countless hours and money put towards research, not many advancements have been made in terms of treatment options for patients with GBM, and it remains incurable. The current treatments available include surgically removing the tumor from the brain, chemotherapy, and radiation. Although these options can prolong the lifespan of a patient with GBM, the life expectancy for people diagnosed with glioblastoma is around 15 to 16 months (3). Any progress towards curable treatments will save hundreds of thousands of lives.

C-C chemokine receptor 1 (CCR1), is a member of the \( \beta \)-chemokine receptor family of proteins and is found on the surface of macrophages. It is a seven-transmembrane receptor, also known as a G protein-coupled receptor, that controls chemotaxis, the movement of cells in response to chemical stimuli. Inhibiting these receptors could retard the growth of the malignant tumor by inhibiting chemotaxis. This research aims to synthesize potential CCR1 antagonists by coupling carboxylic acids with a triazole core. We synthesized these compounds using a simple carboxylic acid coupling and confirmed the identity of the final compounds using nuclear magnetic resonance (NMR) spectroscopy. Further testing of these compounds in inhibition bioassays is planned to be done when lab space becomes accessible again.

**INTRODUCTION**

Glioblastoma multiforme (GBM) is one of the most aggressive and malignant types of tumors of the central nervous system. This tumor spreads primarily through metastasis, which is generally the case with malignant tumors. Through metastasis, cancer cells dissociate from the primary tumor, move through the lymphatic or circulatory systems, and aggregate in other regions of the body (1). In the instance of GBM, tumor invasion is generally restricted to the brain. However, the tumor can spread throughout the brain via the corpus callosum which can eventually lead to new tumors throughout the brain (2).

Due to the lethality of GBM, there has been a dramatic increase in interest in this research. Currently, scientists are working to examine many aspects of the physiology of glioblastoma and its associated cells in the hope of finding a new drug target to either improve treatment options or cure this disease.

Two-to-three adults per every 100,000 are diagnosed with glioblastoma each year (2). This type of cancer also accounts for 52% of all primary brain tumors. GBM makes up 15% of all primary brain tumors and primarily occurs in adults between the ages of 45 and 70 (2). Even with the countless hours and money put towards research, not many advancements have been made in terms of treatment options for patients with GBM, and it remains incurable. The current treatments available include surgically removing the tumor from the brain, chemotherapy, and radiation. Although these options can prolong the lifespan of a patient with GBM, the life expectancy for people diagnosed with glioblastoma is around 15 to 16 months (3). Any progress towards curable treatments will save hundreds of thousands of lives.

C-C chemokine receptor 1 (CCR1), is a member of the \( \beta \)-chemokine receptor family of proteins and is found on the surface of macrophages. It is a seven-transmembrane receptor, also known as a G protein-coupled receptor, that controls chemotaxis, the movement of cells in response to a chemical stimulus. Macrophages appear to play a role in the spread of cancers (4), and this mechanism is not fully understood. Due to CCR1 involvement in chemotaxis, it is possible that the inhibition of CCR1 could potentially reduce the spread of glioblastoma. CCR1 was utilized as a potential therapeutic target for a drug designed to treat rheumatoid arthritis, but this was not successful in human trials (4). CCR1 shows promise as a target for novel compounds designed to treat glioblastoma. By making modifications to CCR1 antagonists, creating a brain-penetrating compound to treat glioblastoma is feasible. Crossing the blood brain barrier is essential to the success of these compounds. However, synthesis of compounds that cross the barrier can be a challenge due to the barrier being highly selective.

Ideally, our CCR1 antagonists should follow Lipinski’s Rule and fall into Egan’s Egg: guidelines that medicinal chemists use to prioritize successful compounds. Lipinski’s Rule of Five describes characteristics that may increase the likelihood that a compound is orally bioavailable (i.e., acts on its target in the desired way when consumed orally). The rule states that an orally active drug violates no more than one of the following criteria: that there are no more than five hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight of less than 500 g/mol, and an octanol-water partition coefficient (ClogP) not greater than 5 in a compound (5). Furthermore, Egan’s Egg refers to an oval-shaped area on a graph plotting the partition coefficient (LogP) versus polar surface area (PSA). Most orally administered
drugs lie within this region—as it provides a model for how well-absorbed into the body the compound would be (6).

This research aims to synthesize new CCR1 antagonists by using Lipinski’s Rules and Egan’s Egg as guidelines for designing compound structures. We coupled carboxylic acids with a triazole core and an amide core, purified the compounds and confirmed their structures with nuclear magnetic resonance (NMR) spectroscopy. We were successful in synthesis and characterization of four novel compounds and future directions include testing these compounds in inhibition assays to confirm their potency.

