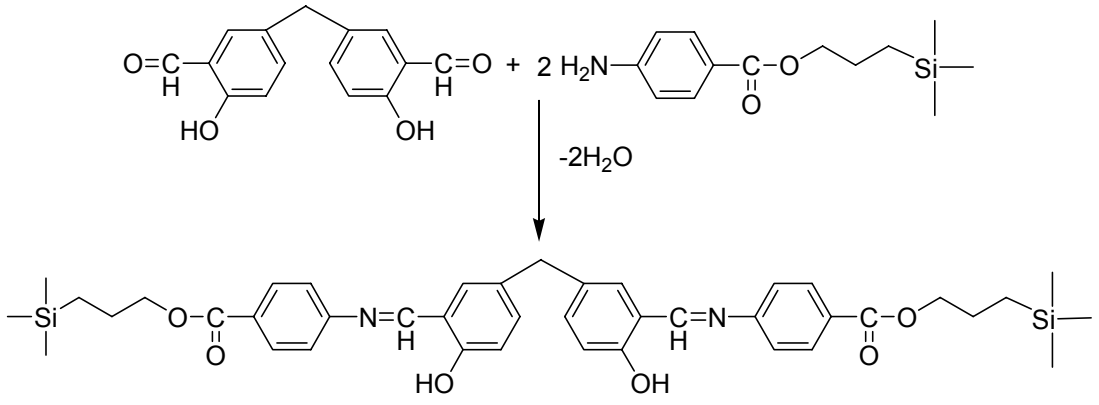
RESULTS AND DISCUSSION

The Schiff base was synthesized by the direct condensation reaction of 5,5'-methylene-bis-salicylaldehyde with *para*-aminobenzoate-propyl-trimethylsilyl (Scheme 1). The compound was obtained as single crystals, the structure being confirmed by FTIR, NMR, ESI-MS, UV-vis and X-ray single-crystal diffraction analyses.

In the Schiff base spectrum, the absence of the characteristic amino and carbonyl stretching bands and the appearance of the imine –CH=N- band at 1622 cm^{-1}, clearly indicates the formation of the Schiff bases. The υ(C=O) absorption of the ester group in the imine spectrum is shifted at higher wavenumber, from 1690 cm^{-1} to 1715 cm^{-1}. The specific bands for Si-CH_3 groups appear at 1248 and 839 cm^{-1}. The purity of the product was further evidenced by elemental analysis. The positive-ion ESI mass spectrum in methanol revealed strong peak at *m*/*z* 745 assigned to the $[M+Na]^+$ ion (see Experimental).

One- and two-dimensional ^1H and ^{13}C NMR spectra confirmed the expected structure (Fig. 1). Thus, in the ^1H-NMR spectrum of the Schiff base, all protons resonated at appropriate positions: a singlet for -OH protons at 12.85 ppm, a singlet for azomethine hydrogen (-CH=N-) at 8.63 ppm , aromatic protons with some modification as compared with starting compounds in the region 8.15-7.03 ppm and a singlet at 3.99 ppm for the methylene group. Aliphatic protons appear in the region 4.35-0.57 ppm while those of trimethylsilyl groups appear at 0.07-0.03 ppm (Fig. 1a). ^{13}C NMR spectrum of the Schiff base also confirms its structure by the signals assigned to all C atoms in the structure (Fig. 1b).

The single-crystal X-ray investigation has demonstrated that the Schiff base compound has a molecular crystal structure formed by the corresponding neutral entities without any co-crystallized solvent molecules. A drawing of molecular structure along with the atom-numbering scheme is shown in Fig. 2. In the crystal structure of the product there are two chemically identical but crystallographic independent molecules on the asymmetric unit, which show the same conformation and very similar geometric parameters. As an example, only one molecular component is depicted in Fig. 2. The Schiff base shows the presence of intramolecular O-H···N hydrogen bond formed by hydroxyl oxygen as donor and nitrogen atom as acceptor.



Scheme 1 – Reaction pathway for obtaining the Schiff base compound.

