Investigating Singly Substituted 2-amino-1,3,4-oxadiazoles as Antibiotic Drug Candidates

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Introduction

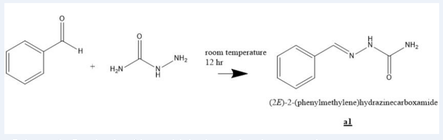
Antibiotic resistance is currently on the rise, as bacteria develop the ability to evade common drugs. Therefore, it is important that synthesis efforts directed towards the development of new antibacterial candidates are continued.

One such candidate is the oxadiazole structure. Oxadiazoles are privileged scaffolds—structures that have been shown to be medicinally active—and they have demonstrated especially promising biological activity against gram positive bacterial strains[1]. In our research, we have synthesized a variety of oxadiazoles from semicarbazones using the 12 Principles of Green Chemistry to make the reactions greener[2]. All of the reactions took place at room temperature and utilized ethyl lactate, water, or acetic acid as the solvent.

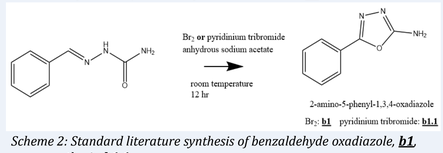
Common synthesis methods for oxadiazoles use Br_2, which is an environmental toxin that is also hazardous for human health[3]. In these syntheses, Br_2 was replaced with pyridinium tribromide, which is much less toxic and therefore makes the reactions much greener[4].

The synthesized oxadiazoles were then tested against the Biosafety Level 1 (BSL 1) gram-positive strain, *Staphylococcus epidermidis*.

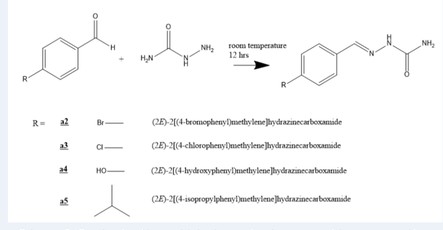
Materials and Methods



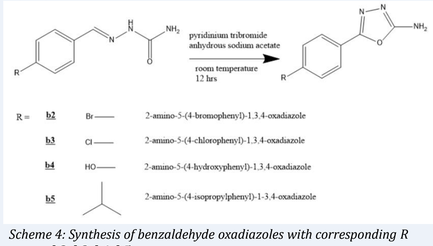
*Scheme 1: Synthesis of benzaldehyde semicarbazone, **a1**.*



*Scheme 2: Standard literature synthesis of benzaldehyde oxadiazole, **b1**, green synthesis, **b1.1**.*



*Scheme 3: Synthesis of benzaldehyde semicarbazones with corresponding R groups **a2**, **a3**, **a4**, **a5**.*



*Scheme 4: Synthesis of benzaldehyde oxadiazoles with corresponding R groups **b2**, **b3**, **b4**, **b5**.*

- IR data collected via the Thermo-Scientific Nicolet iS20 Infrared Spectroscopy machine.
- NMR data collected via JEOL 400 MHz NMR.
- Green semicarbazones synthesized with ethyl lactate and water.
- Standard oxadiazole synthesized with Br_2.
- Green oxadiazoles synthesized with pyridinium tribromide.
- All reactions were run overnight and then vacuum filtered.

Characterization of Products

Compound	Percent Yield (%)	Experimental Melting Point (°C)	Literature Melting Point (°C)
a1	100	199.3 – 200.4	212 – 213
a2	100	207.8 – 210.9	227 – 228
a3	97.84	199.3 – 200.4	202 – 204
a4	100	181.9 – 182.3	186
a5	75.83	182.6 – 186.6	210 – 211
b1	61.17	219 – 220	220 – 222
b1.1	57.37	228 – 229	220 – 222
b2	86.21	253.2 – 260.1	259 – 260
b3	37.37	247.1 – 248.1	243 – 245
b4	52.55	258 – 262	274 – 276
b5	47.51	236 – 239.9	unknown

Table 1: Melting point (experimental and literature) and percent yield data for all synthesized semicarbazones and oxadiazoles.

