



SYNTHESIS AND COMPUTATIONAL STUDIES OF
SYDNONE-BASED DERIVATIVES AS POTENTIAL
ANTI-INFLAMMATORY AGENTS

BY

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ABSTRACT

Various literature sources have documented that sydnones are important bioactive molecules with a wide spectrum of activities involving the anti-proliferative and anti-inflammatory actions. Phenyl styrylketones and their derivatives as members of the chalcone family have also been reported as significant biological agents. The current study was initiated to evaluate *in vitro* cytotoxic and anti-inflammatory activity of sydnone-based compounds including some novel sydnone-styrylketone hybrids. The classical cyclodehydration of *N*-nitroso amino acids was applied in the preparation of the sydnone ring. Aldol condensation was utilized to join two sydnone rings by a styrylketone linker. Compounds identity was confirmed using FTIR, NMR and MS spectroscopy. MTT assay was used to evaluate the cytotoxicity of the synthesized compound. ELISA test was performed to investigate the COX-1/COX-2 inhibitory activity. The binding of sydnones with COX enzymes was examined by Glide docking and the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA). The drug-likeness scores and membrane permeability of the compounds were also *in silico* predicted. Twenty-five sydnone-containing compounds were synthesized. Compounds **46-48** and **56-58** were reported as new sydnone derivatives. Compounds **61-63** were synthesized as novel structures containing two sydnone rings linked via α,β -unsaturated ketone. The structure of the synthesized compounds was confirmed by FTIR, ^1H NMR, ^{13}C NMR and ToF-MS analyses. All compounds exhibited low cytotoxicity especially against normal cell lines (IC_{50} in the range of mM). Only compound **45** had a significant antiproliferative activity against prostate ($\text{IC}_{50} = 42 \mu\text{M}$) and breast ($\text{IC}_{50} = 63 \mu\text{M}$) cancer cell lines. The *in vitro* COX inhibition assay showed varied activity. Compounds **47**, **51**, **58** and **63** showed the most potent COX inhibitory effect at a concentration of 200 μM . Selectivity index showed that only compound **63** was a selective COX-2 inhibitor. Acetylation of the sydnone ring at C4 was detrimental to the cytotoxic activity while prolific for the anti-inflammatory effects (COX inhibition). Docking analysis showed that COX-2 selectivity was due to a favorable positive charged interaction between the sydnone ring of **63** and Arg513 in the catalytic region of COX-2. Compound **51** was hydrogen bonded to the guanidinium group of Arg513. The low inhibitory effect of **63** against COX-1 was due to an unfavorable polar interaction with His513 in the binding pocket of COX-1. Drug-likeness prediction disclosed that the compounds comply with Lipinski's rule and CMC-like rule. Similarity search delineated that sydnone-styrylketone hybrids had common structural features with known anti-inflammatory agents. Prediction of permeability through the physiological membrane revealed a good pharmacokinetic profile with intestinal absorption more than 80% and a potential BBB penetration. In conclusion, the compounds were successfully synthesized and characterized. However, only two compounds **59** and **60** were not successfully prepared. The structure of 3-(4-chloro-3-nitrophenyl)sydnone **45** could be a lead molecule in designing potent chemotherapeutic agents. Compound **63** shared architecture and pharmacophoric characters with known selective COX-2 inhibitors (coxib family) making it a good candidate for designing selective and safe NSAID.

