An Analysis of Aromatic Imines and their Antioxidative and Antibacterial Properties

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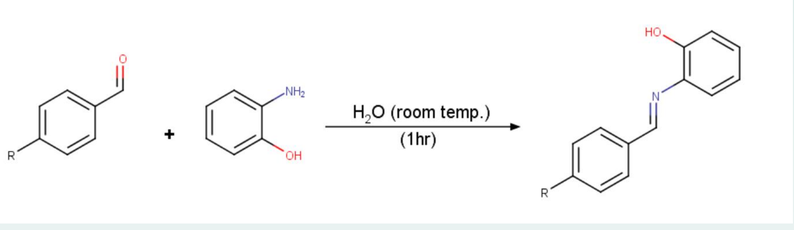
Introduction

Reactive oxygen species are molecules that are byproducts of many cellular transduction pathways produced by oxidative phosphorylation. These oxygen species have the ability to sporadically form single electron radicals that are dangerous in cellular function. Antioxidants are used to find these reactive oxygen species and neutralize them. This study is to find the antioxidative and potential antibacterial properties of several aromatic imines (characterized by 300 MHz 1H NMR, IR and melting point). The imines include 2-[(E)-{[4-(dimethylamino)phenyl] methylidene} amino] phenol (A), 2-[(E) (phenylmethylidene) amino] phenol (B), 2-[(E)-[(4-chlorophenyl) methylidene] amino]phenol (C), 2-[(E)-[(4-fluorophenyl)methylidene]amino] phenol (F), {4-[(E)-[(2-hydroxyphenyl)imino]methyl]phenyl} (oxido)nitroso (N), 2-[(E)-[(4-methoxyphenyl) methylidene] amino]phenol (O), and 2-[(E)-[(4-methylphenyl)methylidene] amino]phenol (T). The imines' antioxidative strengths were tested using DPPH. Disk diffusion assays were also performed.

Materials/Methods

The 7 aromatic imines were synthesized by an aldehyde – amine reaction (Fig. 1). The amine base (2-aminophenol) remained constant while the aldehyde changed for each ligand (p-tolualdehde, 4-chlorobenzaldehyde, 4-fluoro-benzaldehyde, anisaldehyde, (4-formylpheny)(oxido)nitroso, p-dimethylamino benzaldehyde, and benzaldehyde). The 1:1 reaction was achieved open air in an Erlenmeyer flask in water. The products were isolated (with vacuum filtration) and washed with hexane or water (depending on solubility) obtaining 75-80% yield. The products were then confirmed using 1H NMR, IR and melting point characterizations.

Figure 1: Aldehyde-Amine Reaction

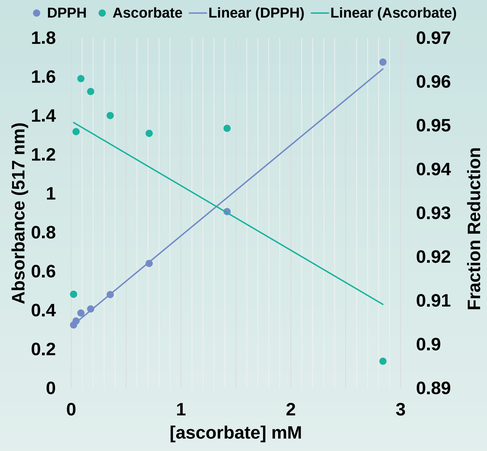


To determine antioxidative strength, DPPH assays are commonly used. Diphenyl-1-picrylhydrazyl (DPPH) contains a free radical that is visibly purple; however, if this radical is reduced by an antioxidant, DPPH loses its color. Because of this, DPPH assays can be used to determine the scavenging radical activity of unknown substances. In preparation for the DPPH assays, ligands were dissolved in degassed methanol - methanol was degassed for ~30 minutes by blowing air into the solvent - at a concentration of ~0.5 mg/ml. A stock solution of ascorbic acid (1 mg/ml or 0.57 mM) and DPPH (0.2 mg/ml or 0.51 mM) was made with methanol, and stocks were diluted to half their concentrations in the plate. A serial dilution was done to fill in the plate. The plate was then covered and put in a dark place for ~5 minutes. The plates were read on a spectrophotometer (using the program SoftMax Pro) at 517 nm.

