Synthesis and Biological Evaluation of Podophyllotoxin Analogues as Potential Chemotherapeutic Agents

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Can we synthesize Aza-Podophyllotoxin derivatives with high potency and selectivity for the treatment of colon cancer?

Introduction

Podophyllotoxin is a natural aryltetralin lignan compound isolated from the rhizomes or leaves of plants within the genus Podophyllum (*P. emodi* and *P. peltatum* respectively). Podophyllotoxin has been under extensive biochemical investigations since the discovery of its biological activity as a strong microtubule destabilizing agent during mitosis[1]. **Podophyllotoxin derivatives such as *etoposide*, *teniposide* and *etopophos*** have since been produced and utilized as chemotherapeutic agents acting on topisomerase II inhibition. Although these antineoplastic compounds have shown significant activity as cancerous cell growth inhibitors (large and small lung, testicular, stomach and pancreatic cancers)[2], they lack selectivity and are thus extremely toxic to healthy cells. This has led to a recent interest in the synthesis of Podophyllotoxin analogues **in hopes of optimizing the biological selectivity and potency of the semi-synthetic** derivatives already on the market. **Our research efforts are directed towards synthesizing a derivative that effectively targets and treats specific colorectal cancers.** Recent studies have shown that the progression of many colorectal cancers is linked to the secondary **up-regulation event of the KRas and WNT oncogenic genes of tumor cells[3,4]**. ***Our goal*** is to synthesize aza-analogues that selectively inhibit this **KRas-WNT synergistic activation in colorectal tumor cells** via subsequent biological investigations with an assay known as the KRAS synthetic lethal phenotypic module provided by the **Eli Lilly pharmaceutical company**.

Methods

The initial approach utilized for the generation of Aza-analogues was an optimized one-pot synthesis (Figure 1 of Image 1) obtained from previous literature. Investigations were pursued to determine a plausible mechanism in the cascade reaction involving tetronic acid with aldehydes and anilines to generate the Aza-podophyllotoxin core structure. In general, it was found that this reaction preceded through a crucial iminium ion intermediate that when considered, lead to a threefold increase in recorded yields[5]; the proposed mechanism can be observed in Figure 2 of Image 1. This mechanism first involves activation of the aldehyde component via condensation (facilitated by LUMO lowering effect of iminium ion) with an electron deficient "sacrificial aniline" to form an electrophilic iminium ion[5]. Upon condensation of the enolized tetronic acid and the resulting iminium ion, the reaction was hypothesized to proceed through formation of a quinone-like species resulting in regeneration of the sacrificial aniline[5]. The aniline thus serves dual purpose in this synthetic cascade as it was proposed (supported with stereochemical evidence) to further react with the quinone-like species, eventually forming the desired Podophyllotoxin analogue[5]. Subsequent biological evaluation of the prepared library (Image 2) through the KRAS synthetic lethal phenotypic module revealed that five analogues possessed promising activity in comparison to the parent Aza compound. Image 3 displays the results of the KRas-WNT inhibition assay for compound **AH-I-80** and the parent derivative **MH-I-002** (for comparison).

Image 1

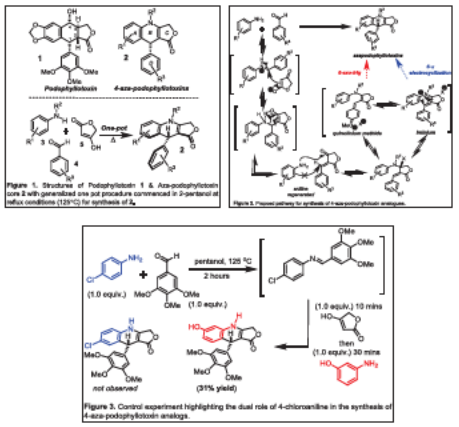


Figure 1. Structures of Podophyllotoxin 1 & Aza-podophyllotoxin core 2 with generalized one pot procedure commenced in 2-pentanol at reflux conditions (125°C) for synthesis of A_n

Figure 3. Control experiment highlighting the dual role of 4-chloroaniline in the synthesis of 4-aza-podophyllotoxin analogs.

