Bacterial Growth Inhibitory Properties of six Amino Acid Derived Schiff-Bases

Kevin Joannou

BIO 488

Professor Deborah Hemmerling

Professor James Titah

Introduction

Schiff-bases are a type of imine first described by Hugo Schiff in the 19th century. These molecules are formed from the condensation of primary amines and carbonyl compounds (Figure 1)(El-Sherif and Aljahdali 2013). The simple process of condensing different primary amines with a variety of carbonyl compounds can be used to synthesize many Schiff-bases. After the binding of Nitrogen to the Carbon in the carbonyl group, the Nitrogen is left with a lone-pair of electrons that can interact with transition metal ions to form complexes (El-Sherif and Aljahdali 2013). Schiff-bases tend to be unstable, but are greatly stabilized by simply including an aryl group directly bonded to either end of the C=N bond (El-Sherif and Aljahdali 2013).

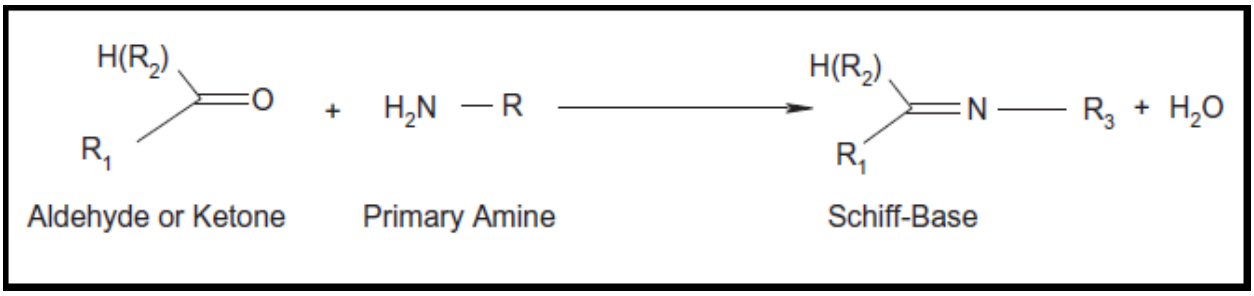

Figure 1: Generic condensation reaction that forms a Schiff-base. Figure adapted from El-Sherif and Aljahdali 2013

The potential medical uses of Schiff-bases have been of interest for the past two decades. Many different Schiff-bases and their transition metal complexes have been tested to reveal potentially useful medical properties such as anti-cancer, antifungal, antiviral and antibacterial effects (Abu-Dief and Mohamed 2015). Schiff-bases synthesized by using amino acids as the primary amine are particularly interesting from a medical standpoint because they can be easily and cheaply synthesized (Singh and Singh 2013; Chohan et al. 2006).

Several studies have examined the antibacterial properties of amino acid derived Schiff-

bases (Chohan et al. 2006; Rimbu et al. 2014; Lekha et al 2013). The standard method for testing antibacterial strength has been to use the Schiff-bases and their transition metal complexes in agar well diffusion experiments that examine the effects of the bases against both Gram positive and Gram negative bacteria (Abu-Dief and Mohamed 2015). It has been found that complexes formed with the same Schiff-bases and different transition metals can have significantly different bacterial growth inhibition strengths (Chohan et al. 2006).

The specific molecular action of different Schiff-bases and their transition metal complexes that result in bacterial growth inhibition are not thoroughly understood, but some experiments have provided evidence that DNA, cell membranes, and cell walls are involved. Abdel-Rahman et al (2014) have found that Schiff-bases synthesized from 5-bromosalicylaldehyde and α- amino acids would intercalate with Calf Thymus DNA. Several studies have found that Schiff-base transition metal complexes tend to have stronger bacterial inhibition than the lone Schiff-bases, which may be due to increased lipophilicity and resulting membrane permeability as a complex (Abu-Dief and Mohamed 2015). Differences in strength of growth inhibition between Gram positive and Negative bacteria also suggests some molecular action involving the bacteria cell wall and/or membranes (Abu-Dief and Mohamed 2015).

