SYNTHESIS, CHARACTERIZATION AND ANTI-PROLIFERATION STUDY OF SOME BENZIMIDAZOLE DERIVATIVES

by

MOHAMMED HADI SAEED AL–DOUH

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

2010
ACKNOWLEDGEMENT

First and foremost, praise be to ALLAH the Great and Almighty surrounded me under His auspices during my PhD study, in the School of Chemical Sciences, Universiti Sains Malaysia. I would like to express my sincere gratitude to my supervisors, Assoc. Prof. Dr. Hasnah Osman (School of Chemical Sciences, Universiti Sains Malaysia) and Assoc. Prof. Dr. Shafida Abd Hamid (Kulliyyah of Science, International Islamic University Malaysia) for their advices, support, direction, and encouragement during experimental work and thesis writing. Their hard work, insights and knowledge have been an inspiration. I would also like to thank to my co-supervisor Dr. Salizawati M. Salhi mi (School of Pharmaceutical Sciences, Universiti Sains Malaysia) for her kind assistance whenever I needed it. Her close work with our group has provided great opportunities to further our studies on the application part of our compounds.

I also thank Assoc. Prof. Dr. Mas Rosemal H. M. Haris (School of Chemical Sciences, Universiti Sains Malaysia) and Dr. Peter Springer (Bruker) for their suggestions of the NMR Experiments. I thank Prof. Dr. Hoong K. Fun, Dr. Reza Kia, Miss. Shea L. Ng and Mr. M. M. Rosli for X–ray crystallography analyses, X–ray crystallography Unit, School of Physics, Universiti Sains Malaysia, and Prof. Dr. David S. Larsen and his team for the HRMS analyses, Department of Chemistry, University Otago, Dunedin, New Zealand.

Special thanks are extended to Dr. Amin Malik Shah bin Abdul Majid in the School of Pharmaceutical Sciences, Universiti Sains Malaysia, and to my friends Mr. Hussain M. Ba-Harethah, Mr. Hayder B. Sahib, Mr. Ahmed Faisal, Mr. Muath
H. Helal, Mr. Abd Al-Raheem, Mr. Mahfoudh Al-Musali and Mr. Anas Horani for their help in the technical experiments of MTT assay. I thank my sister, Miss. Hanani (School of Chemical Sciences, Universiti Sains Malaysia) for her translations to Malay language during my study. Additionally, thanks to my uncle Prof. Dr. Mohammed Yaslam Shobrak (College of Sciences, Biology Dept., Taif University) and Prof. Dr. Mohamed Said Fakeh El-Amodi (College of Sciences, Chemistry Dept., Taif University) for their critical reading of this thesis.

I would like to express my thanks to Hadhramout University of Science and Technology (Mukalla, Hadhramout) for the financial scholarship support, School of Chemical Sciences, and to Universiti Sains Malaysia for founded this work by short-term grants: IRPA [304/PKIMIA/636108], IRPA [304/PKIMIA/638007], FRGS [203/PKIMIA/671046], FRGS [304/PKIMIA/638122] and USM-RU-PGRS [1001/PKIMIA/842024].

Finally great thanks to my mother (Khadijah), father (Hadi), brothers (Omar, Hassan, Aymen, Abdullah, Abdulrahman) and sisters (Heba, Hanadi, Hala, Hana, Hajar). I wish to express my special thanks to my wife (Maymonah), son (Hatem) and daughters (Rawan, Rutana, Rulla) for everything that helped me to complete my study.
DECLARATION

The work described in this thesis was undertaken at School of Chemical Sciences, Universiti Sains Malaysia between June 2005 and May 2010 under the supervision of Assoc. Prof. Dr. Hasnah Osman (School of Chemical Sciences, Universiti Sains Malaysia), Assoc. Prof. Dr. Shafida Abd Hamid (Kulliyyah of Science, International Islamic University Malaysia) and Dr. Salizawati M. Salhimi (School of Pharmaceutical Sciences, Universiti Sains Malaysia). Except where indicated by reference, it is the original work of the author and has not been submitted in support for another degree or qualification of this or any other university, or institute of higher learning.
TABLE OF CONTENTS

Acknowledgement ii
Table of contents v
List of tables xii
List of figures xv
List of schemes xxv
List of abbreviations xxviii
List of appendices xxxi
Abstrak xxxiii
Abstract xxxv

CHAPTER ONE: INTRODUCTION 1
1.1 Schiff bases 1
1.2 Benzimidazoles 4
  1.2.1 Substitution at position 2 8
    1.2.1.1 Reaction of o-arylene diamines with carboxylic acids 8
    1.2.1.2 Reaction of o-arylene diamines with aldehydes 8
    1.2.1.3 Reaction of o-arylene diamines with ketones 10
    1.2.1.4 Benzimidazole derivatives from β-diketone 11
    1.2.1.5 Benzimidazoles from the reaction of o-arylene diamines with quinoxalin-2-one derivatives 11
    1.2.1.6 Benzimidazoles from o-nitroarylamines 12
    1.2.1.7 Benzimidazoles from o-azidoanilines 13
    1.2.1.8 Benzimidazoles from amidines 14
1.2.1.9 Benzimidazoles from resin-bound esters 14

1.2.2 1,2-Disubstitution of benzimidazoles 15

1.2.2.1 Benzimidazoles from \( o \)-arylene amines 15

1.2.2.2 Benzimidazoles from \( o \)-haloarylamines 17

1.2.2.3 Benzimidazoles from \( o \)-nitroarylamines 17

1.2.2.4 Benzimidazoles from \( o \)-halonitrobenzenes 18

1.2.3 Synthesis leading to the formation of both Schiff base and benzimidazoles 19

1.3 Scope of this work 21

1.3.1 The objectives 24

CHAPTER TWO: MATERIALS AND METHODS 25

2.1 Chemicals 25

2.2 Equipments and apparatus for cell proliferation assay 26

2.3 Instrumentation 27

2.4 Synthesis and Characterization 29

2.4.1 2-Benzylloxy-3-methoxybenzaldehyde (benzyl \( o \)-vanillin, 75) 30

2.4.2 2-Amino-\( N \)-(2-hydroxy-3-methoxybenzylidene) benzeneamine (76) and 2-amino-\( N \)-(2-benzyloxy-3-methoxybenzylidene) benzeneamine (82) 31

2.4.3 2-(2-Hydroxy-3-methoxyphenyl)-1\( H \)-benzimidazole (77), \( N \)-1-(2-hydroxy-3-methoxybenzyl)-2-(2-hydroxy-3-methoxyphenyl)-1\( H \)-benzimidazole (78), \( N,N' \)-bis(2-hydroxy-3-methoxybenzylidene)-1,2-diaminobenzene (79), 2-(2-benzyloxy-3-methoxyphenyl)-1\( H \)-benzimidazole (83) and \( N \)-1-(2-benzyloxy-3-methoxybenzyl)-2-(2-benzyloxy-3-methoxyphenyl)-1\( H \)-benzimidazole (84) 32

2.4.4 \( N,N' \)-Bis(2-hydroxy-3-methoxybenzylidene)-1,3-diamino benzene (80) and \( N,N' \)-bis(2-hydroxy-3-methoxybenzylidene)-1,4-diamino benzene (81) 34

2.5 Routine cell culture 35
2.6 Cytotoxicity evaluation

2.6.1 MTT assay

CHAPTER THREE: RESULTS AND DISCUSSION

3.1 2-Benzylloxy-3-methoxybenzaldehyde (benzyl \( o \)-vanillin) (75)

3.1.1 FTIR Spectroscopy

3.1.2 EIMS and HRMS Spectrum

3.1.3 \( ^1 \)H NMR

3.1.4 \( ^{13} \)C NMR

3.1.5 \( ^1 \)H–\( ^1 \)H COSY

3.1.6 \( ^1 \)H–\( ^{13} \)C HMQC

3.1.7 \( ^1 \)H–\( ^{13} \)C HMBC

3.1.8 X–ray crystallography

3.2 2-Amino-\( N \)-(2-hydroxy-3-methoxybenzylidene) benzeneamine (76)

3.2.1 FTIR Spectroscopy

3.2.2 HRMS Spectrum

3.2.3 \( ^1 \)H NMR

3.2.4 \( ^{13} \)C APT NMR

3.2.5 \( ^1 \)H–\( ^1 \)H COSY

3.2.6 \( ^1 \)H–\( ^{13} \)C HMQC

3.2.7 \( ^1 \)H–\( ^{13} \)C HMBC

3.2.8 X–ray crystallography

3.3 2-(2-Hydroxy-3-methoxyphenyl)-1H-benzimidazole (77)

3.3.1 FTIR Spectroscopy

3.3.2 HRMS Spectrum
3.3.3  $^1$H NMR  72
3.3.4  $^{13}$C APT NMR  74
3.3.5  $^1$H–$^1$H COSY  75
3.3.6  $^1$H–$^{13}$C HMQC  76
3.3.7  $^1$H–$^{13}$C HMBC  77
3.3.8  X–ray crystallography  80

3.4  $N$-1-(2-Hydroxy-3-methoxybenzyl)-2-(2-hydroxy-3-methoxyphenyl)-1$H$-benzimidazole (78)  82
3.4.1  FTIR Spectroscopy  82
3.4.2  HRMS Spectrum  83
3.4.3  $^1$H NMR  84
3.4.4  $^{13}$C APT NMR  86
3.4.5  $^1$H–$^1$H COSY  87
3.4.6  $^1$H–$^{13}$C HMQC  89
3.4.7  $^1$H–$^{13}$C HMBC  90
3.4.8  X–ray crystallography  92

3.5  $N,N'$-Bis(2-hydroxy-3-methoxybenzylidene)-1,2-diaminobenzene (79)  97
3.5.1  HRMS Spectrum  97
3.5.2  $^1$H NMR  98
3.5.3  $^{13}$C NMR  99
3.5.4  $^1$H–$^1$H COSY  100
3.5.5  $^1$H–$^{13}$C HMQC  102
3.5.6  $^1$H–$^{13}$C HMBC  102
3.5.7  X–ray crystallography  104

