Investigation of New Metal-Binding Groups For Histone Deacetylase Inhibitors

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Abstract

Histone deacetylases (HDAC) are enzymes that are involved in the regulation of transcription of many genes, such as those that regulate the growth of some cancers. With four compounds already being approved for clinical use by the FDA, histone deacetylase inhibitors work to regulate HDAC enzymes, and thereby to regulate gene transcription. HDAC inhibitors are made up of a cap group, a metal-binding group, and a linker that connects the two (**Figure 1**).

Figure 1: *An HDAC inhibitor essentially consists of three important parts: a cap group, a metal-binding group that binds to the Zn^{2+} ion in the active site of the enzyme, and a linker that connects the two.*

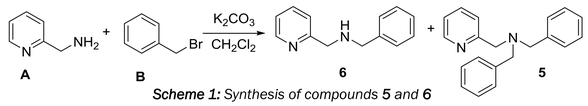
All four HDAC inhibitors in current use are pan-inhibitors. They are not selective and target most HDAC isoforms rather than just a few. This can cause many undesirable side effects. To overcome this problem, it was proposed that new HDAC inhibitors with different metal-binding groups could be synthesized that would allow for more selectivity.

The goal of this project was to use UV and NMR spectroscopy to analyze the binding ability of several metal-binding groups. Some of these compounds were commercially available and others were synthesized in the lab. The compounds identified as having significant binding ability to zinc will be attached to a linker and a cap group to develop new HDAC inhibitors.

Synthesis

Scheme 1

To a mixture of compound **A** (714 µL, 6.93 mmol) and potassium carbonate (638 mg, 4.62 mmol) in dichloromethane (10 mL), compound **B** (275 µL, 2.31 mmol) was added dropwise via a pressure-equalizing dropping funnel. The reaction mixture was stirred at room temperature overnight. The resulting orange-colored reaction mixture was treated with water (10 mL) and extracted with dichloromethane (2 x 10 mL). The DCM extract was concentrated and the residue was purified by chromatography on silica gel in 5-10% methanol/dichloromethane. Compound **6** was isolated as a yellow oil (121.0 mg, 26%) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.59 (d, 1H, *J* =4.38 Hz), 7.67 (m, 1H), 7.39 (d, 3H, *J* =7.14 Hz), 7.35 (t, 3H, *J* =7.5 Hz), 7.20 (m, 1H), 3.96 (s,2H), 3.88 (s,2H), followed by the tertiary amine, compound **5**, (490.0mg) as an orange oil.



Scheme 1: *Synthesis of compounds **5** and **6***

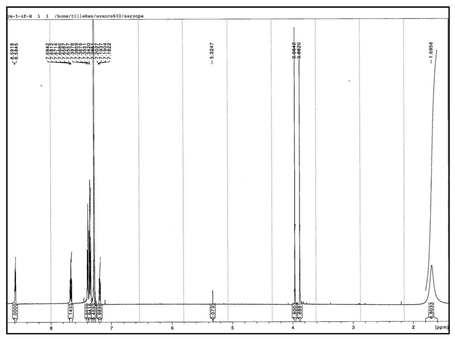
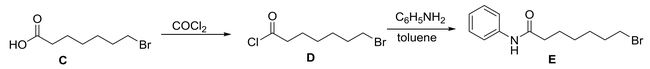


Figure 2: *^{1}H NMR spectrum of compound **6***



Scheme 2: *Synthesis of compound **E***

Scheme 2

A mixture of compound **C** (500 mg, 2.39 mmol) and oxalyl chloride (208 µL, 2.39 mmol) was refluxed at 65°C for 2 hours. The reaction mixture was concentrated on a rotary evaporator to remove any excess oxalyl chloride. Aniline (262 µL, 2.87 mmol) and toluene (4 mL) were added and the reaction mixture was stirred overnight at room temperature. The solid product was collected by vacuum filtration and washed with 20 mL of ethyl acetate. Recrystallization (ethyl acetate/hexanes) then produced orange crystals (337.16 mg, 49.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 2H, J=7.2 Hz), 7.33 (t, 1H, J=7.6 Hz), 7.12 (t, 2H, J=7.6 Hz), 3.43 (t, 2H, J=6.8 Hz), 2.38 (t, 2H, J=7.2 Hz), 1.89 (t, 2H, J=7.2 Hz), 1.77 (t, 2H, J=7.2 Hz).

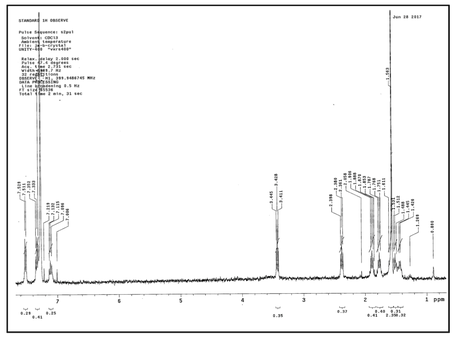
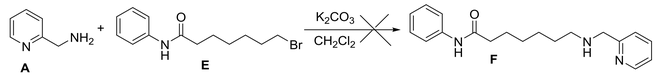


Figure 3: *^{1}H NMR spectrum of compound **E***

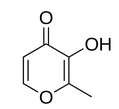


Scheme 3: *Synthesis of compound **F***

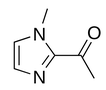
Scheme 3

To a mixture of compound **A** (172 µL, 1.67 mmol), potassium carbonate (53 mg, 1.11 mmol) and dichloromethane (5 mL) was added a solution of compound **E** (150 mg, 0.555 mmol) in dichloromethane (5 mL) via a pressure-equalizing dropping funnel. The reaction mixture was stirred at room temperature overnight. TLC monitoring and ^{1}H NMR spectroscopy confirmed that there was no reaction.