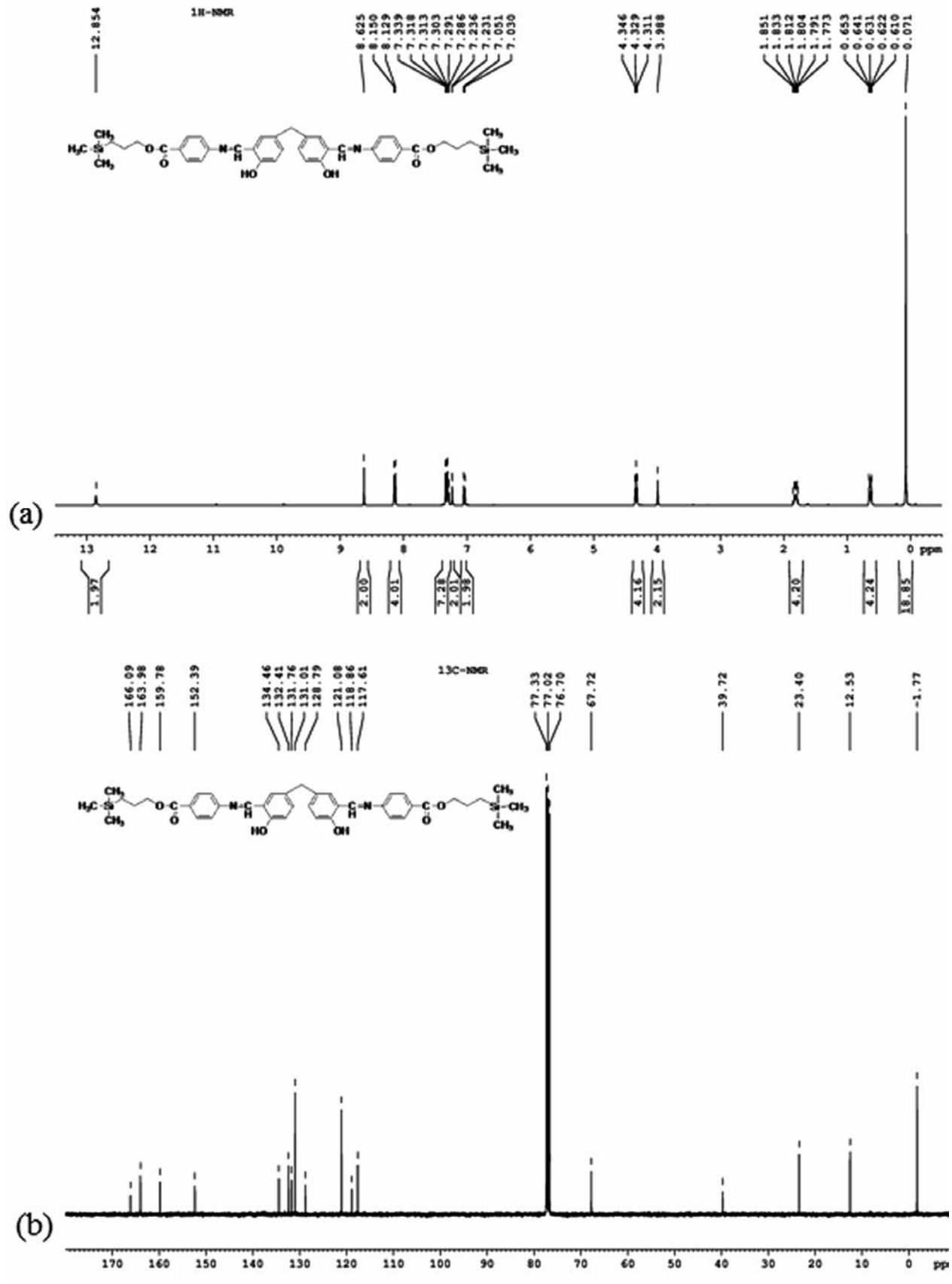


Fig. 1 – NMR spectra of the obtained Schiff base: (a) - ^{1}H NMR; (b) - ^{13}C NMR.