خلاصة البحث

أثبتت مصادر علمية عديدة الأهمية الحيوية لمركبات السيدنون في مجالات متنوعة وخاصة الفعالية المضادة للتكاثر الخلوي والمضادة للالتهاب. تعتبر مركبات الفينيل ستيريل كيتون من عائلة الشالكون وهي ذات فعالية علاجية قوية. الهدف من هذه الدراسة هو تقييم الفعالية الخلوية السمية والمضادة للالتهاب في الزجاج لمركبات ثنائية السيدنون مرتبطة بالفينيل ستيريل كيتون. استخدمت الطريقة التقليدية في تحلق مركبات نتروزو الحموض الأمينية لتحضير حلقة السيدنون. استخدم التكاثر الألدولي لربط حلقتي سيدنون برابط الستيريل كيتون. وتم التأكد من بنية المركبات باستخدام طرائق المطيافية. تم تقييم الارتباط الجزيئي لمركبات السيدنون مع أنزيمات الكوكس باستخدام تقنية غلايد *Glide* والطاقة الحرة للارتباط *MM-GBSA*. بالإجمال تم اصطناع خمسة وعشرين مركباً. وكانت المركبات 46-48 و 56-58 مشتقات جديدة للسيدنون. المركبات 61-63 اعتبرت على أنها بنية جديدة تماماً. أظهرت كل المركبات فعالية ضعيفة سامة للخلايا وخاصة ضد الخلايا الطبيعية. فقط مركب 45 أظهر فعالية قوية كمضاد لسرطان البروستات ($IC_{50} = 42$ ميكرومولار) وسرطان الثدي ($IC_{50} = 63$ ميكرومولار). أظهرت مركبات 47 و 51 و 58 و 63 فعالية مثبطة لأنزيم الكوكس وكان المركب 63 أكثر المركبات انتقائية على الكوكس -2. أظهرت دراسة الارتباط الجزيئي أن المركب 63 يرتبط مع الكوكس -2 من خلال الحمض الأميني Arg513 من خلال تفاعل الشحن الموجبة. انخفاض الفعالية المثبطة لمركب 63 تجاه الكوكس -1 بسبب ارتباط غير قطبي غير ثابت مع الحمض الأميني His513. أثبتت الدراسة أن مركب 63 يمتلك تشابه بنيوي مع المركبات المعروفة المضادة للالتهاب. أظهرت دراسة النفاذ عبر الأغشية عن طريق الحاسب أن المركبات المصطنعة تمتلك حرائك دوائية جيدة من خلال امتصاصية عالية عبر الغشاء المعوي وحاجز الدماغ الوعائي. خلاصة البحث: تم اصطناع المركبات بنجاح باستثناء مركبين 59 و 60. يمكن اعتبار بنية المركب 45 دليلاً جيداً لتطوير مركبات قوية لعلاج السرطان. إن المركب 63 يمتلك خواص دوائية وبنوية مشابهة للمركبات الانتقائية على كوكس -2 ولذلك يمكن اعتباره مركب جيد كدليل لتطوير وتحضير صيغ جديدة مضادة للالتهاب.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Special thanks to you dear great Mom, your selfless spiritual imparted moral support have always been with me helping me in achieving my goals. Tons of thanks to my beloved Dad, you've sacrificed both personally and professionally for me to chase down my dreams. I have to express my feelings of appreciations to you my dear wife, most especially if I remember your love towards me. Millions thanks to my brothers and sisters for their continuous support and motivation.

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TABLE OF CONTENTS

Abstract.....	ii
Abstract in Arabic.....	iii
Approval Page.....	iv
Declaration.....	v
Copyright Page.....	vi
Acknowledgements.....	viii
Table of contents.....	ix
List of Tables.....	xv
List of Figures.....	xix
List of Abbreviations.....	xxiii
CHAPTER ONE: INTRODUCTION.....	1
1.1 RESEARCH BACKGROUND.....	1
1.2 PROBLEM STATEMENT.....	2
1.3 RESEARCH OBJECTIVES.....	3
1.4 RESEARCH HYPOTHESIS.....	3
CHAPTER TWO: LITERATURE REVIEW	4
2.1 CHEMISTRY OF SYDNONE	4
2.1.1 Definition of Sydnone	4
2.1.2 Synthesis of Sydnone	6
2.1.3 Physicochemical Properties of Sydnone	10
2.1.3.1 Electronic Structure of Sydnone Ring	10
2.1.3.2 Sydnones Spectral Studies	12
2.1.3.2.1 Ultraviolet (UV) Spectroscopy	12
2.1.3.2.2 Infrared (ID) Spectroscopy	14
2.1.3.2.3 Nuclear Magnetic Resonance (NMR) spectroscopy ...	14
2.1.3.3 Chemical Properties of Carbon C4 of the Sydnone Ring	15
2.1.3.3.1 Acylation of Formylation of Sydnone Ring	15
2.1.3.3.2 Halogenation of Sydnone Ring	17
2.1.3.3.3 Sydnone Lithiation and Its Application	18
2.2 THE BIOLOGICAL ACTIVITIES OF SYDNONE	19
2.2.1 Anti-inflammatory Activity	20
2.2.2 Cytotoxic and Anticancer Activity	22
2.2.3 Antimicrobial Activity	25
2.2.4 Antioxidant Activity	28
2.3 CONCLUSION	28
CHAPTER THREE: SYNTHESIS OF 3-ARYLSYDNONE, 4-ACETYL -3- ARYLSYDNONE, BIS-SYDNONE PHENYL STYRYL KETON DERIVATIVES	30
3.1 INTRODUCTION	30
3.2 MATERIALS, INSTRUMENTATION, AND METHODS	31
3.2.1 Chemicals	31