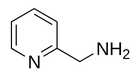
Metal-Binding Studies



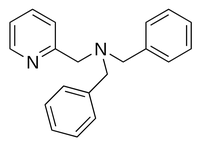
Compound **2**



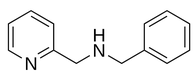
Compound **3**



Compound **4**



Compound **5**



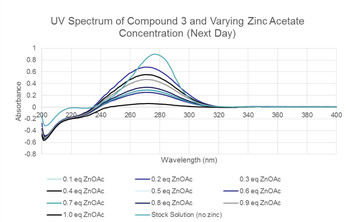
Compound **6**

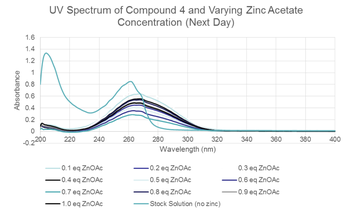
Experimental

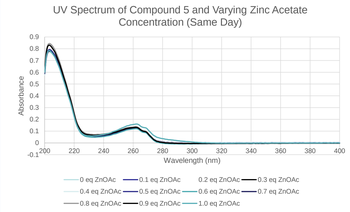
Stock solutions were made in a 50 mL volumetric flask containing each compound dissolved in methanol. The stock solution for compound **2** was 3.01 mM, compound **3** was 3.87 mM, compound **4** was 2.59 mM, compound **5** was 0.628 mM, and compound **6** was 0.717 mM. A stock solution was made containing zinc acetate at the same concentration as each compound. Mixtures of 2.5 mL of the test solution and different volumes of the zinc acetate solutions ranging from 0 mL – 2.5 mL (increasing by 0.25 mL each time) were made in 25 mL volumetric flasks. These solutions were diluted to 25 mL, giving final test solutions with 1/10th the concentration of the stock solution of each test compound and concentrations of the zinc acetate varying from 0.1 – 1.0 molar equivalents. After at least 1 hour, the UV spectra of the final test solutions were recorded using a UV spectrophotometer. Compound **2** was then studied using NMR spectroscopy by dissolving the compound in dimethyl sulfoxide and adding increasing amounts of zinc acetate.

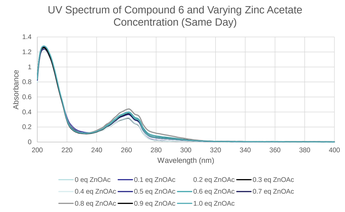
Results

All compounds showed change in UV absorbance with change in zinc acetate concentration. Compound **2** showed a strong correlation between UV peak absorbance and molar equivalents of zinc acetate.

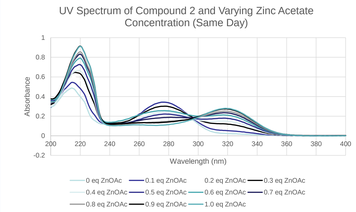


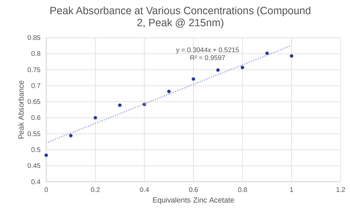






As compound **2** showed a strong correlation between peak absorbance and molar equivalents of zinc acetate, it was also studied using NMR spectroscopy. ^{1}H NMR spectroscopy results showed further evidence of zinc binding with the compound.





Conclusion

The goal of the project was to find new metal-binding groups to be incorporated into HDAC inhibitors to improve selectivity. For this purpose, a list of potential new metal-binding groups was compiled and several were selected for testing. Most of them were commercially available, while a few had to be synthesized in the lab.

The compounds were synthesized, purified using various chromatography techniques, and structures were confirmed by NMR spectroscopy. Compound **6** was successfully obtained along with its tertiary amine, compound **5**. They were both obtained in sufficient amounts to use in the zinc-binding studies. For the synthesis of compound **F**, intermediate **E** was produced successfully. Initial attempt to convert it to the final product **F** was not successful. It needs further investigation.

The synthesized and commercially available metal-binding groups were analyzed for zinc-binding ability using UV and NMR spectroscopy. All compounds showed change in UV absorbance with change in zinc ion concentration to varying degrees, and in the order **2** > **3**> **4**> **6** >**5**. Only compound **2** showed a correlation between UV absorbance and zinc ion concentration. The information obtained during this research project can be used to design new HDAC inhibitors that improve the current understanding of them and hopefully improve their performance as drugs.

Acknowledgments

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