STRUCTURAL AND SYNTHETIC STUDIES OF POTENTIAL ANTITUMOR NATURAL PRODUCTS

Chongming Wu

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institution and State University
in the partial fulfillment of the requirement for the degree of

Doctor of Philosophy
in
Chemistry

Dr. David G. I. Kingston, Chair
Dr. Michael Calter
Dr. Neal Castagnoli
Dr. Harry C. Dorn
Dr. Richard Gandour

August 13, 1998
Blacksburg, Virginia

Keywords: natural product, sesquiterpenoid, furanonaphthoquinone, antitumor

Copyright 1998, Chongming Wu
Bioassay directed fractionation of the methyl ethyl ketone extract of *Chiloscyphus rivularis* yielded eight sesquiterpenoids, and detailed spectroscopic interpretation led to the assignment of their structures as 12-hydroxychiloscyphone, chiloscypha-2,7-dione, 12-hydroxychiloscypha-2,7-dione, chiloscypha-2,7,9-trione, rivulalactone, 4-hydroxy oppositant-7-one, chiloscyphone, and intermedeol. The structure and stereochemistry of rivulalactone, a novel trinorsesquiterpenoid, was confirmed by its synthesis starting from chiloscyphone. 12-Hydroxychiloscyphone, chiloscypha-2,7-dione, 12-hydroxychiloscypha-2,7-dione, chiloscypha-2,7,9-trione, rivulalactone are new. 12-Hydroxychiloscyphone showed selective bioactivity towards DNA repair-deficient yeast mutants and cytotoxicity to human lung carcinoma cells.

In order to improve the activity of cytotoxic furanonaphthoquinones by affixing a hydroxyamino side chain, 2-methyl-2-[(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionyl methyl)amino]-1,3-propanediol and its analogs have been synthesized. Bioassay data showed they act by a different mechanism of action than their parental furanonaphthoquinone derivatives.
ACKNOWLEDGMENTS

I would like to express my sincere gratitude and appreciation to my research advisor, Professor David G. I. Kingston, for his guidance, support, encouragement and patience throughout the completion of this work.

Grateful acknowledgments are made to Professors Michael Calter, Neal Castagnoli, Harry C. Dorn and Richard D. Gandour for their guidance and encouragement.

Thank also go to all the past and present members of the Kingston research group, especially Drs. A. A. L. Gunatilaka, Bingnan Zhou, Xian Liang, Mahendra Chordia, Prakash Jagtap, Lakshman Samala, Maged Abdel-Kader, Mr. Haiqing Yuan, Bill Lennox and Erkan Baloglu for helpful discussions and friendship.

Financial support for this work, provided by National Cancer Institute, is greatly appreciated.

Finally, I would like to thank and acknowledge my wife, Suling, my son, Stanley, my parents, and my family for their support and understanding.
### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF SCHEMES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
</tbody>
</table>

### CHAPTER

#### I. GENERAL INTRODUCTION

1.1 Plant-Derived Drugs

1.1.1 Natural Products as Anticancer Agents

1.2 Bioassay-Guided Fractionation

1.2.1 General Consideration

1.2.2 Yeast-Based Bioassay for DNA-Damaging Agents

#### II. SESQUITERPENOIDS FROM *CHILOSCYPHUS RIVULARIS*

2.1 Introduction

2.1.1 Occurrence of Bioactive Sesquiterpenoids

2.1.2 Chemical Investigation of *Chiloscyphus* Genus

2.1.3 Chiloscyphane Sesquiterpenoids

2.2 Results and Discussion

2.2.1 Isolation of Sesquiterpenoids from *C. rivularis*

2.2.2 Structure Elucidation of Novel Sesquiterpenoids

2.2.2.1 Sesquiterpenoid **2.30**

2.2.2.2 Sesquiterpenoid **2.31**

2.2.2.3 Sesquiterpenoid **2.32**
2.2.2.4 Sesquiterpenoid 2.33
2.2.2.5 Sesquiterpenoid 2.34

2.2.3 Identification of Known Sesquiterpenoids
2.2.3.1 Sesquiterpenoid 2.16
2.2.3.2 Sesquiterpenoid 2.35
2.2.3.2 Sesquiterpenoid 2.36

2.2.4 Synthetic Chemistry Studies of 2.30 and 2.34
2.2.4.1 Semisynthesis of 2.34
2.2.4.2 Modification of 2.30

2.2.5 Biological Evaluation of Compounds 2.30-2.42

2.3 Experimental

III. SYNTHESIS OF POTENTIAL ANTITUMOR DNA INTERCALATORS--FURANONAPHTHOQUINONES WITH HYDROXYAMINO SIDE CHAINS

3.1 Introduction
3.1.1 Antitumor DNA Intercalators
3.1.2 Furanonaphthoquinones with Hydroxyamino Side Chain, Potential Antitumor DNA Intercalators?

