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Research Article

ANTIMICROBIAL ACTIVITY AND CHARACTERIZATION OF SEVEN SYNTHETIC FORMAMIDINE DISULFIDE DERIVATIVES

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ABSTRACT

Objective: This research seeks to synthesize, characterize, and report the antimicrobial activity of seven (7) formamidine disulfide (FMD) derivatives.

Methods: The amine-FMD, a product of the oxidation of thiourea is reacted with seven different aldehydes to yield corresponding FMD Schiff bases (Scheme 1). The simple cup-plate method was employed to carry out the sensitivity test followed by the determination of minimum inhibitory concentration and minimum bactericidal concentration against typed cultures of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The synthetic compounds were characterized by infra-red (IR) spectrum, mass spectrum (MS), ^{13}CNMR, and ^{1}HNMR.

Results: The presence of the characteristic C=N of Schiff bases was observed in the synthetic compounds (Table 1). The compounds show better activity against the Gram-negative organism-*E. coli* ATCC 25923 compared to the Gram-positive organism-*S. aureus* ATCC 25922 (Tables 2 and 3). The least antimicrobial activity was exhibited by 3,4,5-Trimethoxy FMD Schiff base while Anisaldehyde FMD, 3-Nitro FMD, Cinnamaldehyde FMD and Vanillin FMD all show better and comparable antimicrobial activity relative to the other Schiff bases synthesized as reported.

Conclusion: Seven FMD derivatives were successfully synthesized, characterized and the antimicrobial activity determined by IR, MS and NMR.

Keywords: Formamidine disulfide, Antimicrobial activity, Schiff bases, Aldehydes.

INTRODUCTION

Thioureas, containing sulfur and nitrogen atoms, are susceptible to oxidation by a large number of oxidants giving rise to various products including ureas, sulfides, oxides of sulfur, and nitrogen [1]. Earlier it was reported that sulfur-containing compounds show complex free-radical chemistry, which is a very different from that exhibited by carbon-centered free radicals [2]. For example, sulfur-centered radicals are able to form three-electron-bonded intermediates such as RSSR•−, R2SSR•+2, or R2SOH• [3]. The radiation-induced chemistry of thiourea in aqueous solution has been studied [4], emphasizing its potential application as a radio protectant. During the process, formamidine disulfide (FMD) is found to be the major product. In addition to this several sulfur free nitrogen-containing compounds, sulfate ion, and elemental sulfur have also been observed. These products are probably, in most cases, the hydrolysis products of the disulfide [5]. Derivatives of thoiureas have been widely reported to possess antimicrobial activities including anti-HIV activity [6].

EXPERIMENTAL

The Fourier transform infrared spectroscopy (FTIR) spectra were recorded with Shimadzu FTIR 8400 spectrophotometer. The GC-MS spectra analysis was recorded on GCMS-QP 2010 PLUS Shimadzu, Japan. The NMR was run with a 900 MHz NMR instrument with a 21.1 T magnet.

METHODS

Synthesis of FMD and its derivatives

A solution of 2 g of thiourea in 40 ml of concentrated H_2SO_4 was added to an acidified $KMnO_4$ untill the purple color disappeared. The excess acid was neutralized with $NaHCO_3$ until it turns a litmus paper blue. The precipitated Schiff base-FMD was air dried after filtration. The desired aldehyde was added to a solution of FMD weighing 2 g (0.0133 M) in 5 ml of CH_3OH. The mixture was warmed on a water bath for 0.25 hrs with continues stirring. On cooling, the crystals formed were filtered and air dried. The aldehydes used to prepare the respective Schiff bases include 4-methylbenzaldehyde, 4-methoxybenzaldehyde (ansaldehyde), 3-nitrobenzaldehyde, 3,4,5-methoxybenzaldehyde, cinnamaldehyde, piperonal, and vanillin (Scheme 1).

