Spring 5-22-2020

Synthesis and Evaluation of Novel Biologically Active Compounds

Madhurima Das
University of New Orleans, mdas1@uno.edu

Synthesis and Evaluation of Novel Biologically Active Compounds

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Chemistry

by

Madhurima Das

B.S. University of Delhi, 2012
M.S. University of Leeds, 2014
M.S. University of New Orleans, 2018

May, 2020

To my family for their continued love and support

ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my advisor Prof. Mark L Trudell for giving me the opportunity to do research in his laboratory. I would also like to thank him for his continuous support and guidance during my doctoral studies and research, for his patience, motivation, enthusiasm, and immense knowledge. His dynamism, vision, sincerity and motivation have deeply inspired me. He has taught me the methodology to carry out independent research and to present the research as clearly as possible. It was a great privilege and honor to work and study under his guidance. I could not have imagined having a better advisor and mentor for my doctoral research.

I would like to extend my heartfelt thanks to Dr. Stacey Lomenzo for guiding me through my teaching assistant duties. She was always patient, had excellent organizational skills and strong work ethic. I would like to thank her for being so friendly and approachable. She has been like my family and made it easier for me settle down in a new environment.

I would like to thank the rest of my thesis committee: Dr. Branko Jursic, Dr. Steven Rick and Dr. Viktor Poltavets, for their encouragement and insightful comments.

I would like to thank Dr. Nicolas G Bazan and Dr. Surjyadipta Bhattacharjee at Louisiana State University Health Sciences Center for completing all the preliminary biological evaluations for my compounds.

I would like to thank Dr. Phoebe Zito for guiding me through the hydrophobicity studies and for teaching me UV-vis spectroscopy.

I would like to express my thanks to my fellow colleagues in the Trudell group: Maria Lindsay and Ryan McKinnie, who helped me settle in when I first joined the group. They were extremely supportive and I am grateful for their generosity and encouragement. A big thanks to my dear friends and labmates Jumanah Hamdi and Nichole Pianovich who have stood by me through all the ups and down in the past 5 years. I could not have asked for better labmates and friends. I will always cherish the memories of working with them and will be forever grateful for their support and encouragement.

Last but not the least I am extremely grateful to my parents for their love, prayers, and sacrifices for educating and preparing me for my future. I could not have done this without them.

TABLE OF CONTENTS

List of schemes vi

List of figures xi

List of tables xiv

List of abbreviations xvi

Abstract xviii

Chapter 1 1

Chapter 2 62

Chapter 3 114

References 157

Appendix 170

Vita 185

LIST OF SCHEMES

Scheme 1 Synthesis of organic nitrates 5

Scheme 2 Alkaline hydrolysis of nitrate esters 5

Scheme 3 Synthesis of **12** (Route 1) 13

Scheme 4 Synthesis of **12** (Route 2) 13

Scheme 5 Synthesis of SCP-1 (Route A) 14

Scheme 6 Synthesis of SCP-1 (Route B) 15

Scheme 7 Synthesis of SCP-123 16

Scheme 8 Retrosynthetic route for synthesis of NO-SCP-1 analogs 17

Scheme 9 Synthesis of chloroalkanoyl esters of SCP-1 22

Scheme 10 Attempted synthesis of **24b** 24

Scheme 11 Synthesis of nitrate esters **24a-d** using optimized conditions 29

Scheme 12 Attempted synthesis of nitrate ester analogs of SCP-123 (strategy 1) 34

Scheme 13 Attempted synthesis of nitrate ester analogs of SCP-123 (strategy 2) 35

Scheme 14 Synthesis of β-glycoside tetraacetate of **18** using $POCl_3$ as catalyst 34

Scheme 15 Synthesis of O-glycoside tetraacetate of **18** using $BF_3 \bullet OEt_2$ as catalyst.....37

Scheme 16 Synthesis of β-O-glycoside tetraacetate of SCP-1 38

Scheme 17 Synthesis of β-O-glycoside of SCP-1 39

Scheme 18 Synthesis of amides of SCP-1 using amino alcohols **32**-**37**.....40

Scheme 19 Synthesis of amide **44** 41

Scheme 20 Synthesis of amides of SCP-1 using amino acids **45** and **46** 42

Scheme 21 Synthesis of diarylmethane using Friedel-Crafts alkylation 65

Scheme 22 Synthesis of diarylmethanes using indium (III) chloride as catalyst 66

Scheme 23 Bronsted and Lewis acid catalyzed arylation.....67

Scheme 24 Synthesis of diarylmethanes using metal free C-C bond formation.....67

Scheme 25 Synthesis of diarylmethanes using triphenylphosphine ditriflate 68

Scheme 26 Copper catalyzed synthesis of diarylmethanes using Grignard reagent.....68

Scheme 27 Palladium catalyzed synthesis of diarylmethanes using organostannates.....69

Scheme 28 Synthesis of diarylmethanes using Suzuki-Miyaura coupling 69

Scheme 29 Synthesis of diarylmethanes using potassium aryltrifluoroborates.....70

Scheme 30 Synthesis of diarylmethanes using Stille coupling.....70

Scheme 31 Synthesis of diarylmethanes using Kumada coupling.....71

Scheme 32 Synthesis of diarylmethanes using Hiyama coupling 72

Scheme 33 Synthesis of diarylmethanes using Negishi coupling.....72

Scheme 34 Lewis acid mediated synthesis of diarylmethanes 73

Scheme 35 Synthesis of diarylmethanes via benzylic ester rearrangement.....73

Scheme 36 Synthesis of N-substituted azetidine 78

Scheme 37 Synthesis of 1,3-disubstituted azetidine.....78

Scheme 38 Synthesis of 3,3-dichloroazetidine 79

Scheme 39 Synthesis of 3-fluoroazetidine.....79

Scheme 40 Synthesis of AzeInipidine **61** 83

Scheme 41 Synthesis of Cotellic **62**.....84

Scheme 42 Synthesis of 3-aryl-3-azetidinols.....86

Scheme 43 Synthesis of 3,3-diarylazetidine **68a** 86

Scheme 44 Synthesis of 3,3-diarylazetidines **68b-o** 88

Scheme 45 Removal of Cbz group using H_2, Pd/C 90

Scheme 46 Retrosynthetic scheme for synthesis of N-functionalized 3,3-diarylazetidines.....91

Scheme 47 Synthesis of N-Boc-3-phenyl-3-azetidinol **71a**.....92

Scheme 48 Mechanism of nucleophilic addition of phenyllithium 93

Scheme 49 Possible side reaction and formation of homocoupled product **72** 93

Scheme 50 Synthesis of N-Boc-3-aryl-3-azetidinols **71a-c**.....94

Scheme 51 Boc removal using $AlCl_3$.....95

Scheme 52 Friedel-Crafts arylation after Boc removal 96

Scheme 53 Friedel-Crafts arylation before Boc removal 96

Scheme 54 Boc removal after Friedel-Crafts arylation 97

Scheme 55 Synthesis of 3,3-diarylazetidine oxalic acid salts.....98

Scheme 56 N-alkylation of **74a** 100

Scheme 57 N-acylation of **74a** and **74d**.....101

Scheme 58 Dimerization of trimethyl pyruvic acid.....118

Scheme 59 Oxidative decarboxylation of α-keto acid **84**.....119

Scheme 60 Reduction of α-keto acids 119

Scheme 61 Photolysis of α-keto acid **88** 120

Scheme 62 Synthesis of α-keto acid using (cyanomethylene) phosphoranes 121

Scheme 63 Synthesis of α-keto acids using fluorous selenic acid as catalyst.....121

Scheme 64 Synthesis of α-keto acids using AZADO as a catalyst 122

Scheme 65 Silver catalyzed acylation of heteroaromatic bases using α-keto acids.....124

Scheme 66 Pd/Cu-Catalyzed decarboxylative cross-coupling 124

Scheme 67 Hypervalent Iodine(III) and Ru(II)-photocatalyzation of BI-alkyne 126

Scheme 68 Decarboxylation of pyruvic acid.....131

Scheme 69 Oxidation of ethyl lactate to ethyl pyruvate.....132

Scheme 70 Oxidative dehydrogenation of ethyl lactate to ethyl pyruvate 133

Scheme 71 Synthesis of glyceryl tripyruvate.....136

Scheme 72 Retrosynthetic route for a series of pyruvate esters 137

Scheme 73 General scheme for synthesis of ethyl 2,3-dimethyl-2-butenoate.....138

Scheme 74 Optimized reaction scheme for synthesis of **118** 139

Scheme 75 Mechanism for synthesis of **118**.....139

Scheme 76 Synthesis of **120** using LiOH 141

Scheme 77 Synthesis of benzyl ester **125** 143

Scheme 78 Synthesis of esters **122a-f** 144

Scheme 79 Synthesis of diester **122e**.....145

Scheme 80 Synthesis of diester **122f** 145

Scheme 81 Ozonide formation during ozonolysis 147

Scheme 82 Synthesis of pyruvate esters **123a-d** via ozonolysis.....147

LIST OF FIGURES

Fig. 1 Some well-known organic nitrates 4

Fig. 2 Biotransformation of organic nitrate esters 6

Fig. 3 Structures of some well-known NO-NSAIDS....9

Fig. 4 Mechanism of acetaminophen toxicity....11

Fig. 5 SCP series, n = 1 to 5....12

Fig. 6 Structures of SCP-1 and SCP-123....12

Fig. 7 Byproduct **22** 16

Fig. 8 NO-SCP-1 analogs; n = 1-4....17

Fig. 9 Design strategy for water-soluble analogs of SCP-1....19

Fig. 10 Structure of C-acylated product **22**....20

Fig. 11 ^{1}H NMR of **22**....21

Fig. 12 X-ray crystal structures of esters **23b** and **23c** 23

Fig. 13 ^{1}H NMR for (i) **23b** and (ii) **24b** 25

Fig. 14 ^{13}C NMR for (i) **23b** and (ii) **24b** 26

Fig. 15 X-ray crystal structure of chloro and nitrate hybrid product....27

Fig. 16 X-ray crystal structure of **24b**....29

Fig. 17 Comparison of the glutathione depletion caused by nitrate esters **24b** (MD-38) and **24c** (MD-39) over 3 h, 6 h and 12 h with APAP and SCP-1 30

Fig. 18 Comparison of the LDH released by nitrate esters **24b** (MD-38) and **24c** (MD-39) over 3 h, 6 h and 12 h with APAP and SCP-1 31

Fig. 19 CYP450 enzyme profiles for **24b** (MD-39) and **24c** (MD-39) 32

Fig. 20 Antipyretic profile for **24b** (MD-38) and **24c** (MD-39) 33

Fig. 21 Spin Spin coupling of anomeric protons 37

Fig. 22 X-ray crystal structure of **29** 38

Fig. 23 Alkyls amino alcohols used 40

Fig. 24 Structures of amides of SCP-1 **38**-**43** 41

Fig. 25 Absorbance chart for **39** 43

Fig. 26 Calibration curve for **39** at 250 nm 44

Fig. 27 Concentration of all shaken and unshaken samples for each compound 45

Fig. 28 LogP values of the amides compared to SCP-1 and APAP 46

Fig. 29 Some well-known biologically active diarylmethanes 64

Fig. 30 Structures of **53**, **54** and **55** showing the diarylmethane scaffold 74

Fig, 31 Structures of substituted and unsubstituted diarylmethanes **56**-**69** 75

Fig. 32 Structure of URAT1 inhibitor **60** 76

Fig. 33 Top 10 heterocyclic scaffolds in FDA approved drugs 77

Fig. 34 Top four most common four-membered nitrogen heterocycles 77

Fig. 35 Structures for azetidine-based drugs **61** and **62** 80

Fig. 36 Structure of azetidine-based drug **63** 81

Fig. 37 Structures of azetidine-based drugs **64** and **65** 81

Fig, 38 Structure of azetidine-based drug **66** 82

Fig. 39 Bioactive geminal diarylazetidine structures.....85

Fig. 40 Alpha, beta and gamma keto esters 116

Fig. 41 Structures of α-keto acids.....117

Fig. 42 Polymerization of pyruvic acid.....117

Fig. 43 Inhibitor for mGlu5 receptor 126

Fig. 44 Pyruvic acid 127

Fig. 45 General formula for pyruvate 129

Fig. 46 Some biologically active pyruvate esters 130

Fig. 47 Alcohols used to prepare the esters of **120** 143

Fig. 48 Structures of synthesized esters **122a-f** 144

Fig. 49 Structures of synthesized pyruvate esters **123a-d** 148

LIST OF TABLES

Table 1 Optimized yields of chloroalkanoyl esters of SCP-1 24

Table 2 Optimization table for nitrate ester **24b** 28

Table 3 Optimized yields of amides **38**-**43** 40

Table 4 Synthesis of amides of SCP-1 using amino acids **45** and **46** 42

Table 5 Wavelengths used to produce calibration curve for each compound 44

Table 6 Comparison between logP and clogP values 46

Table 7 Friedel-Craft's arylation of **67** with *o*-cresol 87

Table 8 Scope of heteroaromatics and phenol derivatives as nucleophiles 88

Table 9 Optimization studies for synthesis of **71a** 94

Table 10 Optimized yields of 3,3-diarylazetidine oxalic acid salts 98

Table 11 Optimization of N-alkylation of **74a** 99

Table 12 N-alkylated products of **74a** 100

Table 13 Some selected α-keto acids synthesized by Crich and coworkers 122

Table 14 Some α-keto acids synthesized by Firikawa *et al.* 123

Table 15 Selected examples of direct acylation using α-keto acids 125

Table 16 Optimization of reaction conditions for synthesis of **118** 140

Table 17 Reaction conditions for synthesis of **120** 142

Table 18 Reaction condition optimization for synthesis of **122a** 143

Table 19 Optimized yields of esters **122a-f**..146

LIST OF ABBREVIATIONS

APAP	Acetaminophen
Boc	tert-Butyloxycarbonyl
Cbz	Carbobenzyloxy
CNS	Central nervous system
CYP450	Cytochrome P450 enzyme
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DHFR	Dihydrofolate reductase
DIPEA	N,N-Diisopropylethylamine
DMAP	N,N-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMS	Dimethylsulfide
DPPF	Diphenylphosphinyl ferrocene
EDCI	N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide
EtOAc	Ethyl acetate
FAAH	Fatty acid amide hydrolase
GSH	Glutathione
Hex	Hexanes

HFIP	Hexafluoroisopropanol
IDO	Indolamine 2,3-dioxygenase
LDH	Lactate dehydrogenase
MAGL	Monoacylglycerol lipase
MSU	Monosodium urate
NMR	Nuclear magnetic resonance
NO	Nitric oxide
R_f	Retention factor
ROS	Reactive oxygen species
TfOH	Triflic oxide
TFSA	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TPPD	Triphenylphosphine ditriflate

ABSTRACT

SCP-1, a potent derivative of acetaminophen, exhibits significantly diminished hepatotoxicity and nephrotoxicity relative to acetaminophen and nitrate ester derivatives of acetaminophen. It was therefore of interest to explore the development of nitric oxide donor analogs of SCP-1 to identify compounds that could have enhanced analgesic and/or antipyretic activity while taking advantage of the very low liver and kidney toxicity inherent to SCP-1. In this project, a series of nitrate ester analogues of the SCP-1 were prepared as potential nitric oxide donors. The synthesis of SCP analogs was achieved by triflic acid catalyzed O-acylation of SCP-1 with chloroalkanoyl chlorides followed by nitration with silver nitrate. The chloroesters and corresponding nitrate esters were obtained in high yields (>90%). Preliminary hepatotoxicity studies revealed the nitrate esters to be well tolerated by human hepatocytes and had little effect on the three cytochrome P450 enzymes tested (CYP3A4, CYP2E1 and CYP2D6). In addition, the nitrate esters exhibited antipyretic activity similar to acetaminophen.

With the emerging interest in 3,3-diarylazetidines as novel motifs in drug discovery programs, an easy, efficient, scalable synthetic method that could accommodate the introduction of both electron-rich and electron-deficient aryl groups for preparation of diversely substituted 3,3-diarylazetidines would be valuable to medicinal chemists. In this second project, a versatile synthesis of 3,3-diaryl azetidines from N-Boc-3-aryl-3-azetidinols using Friedel-Crafts arylation conditions with $AlCl_3$ is described. A series of substituted diaryl azetidines were readily prepared and isolated as the oxalate salts in high yield and high purity. The 3,3-diaryl azetidine oxalates were then easily converted into *N*-alkyl and *N*-acyl analogues in high overall yields (>85%).

In the last project, A general synthesis of pyruvate esters was developed. Using this method, a series of alkyl pyruvate esters were synthesized in moderate to high yields (56-93%). This was achieved in four steps starting from the readily available triethyl-2-phosphonopropionate which was converted to the alkene ethyl 2,3-dimethyl-2-butenoate using Horner-Wadsworth-Emmons reaction. The ethyl ester was hydrolyzed to the corresponding carboxylic acid after which a series of esters were prepared using Steglich esterification. The esters were then converted to their corresponding pyruvate esters via ozonolysis.

Keywords: Acetaminophen, NO donor, SCP-1, hepatoxicity, azetidines, Friedel-Crafts arylation, butyllithium, N-alkylation, pyruvate esters, α-keto acids, ozonolysis

CHAPTER 1

Synthesis, hepatotoxic evaluation and antipyretic activity of nitrate ester analogs of the acetaminophen derivative SCP-1

ABSTRACT

SCP-1 is a potent saccharin derivative of acetaminophen which is used for acute and chronic pain. SCP-1 demonstrated an analgesic profile similar that to that of acetaminophen. However, SCP-1 exhibited significantly diminished hepatotoxicity and nephrotoxicity relative to acetaminophen and nitrate ester derivatives of acetaminophen. It was therefore of interest to explore the development of nitric oxide donor analogs of SCP-1 to identify compounds that could have enhanced analgesic and/or antipyretic activity while taking advantage of the very low liver and kidney toxicity inherent to SCP-1. In this project, a series of nitrate ester analogues of the SCP-1 were prepared as potential nitric oxide donors. The synthesis of SCP analogs was achieved by triflic acid catalyzed O-acylation of SCP-1 with chloroalkanoyl chlorides followed by nitration with silver nitrate. The chloroesters and corresponding nitrate esters were obtained in high yields (>90%). Preliminary hepatotoxicity studies revealed the nitrate esters to be well tolerated by human hepatocytes and had little effect on the three cytochrome P450 enzymes tested (CYP3A4, CYP2E1 and CYP2D6). In addition, the nitrate esters exhibited antipyretic activity similar to acetaminophen.

INTRODUCTION

1.1 Nitric oxide donors

Nitric oxide donors (NO donors) are compounds that under physiological conditions release nitric oxide (NO). The most popular NO donor is Glyceryl trinitrate (GTN) or nitroglycerin. Although the use of GTN for medicinal purposes dates back to more than 150 years, little had been revealed about its physiological mechanism of action before the 1980s [1]. GTN was first discovered in 1847 by Ascanio Sobrero in Turin, following work with Theophile-Jules Pelouze. Alfred Nobel joined Pelouze in 1851 and recognized the potential of GTN. The invention realized by Alfred Nobel in 1863 paved the way for controlled detonation of GTN. Therefore, when Nobel's physician recommended GTN as treatment of his angina pectoris, Nobel wrote ***"Isn't it the irony of fate that I have been prescribed nitroglycerin to be taken internally! They called it Trinitrin, so as not to scare the chemist and the public"*** [2]. There would not be any irony for Nobel if he knew that it was nitric oxide (NO), released from GTN *in vivo*, that helped to relieve his angina. During the mid-19th century, scientists in Britain took an interest in the newly discovered amyl nitrite, and recognized it as a powerful vasodilator. Lauder Brunton, the father of modern pharmacology, used the compound to relieve angina in 1867, noting the pharmacological resistance to repeated doses. In 1977, Ferid Murad discovered the release of NO from GTN and its action on vascular smooth muscle [3]. In 1980, Robert Furchgott and John Zawadski recognized the importance of the endothelium in acetylcholine-induced vasorelaxation and in 1987, Louis Ignarro and Salvador Moncada identified NO as the endothelial-derived relaxing factor (EDRF) [4] [5]. The surprising and exciting discovery of the multiple roles that NO plays

in physiological and pathophysiological functions in humans earned Furchgott, Ignarro, and Murad the Nobel Prize in 1998.

1.2 Organic nitrates

NO is a colorless gas and may react with a variety of atoms and radicals. For example, it may react readily with O_2 to give nitrogen dioxide (NO_2) [6]; the reaction of NO with superoxide anion generates peroxynitrite ($ONOO^-$), an oxidative species that is responsible for certain types of NO-mediated toxicity *in vivo* [7]. Due to the instability and inconvenient handling of aqueous solutions of authentic NO, there is an increasing interest in using compounds capable of generating NO *in situ*, i.e., NO donors. Organic nitrates, such as GTN (**1**), may be the most well-known and the oldest class of NO donors. Some of the other organic nitrates include pentaerythrityl tetranitrate (PETN, **2**), isosorbide dinitrate (ISDN, **3**), isosorbide 5-mononitrate (ISMO, **4**), and nicorandil (**5**) [2].

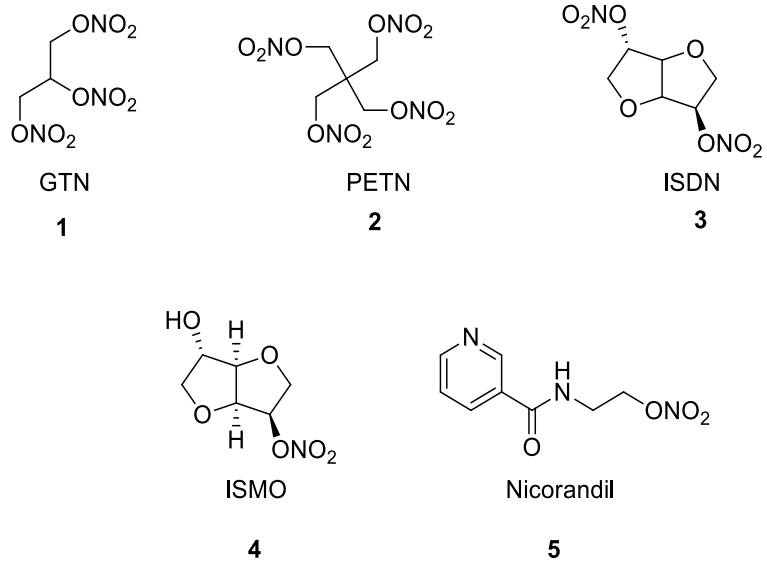
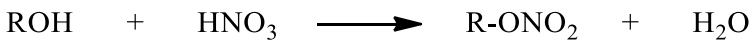
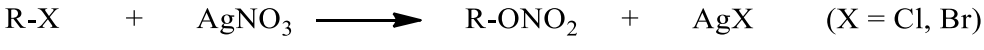


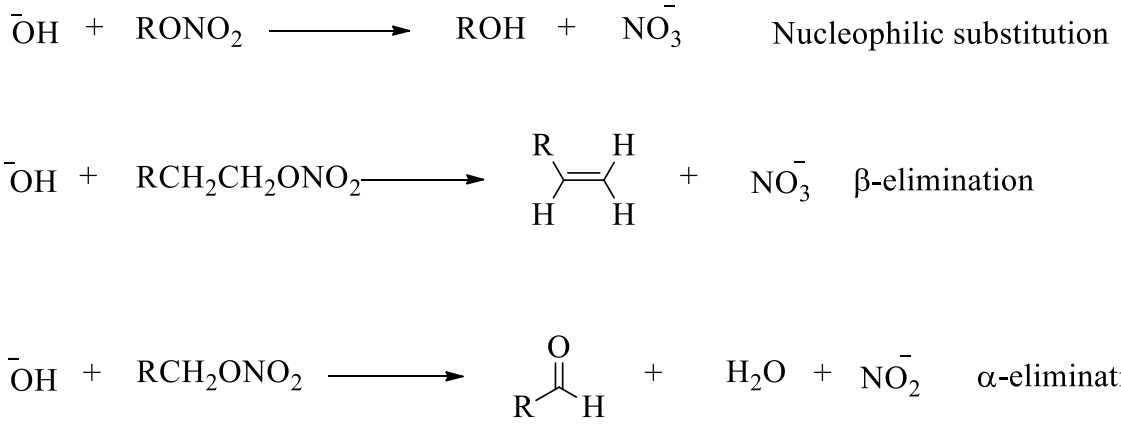
Fig. 1. Some well-known organic nitrates

Organic nitrates can be readily prepared from the esterification of corresponding alcohols or the substitution between reactive alkyl halides and $AgNO_3$ (**Scheme 1**) [8]. They are sparingly soluble in water and are generally stable in neutral or weakly acidic aqueous solutions. Under strong alkaline conditions, they are susceptible to hydrolysis to give alcohol and nitrate (S_N2 substitution), β-elimination to give an alkene, and α-H elimination to give aldehyde and nitrate (**Scheme 2**) [9] [10].





Scheme 1. Synthesis of organic nitrates



Scheme 2. Alkaline hydrolysis of nitrate esters

The NO release from organic nitrates requires enzymatic or non-enzymatic bioactivation. Although the biochemical process of NO release from GTN has not been fully defined, it is likely

that multiple intracellular and extracellular pathways contribute to NO formation from these compounds *in vivo* [11].

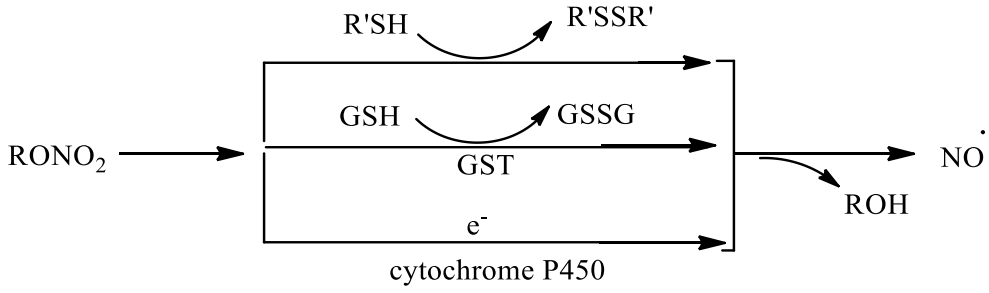


Fig. 2. Biotransformation of organic nitrate esters

It has been suggested that cellular thiols are involved in nonenzymatic formation of NO from GTN [12] [13]. However, since the degradation of GTN by thiols is very slow at room temperature, it appears that the release of NO from reasonable concentrations of GTN and thiols almost certainly involves an enzymatic process [14].

1.3 Biological applications of NO donors

Organic nitrates have long been used to relieve angina pectoris; a disease state caused by constriction of heart arteries. In fact, GTN has a relaxant effect on all types of vessels. Coronary arteries have been found to be more sensitive to GTN than peripheral arteries. GTN has also been used as treatment of acute myocardial infarction [15], congestive heart failure [16], as well as blood pressure control. In addition to relaxation of vascular smooth vessels, GTN is successful in the treatment of children with anal fissures when administered as an ointment [17] and as an alternative to sildenalfil (Viagra) for the treatment of erectile dysfunction [18]. Rectal administration of **1** has been claimed as treatment for prophylaxis of inflammatory bowel disorders [19]. It has been suggested that **3** can be used as a long-term transdermal therapy in preeclamptic

women to avoid maternal hypertension and fetal distress [20]. It was recently reported that organic nitrates eventually inhibited the proliferation of smooth muscle cells (SMC), which was associated with the pathogenesis and progression of atherosclerosis [21] [22] [23].

The role in tumor growth arrest by NO was initially suggested by the observation that murine activated macrophages synthesized nitrite and nitrate leading to cytotoxicity of tumor cells and bacteria [24] [25] [26]. This anti-tumor activity of NO first opened the door to identifying a function of NO as a potential oncologic agent. In general, it has been suggested that at high concentrations NO may have an anti-neoplastic function whereas at low levels it can be pro-angiogenic and pro-tumor formation [27]. NO at high concentrations causes programmed cell death and at low levels protects the cell from apoptosis, which has been suggested to be the result of a dual role of the transformation-related protein 53 (p53) [28]. In this phenomenon, low concentrations of NO may induce *p53* alterations or mutations, which cause tumor cell resistance; however, at high concentrations, the DNA damage induced by NO increases wild-type *p53* leading to programmed cell death [29] [30].

1.4 NO-drug hybrids

The specific characteristics of NO in vasodilatation and a potential role as an anti-neoplastic agent on its own and has led to the development of new hybrid drugs with synergistic activities and minimal side effects that may be caused by either drug alone. For instance, a recent study by Chan *et al.* showed that the regular use of the non-steroidal anti-inflammatory drug (NASAID), aspirin, reduced the risk of colorectal cancers in tumors that over-expressed COX-2 [31]. However, in tumors that showed weak or absent expression of COX-2 the chemo preventive effects were minimal; while the potential side effects of aspirin, including peptic ulcer disease,

were retained in both cohorts. Since established drugs that inhibit carcinogenesis are COX-2 inhibitors, a logical hybrid is one that combines nitric oxide and the non-steroidal anti-inflammatory drugs (NO-NSAIDs). The NO-aspirin analog **6** (**Fig. 3**) developed by Thatcher *et al.*, demonstrated no toxicity when given to human subjects while maintaining COX-1 and antiplatelet activity [32].

NO-NSAIDs are composed of typical non-steroidal anti-inflammatory drugs such as aspirin, salicylic acid, indomethacin, ibuprofen or sulindac to which an NO-releasing moiety has been attached via a covalent bond that is cleaved by non-specific esterase activity. It is the bond between the linker and the NSAID that is cleaved by enzymatic activity. Upon cleavage, the NSAID exerts its COX-inhibitory functions and the generated NO acts in synergy to potentiate the actions of both compounds [32].

In 2000, Swayeh *et al.* reported that nitric oxide (NO) releasing derivatives of NSAID exhibit greater anti-inflammatory, antinociceptive and anti-thrombotic activity than the parent NSAID [33]. With this in mind, they synthesized NO releasing derivative of acetaminophen. This nitrate ester of acetaminophen (NO-APAP, **7**) has been reported to exhibit enhanced anti-nociceptive and anti-inflammatory activity relative to APAP over similar dose ranges. More recently, NO-APAP has been shown to have a reduced hepatotoxicity in human hepatocytes than APAP. Nitric oxide release from NO-APAP is a metabolic and not a spontaneous process. Furthermore, this process causes the production of discrete amounts of NO that do not interfere with blood pressure regulation but could account for the improved pharmacological profile of NO-APAP.

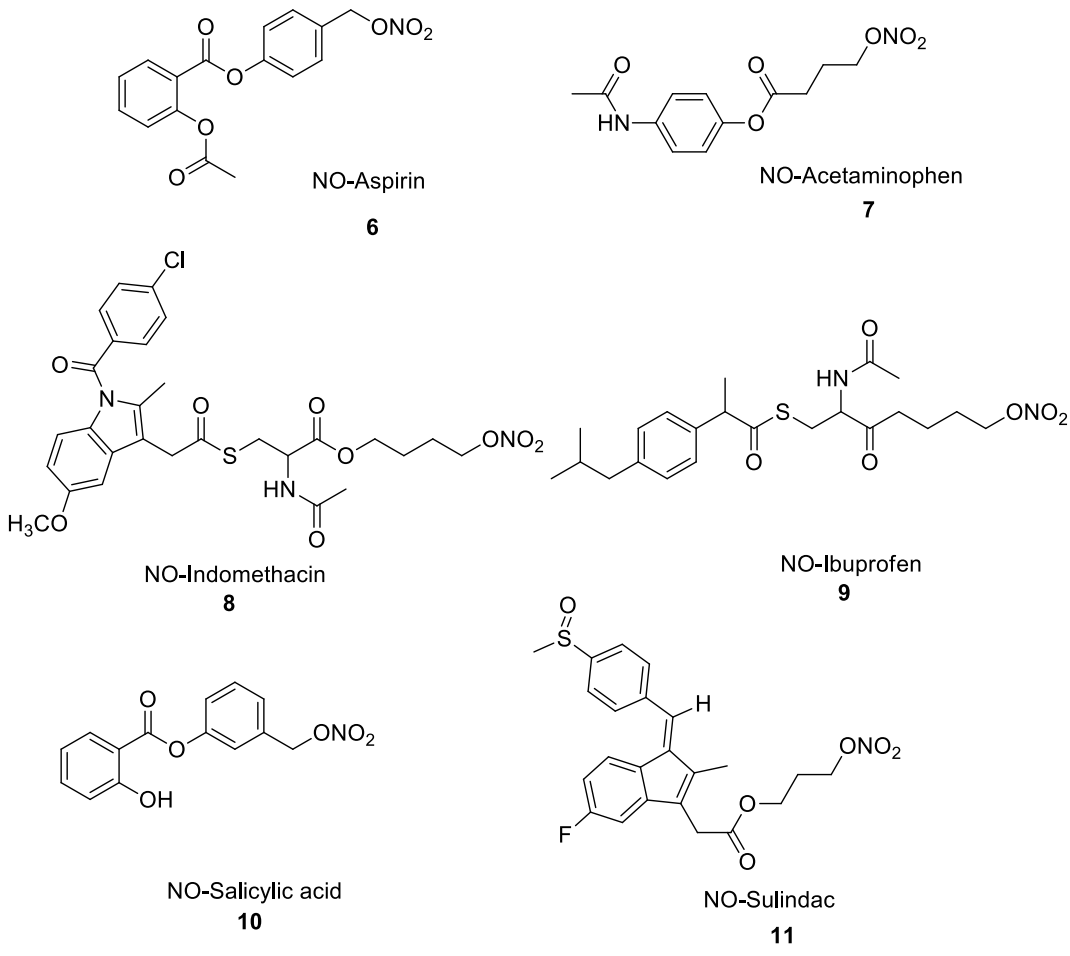


Fig. 3. Structures of some well-known NO-NSAIDS

1.5 Acetaminophen toxicity

The most commonly employed method of managing pain involves the systemic administration of analgesics. Traditionally, analgesics fall into two broad categories: (1) simple, non-narcotic analgesics, such as aspirin, which appear to work by inhibition of prostaglandin synthetase [34], and (2) narcotic analgesics, which appear to work through interaction with the endorphin/enkephalin receptor system of the central nervous system [35]. Narcotic analgesics, can be further divided into two groups, the opioids and non-opioids. The opioid analgesics include among others the phenanthrene alkaloids of opium, comprising morphine, codeine, and thebaine.

The drugs that comprise the group known as the non-opioid analgesics include: (1) N-methyl-D-aspartate (NMDA) receptor antagonists, such as dextromethorphan and ketamine [36]; (2) alpha$_2$ adrenoreceptor agonists, such as clonidine, metomidine, detomidine, dexmetomidine, dexmedetomidine and xylazine, that reduce the release of norepinephrine; (3) other agents, such as tramadol, often mistakenly referred to as an opioid, that produce analgesia by their inhibitory actions on monoamine re-uptake rather than by agonist effect; (4) non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen and other drugs that inhibit cyclooxygenase enzymes [37] and (5) mixed agonist-antagonist analgesics such as buprenorphine, dezocine, nalbuphine. Opioid and non-opioid analgesics may cause a variety of side effects including sedation, constipation, hypotension, nausea, vomiting, elevation of cerebrospinal fluid pressure, respiratory depression, physical dependence and tolerance [38]. Therefore, there is a serious need to develop combinations of drugs that supplement the activity of the opioid and non-opioid analgesics, which allows the use of smaller doses of the opioid and non-opioid analgesics [39].

Analgesics such as acetaminophen and other NSAID type drugs have been used for some time for the treatment of pain and/or as antipyretics**.** However, its high toxicity is the foremost cause of acute liver failure in the western world [40] [41] [42]. In therapeutic use, it is metabolized by hepatic cytochrome P450 enzyme CYP2E1 to N-acetyl-p-benzoquinone imine (NAPQI), which is then immediately conjugated with glutathione (GSH) to form a non-toxic mercapturic acid conjugate which is excreted in urine [43] [44]. In the case of an overdose, glutathione stores are overwhelmed and free NAPQI is able to combine with hepatic macromolecules to produce hepatocellular damage (**Fig. 4**).