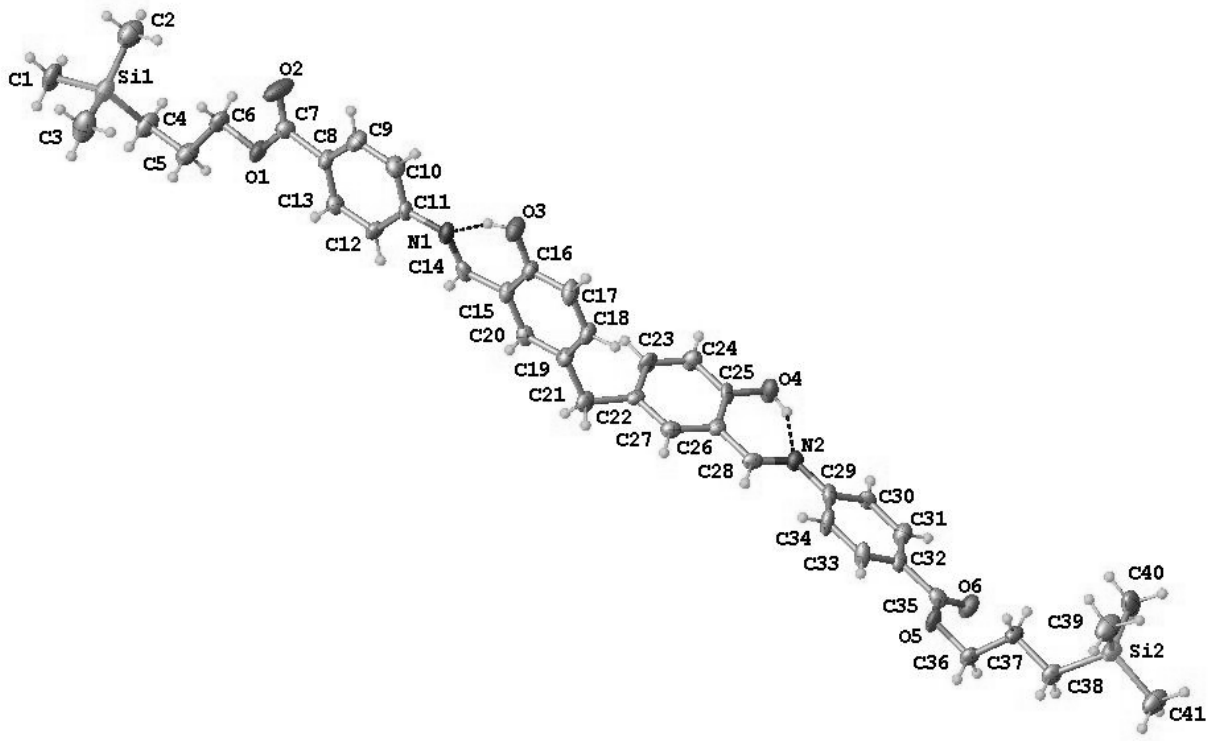


Fig. 2 – X-ray molecular structure of the Schiff base showing the atom-numbering scheme.
The thermal ellipsoids are drawn at 50% probability level.
H-bonds: O3−H···N1 [O3−H 0.82 Å, H···N1 1.87 Å, O1···N1 2.567(5) Å, O3−H···N1 142.5°]; O4−H···N2 [O4−H 0.82 Å, H···N2 1.88 Å, O4···N2 2.55(6) Å, O4−H···N2 145.7°]

Table 1

H-bonds parameters in the crystal structure of the Schiff base

D-H···*A*	*D*-H	H···*A*	*D*···*A*	*D*-H···*A*	Symmetry code
C37A-H···O2B	0.97	2.77	3.658(7)	153.3	1 - *x*, 1 - *y*, -*z*
C39A-H···O2B	0.96	2.52	3.442(7)	161.3	1 - *x*, 1 - *y*, -*z*
C13A-H···O6B	0.93	2.71	3.401(7)	132.0	2 - *x*, 2 - *y*, -*z*
C21B-H···O4B	0.97	2.76	3.566(6)	141.2	2 - *x*, 2 - *y*, -*z*
C37B-H···O2A	0.97	2.55	3.322(8)	136.7	1 - *x*, 1 - *y*, -*z*
C17A-H···*Cg*1*	0.93	2.99	3.875(6)	158.9	1 - *x*, 1 - *y*, -*z*

**Cg*1 is the centroid of C15A-C20A ring

In the crystal structure numerous C-H···O weak hydrogen bonding associated with C-H···π interactions (see Table 1) generate a tree-dimensional supramolecular architecture, as shown in Fig. 3.

