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

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Abstract

"Corresponding pyrroline and pyrrole precursors of Rigidin 35 compound were synthesized and isolated with moderate yields. The new compounds were tested against different cancer cell lines. The compounds exhibit submicromolar antiproliferative activity. Pyrrole and Rigidin 35 show similar killing effects against cancer cells. Correspondent pyrrolines and pyrrolopyrimidines have cytostatic plato and show much stronger toxicity in high concentrations."

Background and Significance

➢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. These tumors can travel away from the area of incidence causing a disruption of multiple, vital physiological mechanisms.
➢14 million people worldwide each year suffer from one of the more than 200 forms of cancer.

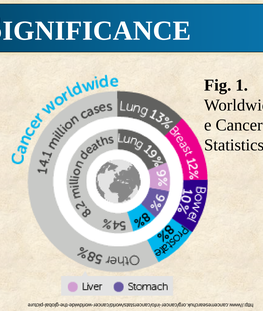

Fig. 1. Worldwide Cancer Statistics

➢The focus of this project was to synthesize intermediates of an existing reaction that created a cancer treating drug, Rigidin 35, in order to improve upon the properties and efficacy of the drug.

➢The Rigidin family was synthesized through novel methods.
➢The Rigidin family exhibited submicromolar to nanomolar antiproliferative potencies against a panel of cell lines including metastatic cell lines and cells lines with dismal prognosis.
➢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.

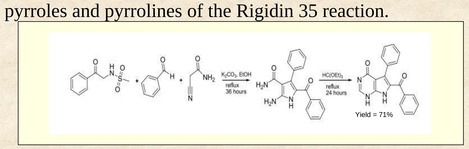

Fig. 2. The reaction scheme used to synthesize Rigidin 35.

Acknowledgements

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References

1. Scott, Robert, and et. all. "Synthetic and Biological Studies of Tubulin Targeting C2-Substituted 7-Deazahypoxanthines Derived from Marine Alkaloid Rigidins." *ChemPubSoc* 9 (2014): 1-10. Print.
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.

Materials and Methods

Rigidin 35 precursors are synthesized from a correspondent sulfonamide. A reaction with methane sulfonyl hydrochloride or 4-methoxyphenyl sulfonyl chloride and acetaphenone hydrochloride each reacting with two equivalents of triethylamine over ice for four hours. Structure was confirmed by ^{1}H NMR.

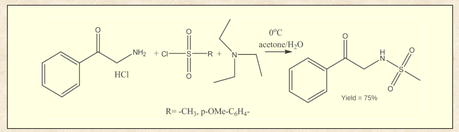


Fig. 3. The reaction scheme of sulfonamide.

Reaction schemes for the pyrroline precursors are driven by a multicomponent process involving the correspondent sulfonamide, benzaldehyde, and either cyanoacetamide, modelled after Rigidin 35, or malonitrile as reagents along with triethylamine as the catalyst. These are one pot reactions, contents were refluxed overnight. The products were separated via column chromatography with hexanes and ethyl acetate. Structures were confirmed by ^{1}H NMR.

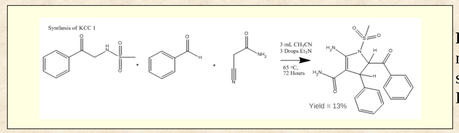


Fig. 4. The reaction scheme for KCC1

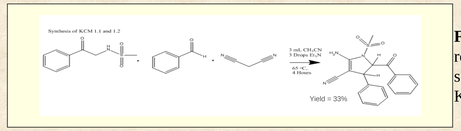


Fig. 5. The reaction scheme for KCM1.1&1.2

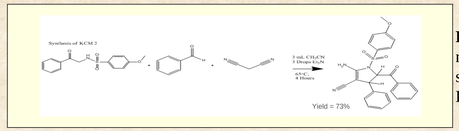


Fig. 6. The reaction scheme for KCM2

The malonitrile pyrrolines were successfully oxidized and cyclized. The oxidation was conducted with DDQ. The cyclizations were conducted with formic acid.

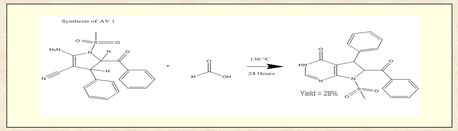


Fig. 7. The reaction scheme of synthesis AV1

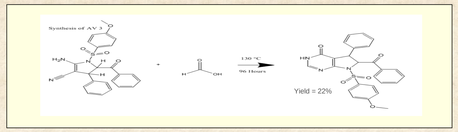


Fig. 8. The reaction scheme of synthesis of AV3

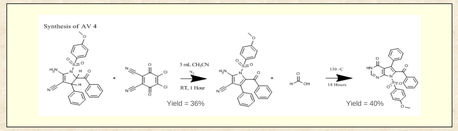


Fig. 9. The reaction scheme of synthesis of AV4

Biological Testing: Toxicity was tested against cervical cancer cell line HeLa 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 concentration for 50% of maximal inhibition of cell proliferation.

