

The Influence of Structure on Antibacterial Activity of Some New Aniline Derived Schiff Bases

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This paper presents the synthesis of some Schiff bases with potential antimicrobial activity. A new ligand was synthesized through the condensation reaction of aniline with various aldehydes: benzaldehyde, salicylic aldehyde, p-hydroxy-benzaldehyde and vanillin. The chemical structure was confirmed by ¹H-NMR and IR spectroscopy. The antimicrobial activity of the Schiff bases and their precursors were tested in comparison with Tetracycline, Nystatin and Ampicillin upon the following strains: Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922) and Candida albicans (ATCC 1023). The compounds were found to be very active against gram-positive and gram-negative bacteria. Some conclusions regarding the chemical structure – antimicrobial activity relationship have been drawn.

Keywords: aniline, Schiff base, antimicrobial activity

The Schiff base compounds represent an important class of ligands that have been extensively studied in coordination chemistry, mainly due to their simple synthesis and easy tunable steric, electronic and catalytic properties [1-8]. Among the reported Schiff bases, salicylaldehyde derivatives showed antibacterial and antifungal activity [9-10], but a systematic study regarding their structure-activity relationship wasn't reported so far. That was the reason why, we have started a complex study by designing Schiff bases that contained the hydroxyl unit in different positions and vicinities, with the aim of clarifying the role of these functionalities in their antimicrobial activity. A model Schiff base without any substituent has been used for comparison, too.

Experimental part

Material and methods

The aniline and aldehyde reagents were purchased from Aldrich and used without further purification. Acetonitrile, methanol and dimethyl formamide were purchased from Carl Roth and used after drying on molecular sieves.

For the study of the antimicrobial activity the following strains were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231. Tetracycline, Ampicillin, Nystatin purchased from Himedia-Spain were used as reference substances.

The infrared (IR) spectra were recorded on a FT-IR Bruker Vertex 70 Spectro-photometer in the transmission mode, by using KBr pellets.

The proton nuclear magnetic resonance (¹H-NMR) spectra were recorded by a BRUKER Avance DRX 400 MHz spectrometer using CDCl₃ as solvent and tetramethylsilane (TMS) as internal reference substance. Chemical shifts were reported in parts per million (ppm).

The azomethine compounds had been synthesized through the condensation reaction of aniline with different aldehydes, in a 1:1 molar ratio (fig. 1.). The reagents were dissolved in acetonitrile to give a 30% solution and the obtained reaction mixture was placed into a round bottom flask fitted with condenser and nitrogen inlet. The reaction mixture has been gently refluxed over night and then concentrated by rotary evaporation. The crude product was recrystallized twice from ethanol to give single crystals by high purity and then dried under vacuum 24 h. The yield was around 90 %.

Testing antimicrobial activity

While testing the *in vitro* qualitative antimicrobial activity, the imine compounds had been codified as BsA1, BsA3, BsA5, BsA6, and the aldehyde reagents had been codified as A1, A3, A5, A6. Testing procedures were validated according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 1990) [11-12]. The reference strains tested were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 that were supplied by the Microbiology Department from "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. Mueller-Hinton agar (Difco) was used for bacteria strains and Sabouraud agar (Difco)

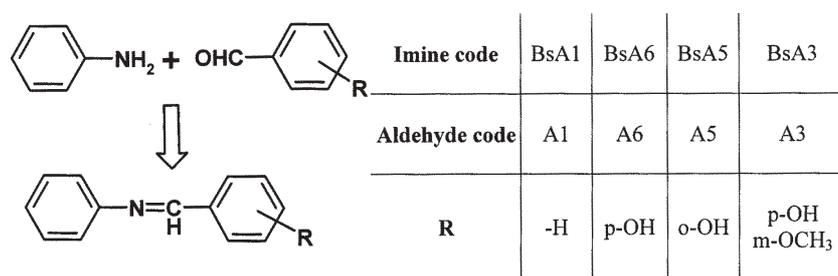


Fig. 1. Synthesis of the target azomethines

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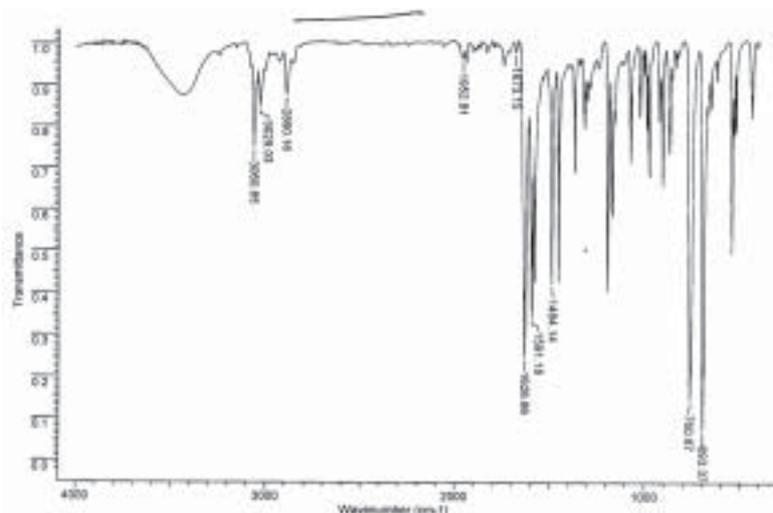