3.2.2 Chemical Synthesis	32
3.2.2.1 Synthesis of Ethyl N-Arylglycinate Esters	32
3.2.2.2 Synthesis of Methyl 4-Aminobenzoate	32
3.2.2.3 Hydrolysis of Ethyl-N-Arylglycinate Esters	33
3.2.2.4 Condensation of Nitro-Containing Anilines with Chloroacetic Acid	33
3.2.2.5 Condensation of Methyl 4-Aminobenzoate with Chloroacetic Acid	34
3.2.2.6 Nitrosation of N-Arylglycine	35
3.2.2.7 Formation of The Sydnone Ring	35
3.2.2.8 Synthesis of Alcohol Sydnone Derivatives	36
3.2.2.9 Synthesis of Aldehyde Sydnone Derivatives	37
3.2.2.10 Acetylation of 3-Arylsydnone	37
3.2.2.11 Synthesis of Bis-Sydnone Phenyl Styrylketones	38
3.2.3 Compounds Purification	39
3.2.4 Thin Layer Chromatography (TLC)	40
3.2.5 Compound Characterization	40
3.2.6 Chemical Shift Calculation	41
3.3 RESULTS AND DISCUSSION	42
3.3.1 Synthesis of Ethyl N-Arylglycinate Esters	42
3.3.2 Synthesis of Methyl 4-Aminobenzoate	49
3.3.3 Hydrolysis of Ethyl-N-Arylglycinate Esters	52
3.3.4 Condensation of Nitro-Containing Anilines with Chloroacetic Acid.....	59
3.3.5 Condensation of Methyl 4-Aminobenzoate with Chloroacetic Acid	67
3.3.6 Nitrosation of N-Arylglycine	69
3.3.7 Formation of The Sydnone Ring	73
3.3.8 Synthesis of Alcohol Sydnone Derivatives	92
3.3.9 Synthesis of Aldehyde Sydnone Derivatives	96
3.3.10 Acetylation of 3-Arylsydnone	99
3.3.11 Synthesis of Bis-Sydnone Phenyl Styrylketones	117
3.4 CONCLUSION	129

**CHAPTER FOUR: IN VITRO EVALUATION OF THE CYTOTOXICITY
AND ANTI-INFLAMMATORY ACTIVITIES OF SYDNONES130**

4.1 INTRODUCTION	130
4.2 MATERIALS AND METHODS	131
4.2.1 In vitro Cytotoxic Activity of Sydnone Derivatives 37-63	131
4.2.1.1 Chemicals, Reagents and Instrumentation	131
4.2.1.2 Cell Lines	131
4.2.1.3 Cell Culture	132
4.2.1.4 Cell Harvesting	132
4.2.1.5 Cell Viability and Counting	132
4.2.1.6 Cell Sub-culturing	133
4.2.1.7 Cell Treatment by Sydnone-based Compounds	133
4.2.1.8 In Vitro Cytotoxicity Assay (MTT assay)	134
4.2.1.9 Calculating of the Cellular Growth	134
4.2.1.10 Statistical Analysis	135

4.2.2 In vitro Anti-inflammatory Activity of Sydnone Derivatives	135
4.2.2.1 Chemicals, Reagents and Instrumentation	135
4.2.2.2 The Principle and Procedures of COX Inhibitor Screening Assay.....	136
4.2.2.3 COX Reactions	138
4.2.2.4 Acetylcholinesterase (AChE) competitive enzyme-linked immunosorbent assay (ELISA)	139
4.3 RESULTS AND DISCUSSION	140
4.3.1 In vitro Cytotoxic Activity of Sydnone Derivatives 37-63.....	140
4.3.2 In vitro Anti-inflammatory Activity of Sydnone Derivatives	151
4.4 CONCLUSION	159

**CHAPTER FIVE: MOLECULAR DOCKING AND PREDICTION OF
THE DRUG-LIKENESS AND MEMBRANE PERMEABILITY OF
SYDNONES**

SYDNONES	160
5.1 INTRODUCTION	160
5.2 MATERIALS AND METHODOLOGY	161
5.2.1 Molecular Docking	161
5.2.1.1 Ligand Preparation	161
5.2.1.2 Protein preparation	161
5.2.1.3 Receptor Grid Generation	162
5.2.1.4 Extra Precision (XP) Molecular Docking	163
5.2.1.5 Calculation of the Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) Biding energy	163
5.2.1.6 Validation of Docking Results using Autodock 4.2 and the in Silico Predicted IC ₅₀ values	163
5.2.2 Prediction of Drug-likeness and Membrane Permeability	165
5.2.2.1 Calculation of Drug-likeness Scores	165
5.2.2.2 Intestine and Blood Brain Barrier (BBB) Permeability of Sydnones	166
5.3 RESULTS AND DISCUSSION	167
5.3.1 Molecular Docking of Sydnones 37-63 as COX-2 Inhibitors	167
5.3.2 Molecular Docking of Sydnones 37-63 as COX-1 Inhibitors	179
5.3.3 Prediction of Drug-likeness and Membrane Permeability	191
5.4 CONCLUSION	198

CHAPTER SIX: CONCLUSION AND FUTURE RECOMMENDATION

6.1 GENERAL CONCLUSION	200
6.2 FUTURE RECOMMENDATIONS	202

REFERENCES

APPENDIX A: FTIR, ¹H NMR, ¹³C NMR and MS SPECTRA

Appendix A-1. Infrared spectrum of 6 in KBr disc	222
Appendix A-2. Infrared spectrum of 7 in KBr disc	223
Appendix A-3. Infrared spectrum of 8 in KBr disc	224
Appendix A-4. Infrared spectrum of 9 in KBr disc	225
Appendix A-5. Infrared spectrum of 10 in KBr disc	226
Appendix A-6. ¹ H NMR spectrum of 6 in DMSO-d ₆	227

