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Synthesis of Silver (I) Coordination of Aspirinate Azo Ligands as Potential Antibacterial Agents

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Abstract. The rise of antimicrobial resistance for infectious bacteria has become an alarming issue to human health. New antimicrobial drugs are in dire need and pivotal to overcome this issue. In this study, aspirinate azo ligands bearing different halogens **L1-5** has been prepared *via* diazo-coupling reaction. The ligands **L1-5** were coordinated with silver, Ag (I) metal to produce Ag(I) aspirin-azo complexes **C1-5**. The antibacterial properties of **L1-5** and **C1-5** were evaluated against *Staphylococcus aureus* and *Escherichia coli* using turbidimetric kinetic method. The complexes **C1-5** showed comparable growth inhibition activity towards *E. coli* (MIC 82-105 ppm) and *S. aureus* (MIC 80-105 ppm) compared to ligands **L1-5** with *E. coli* **(**MIC 83-200 ppm), *S. aureus* (80-131 ppm) and ampicillin (MIC 93 and 124 ppm, respectively). The excellent bacterial resistance of both **L1-5** and **C1-5** indicates the potential of aspirinate azo and their complexes as new antibacterial agents, which significantly benefit to the pharmaceutical industries.

Introduction

The increase of contagious bacteria has led to the emergence of drug resistance which caused many deaths in the society [1]. The development of new drugs with antimicrobial drug resistance properties is in dire need to overcome the growth of pathogenic bacteria. Several types of antimicrobial drugs have been developed through the manufacture of commercial drugs derived from azo dyes such as phenazopyridine [2].

Several studies have been reported on the synthesis of azo dyes with a wide spectrum of biological activity [3]. The azo moieties have the ability to prevent bacteria multiplication and DNA function [4, 5]. Organic dyes featuring azo moieties (N=N) in the molecular network have also been widely studied due to their thermal and energy storage properties [6, 7, 8].

Hybridization of azo dyes with natural product-based compounds with multifunctional groups can increase the efficiency and probability of additional interactions with biological targets/receptors [3]. Our preliminary studies on azo dyes bearing natural product-based compounds such as aspirin and kojic acid showed antibacterial activities [3, 8, 9, 10] and anticancer activity [11, 12].

Complexation of azo dyes with heavy metals namely Ag(I) can also increase the cytotoxic and antibacterial properties [13]. The formation of chelation between a metal ion and ligand able to improve the biological activities compared to the ligands alone [14]. Complexation is able to increase the lipophilicity of ligands, which allow the ligand to enter the bacterial cell and interact with the enzyme receptor or DNA [13]. Ag metal is well known for its antimicrobial properties [15, 16,17]. Ag is non-toxic to humans and has been utilized as antimicrobial agents due to its ability to interact with protein and enzyme of bacteria, which eventually leads to bacterial cell death [18, 19, 20].

In this paper, the synthesis of natural product-based compounds namely aspirinate azo ligands **L1-5** bearing halogens (F, Cl, Br, I) were synthesized *via* diazotization and coupling reaction. The complexes **C1-5** were prepared *via* complexation with Ag(I) in dark condition and at room temperature. The ability of the ligands and complexes to inhibit the growth of *S. aureus* and *E. coli* were tested using turbidimetric kinetic method.

Experimental

Materials and instrumentational

Chemicals and solvents were used as received from Merck. The analysis of melting point was performed using open capillary method on Stuart SMP3. Smart Omni Transmission Nicolet IS10 Perkin Elmers Thermoscientific was used to analyze FTIR spectra. ^{1}H and ^{13}C NMR spectra were obtained from JEOL ECA with ^{1}H at 500 MHz and ^{13}C at 125 MHz using standard reference, DMSO-d_6. The elemental CHNS was analyzed using Thermo-Flash (EA 1112) CHN Analyser. The transmittance values were analyzed on Optima SP-300.

Synthesis of halogenated aspirin-azo ligands (L1-5)

Sodium nitrite (1M, 2mL) was poured into a solution of aniline derivatives (5 mmol) and hydrochloric acid (8 M, 8 mL) in an ice bath. Acetylsalicylic acid (5 mmol) and sodium hydroxide (1 M, 10.0 mL) were added and continue stirring for 45 min. Hydrochloric acid (3 mL, 8 M) was added dropwise onto the solution mixture until the precipitate formed. The solid was filtered, rinsed, recrystallized from hot ethanol and dried under vacuum to obtain the final product **L1-5**.

