Synthesis of Hydrazones as Antibiotics

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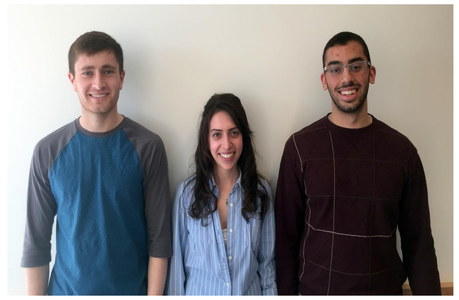
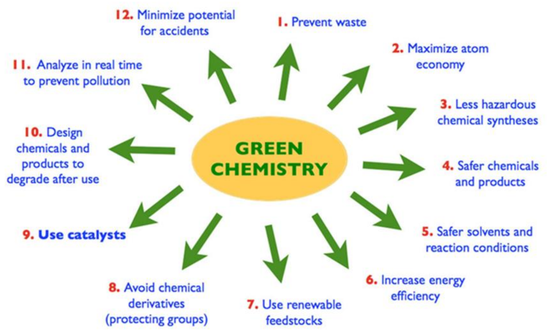


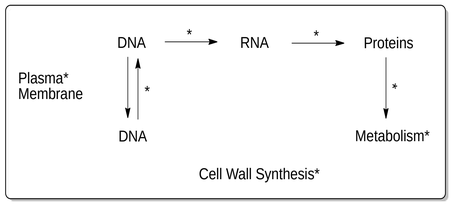
Green Chemistry



http://www.ccvc.umontreal.ca/research/research.html. Accessed on April 1, 2015.

Panel 1. Green chemistry is a concept that aims to reduce, if not eliminate, the use or generation of hazardous materials in the design, manufacture, and application of chemical products. Each year, U.S. factories spew 3 million tons of toxic chemicals into the air, water, and land. By incorporating principles of green chemistry into the laboratory curriculum, we can reduce the amount of generated waste. This academic year, we performed an experiment that adhered to these principles. The combinatorial syntheses and testing of antibiotics were carried out, minimizing excess waste, designing safer products, and increasing synthetic efficiency.

General Mechanisms for Antibiotics



Antibiotics act at all of these points

http://www.medicinenet.com/antibiotic_resistance/page3.htm. Accessed on April 1, 2015

Mechanisms of Action
1. Inhibition of cell wall synthesis
2. Disruption of cell membrane
3. Inhibition of protein synthesis
4. Interference with metabolic processes
5. Interference with nucleic acid synthesis

Panel 2. An antibiotic is a chemical or a substance that inhibits the growth of, or kills bacteria. Many antibiotic drugs have been developed to target different pathways of the bacterial cell.

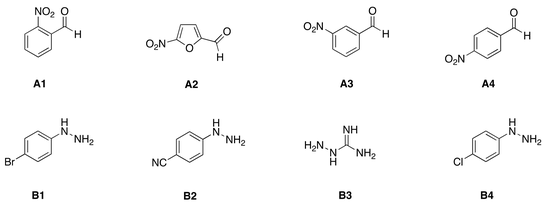
Combinatorial Chemistry

Concepts/techniques:
Combinatorial chemistry; carbonyl chemistry; antibiotics, sterile techniques; biological assays.

Green chemistry messages:
Synthetic efficiency; design of safer products; waste minimization.

1 **2** **3**

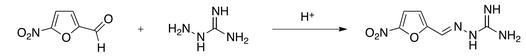
Panel 3. Sixteen different hydrazones (**3**) were formed within eight mixtures by combining four different aldehydes (**1**) with four different hydrazines (**2**) via a nucleophilic acyl substitution reaction.



A1 **A2** **A3** **A4**

B1 **B2** **B3** **B4**

Active Antibiotic Hydrazone



1 **2** **3**

Panel 4. Verification of the green-chemistry experiment in our lab has found that 5-nitro-2-furaldehyde (**A2**) and aminoguanidine (**B3**) reacted in the presence of acid to give the corresponding guanofuracin (**3**). This hydrazone demonstrated the greatest antibacterial activity from all sixteen possible combinations present in the biological assay.

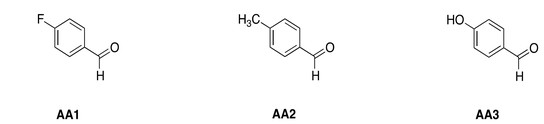
Table 1: Deconvolution of Hydrazone Libraries

Mixture		M1	M2	M3	M4
	Components	A1	A2	A3	A4
M5	B1	A1-B1	A2-B1	A3-B1	A4-B1
M6	B2	A1-B2	A2-B2	A3-B2	A4-B2
M7	B3	A1-B3	A2-B3	A3-B3	A4-B3
M8	B4	A1-B4	A2-B4	A3-B4	A4-B4

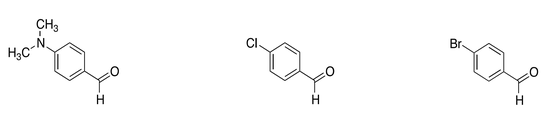
The process of determining the common hydrazone first involves identifying the aldehyde and hydrazine mixtures with the largest zone of inhibition. Subsequently, those identified mixtures are then deconvoluted using a combinatorial table. This guanofuracin product was isolated by discerning the common hydrazone that was visible in the cell cultures of *E. coli* after incubation for 24 hours. The noticeable rings of inhibition in quadrants M2 and M7 on both sets of plates indicated that the common hydrazone found in both mixtures had the most significant antibacterial activity.