Appendix A-7. ¹ H NMR spectrum of 7 in DMSO-d ₆	228
Appendix A-8. ¹ H NMR spectrum of 8 in DMSO-d ₆	229
Appendix A-9. ¹ H NMR spectrum of 9 in DMSO-d ₆	230
Appendix A-10. ¹ H NMR spectrum of 10 in DMSO-d ₆	231
Appendix A-11. FTIR spectrum of 12 in KBr disc	232
Appendix A-12. ¹ H NMR spectrum of 12 in DMSO-d ₆	233
Appendix A-13. FTIR spectrum of 13 in KBr disc	234
Appendix A-14. FTIR spectrum of 14 in KBr disc	235
Appendix A-15. FTIR spectrum of 15 in KBr disc	236
Appendix A-16. FTIR spectrum of 16 in KBr disc	237
Appendix A-17. FTIR spectrum of 17 in KBr disc	238
Appendix A-18. ¹ H NMR spectrum of 13 in DMSO-d ₆	239
Appendix A-19. ¹ H NMR spectrum of 14 in DMSO-d ₆	240
Appendix A-20. ¹ H NMR spectrum of 15 in DMSO-d ₆	241
Appendix A-21. ¹ H NMR spectrum of 16 in DMSO-d ₆	242
Appendix A-22. ¹ H NMR spectrum of 17 in DMSO-d ₆	243
Appendix A-23. FTIR spectrum of 22 in KBr disc	244
Appendix A-24. FTIR spectrum of 23 in KBr disc	245
Appendix A-25. FTIR spectrum of 24 in KBr disc	246
Appendix A-26. FTIR spectrum of 25 in KBr disc	247
Appendix A-27. ¹ H NMR spectrum of 22 in DMSO-d ₆	248
Appendix A-28. ¹ H NMR spectrum of 23 in DMSO-d ₆	249
Appendix A-29. ¹ H NMR spectrum of 24 in DMSO-d ₆	250
Appendix A-30. ¹ H NMR spectrum of 25 in DMSO-d ₆	251
Appendix A-31. FTIR spectrum of 26 in KBr disc	252
Appendix A-32. ¹ H NMR spectrum of 26 in DMSO-d ₆	253
Appendix A-33. FTIR spectrum of 27 in KBr disc	254
Appendix A-34. FTIR spectrum of 28 in KBr disc	255
Appendix A-35. FTIR spectrum of 29 in KBr disc	256
Appendix A-36. FTIR spectrum of 30 in KBr disc	257
Appendix A-37. FTIR spectrum of 31 in KBr disc	258
Appendix A-38. FTIR spectrum of 32 in KBr disc	259
Appendix A-39. FTIR spectrum of 33 in KBr disc	260
Appendix A-40. FTIR spectrum of 34 in KBr disc	261
Appendix A-41. FTIR spectrum of 35 in KBr disc	262
Appendix A-42. FTIR spectrum of 36 in KBr disc	263
Appendix A-43. FTIR spectrum of 37 in KBr disc	264
Appendix A-44. ¹ H NMR spectrum of 37 in CDCl ₃	265
Appendix A-45. ¹³ C NMR spectrum of 37 in CDCl ₃	266
Appendix A-46. FTIR spectrum of 38 in KBr disc	267
Appendix A-47. ¹ H NMR spectrum of 38 in CDCl ₃	268
Appendix A-48. ¹³ C NMR spectrum of 38 in CDCl ₃	269
Appendix A-49. FTIR spectrum of 39 in KBr disc	270
Appendix A-50. ¹ H NMR spectrum of 39 in CDCl ₃	271
Appendix A-51. ¹³ C NMR spectrum of 39 in CDCl ₃	272
Appendix A-52. FTIR spectrum of 40 in KBr disc	273
Appendix A-53. ¹ H NMR spectrum of 40 in CDCl ₃	274
Appendix A-54. ¹³ C NMR spectrum of 40 in CDCl ₃	275
Appendix A-55. FTIR spectrum of 41 in KBr disc	276