Six amino acid derived Schiff-bases were synthesized and analyzed for their melting points, colour, solubility, NMR, and IR spectra at Concordia University of Edmonton (CUE) in 2016: N-Salicylidene Alanine, N-Salicylidene Serine, N-Benzalidene Histidene, N-Benzalidene Leucine, N-4-(dimethylamino) Benzalidene Phenylalaline, and N-4-(dimethylamino) Benzalidene Valine (Chambers and Balogh 2016). The salicylaldehyde Schiff-bases had been previously analyzed for their complex formation equilibria (El-Sherif and Aljahdali 2013). Some Schiff-bases derived from salicylaldehyde and 5-bromosalicylaldehyde with amino acids have

been used to make transition metal complexes and those complexes have been tested for their bacterial growth inhibition properties via agar well diffusion (Rimbu et al. 2014; Lekha et al 2013; Kiruthikajothi et al. 2014). However, a literature review returned no previous tests of the bacterial growth inhibition properties of any of the Schiff-bases synthesized by Chambers and Balogh (2016) without first being complexed with a transition metal (Rimbu et al. 2014).

Therefore, building on the work of Chambers and Balogh (2016), this experiment tested the growth inhibition properties of the six Schiff-bases on species of Gram positive and Gram negative bacteria via Nutrient Agar well diffusion. This is the next logical step in the full biological property characterization of the six amino acid derived Schiff-bases synthesized by Chambers and Balogh (2016).

Methods

Synthesis of Ligands

Some of the needed ligands were already available for testing and were in storage at Concordia University of Edmonton (CUE), but an additional 2 were synthesized to ensure adequate amounts for the experiment. The 6 Schiff-bases used in this experiment are summarized in Table 1.

Synthesis of the ligands was done via the same methods used by Kevin Chambers and Anita Balogh in their 2016 research (Chambers and Balogh 2016). In this method, equimolar amounts of NaOH and the appropriate Amino Acid were dissolved in a solvent (methanol or ethanol), then the appropriate carbonyl compound (salicylaldehyde or 4-dimethylaminobenzaldehyde) was mixed and allowed to react at room temperature for 45 minutes. The solute was evaporated to ~50% of the original volume, and an equimolar amount of

acetic acid was added. The Schiff-bases crystallized and were suction filtered, washed, and air dried.

Table 1: Reagent combinations used to synthesize the Shiff-Bases used in this experiment.

Schiff-Base	Carbonyl Compound	Amino Acid
1	4-Dimethylaminobenzaldehyde	Valine
2	4-Dimethylaminobenzaldehyde	Phenylalanine
3	4-Dimethylaminobenzaldehyde	Leucine
4	Benzaldehyde	Histidine
5	Salicylaldehyde	Serine
6	Salicylaldehyde	Phenylalanine

Synthesis of the ligands was done via the same methods used by Kevin Chambers and Anita Balogh in their 2016 research (Chambers and Balogh 2016). In this method, equimolar amounts of NaOH and the appropriate Amino Acid were dissolved in a solvent (methanol or ethanol), then the appropriate carbonyl compound (salicylaldehyde or 4-dimethylaminobenzaldehyde) was mixed and allowed to react at room temperature for 45 minutes. The solute was evaporated to ~50% of the original volume, and an equimolar amount of acetic acid was added. The Schiff-bases crystallized and were suction filtered, washed, and air dried.

