Novel Anticancer Drugs on the Basis of Diversely Functionalized N-Containing Heterocycles

Kailee Zingler1,2, Liliya Frolova1, Snezna Rogelj2

1 New Mexico Tech, Department of Chemistry, Jones Hall 259, 801 Leroy Place, Socorro, NM 87801

2 New Mexico Tech, Department of Biology, 801 Leroy Place, Jones Annex, Socorro, NM 87801

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Abstract

Corresponding pyrroline and pyrrole precursors of the Rigidin 35 compound were synthesized and isolated , with moderate yields. The new compounds were tested against cervical adenocarcinoma HeLa cells. The compounds exhibit submircromolar antiproliferative activity. Pyrrole and Rigidin 35 show similar killing effects against HeLa cells. Correspondent pyrrolines show much stronger toxicity in high concentrations.

Background and Significance

- Medicinal Chemistry is the science that deals with the discovery and design of new therapeutic chemicals and their development into useful medicines.
- Cancer is a family of diseases in which diseased cells multiply without control.
- The diseased cells can form tumors when they multiply uncontrollably in one area and can travel.
- 14 million people worldwide each year suffer from one of the more than 200 forms of cancer.
- This project focused on synthesizing intermediates of a reaction that created a cancer treating drug, Rigidin 35.

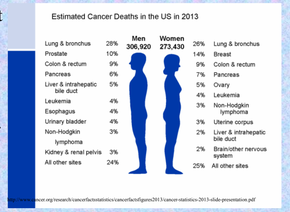


Fig. 1. The statistics for cancer in the United States in 2013, organized by gender.

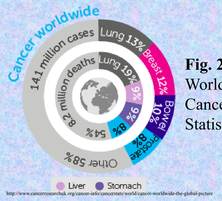


Fig. 2. Worldwide Cancer Statistics

- The Rigidin family was synthesized through novel methods.
- The Rigidin family exhibited submicromolar to nanomolar antiproliferative potencies against a panel of cell lines including in vitro models for drug-resistant tumors, such as glioblastoma and melanoma.
- A selected representative (Rigidin 35) was found to inhibit microtubule dynamics in cancer cells, lending evidence for tubulin targeting as a mode of action for these compounds in cancer cells.
- The impressive killing of cancer cells with Rigidin 35 led to the inquiry about the intermediates of the reaction. This led to this project focusing on synthesizing and testing pyrroles and pyrrolines of the Rigidin 35 reaction.

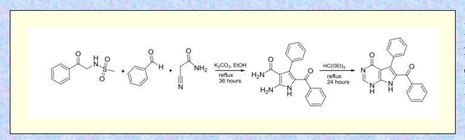


Fig. 3. The reaction used to create the Rigidin 35.

Materials and methods

The first step in the synthesis of Rigidin 35 derivatives is to synthesize correspondent sulfamide. A reaction with methane sulfonyl hydrochloride and acetaphenone hydrochloride each reacting with about two equivalent of triethylamine over ice for four hours. Structure was confirmed by 1H NMR.

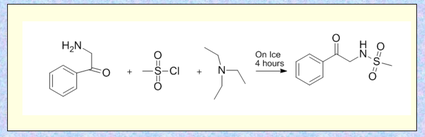


Fig. 4. The reaction scheme of synthesis of sulfamide.

The reaction scheme for the pyrroline derivatives is driven by a multicomponent process involving the correspondent sulfamide, benzaldehyde, and either cyanoacetamide, modelled after Rigidin 35, or malonitrile as reagents and a base catalyst. In a one pot reaction, contents were refluxed at 180 C for two hours. The product precipitated out and filtered off using ethanol and ether. Structure was confirmed by 1H NMR.

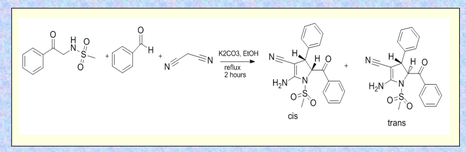


Fig. 5. The reaction scheme for malonitrile pyrrolines

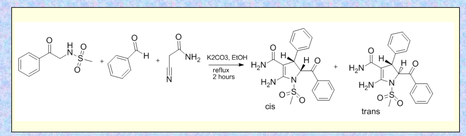


Fig. 6. The reaction scheme for cyanoacetamide pyrrolines

After the synthesis of pyrrolines proved successful, the synthesis of the pyrroles was attempted. The same reaction scheme was used, but this reaction ran for 36 hours. It was successful for cyanoacetamide not for malonitrile.