RESULTS

Previous work showed that amide and triazole scaffolds yield some of the most potent CCR1 antagonists (7, 9). Therefore, we used these scaffolds, shown in Figures 1 and 2, to create novel compounds that could inhibit GBM metastasis. We created new CCR1 antagonists by attaching different carboxylic acids to a triazole base and an amide base, with the goal of improving their compound potency and physical/chemical properties. The synthesis of these compounds was simple, a common carboxylic acid coupling to our starting material which contained either an amide or triazole. The compounds were analyzed and confirmed by NMR.

We made all compounds successfully, and each had a percent yield of 60% or more (Table 1). They follow the criteria for Lipinski’s Rule of Five, with the only exception being that the ClogP for JARSUPAJ-115-29 is greater than 5.

We generated NMR spectra of each compound to confirm the compound was successfully synthesized. Table 2 includes key NMR shifts for each compound. All compounds generated NMR spectra as expected based on their structures (Figure 3). No significant impurity peaks were detected.

DISCUSSION

We created new potential CCR1 antagonists by attaching different carboxylic acids to a triazole base and an amide base with the goal of improving their compound and physical/chemical properties to better fit Lipinski’s rules and Egan’s Egg. The compounds were analyzed and confirmed by NMR. Through NMR testing, we confirmed that the expected compounds were produced as the result of each synthesis. Purity was to be tested by HPLC; however, we were unable to do so due to Covid-19 school closures. The NMR analysis showed that there was little contamination in the compounds and that they can be considered relatively pure.

Our compounds follow the criteria for Lipinski’s Rule of 5 and Egan’s Egg, with the only exception being that the ClogP for JARSUPAJ-115-29 is greater than 5. These properties were measured computationally via ChemDraw. The NMR confirmed the successful synthesis of each compound, with each compound having a greater than 60% yield. These compounds will be sent for further testing in a CCR1 binding assay to determine potency after HPLC is used to determine a more precise purity. If successful, these compounds could inhibit the CCR1 receptor and in turn be used as treatment for the debilitating disease of glioblastoma. To be used as drugs,
these compounds must go through further bioassay studies, pharmacokinetic studies (PK), absorption, distribution, metabolism, and excretion (ADME) studies, as well as animal testing and all phases of clinical trials. In the case that our compound does not inhibit the CCR1 receptor, our research would still greatly inform next steps taken to synthesize more potent compounds by providing us with more data on what r-groups work on our base scaffolds and which r-groups do not.

MATERIALS AND METHODS

To prepare a triazole core (Figure 1), a mixture of Boc-D-Pro (1 equiv.) and 4- chlorobenzylamine (1.1 equiv.) in dimethylformamide (DMF) (30 mL) was cooled to 0°C using an ice bath and stirred. To the reaction mixture, HBTU (1 equiv.) and DIEA (2 equiv.) were added, dropwise, and the reaction mixture was then stirred overnight at room temperature using a magnetic stir plate. Then we dissolved the reaction in ethyl acetate (EtOAc, 150 mL), washed with 1M HCl (2x 50 mL), saturated aqueous sodium bicarbonate (2x 50 mL), and brine, then dried over sodium sulfate and evaporated. The intermediate was purified by ISCO column chromatography, eluted with 0→100% Hexanes (Hex) / Ethyl Acetate (EtOAc).

We used NMR spectroscopy to confirm the presence of the desired product. The purified product was mixed with Lawesson's reagent (0.5 equiv.) in 100 mL of toluene and refluxed under argon for 2 hours before cooling to room temperature. The reaction mixture was dissolved in EtOAc (100 mL), washed with 1N HCl (2x 50 mL), dried over sodium sulfate, and evaporated. This intermediate was then purified by ISCO column chromatography, eluted with 0→100% Hex/EtOAc. NMR was used to confirm the presence of the desired product. The purified product and acetic hydrazide (2 equiv.) were dissolved in acetonitrile (10 mL). To the reaction mixture was added mercury (II) acetate (1.5 equiv.), and the solution was stirred at room temperature overnight. The reaction mixture was filtered using a Celite-fritted filter and washed with acetonitrile. The product was evaporated and purified by ISCO column chromatography, eluted with 0→100% Hex/EtOAc. NMR was used to confirm the presence of the desired product. The purified Boc-protected compound was dissolved in 4N HCl and dioxane and stirred at room temperature for 30 minutes. The solvent was evaporated to produce the triazole core. A solution of the triazole core (55 mg, 0.2 mmol) in dichloromethane (DCM) (2 mL) was treated with EDC (46 mg, 0.24 mmol, 1.2 equiv.) followed by the addition of hydroxybenzotriazole (HOBt) (40 mg, 0.3 mmol, 1.5 equiv.), DIEA (81 μL, 0.46 mmol, 2.3 equiv.), and the carboxylic acid.

R-group (1.5 equiv.). The reaction mixture was stirred at room temperature overnight. The product was taken up in DCM (20 mL) and washed with water (20 mL). The organic phase was recovered, dried over sodium sulfate, and evaporated. After evaporation of the solvent, the residue was purified by flash column chromatography on a 4-gram silica gel column using 0→15% MeOH/DCM as eluent to yield the desired product as an amorphous solid. NMR spectroscopy was used to confirm the presence of the desired product and purity.