The results of the antimicrobial activity and MIC values for the compounds tested are given in Table 2.

It was clear established that the antimicrobial efficacy of different biomolecules is dependent on many cellular pathways as well as physico-chemical and metabolic properties of the various drugs.[14] In the case of Schiff bases, literature studies have indicated that their biological activity can be explained based on the structure of the azomethine CH=N group. Schiff bases possess N, O-donor atoms which can polarize the azomethine group increasing its ability for hydrogen bonding and thus the interaction with the cell membrane of the microorganisms. A great importance on the antimicrobial activity of biomolecules has their hydrophobic/hydrophilic balance which may have a large effect on the transport across the cell membrane. The most important factors influencing the thermodynamics binding affinity between the Schiff base compound and the membrane cell are: the ability for H-bonding of the imine group, the reversibility of the imine bond reaction as a dynamic combinatorial chemistry (DCC) principle, involving molecular or supramolecular inter-changes by its continuously formation and breakage,[15] the lipophilicity, which increases by complexation with metal ions[16] and the dipole moment of the drug.

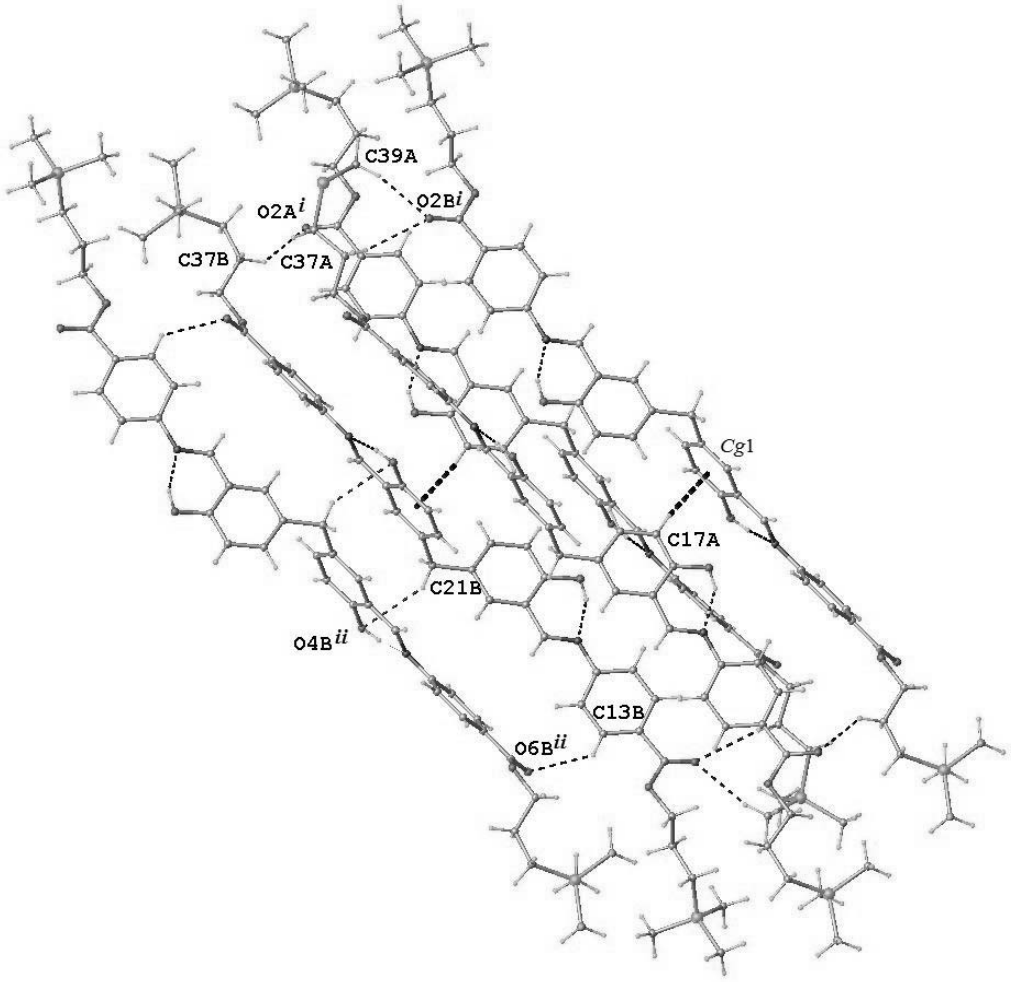


Fig. 3 – 3D supramolecular network.