3.2 Results and Discussion
3.2.1 Synthesis of Furanonaphthoquinone Derivatives
3.2.1.1 Synthesis of naphtho[2,3-b]furan-4,9-dione (3.15)
3.2.1.2 Synthesis of 5-Methoxynaphtho [2,3-b] furan -4,9-dione (3.19)and5,7-Dimethoxynaphtho[2,3-b] furan-4,9-dione (3.2)
3.2.1.3 Biological Activity of Furanonaphthoquinone Derivatives
3.2.2 Synthesis of Furanonaphthoquinones with a Hydroxyamino Side Chain on the Furan Ring (A ring)

3.2.2.1 Synthesis of 2-Methyl-2-[2’-(4’,9’-dihydronaphtho[2’,3’:2,3-b]furan-4’,9’-dionyl-methyl)amino]-1,3-propanediol (3.30) 70

3.2.2.2 Preparation of Analogs of 3.30 73

3.2.2.3 Biological Activity of 3.30 and its Analogs 75

3.2.3 Attempted Synthesis of Furanonaphthoquinones with Hydroxyamino Side Chain at C-Ring (benzene ring)

3.2.3.1 Synthetic Strategy 77

3.2.3.2 Synthetic Scheme 79

3.2.3.3 Summary and Biological Activity of Compounds 3.54 and 3.58 96

3.3 Experimental 98

IV. CONCLUSION 113

V. APPENDIX 114
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>$^1$H NMR Spectrum of 2.30</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Expanded DQCOSY Spectrum of 2.30</td>
<td>26</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Selected HMBC Correlations for 2.30</td>
<td>28</td>
</tr>
<tr>
<td>Figure 4</td>
<td>$^1$H NMR Spectrum of 2.33</td>
<td>36</td>
</tr>
<tr>
<td>Figure 5</td>
<td>$^1$H NMR Spectrum of 2.34</td>
<td>37</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Expanded HMQC Spectrum of 2.34</td>
<td>38</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Selected HMBC Correlations for 2.34</td>
<td>39</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Conformational Structure of 2.34</td>
<td>40</td>
</tr>
<tr>
<td>Figure 9</td>
<td>$^1$H NMR Spectrum of 3.46 and its Acetate</td>
<td>83</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Expanded HMBC Spectrum of 3.46</td>
<td>84</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Selected HMBC Correlations for 3.46</td>
<td>85</td>
</tr>
<tr>
<td>Figure 12</td>
<td>$^1$H NMR Spectrum of 3.52</td>
<td>90</td>
</tr>
<tr>
<td>Figure 13</td>
<td>Selected HMBC Correlations for 3.52</td>
<td>91</td>
</tr>
<tr>
<td>Figure 14</td>
<td>Expanded HMBC Spectrum of 3.52</td>
<td>91</td>
</tr>
</tbody>
</table>
# LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bioassay-Directed Isolation of Active Sesquiterpenoid 2.30</td>
<td>22</td>
</tr>
<tr>
<td>2.</td>
<td>Isolation Procedure of Sesquiterpenoids from <em>C. rivularis</em></td>
<td>23</td>
</tr>
<tr>
<td>3.</td>
<td>Mass Spectrum Fragmentation of 2.30</td>
<td>29</td>
</tr>
<tr>
<td>4.</td>
<td>Mass Spectrum Fragmentation of 2.31</td>
<td>32</td>
</tr>
<tr>
<td>5.</td>
<td>Semi-synthesis of Rivulalactone (2.34) and Possible Mechanisms of Side Chain Degradation</td>
<td>45</td>
</tr>
<tr>
<td>6.</td>
<td>Possible Mechanism for the Formation of 3.24</td>
<td>66</td>
</tr>
<tr>
<td>7.</td>
<td>First Scheme to Synthesize 3.41</td>
<td>78</td>
</tr>
<tr>
<td>8.</td>
<td>Possible Mechanism for the Formation of 3.46</td>
<td>87</td>
</tr>
<tr>
<td>9.</td>
<td>Possible Mechanism for the Formation of 3.52</td>
<td>94</td>
</tr>
<tr>
<td>10.</td>
<td>Synthetic Scheme for Compound 3.58</td>
<td>96</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. $^1$H and $^{13}$C NMR Data for Compound **2.30**  
Table 2. $^1$H NMR Data for Compounds **2.31, 2.32, and 2.33**  
Table 3. $^{13}$C NMR Data for Compounds **2.31, 2.32, and 2.33**  
Table 4. $^1$H and $^{13}$C NMR Data for Compound **2.34**  
Table 5. Bioactivity of Compounds **3.15, 3.19, 3.20, and 3.29**  
Table 6. Bioactivity of Compound **3.30 and its Analogs**  
Table 7. $^1$H and $^{13}$C NMR Data for Compound **3.46**  
Table 8. $^1$H and $^{13}$C NMR Data for Compound **3.52**