Antibacterial strength can be seen through disk diffusions. Three different bacterial cultures were used to test the imine ligands: two gram positive (*B. subtilis* and *B. megaterium*) and one gram negative (*E. coli*). In preparation, cultures were grown in (5 mL) lysogeny broth (LB) at 37° C and 250 rpm for ~24 hrs (stationary phase), and the disks used were made by dissolving the different ligands in methanol *to* 2.5-6 mg/mL and then pipetting 10 µl of the solution onto the disks. Disks were left in a sterile environment to dry. Once cultures were in the stationary phase of growth, they were diluted to the start of the exponential phase. Optical density at 600 nm (OD 600) was measured every 30 minutes. When the OD 600 was between 0.4-0.5 the cultures (150 µl) were plated on Mueller-Hinton agar. Disks were applied to the plates right after the bacteria was spread. The plates were incubated (37° C) for 16-24 hours, and zones of inhibition were measured (Fig. 4).

Results

Figure 2: DPPH vs. Ascorbate



The fraction reduction (FR) shows the amount of DPPH scavenged. It was found using the formula:

$$FR = (A_{DPPH} - A_{Ligand}) / A_{DPPH}$$

where A_{DPPH} is the initial absorbance of DPPH and A_{Ligand} is the absorbance of only DPPH in the ligand solvent, which was calculated by subtracting the $A_{Ligand+DPPH} - A_{Ligand\ control}$.

EC_{50} represents the concentration calculated to cause 50% reduction of the initial DPPH concentration.

Because of very high background and suspected errors in the procedure, and because the ascorbate control EC_{50} did not match the expected value from literature, EC_{50} values tabulated are shown relative to the experimental EC_{50} for ascorbate (Fractional EC_{50} = Ligand EC_{50} /Ascorbate EC_{50}).

Figure 3: Fractional EC50 Values from DPPH Assays

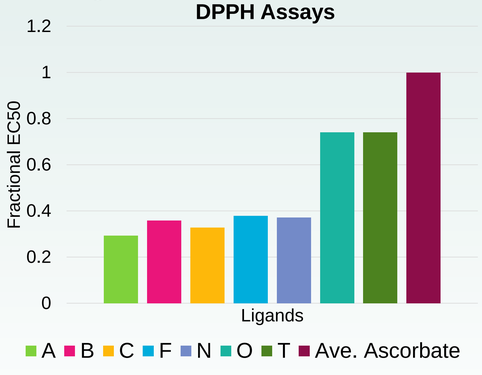


Figure 4: Zones of Inhibition (measured in mm, all disks had a diameter (d) of 6 mm)

Disk Solution	*B. subtilis*		*B. megaterium*		*E. coli*	
	Ring d	Discol. d	Ring d	Discol. d	Ring d	Discol. D
A	*7	6	6	6	*7	10
B	*7	6	6	6	6	9
C	6	8	6	6	6	10
F	*6.5	8	6	6	6	10
N	6	13	6	6	6	9
O	6	6	6	6	6	9
T	6	8	6	6	6	9
Methanol	6	6	6	6	6	11
ampicillin	13	6	21	6	6†	6†

*****Imines that showed obvious antibacterial properties.
Discoloration of Bacteria from disks (Discol.)
† *E. coli* contained a plasmid which was resistant to ampicillin

Conclusions

The antioxidant activity for each compound was calculated relative to ascorbate. The ascorbate had the strongest antioxidant ability; however all ligands did show antioxidant abilities. Specifically ligand O and T displayed the highest antioxidative strength, which were closest in EC_{50} to ascorbate.

The disk diffusion assays showed small antibacterial properties in *B. subtilis* for ligand A, B and F. Discoloration of the bacteria was also seen in *B. subtilis* for ligands C, F, N and T. Discoloration in the bacteria could indicate bacterial toxicity which was overcome by resistance mechanisms, and further investigation is needed. The ligands did not show any antibacterial properties in *B. megaterium* and limited properties in *E. coli.*

Future Work

The errors in data (EC_{50}) were unexpected and made it difficult to analyze. Additional DPPH assays should be performed. FRAP assays will also help confirm the antioxidative properties of the imines. Other disk diffusion assays will further the understanding of the imines' interactions with bacteria.

Acknowledgements

I would like to thank Winona State University for the materials and technology needed to perform this research. I would also like to thank Dr. Emily Ruff & Dr. Joseph West for the guidance and help in performing experiments and analyzing results.

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