Fig. 2. FTIR spectrum of BsA1

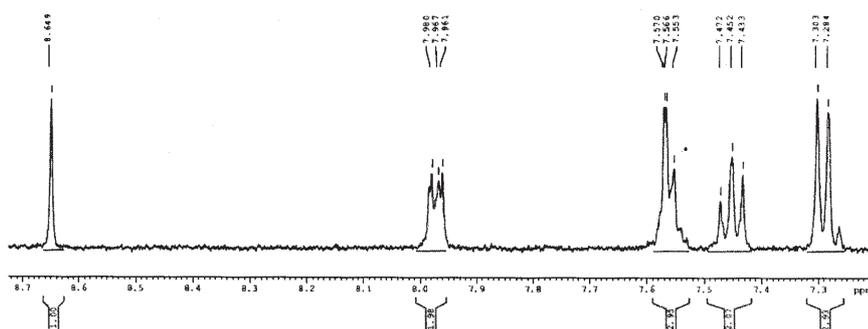


Fig. 3. ¹H-NMR spectrum of BsA1

N ^o	Sample	Diameter of inhibition zone (mm)		
		<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>C. albicans</i> 10231
1	A1	13 ± 0.62	11 ± 0.70	24 ± 0.52
2	BsA1	13 ± 0.47	13 ± 0.62	25 ± 0.51
3	A3	16 ± 0.80	13 ± 0.55	15 ± 0.62
4	BsA3	0	12 ± 0.86	15 ± 0.61
5	A5	0	14 ± 0.72	19 ± 0.47
6	BsA5	15 ± 0.51	7 ± 1.02	21 ± 0.50
7	A6	15 ± 0.52	11 ± 1.18	25 ± 0.57
8	BsA6	16 ± 0.62	20 ± 0.60	26 ± 0.61
9	Ampicillin	32 ± 0.52	20 ± 0.57	-
10	Tetracycline	31 ± 0.32	25 ± 0.62	-
11	Nystatin	-	-	22 ± 0.57

Table 1
IN VITRO ANTIMICROBIAL ACTIVITY OF THE SCHIFF BASES COMPARED TO CORRESPONDING ALDEHYDE REAGENTS

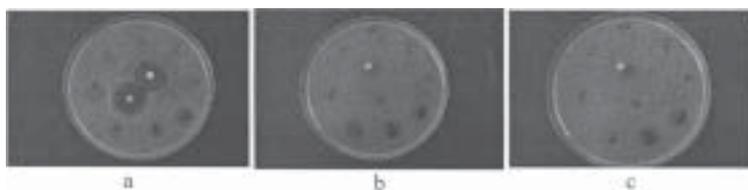


Fig. 4. Antimicrobial activity of the Schiff bases comparative with aldehydes determined by agar-diffusion method against *S. aureus* (a) *E. coli* (b) and *C. albicans* (c)

was used for *Candida*. The inoculums were prepared by diluting over sight cultures of the organisms in sterile 0.9% NaCl and adjusting the turbidity to 0.5 McFarland (about 10^8 cfu/mL). The media was prepared using 0.5 mL of each tested strain mixed with 15 mL portions of molten agar in a sterile Petri dish. After solidification, 0.1 mL solution of each compound was brought into 8 mm wells drilled into the surface of the medium. The final concentration for all tested compounds was 100 μ g/mL. The *in vitro* activity of the compounds was compared to that of standard antibiotic discs of ampicillin 10 μ g, tetracycline 30 μ g and Nystatin 100 μ g. The plates were incubated for 24 h at 37°C and the diameters of the inhibition zones of the microbial growth around the holes were measured [13]. Each assay in that experiment had been done twice.

Results and discussions

The actual structures of the obtained compounds had been confirmed by Fourier transform infrared (FTIR) and ¹H nuclear magnetic resonance (¹H-NMR) spectroscopy. All FTIR spectra clearly showed an intense characteristic absorption band around 1625 cm^{-1} and a low intense one around 2890 cm^{-1} due to the -C=N- vibration and axial stretching of the C-H bond in the newly formed azomethine group, while the bands characteristic for the functional groups of the reagents (the aldehyde group around 1670 cm^{-1} and the amine group around 3350 cm^{-1} , respectively), had disappeared. The weak absorption peaks of the 1952–1673 cm^{-1} range were assigned to the C-H out-of-plane ($\gamma_{\text{C-H}}$) deformations of the phenylene rings, while the strong absorption peaks within the 1591–1484 cm^{-1} and 760–693 cm^{-1} domains were attributed to the $\nu_{\text{C=C}}$ and $\gamma_{\text{C-H}}$

of the 1,4-phenylene rings. The FTIR spectrum of the BsA1 azomethine is given as an example in figure 2.

In the $^1\text{H-NMR}$ spectra, the new azomethine obtained was confirmed by the presence of the chemical shift specific to the azomethine proton around 8.6ppm and the absence of the signals characteristic to carbonyl (around 9.8ppm) and amine (3.55ppm) protons. In the aromatic region (7–8ppm), there were present all the signals belonging to the aromatic protons in the right integral ratio. In figure 3, the $^1\text{H-NMR}$ spectrum of the BsA1 is presented as an example.

The antimicrobial activity of the investigated compounds is shown in table 1 and figure 4.

According to the data from table 1, the tested compounds showed superior antifungal *in vitro* activity, especially in the case of Schiff bases.

Conclusions

Among the new combinations, the activity of BsA6 was greater against *Escherichia coli* and *Candida Albicans*. While the model compound BsA1 presented the same activity as its aldehyde reagent, in the case of imines possessing hydroxyl unit a slight increase of the biological activity against *Staphylococcus aureus* and *Candida albicans*, was observed when compared to its aldehyde reagent. Interesting enough the ortho position of the hydroxyl unit to the carbonyl group led to a decreased activity against *Escherichia coli*, while the same group in para positions led to a drastic increased activity against the same strain. The introduction of a methoxy unit in metha position (BsA3) inhibited the activity against *S. aureus* and maintained the activity against *E. coli* and *C.*

albicans of the corresponding aldehyde reagent. All these data suggested a strong influence of the functional groups and their positions and encouraged us to continue the study by using other substituents.

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