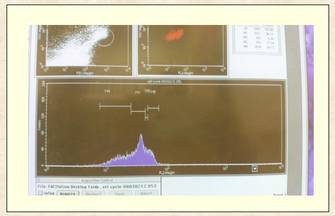
Results

Table 1: GI50s for drugs on HeLa Cells

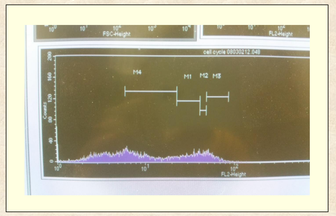
Name	GI50(μM) on HeLa
Rigidin 35	0.02372
Pyrrole 35	19.58
KCC1: Pyrroline 35	13.6542
KCM1.1: isomer 1	>100
KCM1.2: isomer 2	>100
KCM2	43.46
AV1	1.2581
AV3	12.72
AV4	92.19

Fig. 10(a-b): Cell Cycle Analysis via Flow Cytometry

10a: Control

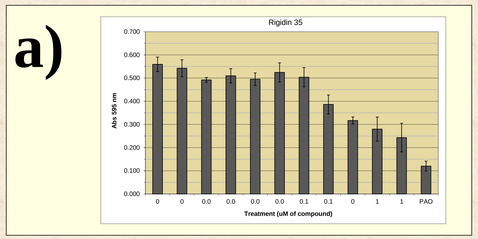


10b: 5μM AV1 Treated



In figures 10(a-b) above are the cell cycle graphs of Jurkat cells treated with 5μM of AV1. The M1 peak is smaller compared to the M3 peak showing that the drug pushed the cells into G2/M. It is important to note a lot of cell death occurs at this concentration and this drug is a tubulin binder leading to deformity

In figures 11(a-d) below are the GI50 graphs of the most active compounds.



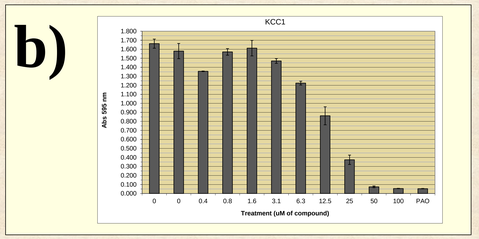
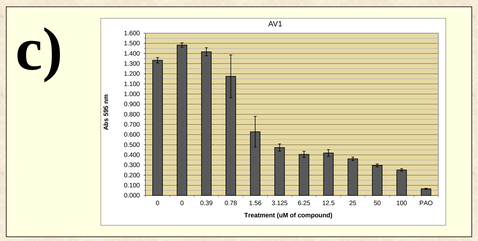


Fig. 11(a-b). 11a**:** Rigidin 35 11b: KCC1



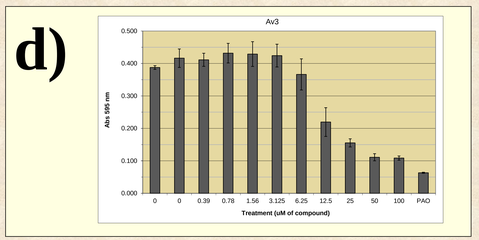


Fig. 11(c-d). 11c: AV1 11d: AV3

These graphs show the clear differences in activity between the structurally very similar compounds. The Rigidin 35 has an excellent GI50 and kills at nanomolar concentrations. The pyrroline based directly on Rigidin, KCC1, has a good GI50, a cytostatic trend similar to Rigidin 35, and kills completely. The cyclized form of the malonitrile based pyrroline, AV1, also shows cytostatic activity and with an even better GI50 than KCC1. The cyclized form of the malonitrile methoxy phenyl pyrroline also has cytostatic activity and with a fairly good GI50.

Conclusions & Future Work

➢ A new multicomponent synthesis for precursors of Rigidin 35 and their penta substituted analogs was developed on the basis of a one pot synthesis using correspondent sulfonamide with organic base catalysts.
➢The reaction of malonitrile pyrrolines and derivatives thereof has been much more successful than that of the cyanoacetamide pyrroline intermediates of the Rigidin 35 reaction.
➢Only one isomer of the malonitrile pyrrolines was able to be cyclized into pyrrolinopyrimidine using formic acid directly, which lead to the first step being oxidation to pyrroles before cyclization, fixing the problem.
➢Antiproliferative activity of all compounds was tested. Oxidation of pyrrolines into pyrroles and cyclization increased activity.
➢Future work for this project will include SAR for pyrrolines, correspondent pyrroles and the products of their cyclization.
➢Perfecting a reaction scheme for the cyanoacetamide pyrrolines will also be created.