Complexation of Ag (I) complexes [Ag(Aspirin-azo)]NO_3 (C1-5)

Silver nitrate (1 mmol) was added dropwise into aspirin-azo ligands **L1-5** (0.5 mmol) in methanol. The mixture of silver nitrate and ligand was stirred for 3 h in the dark at room temperature. Solid precipitates formed were filtered and washed with water followed methanol and dried to afford the final product **C1-5**.

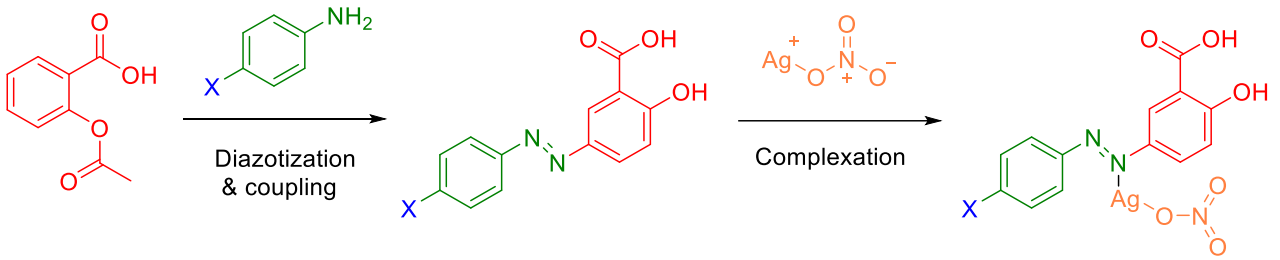
Antibacterial study

Antibacterial studies of the synthesized aspirinate azo ligand **L1-5** and Ag(I) complexes **C1-5** were carried out against *E. coli* (ATCC 25922) and other common types of bacteria, *S. aureus* (S48/81). Both bacteria sub-cultures were grown in Luria-Bertani (LB) broth at 37 °C.10 mL of LB broth was added with ligands and complexes at different concentrations separately (50, 80 and 100 ppm). The media and tested compounds were added with 0.2 mL of sub-cultured bacteria. The mixture was then stirred and incubated at 37 °C. Dimethyl sulfoxide and ampicillin were set as controls, negative and positive, respectively. The transmittance (T) values were recorded for every 1 h (0h – 6h) at wavelength 560 nm. The T values were extrapolated to colony-forming units / mL and articulated in ln Nt[2].

Results and Discussion

Synthesis

Aspirinate azo ligands **L1-5** were initially synthesized *via* diazotization of halogenated anilines derivatives followed by coupling with acetylsalicylic acid with yields 24-85 %. Ag(I) complexes **C1-5** were prepared satisfactorily from direct reaction of **L1-5** with silver nitrate in a molar ratio of 1:2 (Ag: ligand) to produce 26-68 % yields, where azo was acted as nitrogen chelator[1]. The equivalent ratio of Ag(I) and azo ligand gave similar composition and equivalent yields. The synthetic pathway of aspirinate azo ligands **L1-5** and Ag(I) complexes **C1-5** is shown in **Scheme 1**. The structural conformation of ligands **L1-5** and Ag(I) complexes **C1-5** was performed using CHN, FTIR and NMR spectroscopy.



Scheme 1. The synthetic pathway of halogenated azo-aspirin **L1-5** and Ag (I) azo-aspirin complex **C1-5**.

Based on CHN analysis, the data obtained for **L1-5** and **C1-5** corresponded to the proposed structure of the ligands and complex, [Ag (Aspirin-azo)]NO_3. The key functional group present in the IR spectra **L1-5** and **C1-5** is depicted in **Table 1**. The IR spectra of **L1-5** showed a broad band in the region 1437–1487 cm^{-1}, which attributed to the ν(N=N) [3]. The peaks presence at 1619-1688 cm^{-1} and 1660–1676 cm^{-1} were attributed to ν(C=O) vibration, while the appearance of peaks at 1561–1591 cm^{-1} was attributed to ν(Ar) stretching. Upon coordination of azo ligands with silver(I) ions, the FTIR peaks of **C1-5** were significantly shifted indicates successful coordination to produce new silver(I) aspirinate azo compounds. All the absorption peaks attributed to ν(C=O), ν(Ar) and ν(N=N) in **C1-5** were shifted to a higher frequency [21, 22].