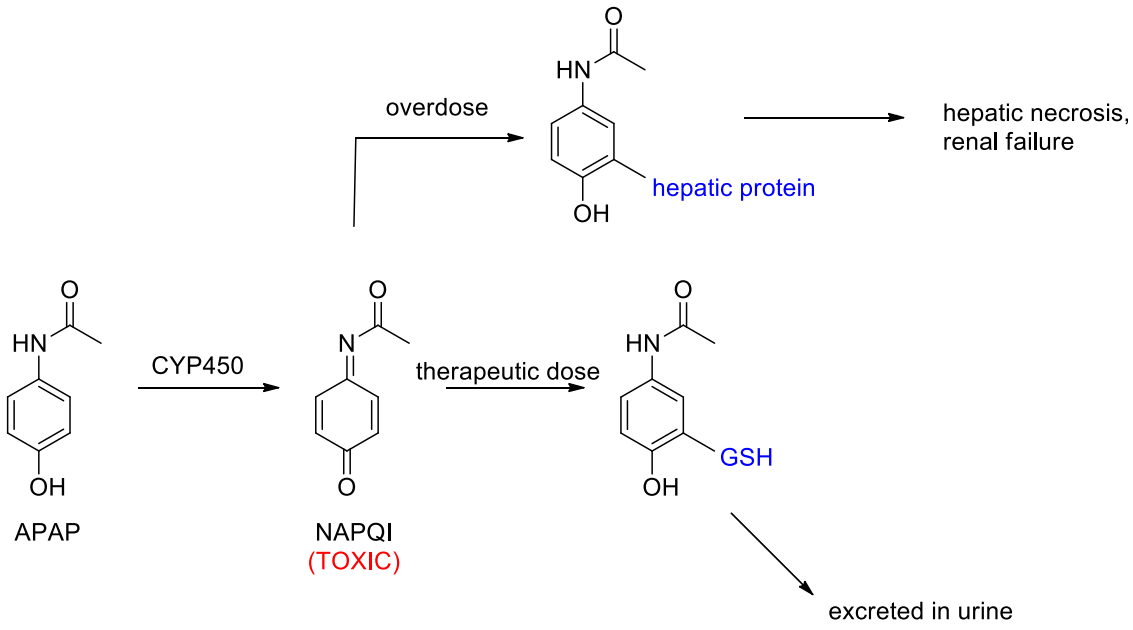


Fig. 4. Mechanism of acetaminophen toxicity

1.6 A series of N-acylated-4-hydroxyphenylamine derivatives (SCP series)

In 1996, Bazan *et al.* synthesized a series of N-acylated-4-hydroxyphenylamine derivatives, linked via an alkyl bridge to the nitrogen atom of a saccharin molecule. This series of compounds was named the "SCP series" (**Fig. 5**) [45]. The SCP series are non-narcotic analgesics that are free from antipyretic activity and have little hepatotoxic effect. The compounds in this series do not produce the metabolite (NAPQI) that is responsible for acetaminophen toxicity and they do not reduce fever. As a result, they are more useful than acetaminophen and other non-narcotic analgesics in the treatment of chronic pain and in situations in which controlling fever is contraindicated, such as after surgery, where fever control can mask infection. Moreover, unlike conventional non-narcotic analgesics, such as aspirin or ibuprofen, the SCP series does not suppress blood coagulation.

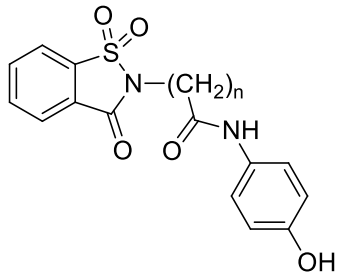


Fig. 5. SCP series, n = 1 to 5

One of them, SCP-1 (n=1) showed analgesic activity equivalent to acetaminophen but, in contrast to the latter, possesses much lower toxicity. Furthermore, SCP-1 (**12**) had the great advantage of not showing antipyretic activity, since antipyresis can mask the presence of latent infections during the post-operative period. It was found to rapidly hydrolyze *in vivo* to give SCP-123 (**13**) which is equipotent on a molar basis with **12**.

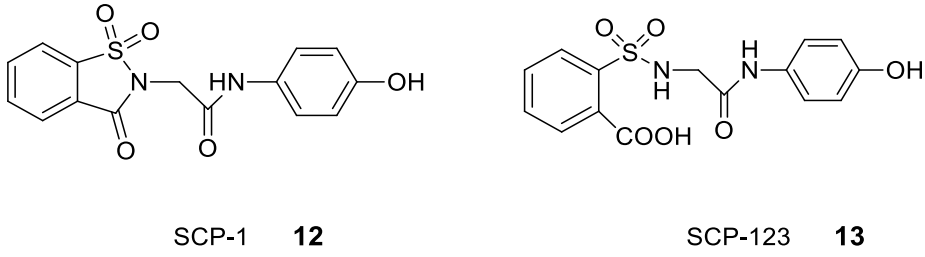
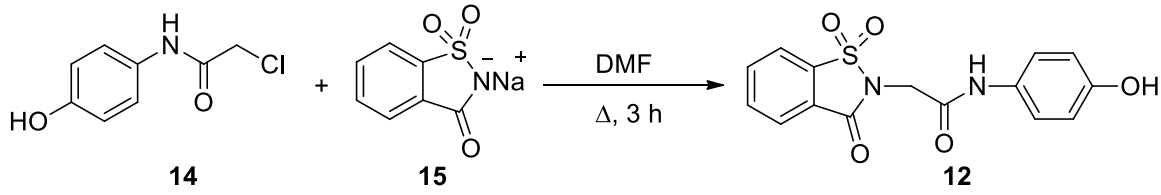


Fig. 6. Structures of SCP-1 and SCP-123

1.7 Synthesis of SCP-1 and SCP-123

In 1996, Bazan *et al.* developed the synthesis of **12** which was prepared by the means of two synthetic routes [45]. In route 1 (**Scheme 3**), 2-chloro-N-(4-hydroxyphenyl)acetamide (**14**) was reacted with the sodium salt of saccharin (**15**) in dimethylformamide (DMF). The mixture was heated at reflux for 3 hours. At the end of this time, the NaCl formed was filtered off and the

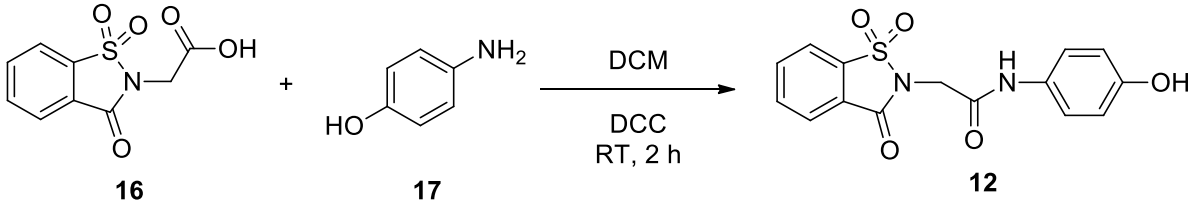
filtrate was evaporated under reduced pressure. The resulting oil was crystallized in 200 mL of n-hexane/acetone (1:1) and the product thereby obtained was recrystallized in ethanol which yields **12** as a white crystalline solid in 86% yield.



14 **15** **12**

Scheme 3. Synthesis of **12** (Route 1)

Alternatively, in route 2 (**Scheme 4**), the acid **16** was reacted with 4-aminophenol (**17**) in the presence of a coupling agent such as dicyclohexylcarbodiimide (DCC). The reaction was carried out in anhydrous methylene chloride (DCM). The reaction was stirred at room temperature for 2 hours. The resulting solid was filtered off and recrystallized in ethanol/water (1:1) to give **12** in 70 % yield.

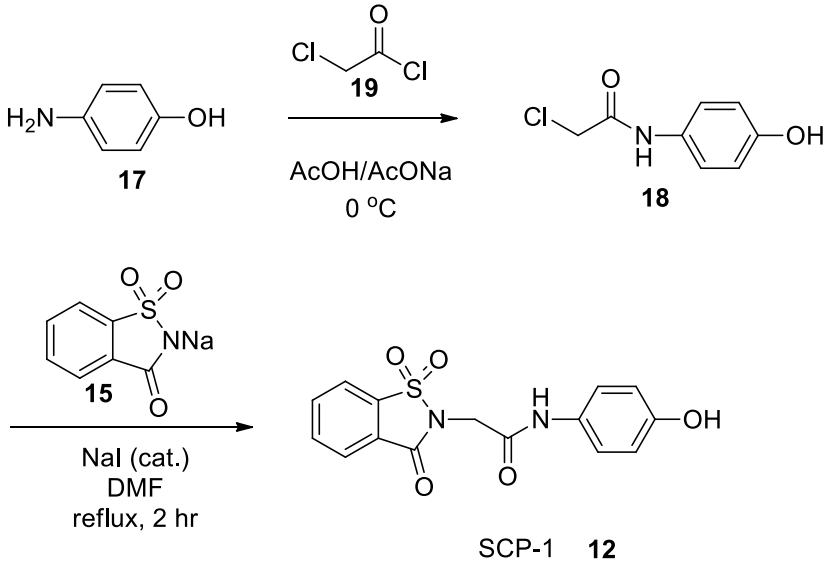


16 **17** **12**

Scheme 4. Synthesis of **12** (Route 2)

In 2009, Miao *et al.* developed the first multigram synthesis of SCP-123 and its sodium salt [46]. Two synthetic routes have been established for its synthesis with two routes differing primarily in the sequence in which the saccharin moiety is added to the acetyl unit. In Route A (**Scheme 5**), the saccharin moiety (**15**) is added in the last step to the 2-chloroacetamide

intermediate **18** that is generated from 4-aminophenol (**17**, 150g) and 2-chloroacetyl chloride (**19**, 155 g) in a solution of acetic acid and sodium acetate. The reaction is kept cold and stirred at ≤ 5 °C. The intermediate **18** precipitates out as a white solid which is then filtered with distilled water to give **18** in a 70% yield.

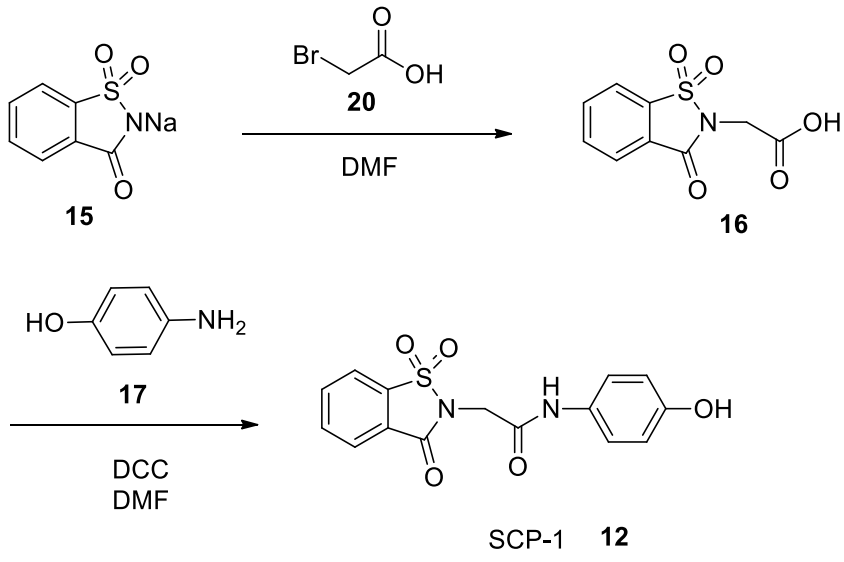


Scheme 5. Synthesis of SCP-1 (Route A)

Chloroacetamide (**18**, 326 g) is then added to the sodium salt of saccharin (**15**, 433 g) in the presence of a catalytic amount of sodium iodide (NaI). The reaction is heated at reflux in DMF for 2 hours. Ice-water is added to the reaction mixture until no more precipitate forms. The sticky white precipitate is then recrystallized in 50% ethanol-water to yield SCP-1 (**12**) in 72% yield.

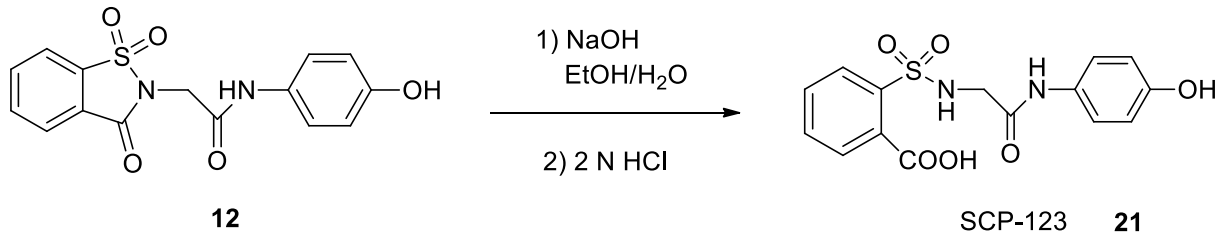
Alternatively, in Route B (**Scheme 6**), the intermediate acetic acid **16** is formed initially from sodium saccharin (**15**) and bromoacetic acid (**20**). The acid **16** is then coupled with **17** to give **12** also via a two-step process. Route A was deemed to be of greater merit due the low cost of the commercially available starting materials and also because both the intermediate **18** and **12** could

be obtained in a state of high purity (>95%) by precipitation or recrystallization. Alternatively, Route B is potentially limited by the hygroscopic intermediate acid **16**, which is difficult to handle, as well the multiple recrystallizations of **12** that are necessary to remove DCC present as a byproduct from the coupling reaction.



Scheme 6. Synthesis of SCP-1 (Route B)

The hydrolysis of **12** was readily achieved with NaOH solution followed by treatment with 2 N HCl (**Scheme 7**). This afforded the corresponding acid **21** (SCP-123) in 93% yield. The hydrolysis step was found to be sensitive to the concentration of the saccharin derivative **12** in the basic solution. If the reaction mixture was not sufficiently dilute, the formation of side product **22**, that resulted from oxidative phenolic coupling, was obtained.



Scheme 7. Synthesis of SCP-123

An optimized concentration of **12** in 0.5 N NaOH was determined to be 0.25 M. At this concentration, the hydrolysis of **12** proceeded cleanly, and the oxidative-coupling product **22** was not observed.

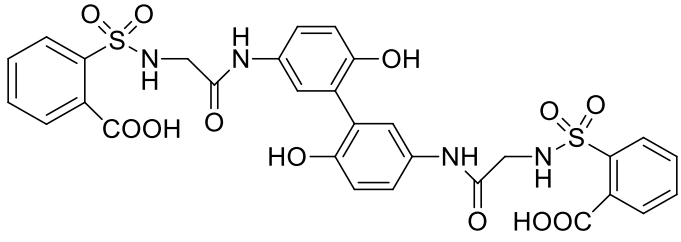


Fig. 7. Byproduct **22**

1.8 Design strategy for NO-SCP-1 hybrids

The goal of this project was to synthesize a series of NO-donor analogs of SCP-1 which could have similar analgesic properties to SCP-1 but also show reduced hepatotoxicity. Since, previously synthesized NO-drugs typically had a linker molecule that attached the NO moiety to the parent NSAID, similar design was incorporated in the synthesis of NO-SCP-1. Comparable to NO-acetaminophen, it was decided that that linker molecule would be an alkyl chain attached to the phenolic OH via an acyl group. **Fig. 8** shows the general formula for the NO-SCP-1 analogs.

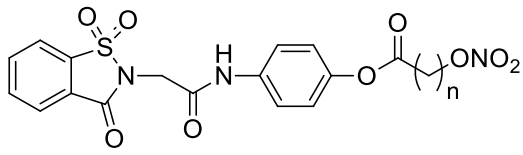
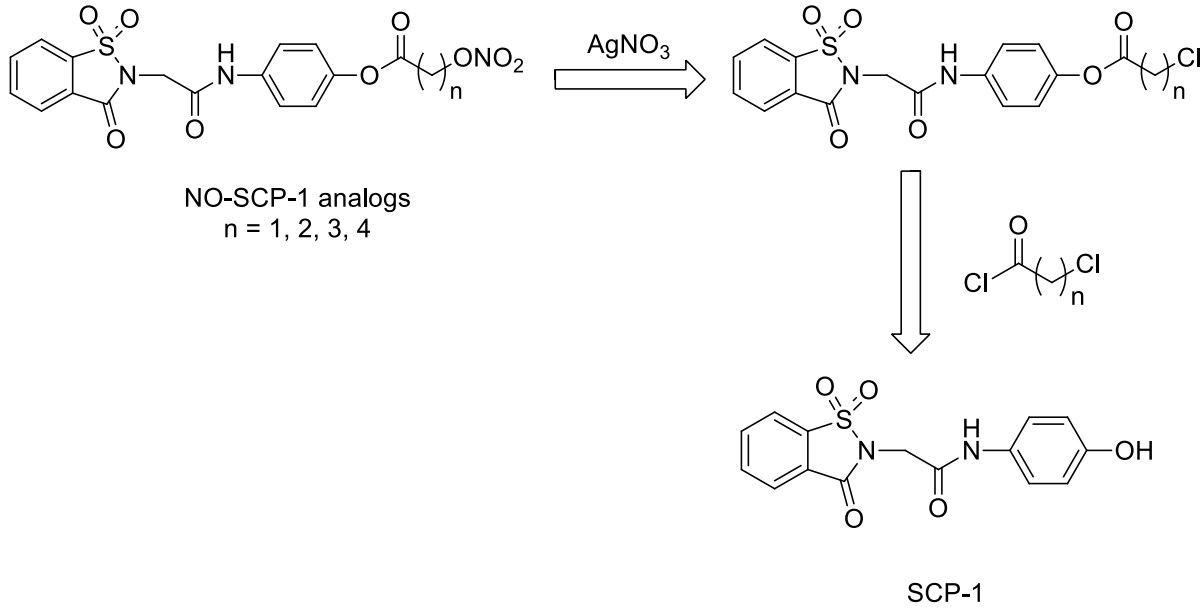


Fig. 8. NO-SCP-1 analogs; n = 1-4

A retrosynthetic route for the synthesis of the NO-SCP-1 analogs is outlined in **Scheme 8**. The synthesis would start from the readily available SCP-1. The linker alkyl chain would be attached to the phenolic OH via *O*-acylation using various chloroalkanoyl chlorides. The chloro group would then be substituted with a nitrate (-ONO_2) group using a simple substitution reaction.



Scheme 8. Retrosynthetic route for synthesis of NO-SCP-1 analogs

1.9 Hydrophobicity in drug design

Hydrophobicity is most commonly understood as the tendency of non-polar molecules to form aggregates in order to reduce their surface of contact with polar molecules such as water [47]. A drug's solubility in water plays a significant role in its design and discovery since the cells in our bodies contain about 65% water. In living matter, water acts as an inert solvent, a dispersing medium for colloidal solutions and as a nucleophilic reagent in numerous biological reactions. Furthermore, hydrogen bonding and hydrophobic interactions in water influence the conformations of biological macromolecules, which in turn affects their biological behavior. It also makes drug toxicity testing and bioavailability evaluation as well as clinical application easier. This means that there is usually a need to design a reasonable degree of water solubility into the structure of a new drug early in the development of that drug [48].

Oral ingestion is the most commonly employed route of drug delivery due to its ease of administration. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability. The most frequent causes of this poor bioavailability are attributed to poor solubility and low permeability. Drugs administered orally have to dissolve in the aqueous gastric fluid before they can be absorbed and transported to their site of action. Drugs that are sparingly soluble in water will be deposited before they reach their site of action. This can clog up blood vessels and damage organs. Water solubility also affects the transport of drugs through membranes such as the blood-brain barrier. The water solubility of an organic compounds depends of the number and nature of polar groups in the structure. In general, higher the number of polar groups, the more water soluble the compound. However, if a drug is too water soluble, it will not be readily transported across lipid membranes [49].

1.9.1 Design of water-soluble analogs of SCP-1

One of the major drawbacks of SCP-1 is its low water solubility. Therefore, there was a need to develop analogs of SCP-1 with increased water solubility. One of the methods to increase water solubility was to attach a polar group to the phenolic moiety such as a glucose molecule. Addition of a carbohydrate molecule to the structure of SCP-1 will not only increase its water solubility but also potentially reduce toxicity [50]. Small molecule drugs, no matter how heavily glycosylated, will always have the potential to pass into the kidneys, through glomerular filtration, and be rapidly cleared.

Alternatively, the water-solubility of SCP-1 could be increased by hydrolyzing the saccharin ring. This can be achieved by synthesizing an array of open ring esters or amides. Amides would be preferred since they are more stable and water-soluble compared to esters.

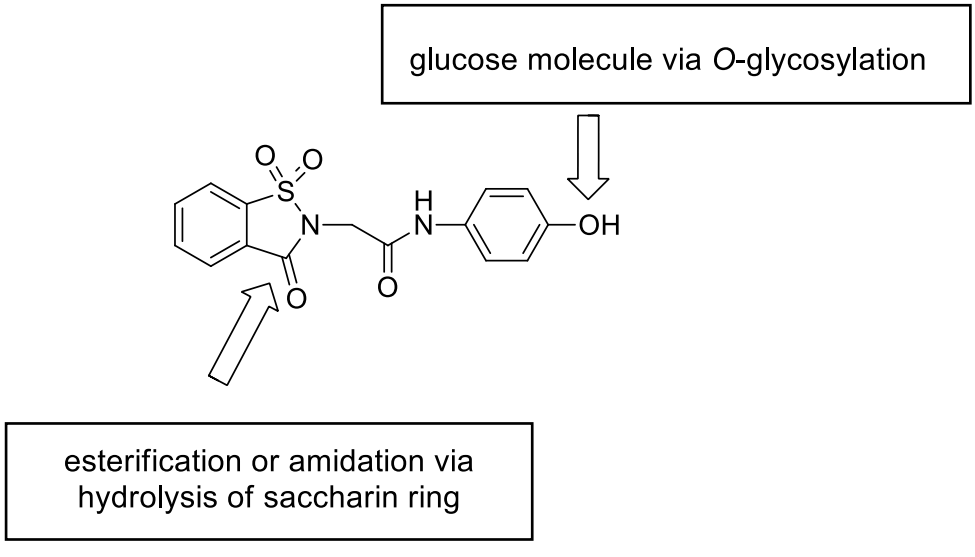


Fig. 9. Design strategy for water-soluble analogs of SCP-1

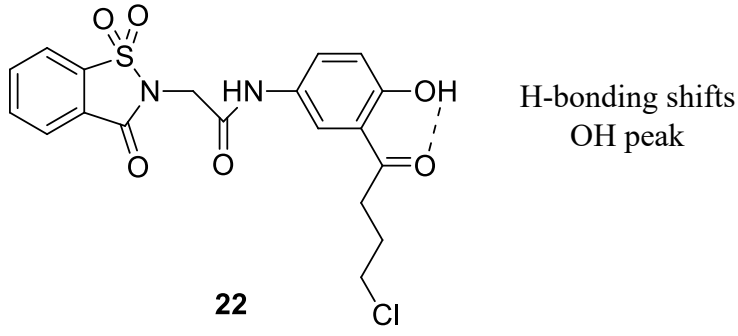
RESULTS AND DISCUSSION

1.10 Nitrate ester analogs of SCP-1 (NO-SCP-1)

1.10.1 Synthesis of chloroalkanoyl ester analogs of SCP-1

As illustrated in **Scheme 9**, the synthesis of the chloroalkanoyl ester analogs of SCP-1 was achieved by the *O*-acylation of the phenolic OH of **12**. Complete *O*-acylation of **22a**, **22b**, **22c** and **22d** was achieved using 2% TfOH/acetonitrile solution [51]. It was observed that lower concentrations of TfOH led to incomplete conversion while higher concentrations of TfOH afforded intractable mixtures.

The *O*-acylation must be carried out in an inert atmosphere and the acid chloride should be added dropwise or in small aliquots. Adding the acid chloride all at once resulted in the formation of the *C*-acylated product. This was observed when 3 equivalents of 4-chlorobutyryl chloride was added to SCP-1. Addition of acid chloride all at once, led to the formation of a mixture of the desired *O*-acylated product **23c** and the *C*-acylated product **22**.



22

Fig. 10. Structure of C-acylated product **22**

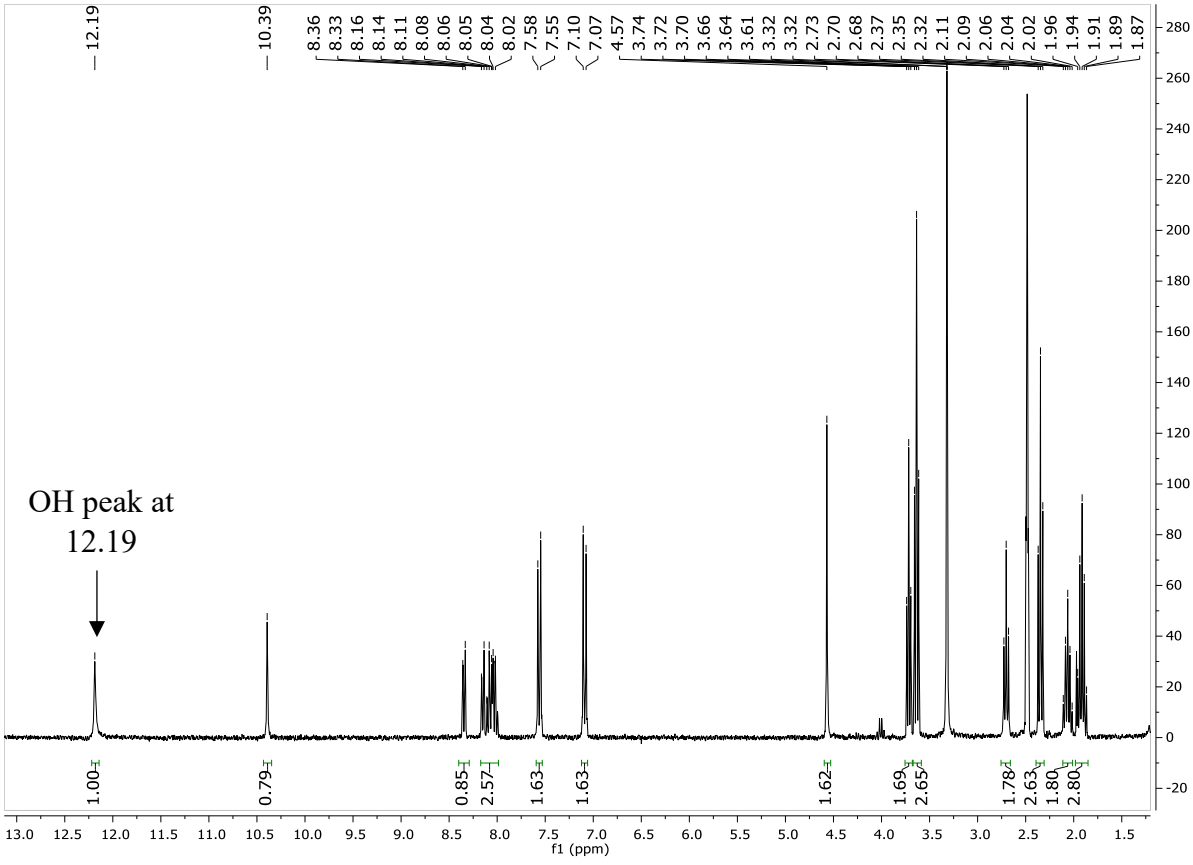
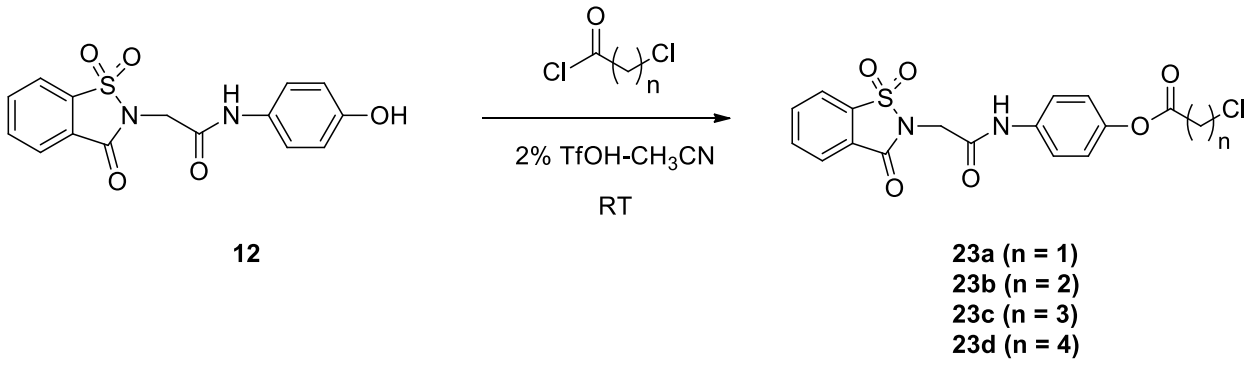


Fig. 11. ^{1}H NMR of **22**

The structure of **22** was confirmed using ^{1}H NMR **(Fig. 11**). The NMR showed a peak at 12.19 ppm which corresponded to the phenolic OH of **22**. The peak for the phenolic OH of SCP-1 appears at ~10.00 ppm. This shift in the OH peak is attributed to the hydrogen bonding between the carbonyl oxygen of the acyl group at the *ortho* position and the hydrogen of the phenolic OH. This hydrogen bonding shifts the signal for the OH peak downfield.

The optimized reactions conditions produced nearly quantitative yields of the corresponding *O*-acylation products (**22a-d**) with no observable *C*-acylation of the electron-rich aromatic ring via Friedel-Crafts acylation.



Scheme 9. Synthesis of Chloroalkanoyl esters of SCP-1

The amount of chloroalkanoyl chloride varied with the length of the carbon chain; the shorter alkyl chain required additional molar equivalents of the chloroalkanoyl chlorides as well as longer reaction times for complete conversion into the esters **23a** and **23b**. Both **23a** and **23b** required 16 hours for complete *O*-acylation with 3 equivalents of the corresponding chloroalkanoyl chloride. However, esters **23c** and **23d** were prepared using only 1.5 and 1.1 equivalents of the chloroalkanoyl chlorides respectively, with shorter reaction times (1 hour). The isolated yields of the chloroesters are reported in **Table 1**. The structure of the esters **23b** and **23c** were unequivocally confirmed by X-ray crystallography (**Fig. 12**) [52].

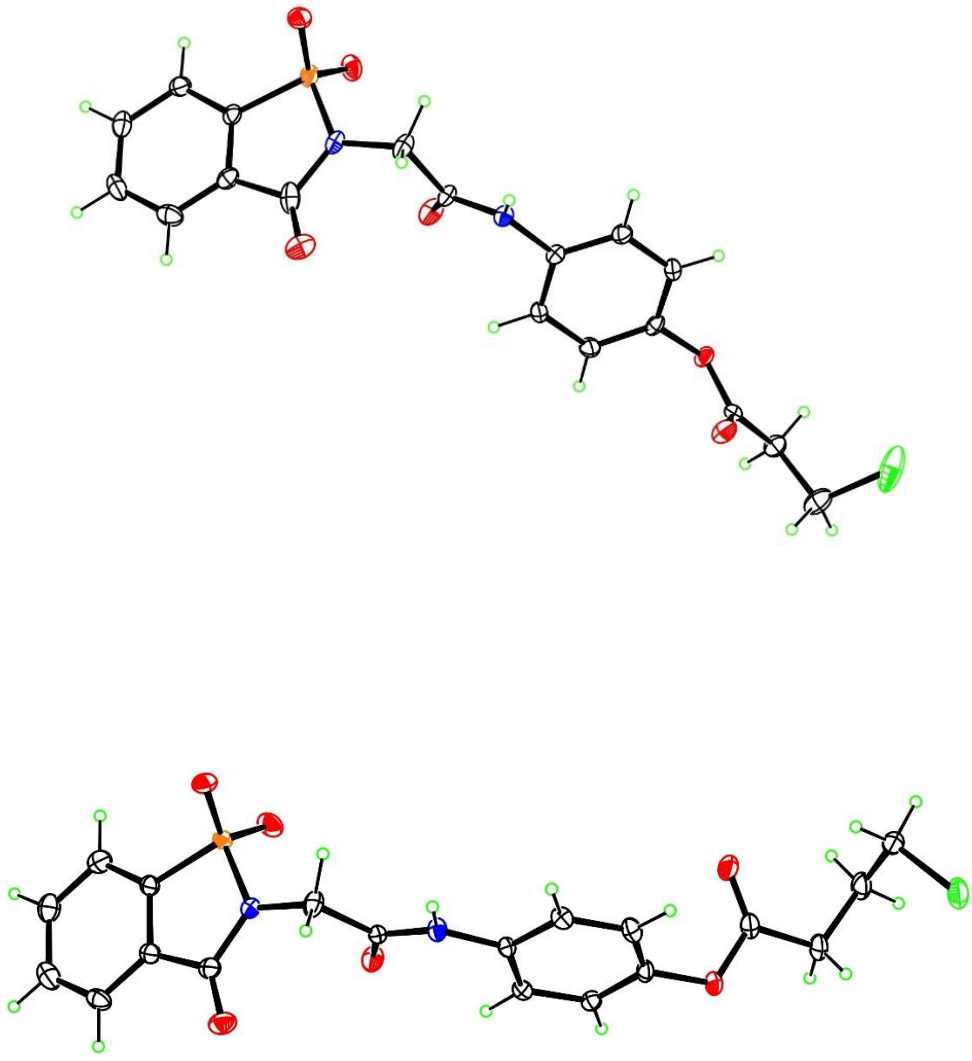


Fig. 12. X-ray crystal structure of esters **23b** and **23c**

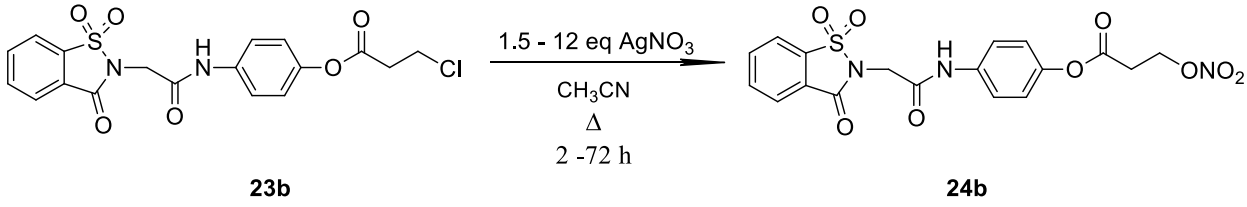
Table 1. Optimized yields of chloroalkanoyl esters of SCP-1

Compound	n	Equivalents of acid chloride	Time (h)	Yield (%)[a]
23a	1	3	16	99
23b	2	3	16	98
23c	3	1.5	1	99
23c	4	1.1	1	99

[a] Isolated yields

1.10.2 Synthesis of Nitrate esters of SCP-1 (NO-SCP-1)

The synthesis of the nitrate esters was attempted by using $AgNO_3$ in acetonitrile (**Scheme 10**). Reactions were set up by varying the number of equivalents of $AgNO_3$ and the reaction time. Initially, 1.5 equivalents of $AgNO_3$ was added to **23c** and the reaction was heated at reflux for 2 hours. The reaction was monitored by Thin Layer Chromatography (TLC) and after 2 hours, there was no visible product formation. The reaction time was increased to 6 hours with 2 equivalents of $AgNO_3$. There was still no product formation by TLC. Reactions were the carried out 3, 4, 6, 8, 10 and 12 equivalents of $AgNO_3$ with different reaction times (**Table 2**).



23b **24b**

Scheme 10. Attempted synthesis of **24b**

It was concluded that the molecular polarities of the chloroesters and nitrate esters were similar and therefore had the same R_f values on the TLC plate and also exhibited nearly identical ^{1}H NMR spectra (**Fig. 13**). ^{13}C NMR was found to be extremely valuable for following the reaction progression. A distinct signal for the (*C*-ONO_2) carbon at δ 73.3 ppm was used to follow the formation of nitrate esters while the (*C*-Cl) signal at δ 44.9 ppm inherent to the chloroesters decreased as **23b** was converted to **24b** (**Fig. 14**).