Antimicrobial activity of FMD Schiff bases

Sensitivity testing using the cup-plate method

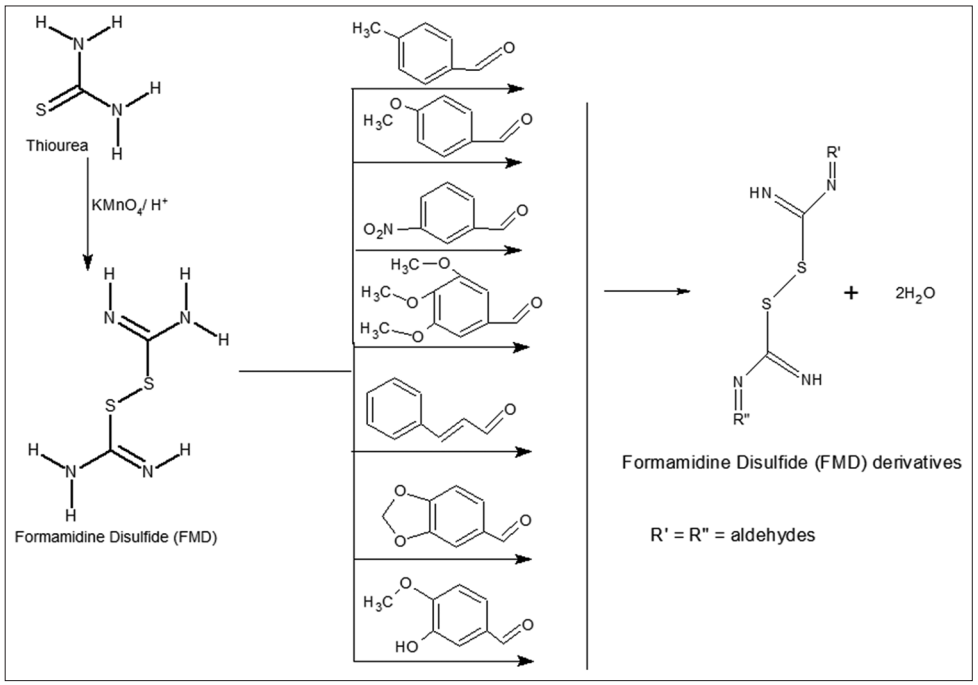
A preparation of freshly prepared peptone water and over-night culture of *Staphylococcus aureus* ATCC 25923 *Escherichia coli* ATCC 25922 was poured onto nutrient agar; the excess was gently and properly discarded and allowed to dry. Several holes were bored on each plate, and adequate quantity of the several dilutions of the drug was added with the aid of a small pipette. After about 0.5 hrs, the plates were incubated in an upright position@37°C for 24 hrs. The zones of inhibition were then measured in "mm" as shown in Tables 4 and 5.

Minimum inhibitory concentration (MIC) of Schiff bases

The solutions of the compounds under investigation were diluted serially with peptone water such that the concentration is halved in each in each tube in a series. The test organisms (*S. aureus* and *E. coli*) were inoculated into nutrient agar and allowed for about 24 hrs. Two drops of the test organisms each were added to the dilutions of the compounds and incubated@37°C for 24 hrs and examined for growth (The control tube does not contain the test organisms) as shown in Tables 2 and 6.

Minimum bactericidal concentration of Schiff bases

A loopful of the test organisms from the test tubes that did not show growth were transferred into plates containing molten nutrient agar with the aid of a standard loop and spread over a quarter of the surface.



Scheme 1: Synthesis of formamidine disulfide and its derivatives

Table 1: IR spectra of the seven synthetic compounds

S.N	Compounds/functional groups cm^{-1}	N-H	C=N	N-C	C=C	C-H	C-H aromatic
1	P-FMD	1580	1690	1320	1520	930	870
2	3-N-FMD	1500	1660	1300	1580	940	860
3	A-FMD	1620	1680	1200	1680	900	750
4	4-M-FMD	1610	1690	1320	1510	830	680
5	3,4,5-T-FMD	1600	1660	1100	1550	950	800
6	C-FMD	1590	1680	1280	1640	920	810
7	V-FMD	1590	1690	1020	1590	720	870

FMD: Formamidine disulfide. IR: Infra-red

Table 2*: Staphylococcus aureus* ATCC 25923

S.N	Compounds/ concentration (mg/dl)	50	33.3	16.7	8.3	4.2	2.2	0.7	0.4
1	P-FMD	-	-	+	+	+	+	+	++
2	3-N-FMD	-	-	-	-	+	+	+	++
3	A-FMD	-	-	-	-	+	+	+	++
4	4-M-FMD	-	-	-	-	+	+	+	++
5	3,4,5-T-FMD	-	-	+	+	+	+	+	++
6	C-FMD	-	-	-	-	-	-	+	++
7	V-FMD	-	-	-	-	+	+	+	++

FMD: Formamidine disulfide

Table 3: *Staphylococcus aureus* ATCC 25923

S.N	Compounds/ concentration (mg/dl)	50	33.3	16.7	8.3	4.2	2.2	0.7	MBC
1	P-FMD	-	-	+	+	+	+	++	100
2	3-N-FMD	-	-	-	-	+	+	++	25
3	A-FMD	-	-	-	-	+	+	++	25
4	4-M-FMD	-	-	-	+	+	+	++	50
5	3,4,5-T-FMD	-	+	+	+	+	+	++	150
6	C-FMD	-	-	-	-	+	+	++	25
7	V-FMD	-	-	-	+	+	+	++	50

MBC: Minimum bactericidal concentration, FMD: Formamidine disulfide

The nutrient agar plates were incubated at 37°C for 24 hrs. The plates were observed for growth as shown in Tables 3 and 7.

RESULTS

Based on the sensitivity test performed, the following zones of inhibition were obtained and tabulated as shown in Table 4.

The MIC of the seven compounds obtained based on visual turbidity is shown in Table 2.