Experimental Set-up

The goal of this experiment was to test the antibacterial effects of six Schiff-Bases on both Gram Negative (*Escherichia coli* KL25 strain) and Gram Positive (*Staphylococcus aureus* undocumented strain on-hand at CUE) bacteria. In this experiment, agar well diffusion using Nutrient Agar made from Difco Nutrient Broth (Catalogue No. 234000) and FLUKA Agar

(Catalogue No. 05040) was used to test the ligands at a concentration of 10 millimolar on the bacteria. This was an effective, easy, and common method used in many similar studies (Abu-Dief and Mohamed 2015). The Nutrient Agar plates were poured, cooled to solidify, had five wells bored through the centre of the gel with a sterilized and trimmed transfer pipette, the well was filled with ~0.0625 ml of a single ligand dissolved in DMSO to the appropriate concentration, and then the remaining Nutrient Agar surface was be streaked with liquid bacterial suspension culture. The Schiff-Bases had to be heated to 55^{o} C to promote dissolution in DMSO to the desired concentration. The Schiff-Base dissolved in DMSO diffused through the gel and bacterial colonies were exposed to it in a gradient of concentration with higher concentrations closer to the well. After 22-24 hours of incubation at 37^{o} C, the plates were be checked for a zone of inhibition around the agar wells. The null hypothesis was that each ligand would cause the same level of bacterial growth inhibition around the wells as DMSO alone.

To test six different ligands on two types of bacteria, twelve different test groups of Nutrient Agar petri dishes was prepared. An additional negative control group consisting of just DMSO, plus a positive control of DMSO and streptomycin (solution concentration 33 millimolar) means that there needed to be fourteen test groups total. Therefore, the total number of experimental units was: 5 replications X 14 groups = 70 experimental units.

Enough replicates were created to be able to detect a minimum 5 mm zone of inhibition with a 95% level of confidence by using a single-tailed T test when the standard deviation within treatment groups is as big as 2 mm. However, no statistical analysis was performed on the data, because all treatment groups had identical averages and standard deviations to the negative control group.

The CUE department of biological and environmental sciences provided all required

resources. All materials needed were on-hand and nothing needed to be ordered to complete the experiment.

Results

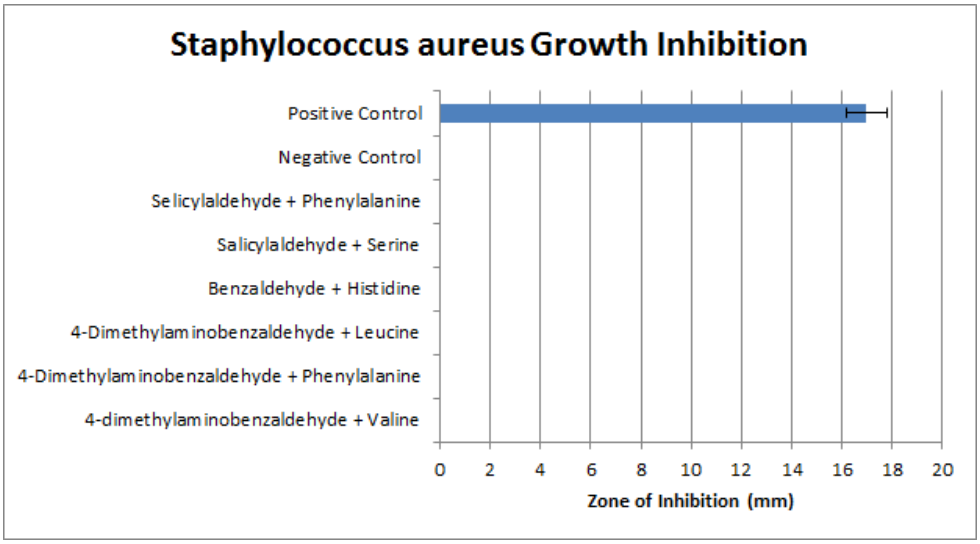

Figure 1: Average zone of growth inhibition around agar wells for treatments and controls on *S. aureus* cultures. Error bar is one standard deviation.