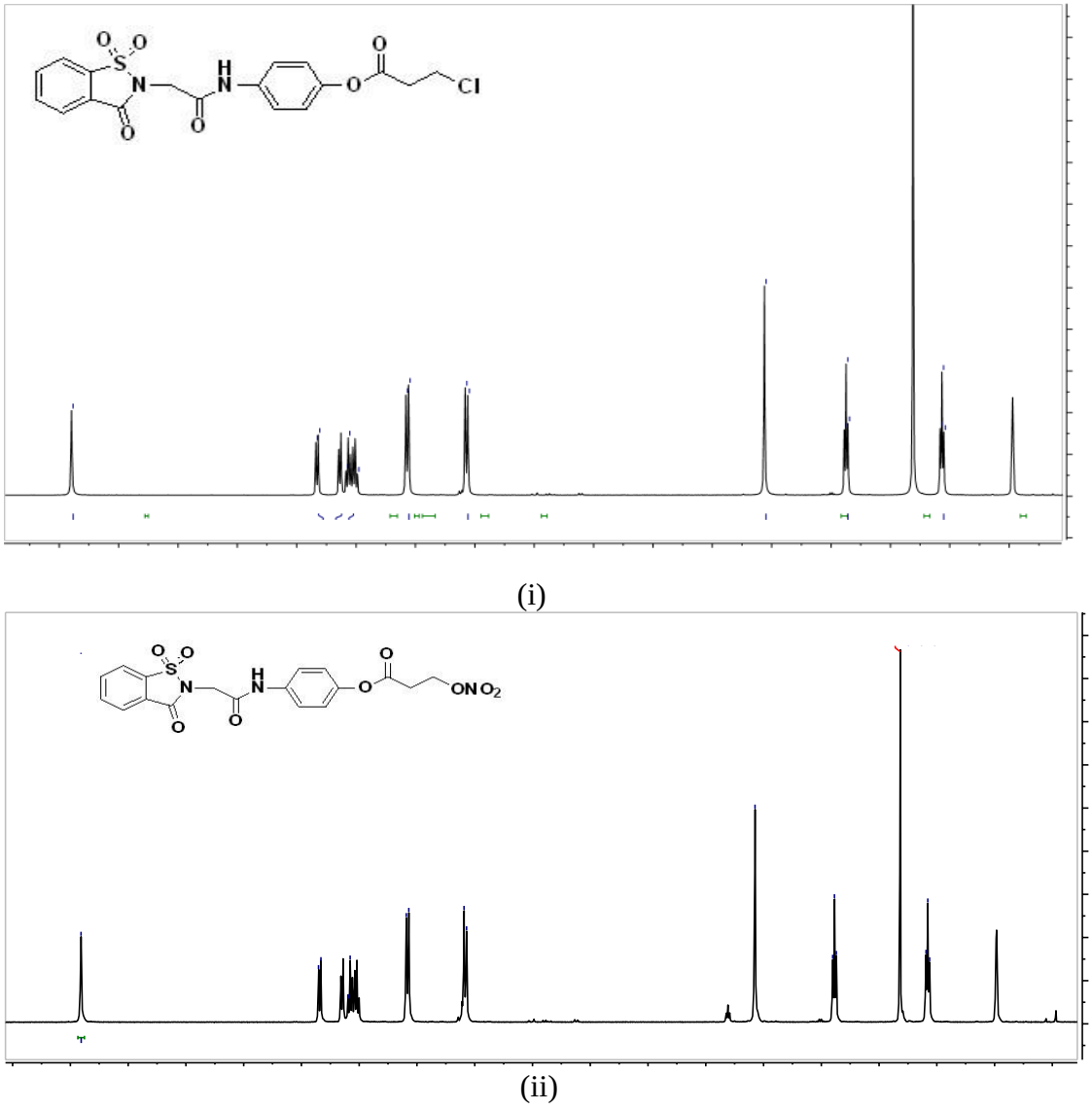
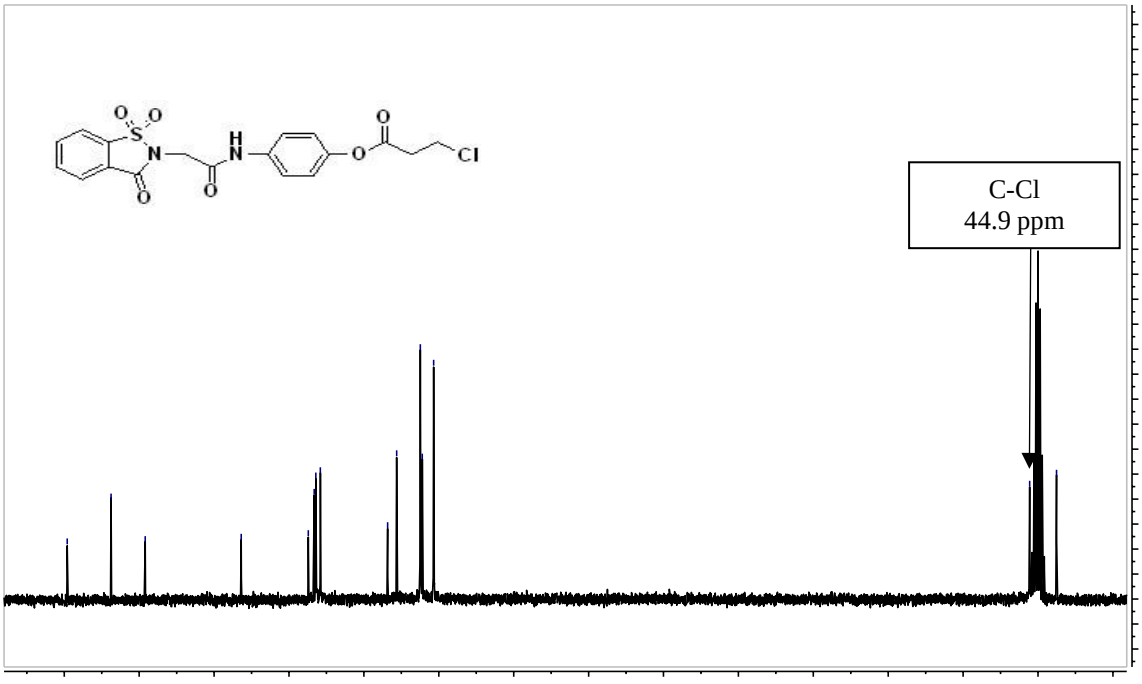
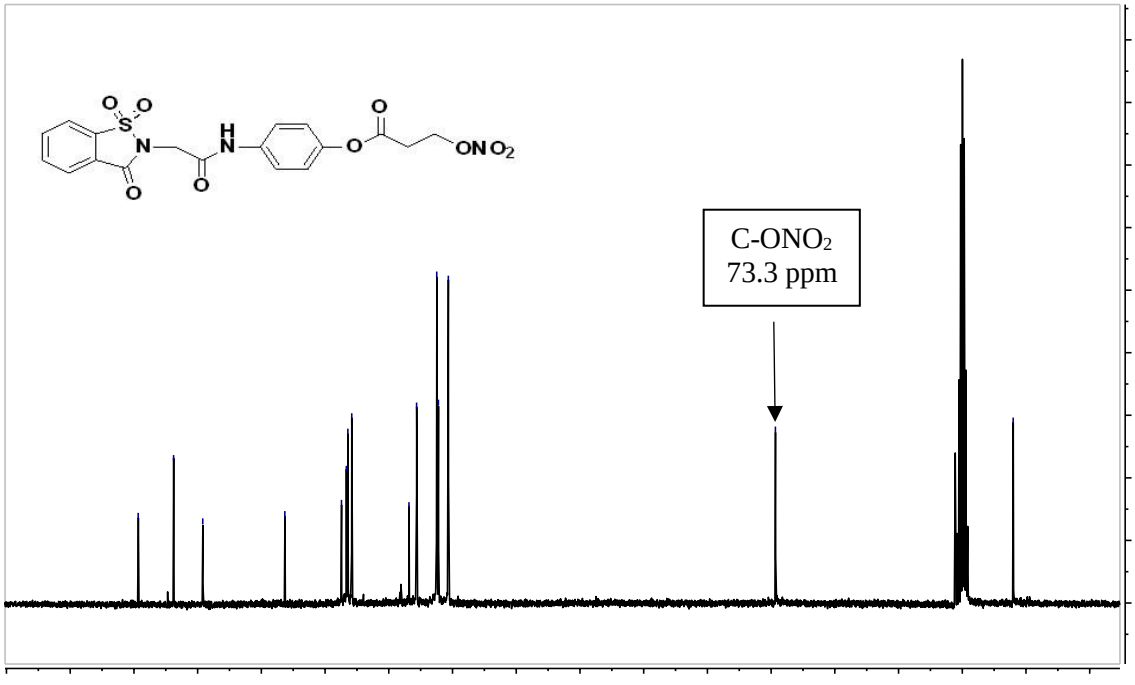


Fig. 13. 1H NMR for (i) **23b** and (ii) **24b**



(i)



(ii)

Fig. 14. ^{13}C NMR for (i) **23b** and (ii) **24b**

Complete conversion of chloroesters to nitrate esters was observed using 12 equivalents of $AgNO_3$ with a reaction time of 72 hours. Using fewer equivalents of $AgNO_3$ resulted in mixtures of chloroesters and nitrate esters. This was confirmed by obtaining the X-ray crystal structure of the product obtained in **entry 5** (**Table 2**). It was observed that the product was a disordered mix of the chloroester and the nitrate ester which means atomic sites in a single crystal contained a hybrid atom that contained either the chloro or the nitrate atom. Refinement of the population parameters yielded 78% chloro and 22% nitrate (**Fig. 15**).

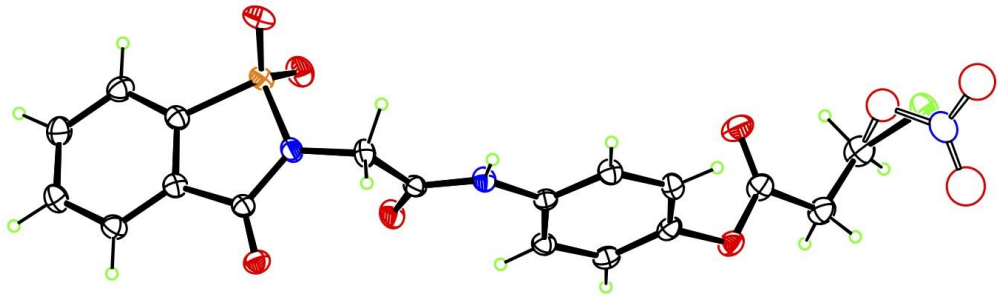


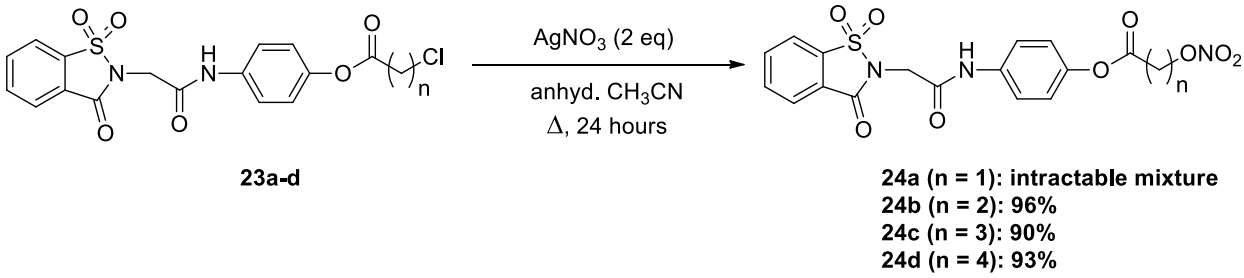
Fig 15. X-ray crystal structure of chloro and nitrate hybrid product

Table 2. Optimization yields for nitrate ester **24b**

Entry	Equivalents of $AgNO_3$	Reaction time (h)	Percent yield (%)
1	1.5	2	86[a]
2	2	6	90[a]
3	3	24	78[a]
4	4	48	80[a]
5	6	48	86[a]
6	8	72	90[a]
7	10	72	88[a]
8	12	72	80[b]

[a] Mixtures of chloroesters and nitrate esters
[b] Isolated yield of nitrate ester **24b**

Reactions were then set up using anhydrous CH_3CN to see if would further optimize the reaction conditions. It was observed that using anhydrous CH_3CN, only 2 equivalents of $AgNO_3$ was required for a full conversion to the nitrate ester **24b** and the product was obtained in 96% yield. Furthermore, the reaction time was also reduced to 24 hours (**Scheme 11**). These optimized conditions were then used to synthesize nitrate esters **24b** to **24d** in good yields (>90%). Attempts to prepare the nitrate ester **24a** were unsuccessful and led to an intractable mixture. However, the longer chain derivatives **24b**–**d** were quite stable and no decomposition was observed over a period of several months. The structure of the nitrate ester **24b** was established by X-ray diffraction (**Fig. 16**).



Scheme 11. Synthesis of nitrate esters **24a-d** using optimized condition

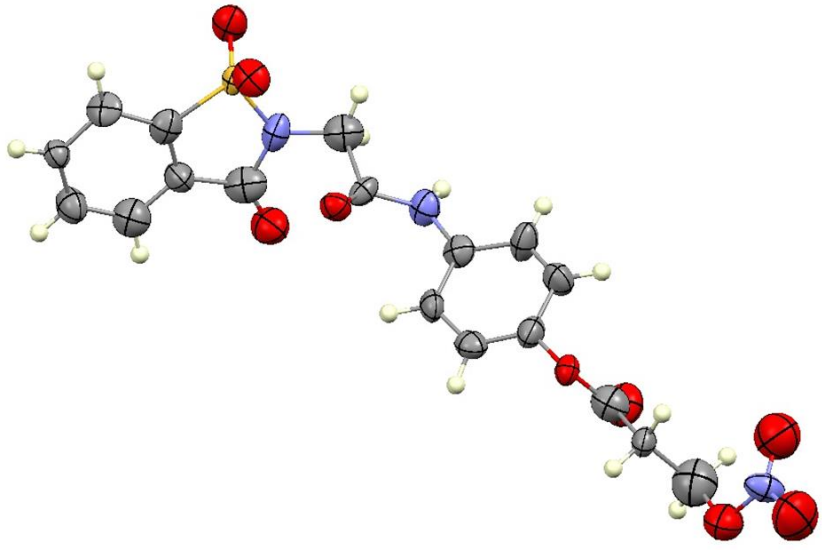


Fig 16. X-ray crystal structure of **24b**

1.10.3 Biological evaluation of nitrate esters 24b and 24c

The nitrate esters **24b** and **24c** were then evaluated for hepatotoxicity. Hepatic GSH (Glutathione) levels were measured in human liver cells over a period of 3, 6 and 12 h for nitrate esters **24b** (MD-38) and **24c** (MD-39). At 500 μM, both **24b** (MD-38) and **24c** (MD-39) led to some GSH depletion as compared to SCP-1 and APAP. However, the level remains constant over a period of 12 h and does not deplete any further (**Fig. 17**). At 1000 μM of **24b** (MD-38) and **24c** (MD-39), GSH levels for both compounds were found to be comparable to SCP-1 and higher than that of APAP after a period of 12 h. Therefore, both **24b** (MD-38) and **24c** (MD-39) were well tolerated by the human hepatocytes and were generally less toxic than APAP.

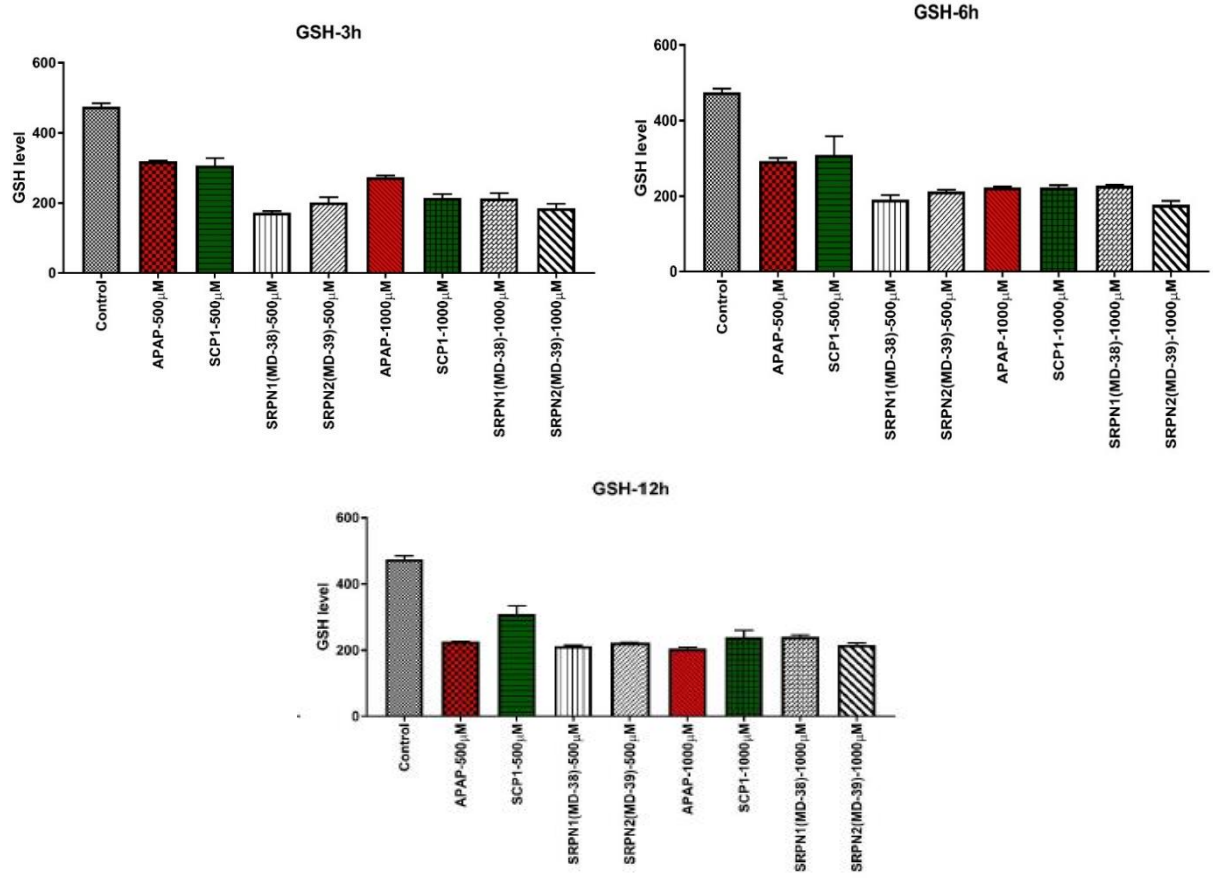


Fig. 17. Comparison of the glutathione depletion caused by nitrate esters **24b** (MD-38) and **24c** (MD-39) over 3 h, 6 h and 12 h with APAP and SCP-1

Nitrate esters **24b** (MD-38) and **24c** (MD-39) were also evaluated for necrotic cell death by measuring LDH (lactate dehydrogenase) release over a period of 12 h (**Fig. 18**). At a concentration of 500 μM, **24c** (MD-39) had higher LDH release than both APAP and SCP-1 over the 12-hour period. However, the nitrate ester **24b** (MD-38, 500 μM) had significantly less LDH release than APAP and with similar activity to SCP-1. At the higher concentration of 1000 μM, **24b** (MD-38) had significantly less LDH release than either APAP or SCP-1. This indicates that that **24b** (MD-38) is better tolerated than APAP and exhibits a similar necrotic profile to that of SCP-1.

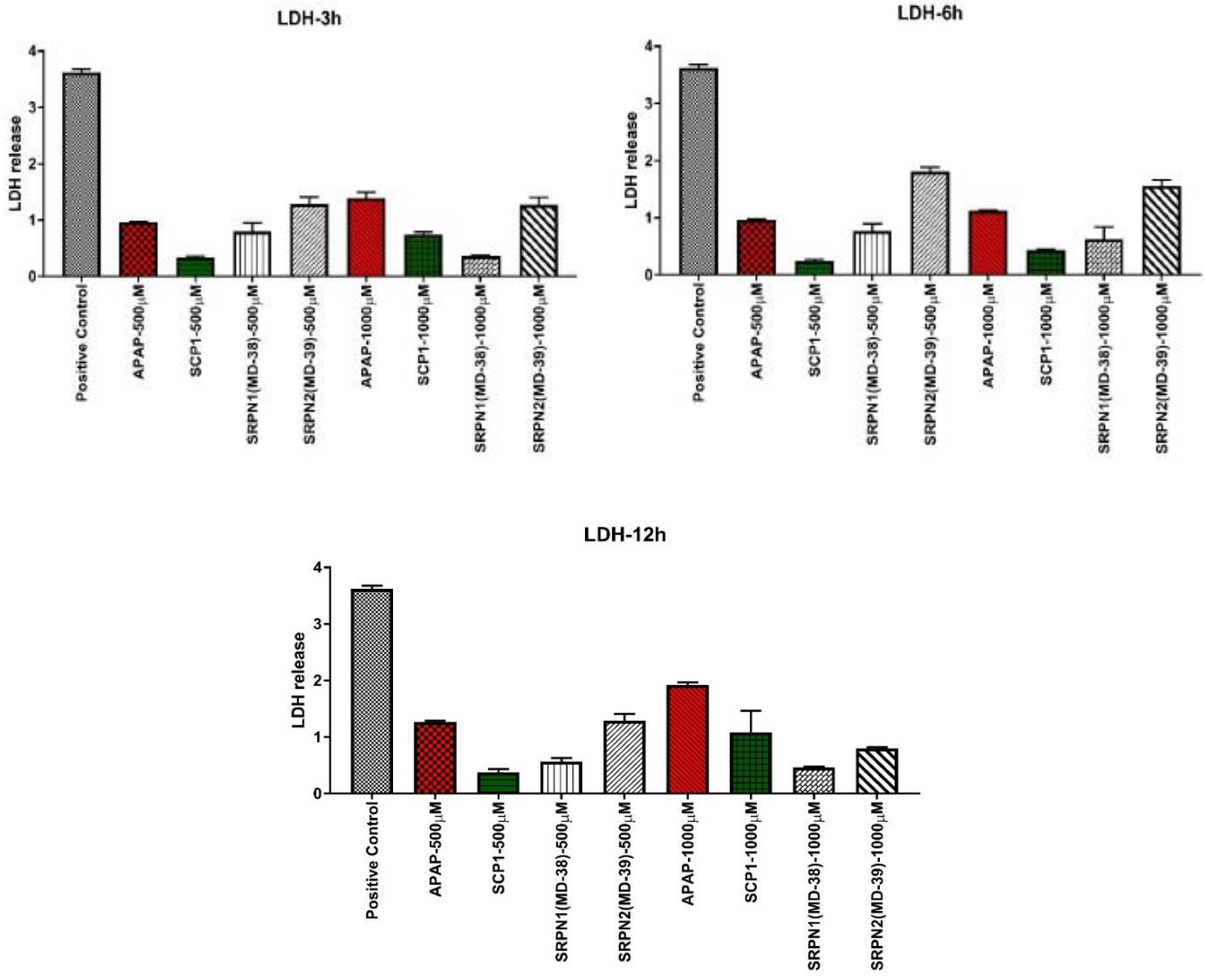


Fig. 18. Comparison of the LDH released by nitrate esters **24b** (MD-38) and **24c** (MD-39) over 3 h, 6 h and 12 h with APAP and SCP-1

As shown in **Fig. 19**, the cytochrome P450 (CYP450) enzyme profiles were also evaluated for the nitrate esters in three human isozymes (CYP3A4, CYP2E1 and CYP2D6) typically involved in hepatic drug metabolism. Enzyme inhibition by **24b** and **24c** was compared to known inhibitors ketoconazole (keto), tranylcypromine (TCP) and quinidine as well as to APAP and SCP-1. At a concentration of 1 μM, the compounds **24b** and **24c** were found not to be enzyme inhibitors and did not damage the three CYP450 system enzymes tested (CYP3A4, CYP2E1 and CYP2D6). The enzyme activity levels were generally higher for **24b** and **24c** than for APAP in CYP3A4 and CYP2E1 enzymes systems and were similar to SCP-1.

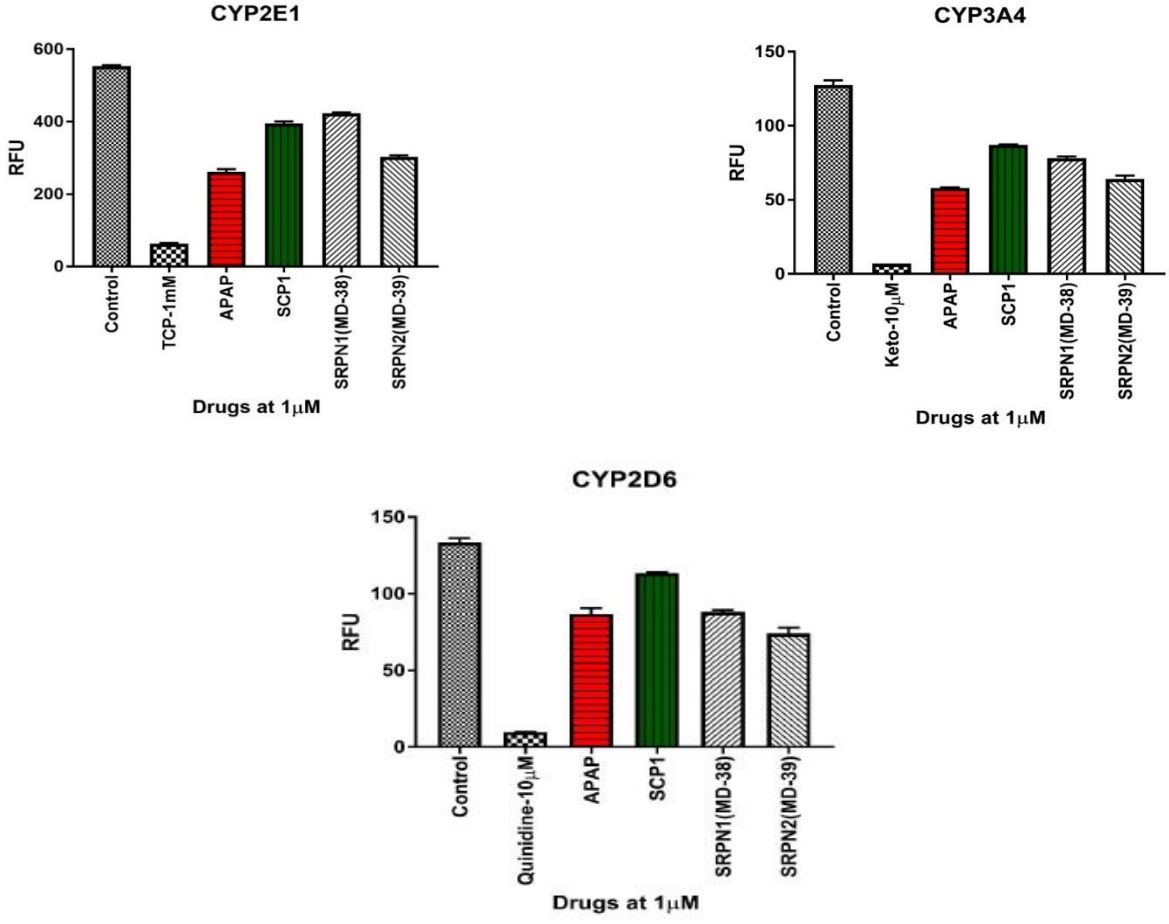


Fig. 19. CYP450 enzyme profiles for **24b** (MD-38) and **24c** (MD-39).

Satisfied that the nitrate esters exhibited a toxicity profile superior to APAP and comparable to SCP-1, it was interesting to evaluate the potential NO-donor capability of terms of possible enhanced anti-inflammatory activity. It was envisaged that the nitrate esters should exhibit enhanced anti-inflammatory activity relative to SCP-1 similar that observed for NO-APAP relative to APAP. To this end, the antipyretic activity was evaluated. SCP-1 does not exhibit antipyretic activity, so any reduction fever could be presumed to be due to NO-release from the nitrate esters., Yeast-induced fever reduction in rats was determine for **24b** and **24c** and compared to APAP. Rectal temperature was measured 2 hours after the LPS injection and every 2 hours after compound administration. **Fig. 20** illustrates that the antipyretic activity of the nitrate ester **24c** (MD-39) was significantly different and comparable to APAP at reducing fever at the dose tested (1 μM). The antipyretic profile of **24b** was not significantly different than vehicle and was slightly less effective than APAP.

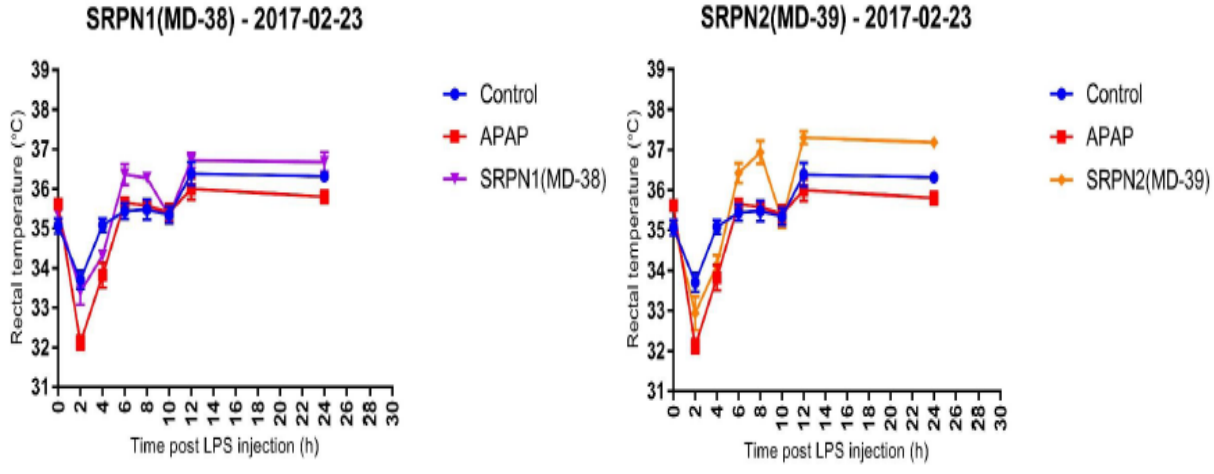
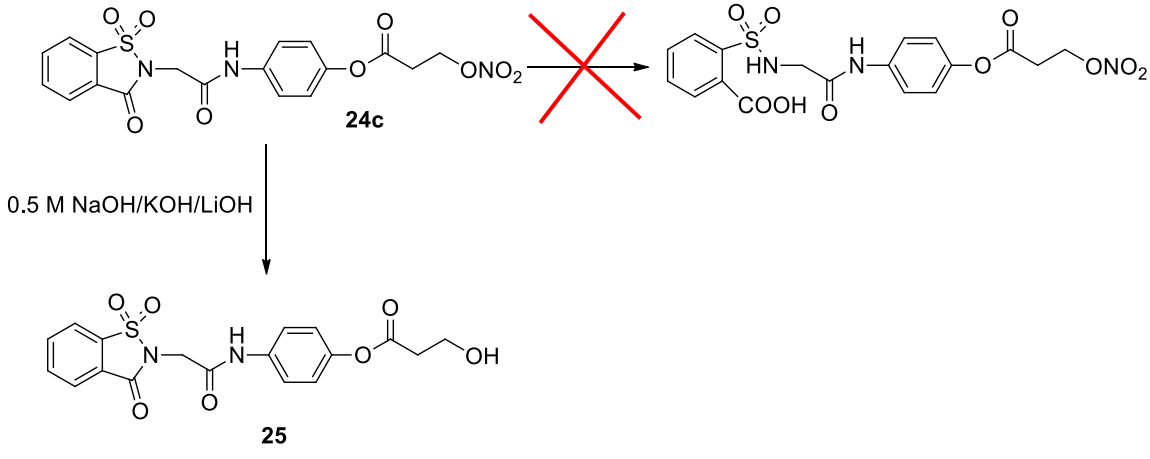


Fig. 20. Antipyretic profile for **24b** (MD-38) and **24c** (MD-39).

1.11 Water-soluble analogs of SCP-1 and SCP-123

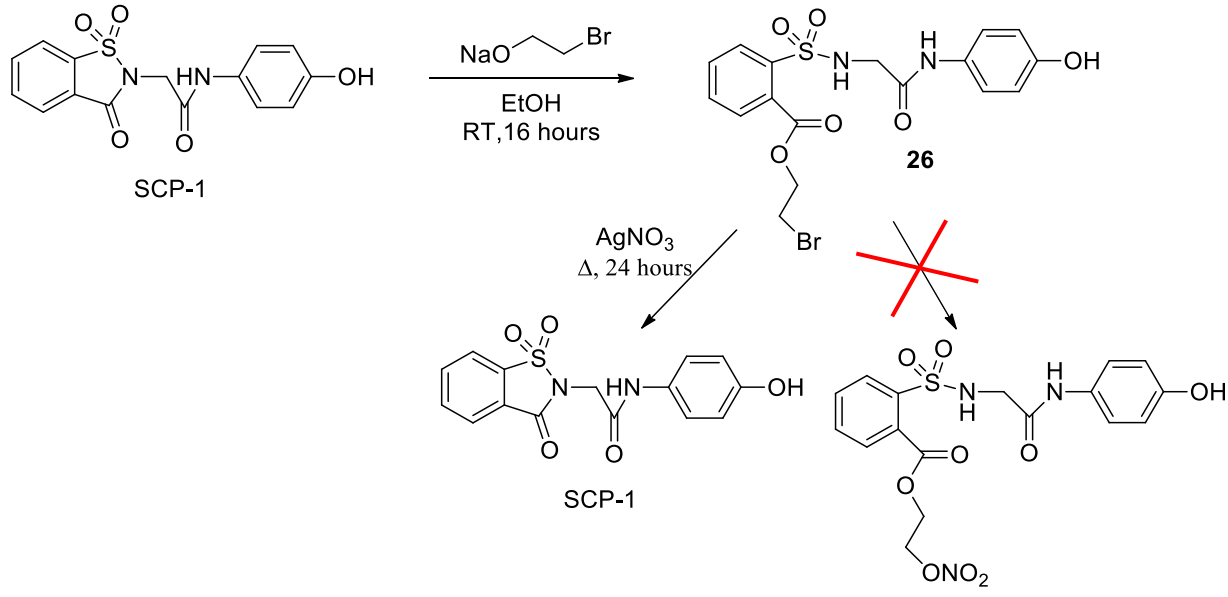
1.11.1 Attempted synthesis of nitrate ester analogs of SCP-123

Strategy 1: Following solubility issues, the focus of the project was then shifted toward the synthesis of nitrate ester analogs of SCP-123. Since it has been previously established that SCP-1 is a prodrug and it is converted to SCP-123 *in vivo*, it was envisioned that the nitrate ester analogs of SCP-123 would be more effective and will be more water-soluble. This strategy involved the hydrolysis of already synthesized nitrate esters of SCP-1. One equivalent of 0.5 N solution of sodium hydroxide was added to **24c** and the reaction was left to stir overnight at room temperature. However, the ^{13}C NMR showed a shift in the C-ONO_2 peak from 73.3 ppm to 56.4 ppm which suggests hydrolysis of the nitrate ester to the alcohol **25** (**Scheme 12**). The reaction was then repeated with 0.5 N solution of LiOH and KOH, however, the result was the same. It was therefore concluded, that even a dilute solution of LiOH, NaOH and KOH resulted in the hydrolysis of the nitrate ester to an alcohol.



Scheme 12. Attempted synthesis of nitrate ester analogs of SCP-123 (Strategy 1)

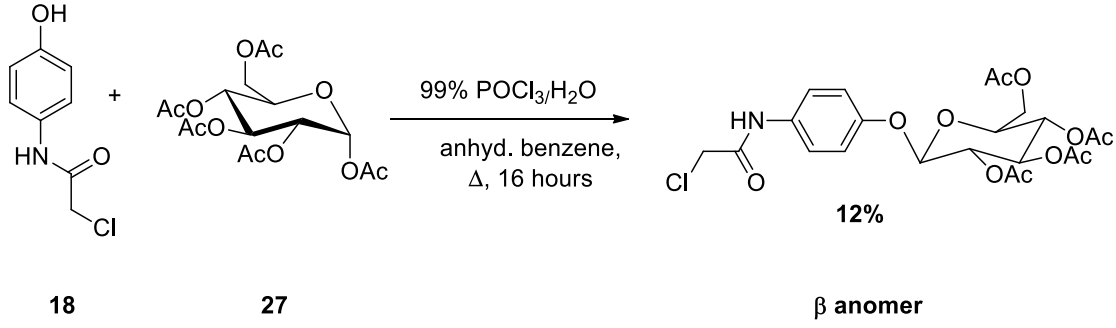
Strategy 2: The next attempt at synthesizing water-soluble analogs involved the hydrolysis of the saccharin ring in SCP-1 while simultaneously forming the ester. To achieve this, sodium 2-bromoethoxide, which was first prepared from sodium hydride and 2-bromo-1-ethanol, was added to SCP-1. This produced the bromoester intermediate **26** in moderate yield (66%) (**Scheme 13**). The intermediate **26** was then reacted with $AgNO_3$. The reaction was followed by TLC and it was observed that the intermediate had converted to SCP-1 instead of the nitrate ester. It was concluded that the heat required for this reaction facilitates ring closure and forms produces SCP-1.



Scheme 13. Attempted synthesis of nitrate ester analogs of SCP-123 (Strategy 2)

1.11.2 O-glycosylation of SCP-1

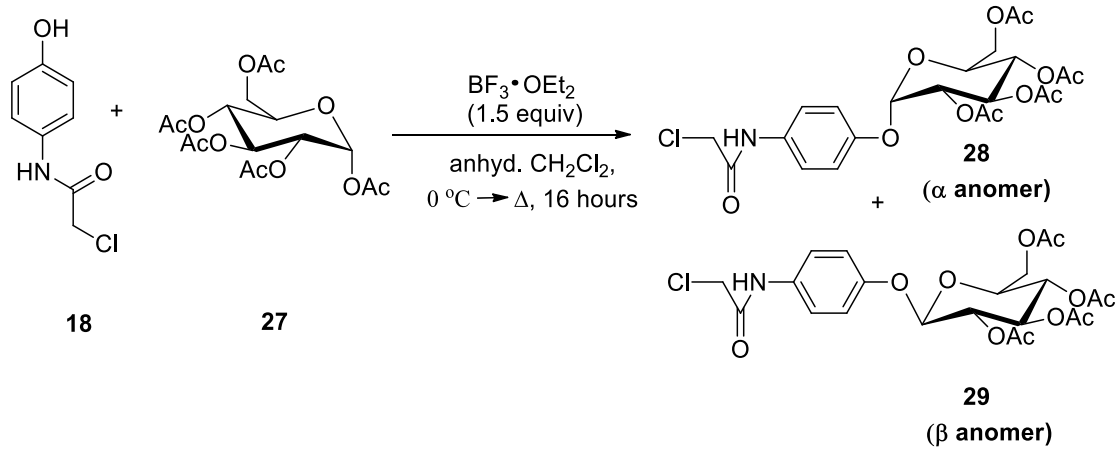
Since the attempts at synthesizing nitrate ester analogs of SCP-123 were not successful, a different approach was sought to synthesize derivatives that were water-soluble. Incorporating a glucose molecule would not only increase the water solubility but also lower the toxicity. It was decided that the phenolic OH could by glycosylated using glucose pentaacetate as the donor. Instead of glycosylating SCP-1, chloroacetamide (**18**) was *O*-glycosylated first after which sodium salt of saccharin (**15**) was added to it. In 1942, Bembry and Powell synthesized phenolic glycosides using fully acylated sugar in the presence of phosphorus oxychloride ($POCl_3$) [53]. Using the same approach, chloroacetamide **18** was dissolved in anhydrous benzene and reacted with *β-D*-glucose pentaacetate (**27**) in the presence of 99% $POCl_3$/water as the catalyst which produced the β anomer of the glycosylated chloroacetamide in 12 % yield (**Scheme 14)**.