3.6  $N,N'$-Bis(2-hydroxy-3-methoxybenzylidene)-1,3-diaminobenzene (80)  107
<table>
<thead>
<tr>
<th>3.6.1</th>
<th>HRMS Spectrum</th>
<th>108</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6.2</td>
<td>$^1$H NMR</td>
<td>108</td>
</tr>
<tr>
<td>3.6.3</td>
<td>$^{13}$C NMR</td>
<td>109</td>
</tr>
<tr>
<td>3.6.4</td>
<td>$^1$H–$^1$H COSY</td>
<td>111</td>
</tr>
<tr>
<td>3.6.5</td>
<td>$^1$H–$^{13}$C HMQC</td>
<td>112</td>
</tr>
<tr>
<td>3.6.6</td>
<td>$^1$H–$^{13}$C HMBC</td>
<td>113</td>
</tr>
<tr>
<td>3.6.7</td>
<td>X–ray crystallography</td>
<td>115</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3.7</th>
<th>$N,N'$-Bis(2-hydroxy-3-methoxybenzylidene)-1,4-diaminobenzene ($81$)</th>
<th>118</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7.1</td>
<td>FTIR Spectroscopy</td>
<td>119</td>
</tr>
<tr>
<td>3.7.2</td>
<td>HRMS Spectrum</td>
<td>120</td>
</tr>
<tr>
<td>3.7.3</td>
<td>$^1$H NMR</td>
<td>121</td>
</tr>
<tr>
<td>3.7.4</td>
<td>$^{13}$C NMR</td>
<td>121</td>
</tr>
<tr>
<td>3.7.5</td>
<td>$^1$H–$^1$H COSY</td>
<td>123</td>
</tr>
<tr>
<td>3.7.6</td>
<td>$^1$H–$^{13}$C HMQC</td>
<td>124</td>
</tr>
<tr>
<td>3.7.7</td>
<td>$^1$H–$^{13}$C HMBC</td>
<td>125</td>
</tr>
<tr>
<td>3.7.8</td>
<td>X–ray crystallography</td>
<td>127</td>
</tr>
<tr>
<td>3.7.9</td>
<td>The cytotoxicity of bis-Schiff bases $79$, $80$ and $81$</td>
<td>129</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3.8</th>
<th>2-Amino-$N$-(2-benzyloxy-3-methoxybenzylidene) benzeneamine ($82$)</th>
<th>131</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8.1</td>
<td>FTIR Spectroscopy</td>
<td>132</td>
</tr>
<tr>
<td>3.8.2</td>
<td>HRMS Spectrum</td>
<td>134</td>
</tr>
<tr>
<td>3.8.3</td>
<td>$^1$H NMR</td>
<td>135</td>
</tr>
<tr>
<td>3.8.4</td>
<td>$^{13}$C NMR</td>
<td>136</td>
</tr>
<tr>
<td>3.8.5</td>
<td>$^1$H–$^1$H COSY</td>
<td>138</td>
</tr>
<tr>
<td>3.8.6</td>
<td>$^1$H–$^{13}$C HMQC</td>
<td>139</td>
</tr>
<tr>
<td>3.8.7</td>
<td>$^1$H–$^{13}$C HMBC</td>
<td>140</td>
</tr>
</tbody>
</table>
3.8.8 X–ray crystallography 143

3.9 2-(2-Benzyloxy-3-methoxyphenyl)-1H-benzimidazole (83) 145
3.9.1 FTIR Spectroscopy 147
3.9.2 EIMS and HRMS Spectrum 148
3.9.3 ¹H NMR 150
3.9.4 ¹³C NMR 152
3.9.5 ¹H–¹H COSY 155
3.9.6 ¹H–¹³C HMQC 158
3.9.7 ¹H–¹³C HMBC 159
3.9.8 X–ray crystallography 161

3.10 N-1-(2-Benzyloxy-3-methoxybenzyl)-2-(2-benzyloxy-3-methoxyphenyl)-1H-benzimidazole (84) 164
3.10.1 FTIR Spectroscopy 167
3.10.2 HRMS Spectrum 168
3.10.3 ¹H NMR 169
3.10.4 ¹³C NMR 171
3.10.5 ¹H–¹H COSY 173
3.10.6 ¹H–¹³C HMQC 174
3.10.7 ¹H–¹³C HMBC 175

3.11 Potential biological activity of benzimidazoles 77, 78, 83 and 84 179
3.11.1 Introduction 179
3.11.2 Cytotoxicity effect of benzimidazoles 77, 78, 83 and 84 on MCF–7 breast cancer cell line and HCT–116 colon cancer cell line 181

CHAPTER FOUR: CONCLUSION 188
REFERENCES 190
APPENDICES

LIST OF PUBLICATIONS

A. International refereed journals

B. Papers presented at international and national conferences
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.1</td>
<td>Benzylation of o–vanillin (1.0 equiv.) by benzyl halides (X) produced via Scheme 3.1</td>
<td>40</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>FTIR spectral data of compound 75 (cm(^{-1}))</td>
<td>41</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>(^1)H and (^{13})C NMR chemical shifts (ppm) and coupling constants (Hz) of 75 in CDCl(_3) and acetone–d(_6)</td>
<td>46</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>2D (^1)H–(^1)H COSY, (^1)H–(^{13})C HMQC and HMBC correlations for 75 in CDCl(_3) and acetone–d(_6)</td>
<td>52</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Hydrogen bond geometry of 75 (Å, ‾)</td>
<td>54</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>FTIR spectral data of 76 (cm(^{-1}))</td>
<td>56</td>
</tr>
<tr>
<td>Table 3.7</td>
<td>(^1)H and (^{13})C APT NMR chemical shifts (ppm) and coupling constants (Hz) of 76 in CDCl(_3)</td>
<td>62</td>
</tr>
<tr>
<td>Table 3.8</td>
<td>2D (^1)H–(^1)H COSY, (^1)H–(^{13})C HMQC and HMBC correlations for 76 in CDCl(_3)</td>
<td>66</td>
</tr>
<tr>
<td>Table 3.9</td>
<td>Hydrogen bond geometry of 76 (Å, ‾)</td>
<td>68</td>
</tr>
<tr>
<td>Table 3.10</td>
<td>FTIR spectral data of benzimidazole 77 (cm(^{-1}))</td>
<td>70</td>
</tr>
<tr>
<td>Table 3.11</td>
<td>(^1)H and (^{13})C APT NMR chemical shifts (ppm) and coupling constants (Hz) of 77 in CD(_3)OD</td>
<td>75</td>
</tr>
<tr>
<td>Table 3.12</td>
<td>2D (^1)H–(^1)H COSY, (^1)H–(^{13})C HMQC and HMBC correlations for 77 in CD(_3)OD</td>
<td>79</td>
</tr>
<tr>
<td>Table 3.13</td>
<td>Hydrogen bond geometry of 77 (Å, ‾)</td>
<td>81</td>
</tr>
<tr>
<td>Table 3.14</td>
<td>FTIR spectral data of benzimidazole 78 (cm(^{-1}))</td>
<td>83</td>
</tr>
<tr>
<td>Table 3.15</td>
<td>(^1)H and (^{13})C APT NMR chemical shifts (ppm) and coupling constants (Hz) of 78 in CDCl(_3)</td>
<td>87</td>
</tr>
<tr>
<td>Table 3.16</td>
<td>2D (^1)H–(^1)H COSY, (^1)H–(^{13})C HMQC and HMBC correlations for 78 in CDCl(_3)</td>
<td>92</td>
</tr>
<tr>
<td>Table 3.17</td>
<td>Hydrogen bond geometry of 78A (Å, ‾)</td>
<td>96</td>
</tr>
<tr>
<td>Table 3.18</td>
<td>Hydrogen bond geometry of 78B (Å, ‾)</td>
<td>96</td>
</tr>
<tr>
<td>Table 3.19</td>
<td>$^1$H and $^{13}$C NMR chemical shifts (ppm) and coupling constants (Hz) of 79 in CDCl$_3$</td>
<td>100</td>
</tr>
<tr>
<td>Table 3.20</td>
<td>2D $^1$H–$^1$H COSY, $^1$H–$^{13}$C HMQC and HMBC correlations for 79 in CDCl$_3$</td>
<td>104</td>
</tr>
<tr>
<td>Table 3.21</td>
<td>Hydrogen bond geometry of 79 (Å, °)</td>
<td>106</td>
</tr>
<tr>
<td>Table 3.22</td>
<td>$^1$H and $^{13}$C NMR chemical shifts (ppm) and coupling constants (Hz) of 80 in CDCl$_3$</td>
<td>110</td>
</tr>
<tr>
<td>Table 3.23</td>
<td>2D $^1$H–$^1$H COSY, $^1$H–$^{13}$C HMQC and HMBC correlations for 80 in CDCl$_3$</td>
<td>114</td>
</tr>
<tr>
<td>Table 3.24</td>
<td>Hydrogen bond geometry of 80 (Å, °)</td>
<td>117</td>
</tr>
<tr>
<td>Table 3.25</td>
<td>FTIR spectral data of bis-Schiff bases 79, 80 and 81 (cm$^{-1}$)</td>
<td>120</td>
</tr>
<tr>
<td>Table 3.26</td>
<td>$^1$H and $^{13}$C NMR chemical shifts (ppm) and coupling constants (Hz) of 81 in CDCl$_3$</td>
<td>122</td>
</tr>
<tr>
<td>Table 3.27</td>
<td>2D $^1$H–$^1$H COSY, $^1$H–$^{13}$C HMQC and HMBC correlations for 81 in CDCl$_3$</td>
<td>127</td>
</tr>
<tr>
<td>Table 3.28</td>
<td>Hydrogen bond geometry of 81 (Å, °)</td>
<td>129</td>
</tr>
<tr>
<td>Table 3.29</td>
<td>The IC$_{50}$ values (µg/mL) of bis-Schiff bases 79, 80 and 81 against MCF–7, T–47D, HepG2, K562 and U937</td>
<td>130</td>
</tr>
<tr>
<td>Table 3.30</td>
<td>FTIR spectral data of 82 (cm$^{-1}$)</td>
<td>133</td>
</tr>
<tr>
<td>Table 3.31</td>
<td>$^1$H and $^{13}$C NMR chemical shifts (ppm) and coupling constants (Hz) of 82 in CDCl$_3$</td>
<td>137</td>
</tr>
<tr>
<td>Table 3.32</td>
<td>2D $^1$H–$^1$H COSY, $^1$H–$^{13}$C HMQC and HMBC correlations for 82 in CDCl$_3$</td>
<td>142</td>
</tr>
<tr>
<td>Table 3.33</td>
<td>Hydrogen bond geometry of 82 (Å, °)</td>
<td>144</td>
</tr>
<tr>
<td>Table 3.34</td>
<td>FTIR spectral data of benzimidazole 83 (cm$^{-1}$)</td>
<td>148</td>
</tr>
<tr>
<td>Table 3.35</td>
<td>$^1$H and $^{13}$C NMR chemical shifts (ppm) and coupling constants (Hz) of 83 in CDCl$_3$ and acetone–$d_6$</td>
<td>155</td>
</tr>
<tr>
<td>Table 3.36</td>
<td>2D $^1$H–$^1$H COSY, $^1$H–$^{13}$C HMQC and HMBC correlations for 83 in CDCl$_3$ and acetone–$d_6$</td>
<td>161</td>
</tr>
<tr>
<td>Table 3.37</td>
<td>Hydrogen bond geometry of 83 (Å, °)</td>
<td>163</td>
</tr>
<tr>
<td>Table 3.38</td>
<td>FTIR spectral data of benzimidazole 84 (cm$^{-1}$)</td>
<td>168</td>
</tr>
</tbody>
</table>
Table 3.39 \(^1\)H and \(^{13}\)C NMR chemical shifts (ppm) and coupling constants (Hz) of 84 in acetone–\(d_6\) 172