Appendix A-56.	¹ H NMR spectrum of 41 in CDCl ₃	277
Appendix A-57.	¹³ C NMR spectrum of 41 in CDCl ₃	278
Appendix A-58.	FTIR spectrum of 42 in KBr disc	279
Appendix A-59.	¹ H NMR spectrum of 42 in DMSO-d ₆	280
Appendix A-60.	¹³ C NMR spectrum of 42 in DMSO-d ₆	281
Appendix A-61.	FTIR spectrum of 43 in KBr disc	282
Appendix A-62.	¹ H NMR spectrum of 43 in DMSO-d ₆	283
Appendix A-63.	¹³ C NMR spectrum of 43 in DMSO-d ₆	284
Appendix A-64.	FTIR spectrum of 44 in KBr disc	285
Appendix A-65.	¹ H NMR spectrum of 44 in DMSO-d ₆	286
Appendix A-66.	¹³ C NMR spectrum of 44 in DMSO-d ₆	287
Appendix A-67.	FTIR spectrum of 45 in KBr disc	288
Appendix A-68.	¹ H NMR spectrum of 45 in DMSO-d ₆	289
Appendix A-69.	¹³ C NMR spectrum of 45 in DMSO-d ₆	290
Appendix A-70.	FTIR spectrum of 46 in KBr disc	291
Appendix A-71.	¹ H NMR spectrum of 46 in DMSO-d ₆	292
Appendix A-72.	¹³ C NMR spectrum of 46 in DMSO-d ₆	293
Appendix A-73.	FTIR spectrum of 47 in KBr disc	294
Appendix A-74.	¹ H NMR spectrum of 47 in DMSO-d ₆	295
Appendix A-75.	¹³ C NMR spectrum of 47 in DMSO-d ₆	296
Appendix A-76.	FTIR spectrum of 48 in KBr disc	297
Appendix A-77.	¹ H NMR spectrum of 48 in DMSO-d ₆	298
Appendix A-78.	¹³ C NMR spectrum of 48 in DMSO-d ₆	299
Appendix A-79.	FTIR spectrum of 49 in KBr disc	300
Appendix A-80.	¹ H NMR spectrum of 49 in CDCl ₃	301
Appendix A-81.	¹³ C NMR spectrum of 49 in CDCl ₃	302
Appendix A-82.	FTIR spectrum of 50 in KBr disc	303
Appendix A-83.	¹ H NMR spectrum of 50 in CDCl ₃	304
Appendix A-84.	¹³ C NMR spectrum of 50 in CDCl ₃	305
Appendix A-85.	FTIR spectrum of 51 in KBr disc	306
Appendix A-86.	¹ H NMR spectrum of 51 in CDCl ₃	307
Appendix A-87.	¹³ C NMR spectrum of 51 in CDCl ₃	308
Appendix A-88.	FTIR spectrum of 52 in KBr disc	309
Appendix A-89.	¹ H NMR spectrum of 52 in CDCl ₃	310
Appendix A-90.	¹³ C NMR spectrum of 52 in CDCl ₃	311
Appendix A-91.	FTIR spectrum of 53 in KBr disc	312
Appendix A-92.	¹ H NMR spectrum of 53 in CDCl ₃	313
Appendix A-93.	¹³ C NMR spectrum of 53 in CDCl ₃	314
Appendix A-94.	FTIR spectrum of 54 in KBr disc	315
Appendix A-95.	¹ H NMR spectrum of 54 in DMSO-d ₆	316
Appendix A-96.	¹³ C NMR spectrum of 54 in DMSO-d ₆	317
Appendix A-97.	FTIR spectrum of 55 in KBr disc	318
Appendix A-98.	¹ H NMR spectrum of 55 in DMSO-d ₆	319
Appendix A-99.	¹³ C NMR spectrum of 55 in DMSO-d ₆	320
Appendix A-100.	FTIR spectrum of 56 in KBr disc	321
Appendix A-101.	¹ H NMR spectrum of 56 in DMSO-d ₆	322
Appendix A-102.	¹³ C NMR spectrum of 56 in DMSO-d ₆	323
Appendix A-103.	FTIR spectrum of 57 in KBr disc	324
Appendix A-104.	¹ H NMR spectrum of 57 in DMSO-d ₆	325

Appendix A-105. ^{13}C NMR spectrum of 57 in DMSO-d6	326
Appendix A-106. FTIR spectrum of 58 in KBr disc	327
Appendix A-107. ^1H NMR spectrum of 58 in DMSO-d6	328
Appendix A-108. ^{13}C NMR spectrum of 58 in DMSO-d6	329
Appendix A-109. FTIR spectrum of 61 in KBr disc	330
Appendix A-110. FTIR spectrum of 62 in KBr disc	331
Appendix A-111. FTIR spectrum of 63 in KBr disc	332
Appendix A-112. ^1H NMR spectrum of 61 in DMSO-d6	333
Appendix A-113. ^1H NMR spectrum of 62 in DMSO-d6	334
Appendix A-114. ^1H NMR spectrum of 63 in DMSO-d6	335
Appendix A-115. ^{13}C NMR spectrum of 61 in DMSO-d6	336
Appendix A-116. ^{13}C NMR spectrum of 62 in DMSO-d6	337
Appendix A-117. ^{13}C NMR spectrum of 63 in DMSO-d6	338
Appendix A-118. High resolution mass spectrum (HRMS) of 61	339
Appendix A-119. High resolution mass spectrum (HRMS) of 62	340
Appendix A-120. High resolution mass spectrum (HRMS) of 63	341

