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Antimicrobial activities of N-(2-hydroxy-1-naphthalidene)-amino acid(glycine, alanine, phenylalanine, histidine, tryptophane) Schiff bases and their manganese(III) complexes

Iffet Sakıyan[1,*], Elif Loğoğlu[1], Seza Arslan[2], Nurşen Sari[3] & Nazmiye Şakıyan[4]
[1]*Ankara University, Science Faculty Department of Chemistry, 06100 Tandogan, Ankara, Turkey,* [2]*Izzet Baysal University, Science and Art Faculty , Department of Biology, Bolu, Turkey,* [3]*Gazi university, Science and Art Faculty Department of Chemistry, Teknikokullar, Ankara, Turkey,* [4]*Ankara University, Science Faculty Department of Biology, 06100 Tandogan, Ankara, Turkey; *Author for correspondence*

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Abstract

The *in vitro* antibacterial and antifungal activities of five different amino acid Schiff bases derived from the reaction of 2-hydroxy-1-naphthaldehyde with glycine, L-alanine L-phenylalanine, L-histidine, L-tryptophane and the manganese(III) complexes of these bases were investigated. Structures of the Schiff bases were proven by ^{1}H-NMR. *In vitro* activities against some Gram-positive (*Staphylococcus aureus* and *Bacillus polymyxa*) and Gram-negative (*Escherichia coli)* bacteria and the fungus *Candida albicans* were determined. The antimicrobial activities tended to decrease with the increasing size of the amino acid residues.

Introduction

Amino acid Schiff bases and their first-row transition metal complexes were reported to exhibit fungicidal, bactericidal, antiviral, and antitubercular activity (Singh *et al*. 2000; Singh *et al.* 1999; Nath *et al*. 2001; El-Said *et al*. 2000; Yao *et al*. 1999; Kohutova *et al*. 2000; Chonan *et al*. 1997, 1998). There also exists a number of reports on the biological activities of 2-hydroxy-1-naphthaldehyde Schiff bases (More *et al*. 2001; Jeewoth *et al.* 2001; Patel *et al*. 1999; Mehta *et al*. 1999; Misra *et al*. 1999; Anacona *et al.* 1999). On the other hand, the synthesis and structures of manganese(III) complexes of various Schiff bases and of other ligands are well established, but there are fewer studies on the biological activities of these complexes against fungi and bacteria (Duarte *et al*. 1997; Singh *et al*. 2001; Dendrinou-Samara *et al*. 2002). It was reported by Singh *et al*. (2001) that the complex [Mn(HSTB)$_3$] (HSTB:salicylaldehyde thiobenzhydrazone) strongly inhibits the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. In other work (Dendrinou-Samara *et al.* 2002), antibacterial screening data showed that manganese metallacrown ethers are more active than the simple, manganese-based herbicide or carboxylate complexes.

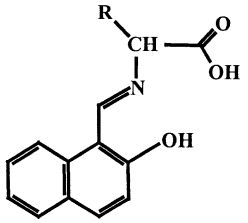
In this work, we report on the synthesis of amino acid Schiff bases using a new method and on the biological activities of these Schiff bases and their manganese (III) complexes against *S. aureus, B. polymyxa, E. coli and C. albicans.*

Material and methods

Physical measurements

The percentages of C, H, and N were obtained from a LECO 932 elemental analyser. The ^{1}H-NMR spectra were acquired from a Bruker GMbH Dpx-400 400 MHz High Performance Digital FT-NMR Spectrometer ($SiMe_4$ with standard). Infrared spectra in the region of 4000–350 cm^{-1} were obtained from KBr-pellets with a Mattson FT-IR 1020 spec-

Table 1. Structures of the Schiff bases investigated.