Table 3.40 2D \(^1\)H–\(^1\)H COSY, \(^1\)H–\(^{13}\)C HMQC and HMBC correlations for 84 in acetone–\(d_6\) 178

Table 3.41 Several benzimidazoles and their IC\(_{50}\) (\(\mu\)M) against MCF–7 180

Table 3.42 The IC\(_{50}\) values of the benzimidazoles 77, 78, 83 and 84 against MCF–7 and HCT–116 187
**LIST OF FIGURES**

| Figure 1.1 | The general formula of Schiff base or imine | 1 |
| Figure 1.2 | Some symmetrical and unsymmetrical bis-Schiff bases | 2 |
| Figure 1.3 | Intramolecular hydrogen bond | 4 |
| Figure 1.4 | The benzimidazole ring | 4 |
| Figure 1.5 | The benzimidazole ring in the chemical structure of vitamin B<sub>12</sub> | 5 |
| Figure 1.6 | The chemical structures of some benzimidazole derivatives evaluated medicinally | 6 |
| Figure 1.7 | Some benzimidazole derivatives evaluated for biological activity | 7 |
| Figure 1.8 | Benzimidazole derivatives evaluated against cancer cell lines | 21 |
| Figure 1.9 | Structure of benzyl o-vanillin | 22 |
| Figure 1.10 | Structures of 2-amino-N-benzylidene benzeneamines 76 and 82 | 22 |
| Figure 1.11 | Structures of bis-Schiff bases 79, 80 and 81 | 23 |
| Figure 1.12 | Structures of benzimidazoles 77, 78, 83 and 84 | 23 |
| Figure 3.1 | The chemical structure and the numbering scheme of 75 | 40 |
| Figure 3.2 | FTIR spectrum of 75 | 41 |
| Figure 3.3 | EIMS spectrum of 75 | 42 |
| Figure 3.4 | HRMS spectrum of 75 | 42 |
| Figure 3.5 | <sup>1</sup>H NMR spectrum of 75 in CDCl<sub>3</sub> | 43 |
| Figure 3.6 | <sup>1</sup>H NMR spectrum of 75 in acetone–d<sub>6</sub> | 44 |
| Figure 3.7 | The correlation between aldehydic proton and H<sub>5</sub> in 75 | 44 |
| Figure 3.8 | <sup>13</sup>C NMR spectrum of 75 in CDCl<sub>3</sub> | 45 |
Figure 3.9  $^{13}$C NMR spectrum of 75 in acetone–d$_6$  
Figure 3.10  $^1$H–$^1$H COSY NMR spectrum of 75 in acetone–d$_6$  
Figure 3.11  The correlations observed in COSY spectrum of 75  
Figure 3.12  $^1$H–$^{13}$C HMQC NMR spectrum of 75 in CDCl$_3$  
Figure 3.13  $^1$H–$^{13}$C HMQC NMR spectrum of 75 in acetone–d$_6$  
Figure 3.14  $^1$H–$^{13}$C HMBC NMR spectrum of 75 in CDCl$_3$  
Figure 3.15  $^1$H–$^{13}$C HMBC NMR spectrum of 75 in acetone–d$_6$  
Figure 3.16  The correlations observed in HMBC spectrum of 75  
Figure 3.17  The crystal structure of 75 showing 50% probability displacement ellipsoids and the atomic numbering. The dashed lines indicate intramolecular hydrogen bonds  
Figure 3.18  The crystal packing of 75, viewed down the b axis. Intermolecular hydrogen bonds are shown as dashed lines  
Figure 3.19  The chemical structure and the numbering scheme of 76  
Figure 3.20  FTIR spectrum of 76  
Figure 3.21  HRMS spectrum of 76  
Figure 3.22  The proposed structures of the prominent peak of 76  
Figure 3.23  $^1$H NMR spectrum of 76 in CDCl$_3$  
Figure 3.24  $^1$H NMR spectrum of 76 in CD$_2$Cl$_2$  
Figure 3.25  $^1$H NMR spectrum of 76 in acetone–d$_6$  
Figure 3.26  The possibility of intramolecular hydrogen bonding in 76  
Figure 3.27  $^{13}$C APT NMR spectrum of 76 in CDCl$_3$  
Figure 3.28  $^{13}$C APT NMR spectrum of 76 in CD$_2$Cl$_2$  
Figure 3.29  $^1$H–$^1$H COSY NMR spectrum of 76 in CDCl$_3$  
Figure 3.30  $^1$H–$^1$H COSY NMR spectrum of 76 in acetone–d$_6$  
Figure 3.31  $^1$H–$^1$H COSY NMR spectrum of 76 in CD$_2$Cl$_2$
Figure 3.32 \(^{1}H–^{13}C\) HMQC NMR spectrum of \(76\) in CDCl\(_3\)  
Figure 3.33 \(^{1}H–^{13}C\) HMQC NMR spectrum of \(76\) in CD\(_2\)Cl\(_2\)  
Figure 3.34 \(^{1}H–^{13}C\) HMBC NMR spectrum of \(76\) in CDCl\(_3\)  
Figure 3.35 The correlations observed in HMBC spectrum of \(76\) in CDCl\(_3\)  
Figure 3.36 The molecular structure of \(76\) showing 50% probability displacement ellipsoids and the atomic numbering. The dashed lines indicate intramolecular hydrogen bonds  
Figure 3.37 The zigzag stacking arrangement of \(76\) viewed along the \(b\) axis. Hydrogen bonds are drawn as dashed lines  
Figure 3.38 The crystal packing of \(76\), viewed down the \(c\) axis showing the molecular stacking. Hydrogen bonds are drawn as dashed lines  
Figure 3.39 The chemical structure and the numbering scheme of \(77\)  
Figure 3.40 FTIR spectrum of \(77\)  
Figure 3.41 HRMS spectrum of \(77\)  
Figure 3.42 The proposed structures of the basic and prominent peaks in HRMS spectrum of \(77\)  
Figure 3.43 \(^{1}H\) NMR spectrum of \(77\) in CD\(_3\)OD  
Figure 3.44 \(^{1}H\) NMR spectrum of \(77\) in acetone–\(d_6\)  
Figure 3.45 \(^{1}H\) NMR spectrum of \(77\) in DMSO–\(d_6\)  
Figure 3.46 \(^{13}C\) APT NMR spectrum of \(77\) in CD\(_3\)OD  
Figure 3.47 \(^{1}H–^{1}H\) COSY NMR spectrum of \(77\) in CD\(_3\)OD  
Figure 3.48 Aromatic protons in \(^{1}H–^{1}H\) COSY NMR spectrum of \(77\) in CD\(_3\)OD  
Figure 3.49 \(^{1}H–^{13}C\) HMQC NMR spectrum of \(77\) in CD\(_3\)OD  
Figure 3.50 Aromatic protons and carbons in \(^{1}H–^{13}C\) HMQC NMR spectrum of \(76\) in CD\(_3\)OD  
Figure 3.51 \(^{1}H–^{13}C\) HMBC NMR spectrum of \(77\) in CD\(_3\)OD
Figure 3.52  Aromatic protons and carbons in $^1$H–$^{13}$C HMBC NMR spectrum of 76 in CD$_3$OD

Figure 3.53  The correlations observed in HMBC spectrum of 77

Figure 3.54  The crystal structure of 77 showing 50% probability displacement ellipsoids and the atomic numbering. The dashed line indicates intramolecular hydrogen bonds

Figure 3.55  The crystal packing of 77, viewed down the b axis. Intermolecular hydrogen bonds are shown as dashed lines

Figure 3.56  The chemical structure and the numbering scheme of 78

Figure 3.57  FTIR spectrum of 78

Figure 3.58  HRMS spectrum of 78

Figure 3.59  The proposed structures of the basic and prominent peaks in HRMS spectrum of 78

Figure 3.60  $^1$H NMR spectrum of 78 in CDCl$_3$

Figure 3.61  $^{13}$C APT NMR spectrum of 78 in CDCl$_3$

Figure 3.62  $^1$H–$^1$H COSY NMR spectrum of 78 in CDCl$_3$

Figure 3.63  Aromatic protons in $^1$H–$^1$H COSY NMR spectrum of 78

Figure 3.64  The correlations observed in COSY spectrum of 78

Figure 3.65  $^1$H–$^{13}$C HMQC NMR spectrum of 78 in CDCl$_3$

Figure 3.66  Aromatic protons and carbons in $^1$H–$^{13}$C HMQC NMR spectrum of 78

Figure 3.67  $^1$H–$^{13}$C HMBC NMR spectrum of 78 in CDCl$_3$

Figure 3.68  The correlations observed in HMBC spectrum of 78

Figure 3.69  The molecular structure of 78A showing 50% probability displacement ellipsoids and the atomic numbering. The disordered methanol solvent molecules were omitted for clarity. Intramolecular hydrogen bonds are drawn as dashed lines

Figure 3.70  The conversion of 78B from 78A
Figure 3.71  The molecular structure of 78B showing 50% probability displacement ellipsoids and the atomic numbering. Intramolecular H bonds are shown as dashed lines

Figure 3.72  Part of the crystal structure of 78B viewed along the $a$-axis showing 1-D extended chains along the $c$-axis. Intermolecular interactions are drawn as dashed lines