LIST OF TABLES

Table 2.1	Acylation of 3-arylsydnone	16
Table 3.1	Yield (%) and melting points of ethyl <i>N</i> -arylglycinate esters, 6-10	44
Table 3.2	FTIR spectra of ethyl <i>N</i> -arylglycinate esters, 6-10	45
Table 3.3	¹ H NMR spectra of ethyl <i>N</i> -arylglycinate esters, 6-10	48
Table 3.4	FTIR and ¹ H NMR spectra of methyl 4-aminobenzoate, 12	52
Table 3.5	Yield (%) and melting points of <i>N</i> -arylglycine, 13-17	53
Table 3.6	FTIR spectra of <i>N</i> -arylglycine, 13-17	55
Table 3.7	¹ H NMR spectra of <i>N</i> -arylglycine, 13-17	58
Table 3.8	Yield (%) and melting points of <i>N</i> -arylglycine, 22-25	60
Table 3.9	FTIR spectra of <i>N</i> -arylglycine, 22-25	62
Table 3.10	¹ H NMR spectra of <i>N</i> -arylglycine, 22-25	66
Table 3.11	FTIR and ¹ H NMR spectra of <i>N</i> -[4-(methoxycarbonyl) phenyl] glycine, 26	69
Table 3.12	Yield (%) and melting points of <i>N</i> -nitroso- <i>N</i> -arylglycine, 27-36	71
Table 3.13	FTIR spectra of <i>N</i> -nitroso- <i>N</i> -arylglycines, 27-36	73
Table 3.14	Yield (%) and melting points of 3-arylsydnone, 37-46	77
Table 3.15	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-phenyl sydnone, 37	79
Table 3.16	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-methylphenyl) sydnone, 38	80
Table 3.17	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-methoxy phenyl) sydnone, 39	81
Table 3.18	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-chlorophenyl) sydnone, 40	82
Table 3.19	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-fluorophenyl)	83

	sydnone, 41	
Table 3.20	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(3-nitrophenyl) sydnone, 42	85
Table 3.21	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-nitrophenyl) sydnone, 43	87
Table 3.22	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-methyl-3-nitrophenyl) sydnone, 44	89
Table 3.23	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-chloro-3-nitrophenyl) sydnone, 45	90
Table 3.24	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-[4-(methoxycarbonyl) phenyl] sydnone, 46	92
Table 3.25	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-[4-(hydroxymethyl) phenyl] sydnone, 47	96
Table 3.26	FTIR, ¹ H NMR and ¹³ C NMR spectra of 3-(4-formylphenyl) sydnone, 48	99
Table 3.27	Yield (%) and melting points of 4-acetyl-3-arylsydnone, 49-60	102
Table 3.28	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-phenylsydnone, 49	104
Table 3.29	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-methylphenyl) sydnone, 50	105
Table 3.30	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-methoxy phenyl) sydnone, 51	106
Table 3.31	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-chlorophenyl) sydnone, 52	107
Table 3.32	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-fluorophenyl) sydnone, 53	108
Table 3.33	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(3-nitrophenyl) sydnone, 54	109
Table 3.34	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-nitrophenyl) sydnone, 55	110
Table 3.35	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-methyl-3-nitro phenyl) sydnone, 56	113
Table 3.36	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-(4-	115

	chloro-3-nitro phenyl) sydnone, 57	
Table 3.37	FTIR, ¹ H NMR and ¹³ C NMR spectra of 4-acetyl-3-[(4-(methoxy carbonyl)phenyl)] sydnone, 58	117
Table 3.38	Yield (%) and melting points of <i>bis</i> -sydnone styrylketone, 61-63	120
Table 3.39	FTIR spectral data of <i>bis</i> -sydnone styrylketone, 61-63	122
Table 3.40	¹ H NMR spectral data of <i>bis</i> -sydnone styrylketone, 61-63	124
Table 3.41	¹³ C NMR spectral data of <i>bis</i> -sydnone styrylketone, 61-63	127
Table 3.42	HRMS spectra of <i>bis</i> -sydnone styrylketone, 61-63	128
Table 4.1	Performing COX reactions	139
Table 4.2	<i>In vitro</i> cytotoxic activity (IC ₅₀ , mM) of 3-arylsydnone 37-48	142
Table 4.3	<i>In vitro</i> cytotoxic activity (IC ₅₀ , mM) of 4-acetyl-3-arylsydnone	145
Table 4.4	<i>In vitro</i> cytotoxic activity (IC ₅₀ , mM) of <i>bis</i> -sydnone styrylketone 61-63	148
Table 4.5	<i>In vitro</i> cyclooxygenase inhibitory activity of 3-arylsydnone 37-48	153
Table 4.6	<i>In vitro</i> cyclooxygenase inhibitory activity of 4-acetyl-3-arylsydnone 49-58	154
Table 4.7	<i>In vitro</i> cyclooxygenase inhibitory activity of <i>bis</i> -sydnone styrylketone 61-63	155
Table 5.1	Extra precision (XP) binding scores and molecular mechanics based on generalized Born/surface area (MM - GBSA) for docking poses of sydnone analogs 37-63 into cyclooxygenase-2 (PDB code: 3NT1)	168
Table 5.2	Validation of the docking results using Autodock 4.2 and the <i>in silico</i> predicted IC ₅₀ values	169
Table 5.3	Types of interactions of sydnone analogs 37-63 and celecoxib with Arg513 residue of the binding site of COX-2	172
Table 5.4	Hydrogen bonding and hydrophobic interactions of sydnone analogues 37-63 and celecoxib with the binding site of COX-2	173
Table 5.5	Extra precision (XP) binding scores and molecular mechanics based on generalized Born/surface area (MM -	181