DISCUSSION

The oxidation of thiourea with alkaline permanganate (or alkaline peroxide) converts thiourea into urea (Scheme 2). Sulfhydryl groups are known to catenate and in this case to FMD. The subsequent condensation reaction of FMD with various aldehydes to yield FMD Schiff bases is summarized in Scheme 1 above. The antimicrobial activity reveals that cinnamaldehyde, vanillin, and methyl benzaldehyde Schiff bases have similar MIC $^{@}$4.2 mg/dl against *E. coli* ATCC 25922 while $^{@}$8.3 mg/dl against *S. aureus* ATCC 25923. This projects a better activity against Gram-negative organisms. The activity was exhibited by trimethoxy FMD. The summary of the antimicrobial activity of the synthetic compounds is summarized in Tables 4-7. All the synthetic compounds show the presence of C=N functional group in their IR chat which is characteristic of all Schiff bases. The peaks observed around 1100 and 1300 cm^{-1} confirm the presence of ether linkage in some of the compounds like

Table 4: *Staphylococcus aureus* ATCC 25923

Diameter of zones of inhibition in "mm"						
S.N	**Compounds/concentration (mg/dl)**	**50**	**33.3**	**16.7**	**8.3**	**1.5 +ve control**
1	P-FMD	9	9	6	6	20
2	3-N-FMD	20	19	14	17	19
3	A-FMD	9	8	8	8	19
4	4-M-FMD	9	11	11	-	20
5	3,4,5-T-FMD	4	6	8	4	22
6	C-FMD	13	12	9	13	19
7	V-FMD	12	8	8	-	20

FMD: Formamidine disulfide

Table 5: *Escherichia coli* ATCC 25922

Diameter of zones of inhibition in "mm"						
S.N	**Compounds/concentration (mg/dl)**	**50**	**33.3**	**16.7**	**8.3**	**1.5 +ve control**
1	P-FMD	11	10	4	8	14
2	3-N-FMD	12	10	6	12	14
3	A-FMD	4	4	4	4	15
4	4-M-FMD	11	13	4	5	15
5	3,4,5-T-FMD	-	4	3	4	14
6	C-FMD	9	10	-	-	14
7	V-FMD	11	12	10	12	14

The diameter of the cup borer is 6 mm and these zones of inhibitions recorded are zones of inhibition less diameter of cup borer. FMD: Formamidine disulfide

Table 6: *Escherichia coli* ATCC 25922

S.N	**Compounds/ concentration (mg/dl)**	**50**	**33.3**	**16.7**	**8.3**	**4.2**	**2.2**	**0.7**	**0.4**
1	P-FMD	-	-	-	+	+	+	+	++
2	3-N-FMD	-	-	-	+	-	+	+	++
3	A-FMD	-	-	-	+	-	+	+	++
4	4-M-FMD	-	-	-	+	-	+	+	++
5	3,4,5-T-FMD	-	-	-	-	+	+	+	++
6	C-FMD	-	-	-	+	-	+	+	++
7	V-FMD	-	-	-	+	-	+	+	++

-: Turbid, +: Slightly turbid, ++: Very turbid. FMD: Formamidine disulfide

trimethoxy FMD and piperonal FMD. Table 1 is a summary of other important functional groups obtained from the IR spectrum of the compounds. The mass spectrum of the synthetic compounds reveals that the disulfide bond is a common point of symmetry of FMD Schiff bases [7]. The ^{13}C NMR spectra of all the compounds have constant overlapping peaks at 190 ppm. These peaks are attributed to the two imine carbons. The aromatic substituents on the nucleus however help at differentiating the various carbons as described by Afolabi and Jegede [8].

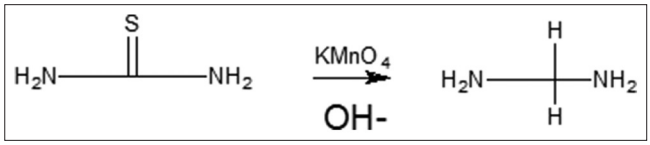
CONCLUSION

Seven FMD Schiff bases were synthesized, characterized and the anti-microbial activity against Gram-positive and Gram-negative organisms was successfully determined and reported.

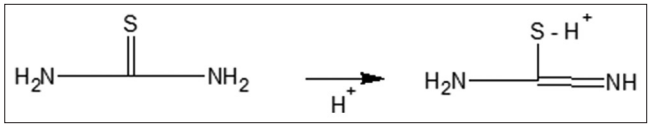
Table 7: *Escherichia coli* ATCC 25922

S.N	**Compounds/ concentration (mg/dl)**	**50**	**33.3**	**16.7**	**8.3**	**4.2**	**2.2**	**0.7**	**MBC**
1	P-FMD	-	-	-	+	+	+	++	50
2	3-N-FMD	-	-	-	+	+	+	++	50
3	A-FMD	-	-	-	-	-	+	++	12.5
4	4-M-FMD	-	-	-	-	+	+	++	25
5	3,4,5-T-FMD	-	+	+	+	+	+	++	150
6	C-FMD	-	-	-	-	+	+	++	25
7	V-FMD	-	-	-	+	+	+	++	50

-: Absence of growth, +: Presence of growth, ++: Presence of much growth. MBC: Minimum bactericidal concentration, FMD: Formamidine disulfide



Scheme 2: Thiourea in alkaline medium is converted to urea But in acidic conditions, thiourea is said to have a free sulfhydryl group



Scheme 3: Thiourea in acidic medium

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