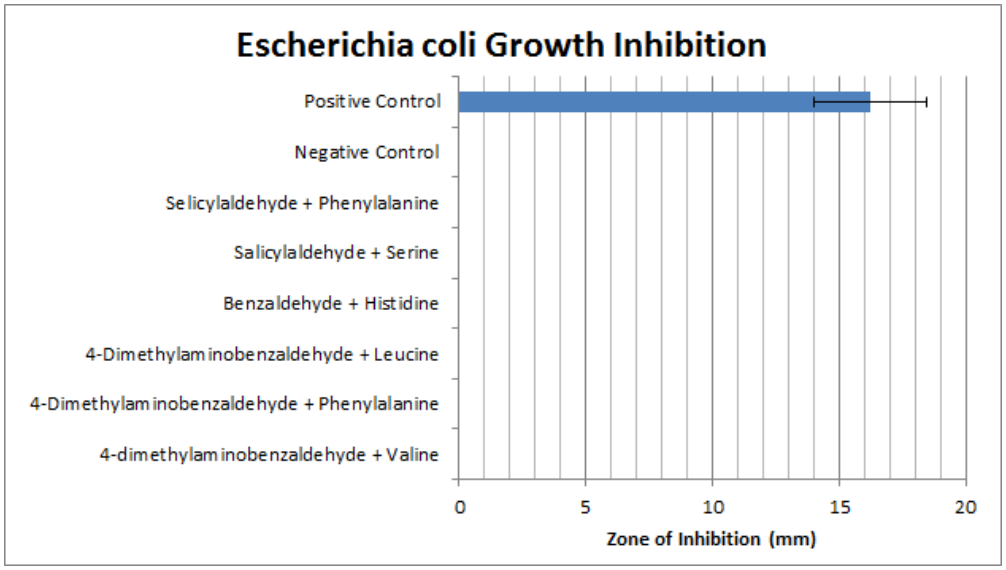

Figure 2: Average zone of growth inhibition around agar wells for treatments and controls on *E. Coli* cultures. Error bar is one standard deviation.

All treatments and the negative control group had zero millimeters of growth inhibition for either bacteria tested (Figure 1, Figure 2), and lawns of bacterial colonies were successfully grown. The broad-spectrum antibiotic positive control produced large zones of inhibition, in fact, the zones were so large that the plates were nearly colony-free and the zone around the centre test well could not be measured. Only the outer four wells on each plate were spread out enough to allow some colony growth between them, and thus allow measurement of zone of inhibition.

Discussion

The results of the experiment were extraordinarily clear: there was no detected bacterial growth inhibitory effect of any of the tested Schiff-Base ligands. The positive control group demonstrated that the agar well diffusion was an effective method for testing the Schiff-Bases, as was expected (Abu-Dief and Mohamed 2015). All treatment wells had zero millimeters of growth inhibition around them, which is an identical result and sample size to the negative control, and renders statistical analysis superfluous. This result supports the null hypothesis that the tested Schiff-Bases do not inhibit Gram positive or negative bacterial growth any more than DMSO.

It is possible that the tested Schiff-Bases do not inhibit the growth of all or most bacteria taxa. The results from this experiment support the hypothesis that the tested Schiff-Bases do not inhibit bacterial growth via any molecular mechanism essential to either Gram positive or negative bacteria, but only one species of Gram positive and negative bacteria were tested. It may be that the tested Schiff-Bases do inhibit the growth of some yet untested bacteria species, but the results from this experiment neither support nor falsify that possibility.

It was somewhat surprising that none of the tested Schiff-Bases showed any bacterial

growth inhibition effect despite being in a saturated solution. Other Amino Acid derived Schiff-Bases have been known to have a bacterial growth inhibitory power similar to streptomycin (Chohan et al. 2006). It seems that the tested Schiff-Bases in this experiment do not have the same cell membrane or DNA interactions that other Schiff-Bases have (Abdel-Rahman et al. 2014, Abu-Dief and Mohamed 2015).