Scheme 14. Synthesis of *β*-glycoside tetraacetate of **18** using $POCl_3$ as catalyst

In order to achieve higher yields and cleaner reaction conditions, the Schmidt glycosylation method was utilized [54]. This method involves the use of a Lewis acid like BF_3•OEt_2 as the catalyst. Using this approach, **18** was dissolved in anhydrous dichloromethane (DCM) at 0 ℃ followed by the addition of BF_3•OEt_2 and **27** (**Scheme 15**). The low temperature

facilitates the formation of the ortho ester which when heated up gave the *O*-glycoside as a mixture of α and β anomers in 43% and 57% yield respectively. The mixture of anomers was separated by column chromatography.



Scheme 15. Synthesis of *O*-glycoside tetraacetate of **18** using BF_3•OEt_2 as catalyst

The α and β anomers were characterized using ^{1}H NMR spectroscopy. One distinguishable characteristic is the spin spin coupling ($J_{H\text{-}H}$) of the anomeric proton. The *alpha* anomeric proton (equatorial) has a coupling constant of about 3 Hz, while the *beta* anomeric proton (axial) has a coupling constant of about **7**.9 Hz (**Fig. 21**).

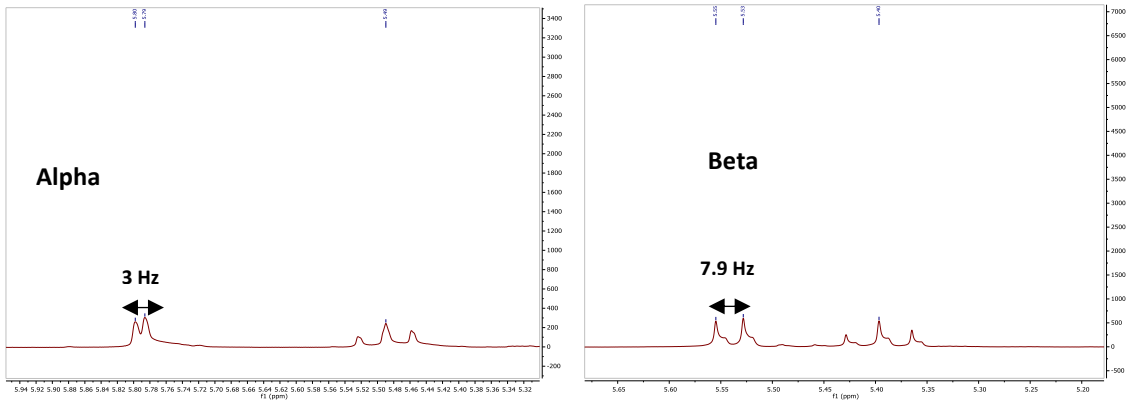


Fig 21. Spin spin coupling of anomeric protons

The structure of **29** was also confirmed using X-ray crystallography (**Fig. 22**).

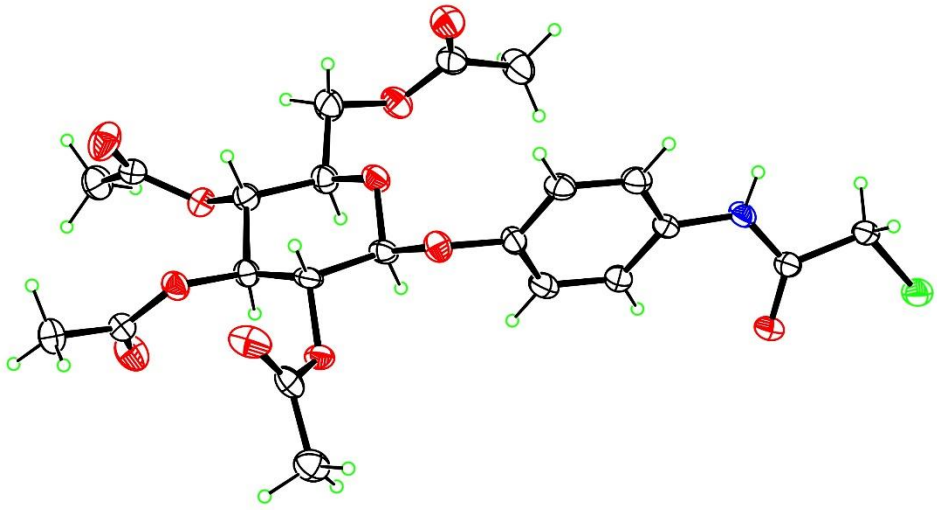
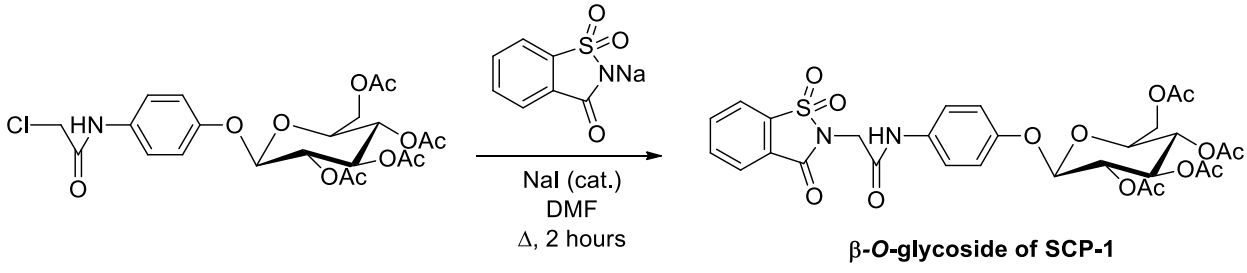


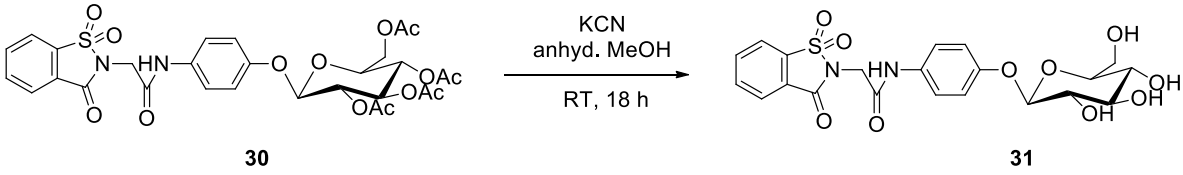
Fig. 22. X-ray crystal structure of **29**

29 was then treated with the **15** in the presence of a catalytic amount of sodium iodide (NaI). The reaction was heated for 2 hours after which it was poured over ice. The *β*-glycoside of SCP-1 (**30**) precipitated in quantitative yield as an off-white solid (**Scheme 16**). The product was characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry.



Scheme 16. Synthesis of *β-O*-glycoside tetraacetate of SCP-1

The final step involves the deacetylation of the glucose molecule. This was achieved by treating the *β-O*-glycoside tetraacetate of SCP-1 (**30**) with potassium cyanide in anhydrous methanol (**Scheme 17**). The reaction was monitored by TLC which showed the deacetylation of every acetyl group one by one as multiple product spots developed. Only one spot remained after 18 hours which indicated complete deacetylation. The product (**31**) was obtained as a yellow solid in 67% yield.



30 **31**

Scheme 17. Synthesis of *β-O*-glycoside of SCP-1

1.11.3 Synthesis of amides of SCP-1

A series of amide analogues of SCP-1 were synthesized. This was achieved by reacting SCP-1 with various alkyl amino alcohols and amino acids. The nucleophilic amines attacked the carbonyl group on the saccharin moiety and then formed the respective amides. First, a series of alkyl amino alcohols **32-37** (**Fig. 23**) were reacted with SCP-1 in ethanol. The reactions were heated at reflux overnight. The products **38-43** (**Fig. 24**) were extracted in ethyl acetate and obtained as shiny white solids in low to moderate yields (23-48%) (**Scheme 18**). These compounds were found to be very hygroscopic and water-soluble. This resulted in low yields as a large amount of the product dissolved in the aqueous layer during the liquid/liquid extraction. The compounds were obtained in high purity and no further purification was required.

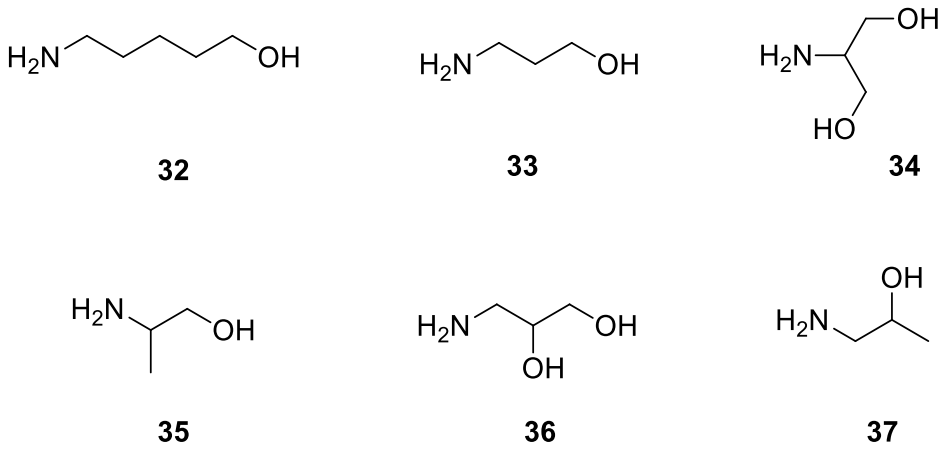
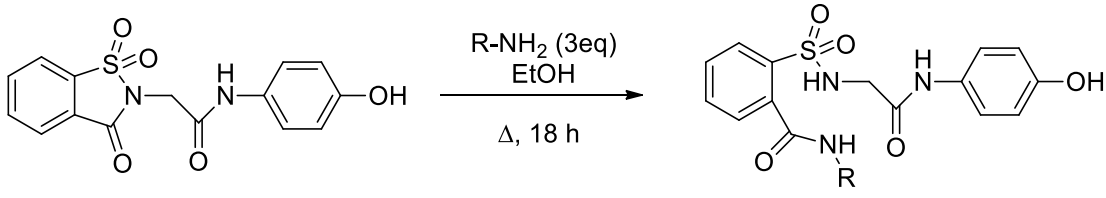


Fig. 23. Alkyl amino alcohols used



Scheme 18. Synthesis of amides of SCP-1 using amino alcohols **32-37**

Table 3. Optimized yields of amides 38-43

Compound no.	Amine used	Isolated Yield (%)
38	32	23
39	33	24
40	34	24
41	35	30
42	36	47
43	37	48

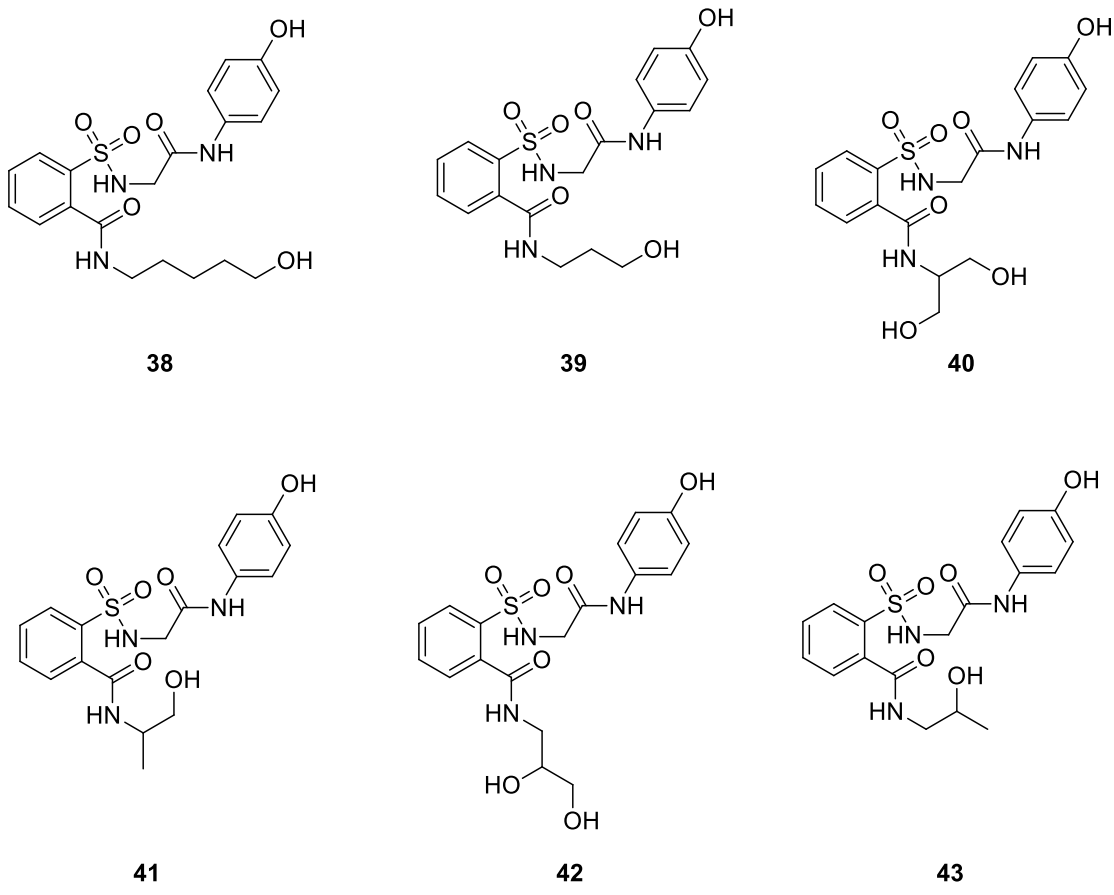
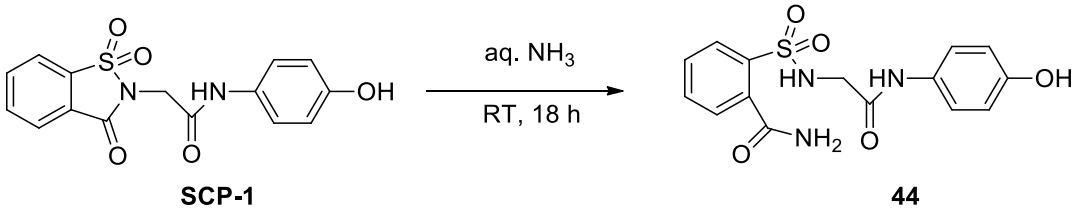


Fig. 24. Structures of amides of SCP-1 **38-43**

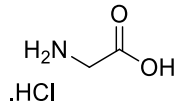
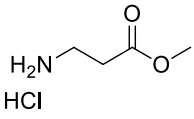
The same procedure was also used to synthesize **44** by reacting SCP-1 with ammonia (NH_3). The reaction was carried out at room temperature without any solvent and gave the product in 78% yield as a colorless oil (**Scheme 19**).

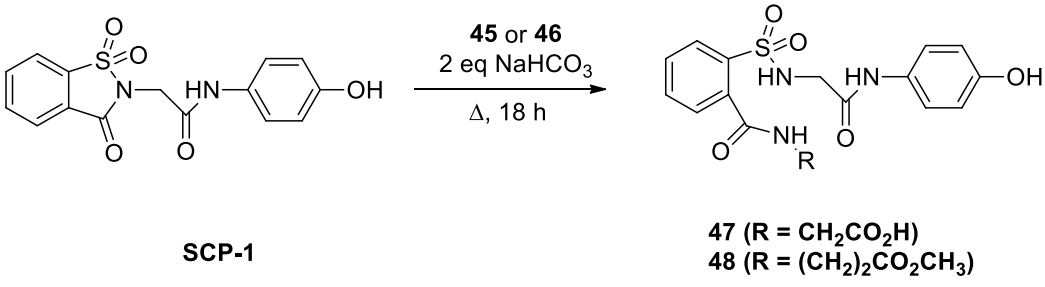


Scheme 19. Synthesis of amide **44**

The scope of the reaction was further explored by using amino acids glycine hydrochloride (**45**) and β-alanine methyl ester hydrochloride (**46**). These reactions required the addition of a base which converted the hydrochloride salt into the free base. Two equivalents of sodium bicarbonate ($NaHCO_3$) was used for this purpose. Reactions were carried out in methanol and DMF respectively to give products **47** and **48** as white solids in moderate yields (49-50%) (**Scheme 20**).

Table 4. Synthesis of amides of SCP-1 using amino acids **45** and **46**

Amino acid	Amino acid structure	Solvent	Isolated yield (%)
45		MeOH	49
46		DMF	50



Scheme 20. Synthesis of amides of SCP-1 using amino acids **45** and **46**

1.11.4 Hydrophobicity studies

Hydrophobicity studies were conducted on compounds **39-43**, **47** and **48**. The hydrophobicity of any compound can be measured by calculating the logP value where P is the partition coefficient between octanol and water. Lower the logP value, the less hydrophobic the compound. In general, water-soluble compounds have a logP value of less than 1.

Equal amounts of octanol and water needed to be stirred overnight. The water-saturated octanol layer and octanol-saturated water layer were then stored in separate bottles. The Octanol-saturated water was used to prepare 5μM, 10 μM, 20 μM, 40 μM and 60 μM solutions of each compound. UV-vis spectroscopy was used to measure the absorbance between 200-450 nm (**Fig. 25**) for each concentration and a calibration curve was produced (**Fig. 26**).

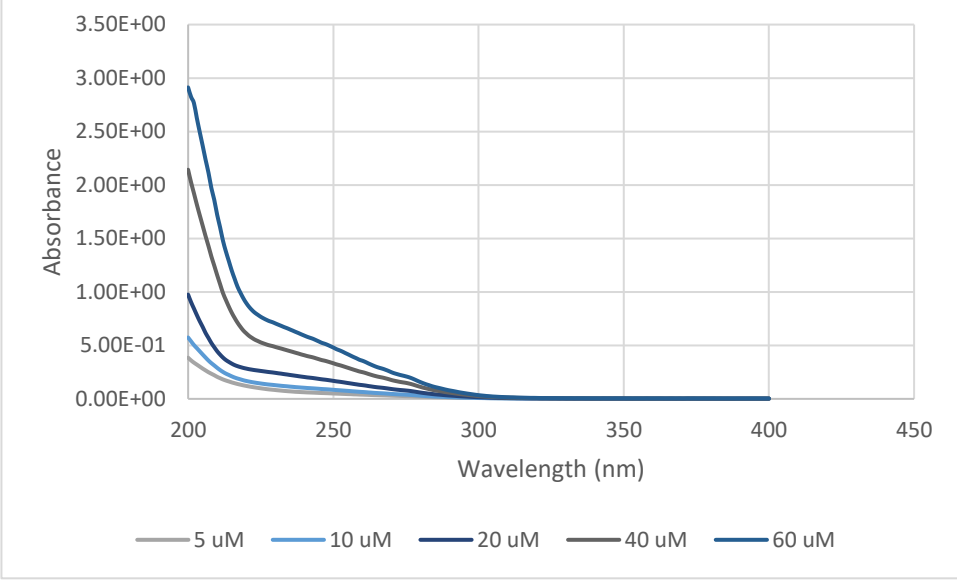


Fig. 25. Absorbance chart for **39**

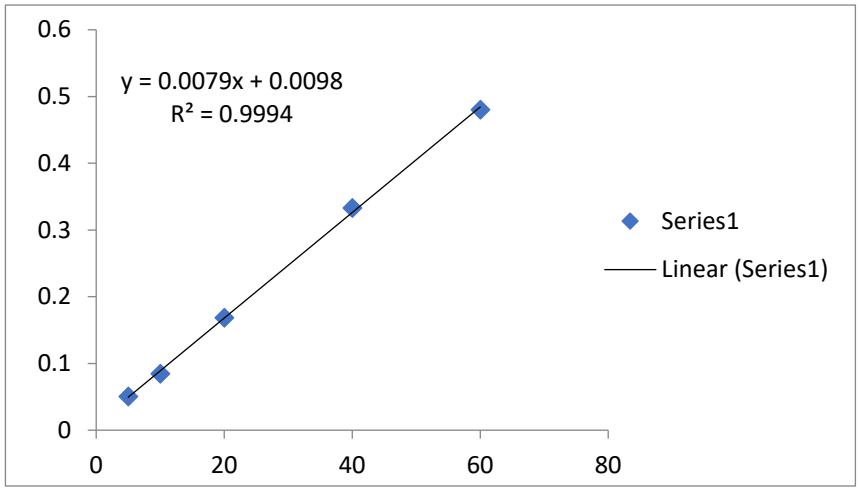


Fig. 26. Calibration curve for **39** at 250 nm

Table 5. Wavelengths used to produce calibration curve for each compound

Compound	Wavelength used for calibration curve
39	250
40	248
41	250
42	240
43	250
47	230
48	225

Next, seven samples of 50 μM solutions were prepared. Six of these samples were shaken with water-saturated Octanol for 4 hours. Once it was shaken, some of the sample dissolved in the octanol layer. One sample was left unshaken. UV-vis spectroscopy was used to measure the absorbance for octanol-saturated water for all seven samples. Using the calibration curve, the

concentration of the compound in the octanol-saturated water was calculated (**Fig. 27**) after which the average of the six shaken samples were taken. The concentration of the compound in the octanol layer can be calculated by subtracting the concentration of the sample in the water layer of the shaken sample from the concentration in water of the unshaken sample.

The partition coefficient (P) of each compound was then calculated using the formula:

$$P = \frac{concentration\ in\ Octanol}{concentration\ in\ water}\ x\ 100$$

Therefore, $P = \frac{concentration\ in\ unshaken - concentration\ in\ shaken}{concentration\ in\ shaken}\ x\ 100$

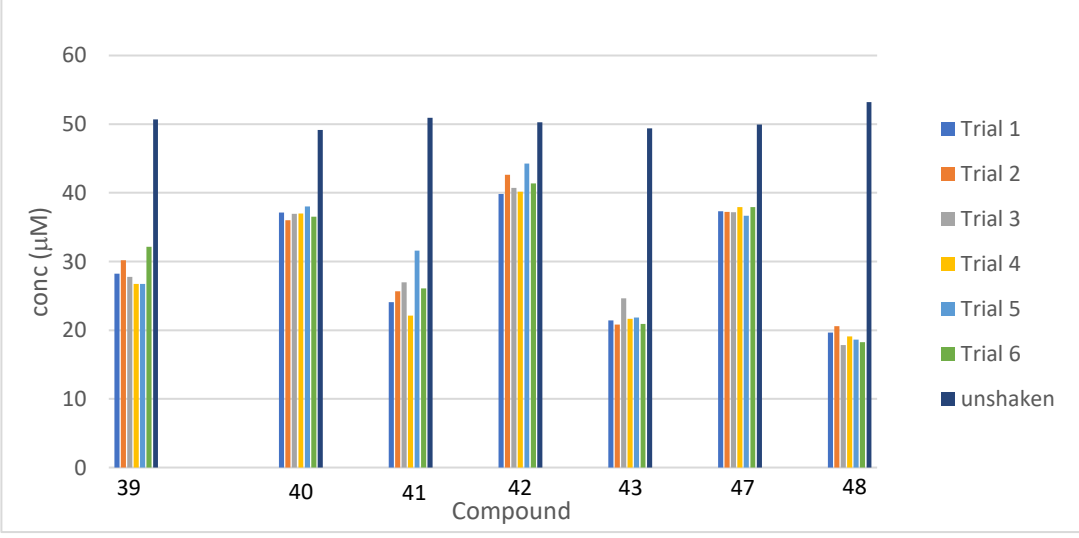


Fig. 27. Concentration of all shaken and unshaken samples for each compound

The logP values of compounds **39-43**, **47** and **48** are depicted in the chart below (**Fig. 28**).

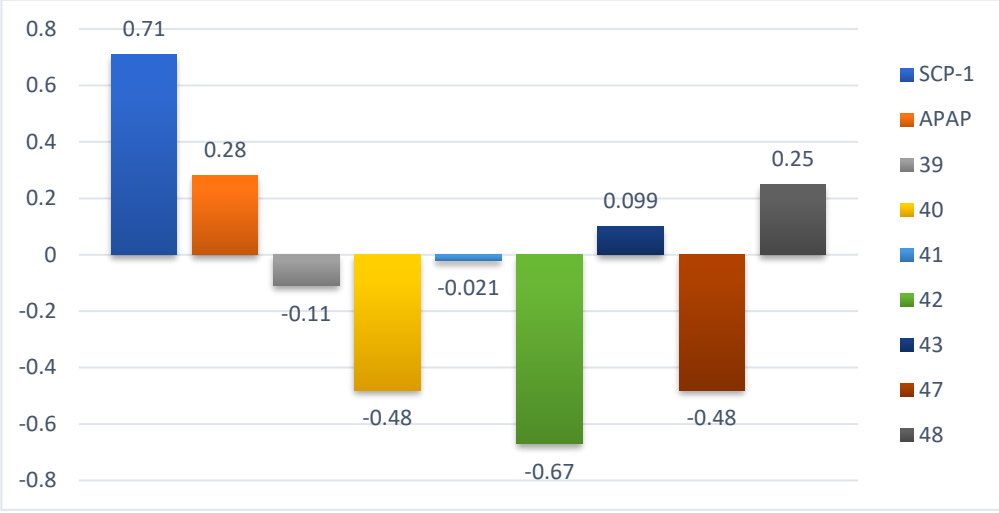


Fig. 28. LogP values of the amides compared to SCP-1 and APAP

Table 6. Comparison between logP and clogP values [55]

Compound	LogP	clogP
39	-0.11	-0.13
40	-0.48	-0.77
41	-0.021	0.08
42	-0.67	-0.77
43	0.099	0.08
47	-0.48	-0.45
48	0.25	0.1

As shown in **Fig. 28**, the logP values of SCP-1 and APAP are 0.71 and 0.27 respectively. With a logP value of greater than 0.5, SCP-1 has very low water solubility and therefore has poor bioavailability and also affects the transport of the drug between cell membranes. APAP has a lower logP value and therefore is more water-soluble than SCP-1. The amides **39-43**, **47** and **48** have very low logP values with **39-42** and **47** having negative logP values (-0.11, -0.48, -0.021 and -0.67) and **43** having logP value close to zero. The logP values of the amides are very close and comparable to the computational logP (clogP) values (**Table 6)**. These values prove that these amides are highly water-soluble and can prove to be ideal drug candidates. This will undoubtedly increase their oral bioavailability and possibly reduce toxicity.

CONCLUSION

In summary, a series of novel nitrate ester congeners of the acetaminophen derivative SCP-1 were synthesized. The nitrate esters **24b** (MD-38) and **24c** (MD-39) exhibited a hepatotoxicity profile similar to SCP-1. In addition, the nitrate esters exhibited little effect on the three cytochrome P450 isozymes tested (CYP3A4, CYP2E1 and CYP2D6). The nitrate ester **24c** (MD-39) exhibited antipyretic activity similar to APAP. Overall, these preliminary studies demonstrated that attachment of a NO-donor moiety to the SCP-1 ring system can lead to compounds with enhanced antipyretic activity compared to SCP-1 without increased toxicity.

Following solubility issues, a series of water-soluble analogs of SCP-1 were synthesized. These was achieved by synthesizing O-glycosylated analogs and amide derivatives of SCP-1 A series of amides were prepared by hydrolysis of the saccharin moiety. Amides **39-43, 47** and **48** underwent hydrophobicity studies. All amides were found to be highly water-soluble having low logP values comparable to the clogP values. Further testing of these compounds is underway and results will be reported in due course.

EXPERIMENTAL

General information

All reactions were carried out in oven-dried glassware under a N_2 atmosphere unless otherwise noted. All chemicals were purchased from Alfa Aesar, Sigma Aldrich, or VWR and were used as received without further purification. Chromatography refers to flash silica gel column chromatography (0.060–0.200mm (60 Å) silica gel from Sorbent Technology was used as the stationary phase). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature in DMSO-d_6 on a Bruker 300 MHz instrument. ^{1}H chemical shifts were referenced to the DMSO solvent signal (2.48 ppm). ^{13}C chemical shifts were referenced to the DMSO solvent signal (40.00 ppm). Atlantic Microlab Inc., Norcross, GA performed all CHN microanalyses.

General procedure A: Preparation of chloroesters of SCP-1 (23a-d)

SCP-1 (1.00 g, 3.00 mmol) was dissolved in 2% solution of triflic acid in acetonitrile (15 mL) (A 1% solution was used for the preparation of acetyl ester **23a**) followed by the indicated amount of acid chloride. The mixture was stirred at room temperature and the reaction was followed by TLC (70% EtOAC-Hex). When no more starting material was seen by TLC, the mixture was poured over ice-cold water (50 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with 1N HCl (20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was then dried over anhydrous sodium sulfate. The sodium sulfate was filtered and the solvent was removed *in vacuo* to yield a white solid.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl-2-chloroacetate (23a)

General procedure A. Product was prepared using chloroacetyl chloride (0.720 mL, 9.00 mmol, 3 equiv., 16 h) and was isolated as a white solid (1.21 g, 99% yield). mp 199.1-200.0 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.45 (s, 1H), 8.36 (d, J = 7.7, 1H), 8.17 (d, J = 7.7Hz, 1H), 8.12-8.01 (m, 2H), 7.61 (d, J = 8.8, 2H), 7.15 (d, J = 8.8, 2H), 4.68 (s, 2H), 4.59 (s, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 166.8, 163.7, 159.1, 146.3, 137.4, 136.8, 136.4, 135.7, 126.8, 125.6, 122.2, 122.1, 120.7, 41.7.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl-3-chloropropionate (23b)

General procedure A. Product was prepared using 3-chloropropionyl chloride (0.86 mL, 9.0 mmol, 3 equiv., 16 h) and was isolated as a white solid (1.24 g, 98% yield). mp 195.3-197.0 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.41 (s, 1H), 8.34 (d, J = 7.7 Hz. 1H)), 8.15 (d, J = 7.4 Hz, 1H), 8.05 (dt, J = 23.1, 7.4 Hz, 2H), 7.58 (d, J = 8.9, 2H), 7.08 (d, J = 8.9, 2H), 4.57 (s, 2H), 3.88 (t, J = 6.2 Hz, 2H), 3.08 (t, J = 6.2 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 169.6, 163.7, 159.1, 146.3, 137.4, 136.6, 136.4, 135.8, 126.8, 125.6, 122.4, 122.2, 120.6, 41.1, 37.5.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl-4-chlorobutanoate (23c)

General procedure A. Product was prepared using 4-chlorobutyryl chloride (0.5 mL, 4.5 mmol, 1.5 equiv., 1 h) and was isolated as a white solid (1.30g, 99% yield). mp 189.2-190.6 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.40 (s, 1H), 8.34 (d, J = 9 Hz, 1H), 8.15 (d, J = 7.3 Hz, 1H), 8.11-8.00 (m, 2H), 4.57 (s, 2H), 3.72 (t, J = 6.4 Hz, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.06 (p, J = 7.0 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 171.5, 163.7, 159.1, 146.5, 137.4, 136.3, 135.7, 126.8, 125.6, 122.5, 122.1, 120.6, 44.8, 41.1, 31.3, 27.8.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl-5-chloropentanoate (23d)

General procedure A. Product was prepared using 5-chlorovaleroyl chloride (0.43 mL, 3.3 mmol, 1.1 equiv., 1 h) and was isolated as a white solid (1.34g. 99% yield). mp (154.4-155.9 ℃).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.40 (s, 1H), 8.35 (d, J = 7.5 Hz, 1H), 8.15 (d, J = 7.0 Hz, 1H), 8.00-8.17 (m, 2H), 7.57 (d, J = 8.9 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 4.57 (s, 2H), 3.67 (t, J = 6.1 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.82-1,70 (m, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 172.1, 163.7, 159.1, 146.5, 137.4, 136.4, 136.4, 133.8, 126.8, 125.6, 122.5, 122.2, 120.6, 45.4, 41.0, 33.0, 31.7, 22.1.

General procedure B: Preparation of nitrate esters of SCP-1 (24b-d)

The chloroester (3.00 mmol) was dissolved in dry acetonitrile (20 mL) and silver nitrate (1.02g, 6.00 mmol) was then added to the solution. The mixture was heated at reflux for 24 h. The precipitate was filtered through a pad of Celite. The filtrate was then poured into water (50 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine (3 x 20 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was filtered and the solvent was removed *in vacuo.* The residue was recrystallized from ethyl acetate/hexanes to yield a yellow solid.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl 3-(nitrooxy)propionate (MD-38, 24b)

General procedure B. Product was isolated as a yellow solid (1.29g, 96% yield). mp 174.3 ℃ (decomp).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.41 (s, 1H), 8.34 (d, J = 8.3 Hz, 1H), 8.15 (d, J = 7.4 Hz, 1H), 8.11-7.99 (m, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 4.57 (s, 2H), 3.89 (t, J = 6.2 Hz, 2H), 3.08 (t, J = 6.2 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 169.3, 163.7, 159.1, 146.3, 137.4, 136.6, 136.4, 135.8, 126.8, 125.6, 122.4, 122.2, 120.6, 69.3, 32.0.

Anal. calcd. for $C_{18}H_{15}N_3O_9S$: C, 48.11; H, 3.36; N, 9.35. Found: C, 48.02; H, 3.45; N, 9.10.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl 4-(nitrooxy)butanoate (MD-39, 24c)

General procedure B. Product was isolated as a yellow solid (1.25g, 90% yield). mp 159.1 ℃ (decomp.).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.42 (s, 1H), 8.36 (d, J = 7.7 Hz, 1H), 8.16 (d, J = 7.3 Hz, 1H), 8.06 (dt, J = 15.4, 7.4 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 4.61 (t, J = 5.8 Hz, 2H), 4.59 (s, 2H), 2.70 (t, J = 7.0, 2H), 2.03 (p, J = 6.9 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 171.6, 163.7, 159.1, 146.5, 137.4, 136.5, 126.8, 125.6, 122.5, 122.2, 120.5, 73.2, 30.2, 22.1.

Anal. calcd. for $C_{19}H_{17}N_3O_9S$: C, 49.24; H, 3.70; N, 9.07. Found: C, 49.18; H, 3.75; N, 9.00.

4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenyl 5-(nitrooxy)pentanoate (24d)

General procedure B. Product was isolated as a yellow solid (1.33g, 93% yield). mp 144.6 ℃ (decomp.).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.41 (s, 1H), 8.34, (d, J = 7.7, 1H), 8.15 (d, J = 8.0, 1H), 8.11-7.99 (m, 2H), 7.57 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H), 4.58 (s, 1H), 4.55 (t, J = 6.1 Hz, 2H), 2.62 (t, J = 6.8 Hz, 2H), 1.81-1.63 (m, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz), δ: 172.0, 163.7, 159.1, 146.5, 137.4, 136.4, 135.6, 126.8, 125.6, 122.5, 122.1,121.4,120.6, 73.8, 33.1, 25.8, 21.0.

Anal. calcd. for $C_{20}H_{19}N_3O_9S$: C, 50.31; H, 4.01; N, 8.80. Found: C, 50.06; H, 4.12; N, 8.67.

Preparation of 2-(acetoxymethyl)-6-(4-(2-chloroacetamido)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (O-glycoside of chloroacetaminophen, 29)

2-Chloroacetaminophen (0.501g, 2.70 mmol) was dissolved in anhydrous dichloromethane (20 mL). followed by the addition of β-D-Glucose pentaacetate (0.952g, 2.44 mmol). The mixture was cooled in an ice bath to 0 ℃. BF_3•OEt_2 (0.452 mL, 3.66 mmol) was then added to the solution dropwise and the reaction was stirred at 0 ℃ for 30 minutes. The reaction was then heated at reflux for 18 hours. The reaction was quenched with 1N NaOH solution and the layers were separated. The aqueous layer was extraction with dichloromethane (3 x 10 mL). The organic layers were combined and washed with brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under vacuum and the residue was purified using column chromatography (50% EtOAc/Hexanes). The β anomer was isolated as a colorless oil (0.717g, 57% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.23 (s, 1H), 7.50 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 10.3 Hz, 2H), 6.15 (d, J = 3 Hz, 1H), 5.45 (t, J = 9.9 Hz, 1H), 5.32 (t, J = 9.9 Hz, 1H), 5.10-4.97 (m, 2H), 4.21 (s, 2H), 4.02-3.97 (m, 2H), 2.16 (s, 12H).