Table 2

Antibacterial and antifungal screening results

Sample	**MIC (µg/mL)**				
	Fungi			**Bacteria**	
	Aspergillus fumigatus ATCC 66567	Penicillium chrysogenum ATCC 20044	Fusarium ATCC 20327	Bacillus sp. ATCC 31073	Pseudomonas sp. ATCC 15780
para-aminobenzoate-propyl-trimethylsilyl	1.5	1.5	1.5	48	48
Schiff base compound	0.16	0.16	0.16	<256	<256
Caspofungin[b]	0.3	0.3	0.3	-	-
Kanamycin[b]	-	-	-	4	4

[b] Standard compound

According to the results presented in Table 2, a very good antifungal activity was evidenced for the obtained Schiff base as compared with the starting amine and the reference compound, Caspofungin. The lipophilicity of the Schiff base is due to the trimethylsilyl groups in the structure, while the calculated dipole moment by using Hyper Chem program is 3.30 D. These structural features and the value of dipole moment are elements that ensure the electrostatic and hydrophobic interactions between the polar (azomethine and ester) groups and non-polar (propyl and

trimethylsilyl) moieties of the synthesized Schiff base and the transporter proteins in the membrane cell[14] as well as the direct diffusion through the lipid bilayer due to the lipophilicity.[17]

EXPERIMENTAL

Materials and measurements

para-aminobenzoate-propyl-trimethylsilyl was obtained by a procedure already described,[18] 5,5'-methylene-bis-salicylaldehyde was prepared according to a known procedure,[19,20] methanol (Chimopar), chloroform (Chimopar), dimethylformamide (Aldrich) were used as received.

Fourier transform infrared (FT-IR) spectra were recorded using a Bruker Vertex 70 FT-IR spectrometer. Registrations were performed in the transmission mode in the range 400-4000 cm^{-1} at room temperature with a resolution of 2 cm^{-1} and accumulation of 32 scans.

The NMR spectra were recorded on a Bruker Avance DRX 400 MHz Spectrometer equipped with a 5 mm QNP direct detection probe and z-gradients. Spectra were recorded in $CDCl_3$, at room temperature. The chemical shifts are reported as δ values (ppm), referenced to the solvent residual peak (7.26 ppm). The atom labeling for the NMR assignments is that from XRD structures. The assignments of all the signals in the 1D NMR spectra were done using 2D NMR experiments like H,H-COSY, H,C-HMQC and H,C-HMBC.

UV-Vis absorption spectra measurements were carried out in DMF solution on a Specord 200 spectrophotometer.

The study of antimicrobial activity of the new synthesized compound was carried out using three species fungi (Aspergillus fumigatus ATCC 66567, Penicillium chrysogenum ATCC 20044, Fusarium ATCC 20327) from pure culture and two bacteria (Pseudomonas sp. ATCC 15780 and Bacillus sp. ATCC 31073) species according to previously reported standard procedures.[18]

X-ray crystallography

Crystallographic measurements were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer equipped with graphite-monochromated Mo-Kα radiation. Single crystals were positioned at 40 mm from the detector and 399 frames were measured each for 90s over 1° scan width. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction.[21] All the structures were solved by direct methods using Olex2[22] software with the SHELXS structure solution program and refined by full-matrix least-squares on F² with SHELXL-97.[23] Atomic displacements for non-hydrogen atoms were refined using an anisotropic model. Hydrogen atoms have been placed in fixed, idealized positions accounting for the hybridation of the supporting atoms and the possible presence of hydrogen bonds in the case of donor atoms. The molecular plots were obtained using the Olex2 program. The positional parameters of the DMF and water molecules were refined in combination with PART and SADI restrains using anisotropic/isotropic model for non-H atoms. Table 3 provides a summary of the crystallographic data together with refinement details. CCDC 1521679 contains the supplementary crystallographic data for this contribution. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 3

Crystallographic data, details of data collection and structure refinement parameters for Schiff base product