No acute toxicity for the tested bacteria is promising news for the toxicity of the Schiff-Bases for humans working with these chemicals. However, it is possible that the significant biochemical differences between bacteria and humans would allow for the tested Schiff-Bases to be toxic to humans via an unknown mechanism. It would be prudent to do further testing on eukaryotes, and human tissue cultures in particular before declaring these Schiff-Bases safe for human contact. That type of research may also uncover currently unknown but medically useful effects that the Schiff-Bases may have.

There are two logical next steps in research for the six Schiff-Bases used in this experiment. First, as stated above, the Schiff-Bases should be tested on eukaryotes to identify any acute toxic effects they may have. Second, the Schiff-Bases should be complexed with transition metal ions and those coordination complexes should be tested for prokaryote and eukaryote toxicity. It is common for Schiff-Bases to have significantly different biological effects when in a coordination complex with transition metals (Chohan et al. 2006).

Overall, this experiment demonstrated with a high degree of clarity and confidence that the Schiff-Base products of 4-Dimethylaminobenzaldehyde + Valine, 4-Dimethylaminobenzaldehyde + Phenylalanine, 4-Dimethylaminobenzaldehyde + Leucine, Benzaldehyde + Histidine, salicylaldehyde + Serine, and Salicylaldehyde + Phenylalanine do not inhibit the growth of either *E. coli* or *S. aureus* when in a saturated solution with DMSO at a

concentration of up to 10 millimolar.

Acknowledgments

Many thanks to Dr. Hemmerling and Dr. Titah for guidance and supervision in planning and completing this research project, as well as the departments of Chemistry, and Biological and Environmental Sciences for providing all required materials, equipment, and space.

Literature Cited

Abdel-Rahman LH, El-Khatib RM, Nassr LAE, Abu-Dief AM, Ismael M, Seleem AA. 2014. Metal based pharmacologically active agents: synthesis, structural characterization, molecular modeling, CT-DNA binding studies, and *in vitro* antimicrobial screening of iron(II) bromosalicylidene amino acid chelates. Spectrochim Acta A Mol Biomol Spectrosc. 117: 366-378.

Abu-Dief AM, Mohamed IMA. 2015. A review on versatile applications of transition metal complexes incorporating Schiff bases. Beni-Sueff University Journal of Basic and Applied Sciences 4: 119-133.

Alias M, Kassum H, Shakir C. 2014. Synthesis, physical characterization and biological evaluation of Schiff base M(II) complexes. Journal of the Association of Arab Universities for Basic and Applied Sciences 15: 28-34.

Chambers K, Balogh A. 2016. Synthesis and characterization of Schiff base ligands derived from amino acids. Poster session presented at: Concordia University of Edmonton Student Research Celebration 2016; Edmonton, Alberta.

Chohan ZH, Arif M, Akhtar MA, Supuran CT. 2006. Metal-based antibacterial and antifungal agents: synthesis, characterization and In vitro biological evaluation of Co(II), CU(II), Ni(II), and Zn(II) complexes with amino acid-derived compounds. Bioinorganic Chemistry and Applications 2006: 1-13.

Kiruthikajothi K, Chandramohan G, Muniyappan K. 2014. Synthesis and antimicrobial evaluation of copper (II) complexes with some amino acids. Chemical Science Review and Letters 3(10): 247-251.

Lekha L, Kanmaniraja K, Rajagopal G, Sivakumar D, Easwaramoorthi D. Synthesis, spectral characterization and antimicrobial assessment of Schiff Base ligand derived from amino acid and its transition metal complexes. International Journal of Chemical and Pharmaceutical Sciences 4(2): 48-54.

Rimbu C, Danac R, Pui A. 2014. Antibacterial activity of Pd(II) complexes with salicylaldehyde-amino acids Schiff Bases ligands. Chem Pharm Bull. 62(1): 12-15.

Singh HL, Singh J, Mukherjee A. 2013. Synthesis, spectral, and *In Vitro* antibacterial studies of organosilicon (IV) complexes with Schiff bases derived from amino acids. Bioinorganic Chemistry and Applications 2013: 9 p.