GBSA) for docking poses of sydnone analogs 37-63 into cyclooxygenase-1 (PDB code: 2N8Z)

Table 5.6	Types of interactions of sydnone analogs 37-63 and celecoxib with His513 residue of the binding site of COX-1	182
Table 5.7	Hydrogen bonding and hydrophobic interactions of sydnone analogs 37-63 and celecoxib with the binding site of COX-1	183
Table 5.8	The calculated physicochemical properties of selected sydnone-based derivatives	192
Table 5.9	Drug-likeness prediction of selected sydnone-based derivatives	193
Table 5.10	Predicted membrane permeability of selected sydnone derivatives	193
Table 5.11	Qualifying and preferred ranges for CMC-like drug-likeness rule	196

LIST OF FIGURES

Figure 1. 1	Sydnone Structure	1
Figure 2.1	Proposed structures of sydnone ring	4
Figure 2.2	Bipolar and tetrapolar structures of sydnones	5
Figure 2.3	Aromaticity of the sydnone ring	6
Figure 2.4	Preparation of -nitroso analogues in neutral conditions	7
Figure 2.5	Mechanism of ring closure and sydnone formation	8
Figure 2.6	Examples of sydnone derivatives	9
Figure 2.7	Structures of <i>N,N,N',N'</i> -tetrabromobenzene-1,3-disulfonamide XXIII (TBBDS) and 1,3-dibromo-5,5-dimethylhydantoin XXIV (DBH)	10
Figure 2.8	Electronic Structure of Sydnone Ring	11
Figure 2.9	Examples on sydnones whose UV absorption wavelengths are unusually high	13
Figure 2.10	The chemical shift of sydnone CO peak changes within a narrow range from 160-170 ppm regardless of the nature of the substituent at C4. As examples, compounds XXXII, XXXIII, XXXIV have their CO peaks at 169.19 ppm, 165.80 ppm, and 163.20 ppm	15
Figure 2.11	Formation of a fused-ring sydnone via direct acylation of the sydnone ring.	16
Figure 2.12	Halogenation of sydnone at carbon C4	17
Figure 2.13	The introduction of heteroatom groups to 3-arylsydnone via 4-lithiosydnone as an intermediate	18
Figure 2.14	One-Pot <i>o</i> -Acylation and Subsequent Sydnone 4-Substitution	19
Figure 2.15	Anti-inflammatory sydnones	21
Figure 2.16	Cytotoxic sydnones	23
Figure 2.17	tridentate palladium Pd (II) complexes with thiosemicarbazones and phenyl sydnone	24

Figure 2.18	Antimicrobial sydnones 1	26
Figure 2.19	Antimicrobial sydnones 2	27
Figure 2.20	Antimicrobial sydnones 3	27
Figure 2.21	Antioxidant sydnones	28
Figure 3.1	Sydnone structure	31
Figure 3.2	Synthesis of ethyl <i>N</i> -arylglycinate esters	32
Figure 3.3	Synthesis of methyl esters of 4-aminobenzoic acid	33
Figure 3.4	Hydrolysis of ethyl- <i>N</i> -arylglycinate esters	33
Figure 3.5	Condensation of para-substituted anilines with chloroacetic acid	34
Figure 3.6	Condensation of methyl 4-aminobenzoate with chloroacetic acid	35
Figure 3.7	Preparation of <i>N</i> -nitroso- <i>N</i> -arylglycine	35
Figure 3.8	Ring closure and formation of the sydnone ring	36
Figure 3.9	Preparation of alcohol sydnone derivative	37
Figure 3.10	Preparation of aldehyde sydnone derivative	37
Figure 3.11	Preparation of 4-acetyl-3-arylsydnone derivatives	38
Figure 3.12	Synthesis of bis-sydnone phenyl styrylketones	39
Figure 3.13	Proposed Mechanism of the formation of ethyl <i>N</i> -phenyl glycinate ester	43
Figure 3.14	Proposed Mechanism of the preparation of methyl 4-amino benzoate 12	50
Figure 3.15	Proposed Mechanism of the preparation of <i>N</i> -(nitrophenyl) glycine derivatives, 22-25.	60
Figure 3.16	Analysis and expansion of the aromatic ring proton multiplets of compound 24 (a) and compound 25 (b)	67
Figure 3.17	Proposed Mechanism of the formation of nitrosonium ion and the <i>N</i> -nitroso derivatives therefrom	70
Figure 3.18	Proposed Mechanism of ring closure and sydnone formation	75