Empirical formula	$C_{41}H_{50}N_2O_6Si_2$
Formula weight	723.01
Temperature/K	150
Crystal system	triclinic
Space group	*P*-1
a/Å	10.1797(14)
b/Å	12.147(3)
c/Å	32.926(4)
α/°	100.137(14)
β/°	95.302(10)
γ/°	98.206(14)
V/Å^3	3937.7(11)
Z	4
D_{calc}/mg/mm^3	1.220
μ/mm^{-1}	0.138
Crystal size/mm^3	0.15 × 0.05 × 0.05
$\theta_{min,}$ θ_{max}(°)	4.62 to 48.52°
Reflections collected	29487
Independent reflections	12692[R_{int} = 0.1196]
Data/restraints/parameters	12692/0/931
R_1[a](I>2σ(I))	0.0864
wR_2[b](all data)	0.1372
GOF[c]	0.968
Largest diff. peak/hole/e Å^{-3}	0.28/-0.27

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, [b] $wR_2 = \{\Sigma[w\,(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.
[c] GOF = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

Synthesis of the Schiff base compound

A mixture of *para*-aminobenzoate-propyl-trimethylsilyl (0.251 g, 1.000 mmol) and 5,5'-methylene-bis-salicylaldehyde (0.128 g, 0.500 mmol) in methanol/chloroform (2:1 v/v) was stirred at room temperature for 2 h and then was heated to reflux for 8 hours. Orange crystals appeared after three days. (0.232 g, 92 %); m.p. 124-126 ^{o}C. Anal. Calcd for $C_{41}H_{50}N_2O_6Si_2$ (M_r 723.01 g/mol): C, 68.11; H, 6.97; N, 3.87. Found: C, 68.45; H, 7.08; N, 3.82. ESI-MS (methanol), positive: *m/z* 745.3 ([M + Na]$^+$). UV-vis (DMF), λ_{max} (ε, M^{-1}cm^{-1}): 304 (24682), 357 (19036).

IR ν_{max} (KBr), cm^{-1}:3410vw, 2953m, 2895w, 2882w, 1715vs, 1659w, 1622m, 1601s, 1578s, 1487s, 1462w, 1445w, 1414m, 1389w, 1358m, 1310m, 1275vs, 1248s, 1202m, 1171s, 1159s, 1101s, 1011w, 997w, 972w, 955w, 920w, 858s, 839s, 772s, 750m, 737m, 694m, 627w, 523w, 503w, 490vw, 430vw.

^{1}H-NMR ($CDCl_3$, 400.13 MHz, δ, ppm): 12.85 (s, 2H, -OH), 8.63 (s, 2H, H14, 28), 8.14 (d, J=8.4 Hz, 4H, H9, 13, 31, 33), 7.34-7.29 (m, 6H, H10, 12, 18, 23, 30, 34), 7.24 (d, J=2 Hz, 2H, H 20, 27), 7.04 (d, J=8.4 Hz, 2H, H17, 24), 4.3 (t, J=7 Hz, 4H, H6, 36), 3.99 (s, 2H, H21), 1.85-1.77 (m, 4H, H5, 37), 0.65-0.61 (m, 4H, H4, 38), 0.07 (s, 18H, H1-3, 39-41).

^{13}C-NMR ($CDCl_3$, 100.6 MHz, δ, ppm): 166.09 (C 7, 35), 163.98 (C14, 28), 159.78 (C16, 25), 152.39 (C11, 29), 134.46 (C18, 23), 132.41 (C20, 27), 131.76 (C19, 22), 131.01 (C9, 13, 31, 33), 128.79 (C8, 32), 121.08 (C10, 12, 30, 34), 118.86 (C15, 26), 117.61 (C17, 24), 67.72 (C6, 36), 39.72 (C21), 23.40 (C5, 37), 12.53 (C4, 38), -1.77 (C1-3, 39-41).

CONCLUSIONS

A Schiff base containing trimethylsilyl units in the structure has been synthesized and structurally characterized. Single crystal X-ray diffraction revealed the molecular structure of the Schiff base and its ability to self-assembly in a 3D structure by C-H···O contacts and C-H···π interactions. The biocidal activity of Schiff base product was evaluated against bacteria and fungi species and the tests indicated a higher antifungal activity as compared with the standard used.

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