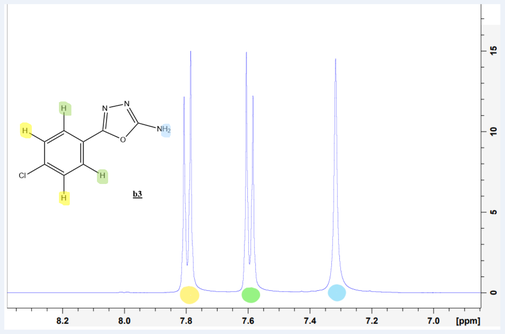


*Figure 1: ^1H NMR spectra for compound **b3**.*

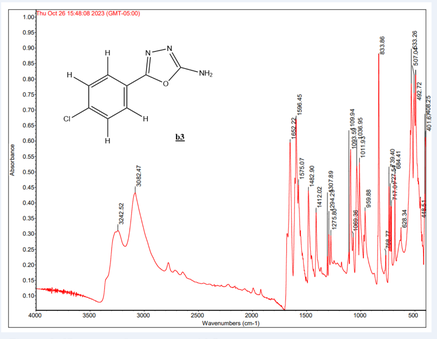


*Figure 2: IR spectra for compound **b3**.*

- Compounds **a1**, **a2**, **a3**, **a4**, **b1**, **b1.1**, **b2**, and **b3** were made successfully, as confirmed by TLC, NMR, and IR.
- Semicarbazones **a1**-**a4** all had IR peaks around 3400, 3000, and 1600, which are the expected peaks for the functional groups of those molecules. **a5** had additional peaks and was likely impure.
- Oxadiazoles **b1**, **b1.1**, **b2**, and **b3** all exhibited the correct IR peaks for the oxadiazole ring, benzene ring, and primary amine. Peaks for **b4** and **b5** were shifted upfield, and there were likely impurities in both of those compounds.
- The melting point for **b4** did not match the literature melting point, further supporting the claim that that sample contains impurities.

Biological Testing

*Figure 3: MIC plate for compounds **b1** and **b1.1**.*

- MIC run against BSL-1 *Staph. epi.* with compounds **b1** – **b5**.
- No oxadiazoles exhibited biological activity against this strain.
- Possibility that thiadiazoles or larger oxadiazoles would have some activity[5].

Compound	Active against Staphylococcus epidermidis?
b1	No
b1.1	No
b2	No
b3	No
b4	No
b5	No

Table 2: Biological activity of each synthesized oxadiazole against BSL-1 strain of Staph. epi., determined via an MIC assay.

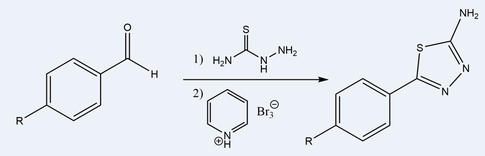
Conclusion

Five semicarbazones were successfully synthesized (**a1**, **a2**, **a3**, **a4**, **a5**) using a green solvent system and while running the reactions at room temperature. We were also successful in replicating a published synthesis of benzaldehyde oxadiazole using the published procedure (**b1**)[3]. Additionally, four oxadiazoles were successfully synthesized (**b1.1**, **b2**, **b3**, **b5**) using the greener method (confirmed by TLC, NMR, and IR).

After running MIC assays with the target compounds against *Staphylococcus epidermidis*, it was determined that the compounds do not have activity against that strain, although there is still promise that larger structures containing the oxadiazole scaffold might have some biological activity.

Future Directions

- Synthesize and purify thiosemicarbazones and thiadiazoles, then test them for antimicrobial activity and COVID-19-to-human cell adhesion interference.



Scheme 5: General reaction of substituted benzaldehyde with 1) thiosemicarbazone and 2) pyridinium tribromide to form substituted thiadiazoles.

References

[1] Boudreau, M.A.; Ding, D.; Meisel, J.E.; Janardhanan, Spink, E.; Peng, Z.; Qian. Y.; Yamaguchi, T.; Testero, S.A.; O'Daniel, P.I.; Leemas, E.; Lastochkin, E.; Song, W.; Schroeder, V.A.; Wolter, W.R.; Suckow, M.A.; Mobashery, S.; Chang, M. Structure—Activity Relationship for the Oxadiazole Class of Antibacterials. *ACS Medicinal Chemistry Letters*. **2019**, *11(3)*, 322-326. Referenced 9/30/2023.

[2] ACS 12 Principles of Green Chemistry. https://www.acs.org/greenchemistry/principles/12-principles-of-green-chemistry.html. Referenced 11/26/2023.

[3] Bansal, R.K.; Bhagchandani, G. Synthesis and Mass Spectral Studies of Some New 1,3,4-Oxadiazoles. *J. Indian Chem.*, **1982**, *59*, 277-279. DOI: https://doi.org/10.1002/chin.198230194. Referenced 11/26/2023.

[4] Pyridinium Tribromide, CAS RN 39416-48-3; SDS No. 133248; Sigma Aldrich, St. Louis, MO, September 13, 2023. https://www.sigmaaldrich.com/US/en/sds/aldrich/133248. Referenced 11/26/2023. Referenced 11/26/2023.

[5] Yadav, V.D.; Boshoff, H.I.; Trifonov, L.; Roma, J.S.O.; Ioerger, T.R.; Barry, C.E.; Oh, S. Synthesis and Structure—Activity Relationships of a New Class of Oxadiazoles Targeting DprE1 as Antitubercular Agents. *ACS Medicinal Chemistry Letters*, **2023**, *14* (9), 1275-1283. DOI: https://doi.org/10.1021/acsmedchemlett.3c00295. Referenced 10/15/2023.

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