Preparation of 2-(acetoxymethyl)-6-(4-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetamido)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (β-D-glycoside of SCP-1, 30)

29 (1.50 g, 3.00 mmol) and **15** (0.743g, 3.62 mmol) were dissolved in anhydrous DMF (20 mL). Sodium iodide (0.0500g, 0.300 mmol) was then added to it. The reaction was stirred at 110 ℃ for 2 h. Any solids were filtered off and the filtrate was poured over ice-water. More ice was

added until no more precipitate formed. The precipitate was filtered off and dried under vacuum to give **30** as a yellow solid (2.00 g, 100% yield). mp 196-198 ℃

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.28 (s, 1H), 8.34 (d, *J* = 7.7 Hz, 2H), 8.20 – 7.98 (m, 3H), 7.50 (d, *J* = 8.1 Hz, 2H), 6.96 (d, *J* = 9.0 Hz, 2H), 5.47 (d, *J* = 7.8 Hz, 1H), 5.38 (t, *J* = 9.6 Hz, 1H), 5.08 – 4.93 (m, 2H), 4.54 (s, 2H), 4.21 (s, 2H), 4.05 (d, *J* = 10.8 Hz, 1H), 2.02 – 1.94 (m, 12H).

Preparation of 2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)-N-(4-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)acetamide (31)

30 (2.00g, 3.00 mmol) was dissolved in anhydrous MeOH (20 mL) and potassium cyanide (0.100 g, 1.51 mmol) was added to it. The reaction was stirred at room temperature for 18 h. The mixture was passed through a silica gel plug. The solvent was removed under vacuum to give **32** as a yellow solid (1.00 g, 67% yield). mp 152 ℃ (decomp.)

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.27 (s, 1H), 8.33 (d, J = 7.7 Hz, 2H), 8.11-7.95 (m, 3H), 7.53 (d, J = 9.0 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 5.83 (d, J = 7.8 Hz, 1H), 4.21 (s, 2H), 3.96-3.42 (m, 10 H).

General procedure C: Preparation of amides of SCP-1 (38-43, 47-48)

SCP-1 (1.00g, 3.00 mmol) was dissolved in absolute ethanol (30 mL). The amino alcohol **32-37** (9.00 mmol) was added to the reaction mixture. The solution was stirred at reflux for 24 h. The reaction mixture was cooled down to room temperature and then diluted with deionized water (50 mL). The aqueous solution was extracted with ethyl acetate (3 x 20 mL). The organic layers were

combined and washed with 2N HCl (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under vacuum to give amides **38-43** and **47-48**.

N-(5-hydroxypentyl)-2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)benzamide (38)

General procedure C. The product was prepared using **32** (1.00 mL, 9.00 mmol) and was isolated as a light pink solid (0.300 g, 23 % yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.75 (s, 1H), 9.18 (s, 1H), 8.75 (t, *J* = 5.6 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.71 – 7.5 (m, 2H), 7.52 (d, *J* = 7.3 Hz, 1H), 7.42 (t, *J* = 5.9 Hz, 1H), 7.19 (d, *J* = 8.9 Hz, 2H), 6.63 (d, *J* = 8.9 Hz, 2H), 3.65 (d, *J* = 5.9 Hz, 2H), 3.39 (t, *J* = 6.4 Hz, 2H), 3.23 (q, *J* = 6.8 Hz, 2H), 1.45 (dtd, *J* = 33.9, 14.6, 5.9 Hz, 9H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.7, 165.8, 154.0, 138.0, 137.3, 136.3, 133.2, 130.3, 129.7, 129.0, 121.4, 115.5, 61.1, 46.4, 32.7, 29.0, 23.4.

2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)-N-(3-hydroxypropyl)benzamide (39)

General procedure C. The product was prepared using **33** (0.700 mL, 9.00 mmol) and was isolated as a white solid (0.300 g, 24 % yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.75 (s, 1H), 8.72 (t, J = 5.6 Hz, 1H), 7.86 (d, J = 9.0 Hz, 1H), 7.70 – 7.57 (m, 2H), 7.52 (d, J = 8.8 Hz, 1H), 7.43 (t, J = 5.9 Hz, 1H), 7.19 (d, J = 8.9 Hz, 2H),

6.63 (d, J = 6.8 Hz, 2H), 3.65 (d, J = 5.9 Hz, 2H), 3.48 (t, J = 6.3 Hz, 2H), 3.30 (q, J = 6.6 Hz, 2H), 1.68 (p, J = 6.5 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.7, 165.8, 154.0, 137.3, 136.3, 133.2, 130.3, 129.7, 129.0, 121.4, 115.5, 59.0, 46.4, 37.2, 32.4.

Anal. Calcd. for $C_{18}H_{21}N_3O_6S•H_2O$: C, 50.81; H, 5.45; N, 9.88. Found: C, 50.80; H, 5.47; N, 9.78

N-(1,3-dihydroxypropan-2-yl)-2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)benzamide (40)

General procedure C. The product was prepared using **34** (0.820 mL, 9.00 mmol) and isolated as a white solid (0.305g, 24 % yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.78 (s, 1H), 9.19 (s, 1H), 8.56 – 8.41 (m, 1H), 7.84 (d, *J* = 8.7 Hz, 1H), 7.74 (t, *J* = 6.0 Hz, 1H), 7.67 – 7.61 (m, 1H), 7.59 – 7.52 (m, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 4.86 (t, *J* = 5.3 Hz, 2H), 3.93 (dt, *J* = 7.9, 5.7 Hz, 1H), 3.76 – 3.70 (m, 2H), 3.53 (t, *J* = 5.4 Hz, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.1, 166.2, 154.1, 137.1, 136.7, 133.1, 130.2, 130.0, 129.80, 129.0, 121.7, 115.48, 60.6, 54.3, 46.1.

Anal. Calcd. for $C_{18}H_{21}N_3O_7S•H_2O$: C, 48.97; H, 5.25; N, 9.52. Found: C, 48.97; H, 5.25; N, 9.50

N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)-N-(1-hydroxypropan-2-yl)benzamide (41)

General procedure C. The product was prepared using **35** (0.700 mL, 9.00 mmol) and isolated as a white solid (0.366 g, 30 % yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.78 (s, 1H), 8.76 (t, *J* = 6.3 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.68 – 7.51 (m, 5H), 7.12 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 8.9 Hz, 2H), 3.81 (q, *J* = 5.9 Hz, 1H), 3.69 (d, *J* = 5.9 Hz, 2H), 3.53 (s, 2H), 3.40 (s, 3H), 3.24 –3.15 (m, 1H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.1, 165.7, 153.6, 136.1, 132.8, 129.8, 129.3, 128.6, 121.2, 115.1, 65.1, 47.0, 45.8, 21.1.

Anal. Cald. for $C_{18}H_{21}N_3O_6S•H_2O$: C, 50.81; H, 5.45; N, 9.88. Found: C, 50.81; H, 5.49; N, 9.86.

N-(2,3-dihydroxypropyl)-2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)benzamide (42)

General procedure C. The product was prepared using **36** (0.700 mL, 9.00 mmol) and isolated as a white solid (0.600 g, 47% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.76 (s, 1H), 9.18 (s, 1H), 8.72 (t, *J* = 6.3 Hz, 1H), 7.85 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.66 (td, *J* = 7.6, 1.4 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.56 – 7.52 (m, 1H), 7.13 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 8.9 Hz, 2H), 4.94 (d, *J* = 4.8 Hz, 1H), 4.57 (t, *J* = 5.2 Hz, 1H), 3.72 – 3.68 (m, 2H), 3.38 (t, *J* = 5.6 Hz, 2H), 3.29 (s, 2H), 3.19 – 3.03 (m, 1H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.6, 166.0, 154.0, 137.2, 136.4, 133.1, 130.2, 129.8, 129.0, 121.6, 115.5, 70.6, 64.2, 46.2, 43.3.

Anal. Calcd. for $C_{18}H_{21}N_3O_7S$: C, 51.06; H, 5.00; N, 9.92. Found: C, 50.88; H, 5.25: N, 9.76.

2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)-N-(2-hydroxypropyl)benzamide (43)

General procedure C. The product was prepared using **37** (0.700 mL, 9.00 mmol) and isolated as a white solid (0.600 g, 48% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.78 (s, 1H), 8.76 (t, *J* = 6.3 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.68 – 7.51 (m, 5H), 7.12 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 8.9 Hz, 2H), 3.81 (q, *J* = 5.9 Hz, 1H), 3.69 (d, *J* = 5.9 Hz, 2H), 3.53 (s, 2H), 3.40 (s, 3H), 3.24 –3.15 (m, 1H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.1, 165.7, 153.6, 136.1, 132.8, 129.8, 129.3, 128.6, 121.2, 115.1, 65.1, 47.0, 45.8, 21.1.

Anal. Calcd. for $C_{18}H_{21}N_3O_6S$: C, 53.06; H, 5.20; N, 10.31. Found: C, 52.99; H, 5.40; N, 10.07.

2-(2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)benzamido)acetic acid (47)

SCP-1 (1.00g, 3.00 mmol) was dissolved in absolute MeOH (30 mL). Glycine hydrochloride (1.00 g, 9.00 mmol) and $NaHCO_3$ (0.504 g, 6.00 mmol) were added to the reaction mixture. The solution was stirred at reflux for 24 h. The reaction mixture was cooled down to room temperature and then diluted with deionized water (50 mL). The aqueous solution was extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and washed with 2N HCl (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under vacuum to give amide **47** as a white solid (0.600 g, 49% yield).

^{1}H NMR (DMSO-d_6, 300 MHZ) δ: 9.73 (s, 1H), 9.18 (s, 1H), 9.07 (t, *J* = 5.9 Hz, 1H), 7.93 – 7.81 (m, 1H), 7.74 – 7.66 (m, 1H), 7.65 – 7.55 (m, 2H), 7.48 (t, *J* = 5.9 Hz, 1H), 7.15 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 3.98 (d, *J* = 5.8 Hz, 2H), 3.69 (d, *J* = 6.0 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 171.6, 168.7, 165.9, 154.0, 137.4, 135.7, 133.2, 130.5, 129.9, 129.1, 121.5, 115.5, 46.2, 41.5.

Anal. Calcd. for $C_{17}H_{17}N_3O_7S•H_2O$: C, 48.00; H, 4.50; N, 9.88. Found: C, 47.95; H, 4.60; N, 9.78.

Methyl 3-(2-(N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)sulfamoyl)benzamido) propanoate (48)

SCP-1 (1.00g, 3.00 mmol) was dissolved in anhydrous DMF (30 mL). *β*-Alanine methyl ester hydrochloride (1.26 g, 9.00 mmol) and $NaHCO_3$ (0.504 g, 6.00 mmol) were added to the reaction mixture. The solution was stirred at 90 ℃ for 24 h. The reaction mixture was cooled down to room temperature and then diluted with deionized water (50 mL). The aqueous solution was extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and washed with 2N HCl (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under vacuum to give amide **48** as a white solid (0.653 g, 50% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.73 (s, 1H), 9.18 (s, 1H), 8.89 – 8.78 (m, 1H), 7.86 (d, *J* = 7.5 Hz, 1H), 7.63 (dq, *J* = 14.1, 6.9 Hz, 2H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.39 (s, 1H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.66 – 6.58 (m, 2H), 3.66 (s, 2H), 3.60 (s, 3H), 3.47 (q, *J* = 6.7 Hz, 2H), 2.61 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 171.8, 168.5, 165.4, 153.6, 136.9, 135.6, 132.8, 130.0, 129.2, 128.6, 121.1, 115.1, 51.5, 45.9, 35.4, 33.1.

Anal. Calcd. for $C_{19}H_{21}N_3O_7S$: C, 52.41; H, 4.86; N, 9.65. Found: C, 52.33; H, 4.90; N, 9.47.

CHAPTER 2

Scalable synthesis and functionalization of a series of novel 3,3-diarylazetidines

ABSTRACT

The 3,3-diarylazetidine ring has recently emerged as an important molecular scaffold in medicinal chemistry. The constrained 3,3-diarylazetidine ring system provides a well-defined positioning of the arene substituents, increased rigidity, fewer rotatable bonds relative to corresponding acyclic system and can be isosteric with N-diarylmethylamines and geminal diaryl functional groups. With the emerging interest in 3,3-diarylazetidines as novel motifs in drug discovery programs, an easy, efficient, scalable synthetic method that could accommodate the introduction of both electron-rich and electron-deficient aryl groups for preparation of diversely substituted 3,3-diarylazetidines would be valuable to medicinal chemists. In this second project, a versatile synthesis of 3,3-diaryl azetidines from N-Boc-3-aryl-3-azetidinols using Friedel-Crafts arylation conditions with $AlCl_3$ is described. A series of substituted diaryl azetidines were readily prepared and isolated as the oxalate salts in high yield and high purity. The 3,3-diaryl azetidine oxalates were then easily converted into *N*-alkyl and *N*-acyl analogues in high overall yields (>85%).

INTRODUCTION

2.1 Diarylmethanes

The importance of diarylalkanes as pharmaceutically active compounds has been well documented. Various diarylmethane based molecules like podophyllotoxin (**49**), peperomin B (**50**), tolterodine (**51**) and lasofoxifene (**52**) exhibit antibacterial, antiprotozoal, anti-inflammatory, antimuscarinic and anticonvulsant activity [56] [57] [58] [59].

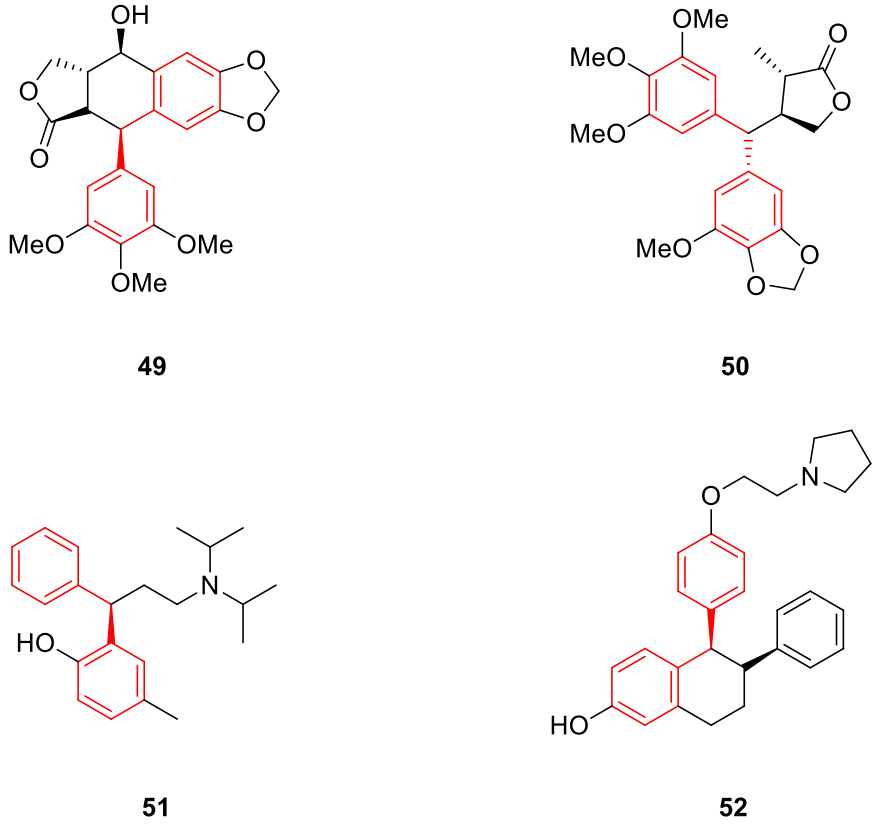


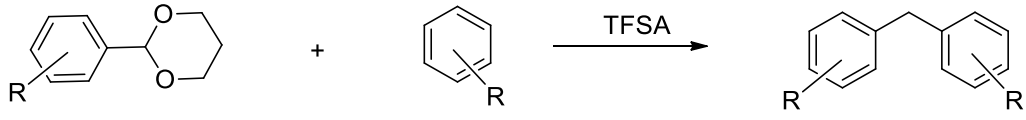
Fig. 29. Some well-known biologically active diarylmethanes

2.1.1 Synthesis of diarylmethanes

Diarylmethanes have mostly been synthesized via Friedel–Craft's alkylation of the corresponding benzyl alcohols with another arene, metal catalyzed cross coupling of aryl halides with benzyl nucleophiles, metal catalyzed cross coupling of benzyl halides with aryl nucleophiles and C–C bond formation between tosyl hydrazones and aryl boronic acids.

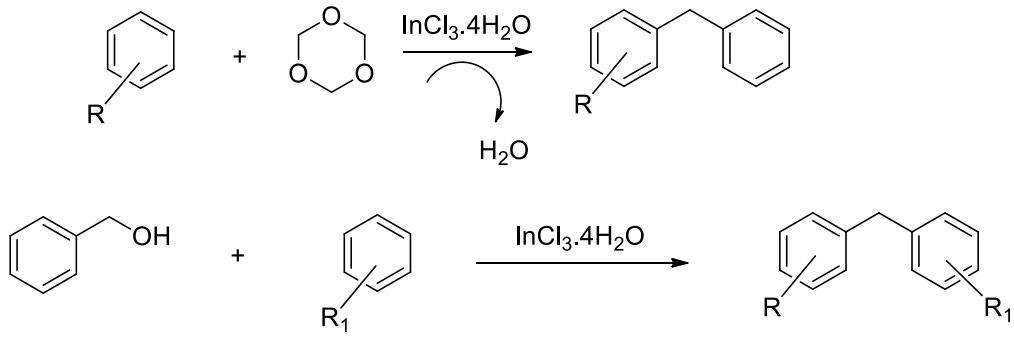
2.1.1.1 Friedel–Crafts reaction

In 1997, S. Fukuzawa *et al.* reported trifluoromethane sulfonic acid (TFSA) catalyzed reductive Friedel–Craft's alkylation of benzaldehyde acetals for the preparation of diarylmethanes (**Scheme 21**) [60]. When low boiling arenes were used, the arene served as the solvent as well as the reagent while for high boiling arenes 1,2-dichloroethane or nitromethane was used as a solvent. Though both electron rich as well as electron poor aldehyde acetals responded to this reaction, the reaction rate was faster with the electron rich aldehyde acetals with the ratio of ortho:meta:para ratio being same as that observed for general Lewis acid catalyzed Friedel–Craft's alkylation reaction.



Scheme 21. Synthesis of diarylmethane using Friedel-craft's alkylation

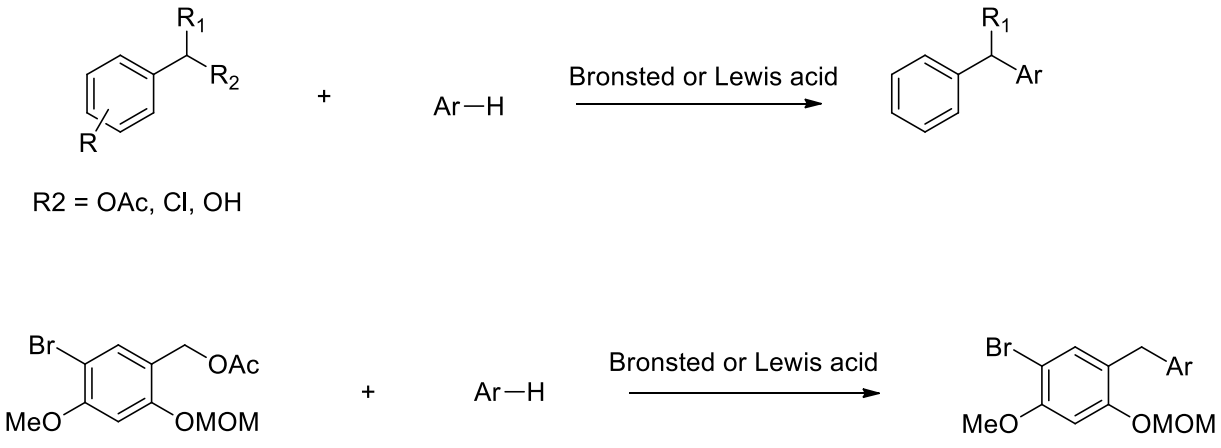
Sun *et al.* in 2006 reported indium (III) chloride catalyzed electrophilic substitution of trioxanes with arenes for the preparation of diarylmethanes [61]. The authors found that the reaction was high yielding only with electron rich arenes while electronically poor ones gave low yield. Later, the authors reported indium trichloride acetyl acetone to be an efficient Lewis catalyst for the Friedel–Craft's arylation of benzyl alcohols for the efficient preparation of diarylmethanes (**Scheme 22**) [62].



Scheme 22. Synthesis of diarylmethanes using indium (III) chloride as catalyst

In 2009, Myrboh *et al.* reported $AlCl_3$ catalyzed Friedel–Craft's alkylation of benzylazines with polynuclear hydrocarbons to form diarylmethanes in aprotic solvents [63]. The corresponding azines were accessed from the respective carbonyl compounds through condensation with aqueous solution of hydrazines.

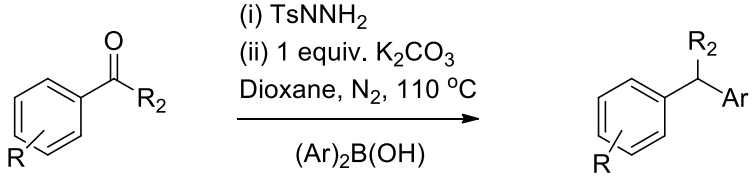
Mendoza *et al.* in 2011 reported Bronsted acid catalyzed arylation of benzyl acetates and anisyl acetates with arenes for the preparation of diarylmethanes (**Scheme 23**) [64]. Trifluoromethane sulfonic acid and triflimide respectively were used as the Bronsted acid catalyst.



Scheme 23. Bronsted and Lewis acid catalyzed arylation

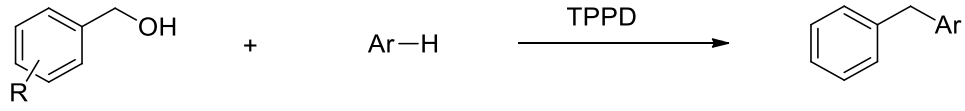
2.1.1.2 Metal-free C-C bond formation

In 2009, Barluenga *et al*. reported metal free C-C bond formation for the synthesis of diarylmethanes from tosyl hydrazones [65]. Boronic acids were used as the aryl source. The authors proposed heating the tosyl hydrazone salts in presence of a base generates the diazo intermediate. This intermediate reacts with aryl boronic acids to form the benzyl boronic acid. The formation of benzyl boronic acid can be explained either through the intermediate formation of a boronate or through the formation of a carbene (through the loss of nitrogen molecule). Reaction of the carbene with the boronic acid forms the benzyl boronic acid. Protodeboronation of the benzyl boronic acid led to the formation of the product (**Scheme 24**).



Scheme 24. Synthesis of diarylmethane using metal free C-C bond formation

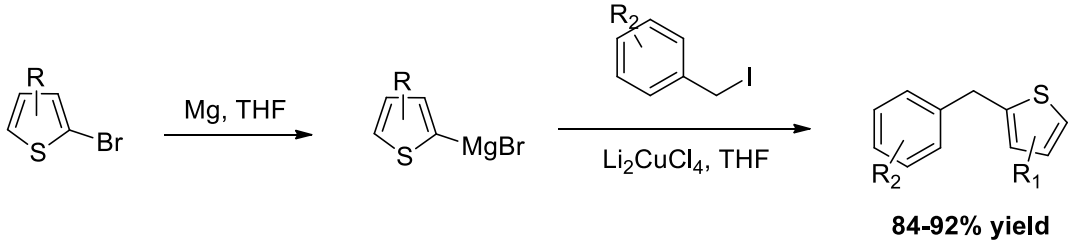
In 2012, Khodaei *et al.* reported triphenyl phosphine ditriflate (TPPD) mediated Friedel–Craft's alkylation of benzyl alcohols with arenes for the facile preparation of diarylmethanes (**Scheme 25**) [66].



Scheme 25. Synthesis of diarylmethanes using triphenyl phosphine ditriflate

2.1.1.3 Copper and Palladium catalyzed cross coupling reactions

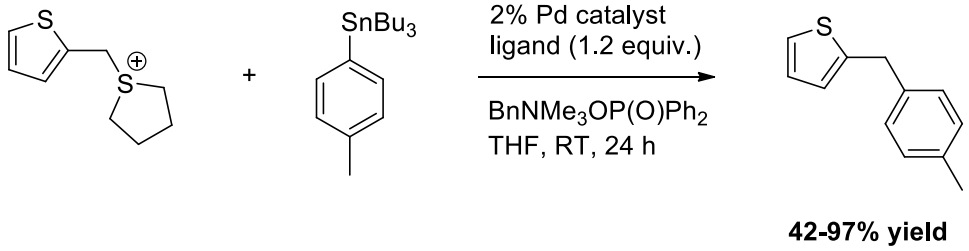
In 1996, Ku *et al.* reported copper catalyzed cross coupling of aryl Grignard reagents with benzyl halides for the preparation of diarylmethanes [67] (**Scheme 26**). This was a high yielding, easily scalable reaction for the preparation of diarylmethanes for multigram industrial scale.



Scheme 26. Copper catalyzed synthesis of diarylmethanes using Grignard reagent

Leibeskind *et al.* reported in 1997 and 1999, metal catalyzed cross coupling reactions of heterobenzylic sulfonium salts with organostannanes, organoboronic acids as well as organozinc reagents [68] [69] (**Scheme 27**). For the cross coupling with organostannanes and organoboronic acids, palladium was used while for organozinc halides, nickel was used. For improving the

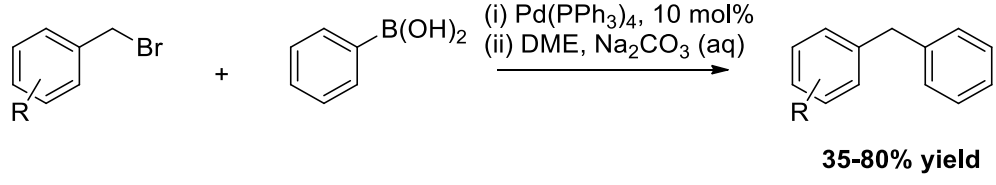
efficiency of the organostannanes, $Ph_2P(O)O^-Bu_4N^+$ was used as Bu_3Sn scavenger. The use of highly nucleophilic phosphine ligands in order to stabilize the metal catalyst and the electrophilic sulfonium salts leads to the competing side reactions which were overcome by using essentially non-nucleophilic triaryl phosphites as ligands.



Scheme 27. Palladium catalyzed synthesis of diarylmethanes using organostannates

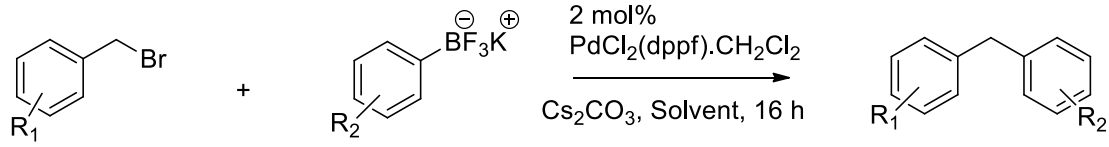
2.1.1.4 Suzuki-Miyaura coupling

Georghiou *et al.* in 1999 reported the Suzuki–Miyaura coupling of benzyl halides with aryl and naphthyl boronic acids to form the respective diarylmethanes [70] (**Scheme 28**). The authors observed using benzyl iodides caused a minor increase in the reaction yield compared to benzyl bromides. Using benzyl bromides required a higher catalyst loading and the reaction took longer to reach completion.



Scheme 28. Synthesis of diarylmethanes using Suzuki-Miyaura coupling

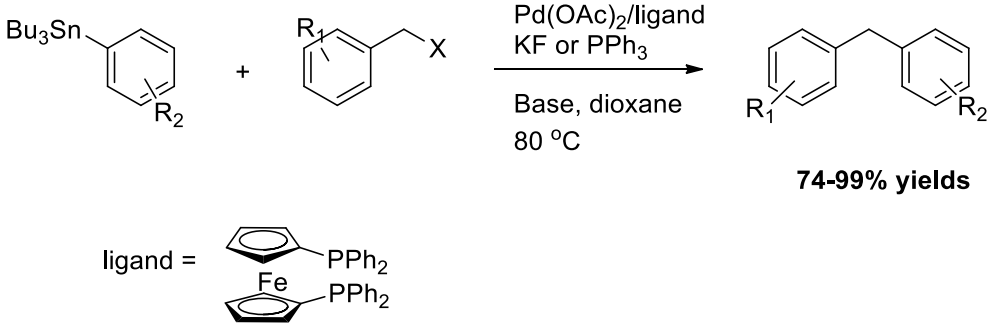
In 2006, Molander *et al.* reported Suzuki coupling of benzyl halides with potassium aryltrifluoroborates for the preparation of diarylmethanes [71]. The pre-catalyst used was $PdCl_2(dppf).CH_2Cl_2$ (**Scheme 29**).



Scheme 29. Synthesis of diarylmethanes using potassium aryltrifluoroborates

2.1.1.5 Stille coupling

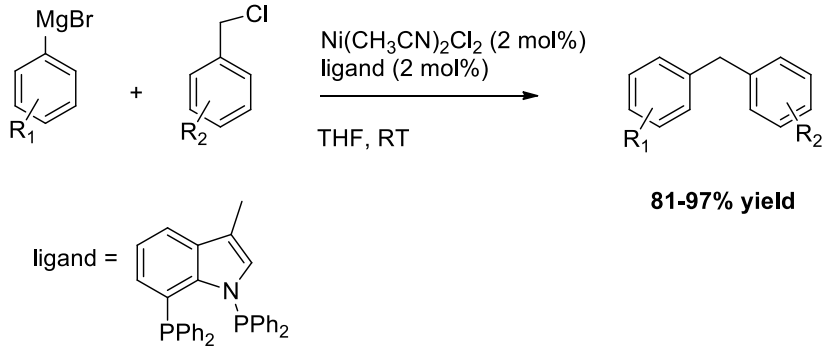
Monteiro *et al.* reported Stille coupling of benzylic halides with aryl tributyl tin for the preparation of diarylmethanes with palladium acetate as the palladium source, diphenyl phosphinyl ferrocene (dppf) as the ligand and potassium fluoride as a base in dioxane as solvent [72]. However, when the reaction was carried out in the absence of a base, triphenyl phosphine was found to be a better ligand (**Scheme 30**).



Scheme 30. Synthesis of diarylmethanes using Stille coupling

2.1.1.6 Kumada coupling

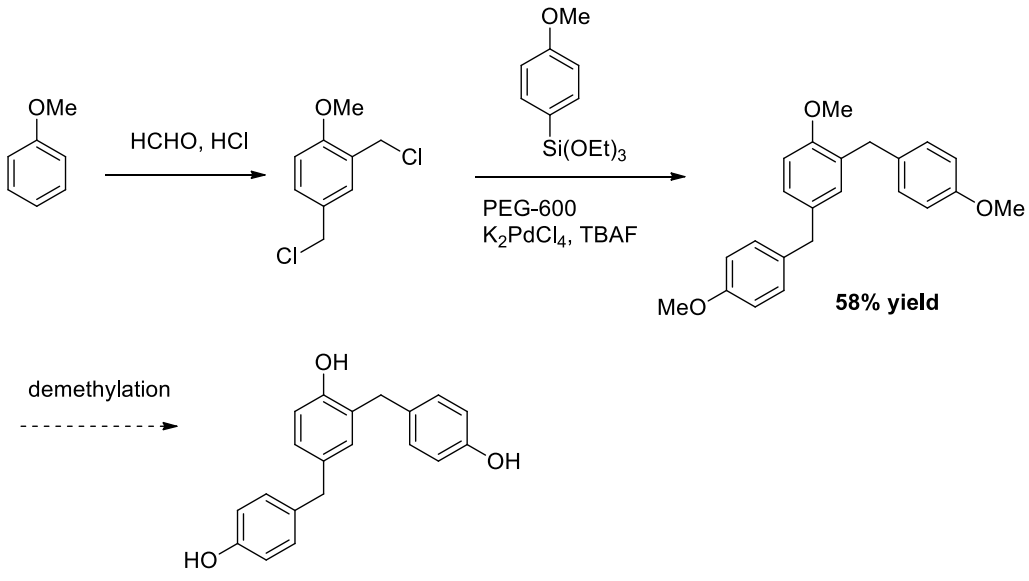
Sarkar and coworkers in 2010 developed a novel air stable bidentate ligand having an indole moiety and N–P donor atom for the Nickel catalyzed Kumada coupling of aryl and benzyl chlorides with aryl magnesium halides [73]. The Kumada coupling reactions of the benzyl chlorides with the aryl magnesium halides were highly reactive yielding the corresponding diarylmethanes in high yields (up to 90% yield) (**Scheme 31**).



Scheme 31. Synthesis of diarylmethanes using Kumada coupling

2.1.1.7 Hiyama coupling

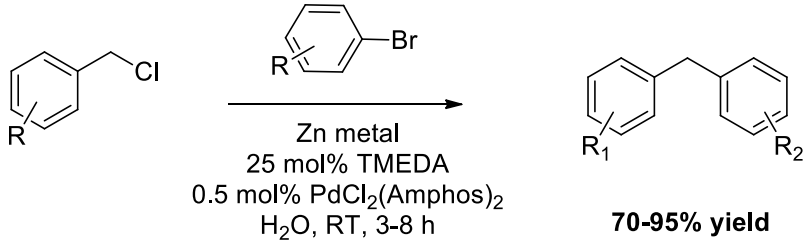
Sarkar *et al.* in 2010 reported palladium nanoparticles as catalyst for the Hiyama coupling of benzylic halides with aryl trialkoxy silanes [74]. The authors further demonstrated the importance of this methodology by synthesizing the natural product 2,4-bis(4-hydroxybenzyl) phenol in only 3 steps starting from the readily available anisole (**Scheme 32**).



Scheme 32. Synthesis of 2,4-bis(4-hydroxybenzyl) phenol using Hiyama coupling

2.1.1.8 Negishi coupling

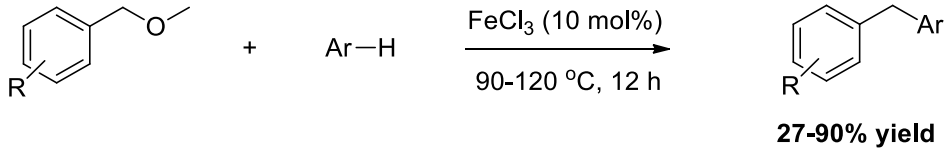
In 2012, Lipshutz *et al.* reported a remarkably simple method to develop unsymmetrical diarylmethanes that relies on an *in situ* organozinc-mediated, palladium-catalyzed cross-coupling [75]. Thus, by mixing a benzyl and aryl halide together in the presence of Zn metal and a Pd catalyst, diarylmethanes are formed at room temperature without assistance by a surfactant (**Scheme 33**).



Scheme 33. Synthesis of diarylmethanes using Negishi coupling

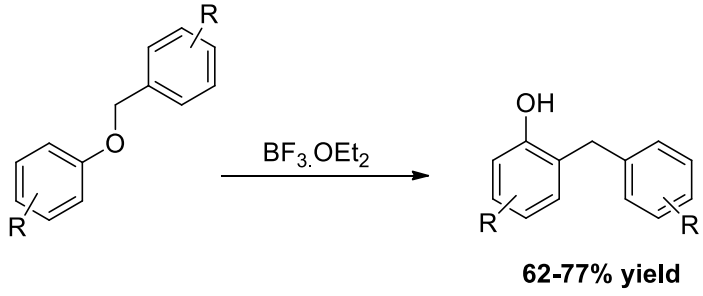
2.1.1.9 Lewis acid mediated sp^3 C–O bond activation

Shi *et al.* in 2008 reported $FeCl_3$ mediated sp^3 C–O activation of benzyl ethers for the Friedel–Craft's alkylation of benzyl ethers with arenes [76] (**Scheme 34**).



Scheme 34. Lewis acid mediated synthesis of diarylmethanes

In 2012, Kraus *et al.* reported Lewis acid catalyzed rearrangement of benzylic ethers for the facile preparation of diarylmethanes [77] (**Scheme 35**).



Scheme 35. Synthesis of diarylmethanes via benzylic ether rearrangement

2.1.2 Diarylmethanes in drug discovery

Diarylmethanes are very important scaffolds in organic and medicinal chemistry. Some have been used as dyes [78] [79], protecting groups for nucleosides, carbohydrates [80] [81] anticancer drugs [82] epoxy resins, and antioxidants [83]. Piritrexim (**53**), a lipophilic inhibitor of the key metabolic enzyme dihydrofolate reductase (DHFR) has been studied intensively as an anticancer drug (**Fig. 28**). A noble structural feature of **53** is a methylene (CH_2) bridge between the two halves of the molecule. This bridge is also present in trimethoprim (**54**), another lipophilic DHFR inhibitor widely used for therapy in AIDS patients, usually in combination with a sulfa drug to enhance efficacy. Another member of this class that has been used clinically against these infections is trimetrexate (**55**), which contains a longer CH-NH bridge [84] (**Fig. 30**).