The significance resonance of ^{1}H and ^{13}C NMR corresponded to the **L1-5** and **C1-5** structure is shown in **Table 2** and **Table 3**. The coordination of Ag(I) complex **C1-5** is shown in Fig. 1.

The coordination of Ag(I) with aspirinate azo ligands **L1-5** was confirmed by the shifted of peaks in the spectra. The peaks corresponded to H_6 in **L1-5** were assigned as a doublet at δ 8.30 -8.33 ppm and H_4 as doublet to doublet at δ 7.92-8.07 ppm, which strongly indicated on the successful coupling reaction *via* the formation of N=N at the *para* position of OH group. Upon coordination with Ag(I) ions, H_4 was shifted to the upfield at δ 0.06-0.21 ppm. While $H_{2'}$ was shifted upfield at δ 0.01-0.10 ppm. The shift of ^{1}H peaks was also contributed by the existence of nitrate ions (NO_3^-) in the complexes. The nitrate ions worked as a counter ion and established a small or minor interaction with an aromatic proton [23].

The ^{13}C NMR spectra of **L1-5** and **C1-5** were in agreement with the target structures as indicated by the occurrence of resonances at δ 148.5-152.2 ppm and δ 142.7-144.4 ppm attributed to $C_{1'}$ and C_5, respectively. Upon coordination with Ag(I) ions, C_5 has shifted upfield in the range of δ 0.82-1.58 ppm. While $C_{1'}$ was shifted downfield in the range of δ 0.21-0.49 ppm (Table 3). Ag(I) was envisaged to coordinate with N bonded to C_5 due to the strong shifting effect [24, 25].

Table 1. Summary of main FTIR band of azo ligand **L1-5** and Ag(I) complex **C1-5.**

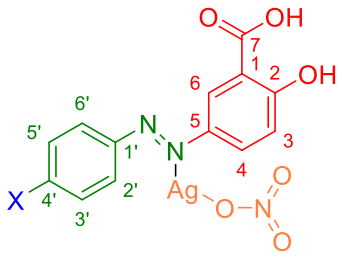
Compound	Wavelength (cm^{-1})		
	ν(C=O)	ν(Ar)	ν(N=N)
L1	1688	1587	1480
C1	1661	1561	1450
L2	1666	1591	1487
C2	1624	1561	1444
L3	1659	1576	1478
C3	1661	1580	1458
L4	1665	1574	1455
C4	1619	1561	1437
L5	1661	1584	1465
C5	1622	1563	1440

Table 2. Summary of ^{1}H NMR resonances nearest to the N=N moieties.

Compound	^{1}H NMR shifts in ppm		
	H_4	H_6	$H_{2'}$
L1	7.920	8.306	7.840
C1	7.857	8.330	7.821
Shift value (ppm)	0.063	0.024	0.019
L2	8.071	8.321	7.947
C2	7.862	8.326	7.896
Shift value (ppm)	0.209	0.005	0.051
L3	8.077	8.332	7.880
C3	7.991	8.318	7.870
Shift value (ppm)	0.086	0.014	0.010
L4	8.082	8.330	7.808
C4	7.881	8.316	7.772
Shift value (ppm)	0.201	0.014	0.036
L5	8.077	8.234	7.951
C5	7.915	8.310	7.848
Shift value (ppm)	0.162	0.080	0.103

Table 3. The resonances of ^{13}C NMR for **L1-5** and **C1-5.**

Compound	^{13}C NMR peak shifts in ppm	
	$C_{1'}$-N	C_5-N
L1	151.814	144.460
C1	152.205	142.895
Shift value (ppm)	0.391	1.385
L2	148.580	144.288
C2	148.914	142.924
Shift value (ppm)	0.334	1.364
L3	150.412	144.298
C3	150.621	143.477
Shift value (ppm)	0.209	0.821
L4	150.707	144.326
C4	151.117	142.829
Shift value (ppm)	0.410	1.497
L5	151.117	144.298
C5	151.604	142.714
Shift value (ppm)	0.487	1.584



C1-5

X : 1 = H, 2 = F, 3 = Cl, 4= Br, 5 = I

Figure 1. Ag(I) azo-aspirin complex **C1-5** structure.