Figure 3.19	Proposed Mechanism of the synthesis of alcohol sydnone derivative, 47	93
Figure 3.20	Proposed Mechanism of the synthesis of aldehyde sydnone derivative, 4	97
Figure 3.21	Proposed Mechanism of sydnone acylation at carbon C4.	100
Figure 3.22	Proposed Mechanism of the synthesis of bis-sydnone styrylketone	119
Figure 4.1	The chemical structure of SC-560 (selective COX-1 inhibitor) and DuP-697 (selective COX-2 inhibitor)	136
Figure 4.2	Steps of AChE ELISA. (a) plate wells pre-coated with mouse anti-rabbit IgG and blocked with proper proteins. (b) Incubation of tracer, antiserum, free PG and COX inhibitor. (c) wash to remove all unbound reagents. (d) The addition of Ellman's reagent	137
Figure 4.3	Reactions of acetylcholine in the existence of AChE and its substrate (Ellman's reagent)	138
Figure 4.4	Dose-response curve of 3-(4-chloro-3-nitrophenyl) sydnone 45 against MCF (a), PC3 (b), and NHDF (c).	143
Figure 4.5	Dose-response curve of 4-acetyl-3-(4-chloro-3-nitrophenyl) sydnone 57 against MCF (a), PC3 (b), and NHDF (c).	146
Figure 4.6	A comparison between the cytotoxic activity of 3-arylsydnone (37-46) and 4-acetyl-3-arylsydnone (49-58).	147
Figure 4.7	Dose-response curve of sydnone 63 against MCF (a) and PC3 (b). Data presented as the mean of triplicates \pm the standard deviation.	148
Figure 4.8	Examples on the structure-toxicity relationship of sydnones	150
Figure 4.9	Sydnone-substituted chalcone (IV) and sydnone-cis stilbene (V)	151
Figure 4.10	Examples on anti-inflammatory sydnones	156
Figure 4.11	Structural comparison between diaryl heterocycles (coxibs) and <i>bis</i> -sydnone styrylketones.	157
Figure 5.1	The calculated binding free energy (MM-GBSA, kcal/mol) and Glide docking scores plotted vs the experimental COX-2 inhibitory activity (%) of compounds 37-63	170

Figure 5.2	(a) 3D representation of compound 63 inside the binding site of COX-2 (b) Schematic representation of the interactions between compound 63 and COX-2 active site	175
Figure 5.3	(a) 3D representation of compound 51 inside the binding site of COX-2. (b) Schematic representation of the interactions between compound 51 and COX-2 active site	177
Figure 5.4	(a) 3D representation of celecoxib inside the binding site of COX-2. (b) Schematic representation of the interactions between celecoxib and COX-2 active site	179
Figure 5.5	The calculated binding free energy (MM-GBSA, kcal/mol) and Glide docking scores vs the experimental COX-1 inhibitory activity (%) of compounds 37-63	180
Figure 5.6	(a) 3D representation of compound 63 inside the binding site of COX-1. (b) Schematic representation of the interactions between compound 63 and COX-1 active site.	185
Figure 5.7	(a) 3D representation of compound 51 inside the binding site of COX-1. (b) Schematic representation of the interactions between compound 51 and COX-1 active site.	186
Figure 5.8	Schematic representation of the interactions between COX-1 active site (PDB code: 3N8Z) and (a) 3-(4-fluorophenyl)sydnone 41, (b) 3-(4-methoxyphenyl)sydnone 39.	188

LIST OF ABBREVIATIONS

^1H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
AA	Arachidonic acid
AChE	Acetyl choline esterase
AD	Alzheimer's disease
ANOVA	Analysis of variance
BA	B-amyloid
BBB	Blood-brain barrier
BHA	Butyl hydroxy anisole
CADD	Computer-aided drug design
CGM	Complete growth media
CNS	Central nervous system
COX	Cyclooxygenase
DBH	1,3-Dibromo-5,5-dimethylhydantoin
DCM	Dichloromethane
DF	Dilution factor
DME	Dimethoxy ethane
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
ER	Endothelial reticulum
ESI	Electrospray ionization
FTIR	Fourier transform infrared spectroscopy
GABA	Gamma-aminobutyric acid

GIT	Gastro-intestinal tract
GLDH	Glutamate dehydrogenase
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HOMO	High occupied molecular orbital
IA	Intestinal absorption
IAN	Iso-amyl nitrite
IR	Infrared
LUMO	Lowest unoccupied molecular orbital
MBD	Membrane binding domain
MM-GBSA	Molecular Mechanics-Generalized Born Surface Area
MO	Molecular orbital
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
NSB	Non-specific binding
OD	Optical density
OPLS	Optimized Potential for Liquid Simulations
PBS	Phosphate buffer saline
PDB	Protein data bank
PG	Prostaglandin
PMA	Phorbol myristate acetate ester
RMSD	Root-mean-square deviation