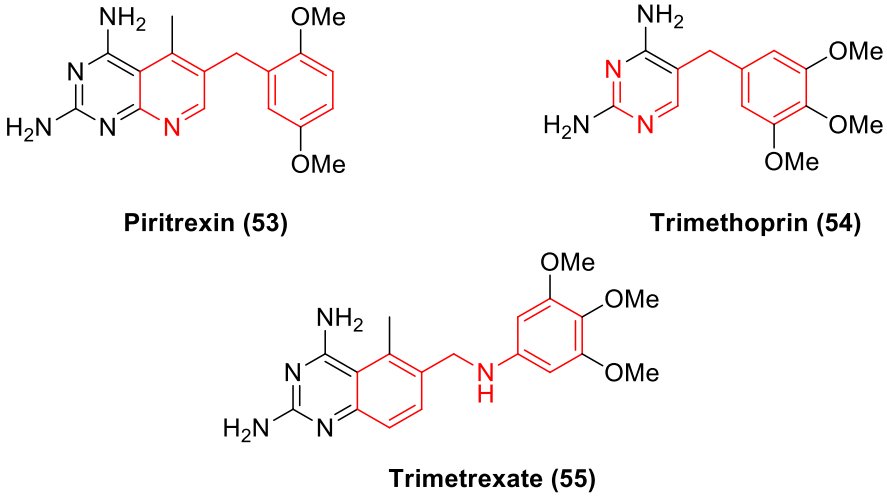


Fig. 30. Structures of **53**, **54 and 55** showing the diarylmethane scaffold

In 2006, Panda *et al.* reported the synthesis of a series of aminoalkyl derivatives of substituted and unsubstituted diarylmethanes (**Fig. 31**). These compounds were evaluated for antitubercular activity. Indole and phenanthrene substituted derivatives showed promising activity against *M. tuberculosis* $H_{37}R_v$.

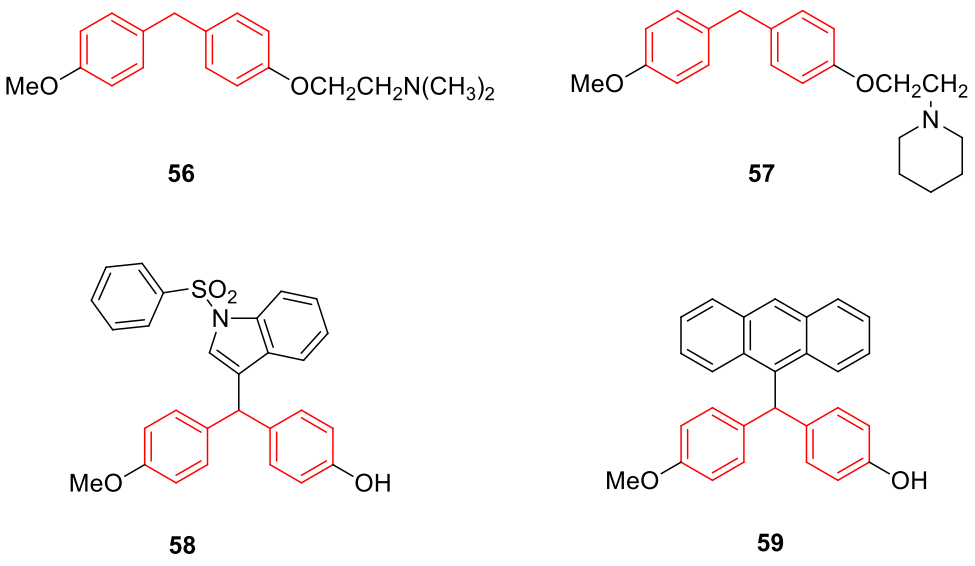
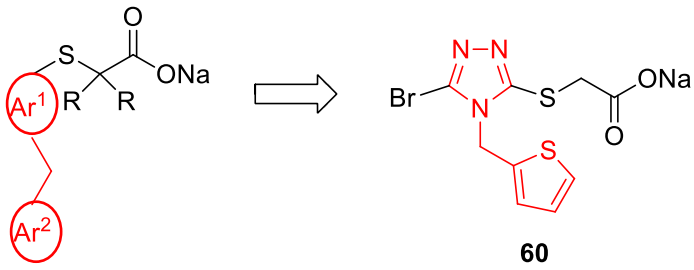


Fig. 31. Structures of substituted and unsubstituted diarylmethanes **56-59**

Gout is the most common inflammatory arthritis caused by the deposition of monosodium urate (MSU) in articular and periarticular tissues and characterized by recurrent joint swelling, redness, warmth and severe pain [85]. If left untreated or poorly managed, tophaceous gout will ultimately lead to permanent joint destruction, bone erosion and kidney impairment, dramatically affecting patients' quality of life and even threatening their lives [86]. Recently, Cai and coworkers developed uric acid transporter 1 (URAT1, SLC22A12) inhibitors, for the treatment of gout, that contained a diarylmethane backbone. Structure-activity relationship (SAR) studies were carried out on the general backbone. The authors synthesized 33 compounds out of which **60** was found to be the most potent (**Fig. 32**).



60

Fig. 32. Structure of URAT1 inhibitor **60**

2.2 Heterocycles in drug discovery

Heterocycles are key scaffold components in medicinal chemistry. They are fundamental building blocks of most drugs on market today (**Fig. 33**). The importance of heterocycles is well understood by modern medicinal chemists, since they play a significant role in molecular properties such as the electronic distribution, three dimensionality, and scaffold rigidity. They are often key factors in whole molecule properties such as lipophilicity or polarity and can determine molecular reactivity, metabolic stability, and toxicity [87]. Most frequently, nitrogen heterocycles or various positional combinations of nitrogen atoms, sulphur, and oxygen in five or six-membered rings can be found in many drugs. According to statistics, more than 85% of all biologically-active chemical entities contain a heterocycle [88].

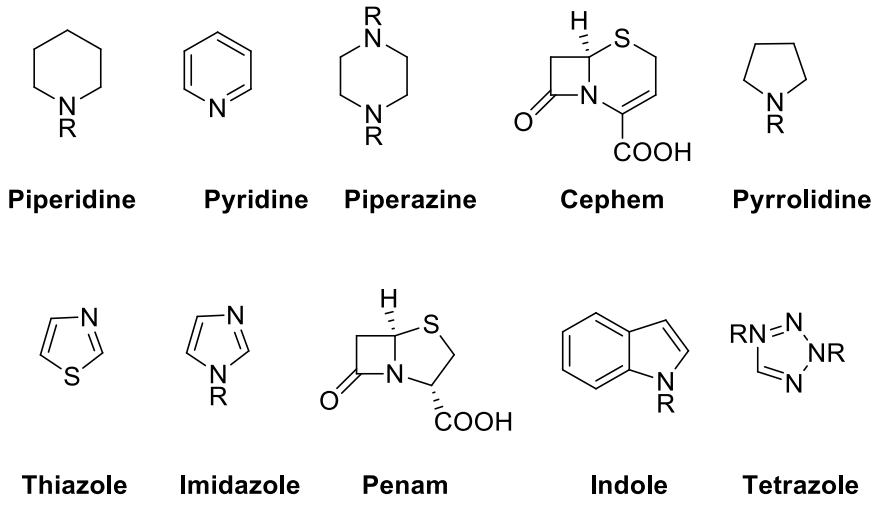


Fig. 33. Top 10 heterocyclic scaffolds in FDA approved drugs

2.2.1 Four-membered nitrogen heterocycles: Azetidines

With the exception of a single aziridine-containing drug (mitomycin), the most commonly found for-membered nitrogen heterocycles are all *β*-lactams, of which 95% are fused to another ring with the nitrogen atom shared (**Fig. 34**) [88]. They are least diverse in terms of diseases they are used to treat. Case in point, all but the cholesterol lowering agent ezetimibe [89] of the β-lactam drugs [90] are antibiotics or used in combination with antibiotics.

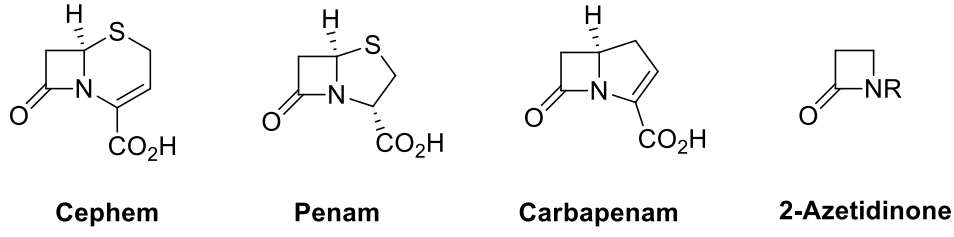
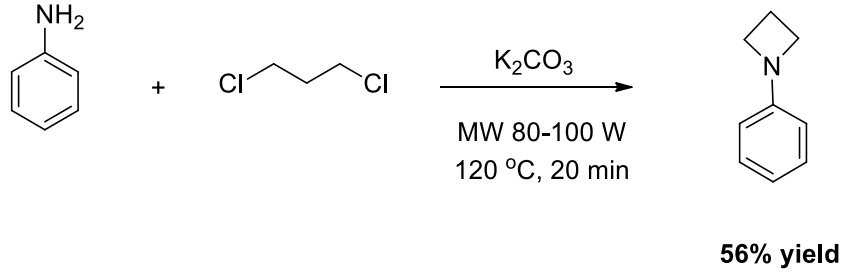


Fig. 34. Top four most common four-membered nitrogen heterocycles

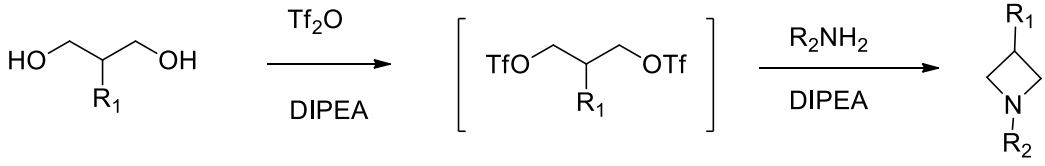
2.2.1.1 Syntheses of azetidines

Cyclization of a preformed chain by nucleophilic displacement of a leaving group by a nitrogen nucleophile is, by far, the most common method to produce azetidines. Amines are the most common nitrogen nucleophiles [91]. In 2006, Ju *et al*. reported the synthesis of N-substituted azetidine by cyclization of aniline with 1,3-dichloropropane. The reaction was carried out in water by the assistance of microwave (**Scheme 36**) [92].



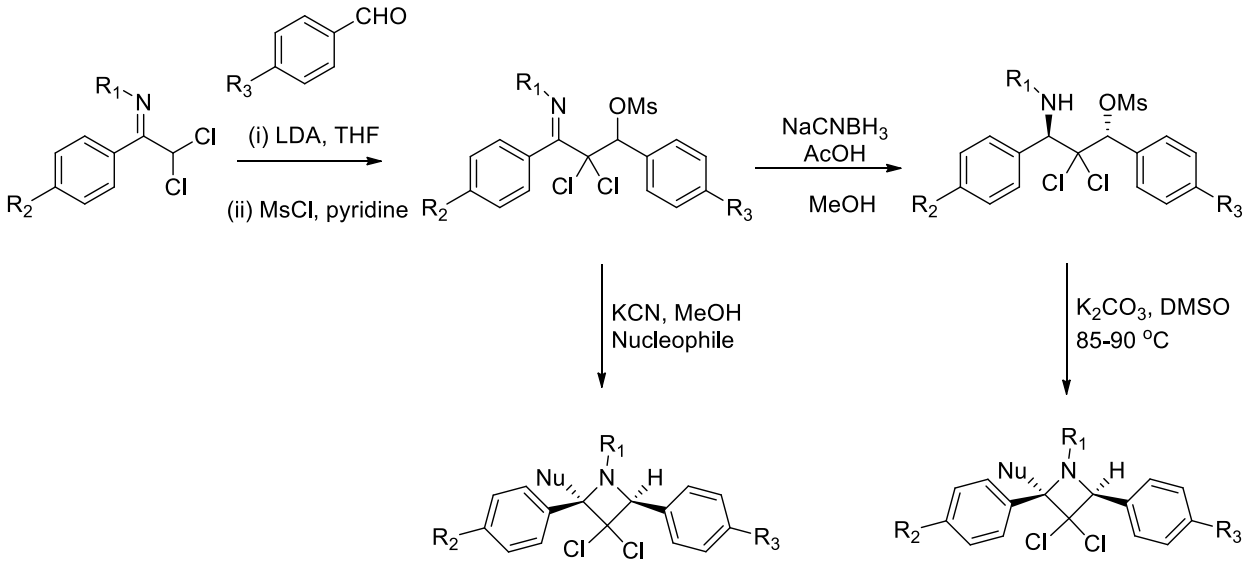
Scheme 36. Synthesis of N-substituted azetidine

Traditional protocols in the presence of alcoholic intermediates involve the transformation of the alcohol in sulfonic ester, mainly mesylate [93] [94] [95] tosylate [96] [97] [98] and triflates [99] followed by base mediated cyclization. Hillier and co-worker reported the synthesis of 1,3-substituted azetidines from 2-substituted-1,3-propanediols in a one-pot reaction (**Scheme 37**).

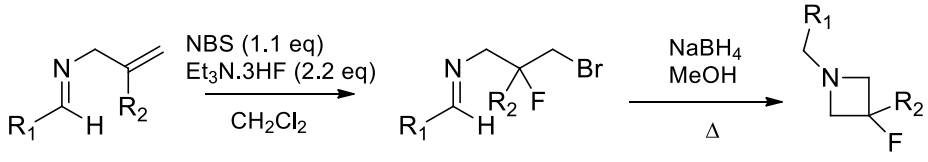


Scheme 37. Synthesis of 1,3 substituted azetidine

De Kimpe's group provided the synthesis of uncommon 3,3-dichloroazetidines (**Scheme 38**) [100] and 3-fluoroazetidines (**Scheme 39**) [101] starting from imines.



Scheme 38. Synthesis of 3,3-dichloroazetidines

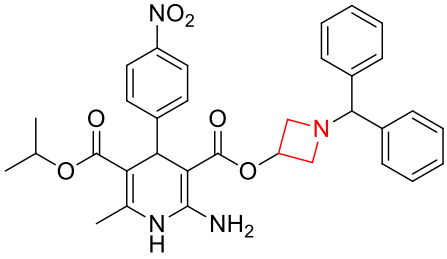


Scheme 39. Synthesis of 3-fluoroazetidines

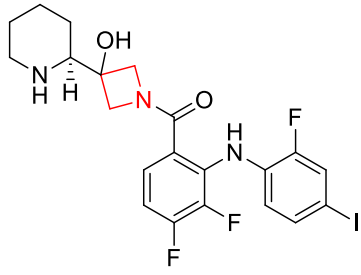
2.3 Azetidines as a pharmaceutical scaffold

Azetidine is considered a privileged scaffold in drug discovery. It is a good compromise between a satisfactory stability and a strong molecular rigidity, allowing an efficient tuning of pharmacological properties displayed by molecules bearing this moiety [102]. Azetidines, despite

their indisputable importance as bioactive compounds and pharmaceutical tools, have received little attention by the chemical community compared to the higher homologous counterparts. There are very few azetidine containing drugs are currently on the market. Dihydropyridine azelnipidine (Calblock, **61**) is Sankyo's calcium channel blocker [103]. Exelixis' cobimetinib (Cotellic, **62**), as a targeted cancer therapy, is a mitogen-activated protein kinase-1/2 (MEK1/2) inhibitor (**Fig. 35**) [104].



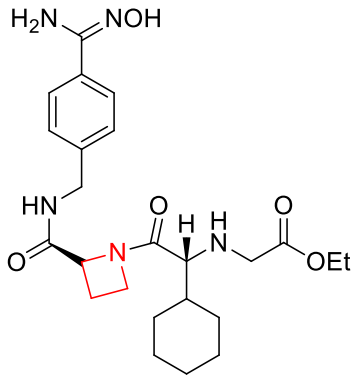
azelnipidine (Calblock, 61)
Daiichi-Sankyo, 1989
calcium channel blocker



cobimetinib (Cotellic, 62)
Exelixis/Genentech, 2015
MEK1/2 inhibitor

Fig. 35. Structures of azetidine-based drugs **61** and **62**

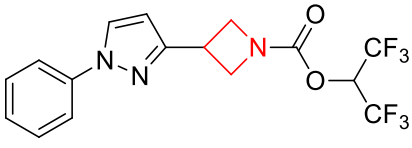
Another azetidine-containing drug ximelagatran (Exanta, **63**) as a direct thrombin inhibitor was discovered by AstraZeneca. Initially sold as an anticoagulant, it was pulled off the market in 2006 due to hepatoxicity (**Fig. 36**) [105].



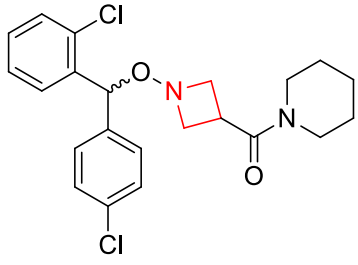
ximelagatran (Exanta, 63)
AstraZeneca
direct thrombin inhibitor

Fig. 36. Structure of azetidine-based drug **63**

Azetidine carbamate **64** is an efficient, covalent inhibitor of monoacylglycerol lipase (MAGL) discovered by Pfizer [106]. The hexafluoroisopropanol (HFIP) group here serves as the leaving group when attacked by the key serine residue (Ser122) at the enzyme's active site. Fatty acid amide hydrolase (FAAH) inhibitors are potential treatment for pain. Vernalis discovered a mixture of chiral azetidine-ureas VER-24052 (**65**) as a FAAH inhibitor (**Fig. 37**) [107].



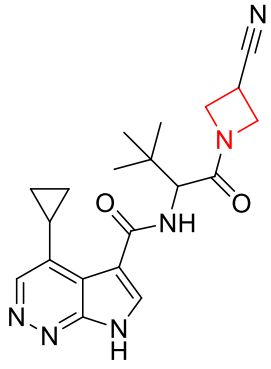
64
Pfizer
monoacylglycerol lipase inhibitor



VER-24052, 65
Vernalis
Fatty acid amide hydrolase inhibitor

Fig. 37. Structures of azetidine-based drugs **64** and **65**

In 2013, Roche reported an azetidine-containing bis-amide **66** as a selective JAK3 (Janus kinase) inhibitor (IC_{50} = 0.26 nM) with a 10-fold selectivity over JAK1 (IC_{50} = 3.2 nM). In addition, the combination of its selectivity over the kinome, good solubility and reasonable exposure was translated to *in vivo* potency and selectivity in an acute PK/PD mouse model (**Fig. 38**) [108].

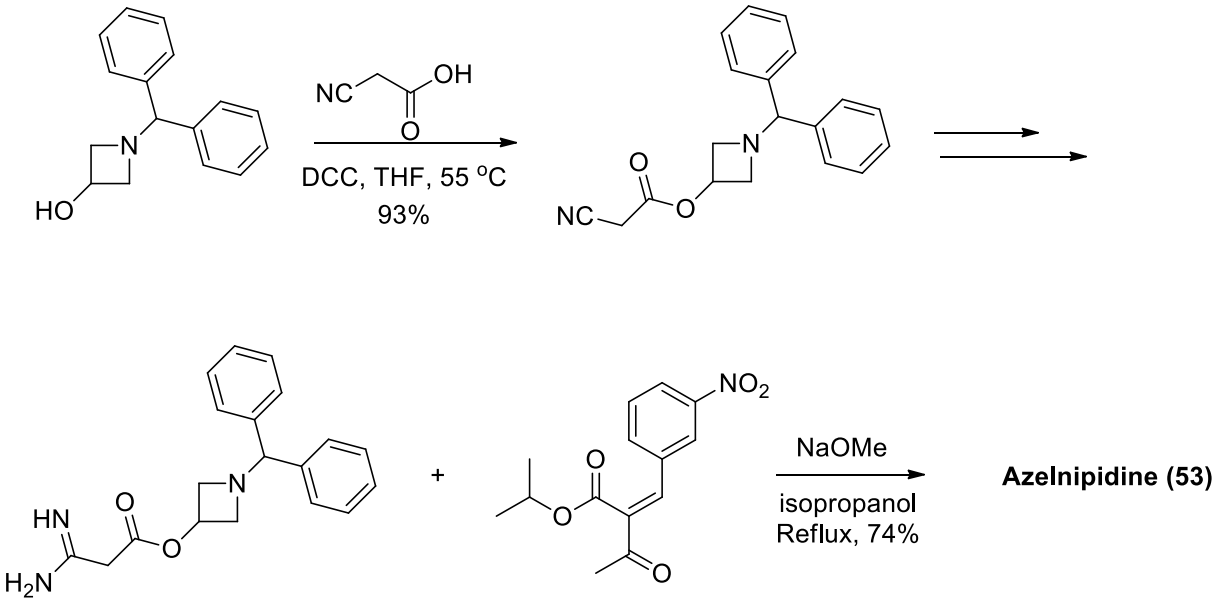


66
Pfizer
JAK inhibitor

Fig. 38. Structure of azetidine-based drug **66**

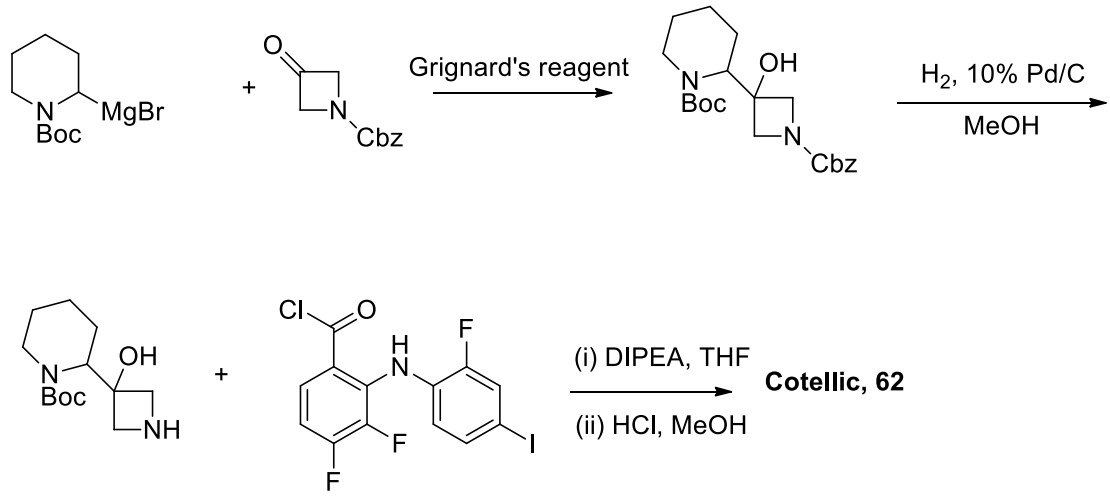
2.3.1 Synthesis of bioactive azetidines

Sankyo's synthesis of azelnipidine (Calblock, **61**) began with 1-benzhydryl- 3-hydroxyazetidine, readily assembled from condensation of benzhydrylamine with epichlorohydrin. Subsequent 1,3- dicyclohexylcarbodiimide (DCC)-mediated esterification with cyanoacetic acid produced ester, which was converted to an amidine in two additional steps. A Hantzsch dihydropyridine synthesis between the amidine and an enone **t**hen delivered azelnipidine **61** (**Scheme 40**).



Scheme 40. Synthesis of AzeInipidine **61**

Exelixis' synthesis of cobimetinib (Cotellic, **62**) commenced with addition of a piperidine-Grignard reagent to N-Cbz-3-azetidinone. The resulting 3-hydroxy azetidine underwent Palladium-catalyzed hydrogenation, removing the Cbz protection, to afford the exposed azetidine. Ester formation from the coupling between the free azetidine and an acid chloride in the presence of diisopropylethylamine (DIPEA) produced cobimetinib (**62**) after deprotection of the Boc group.



Scheme 41. Synthesis of Cotellic **62**

2.4 Geminal 3,3-diaryl azetidines

The combination of saturated heterocycles with aromatic substituents provide particularly attractive screening compounds as fragments or lead-like compounds due to the different potential binding interactions available. However there remain challenges in readily accessing a broad array of substituted heterocycles under mild conditions, as is appropriate in divergent and iterative medicinal chemistry investigations [109]. The sp^3-sp^2 coupling of small rings or saturated heterocycles with aromatic components presents a valuable transformation that can facilitate the construction of important compound types in drug discovery. 3,3-Diarylazetidines are relatively little studied but can be found in biologically active compounds in the patent literature (**Fig. 39**) [110] [111].

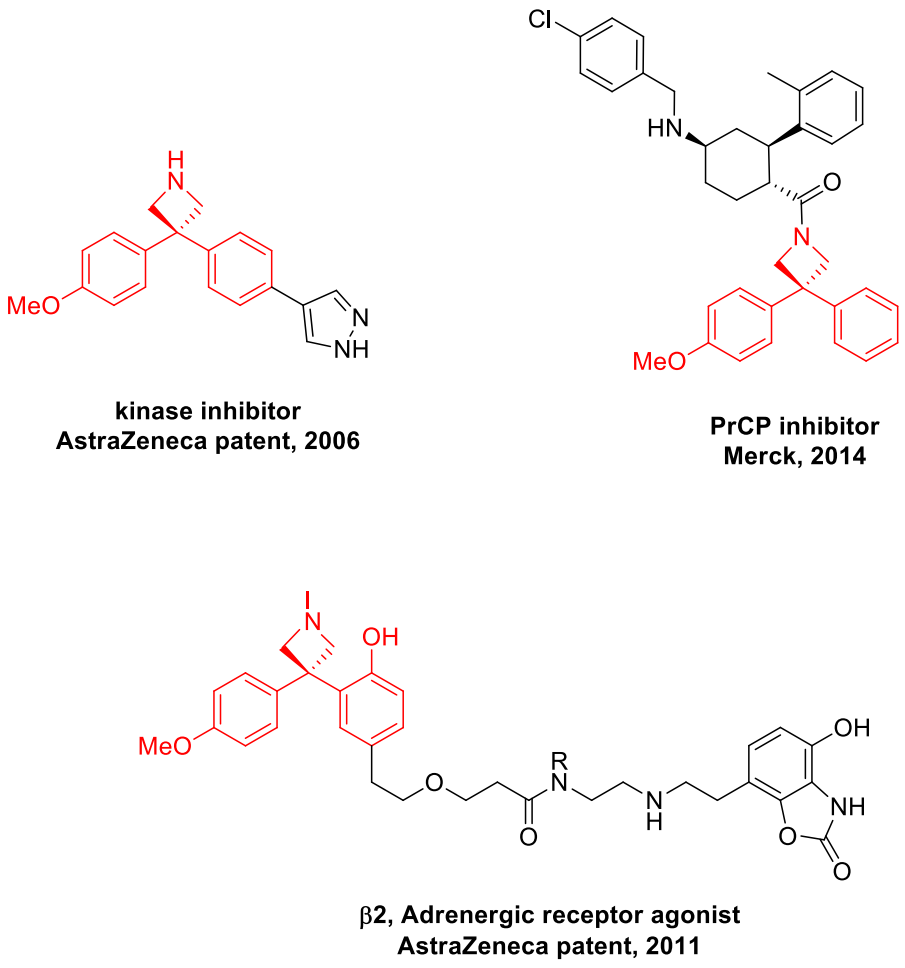
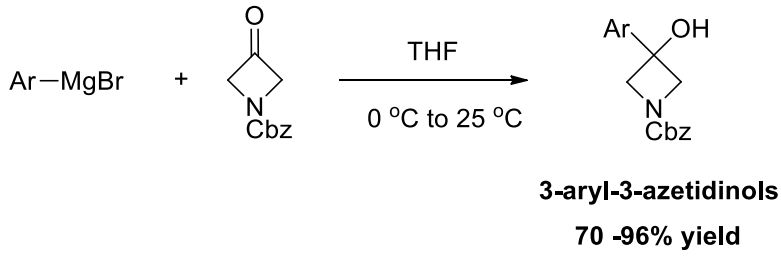


Fig. 39. Bioactive geminal diaryl azetidine structures

2.5 Synthesis of 3,3-diarylazetidines: Recent developments and scope

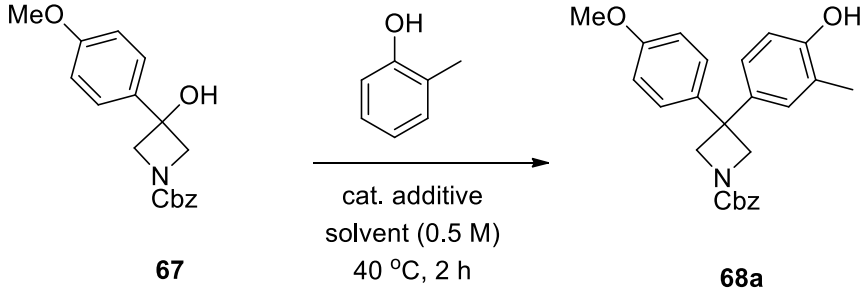
In 2019, Bull and coworkers reported the synthesis of 3,3-diarylazetidines by Fridel-Craft's alkylation of electron rich aromatics with azetidinols, using a calcium triflimide catalyst [112]. A calcium catalyst generates azetidine carbocations from 3-arylazetidin-3-ols under mild conditions. The reaction was compatible with functionalized substrates and a wide range of electron-rich aromatic and heteroaromatic nucleophiles. The azetidinols were prepared using Grignard's

reagents (**Scheme 42**). The resulting 3-aryl-3-azetidinols were then reacted with various aromatic nucleophiles to give the corresponding 3,3-diarylazetidines.



Scheme 42. Synthesis of 3-aryl-3-azetidinols

Initially, the authors examined N-Cbz azetidinols bearing a 4-methoxyphenyl group using catalytic amounts of various Lewis acids and phenols such as *o*-cresol as nucleophiles (**Scheme 43**). Optimization studies for Fridel-Craft's arylation of azetidinol **67** with *o*-cresol is shown in **Table 5**.

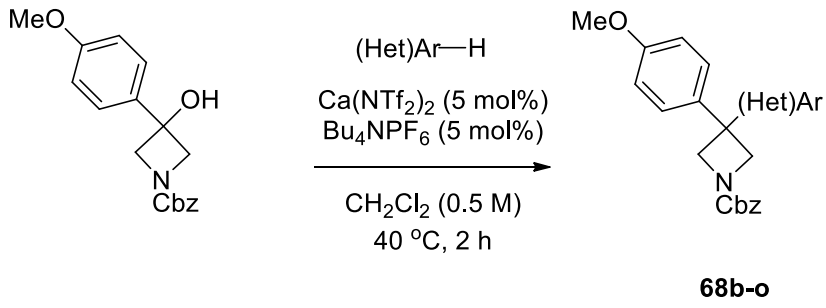


Scheme 43. Synthesis of 3,3-diarylazetidine **68a**

Table 7. Fridel-Craft's aryltion of **67** with o-cresol.

Entry	Cat (mol%)	Equiv of o-cresol	Solvent	Yield of 68a (%)
1	$Li(NTf_2)/Bu_4NPF_6$ (11/5.5)	5	CH_2Cl_2	45
2	$FeCl_3$ (5)	5	CH_2Cl_2	75
3	$Ca(NTf_2)_2/Bu_4NPF_6$ (5/5)	5	CH_2Cl_2	92
4	$Ca(NTf_2)_2$ (5)	5	CH_2Cl_2	0
5	$Ca(NTf_2)_2/Bu_4NPF_6$ (5/5)	3	CH_2Cl_2	94
6	$Ca(NTf_2)_2/Bu_4NPF_6$ (5/5)	3	PhMe	96
7	$Ca(NTf_2)_2/Bu_4NPF_6$ (5/5)	3	Heptane	91

The Bu_4NPF_6 additive was crucial, with no reaction in its absence (Table 5-entry 4). Decreasing the equivalents of the phenol from 5 to 3 maintained high yield (94%). Solvents like toluene and heptane were suitable but dichloromethane was used because of improved substrate solubility. These optimized conditions were then used to investigate the scope of the reaction. A wide range of aromatic and heteroaromatic nucleophiles were used (**Scheme 44**).



Scheme 44. Synthesis of 3,3-diarylazetidines **68b-o**

Table 8. Scope of heteroaromatics and phenol derivatives as nucleophiles

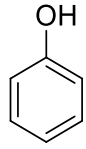
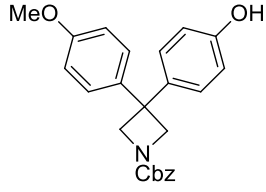
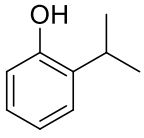
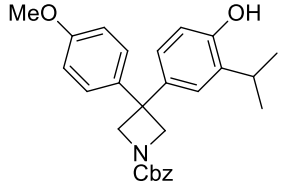
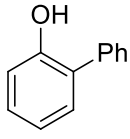
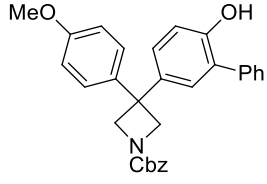
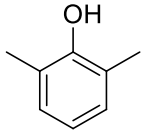
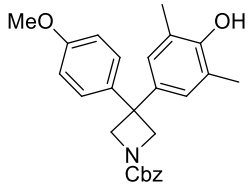
Compound no.	(Het)Ar nucleophile	Product
68b		
68c		
68d		
68e		

Table contd.

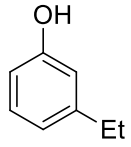
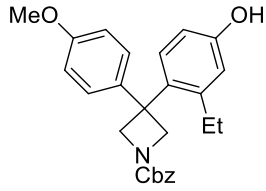
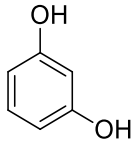
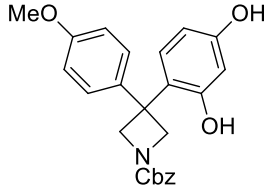
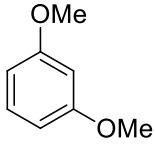
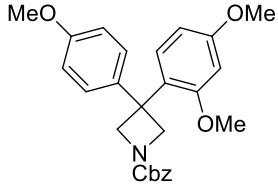
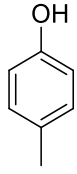
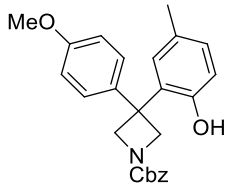
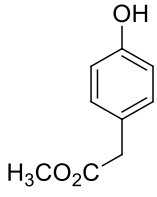
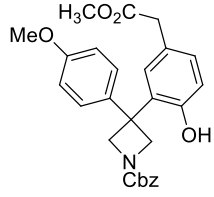
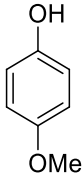
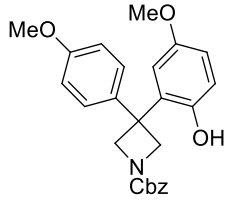
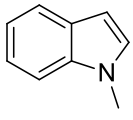
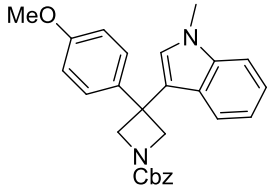
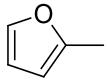
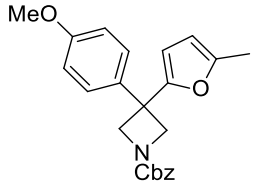
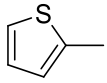
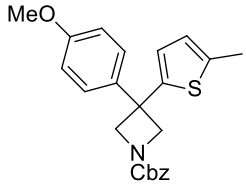
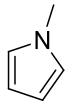
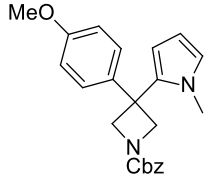
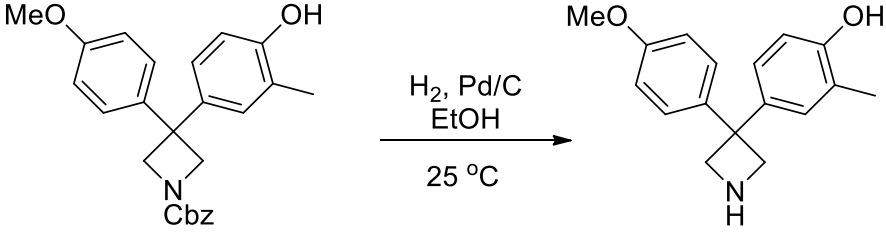
68f		
68g		
68h		
68i		
68j		
68k		
68l		

Table contd.

68m		
68n		
68p		

The Cbz group was removed by using H_2, Pd/C catalyst to give the free-base azetidine **69** (**Scheme 45**), important for further functionalization.



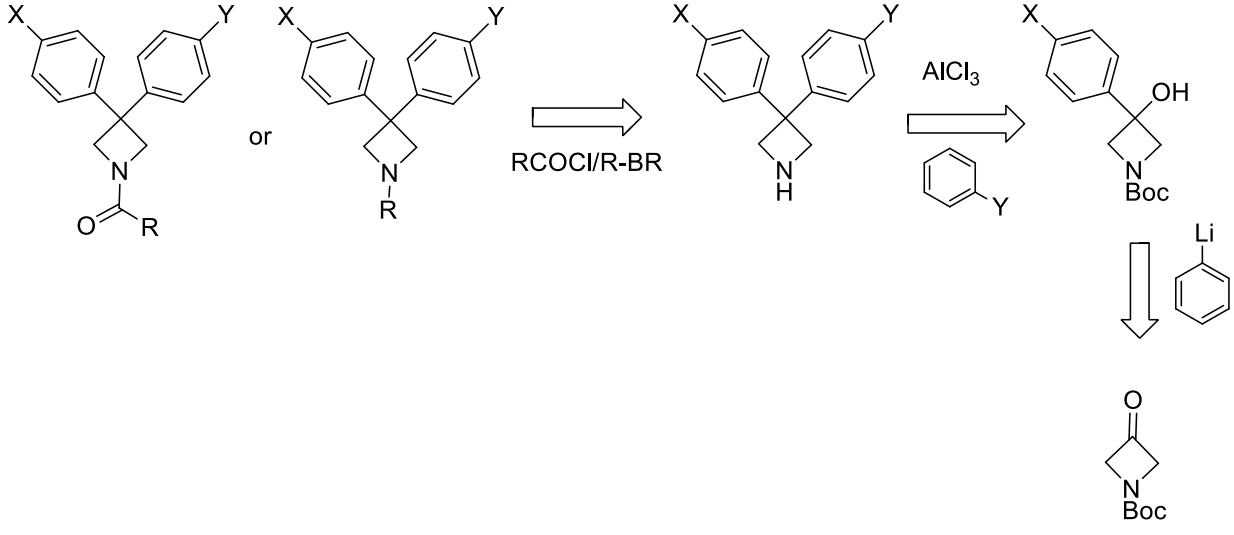
68a **69**

Scheme 45. Removal of Cbz group using H_2, Pd/C

2.5 Design strategy for synthesis of 3,3-diarylazetidines and subsequent derivatization

Previous studies in our group have reported the synthesis of 3,3-diarylazetidines from N-Boc-3-azetidinols [113]. The N-Boc-3-azetidinols were prepared from the readily available N-Boc-3-azetidinone. This was achieved by reacting the azetidinone with aryllithium reagents. Subsequently, the azetidinols underwent Friedel-Craft's arylation using aluminum chloride ($AlCl_3$) as the Lewis acid. It was observed that the $AlCl_3$ not only assists in the nucleophilic attack of the aryl group but also deprotects the Boc group to give the free-base azetidine.