Image 2

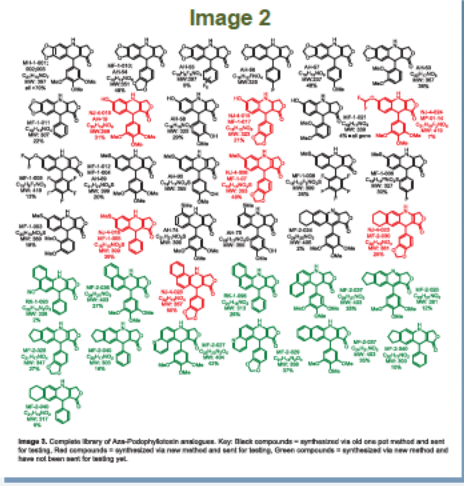
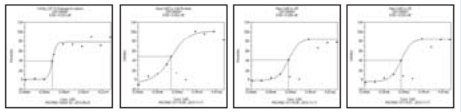


Image 2. Complete library of Aza-Podophyllotoxin analogues. Key: Black compounds = synthesized via old one pot method and sent for testing, Red compounds = synthesized via new method and sent for testing, Green compounds = synthesized via new method and have not been sent for testing yet.

Image 3

KRAS Synthetic Lethal Phenotypic Module Results:

Parent compound MH-I-002 assay results:



Compound AH-I-80 assay results:

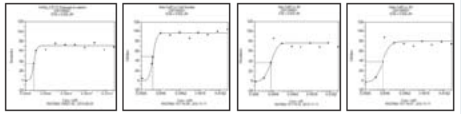
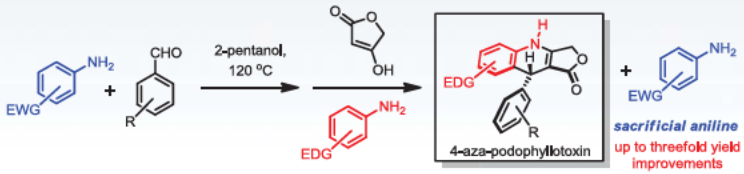


Image 4



Results

Compounds **AH-I-80, AH-I-90, AH-I-89 (MF-1-004, MF-1-012), MF-1-005 (NJ-4-016), and MF-1-014 (NJ-4-024)** showed exceptional inhibitory activity of the synergistic KRas-WNT pathway. It can be seen from Image 3 that analogue **AH-I-80** displays almost synonymous activity to the parent compound (**MH-I-002**) at significantly lower concentrations. The observed half maximal inhibitory concentration (IC_{50}) of **AH-I-80** ranges from 0.002 μM – 0.004 μM while the parent compounds displays an IC_{50} range from 22 nM to 36 nM. The other active compounds displayed relatively important inhibitory activity but at slightly higher or similar concentrations than that of the parent compound.

Discussion

The KRAS synthetic lethal phenotypic module conclusions exhibit favorable results for compound **AH-I-80**. Podophyllotoxin and its analogues are potent anticancer agents that lack selectivity and thus, are toxic to healthy host cells. The IC_{50} range of this compound (2 nM – 4 nM) reveals that **AH-I-80** is about 10 times more potent than the parent compound with slightly lower inhibitory activity. Thus, a lower dose of compound **AH-I-80** would yield similar activity to **MH-I-002** towards inhibition of the KRas-WNT expression pathways in colorectal tumor cells. As a result of this, less healthy host cells would be effected by this activity and the overall toxicity of the compound would be notably less than that of the parent compound. These results are extremely remarkable and provide insight into possible chemotherapeutic applications of this compound. Although these results did not display the intended increase in selectivity towards cancerous cells, this increase in potency is extremely valuable for the design of a second generation compound-library. In conclusion, **over 30 novel Aza-podophyllotoxin derivatives** have been synthesized and several lead compounds have been determined to possess significant anticancer activity with increased potency in comparison to the parent (reference) compound which in the past has displayed superior activity. Not only has this research provided positive results for future applications but it has also provided me during my undergraduate experience with a vast amount of knowledge and exposure to modern chemical and biological research.

References

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