Figure 3.73  The chemical structure and the numbering scheme of 79

Figure 3.74  The HRMS spectrum of 79

Figure 3.75  The proposed structures of the prominent peak in HRMS spectrum of 79

Figure 3.76  $^1$H NMR spectrum of 79 in CDCl$_3$

Figure 3.77  $^{13}$C NMR spectrum of 79 in CDCl$_3$

Figure 3.78  $^1$H–$^1$H COSY NMR spectrum of 79 in CDCl$_3$

Figure 3.79  $^1$H–$^1$H COSY NMR spectrum of aromatic protons of 79

Figure 3.80  The correlations observed in COSY spectrum of 79

Figure 3.81  $^1$H–$^{13}$C HMQC NMR spectrum of 79 in CDCl$_3$

Figure 3.82  $^1$H–$^{13}$C HMBC NMR spectrum of 79 in CDCl$_3$

Figure 3.83  The correlations observed in HMBC spectrum of 79

Figure 3.84  The crystal structure of 79 showing 50% probability displacement ellipsoids and the atomic numbering. The dashed lines indicate intramolecular hydrogen bonds

Figure 3.85  The crystal packing of 79, viewed down the $b$ axis. Intramolecular hydrogen bonds are shown as dashed lines

Figure 3.86  The chemical structure and the numbering scheme of 80

Figure 3.87  HRMS spectrum of 80

Figure 3.88  The proposed structures of the prominent peak of 80

Figure 3.89  $^1$H NMR spectrum of 80 in CDCl$_3$

Figure 3.90  $^{13}$C NMR spectrum of 80 in CDCl$_3$

Figure 3.91  $^1$H–$^1$H COSY NMR spectrum of 80 in CDCl$_3$
Figure 3.92  $^1$H–$^1$H COSY NMR spectrum of aromatic protons of 80  

Figure 3.93  The correlations observed in COSY spectrum of 80  

Figure 3.94  $^1$H–$^{13}$C HMQC NMR spectrum of 80 in CDCl$_3$  

Figure 3.95  $^1$H–$^{13}$C HMBC NMR spectrum of 80 in CDCl$_3$  

Figure 3.96  Aromatic protons and carbons in $^1$H–$^{13}$C HMBC NMR spectrum of 80  

Figure 3.97  The correlations observed in HMBC spectrum of 80  

Figure 3.98  The crystal structure of 80 showing 50% probability displacement ellipsoids and the atomic numbering. The dashed lines indicate intramolecular hydrogen bonds  

Figure 3.99  The crystal packing of 80, viewed down the $a$ axis. Intramolecular hydrogen bonds are shown as dashed lines  

Figure 3.100  The chemical structure and the numbering scheme of 81  

Figure 3.101  FTIR spectra of a) 79, b) 80 and c) 81  

Figure 3.102  HRMS spectrum of 81  

Figure 3.103  The proposed structures of the prominent peak of 81  

Figure 3.104  $^1$H NMR spectrum of 81 in CDCl$_3$  

Figure 3.105  $^{13}$C NMR spectrum of 81 in CDCl$_3$  

Figure 3.106  $^1$H–$^1$H COSY NMR spectrum of 81 in CDCl$_3$  

Figure 3.107  $^1$H–$^1$H COSY NMR spectrum of aromatic protons of 81  

Figure 3.108  The correlations observed in COSY spectrum of 81  

Figure 3.109  $^1$H–$^{13}$C HMQC NMR spectrum of 81 in CDCl$_3$  

Figure 3.110  $^1$H–$^{13}$C HMBC NMR spectrum of 81 in CDCl$_3$  

Figure 3.111  Aromatic protons and carbons in $^1$H–$^{13}$C HMBC NMR spectrum of 81  

Figure 3.112  The correlations observed in HMBC spectrum of 81
Figure 3.113 The molecular structure of 81 showing 50% probability displacement ellipsoids and the atomic numbering [symmetry code of unlabelled atoms – \(x + 1\), \(-y\), \(-z + 2\)]. Intramolecular H bonds are drawn as dashed lines

Figure 3.114 The crystal packing of 81, viewed down the c axis showing of molecules along the c axis. Intramolecular hydrogen bonds are shown as dashed lines

Figure 3.115 The chemical structure and the numbering scheme of 82

Figure 3.116 FTIR spectrum of 82

Figure 3.117 The possibility of intramolecular hydrogen bonding in 82

Figure 3.118 High-resolution mass spectrum of 82

Figure 3.119 The proposed structures of the basic peaks of 82

Figure 3.120 \(^1\)H NMR spectrum of 82 in CDCl\(_3\)

Figure 3.121 \(^{13}\)C NMR spectrum of 82 in CDCl\(_3\)

Figure 3.122 \(^1\)H–\(^1\)H COSY NMR spectrum of 82 in CDCl\(_3\)

Figure 3.123 \(^1\)H–\(^1\)H COSY NMR spectrum of aromatic protons of 82

Figure 3.124 \(^1\)H–\(^{13}\)C HMQC NMR spectrum of 82 in CDCl\(_3\)

Figure 3.125 Aromatic protons and carbons in \(^1\)H–\(^{13}\)C HMQC NMR spectrum of 82

Figure 3.126 \(^1\)H–\(^{13}\)C HMBC NMR spectrum of 82 in CDCl\(_3\)

Figure 3.127 Aromatic protons and carbons in \(^1\)H–\(^{13}\)C HMBC NMR spectrum of 82

Figure 3.128 The correlations observed in HMBC spectrum of 82

Figure 3.129 The asymmetric unit of 82. Displacement ellipsoids are drawn at the 30% probability level. Intramolecular interactions are shown as dashed lines

Figure 3.130 The chemical structure and the numbering scheme of 83

Figure 3.131 FTIR spectrum of 83

Figure 3.132 EIMS spectrum of 83
Figure 3.133  HRMS spectrum of 83

Figure 3.134  The proposed structures of the prominent peaks of 83

Figure 3.135  $^1$H NMR spectrum of 83 in CDCl$_3$

Figure 3.136  $^1$H NMR spectrum of 83 in acetone–$d_6$

Figure 3.137  $^{13}$C NMR spectrum of 83 in CDCl$_3$

Figure 3.138  $^{13}$C NMR spectrum of 83 in acetone–$d_6$

Figure 3.139  $^1$H–$^1$H COSY NMR spectrum of 83 in CDCl$_3$

Figure 3.140  $^1$H–$^1$H COSY NMR spectrum of aromatic protons of 83 in CDCl$_3$

Figure 3.141  $^1$H–$^1$H COSY NMR spectrum of 83 in acetone–$d_6$

Figure 3.142  $^1$H–$^1$H COSY NMR spectrum of aromatic protons of 83 in acetone–$d_6$

Figure 3.143  The correlations observed in COSY spectrum of 83

Figure 3.144  $^1$H–$^{13}$C HMQC NMR spectrum of 83 in CDCl$_3$

Figure 3.145  $^1$H–$^{13}$C HMQC NMR spectrum of 83 in acetone–$d_6$

Figure 3.146  $^1$H–$^{13}$C HMBC NMR spectrum of 83 in CDCl$_3$

Figure 3.147  $^1$H–$^{13}$C HMBC NMR spectrum of 83 in acetone–$d_6$

Figure 3.148  The correlations observed in HMBC spectrum of 83

Figure 3.149  The crystal structure of 83 showing 50% probability displacement ellipsoids and the atomic numbering. The dashed line indicates intramolecular hydrogen bond

Figure 3.150  The crystal packing of 83, viewed down the b axis. Intramolecular hydrogen bonds are shown as dashed lines

Figure 3.151  The chemical structure and the numbering scheme of 84

Figure 3.152  The crystal structure of the bis-Schiff base 79

Figure 3.153  FTIR spectrum of 84

Figure 3.154  HRMS spectrum of 84
Figure 3.155  The proposed structures of intense peaks of 84

Figure 3.156  $^1$H NMR spectrum of 84 in acetone–$d_6$

Figure 3.157  $^{13}$C NMR spectrum of 84 in acetone–$d_6$

Figure 3.158  $^1$H–$^1$H COSY NMR spectrum of 84 in acetone–$d_6$

Figure 3.159  $^1$H–$^1$H COSY NMR spectrum of aromatic protons range of 84

Figure 3.160  The correlations observed in COSY spectrum of 84

Figure 3.161  $^1$H–$^{13}$C HMQC NMR spectrum of 84 in acetone–$d_6$

Figure 3.162  Aromatic protons and carbons in $^1$H–$^{13}$C HMQC NMR spectrum of 84

Figure 3.163  $^1$H–$^{13}$C HMBC NMR spectrum of 84 in acetone–$d_6$

Figure 3.164  Aromatic protons and carbons in $^1$H–$^{13}$C HMBC NMR spectrum of 84

Figure 3.165  The correlations observed in HMBC spectrum of 84

Figure 3.166  The chemical structure of benzimidazole 89

Figure 3.167  The chemical structure of benzimidazoles 73, 74 and 98

Figure 3.168  The structure of the evaluated benzimidazoles 77, 78, 83 and 84

Figure 3.169  Dose response curve of the evaluated benzimidazole 84 with MCF–7 cancer cell

Figure 3.170  Dose response curve of the evaluated benzimidazole 77 with MCF–7 cancer cell

Figure 3.171  Dose response curve of the evaluated benzimidazole 78 with MCF–7 cancer cell

Figure 3.172  Dose response curve of the evaluated benzimidazole 83 with MCF–7 cancer cell

Figure 3.173  Dose response curve of the evaluated benzimidazole 78 with HCT–116 cancer cell

Figure 3.174  Dose response curve of the evaluated benzimidazole 77 with HCT–116 cancer cell
Figure 3.175  Dose response curve of the evaluated benzimidazole 84 with HCT–116 cancer cell  186