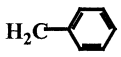
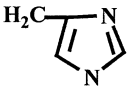
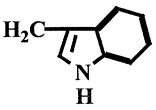
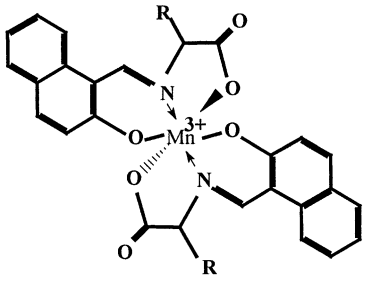
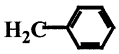
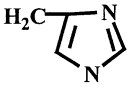
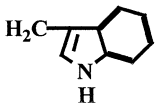
Schiff bases		R	
1	H_2L^1	H	(Gly)
2	H_2L^2	CH_3	(Ala)
3	H_2L^3		(Phe)
4	H_2L^4		(His)
5	H_2L^5		(Trp)

Table 2. Structures of the Mn^{3+} complexes investigated.



Complexes		R	
6	$Na[MnL_2^1]$	H	(Gly)
7	$Na[MnL_2^2]$	CH_3	(Ala)
8	$Na[MnL_2^3]$		(Phe)
9	$Na[MnL_2^4]$		(His)
10	$Na[MnL_2^5]$		(Trp)

trophotometer. Magnetic susceptibilities were determined at room temperature (20°) with a model MKI, MSBI/24093/6232 Sherwood Scientific, Magnetic Susceptibility (Cambridge, U.K.). Balance that was calibrated with $HgCo(SCN)_4$. Thermo-Gravimetric measurements were obtained from on a Rigaku Thermoflex TG 8110 system. The UV-visible spectra were acquired from with a Unicam UV2-100 UV/Visible spectrophotometer.

Chemicals

The amino acids (glycine, L-alanine, L-phenylalanine, L-histidine, and L-tryptophane) were purchased from Sigma. 2-Hydroxy-1-naphthaldehyde from Aldrich (recrystallized from 70% acetic acid). Methanol and acetic acid were purchased from Merck AG and used without further purification.

Synthesis of the Schiff bases

The 2-hydroxy-1-naphthaldehyde (10 mmol 1.72 g) was dissolved in 100 ml methanol and then added to the amino acid (10 mmol) (glycine, L-alanine, L-phenylalanine, L-histidine, L-tryptophane) solution in methanol (50 ml). The mixture was refluxed for 3 h, the solvent was removed on a rotary evaporator and the residue crystallized at room temperature. After a day

Table 3. ^{1}H-NMR data of the Schiff bases (solvent d_4-DMSO, except alanine and Phenylalanine Schiff bases).

Schiff bases	Phenolic OH (10)	*H*C=N (7)	Imidazole C-*H*	Indol ring C-*H*	Naphthyl protons C-*H* (four *d*, two *t*) (1-6)	C-*H* (8)	CH_2 (9)
	13.6	8.9				4,3	4.26
1	(s)	(s)	–	–	6,5–7.6	(Ch_2;s)	
2						4.4 (*d* − *d*)	(CH_3)
solvent	Not	9.0	–	–	6.8–8.0	^{3}J=7.1 Hz	1,6
$CDCl_3$	observed	(s)					(d)
3						4.7 (*d* − *d*)	3.2
solvent	14.2	8.9	–	–	6.8–8.9	^{3}J=3.8 Hz;	(two *d*-*d*)
D_2O	(s)	(s)			(phenyl:∼7.3)	10.0 Hz	
			7.5 and 6.7			4.5 (*d* − *d*)	2,9-3.1
4	13.9	8.8	(two	–	6.5–7.7	^{3}J=4.7 Hz; 8.0	(two *d*-*d*)
	(*s*)	(*s*)	distinct; *s*)			Hz	
				6,8–7.5		4.6 (*d*-*d*)	3.2-3.3
5	14,1	8.7	–	(benzo:two d, two *t*)	6.6–7.6	^{3}J=4.1 Hz,	(two *d*-*d*)
	(*s*)	(*s*)		7.6 (pyrol: *s*)		7.8 Hz	^{2}J=147 Hz

Key: *s*: singlet; *d*: doublet; *d*-*d*: doublet of doublets; *t*: triplet; *m*: multiplet.