The goal of this project was to functionalize 3,3-diarylazetidines previously synthesized in the group. **Scheme 46** shows the she retrosynthetic approach for the synthesis of the functionalized 3,3-diarylazetidines. The 3,3-diarylazetidines will have to be prepared first. These diarylazetidines will be functionalized by adding acyl and alkyl groups to the azetidine nitrogen.

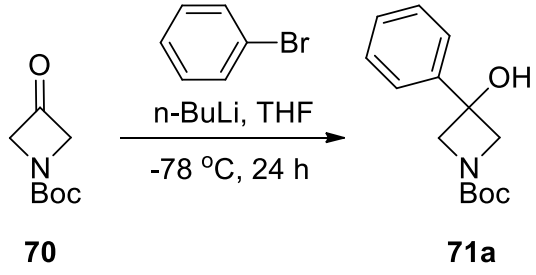


Scheme 46. Retrosynthetic scheme for synthesis of N-functionalized 3,3-diarylazetidines

RESULTS AND DISCUSSION

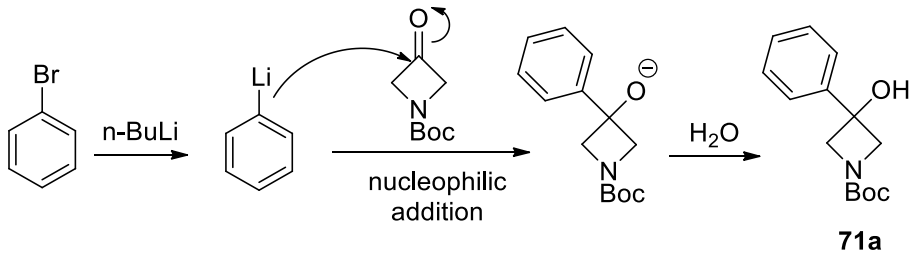
2.6 Synthesis of N-boc-3-aryl-3-azetidinols

Previously used synthetic methodology was used to synthesize the diarylazetidines. The series of diarylazetidizes was synthesized in two steps. The first step involved the reaction of an aryllithium compound with N-Boc-3-azetidinone (**70**). The arryllithium was prepared *in situ* using a metal-halogen exchange between n-butyllithium (n-BuLi) and an aryl halide. Synthesis of N-Boc-3-phenyl-3-azetidinone was attempted first. Bromobenzene was treated with n-BuLi in Tetrahydrofuran (THF) at -78 ℃ to give phenyllithium after which **70** was added to the reaction mixture and stirred at room temperature overnight to give N-Boc-3-phenyl-3-azetidinol (**71a**) (**Scheme 47**).



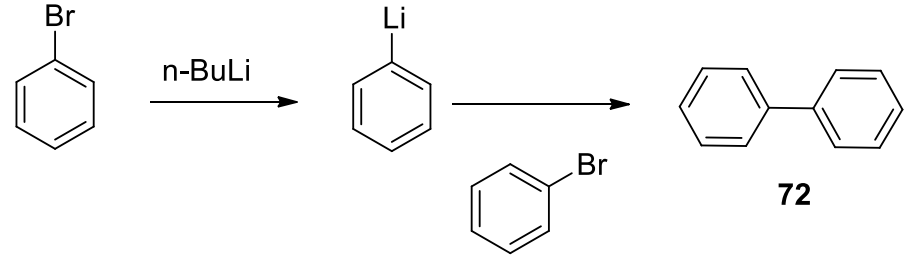
Scheme 47. Synthesis of N-Boc-3-phenyl-3-azetidinol **71a**

The mechanism for this reaction is shown in **Scheme 48**. The reaction takes place via a nucleophilic addition of the phenyllithium on the carbonyl of the azetidinone.



Scheme 48. Mechanism of nucleophilic addition of phenyllithium

71a was isolated by column chromatography. The first few attempts resulted in very low yields (42%). The major product of the reaction was found to be homocoupled product **72** (presumably biphenyl). Initially, **72** was mistaken as the desired product on the TLC plate (R_f = 0.5) and isolated using column chromatography. However, ^{1}H NMR showed peaks only in the aromatic region (~7-8 ppm). This homocoupled product could have formed via Wurtz coupling. This happens when the lithiated product (phenyllithium) reacts with the aryl halide (bromobenzene) (**Scheme 49**). This required us to reevaluate the TLC plate. There was a faint UV active spot with an R_f value of 0.3 which was isolated using column chromatography. ^{1}H NMR confirmed its identity as **71a**.



Scheme 49. Possible side reaction and formation of homocoupled product **72**

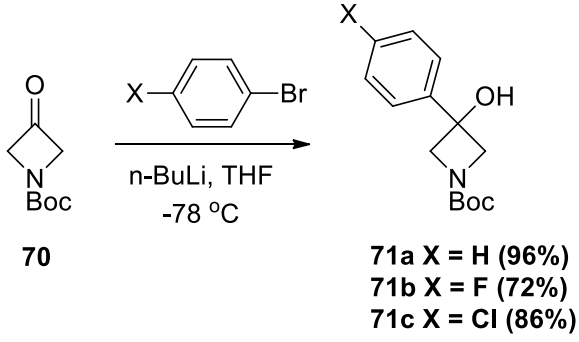
To increase the yield of **71a** and reduce the formation of **72**, the amount of bromobenzene added was reduced from 3 equivalents to 2 equivalents. This reduced the formation of the

homocoupled product **72** but didn't increase the yield of the azetidinol **71a** drastically (62% yield). Next, the concentration of phenyllithium in THF was investigated. It was concluded that using 1.3 equivalents and 0.25 M solution of the aryllithium gave a small amount of **72** and **71a** was obtained in high yield (96%). **Table 7** shows the optimization studies for this reaction.

Table 9. Optimization studies for the synthesis of **71a**

Entry	Equiv. of n-BuLi	Molarity of n-BuLi in THF (M)	Yield of 63a (%)
1	3	1.1	42
2	3	0.44	62
3	2	0.12	63
4	2	0.30	72
5	1.3	0.25	96

Using the optimized conditions azetidinols **71a-c** were prepared. Using electron withdrawing groups like chlorine and fluorene resulted in lower yields of the azetidinols (**Scheme 50**).



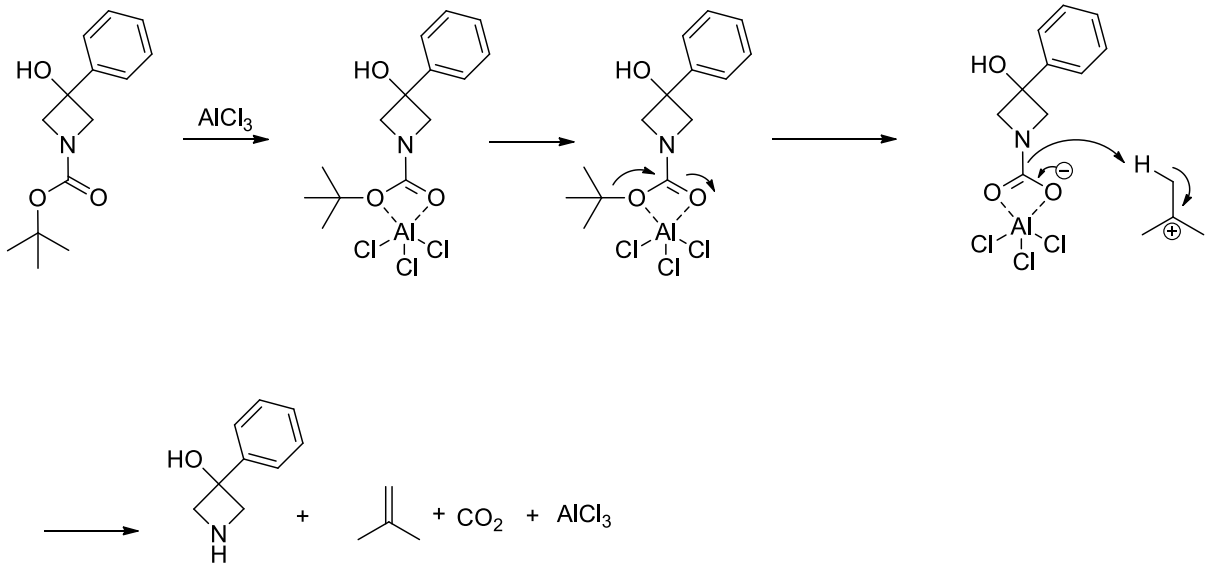
70

71a X = H (96%)
71b X = F (72%)
71c X = Cl (86%)

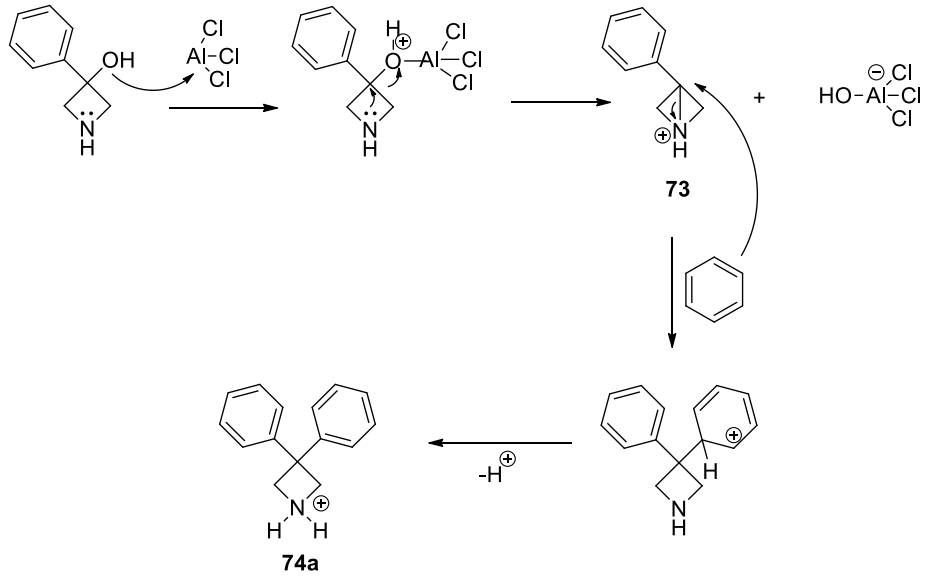
Scheme 50. Synthesis of N-Boc-3-aryl-3-azetidinols **71a-c**

2.7 Synthesis of 3,3-diarylazetidines

The 3,3-diarylazetidines were prepared from the azetidinols **71a-c** by Friedel-Crafts arylation reaction using $AlCl_3$ as the Lewis catalyst. This reaction can proceed via two mechanisms. The $AlCl_3$ could first remove the Boc group to give the free base azetidinol. It then enhances the electrophilicity of the azetidinol by creating a complex with it. The O-H group is removed by $AlCl_3$ creating a bicyclic compound **73**. The nucleophilic benzene attacks the electrophilic carbocation to give the desired diaryl product **74a** (**Scheme 51** and **52**).

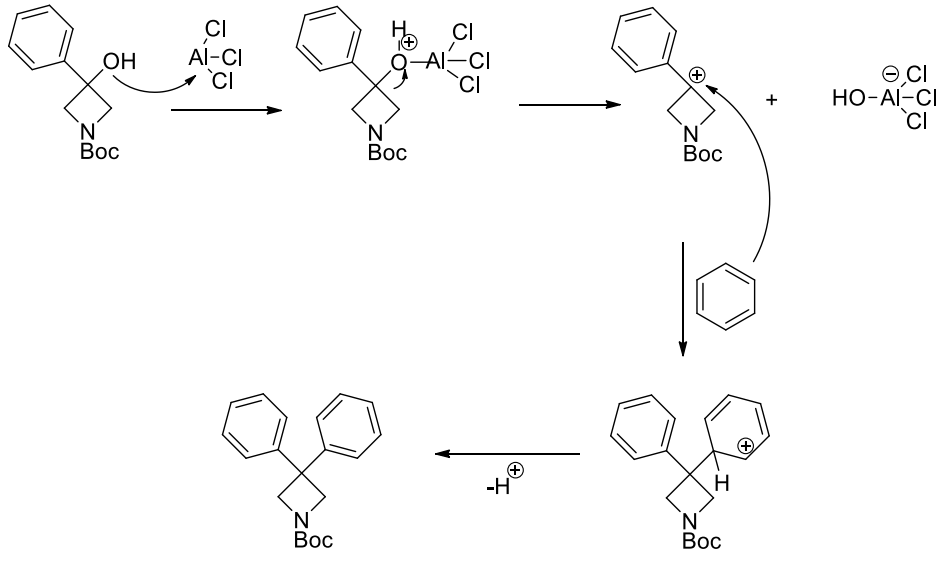


Scheme 51. Boc removal using $AlCl_3$

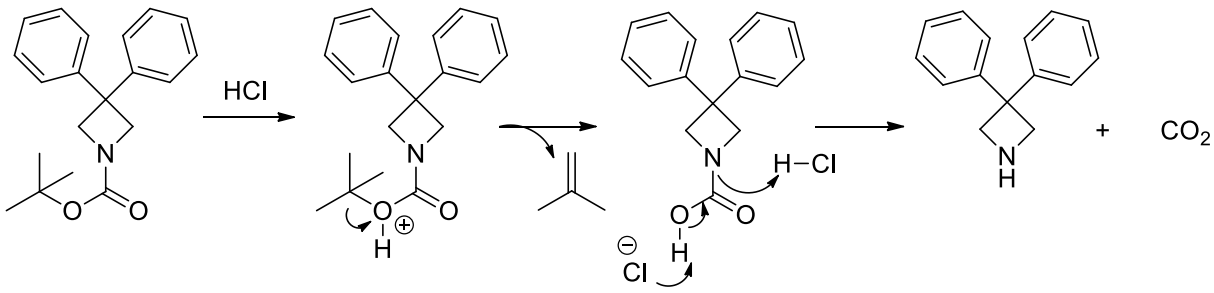


Scheme 52. Friedel-Crafts arylation mechanism after Boc removal

Alternatively, the N-Boc-3-azetidinol could get arylated first via Friedel-Crafts arylation after which the Boc group would be removed (**Scheme 53** and **54**).



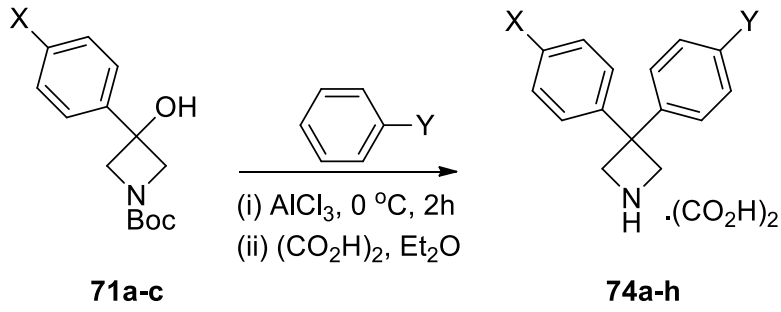
Scheme 53. Friedel-Crafts arylation before Boc removal



Scheme 54. Boc removal after Friedel-Crafts aylation

At the end of the reaction, The TLC plate showed a UV active spot corresponding to the N-Boc-3-arylazetdinol as well as a spot on the baseline which could either correspond to the free base 3-arylazetidinol or some other impurities. It was concluded that it is likely that the Boc group was removed after the Friedel-Crafts arylation which would explain the N-Boc-3-arylazetidinol on the TLC plate at the end of the reaction.

As summarized in **Table 8**, the azetidinols **71a-c** and a variety of substituted benzenes (20 equiv.) were treated with $AlCl_3$ without solvent at 0 °C for 2h to furnish the Friedel-Crafts arylation products **74a-h** (**Scheme 55**). The Boc-protecting group was removed during this step, which allowed the azetidine to be easily isolated as the oxalic acid salt. This avoided the need for chromatography and any additional purification steps. As the oxalate salts the 3,3-diarylazetidines were crystalline solids that could be stored on the bench indefinitely.



71a-c **74a-h**

Scheme 55. Synthesis of 3,3-diarylazetidine oxalic acid salts

Table 10. Optimized yields of 3,3-diarylazetidine oxalic acid salts

Entry	71	X	Y	Yield of 74 (%)
a	71a	H	H	86
b	71a	H	CH_3	83
c	71a	H	F	60
d	71a	H	Cl	86
e	71a	H	OCH_3	72
f	71b	F	H	63
g	71c	Cl	H	70
h	71c	Cl	Cl	81

As seen in **Table 10**, both electron withdrawing and donating groups were tolerated and the 3,3-diarylazetidines were synthesized in moderate to good yields (60-86%). However, it was observed that halogenated benzenes with electron withdrawing groups like fluorine gave lower yields. This is seen in **entry c** when adding fluorobenzene lowers the yield to 60%. Activating groups like toluene and 4-methoxybenzene (**entries b and e**) gave higher yields of 83% and 72% respectively.

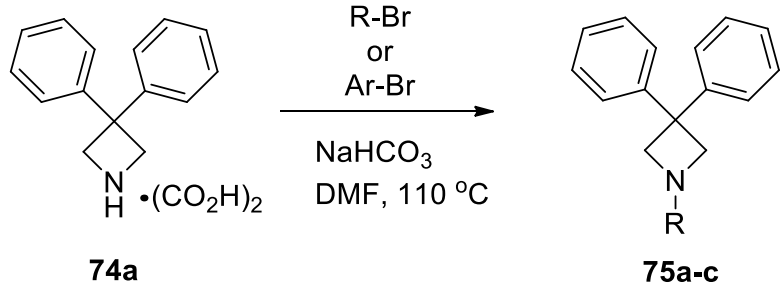
2.8 Synthesis of N-functionalized 3,3-diarylazetidines

To demonstrate versatility of the 3,3-arylazetidine salts **74** toward further functionalization at nitrogen, the preparation of a series of *N*-substituted analogues was explored. Initially, N-alkylation of 3,3-diarylazetidine oxalic acid salt **74a** was carried out with benzyl bromide. As illustrated in **Table 11**, a variety of bases (Et_3N; $NaHCO_3$, Na_2CO_3, *t*-BuOK, NaH, *n*-BuLi) and solvents (EtOH, THF and DMF) were used.

Table 11. Optimization of N-alkylation of **74a**

Entry	Base	Solvent	Yield of 75a (%)
1	Et_3N	EtOH	46
2	Et_3N	DMF	52
3	Na_2CO_3	DMF	56
4	*t*-BuOK	THF	32
5	NaH	EtOH	63
6	NaH	DMF	82
7	$NaHCO_3$	DMF	90

While many combinations of base and solvent gave modest yields of the *N*-alkylation product, it was determined that using $NaHCO_3$ (3 equivalents) in DMF at 110 °C furnished **75a** in 90% yield. It was also determined that using DMF as the solvent in general gave higher yields as the reaction could be heated to a higher temperature when compared to THF and EtOH. The optimized conditions were then applied to N-alkylation with 3-bromoethylpropionate to give **75b** in 94% yield. The reaction conditions were also tolerated with aromatic compounds like 2-bromopyridine. However, the N-arylated product **75c** was obtained in only 54% yield (**Scheme 56**).

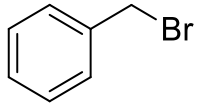
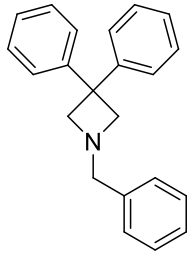
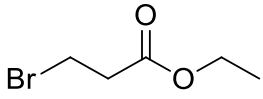
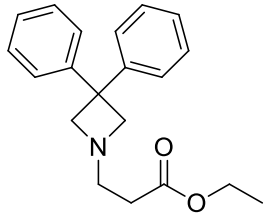
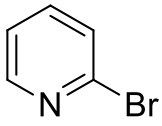
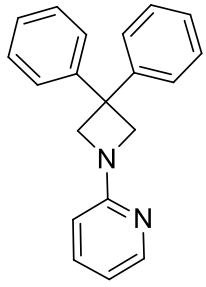


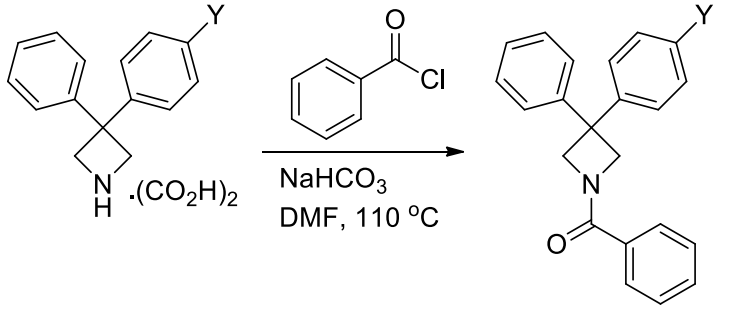
74a **75a-c**

Scheme 56. N-alkylation of **74a**

To further investigate the scope of this reaction, the 3,3-diarylazetidines were acylated at the nitrogen using benzoyl chloride. Both symmetrical and unsymmetrical 3,3-diarylazetidines were acylated and the N-acylated products were obtained in high yields (**Scheme 57**).

Table 12. N-alkylated products of **74a**

Entry	R-Br/ Ar-Br	Product 75	Yield of 75 (%)
a			91
b			94
c			54



74a (Y = H)
74d (Y = Cl)

76a X = H (93%)
76b X = Cl (89%)

Scheme 57. N-acylation of **74a** and **74d**

CONCLUSION

In summary, we have developed a simple method for the preparation of 3,3-diarylazetidines using $AlCl_3$-mediated Friedel-Crafts arylation of *N*-Boc-3-aryl-3-azetidinols. The conditions allowed for incorporation of both electron-rich and electron-deficient aryl groups to furnish the 3,3-diarylazetidines in good yield as stable oxalic acid salts. The salts exhibit exceptional shelf life and can be efficiently converted into N-alkylated, N-arylated and N-acylated derivatives of the 3,3-diarylazetidines. With easy access to the 3,3-diarylazetidines, this moiety will undoubtedly be prominent isostere in future drug discovery programs.

EXPERIMENTAL

General information

All reactions were carried out in oven-dried glassware under a N_2 atmosphere unless otherwise noted. All chemicals were purchased from Alfa Aesar, Sigma Aldrich, VWR and CNH Technologies and were used as received without further purification. Chromatography refers to flash silica gel column chromatography (0.060–0.200mm (60 Å) silica gel from Sorbent Technology was used as the stationary phase). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature in DMSO-d_6 on a Bruker 300 MHz instrument. ^{1}H chemical shifts were referenced to the DMSO solvent signal (2.48 ppm). ^{13}C chemical shifts were referenced to the DMSO solvent signal (40.00 ppm). Atlantic Microlab Inc., Norcross, GA performed all CHN microanalyses.

General procedure A: Preparation of N-Boc-3-aryl-3-azetidinol (71a-c)

Bromobenzene or halogenated bromobenzene (40 mmol) was dissolved in anhydrous THF (144 mL) and cooled to -78 ℃. To this flask, *n*-BuLi (2.5 M, 40 mmol, 32 mL) was added dropwise. The mixture was stirred at -78 ℃ for 10 minutes. *N*-boc-3-azetidinone (**1**, 3.4 g, 20 mmol) was dissolved in anhydrous THF (4 mL) and then slowly added via syringe to the reaction mixture. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with water, extracted with diethyl ether (3 x 100 mL) and the combined organic layers were washed with brine (100 mL). The organic portion was dried over anhydrous sodium sulfate,

filtered and the solvent was removed under vacuum. The crude oil was purified by column chromatography (30% EtOAc:hexanes) to afford the 3-arylazetidinol **63a-c** as a pale-yellow solid.

N-Boc-3-phenyl-3-azetidinol (71a)

According to **General Procedure A**, the reaction was performed with bromobenzene (6.2 g, 40 mmol). The azetidinol **63a** was obtained as a pale-yellow solid (4.8 g, 96% yield). mp 85.3-87.8 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.48 (d, J = 7.7 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.27 (m, 1H), 6.31 (s, 1H), 4.02 (s, 4H), 1.40 (s, 9H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 156.3, 145.1, 128.6, 127.5, 125.0, 79.2, 70.1, 65.1, 28.5.

N-Boc-3-(4-fluorophenyl)-3-azetidinol (71b)

According to **General Procedure A**, the reaction was performed with 4-fluorobromobenzene (7.0 g, 40 mmol). The azetidinol **63b** was obtained as a pale-yellow solid (3.8 g, 72% yield). mp 90.8-92.8 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.53-7.49 (m, 2H), 7.17-7.12 (m, 2H), 6.39 (s, 1H), 4.02 (s, 4H), 1.39 (s, 9H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 163.3, 160.1, 156.3, 141.3, 127.1, 127.0, 115.4, 115.1, 79.2, 69.8, 65.3, 28.4.

N-Boc-3-(4-Chlorophenyl)-3-azetidinol (71c)

According to **General Procedure A**, the reaction was performed with 4-chlorobromobenzene (7.6 g, 40 mmol). The azetidinol **63c** was obtained as a pale-yellow solid (4.8 g, 86% yield). mp 139.0-140.6 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.50 (d, J = 8.7 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 6.43 (s, 1H), 4.00 (s, 4H), 1.40 (s, 9H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 156.3, 144.2, 132.2, 128.6, 127.0, 79.4, 69.7, 65.2, 28.5.

General Procedure B: Preparation of 3,3-diarylazetidine oxalate salts (74a-h)

Aluminum chloride (4.0 g, 30 mmol) was suspended in benzene or halogenated benzene (20 mmol) and cooled to 0 ℃ in an ice bath under an atmosphere of nitrogen. N-Boc-3-aryl-3-azetidinol (**2**, 10 mmol) was dissolved in benzene or substituted benzene and added to the reaction mixture via syringe. The reaction mixture was then stirred at 0 ℃ for 2 h under nitrogen. The reaction was quenched with ice and stirred for 30 minutes. Saturated sodium bicarbonate solution (20 mL) was added to the mixture followed by ammonium hydroxide to achieve a pH of 11. The mixture was the extracted with diethyl ether (3 x 20 mL) and washed with brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent removed under vacuum. The residue was then dissolved in a minimum amount of diethyl ether and an ether solution of oxalic acid (10 mmol) was added dropwise to the residue solution. The oxalate salt precipitated as a white solid which was collected by vacuum filtration.

3,3-Diphenylazetidine hydrogen oxalate (74a)

General procedure B. The product was obtained from **71a** and benzene as a white solid (2.57 g, 86% yield). mp 230.5-232.3 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.30 (s, 1H), 7.43 (d, *J* = 7.5 Hz, 4H), 7.35 (t, *J* = 7.6 Hz, 4H), 7.23 (t, *J* = 7.3 Hz, 2H), 4.62 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 162.8, 146.6, 129.0, 127.0, 126.6, 61.3, 60.4, 48.3.

Anal. Calcd. for $C_{15}H_{15}N•(CO_2H)_2$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.01; H, 5.88; N, 4.55.

3-(4-Methylphenyl)-3-phenyl-azetidine hydrogen oxalate (74b)

General procedure B. The product was obtained from **71a** and toluene as a white solid (2.23 g, 71% yield). mp 185.6-187.6 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.40, (d, J = 7.8 Hz, 2H), 7.63-7.27 (m, 4H), 7.25-7.15 (m, 3H), 4.58, (s, 4H), 2.23 (s, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 165.3, 146.2, 143.1, 136.7, 129.3, 127.4, 126.4, 115.6, 57.3, 48.9, 21.1.

Anal. Calcd. for $C_{16}H_{17}N•(CO_2H)_2$: C, 69.00; H, 6.11; N, 4.47. Found: C, 68.93; H, 6.30; N, 4.40.

3-(4-Fluorophenyl)-3-phenylazetidine hydrogen oxalate (74c)

General procedure B. The product was obtained from **71a** and fluorobenzene as a white solid (1.90 g, 60% yield). mp 164.2-166.4 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.51-7.41 (m, 4H), 7.35 (t, J = 7.5 Hz, 2H), 7.25-7.15 (m, 3H), 4.61 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 164.4, 162.8, 160.4, 145.9, 142.2, 129.4, 128.8, 128.7, 127.5, 126.5, 126.2 116.2, 116.0, 57.3, 48.7, 31.7.

Anal. Calcd. for $C_{15}H_{14}NF•(CO_2H)_2$: C, 64.35; H, 5.09; N, 4.41. Found: C, 64.13; H, 5.15; N, 4.25.

3-(4-Chlorophenyl)-3-phenylazetidine hydrogen oxalate (74d).

General procedure B. The product was obtained from **71a** and chlorobenzene as a white solid (2.86 g, 86% yield). mp 214.6-217.6 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.47 (d, *J* = 8.6 Hz, 2H), 7.43-7.40 (m, 4H), 7.38 – 7.33 (m, 2H), 7.27 – 7.21 (m, 1H), 4.60 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 164.8, 145.2, 144.7, 132.0, 129.2, 129.1, 128.3, 127.4, 126.2, 57.0, 48.6.

Anal. Calcd. for $C_{15}H_{14}NCl•(CO_2H)_2$: C, 61.18; H, 4.48; N, 4.20. Found: C, 60.95; H, 4.78; N, 3.94.

3-(4-Methoxyphenyl)-3-phenylazetidine hydrogen oxalate (74e).

General procedure B. The product was obtained from **71a** and anisole as a white solid (2.36 g, 72% yield). mp 160.0-162.8 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.49 (s, 1H), 7.49-7.32 (m, 6H), 7.23 (m 1H), 6.89 (d, J = 8.6 Hz, 2H), 4.57 (s, 4H), 3.70 (s, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 158.4, 146.1, 137.7, 129.1, 127.6, 127.1, 126.2, 114.2, 57.1, 55.6, 48.6.

Anal. Calcd. for $C_{16}H_{17}NO•(CO_2H)_2•H_2O$: C, 62.24; H, 6.09; N, 4.03. Found: C, 62.03; H, 6.37; N, 3.75.

3-Phenyl-3-(4-fluorophenyl)azetidine hydrogen oxalate (74f)

General procedure B. The product was obtained from **71b** and benzene (1.99 g, 63% yield). mp 164.2-166.4 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.51-7.41 (m, 4H), 7.35 (t, J = 7.5 Hz, 2H), 7.25-7.15 (m, 3H), 4.61 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 164.4, 162.8, 160.4, 145.9, 142.2, 129.4, 128.8, 128.7, 127.5, 126.5, 126.2 116.2, 116.0, 57.3, 48.7, 31.7.

Anal. Calcd. for $C_{15}H_{14}NF•(CO_2H)_2$: C, 64.35; H, 5.09; N, 4.41. Found: C, 64.13; H, 5.15; N, 4.25.

3-Phenyl-3-(4-chlorophenyl)azetidine hydrogen oxalate (74g)

General procedure B. The product was obtained from **71c** and chlorobenzene as a white solid (2.33 g, 70% yield). mp 214.6-217.6 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.47 (d, *J* = 8.6 Hz, 2H), 7.43-7.40 (m, 4H), 7.38 – 7.33 (m, 2H), 7.27 – 7.21 (m, 1H), 4.60 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 164.8, 145.2, 144.7, 132.0, 129.2, 129.1, 128.3, 127.4, 126.2, 57.0, 48.6.

Anal. Calcd. for $C_{15}H_{14}NCl•(CO_2H)_2$: C, 61.18; H, 4.48; N, 4.20. Found: C, 60.95; H, 4.78; N, 3.94.

3,3-Di(4-chlorophenyl)azetidine hydrogen oxalate (74h)

General procedure B. The product was obtained from **71c** and chlorobenzene as a white solid (2.87 g, 81% yield). mp 219.6-221.1 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.63 (s, 1H), 7.47 (d, J = 8.1 Hz, 4H), 7.41 (d, J = 7.9 Hz, 4H), 4.54 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 144.5, 135.5, 132.32, 129.4, 128.6, 56.9, 48.3.

Anal. Calcd. for $C_{16}H_{16}NCl•(CO_2H)_2$: C, 55.45; H, 4.11; N, 3.80. Found: C, 55.40; H, 4.12; N, 3.71.

General procedure C: Preparation of N-substituted 3,3-diarylazetidines

3,3-Diarylazetidine oxalate salt (1 equiv.) was dissolved in anhydrous DMF (20 mL) followed by the addition of the alkyl, aryl or acyl bromide (3 equiv) and $NaHCO_3$ (4 equiv). The mixture was heated at 110 ℃ for 16 h. The reaction was poured into water (100 mL) and extracted with diethyl ether (3 x 20 mL). The combined organic layers were washed with water (5 x 10 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under vacuum. The residue was then purified.

N-Benzyl-3,3-diphenylazetidine (75a)

General procedure C. The product was prepared using **74a** (0.500 g, 1.67 mmol), benzyl bromide (0.600 mL, 5.01 mmol) and $NaHCO_3$ (0.561 g, 6.68 mmol) and was purified by column chromatography (50% EtOAc:hexanes). The product was obtained as a pale-yellow oil. (0.450 g, 90% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.38-7.20 (m, 15 H), 5.47 (s, 2H), 3.48 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 139.6, 128.8, 128.7, 128.3, 128.3, 127.4, 115.1, 57.4.

Anal. Calcd for $C_{22}H_{21}N$: C, 88.25; H, 7.07; N, 4.68. Found: C, 88.19; H, 7.00; N, 4.53.

Ethyl-3-(3,3-diphenylazetidinyl)-propionate hydrogen oxalate (75b)

General procedure C. The product was prepared using **74a** (0.500 g, 1.67 mmol), ethyl 3-bromopropionate (0.630 mL, 5.01 mmol) and $NaHCO_3$ (0.561 g, 6.68 mmol). The residue was dissolved in a minimal amount of diethyl ether. Oxalic acid dissolved in a minimal amount of

diethyl ether was then added to it. The oxalate salt precipitated out as an off-white solid and was collected by vacuum filtration. (0.614 g, 92% yield). mp 185.0 ℃ (decomp.)

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.39 (d, *J* = 7.4 Hz, 4H), 7.32 (t, J = 6.6 Hz, 4H), 7.20 (t, *J* = 6.6 Hz, 2H), 4.46 (s, 4H), 4.06 (q, J = 6.6 Hz, 2H), 3.17 (t, J = 7.4 Hz, 3H), 2.52(t, J = 7.2 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 170.6, 163.0, 145.7, 128.6, 126.6, 126.0, 64.8, 60.4, 51.7, 46.6, 30.5, 14.1.

Anal. Calcd for $C_{20}H_{23}NO_2 \bullet C_2H_2O_4 \bullet H_2O$: C, 63.30; H, 6.52; N, 3.36. Found: C, 63.10; H, 6.50; N, 3.21.

N-(2-Pyridinyl)-3,3-diphenylazetidine (75c)

General procedure C. The product was prepared using **74a** (0.200 g, 0.668 mmol), 2-bromopyridine (0.200 mL, 2.00 mmol) and $NaHCO_3$ (0.300 g, 2.67 mmol). The product was isolated and purified using column chromatography (50% Ethyl acetate: Hexanes) to obtain a colorless oil (0.103 g, 54% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.07 (d, J = 6Hz, 1H), 7.52 (t , J = 6 Hz, 1H), 7.39-7.29 (m, 8H), 7.19 (t, J = 6 Hz, 2H), 6.65 (t, J = 6 Hz, 1H), 6.47 (d, J = 6 Hz, 1H), 4.45 (s, 4H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 160.6, 147.7, 146.9, 137.1, 128.5, 126.2, 113.3, 106.2, 63.7, 46.8.

N-Benzoyl-3,3-diphenylazetidine (76a)

General procedure C. The product was prepared using **74a** (0.500 g, 1.67 mmol), benzoyl chloride (0.600 mL, 5.01 mmol) and $NaHCO_3$ (0.561 g, 6.68 mmol). The product was isolated and purified using column chromatography (50% Ethyl acetate: Hexanes) to obtain a white solid. (0.502g, 96% yield). mp 179.4-181.0 ℃.