Figure 3.176  Dose response curve of the evaluated benzimidazole 83 with HCT–116 cancer cell  186
## LIST OF SCHEMES

| Scheme 1.1 | First benzimidazole 6, prepared by Hobrecker in 1872  | 4 |
| Scheme 1.2 | The Phillips-type to prepare the benzimidazole | 8 |
| Scheme 1.3 | Preparation of benzimidazoles using the method of Wang et al. | 8 |
| Scheme 1.4 | Benzimidazoles preparation in the presence of Na$_2$S$_2$O$_5$ | 9 |
| Scheme 1.5 | Benzimidazoles preparation in the presence of NaHSO$_3$ | 9 |
| Scheme 1.6 | Preparation of benzimidazoles using the method of Bahrami et al. | 10 |
| Scheme 1.7 | The preparation of the benzimidazoles from MW irradiation | 10 |
| Scheme 1.8 | Preparation of benzimidazoles using the method of Elderfield and Kreysa | 11 |
| Scheme 1.9 | Benzimidazoles from $\beta$-diketones | 11 |
| Scheme 1.10 | Preparation of benzimidazoles using the method of Mamedov et al. | 12 |
| Scheme 1.11 | Benzimidazoles from $o$-nitroarylamines | 12 |
| Scheme 1.12 | Benzimidazoles from $o$-azidoarylamines | 13 |
| Scheme 1.13 | Benzimidazoles from amidines | 14 |
| Scheme 1.14 | Synthesize benzimidazoles by MW assay using the method of Lim et al. | 14 |
| Scheme 1.15 | Synthesize benzimidazoles with Na$_2$S$_2$O$_5$ using the method of Ozden et al. | 15 |
| Scheme 1.16 | Synthesize benzimidazoles with NaHSO$_3$ using the method of Kilcigil et al. | 16 |
| Scheme 1.17 | Synthesize benzimidazoles with $p$-toluenesulfonic acid using the method of Rao et al. | 16 |
| Scheme 1.18 | Benzimidazoles from MW irradiation by K-10 clay | 17 |
Scheme 1.19  Synthesize benzimidazoles with 5-10% Pd(PPh₃)₄ using the method of Brain and Brunton 17

Scheme 1.20  Synthesize benzimidazoles using the method Vazquez et al. 18

Scheme 1.21  Synthesize benzimidazoles using the method of Goker et al. and Page et al. 19

Scheme 1.22  Synthesize Schiff bases and benzimidazoles using the method of Smith and Ho 19

Scheme 1.23  Synthesize Schiff bases and benzimidazoles using the method of Latif et al. 20

Scheme 2.1  Synthetic route towards compound 75 30

Scheme 2.2  Synthetic route towards compounds 76 and 82 31

Scheme 2.3  Synthetic route towards compounds 77, 78, 79, 83 and 84 33

Scheme 2.4  Synthetic route towards compounds 80 and 81 34

Scheme 3.1  Synthetic route towards the preparation of compound 75 39

Scheme 3.2  Synthesize Schiff base 85 using the method of Danheiser et al. 55

Scheme 3.3  Formation of compound 76 55

Scheme 3.4  Preparation of compounds 77, 78 and 79 from 76 69

Scheme 3.5  Preparation of bis-Schiff base 80 107

Scheme 3.6  Preparation of bis-Schiff base 81 118

Scheme 3.7  Formation of benzeneamine 82 131

Scheme 3.8  The tetrahedral mechanism to form 76 or 82 132

Scheme 3.9  Formation of benzimidazoles 83 and 84 145

Scheme 3.10  Formation of benzimidazoles 77, 78, 83 and 84 from amino benzeneamines 76 or 82 146

Scheme 3.11  The proposed mechanism for the formation of bis-Schiff bases 79 or 86 from 2-amino benzeneamines 76 or 82 165

Scheme 3.12  The proposed mechanism to form a tetrahedral intermediate 87 by the cyclization of the bis-Schiff bases 79 or 86 166
Scheme 3.13  The proposed mechanism to form the benzimidazoles 78 or 84 by 1,3-H sigmatropic rearrangement of a tetrahedral intermediate 87
LIST OF ABBREVIATIONS

PPA = Polyphosphoric acid
DMF = Dimethyl formamide
DMA = \(N,N\)-Dimethyl acetamide
AcOH = Acetic acid
EtOH = Ethanol
MeOH = Methanol
CAN = Ceric ammonium nitrate
DCM = Dichloromethane
NMP = \(N\)-Methyl-2-pyrrolidone
MW = Microwave irradiation
Py. = Pyridine
MS = Molecular sieves
K-10 clay = montmorillonite
HATU = \(N,N,N',N'-\)Tetramethyl-O-(7-azabenzotriazole-1-yl) uranum hexafluorophosphate
DIPEA = \(N,N\)-Diisopropylethylamine
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TBAI = Tetra-\(n\)-butylammonium iodide
DMAP = \(N,N'\)-4-Dimethyl aminopyridine
PTC = Phase-transfer catalysts
TBAB = Tetra-\(n\)-butylammonium bromide
equiv. = equivalent
Temp. = Temperature
TLC = Thin layer chromatography
\(R_f\) = Retention factor
hr = hour
min = minute
mol = mole
\(\Delta\) = heat
rt = room temperature
FTIR = Fourier-Transform Infrared
as = asymmetric
sy = symmetric
\( \nu \) = stretching band
\( \delta \) = bending band

EIMS = Electron-Ionization Mass Spectrum
HRMS = High-Resolution Mass Spectrum
NMR = Nuclear Magnetic Resonance
\( \delta \) = chemical shift
ppm = part per million
TMS = tetramethylsilane

\( J \) = coupling constant
Hz = Hertz
MHz = Megahertz

s = singlet
d = doublet
t = triplet
q = quartet
m = multiplet
dd = double doublet
dq = double quartet

\( \text{br} \) = broad

APT = Attached Proton Test
DEPT = Distortionless Enhancement by Polarization Transfer
COSY = Correlation Spectroscopy
HMQC = Heteronuclear Multiple Quantum Coherence
HMBC = Heteronuclear Multiple Bond Coherence

\( ^{\circ} \) = degree
Å = angstrom unit (\( \equiv 10^{-10} \) m)
\( \mu \) = absorption coefficient
\( \theta \) = scattering angle
\( \lambda \) = X-ray wavelength
\( \sigma \) = standard error
\(hkl\) = Miller indices
\(F\) = structure factor
\(F_o\) = observed structure factor
\(I\) = reflection intensity
\(R\) = conventional residual, calculated from \(F_o\)-data
\(S\) = sign of a structure factor
\(T\) = temperature
\(V\) = volume
\(w\) = weight of a structure factor
\(wR\) = weighted residual, calculated from \(F_o\)-data
\(wR_2\) = weighted residual, calculated from \(F_o^2\)-data
\(Z\) = number of formula units per unit cell
\(MTT\) = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
\(DMEM\) = Dulbecco's Modified Eagle Medium
\(PBS\) = Phosphate Buffer Saline
\(HIFCS\) = Heat inactivated fetal calf serum
\(OD\) = optical density
\(IC_{50}\) = 50% inhibitory concentration
\(SD\) = Standard deviation
\(MCF–7\) = Breast cancer cell line
\(HCT–116\) = Colon cancer cell line
\(HT–29\) = Colon cancer cell line
\(MDA–MB–231\) = Human Breast cancer cell line
\(T–47D\) = Human Breast cancer cell line (Human breast ductal carcinoma cell)
\(HepG2\) = Liver cancer cell line (Human hepatocellular carcinoma)
\(K562\) = Leukaemia cell line (Human erythromyeloblastoid leukaemia cell line)
\(U937\) = Leukaemia cell line (Human leukemic monocyte lymphoma cell line)
## LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix A-X</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix A-1</td>
<td>Crystal Data of 75</td>
<td>207</td>
</tr>
<tr>
<td>Appendix A-2</td>
<td>Crystal Data of 76</td>
<td>208</td>
</tr>
<tr>
<td>Appendix A-3</td>
<td>Crystal Data of 77</td>
<td>209</td>
</tr>
<tr>
<td>Appendix A-4</td>
<td>Crystal Data of 78A</td>
<td>210</td>
</tr>
<tr>
<td>Appendix A-5</td>
<td>Crystal Data of 78B</td>
<td>211</td>
</tr>
<tr>
<td>Appendix A-6</td>
<td>Crystal Data of 79</td>
<td>212</td>
</tr>
<tr>
<td>Appendix A-7</td>
<td>Crystal Data of 80</td>
<td>213</td>
</tr>
<tr>
<td>Appendix A-8</td>
<td>Crystal Data of 81</td>
<td>214</td>
</tr>
<tr>
<td>Appendix A-9</td>
<td>Crystal Data of 82</td>
<td>215</td>
</tr>
<tr>
<td>Appendix A-10</td>
<td>Crystal Data of 83</td>
<td>216</td>
</tr>
<tr>
<td>Appendix B-1</td>
<td>Tables of ELISA spectroscopy results for MCF–7 growth inhibition assay with the increase in 77 concentrations</td>
<td>217</td>
</tr>
<tr>
<td>Appendix B-2</td>
<td>Tables of ELISA spectroscopy results for HCT–116 growth inhibition assay with the increase in 77 concentrations</td>
<td>218</td>
</tr>
<tr>
<td>Appendix B-3</td>
<td>Tables of ELISA spectroscopy results for MCF–7 growth inhibition assay with the increase in 78 concentrations</td>
<td>219</td>
</tr>
<tr>
<td>Appendix B-4</td>
<td>Tables of ELISA spectroscopy results for HCT–116 growth inhibition assay with the increase in 78 concentrations</td>
<td>220</td>
</tr>
<tr>
<td>Appendix B-5</td>
<td>Tables of ELISA spectroscopy results for MCF–7 growth inhibition assay with the increase in 83 concentrations</td>
<td>221</td>
</tr>
<tr>
<td>Appendix B-6</td>
<td>Tables of ELISA spectroscopy results for HCT–116 growth inhibition assay with the increase in 83 concentrations</td>
<td>222</td>
</tr>
<tr>
<td>Appendix B-7</td>
<td>Tables of ELISA spectroscopy results for MCF–7 growth inhibition assay with the increase in 84 concentrations</td>
<td>223</td>
</tr>
</tbody>
</table>
Appendix B-8  Tables of ELISA spectroscopy results for HCT–116 growth inhibition assay with the increase in concentrations
SINTESIS, PENCIRIAN DAN KAJIAN ANTI-PROLIFERASI TERBITAN BENZIMIDAZOL