A solution of the amide core (synthesis shown in Merritt et al (7)) (50 mg, 0.2 mmol) in DCM (2 mL) was treated with EDC (46 mg, 0.24mmol, 1.2 equiv.). This was followed by the addition of HOBr (40 mg, 0.3 mmol, 1.5 equiv.), DIEA (81 μL, 0.46 mmol, 2.3 equiv.), and the carboxylic acid R-group (0.3 mmol, 1.5 equiv.). The reaction mixture was stirred at room temperature for 2 days. The organic phase was extracted with

### Table 1. Compound properties (compound name, molecular weight, H bond donors, H bond acceptors, ClogP (the measurement of how hydrophobic a compound is and its ability to dissolve in octanol over water (c_{octanol}/c_{water}) - calculated by chemdraw), polarizability - calculated by chemdraw, and yield.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>MW (g/mol)</th>
<th>H-bond Donors</th>
<th>H-bond Acceptors</th>
<th>ClogP</th>
<th>Polarizability</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>JARSU-115-27</td>
<td>497.34</td>
<td>0</td>
<td>5</td>
<td>4.12</td>
<td>48.27</td>
<td>61.1 mg</td>
<td>82.68%</td>
</tr>
<tr>
<td>JARSU-115-29</td>
<td>473.32</td>
<td>0</td>
<td>4</td>
<td>5.52</td>
<td>40.62</td>
<td>61.5 mg</td>
<td>64.97%</td>
</tr>
<tr>
<td>ENKH-115-26</td>
<td>462.90</td>
<td>0</td>
<td>5</td>
<td>3.40</td>
<td>36.74</td>
<td>71.0 mg</td>
<td>61.43%</td>
</tr>
<tr>
<td>ENKH-115-28</td>
<td>438.88</td>
<td>0</td>
<td>4</td>
<td>4.81</td>
<td>40.60</td>
<td>51.8 mg</td>
<td>63.90%</td>
</tr>
</tbody>
</table>

### Table 2. Compounds and key NMR shifts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Key 'H NMR shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>JARSU-115-27</td>
<td>δ 7.58 (m, 1H), 7.33-7.284 (m, 3H), 6.875 (m, 3H), 5.62 (d, 1H), 5.25 (s, 1H), 4.987 (m, 2H), 3.979-3.483 (m, 3H), 2.39 (s, 3H), 2.20-2.0 (m, 4H), 1.98 (m, 1H)</td>
</tr>
<tr>
<td>JARSU-115-29</td>
<td>δ 7.70-7.50 (m, 1H), 7.40-7.20 (m, 2H), 6.99-6.80 (m, 4H), 5.62-5.59 (d, 1H), 5.05-4.95 (m, 3H), 4.00-3.85 (m, 2H), 3.80-3.65 (m, 2H), 2.45-2.20 (s, 3H), 2.19-1.99 (m, 4H)</td>
</tr>
<tr>
<td>ENKH-115-26</td>
<td>δ 7.40-7.19 (m, 3H), 6.99-6.80 (m, 1H), 5.75-5.60 (m, 1H), 5.10-4.85 (m, 3H), 3.95-3.6 (m, 5H), 2.80-2.60 (m, 4H), 2.40-2.30 (m, 3H), 2.20-1.91 (m, 2H)</td>
</tr>
<tr>
<td>EN-115-28</td>
<td>δ 7.362-7.206 (m, 4H), 6.9 (m, 2H), 5.695 (d, 2H), 5.25 (s, 1H), 5.061-4.987 (m, 2H), 3.979-3.483 (m, 3H), 2.4 (s, 3H), 2.05-2.0 (m, 4H), 1.8 (m, 1H)</td>
</tr>
</tbody>
</table>
20 mL DCM and 20 mL deionized water, dried over sodium sulfate, and evaporated. The resulting residue was purified by ISCO, eluted with 0→15% MeOH/DCM to give the desired product. NMR was performed to confirm the presence of the desired product and purity.

We created new CCR1 antagonists by attaching different carboxylic acids to a triazole base and an amide base, with the goal of improving previous compound potency and physical/chemical properties. These compounds were analyzed and confirmed by NMR. Figure 1 depicts the synthesis of starting material as it follows the procedure illustrated. The triazole was cyclized by coupling acetic hydrazide, mercury (II) acetate, acetonitrile and starting material from a previously started reaction. Figure 2 shows the synthesis of compounds following the procedure illustrated. Amide bonds were formed by coupling the pyrrolidine amine group to carboxylic acids using HOBt, EDC, and DIEA. Figure 3 shows the finalized NMRs of the four compounds synthesized. The NMRs were done by a Varian brand NMR at 400 mHz.

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