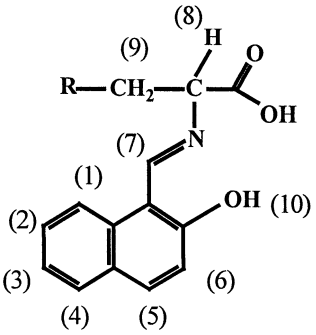


Fig. 1. Structure of Schiff bases.

or so, yellow crystals were obtained and recrystallized from methanol/n-heptane.

Synthesis of the complexes

Manganese(III) complexes were synthesized according to Sakiyan *et al.* 2001. The amino acid (Glycine, L-alanine, L-phenylalanine, L-histidine, L-tryptophane) (10 mmol) was added to a methanol solution (50 ml) of NaOH (20 mmol, 0.8 g). A methanol-solution (50 ml) of 2-hydroxy-1-napthaldehyde (10 mmol, 2.44 g) was then added to this mixture and stirred magnetically. After 2 min, solid $Mn(OOCCH_3)_3 \cdot 2H_2O$ (5 mmol, 1.34 g) was added and the mixture was kept at room temperature for 3 h with continuous stirring. The volume of the solution was reduced to 1/4th of its original value on a rotary evaporator. The orange-brown-coloured complex was filtered and recrystallized from a 2:1 mixture of methanol/ethanol.

Microbiological studies

Test microorganisms and medium

The bacterial subcultures for *Staphylococcus aureus, Bacillus polymyxa* and *Escherichia coli* were obtained from Gazi University Gazi Hospital, Microbiology Department. An antifungal susceptibility test was used *Candida albicans* (ATCC 90028). *Staphylococcus aureus, Bacillus polymyxa, Escherichia coli* and *Candida albicans* were cultured on Brain Heart Infusion Broth(BHI) for the antibacterial and antifungal activity tests.

Method

The compounds were tested for their antimicrobial activity by the well-diffusion method (Raman *et al.* 2001). Each compound was dissolved in dimethyl sulfoxide at a concentration of 5 mg/ml. DMSO was used as solvent and also for control. DMSO was found to have no antimicrobial activity against any of the test organisms. 1 cm^3 of a 24 h broth culture containing 10^6CFU/cm^3 was placed in sterile Petri-dishes. Molten nutrient agar (15 cm^3) maintained at ca. 45 °C was then poured into the Petri-dishes and allowed to

Table 4. The *in vitro* antimicrobial and antifungal activities of Schiff bases.

No	Schiff bases	Diameter of zone (mm)			
		S. aureus	*E. coli*	*B. polymxa*	*C. albicans*
1	$H_2C_{13}H_9O_3N$ H_2O (Gly)	6 mm	3 mm	8 mm	18 mm
2	$H_2C_{14}H_{11}O_3N$ (Ala)	5 mm	4 mm	7 mm	16 mm
3	$H_2C_{20}H_{15}O_3N$ $2.5H_2O$ (Phe)	4 mm	2 mm	6 mm	14 mm
4	$H_2C_{17}H_{13}O_3N_3$ $1/2H_2O$ (His)	3 mm	–	3 mm	9 mm
5	$C_{22}H_{18}O_3N_2$ $2H_2O$ (Trp)	2 mm	4 mm	8 mm	10 mm
	Control (DMSO)	–	–	–	–

Table 5. The *in vitro* antimicrobial and antifungal activities of Mn^{3+} complexes.

No	Complexes	Diameter of zone (mm)			
		S. aureus	*E. coli*	*B. polymyxa*	*C. albicans*
6	$Na[Mn(C_{13}H_9O_3N)_2]$ $2.5H_2O$ (Gly)	9 mm	0.5 mm	3 mm	8 mm
7	$Na[Mn(C_{14}H_{11}O_3N)_2]$-$2H_2O$ (Ala)	2 mm	4 mm	5 mm	8 mm
8	$Na[Mn(C_{20}H_{15}O_3N)_2]$-$4.5H_2O$ (Phe)	8 mm	0.5 mm	–	1 mm
9	$Na[Mn(C_{17}H_{13}O_3N_3)_2]$-$3H_2O$ (His)	–	–	–	–
10	$Na[Mn(C_{22}H_{18}O_3N_2)_2].4.5H_2O$ (Trp)	–	–	–	–
	Control (DMSO)	–	–	–	–

solidify. Then holes of 6 mm diameter were formed in the agar using a sterile cork borer and these holes were completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. The tests were carried out in triplicate.