ABSTRAK

bes bis-Schiff disahkan gelang enam- *illusory*, yang dihasilkan daripada pembentukan intramolekul ikatan hydrogen antara ikatan N=C–H dan kumpulan O–H pada gelang tritertukarganti yang disahkan dengan menggunakan analisis kristalografi sinar-X. Sebatian jenis keempat adalah benzimidazol 77, 78, 83 dan 84, yang telah dihasilkan daripada amino benzenamina 76 and 82, masing-masing, dalam media berakues atau berbes dengan mekanisme penambahan penyingkiran diikuti oleh mekanisme pensiklikan. Eksperimen NMR $^1$H, $^{13}$C, $^1$H–$^1$H COSY, HMQC dan HMBC dijalankan untuk mengesahkan kedudukan jalur pada 77, 78, 83 dan 84. Struktur kristal benzimidazol 77, 78 dan 83 disahkan dengan menggunakan analisis kristalografi sinar-X. Semua benzimidazol telah dinilaikan terhadap titisan sel kanser payudara MCF–7 dan titisan sel kanser kolon HCT–116 menggunakan kaedah MTT. Keputusan benzimidazol 84 menunjukkan aktiviti sitotoksik yang tinggi terhadap titisan sel *MCF–7* dengan IC$_{50}$ = 8.86 ± 1.10 µg/mL, dan aktiviti sitotoksik yang sederhana terhadap titisan sel *HCT–116* dengan IC$_{50}$ = 24.08 ± 0.31 µg/mL. Manakala benzimidazol 78 menunjukkan IC$_{50}$ rendah bernilai 16.18 ± 3.85 µg/mL terhadap titisan sel *HCT–116*. Kedua benzimidazol 77 dan 78 menunjukkan aktiviti sitotoksik yang sederhana terhadap titisan sel *MCF–7*, manakala benzimidazol 83 tidak menunjukkan sebarang aktiviti terhadap titisan sel *MCF–7* dan *HCT–116*.
SYNTHESIS, CHARACTERIZATION AND ANTI-PROLIFERATION STUDY OF SOME BENZIMIDAZOLE DERIVATIVES

ABSTRACT

Four different types of compounds were successfully synthesized and characterized. The syntheses of these compounds involved benzylation, addition elimination and cyclization reactions. The structures of the synthesized compounds were confirmed by melting points, FTIR, HRMS, 1D and 2D NMR spectroscopy and X–ray crystallography. The first type of these compounds is 2-benzyloxy-3-methoxybenzaldehyde 75, which was synthesized by the reaction of o-vanillin with benzyl bromide in acetone as the solvent and K$_2$CO$_3$ as a base in the presence of tetra-n-butylammonium iodide (TBAI) as catalyst. The second type of the studied compounds is 2-amino-N-benzylidene benzeneamines 76 and 82, which were synthesized from the reaction of o-phenylenediamine with o-vanillin or 75 in dichloromethane at cold conditions. Both $^1$H–$^1$H COSY and HMBC NMR experiments were performed to further confirm the assigned peaks of compounds in CDCl$_3$, CD$_2$Cl$_2$ and acetone-$d_6$. Some effect from those six– and five–membered illusory rings, which were formed from the intramolecular hydrogen bonding between N–H in the amino ring and the N atom in N=C–H bond and O–H with N atom of N=C–H of 76 or between N–H in the amino ring and the N atom in N=C–H bond and the bond of the methine C–H of 82 with oxygen atom in the benzyloxy ring. The crystal structures of both 76 and 82 confirmed the hydrogen bonds by using the X–ray crystallographic analysis. The third type of the studied compounds is bis-Schiff bases 79, 80 and 81, which are formed from the reaction between o-vanillin with o-, m- and p-phenylenediamines, respectively, by a tetrahedral mechanism or
addition/elimination mechanism. The crystal structures of those bis-Schiff bases confirmed six–membered illusory rings, which formed from the intramolecular hydrogen bonding between N=C–H bonds and O–H groups of the trisubstituted rings by using the X–ray crystallographic analysis. The fourth type of the studied compounds is benzimidazoles 77, 78, 83 and 84, which are converted from amino benzeneamines 76 and 82, respectively, in aqua or basic media by addition/elimination mechanism followed by cyclization. Those benzimidazoles have been evaluated against both breast cancer cell line MCF–7 and colon cancer cell line HCT–116 using MTT assay. The results of benzimidazole 84 showed high cytotoxic activity against MCF–7 cell lines with IC_{50} = 8.86 ± 1.10 μg/mL, and moderate cytotoxic activity against HCT–116 cell lines with IC_{50} = 24.08 ± 0.31 μg/mL. Benzimidazole 78 showed the lowest IC_{50} value at 16.18 ± 3.85 μg/mL against HCT–116 cell lines. Both benzimidazoles 77 and 78 showed moderate cytotoxic activity against MCF–7 cell lines, while benzimidazole 83 showed no cytotoxic effect with both MCF–7 and HCT–116 cell lines.
CHAPTER ONE

INTRODUCTION

1.1 Schiff bases

Schiff bases or imines are compounds formed from the condensation reaction between aldehydes or ketones and primary amines or amino acids in the presence of an acid catalyst (Figure 1.1). The product containing an azomethine, C=N linkage was discovered by Hugo Joseph Schiff (Layer, 1963; Cozzi, 2004). Many nomenclatures are used to describe the formation of Schiff bases, for example: aldimines, anils, benzanils and ketimines, derived from aldehydes, aniline, aromatic aldehydes and ketones, respectively (Layer, 1963; El–Bayoumi et al., 1971a,b).

![General formula of Schiff base or imine](image)

Figure 1.1: The general formula of Schiff base or imine.

These compounds are ranked among the most versatile synthetic organic intermediates, which are important for the synthesis of biologically important compounds. They are widely used as medical, pharmaceutical and industrial materials and were reported to show a variety of biological activities including as antifungal (Singh and Dash, 1988; More et al., 2001), antibacterial (Baseer et al., 2000; El-Masry et al., 2000; Kabeer et al., 2001) and anticancer (Kuz'min et al., 2000; Desai et al., 2001), among others. They also showed moderate activity against *Staphylococcus aureus* and *Bacillus subtilis* (Jarrahpour et al., 2004).
They have also been used extensively in inorganic and coordination chemistry fields for the synthesis of new organometallic compounds. The imines formed from the reaction of the aldehydes and amines also proved to be the source of versatile compounds for many transition metals where they act as donor groups to bind the metal ions (Vigato and Tamburini, 2004). Besides that, Schiff bases are also used to produce new azo dyes (Jarrahpour and Zarei, 2004, Naeimi et al., 2007). In another application, So et al. (2007) synthesized and characterized a series of Schiff base derivatives, which exhibited liquid crystal properties.

Bis-Schiff bases are produced from two equivalents of aldehyde or ketone with a diamine. The bases can be either symmetric or unsymmetric (asymmetric) structures, depending on the reacted aldehydes and diamines. Figure 1.2 shows examples of symmetric and unsymmetric bis-Schiff bases 1-4 (Lopez et al., 1998a,b). Compounds 1 and 2 started off with symmetrical aromatic diamine ring, but the aldehyde rings are different. Compound 3 has symmetrical cyclic diamine ring with symmetrical aldehyde rings, while both diamine and aldehyde in compound 4 are unsymmetric.

Figure 1.2: Some symmetrical and unsymmetrical bis-Schiff bases.
Both symmetrical and unsymmetrical bis-Schiff bases are interesting compounds particularly when they coordinate around the central metal ion to form transition metal ion complexes. For example, the symmetric bis-Schiff base complexes are normally used as macrocyclic models, while the unsymmetric complexes are used as irregular binding model with peptides (Atkins et al., 1985). However, the unsymmetrical compounds are more advanced than their symmetrical counterparts in the explaining of the composition and geometry of metal ion binding sites in metalloproteins, leading to the enzymatic selectivity of the natural systems with synthetic materials (Bu et al., 1997). Intercalation of other derivatives of symmetrical bis-Schiff bases with DNA by UV spectroscopy has also been studied (Parra et al., 2007).

Various molecular structural studies on bis-Schiff bases and their complexes with different metal ions have been reported. Bis-Schiff bases and others were found to form suitable inner coordination sphere between tin atom with O and N atoms as quadridentate chelates (Teoh et al., 1997). Ruthenium complexes of bis-Schiff bases derived from o-vanillin and salicyldehyde however, showed dibasic tetradentate chelates (Viswanathamurthi et al., 1998). An intramolecular hydrogen bond between the O and N atoms in Schiff bases is one of the important factors leading to the formation of metal complexes (Cohen et al., 1964). Kabak et al. (2000) prepared the derivatives of bis-Schiff bases and studied their photochromic conformational properties. The intramolecular hydrogen bond causes a six–membered illusory ring to be formed between the O–H group at ortho position of the aldehydic moiety and N atom of the aminic moiety (Kawasaki et al., 1999; Fukuda et al., 2003); (Figure 1.3).
1.2 Benzimidazoles

Benzimidazole (Figure 1.4) is a heterocyclic compound containing an imidazole ring fused at the 4,5-position with benzene ring. Hobrecker prepared the first benzimidazole in 1872 when he reduced 2-nitro-4-methylacetanilide, 5 to obtain the tautomers 2,5 (or 2,6)-dimethyl benzimidazole (6), (White, 1951; Hofmann, 1953); (Scheme 1.1).

Since benzimidazoles have similar structures to purines, whose derivatives play important roles in biological systems; substituted benzimidazoles also showed interesting biological activities. For example, the ring of the benzimidazoles is found
as an integral part in the chemical structure of vitamin B₁₂, 7 (Bonnett, 1963; Preston, 1974); (Figure 1.5).

![Figure 1.5: The benzimidazole ring in the chemical structure of vitamin B₁₂, 7.](image)

Many benzimidazoles are pharmaceutical agents and used widely in biological system applications (Townsend and Revankav; 1970; Trivedi et al., 2006). Some derivatives of benzimidazoles were reported and used as antiviral agents (8-9, Gudmundsson et al., 2000; Cheng et al., 2005), topoisomerase I inhibitors (10-11, Kim et al., 1996, 1997; Rangarajan et al., 2000; Mekapati and Hansch, 2001) and as antiproliferative agent (12, Hong et al., 2004), (Figure 1.6).

Some of 4,5,6,7-tetrahalo-1H-benzimidazoles (13) were synthesised and showed antiprotozoal activity against *Acanthamoeba castellanii* (Kopanska et al., 2004), antimycobacterial activity against *Mycobacterium* strains (14, Kazimierczuk et al., 2005). In other studies, they can also act as antibacterial agents (15-16, Ozden
et al., 2005; Nezhad et al., 2005), and showed anthelmintic activity against *Trichinella spiralis* (Mavrova et al., 2005), anti-inflammatory and analgesic activities (17, Sondhi et al., 2006) and as inhibitors for hepatitis B (Li et al., 2006) and C viruses (18, Beaulieu et al., 2004). Some benzimidazoles were also tested as anti-HIV (19, Roth et al., 1997; Smith et al., 2003) and anticancer agents (20-21, Craigo et al., 1999; Rida et al., 2006).

