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INVESTIGATION OF ANTICOAGULATION PROPERTIES OF SULFATED GLYCOSAMINOGLYCAN MIMETICS

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

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Abstract

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The existence of thrombosis in numerous pathophysiological situations formed a vast necessity for anticoagulation therapy. Thrombin and factor Xa are the only two factors of the entire coagulation cascade that have been major targets for regulation of clotting via the direct and indirect mechanism of inhibition. Our recent discovery of sulfated non-saccharide glycosaminoglycan mimetics, especially G2.2, that demonstrates highly selective cancer stem-like cells (CSCs) inhibition activity. G2.2 inhibited the growth of CSCs from multiple cancer cell lines. To evaluate its in vivo anticoagulation effect, we asked a contract research organization (CRO) to produce 20 g of material, labelled as G2.2Y. Evaluation of G2.2C in HT-29 xenograft mouse model showed a significant reduction in tumor volume and CSC markers, but unexpected bleeding consequences in some animals were observed. Also in a tail bleeding experiment, G2.2Y showed a significant enhancement in bleeding volume. Comparable studies with G2.2 synthesized in our laboratory had shown no bleeding effects. To investigate the difference between the two G2.2

samples (G2.2W (white) and G2.2Y (Yellow) that were performed using UPLC-MS characterization, we were able to determine that the G2.2Y sample was an 85:15 blend of two compounds. Elemental, NMR and MS data revealed that G2.2W was fully sulfated flavonoid derivative, as expected, but G2.2Y contained one less sulfate group. We tested both agents for their inhibition of various coagulation factors and revealed that G2.2Y inhibited fXIa nearly 2-fold better in comparison to G2.2W. Furthermore, activated partial thromboplastin time assay (APTT) indicated that G2.2W exhibited almost 3-4-fold less anticoagulant activity compared to G2.2Y. This indicates that the loss of just one sulfate group could induce substantial side effects and lead to a discovery of new anticoagulant agent. Such structure–activity relationship is important to understand if the in vivo metabolism of the agents leads to accumulation of de-sulfated products.

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