Results and discussion

Synthesis

The manganese(III) complexes of the Schiff bases were synthesized by the template method described by Sakiyan *et al*. (2001) (Table 2). Their structures were investigated by elemental analyses, IR, UV, TGA, and -magnetic susceptibility measurements. Amino acid Schiff bases were synthesized using a new method and the samples obtained were found to be more stable than those reported in previous work (Sakiyan *et al*. 2001). Their structures were identified with elemental analyses, IR, ^{1}H- NMR, and UV (Table 1).

NMR spectra of Schiff bases

The NMR data of the five Schiff bases studied are summarized in Table 3 and Figure 1. The phenolic OH protons are intramolecularly bonded in all the structures and appear in the range 13.6–14.2 ppm. As to the carboxyl protons, there appears to be no discrete signal assignable to them, which is probably caused by the rapid exchange between the carboxyl group and water molecules present in DMSO. The azomethyn protons appear in the range, 8.7–9.0 ppm, which is characteristic of these protons. Schiff bases derived from glycine and alanine display six aromatic protons, as expected. In the spectrum of phenylalanine, the integral of the aromatic region corresponds to eleven protons; five protons on the phenyl ring are recognizable at ca. 7.3 ppm. With histidine and tryptophane, protons on the five-membered rings of the imidazole and indol moieties give rise to signals partially overlapped with the aromatic proton-signals.

However, these signals are still recognizable. Methyn (for glycine, methylene) protons on the α-carbon of the carboxylic acid moieties appear at 4.4–4.7 ppm. This signal is a singlet for (1), a quartet for (2), and doublet of doublets for (3), (4) and (5), all of which arise from the non-equivalent methylene protons in structures (3), (4) and (5).

Full assigment of the aromatic protons was not made. Ortho- couplings for these protons occur in the range of 7–9 Hz and meta and para couplings were not detected. Other couplings are given in Table 3.

Antimicrobial activities

Amino acid Schiff bases and manganese(III) complexes were assayed in vitro for their ability to inhibit the growth of representative Gram-positive (*Staphylococcus aureus* and *Bacillus polymyxa*) and Gram-negative (*Escherichia coli)* bacteria and the fungus *Candida albicans.* The susceptibilities of certain strains of bacteria and a fungus to the amino acid Schiff bases and their complexes were evaluated by measuring the size of the bacteriostatic diameter. The results are given in Tables 4 and 5 for the Schiff bases and the manganese complexes, respectively.

In the literature, most of the amino acid Schiff base derivatives are reported to be more active against representative bacteria and fungus than their complexes under identical conditions (Raman *et al*. 2001; Koksal *et al*. 2001). In our work, we also observed that the Schiff bases in general are more active against the fungus, *Candida albicans* than their complexes. However the manganese(III) complexes, 6 and 8 (Table 5) were found to be more active against, *S. aureus* than the corresponding Schiff bases. Against the bacteria *E. coli* and *B. polymyxa* the Schiff bases, 1, 2, 3, 4 and 5 are more or equally active than or as the complexes. Manganese(III) complexes of the Schiff bases of histidine and tryptophan (9 and 10) were totally inactive against all the bacteria and the fungus. An inverse correlation between pesticide activity and amino acid residue exists. The in vitro, antimicrobial activities of the compounds with phenyl, imidazole, and indol groups on the α-carbon were fewer compared to those bearing H and CH_3.

Apparently, a bulky group on the asymmetric carbon center reduces the activity of the compound. Further studies with other similar structures would better clarify this issue.

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