Recent publication also reported the use of phenolic and anisolic benzimidazole derivatives in vasodilator and antihypertensive studies (Soto et al., 2006), while other alkylxoyaryl benzimidazole derivatives have been tested for the spasmolytic activity (Vazquez et al., 2006). Figure 1.7 shows the chemical structures of many useful benzimidazole derivatives evaluated for biological activities.
CHAPTER ONE                                                                     INTRODUCTION

Figure 1.7: Some benzimidazole derivatives evaluated for biological activity.

The preparation of benzimidazole derivatives is well established. Monosubstitution occurs mainly at position 2, while the disubstitution at positions 1 and 2.
1.2.1 Substitution at position 2

1.2.1.1 Reaction of o-arylene diamines with carboxylic acids

The Philips-type method follows the reaction of o-arylene diamines with carboxylic acids or their derivatives using hydrochloric acid or polyphosphoric acid (PPA) as a catalyst to produce the respective benzimidazoles 22, (Scheme 1.2). The benzimidazoles can also be produced from both reactants at 18 °C for 120 hr at pH 0.5 (Haley and Maitland, 1951).

\[
\begin{array}{c}
\text{NH}_2 \hspace{1cm} \text{NH}_2 \\
\text{HO} \hspace{1cm} \text{O} \\
\text{N} \hspace{1cm} \text{N} \\
\text{R} \hspace{1cm} \text{HCl or PPA} \\
\text{R} = \text{H, CH}_3, \text{CHCl}_2, \text{CCl}_3, \text{CF}_3, \text{Ph}, \text{CH}_2\text{Ph}
\end{array}
\]

Scheme 1.2: The Phillips-type to prepare the benzimidazole.

In solvent-free conditions with 7-10 mol % 8N HCl as a catalyst, a mixture of o-arylene diamines with carboxylic acids or their derivatives reacted in a domestic microwave oven at 600 W for 10 min to produce benzimidazoles 23 (Wang et al., 2007; Scheme 1.3).

\[
\begin{array}{c}
\text{NH}_2 \hspace{1cm} \text{NH}_2 \\
\text{HO} \hspace{1cm} \text{O} \\
\text{N} \hspace{1cm} \text{N} \\
\text{R}_1 \hspace{1cm} \text{R}_2 \\
\text{MW, HCl} \hspace{1cm} \text{Solvent-free}
\end{array}
\]

Scheme 1.3: Preparation of benzimidazoles using the method of Wang et al.

1.2.1.2 Reaction of o-arylene diamines with aldehydes

Kamal et al. (2004) prepared benzimidazoles 24 by reacting o-arylene diamines with 4-hydroxybenzaldehydes in ethanolic solution under reflux in the presence of Na\(_2\)S\(_2\)O\(_5\) as oxidizing reagent, while Falco et al. (2006) used the same oxidizing reagent to prepared benzimidazoles 25 in dimethylformamide (DMF) under reflux for 12 hr, (Scheme 1.4).
**Scheme 1.4: Benzimidazoles preparation in the presence of Na$_2$S$_2$O$_5$.**

Other benzimidazole derivatives 26-27 were yielded by refluxing o-arylene diamines with benzaldehydes in the presence of NaHSO$_3$ as oxidizing reagent at 140 °C in $N,N$-dimethylacetamide (DMA) or in EtOH/H$_2$O at 60 °C, respectively (White et al., 2004; Safonov et al., 2006); (Scheme 1.5).

**Scheme 1.5: Benzimidazoles preparation in the presence of NaHSO$_3$.**
Meanwhile, Bahrami et al. (2008) described a new convenient method for the synthesis of benzimidazole derivatives 28 by the reaction of substituted o-arylene diamines with aromatic aldehydes in the presence of ceric ammonium nitrate (CAN) as a catalytic redox reagent and H$_2$O$_2$ in free solvent for 10-70 min at 50 °C; (Scheme 1.6). Under microwave irradiation at 1000 W, the reaction between aldehydes and o-arylene diamines afforded the benzimidazole derivatives 29 (Soto et al., 2006; Scheme 1.7).

\[
\begin{align*}
\text{Scheme 1.6: Preparation of benzimidazoles using the method of Bahrami et al.}
\end{align*}
\]

\[
\begin{align*}
\text{Scheme 1.7: The preparation of the benzimidazoles from MW irradiation.}
\end{align*}
\]

1.2.1.3 Reaction of o-arylene diamines with ketones

2-Disubstituted benzimidazoline 31 was formed from the reaction between o-arylene diamines and ketones by elimination of water, which decomposed under heat to produce 2-substituted benzimidazoles, 32-33 and hydrocarbons (Elderfield and Kreysa, 1948); (Scheme 1.8). The intermediate 31 was isolated with its tautomer 30 (Elderfield and Meyer, 1954a,b).
1.2.1.4 Benzimidazole derivatives from $\beta$-diketone

Benzimidazole derivatives can also be synthesized from $\beta$-diketone. This was described by Rossi et al. (1960) when $\alpha$-phenylenediamine was heated with $\beta$-diketone derivatives in xylene with acidic media to furnish good yields of 34-35 (Scheme 1.9).

1.2.1.5 Benzimidazoles from the reaction of $\alpha$-arylene diamines with quinoxalin-2-one derivatives

Novel benzimidazoles were obtained from the reaction between $\alpha$-arylene diamines and quinoxalin-2-one derivatives under reflux in acetic acid for 2 hr to afford 2-benzimidazolylquinoxaline derivatives, 35. Good yield of 35 was obtained,
which depends on the position of $R$ in $o$-phenylenediamine (Mamedov et al., 2008); (Scheme 1.10).

\[ \text{NH}_2 \quad \text{NO}_2 \quad \text{R} \]

\[ \text{O} \quad \text{N} \quad \text{H} \]

\[ \text{R}_1, \text{R}_2 = \text{H}, \text{CH}_3, \text{NO}_2 \]

\[ \text{R}_3 = \text{H}, \text{CH}_3 \]

Scheme 1.10: Preparation of benzimidazoles using the method of Mamedov et al.

1.2.1.6 Benzimidazoles from $o$-nitroarylamines

The reaction between $o$-nitroarylamines and aldehydes forms $N$-benzylidene-2-nitroaniline derivatives 36, which was converted to 2-substituted benzimidazole derivatives 37 by the reductive cyclization using trialkyl phosphite (Smith and Suschitzky, 1961; Scheme 1.11). The trialkyl phosphite which acts as a reducing agent reduced the nitro group, followed by intramolecular cyclization process to produce 37 (Grimmett, 1997).

\[ \text{NH}_2 \quad \text{NO}_2 \quad \text{R} \]

\[ \text{O} \quad \text{N} \quad \text{H} \]

\[ \text{R}_1, \text{R}_2 = \text{H}, \text{CH}_3, \text{NO}_2 \]

\[ \text{R}_3 = \text{H}, \text{CH}_3 \]

Scheme 1.11: Benzimidazoles from $o$-nitroarylamines.
1.2.1.7 Benzimidazoles from o-azidoanilines

Thermolysis method is used to react o-azidoarylamines with aldehydes to produce good yields of N-benzylidene-2-azidoaniline derivatives, 38, which were converted to 2-substituted benzimidazole derivatives, 39 (Krbechek and Takimoto, 1964; Hall and Kamm, 1965). Recently, Shen and Driver (2008) synthesized benzimidazoles from o-azidoanilines in two steps; using MgSO₄ in dichloromethane (DCM) at room temperature for 36 hr, followed by the addition of 30 mol % FeBr₂ as a catalyst with 4 Å molecular sieves in DCM at 40 °C for 12 hr (Scheme 1.12). Ferrous bromide, a Lewis acid was coordinated to the imine nitrogen to increase the electronegativity of 38, followed by the reduction to generate 40, which dissociated from iron catalyst to produce 41. The tautomerisation of 41 gave 39.

Scheme 1.12: Benzimidazoles from o-azidoarylamines.
1.2.1.8 Benzimidazoles from amidines

There are two ways of synthesizing benzimidazole derivatives 42 from amidines; i) by the reaction of \(N\)-arylamidines with benzenesulfonyl chloride in a base under anhydrous conditions (Preston, 1974), ii) by the oxidation of the amidines using sodium hypochlorite in basic media via \(N\)-chlooramidines 43 (Grenda et al., 1965); (Scheme 1.13).

\[
\begin{align*}
\text{N} & \text{OH} \quad \text{R} & \text{NH}_2\text{Cl} \\
\text{O} & \text{Cl} & \text{S} & \text{OO} \\
\text{N} & \text{H} & \text{R} & \text{NH}_2\text{Cl} \\
\text{H} & \text{R} & \text{Cl} & \text{OH} \\
\text{NaOCl}, \text{OH}^- & & & \\
& \text{Py}. \\
\text{OH}^- & & & \\
\text{NaOCl} \\
\end{align*}
\]

Scheme 1.13: Benzimidazoles from amidines.

1.2.1.9 Benzimidazoles from resin-bound esters

Lim et al. (2008) prepared 2-substituted benzimidazoles 44 from the condensation process between \(o\)-arylene diamines and resin-bound esters under microwave irradiation condition in the presence of PPA and \(N\)-Methyl-2-pyrrolidone (NMP) at 150 °C for 10 min and then 230 °C for 30 min to give an excellent yield (Scheme 1.14).

\[
\begin{align*}
\text{NH}_2 & & \text{O} & \text{O} & \text{NH}_2 \\
\text{R}_1 & & \text{R}_2 & & \\
\text{MW,} & & \text{150 °C, 10 min;} & & \text{230 °C, 30 min} \\
\text{15% PPA in NMP} & & & & \\
\text{R}_1 & = & \text{H, 4-CH}_3, \text{4-F}, & & \\
\text{R}_2 & = & & & \\
\end{align*}
\]

Scheme 1.14: Synthesize benzimidazoles by MW assay using the method of Lim et al.
1.2.2 1,2-Disubstitution of benzimidazoles

1.2.2.1 Benzimidazoles from \( \sigma \)-arylene amines

The protection of one of the amino groups in \( \sigma \)-arylene amines produced a derivative of \( N \)-substituted \( \sigma \)-arylenediamine 45. A cyclization of 45 with the second molecule of aldehyde derivatives in the presence of \( \text{Na}_2\text{S}_2\text{O}_5 \) as an oxidizing reagent produced the benzimidazole derivative 46 (Ozden et al., 2005); (Scheme 1.15).

\[
\text{R}_1 = \text{H, } n\text{-propyl, benzyl, 2,4-Cl benzyl,}
\text{R}_2 = \text{CH}_3, \text{CH}_2\text{CH}_3,
\text{R}_3 = \text{COOH, CN.}
\]

Scheme 1.15: Synthesize benzimidazoles with \( \text{Na}_2\text{S}_2\text{O}_5 \) using the method of Ozden et al.