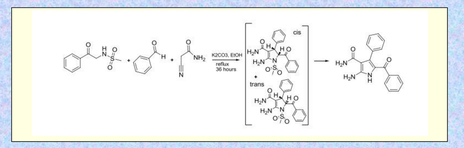


Fig. 7. The reaction scheme for cyanoacetamide pyrroles

Results

The yields of the reactions were calculated in order to determine the success of the reactions: $PercentageYield = \frac{ActualYield}{TheoreticalYield} \times 100\%$

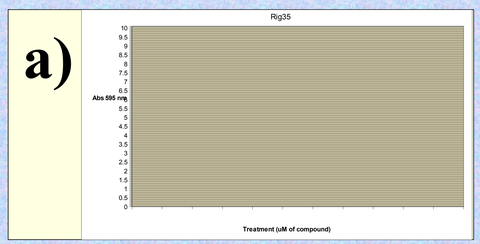
$$\%YieldPyrroline35 = \frac{15mg}{115mg} \times 100\% = 13\%yield$$

$$\%YieldMalonitrilePyrroline = \frac{36mg}{110mg} \times 100\% = 33\%yield$$

$$\%YieldPyrrole35 = 62\%$$

$$\%YieldRigidin35 = 71\%$$

Toxicity was tested against HeLa Cells which were seeded at a density of ≈4,000cells/well in a 96 well plate. Cells were treated with the drugs for 48 hours. Cell viability was tested with MTT assays. The testing was done to determine the GI50. The GI50 is the concentration for 50% of maximal inhibition of cell proliferation (smaller GI50 is best).



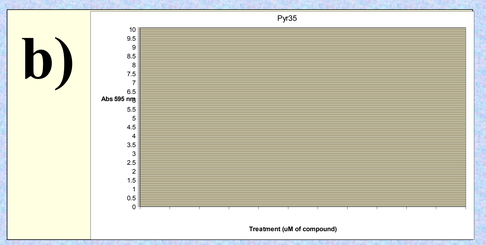
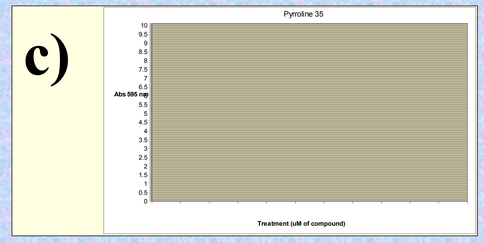


Fig. 8(a-b). 8a: Rig35, GI50=0.237uM; 8b: Pyr35, GI50 =19.579uM.



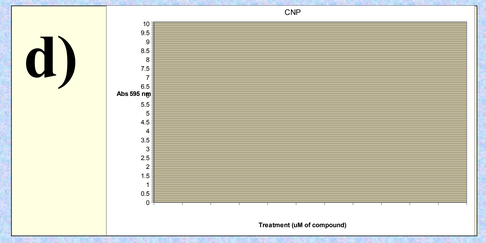


Fig. 8(c-d). 8c: Pyrroline35, the cyanoacetamide pyrroline GI50=13.654uM; 8d: CNP, the malonitrile pyrroline GI50>100uM.

These graphs show the clear differences in activity between these similar compounds. The Rigidin 35 has an excellent GI50 and kills at nanomolar concentrations. The pyrrole 35 has a good GI50 and shows a cytotoxic trend, rather similar to Rigidin 35. The pyrroline 35 shows the best minimum inhibitory concentration (MIC) at fifty micromolar, the drug kills completely, and has an impressive GI50. Malonitrile pyrroline has the lowest killing graph and the highest GI50.

Conclusions & Future Work

- A new multicomponent synthesis for precursors of Rigidin 35 was developed on the basis of a one pot synthesis using sulfamide and inoganic base catalysts.
- It is interesting that the cynanoacetamide pyrrolines kills completely and with little amounts of drug, where the malonitrile pyrrolines did not kill at all.
- The reaction of both pyrrolines was successful and with poor yields in comparison to the known yields of Rigidin 35 and the correspondent pyrroles. Most likely due to imperfections in the current synthetic scheme.
- The reaction of the malonitrile pyrrole was not successful.
- Future work for this project will involve cyclizing the pyrrolines and testing for biological activity. Cyclizing them should improve their killing of cancer cells. The synthesis of malonitrile pyrroles will be attempted again and also tested for biological activity.

References

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2. Magedov, Igor, and et. all. "Novel three-component synthesis and antiproliferative properties of diversly functionalized pyrrolines." *Bioorganic & Medicinal Chemistry Letters* 18 (2008): 1392-1396. Print.

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