Antibacterial analysis

Aspirinate azo ligands **L1-5** and Ag(I) aspirinate azo complexes **C1-5** showed antimicrobial properties screened on bacteria *S. aureus* and *E. coli* at the various concentration [26]. In both bacteria, MIC values were obtained by inducing the growth rate concentration to zero for both bacteria as shown in **Table 4**.

Table 4. MIC values of **L1-5** and **C1-5** for *E. coli* and *S. aureus*.

Ligand/complex	*E. coli*	*S. aureus*
L1	117	104
C1	82	104
L2	>200	125
C2	105	131
L3	85	98
C3	91	80
L4	86	70
C4	91	81
L5	83	80
C5	90	72
Ampicillin	93	124

Ligands **L1, L3-L5** demonstrated good antibacterial activities with MIC values 83-117 ppm and 70-104 ppm against *E. coli* and *S. aureus* ppm respectively, which are comparable to ampicillin (MIC 93 ppm and 124 ppm, respectively). Aspirinate azo dyes **L5** bearing iodine exhibited excellent inhibition against *E. coli* with MIC 83 ppm, while **L4** bearing bromine showed excellent growth inhibition against *S. aureus* with MIC 70 ppm. The presence of azo moieties in the ligands has contributed to the strong inhibition of bacteria growth [27, 28]. Additionally, the presence of hydroxyl (OH) and carboxyl group (COOH) in the molecular network has contributed to the hydrophilic properties of the molecules which allowed the ligand to interact with bacteria receptors or DNA *via* hydrogen bonds interaction. The halogen in the ligand has contributed to the increase of antimicrobial activity. Moreover, the halogen has the capability to upsurge the lipophilicity of ligand for facile diffusion into the bacterial cell wall and membrane *via* the interaction of halogen bond towards bacterial receptor or DNA [29, 30, 31].

The formation of Ag(I) complexes, however, showed the increase and decrease in the antimicrobial activities (Table 4). The complexes **(C1-5)** gave comparable and lower MIC values of 82-105 ppm and 72-131 ppm against *E. coli* and *S. aureus*, respectively compared to ampicillin (MIC 93 ppm and 124 ppm, respectively) and the ligands **L1-5**. While the complexes **(C1-2)** demonstrated a stronger ability to inhibit the growth of *E. coli* with MIC 82 ppm and 105 ppm, respectively compared to their free ligands **L1** (117 ppm) and **L2** (>200 ppm). While the growth inhibition activity against *S. aureus* demonstrated compound **C3** and **C5** with excellent MIC values of 80ppm and 72 ppm, respectively, in comparison to their ligands **L3** (MIC 98 ppm) and **L5** (MIC 80 ppm).

Upon coordination with Ag, the antibacterial activity was increased as the lipophilicity of the compound increased [31]. Furthermore, Ag(I) ions have also improved the π-electrons delocalization throughout the ligand molecule, thus increase its ability to penetrate the lipid cell membrane of the bacteria [32, 33]. The decrease in the inhibition activities is believed due to the high lipophilic properties of the complexes after coordinated with Ag that reduced the solubility of complexes to dissolve and react with enzyme and protein [34]. It can be concluded that the combined interaction of hydrophilicity and lipophilicity of a molecule plays an essential part in the antibacterial properties of the compounds [3, 32].

Conclusion

Aspirinate azo derivatives bearing series of halogens **L1-5** were successfully synthesized and coordinated with Ag to form complexes **C1-5**. The azo derivatives **L4** gave excellent inhibition against *S. aureus* (MIC 70 ppm) while complex **C1** showed excellent inhibition against *E. coli* featuring the lowest MIC value (MIC 82 ppm) as compared to other ligands, complexes and standard ampicillin antibiotic drug. The presence of halogens in **L2-5** exhibited excellent antimicrobial activities compared to **L1**. Coordination of ligands with Ag(I) has increased the lipophilicity of the overall complexes and increased the antibacterial activity. **C3-5** had a marginal decrease in activity for growth inhibition against *E. coli* as compared to the ligands. The excellent antibacterial properties of aspirinate azo dyes and Ag coordination have shown promising potential for the development of new antibiotic drugs in the pharmaceutical industry.

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