Kilcigil et al. (1999, 2003) used oxidative condition by adding \( \text{NaHSO}_3 \) in DMF at 110 \( ^\circ \text{C} \) for 4.5 hr to prepare the benzimidazoles 47 from 45 (Scheme 1.16). Both 46 and 47 were evaluated as having antimicrobial activity against for \( \text{Candida albicans} \). The reaction of two moles of aldehyde derivatives with one mole of \( \sigma \)-arylene amine in the presence of a catalytic amount of \( \text{p-toluene} \text{ sulfonylic acid} \) produces 2-arylbenzimidazoline 48, which is converted to alcohols and 2-arylbenzimidazole derivative 49 by redox process. Benzimidazole derivative 50 is generated when 49 is heated in ethanolic solution. The product was then tested as an anti-HIV agent (Rao et al., 2002); (Scheme 1.17).
Scheme 1.16: Synthesize benzimidazoles with NaHSO₃ using the method of Kilcigil et al.

Scheme 1.17: Synthesize benzimidazoles with p-toluenesulfonic acid using the method of Rao et al.

In another method, Perumal et al. (2006) reacted two moles of various aldehydes with o-phenylenediamines in the presence of montmorillonite K-10 under microwave irradiation and in the absence of solvent to produce bis-Schiff bases 51, which is cyclized thermally to benzimidazole derivatives 52; (Scheme 1.18).
1.2.2.2 Benzimidazoles from o-haloarylamines

The amidation of o-haloarylamine to 53 occurred by heating a mixture of 2-bromoanilines with the amides in the presence of POCl₃ in toluene. It was then treated with 5–10% Pd(PPh₃)₄ catalyst mixed with NaO'Bu and K₂CO₃ in toluene for 18 hr under reflux to yield benzimidazole 54 (Brain and Brunton, 2002); (Scheme 1.19).

![Scheme 1.19: Synthesize benzimidazoles with 5-10% Pd(PPh₃)₄ using the method of Brain and Brunton.](image)

1.2.2.3 Benzimidazoles from o-nitroarylamines

The acetylation of o-nitroarylamines with Ac₂O using H₂SO₄ as catalyst produced the acetanilide 55, which was treated with Me₂SO₄ and KOH to form N-methylated acetamide 56. Addition of H₂SO₄, hydrolyzed 56 to N-methyl-2-
nitroaniline, 57, which was reduced by H₂ and Ni-Raney to afford o-phenylenediamine 58. The benzimidazole 59 was produced by refluxing 58 with CF₃COOH. Compound 59 showed antiparasitic activity against *Giardia intestinalis*, *Trichomonas vaginalis* and *Plasmodium falciparum* (Vazquez et al., 2006); (Scheme 1.20).

Scheme 1.20: Synthesize benzimidazoles using the method of Vazquez *et al.*

### 1.2.2.4 Benzimidazoles from o-halonitrobenzenes

Many researchers have reported the methods to synthesize benzimidazoles 62 and 63 starting from o-halonitrobenzene derivatives, which was then aminated to produce o-nitroarylamine 60. The reduction of 60 with NiCl₂/Zn or with H₂ and Pd/C afforded o-phenylenediamine derivative 61. The cyclization of 61 with corresponding benzaldehydes in the presence of Cu(AcO)₂/H₂S, or in the presence of Na₂S₂O₅ in DMF gave benzimidazoles 62 and 63 (Goker *et al.*, 1998, 2002). However, using carboxylic acid derivatives in the presence of *N*,*N*,*N′*,*N′*-tetramethyl-*O*-(7-azabenzotriazole-1-yl) uranium hexafluorophosphate (HATU) as a coupling reagent and a base *N*,*N*-diisopropylethylamine (DIPEA) or Hunig’s base in DMF under acidic condition, afforded benzimidazole 63 (Page *et al.*, 2008; Scheme 1.21).
1.2.3 Synthesis leading to the formation of both Schiff base and benzimidazoles

Smith and Ho (1971) described the reaction of o-phenylenediamine with benzaldehyde by oxidative process in their preparation of 2-amino-N-benzylidene aniline, 64, N,N'-dibenzal-o-phenylenediamine, 65, 2-phenylbenzimidazole, 66 and 1-benzyl-2-phenylbenzimidazole, 67 (Scheme 1.22).
Latif et al. (1983) reported in great detail the reaction of some phenolic aldehydes with \( o \)-phenylenediamine in boiling ethanolic solution, and they isolated the intermediates, 68. Bis-Schiff bases 69 and benzimidazoles 70-71 produced from the reaction in most cases depended largely on the substituents in the phenyl ring of the aldehyde and the solvent used in the reflux process (Scheme 1.23).

Both methods resulted in the formation of intermediates 64 and 68 from the reaction of one molecule of aldehyde derivatives with \( o \)-phenylenediamine under low temperature. However, the bis-Schiff bases 65 and 69, including the benzimidazoles 66, 67, 70 and 71 were formed when 64 or 68 reacted with the second molecule of aldehyde derivative under reflux with nitrobenzene or 1-butanol.

Benzimidazole 73 is an analogue of the natural products 72, which were synthesized from benzoic acid in multiple steps by Kumar et al. (2002) which produced moderate yields. Other benzimidazole derivative 74 was also prepared from the condensation of benzoic acid with \( o \)-phenylenediamine derivative. The natural products 72 were isolated from acetone extract of *Streptomyces* sp. 517-02.
(Shibata et al., 1993; Ueki et al., 1993; Ueki and Taniguchi, 1997) and from Streptomyces sp. AJ956 (Sato et al., 2001, Figure 1.8). Both 73 and 74 lacked of cytotoxicity activity against a range of cancer cell lines (Kumar et al., 2002; Huang et al., 2006).

![Figure 1.8: Benzimidazole derivatives evaluated against cancer cell lines.](image)

1.3 **Scope of this work**

In the current project, four types of different structures were targeted. 2-Benzylkoxy-3-methoxy-benzaldehyde or benzyl o-vanillin 75, two of 2-amino-N-benzylidene benzeneamines 76 and 82, three of bis-Schiff bases 79, 80 and 81 and four of benzimidazoles 77, 78, 83 and 84 were synthesized with modified methods and characterized by FTIR, HRMS, 1D and 2D NMR spectroscopy and X-ray crystallography (Figures 1.9-1.12).

Benzyl o-vanillin 75 was prepared to protect the hydroxyl group of o-vanillin, which was benzylated using modified preparation method. The comparison of the o-vanillin products and its derivative 75 reactions with o-phenylenediamine was studied. Both aldehydes o-vanillin and 75 were used to prepare benzimidazole derivatives 77, 78, 83 and 84 via amino benzeneamines intermediates 76 and 82, respectively.
Bis-Schiff base 79 was also found as a main product from the reaction between o-vanillin with o-phenylenediamine, while both bis-Schiff bases 80 and 81 were prepared from the reaction of o-vanillin with m-phenylenediamine and p-phenylenediamine, respectively. The benzimidazoles 77, 78, 83 and 84 were evaluated as anti-proliferation with MTT assay.
Figure 1.11: Structures of bis-Schiff bases 79, 80 and 81.

Figure 1.12: Structures of benzimidazoles 77, 78, 83 and 84.
1.3.1 The objectives

The objectives of this work are listed as follows:

(a) To synthesize 2-benzyloxy-3-methoxybenzaldehyde, 75.

(b) To synthesize both 2-amino-N-benzylidene benzeneamines 76 and 82.

(c) To synthesize bis-Schiff bases, 79, 80 and 81.

(d) To synthesize the benzimidazole derivatives, 77, 78, 83 and 84.

(e) To elucidate the structures of the synthesized compounds using FTIR, HRMS, NMR and X–ray crystallography techniques (if applicable).

(f) To evaluate the benzimidazoles 77, 78, 83 and 84 as anti-proliferation with MTT assay.
2.1 Chemicals

The chemicals that were used throughout the project are listed as follows, which were used without further purification:

- **Acetone** (AR grade) Riedel-de Haën, Germany.
- **Acetone-D6** (acetone-\(d_6\)) contains 0.03 v/v % TMS (for NMR spectroscopy) Sigma-Aldrich, USA.
- **Anhydrous Magnesium sulfate** Fluka Chemika, Switzerland.
- **Benzyl bromide** Fluka Chemika, Switzerland.
- **Chloroform** (\(\text{CHCl}_3\)), (AR grade) Riedel-de Haën, Germany.
- **Chloroform-D** (\(\text{CDCl}_3\)) contains 0.03 v/v % TMS (for NMR spectroscopy) Sigma-Aldrich, USA.
- **Dichloromethane** (DCM), (AR grade) Riedel-de Haën, Germany.
- **Dichloromethane-D2** (\(\text{CD}_2\text{Cl}_2\)) contains 0.03 v/v % TMS (for NMR spectroscopy) Sigma-Aldrich, USA.
- **Diethyl ether** Riedel-de Haën, Germany.
- **Dimethyl sulfoxide** (DMSO) Sigma-Aldrich, USA.
- **Dulbecco's Modified Eagle Medium** (DMEM) Gibco, Life technology, UK.
- **Ethanol** Riedel-de Haën, Germany.
- **HCT–116** ATCC, Rockville, MD, USA.
- **Heat inactivated fetal calf serum** (HIFCS) Sigma-Aldrich, Germany.
- **\(n\)-Hexane** Riedel-de Haën, Germany.
- **2-Hydroxy-3-methoxybenzaldehyde** (\(o\)-vanillin) Sigma-Aldrich, USA.
- **McCoy’s (growth medium)** Gibco, Life technology, UK.