Para-Substituted Benzaldehydes

Panel 5. Hydrazones are a family of compounds that display a wide spectrum of biological activities including antibiotic activity. We want to understand how the chemical structure influences the antibiotic ability of these compounds. A small library of hydrazones from various mixtures of aromatic benzaldehydes substituted with electron-donating and electron-withdrawing substituents at the *para*-position have been synthesized via combinatorial chemistry and screened for antibiotic activity against *E. coli* in order to contribute to an extensive structure-activity relationship.



AA1 **AA2** **AA3**



AA4 **AA5** **AA6**

The formation of analogues by the introduction of new substituents into the structure of a lead may result in an analogue with significantly different chemical and hence different pharmacokinetic properties. For example, the introduction of a new substituent may cause changes in lipophilicity, shape, and may introduce a new metabolic pathway for the analogue.

Interpretation of Results

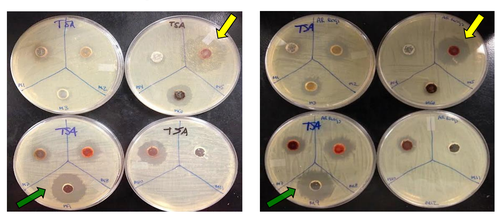
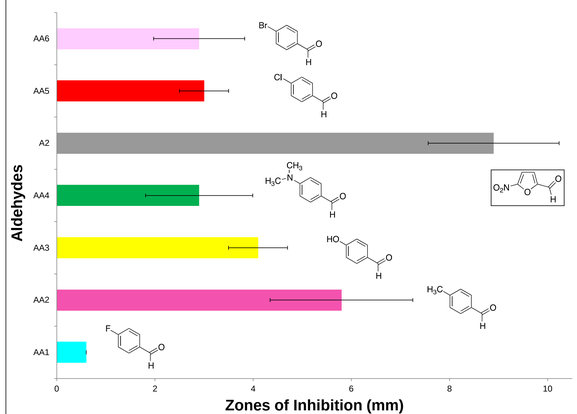


Table 1: Deconvolution of Hydrazone Libraries

Mixtures (M)	M8	M9	M10	M11
M1	AA1-B1	AA1-B2	AA1-B3	AA1-B4
M2	AA2-B1	AA2-B2	AA2-B3	AA2-B4
M3	AA3-B1	AA3-B2	AA3-B3	AA3-B4
M4	AA4-B1	AA4-B2	AA4-B3	AA4-B4
M5	A2-B1	A2-B2	A2-B3	A2-B4
M6	AA5-B1	AA5-B2	AA5-B3	AA5-B4
M7	AA6-B1	AA6-B2	AA6-B3	AA6-B4

Panel 6. Using an agar cup diffusion method, the antibacterial activity of the eleven hydrazone mixtures in 1% DMSO were tested against *E. coli.* The rings of inhibition as indicated by the yellow and green arrows in the images above indicate that the common hydrazone present in those two mixtures was **A2–B3** (guanofuracin). It can be inferred from these multiple assays that this hydrazone combination exhibits the greatest antibacterial activity. In these assays, mixtures M1–M7 represented the aromatic aldehydes (AA1–AA6, and A2); and mixtures M8–M11 represented the hyrdazines used (B1–B4).

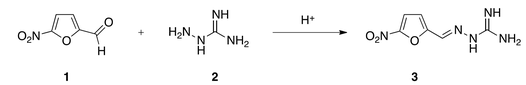
Comparison of Zones of Inhibition



Note: Error bars in the graph above represent the standard error of the mean

Panel 7. A small library of hydrazones from various mixtures of aromatic aldehydes and hydrazines have been synthesized via combinatorial chemistry and screened for antibiotic activity against *E. Coli.* This graph shows the average degree of inhibition and potency of each aldehyde from its respective mixture. Our results indicate that the most active hydrazone is guanofuracin, which is composed of 5-nitro-2-furaldehyde (A2) and aminoguanidine (B3). Our data shows that a 5-nitro substituent in conjunction with the heterocyclic furan aldehyde will be more biologically active than its benzene aldehyde counterparts with various electron-donating or electron-withdrawing groups at the *para*-position (AA1-AA6).

Future Directions



1 **2** **3**

Panel 8. Future research will involve the screening of benzaldehyde derivatives with different electron-donating or electron-withdrawing groups. In addition, we plan on synthesizing the most active hydrazone, guanofuracin, via solution-phase synthesis, purifying it via chromatography and screening for antibiotic activity. We can then compare this product to the mixture of guanofuracin in the combinatorial library.

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