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.66 (d, J = 7.3 Hz, 2H), 7.56 – 7.43 (m, 3H), 7.39 (d, J = 8.1 Hz, 4H), 7.32 (t, J = 7.5 Hz, 4H), 7.20 (t, J = 6.7 Hz, 2H), 4.93 (s, 2H), 4.71 (s, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 169.2, 146.5, 131.2, 128.6, 128.5, 127.9, 126.5, 126.3, 65.9, 61.67, 46.7.

Anal. Calcd for $C_{22}H_{19}NO$: C, 84.31; H, 6.11; N, 4.47. Found: C, 84.08; H, 5.96; N, 4.40.

N-Benzoyl-3-(4-chlorophenyl)-3-phenylazetidine (76b)

General procedure C. The product was prepared using **74d** (0.360 g, 1.08 mmol), benzoyl chloride (0.376 mL, 3.24 mmol) and $NaHCO_3$ (0.363 g, 4.32 mmol). The product was isolated and purified using column chromatography (50% Ethyl acetate: Hexanes) to obtain a colorless oil (0.323 g, 86% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.69 – 7.61 (m, 2H), 7.50 (d, *J* = 6.9 Hz, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.37 (d, *J* = 14.7 Hz, 7H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.21 (q, *J* = 7.1, 5.9 Hz, 1H), 4.90 (s, 2H), 4.68 (s, 2H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 169.5, 146.4, 145.8, 133.2, 131.6, 129.1, 128.9, 128.7, 128.3, 127.1, 126.7, 66.1, 61.9, 46.7.

Anal. Calcd for $C_{22}H_{18}ClNO$: C, 75.97; H, 5.22; N, 4.03. Found: C, 75.81; H, 5.01; N, 3.95

CHAPTER 3

General Synthesis of Alkyl Pyruvate Esters

ABSTRACT

A general synthesis of pyruvate esters was developed. Using this method, a series of alkyl pyruvate esters were synthesized in moderate to high yields (56-93%). This synthesis can be used to synthesize both mono-pyruvate and di-pyruvate esters. This was achieved in four steps starting from the readily available triethyl-2-phosphonopropionate which was converted to the alkene ethyl 2,3-dimethyl-2-butenoate using Horner-Wadsworth-Emmons reaction. The ethyl ester was hydrolyzed to the corresponding carboxylic acid after which a series of esters were prepared using Steglich esterification. The esters were then converted to their corresponding pyruvate esters via ozonolysis.

INTRODUCTION

3.1 Keto acids

Keto acids contain two oxygen-containing functional groups: the carboxylic acid (COOH) and ketone group (C=O) [114]. There are three classes of keto acids: alpha-keto acid, beta-keto acid and gamma-keto acid (**Fig. 40**). Out of these three, particularly important in biology are the *α*-keto acids (keto group adjacent to the carboxylic acid), since they are involved in the Krebs cycle and in glycolysis [115].

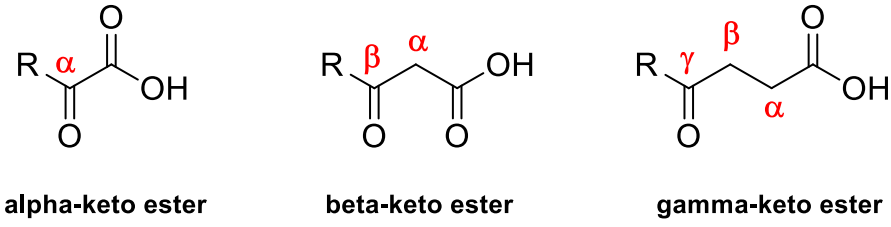


Fig. **40.** Alpha, beta and gamma keto esters

α-Keto acids, especially the *α*-keto acid analogues of the naturally occurring amino acids, are of major importance in intermediary metabolism. Thus, pyruvic acid is a metabolite involved in a number of enzyme-catalyzed intracellular phenomena, and oxaloacetic acid (**77**), *α*-ketoglutaric acid (**78**), and oxalosuccinic acid (**79**) are intermediates in the tricarboxylic acid cycle (Krebs's cycle) (**Fig. 40**) [116] [117] [118].

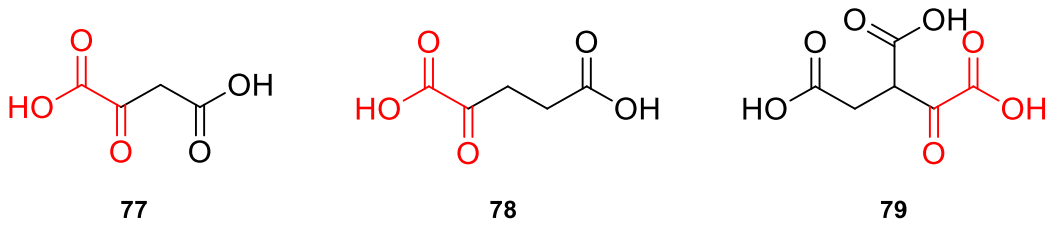


Fig. 41. Structures of α-keto acids

3.1.1 Properties of keto acids

The straight-chain α-keto acids are either liquids or low-melting solids. The branched-chain and phenyl-substituted α-keto acids vary from liquids to high-melting solids [119] [120]. Some a-keto acids are unstable as the free acid and exhibit a tendency to decarboxylate and polymerize. The majority are relatively stable as salts, of which the sodium and barium salts have most often been prepared. In general, α-keto acids are relatively stable in neutral solution and can be stored frozen at -20 ℃ with little decomposition, but there are notable exceptions. It is well-known that pyruvic acid **(80)** readily polymerizes on storage in aqueous solution to DL-γ-hydroxy-γ-methyl-α-ketoglutaric acid (**81)** and higher molecular weight compounds [121].

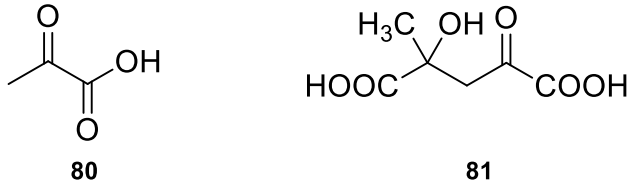
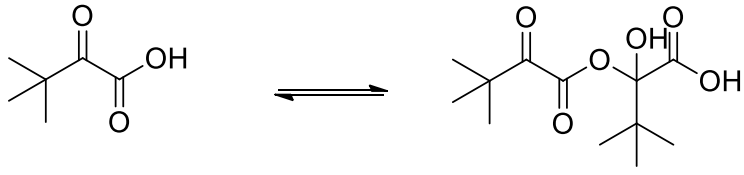


Fig. 42. Polymerization of Pyruvic acid

Schellenberger and coworkers noted that the molecular weight of pure trimethyl pyruvic acid **(82)** when measured cryoscopically in dioxane, up to 0.01 mol fraction was 10% greater than expected [122]. Since association by hydrogen bonding is unlikely, it was concluded that some dimerization occurred (**Scheme 58**). Subsequently, crystalline dimer was obtained from cold samples of trimethyl pyruvic acid. Glucksmann first isolated the dimer in 1989 [123]. It was concluded from dipole measurements and from infrared spectroscopic data that the dimer was an "activated ester" (**83**).

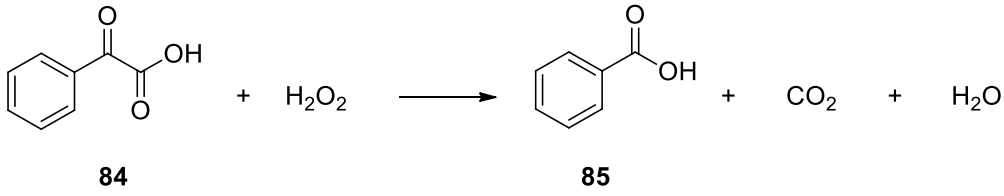


82 **83**

Scheme 58. Dimerization of trimethyl pyruvic acid

It is well-known that many α-keto acids are metabolized via enzyme-catalyzed oxidative decarboxylation (i.e. via pyruvate, α-ketoglutarate, and branched-chain α-keto acid dehydrogenases) [124]. It is also well-known that α-keto acids are rapidly and quantitatively decarboxylated by such mild oxidizing reagents as ceric sulfate [125], hydrogen peroxide [126], peroxyphthalic acid [127], potassium permanganate [128], and lead tetraacetate [129]. In 1979, Seigel and coworkers reported decarboxylation of α-keto acids by hydrogen peroxide (H_2O_2) is base catalyzed and is accelerated in the presence of Fe^{2+} (but not other cations) [130]. In aqueous solutions of H_2O_2, **84** undergoes relatively slow decarboxylation to form **85** in

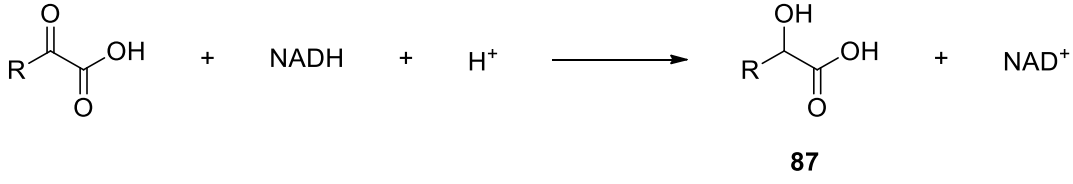
quantitative yields. The addition of catalytic amounts of ferrous (Fe^{2+}) salts to the reaction mixture cause a dramatic rate enhancement, without reducing the yield of **85** (**Scheme 59**).



84 **85**

Scheme 59. Oxidative decarboxylation of α-keto acid **84**

In 1947, Waters reported that α-keto acids can be reduced by hydrogen to the corresponding α-hydroxy acid [119]. It has been known for more than 30 years that lactate dehydrogenase is nonspecific and that it catalyzes the reduction of a large number of α-keto acids to the corresponding α-hydroxy acids **(87)**, some of these α-keto acids are reduced at rates similar to that found with pyruvate (**Scheme 60**) [131].

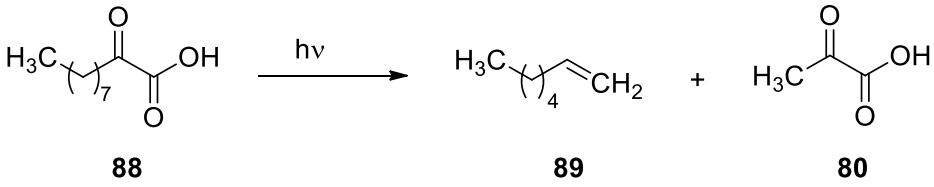


87

Scheme 60. Reduction of α-keto acids

In addition to decarboxylation and reduction, α-keto acids are also susceptible to free-radical induced fragmentation. In 1968, Evans and Leermakers reported photolysis reactions of α-

ketodecanoic acid [132]. α-ketodecanoic acid (**88**) on photolysis, was found to split into 1-heptene (**89**) and pyruvic acid (**80**) (**Scheme 61**).



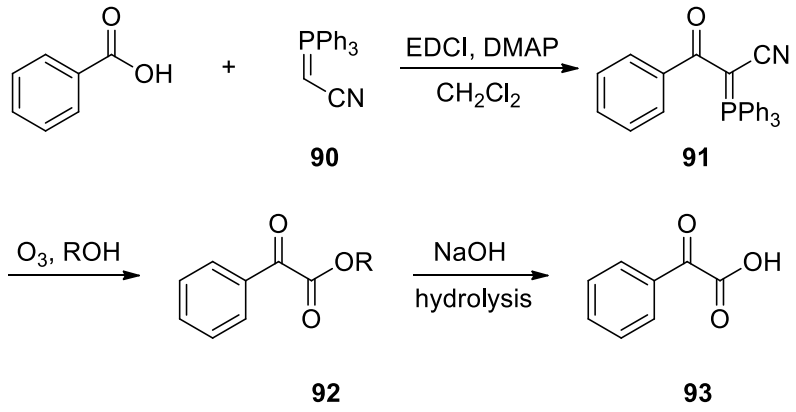
88 **89** **80**

Scheme 61. Photolysis of α-keto acid **88**

3.1.2 Synthesis of α-keto acids

The first α-keto acid to be prepared was pyruvic acid (**80**). This compound was prepared by Berzelius in 1835 [133]. Many of the more biologically important a-keto acids were prepared over 75 years ago**,** e.g., oxaloacetic (oxalacetic) (**77**) and a-ketoglutaric (**78**). Waters lists the methods of synthesis of 36 α-keto acids**.** Many of the α-keto acids have been prepared by unique methods [119]. Some of the recent developments for the synthesis of α-keto acids have been described below.

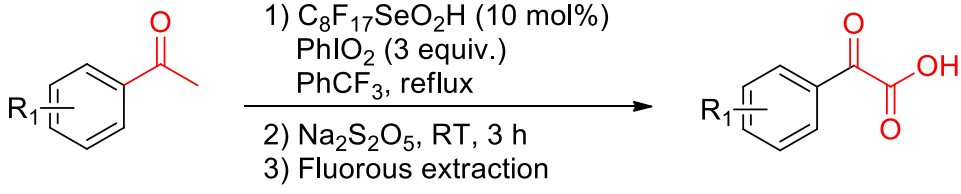
In 1994, Wasserman and Ho reported the synthesis of α-keto acids using (cyanomethylene)phosphoranes [134]. In this method, (cyanomethylene)phosphoranes (**90**) was coupled with carboxylic acids or acid chlorides to give cyano keto phosphoranes (**91**). **91** then undergoes oxidation and subsequent nucleophilic attack to give an α-keto ester (**92**) which can be hydrolyzed to the acid (**93**) (**Scheme 62**).



Scheme 62. Synthesis of α-keto acid using (cyanomethylene)phosphoranes

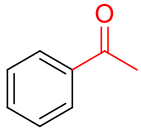
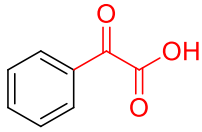
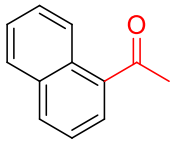
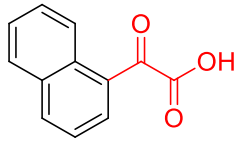
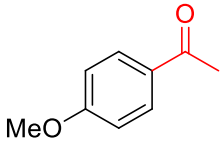
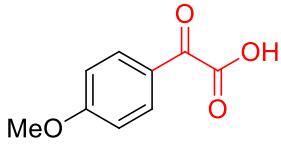
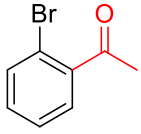
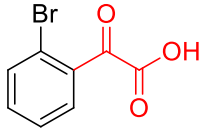
This method however, produced stoichiometric amount of waste from phosphorus reagents. Therefore, a more atom-economical approach was necessary.

Crich and coworkers developed a selenium-catalyzed oxidation of ketones to prepare aryl-substituted glyoxylic acids using iodoxybenzene ($PhIO_2$) as the oxidizing agent and fluorous selenic acid (10 mol %) as the catalyst [135]. By using a biphasic fluorous system, the fluorous phase containing the catalyst could be recovered and reused in new reactions. The method was successfully applied in the synthesis of five different aryl-substituted glyoxylic acids in 89−92% yield after 3 h at room temperature (**Scheme 63**).

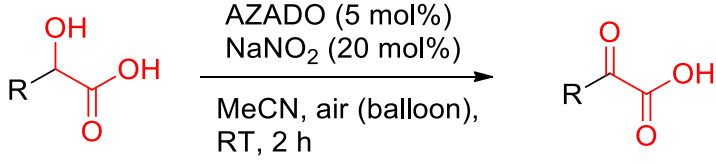


Scheme 63. Synthesis of α-keto acids fluorous selenic acid as catalyst

Table 13. Some selected α-keto acids synthesized by Crich and coworkers [135]

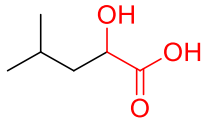
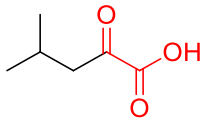
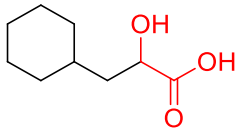
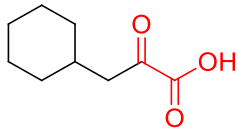
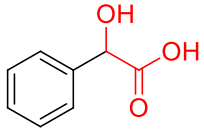
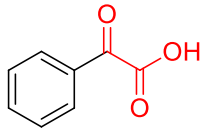
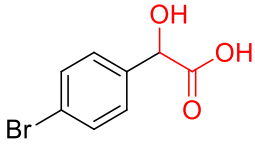
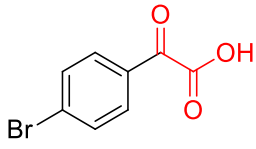
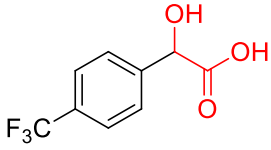
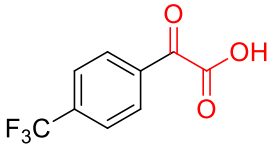
Entry	Ketone	α-keto acid	Yield (%)
1			89
2			92
3			90
4			92

In 2016, Shibuya *et al.* reported the synthesis of α-keto esters from α-hydroxy acids using 2-azaadamantane N-oxyl (AZADO), a nitroxyl radical catalyst [136]. This was a promising approach and highly atom-economical which does not require deprotection and hydrolysis (**Scheme 64**). A broad range of α-hydroxy acids were efficiently oxidized to provide the corresponding α-keto acids in high yield (**Table 14**).



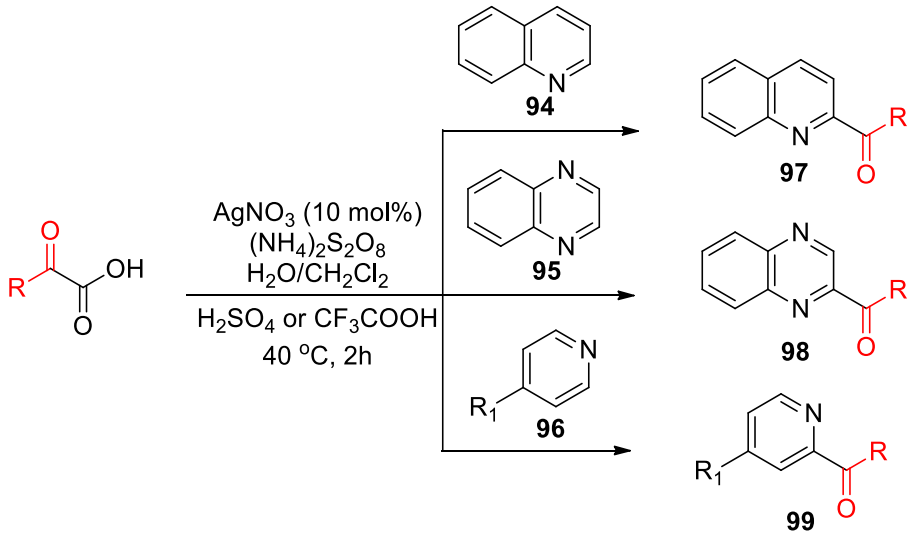
Scheme 64. Synthesis of α-keto acids using AZADO as a catalyst

Table 14. Some α-keto acids synthesized by Furukawa *et al.* [136]

Entry	α-hydroxy acid	α-keto acid	Yield (%)
1			89
2			86
3			95
4			98
5			99

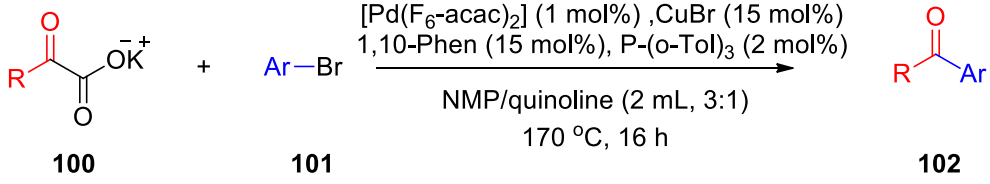
3.1.3 α-keto acids as acylating agents

In 1991, Fontana and coworkers [137] reported for the first time the use of α-keto acids as acylating agents in the selective acylation of heteroaromatic bases **94**–**96**. A silver-catalyzed oxidative decarboxylation of α-keto acids by $(NH_4)_2S_2O_8$ in a biphasic system (H_2O/CH_2Cl_2) or in water was used to prepare mono and diacyl derivatives **97**–**99** (**Scheme 65**).



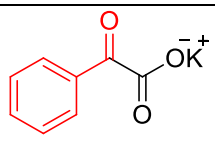
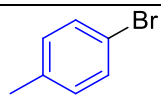
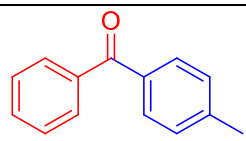
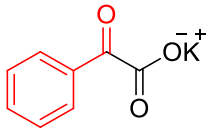
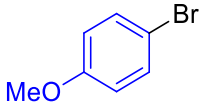
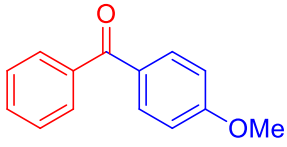
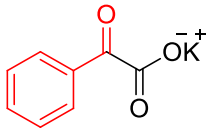
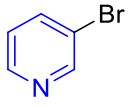
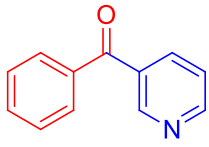
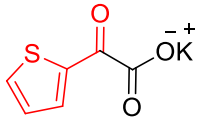
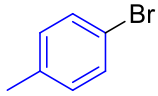
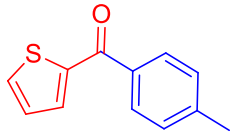
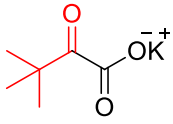
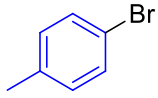
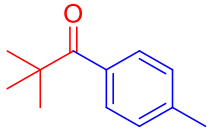
Scheme 65. Silver catalyzed acylation of heteroaromatic bases using α-keto acids

In 2008, Gooβen and coworkers reported synthesis of unsymmetrical ketones using α-keto acids by direct acylation of aromatic rings [138]. Twenty-six differently substituted unsymmetrical ketones **102** were prepared in 5−99% yields after 16−36 h of reaction at 170 °C, by the cross-coupling reaction of α-keto acid potassium salts **100** and aryl bromides **101**. The reaction was catalyzed by Cu/Pd, in the presence of P-(*o*-Tol)$_3$ and 1,10-phenanthroline as ligands and using a mixture of NMP/quinoline (3:1) as the solvent (**Scheme 66**).

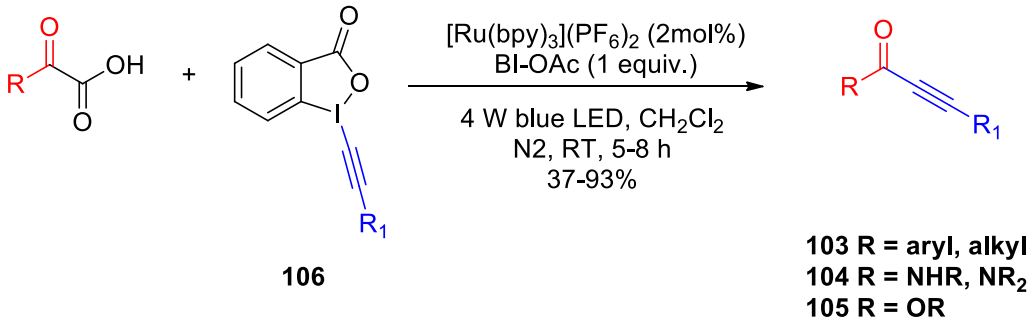


Scheme 66. Pd/Cu-catalyzed decarboxylative cross-coupling

Table 15. Selected examples of direct acylation using α-keto acids [138]

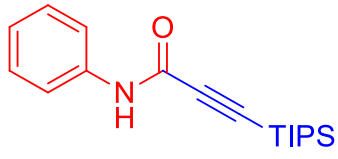
Entry	100	101	102	Yield of 102 (%)
a				83
b				82
c				57
d				45
e				90

Chen and coworkers [139] have developed an interesting way to prepare ynones **103**, ynamides **104**, and ynoates **105**, respectively, by the reaction of alkynes with α-keto acids, carbamoyl keto acids, and alkoxycarbonyl keto acids. The authors have combined the use of photoredox catalysis with hypervalent iodine(III) reagents, i.e., Benziodoxole acetate (BI-OAc) and alkynyl benziodoxole (BI-alkyne, **106**), under 4 W blue LED irradiation (**Scheme 67**).



Scheme 67. Hypervalent Iodine(III) and Ru(II)-Photocatalyzation of BI-alkyne

The usefulness of this method was demonstrated in the synthesis of ynamide **104a** (**Fig. 43**), which is an effective inhibitor for the metabotropic glutamate receptor 5 (mGlu5 receptor) with a therapeutic potential for both peripheral and CNS disorders [140].



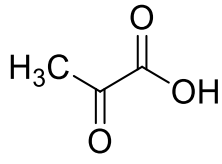
104a

(TIPS = triisopropylsilyl)

Fig. 43 Inhibitor for mGlu5 receptor

3.2 Pyruvic acid

Pyruvic acid also known as 2-oxopropanoic acid (**80**) is the simplest alpha-keto acid (**Fig. 44**). It plays a central role in energy metabolism in living organisms. It is widely used in drug, agrochemical, chemical and food industries. Some applications of pyruvic acid include significantly increasing fat and weight loss [141], improving exercise endurance capacity [142], effectively reducing cholesterol [143], serving as a potent antioxidant [144], and reducing anoxic injury and free radical generation [145]. Pyruvate, the conjugate base, CH_3COCOO^-, is a key intermediate in several metabolic pathways throughout the cell.



80

Fig. 44. Pyruvic acid

3.2.1 Chemical syntheses of pyruvic acid

In 1932, Howland and Fraser developed a method for the synthesis of pyruvic acid on an industrial scale [146]. In this process, pyruvic acid is distilled from a mixture of tartaric acid and potassium hydrogen sulfates at 220°C. The crude acid obtained is then distilled under vacuum. This process is simple but not cost-effective. The total cost is estimated to be about US $8,000–9,000/ton.

3.2.2 Biotechnological production of pyruvic acid

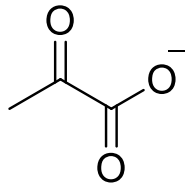
Compared to the chemical method, production of pyruvic acid by using biotechnological methods is an alternative approach to reduce the production cost. There are three methods for biotechnological production of pyruvate: the direct fermentation method, the resting cell method, and the enzymatic method. Of these, direct fermentative production of pyruvate from a carbon source (such as glucose) has merits in terms of both cost-effectiveness and the high purity of the product. However, as pyruvate is located at a vital junction of cell metabolism, it is usually difficult to obtain strains that can accumulate large amounts of pyruvate extracellularly.

In 1989, researchers in Toray industries, Japan, identifiede some yeast strains belonging to the genus *Tirulopsis*, that produced more than 50 g/l pyruvate [147]. This result indicated that the fermentative production of pyruvate can be commercialized. Pyruvate production using the fermentative method, with a scale of about 400 tons per year, was industrialized by Toray Industries in 1992 and the fermentation has now been scaled-up to 50 m^3 fermentors [148].

3.3 Pyruvate esters

Pyruvate is a three-carbon (triose) ketoacid (**107, Fig. 45**) that is produced in biological systems in the end stages of glycolysis, a product of sugar metabolism. It is also a breakdown product of certain amino acids (alanine, glycine, cysteine, Serine). Pyruvate can be reduced to lactate in the cytoplasm, a fermentative event in mammalian cells, or oxidatively decarboxylated to acetyl CoA in the mitochondrion [149]. It has been suggested that pyruvate and certain pyruvate derivatives may have utility in treating certain disorders and promoting health. For example,

pyruvate is sold as a dietary supplement for use in promoting weight loss and enhancing energy. It has also been suggested as a therapeutic intervention for clinical management of myocardial insufficiency [150] and to prevent the adverse effects of myocardial ischemia [151].



107

Fig. 45 General formula for pyruvate

U.S. Pat. No. 6,086,789 describes certain pyruvate derivatives as useful for dermatologic indications as well as for treating diabetic ketosis, myocardial ischemia, injured organs and hypercholesterolemia. Specifically, it ascribes these activities to various esters of pyruvate, including polyol-pyruvate esters, pyruvate thioesters, glycerol pyruvate esters, and dihydroxyacetone-pyruvate ester **(Fig. 46**). Related U.S. Pat. No. 5,968,727 describes the use of pyruvate thioesters, such as cysteine, methionine and homocysteine, and glycerol pyruvate esters and dihydroxyacetone-pyruvate esters, in organ preservation solutions and for treating ischemia. Similarly, certain pyruvate and pyruvyl amino acid conjugates have been suggested for use in diabetes [152] [153].

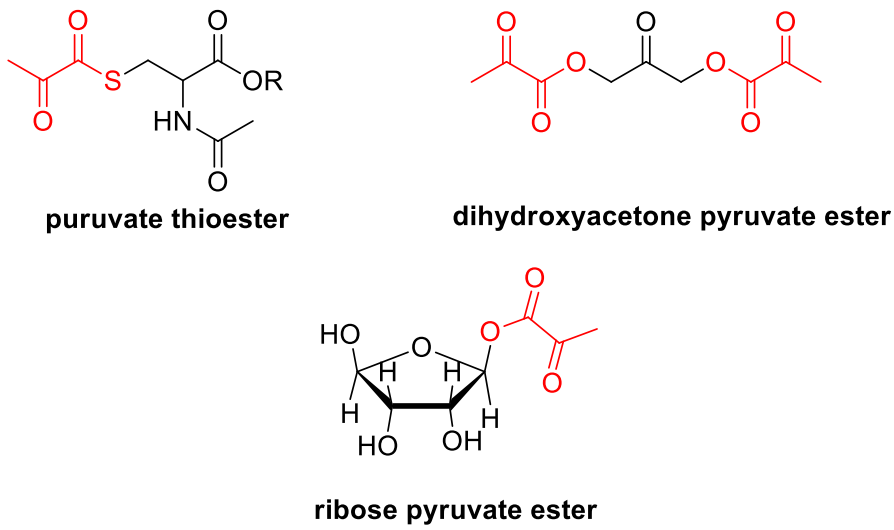
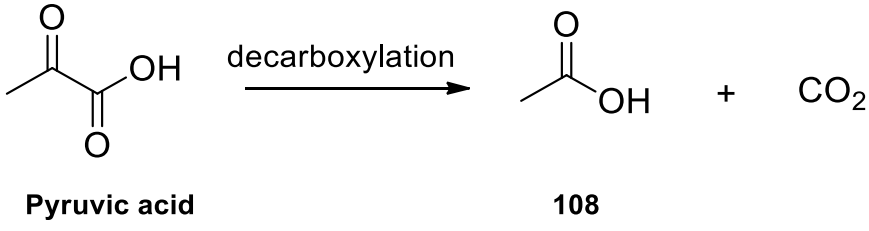


Fig. 46. Some biologically active pyruvate esters

U.S. Pat. No. 5,283,260, describes the treatment of diabetes with a physiologically acceptable form of pyruvate. The patent discloses a pyruvate compound in the form of a covalently linked pyruvyl-amino acid. However, administration of large amounts of pyruvate-amino acid may result in nitrogen overload which could harm patients with liver and/or kidney pathology. Notwithstanding the acceptance of pyruvate as an effective component of a reperfusion solution or other varied applications, pyruvic acid is a strong and unstable acid which cannot be infused as such. Furthermore, it has been recognized that traditional pharmacological pyruvate compounds, such as salts of pyruvic acid, are not particularly physiologically suitable. For example, these com pounds lead to the accumulation of large concentrations of ions (calcium or sodium) in the patient's body fluids. Similarly, amino acid compounds containing pyruvate can lead to excessive nitrogen loads [154].

3.3.1 Preparation of ethyl pyruvate: Recent developments

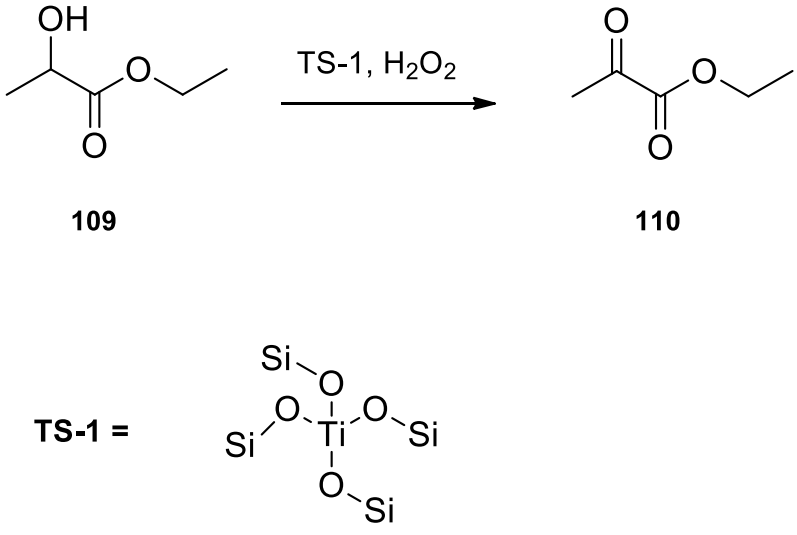
Pyruvate (**107**), is an effective scavenger of reactive oxygen species (ROS) and has been shown to be salutary in numerous models of redox-mediated tissue or organ injury. However, it is unstable in solution and, hence, not attractive for development as a therapeutic agent. Pyruvate esters like ethyl pyruvate are thought to be more stable and has been reported to mitigate the damage caused by various stressors, such as, hemorrhagic shock, stroke, sepsis, and acute pancreatitis [155] [156] [157] [158]. Preparation of pyruvate esters directly from pyruvic acid has proven to be difficult. Pyruvic acid easily undergoes decarboxylation to give acetic acid (**108**) and carbon dioxide (**Scheme 68**). Therefore, an alternate approach must be taken for preparation of these esters.



Scheme 68. Decarboxylation of pyruvic acid

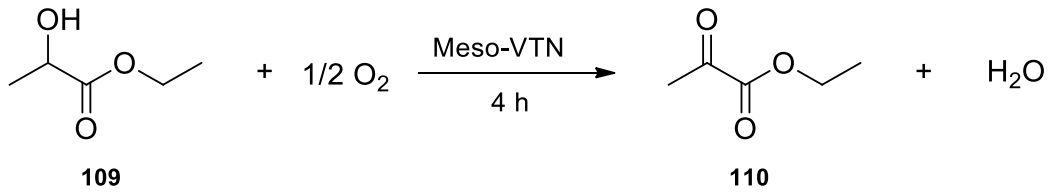
Currently, the commercial production of pyruvate esters is realized by the dehydrative decarboxylation of tartaric acid. Excess $KHSO_4$ as a dehydrating agent is required in this process, leading to low atom efficiency and environmental pollution [159]. Thus, the development of a green, efficient, alternative route for the production of pyruvate is necessary. In 2018, Zhou *et al.* reported a highly efficient oxidation of ethyl lactate (109) to ethyl pyruvate (110) in the presence of TS-1 catalyst (a Titanium-substituted zeolite) and aqueous 30% hydrogen peroxide (H_2O_2)

[160]. The reaction was carried out under mild conditions without any solvent (**Scheme 69**). At 50 ℃, **109** can be completely oxidized to **110** with ≥95% yield. However, the prolongation of reaction time and higher temperature led to the hydrolysis and eventual decarboxylation of **110**.



Scheme 69. Oxidation of ethyl lactate to ethyl pyruvate

In the same year, Shiju *et al.* reported the direct oxidative dehydrogenation of lactates with molecular oxygen is a "greener" alternative for producing pyruvate esters [161]. The authors reported a one-pot synthesis of mesoporous vanadia−titania (Meso-VTN), acting as highly efficient and recyclable catalysts for the conversion of ethyl lactate to ethyl pyruvate (**Scheme 70**). Mesoporous vanadia−titania nanocrystals (meso-VTN) were synthesized via the coassembly of vanadium and titanium precursors in the presence of an amphiphilic triblock copolymer as a templating agent. These new materials feature a large surface area and a high density of isolated vanadium species.



Scheme 70. Oxidative dehydrogenation of ethyl lactate to ethyl pyruvate

3.3.2 Biological applications of ethyl pyruvate

Ethyl pyruvate was originally regarded as simply a way to administer pyruvate anion, whilst avoiding some of the problems associated with the instability of pyruvate in aqueous solutions. Increasingly, however, it is becoming apparent that certain pyruvate esters, including ethyl pyruvate, have pharmacological effects, such as suppression of inflammation, that are quite distinct from those exerted by pyruvate anion.

Sims *et al.* found that treatment with ethyl pyruvate restores much of the structural and functional damage to the intestinal mucosa that normally occurs when rats are subjected to mesenteric ischemia/reperfusion [162]. Treatment with ethyl pyruvate seemed to be more effective than treatment with an equimolar dose of sodium pyruvate. Similar findings indicating that ethyl pyruvate is more effective than pyruvate, were reported by Varma *et al.*., who compared the two compounds *an in vitro* study of redox-mediated cellular injury [163].

Sepsis, a lethal syndrome that develops in response to infection, occurs in 750,000 patients per year in the United States and is fatal in 20–40% of cases [164] [165]. It is mediated by an early (e.g., tumor necrosis factor) and late [e.g., high mobility group B-1 (HMGB1)] proinflammatory cytokine response to infection. Fink and coworkers identified ethyl pyruvate, a stable lipophilic,

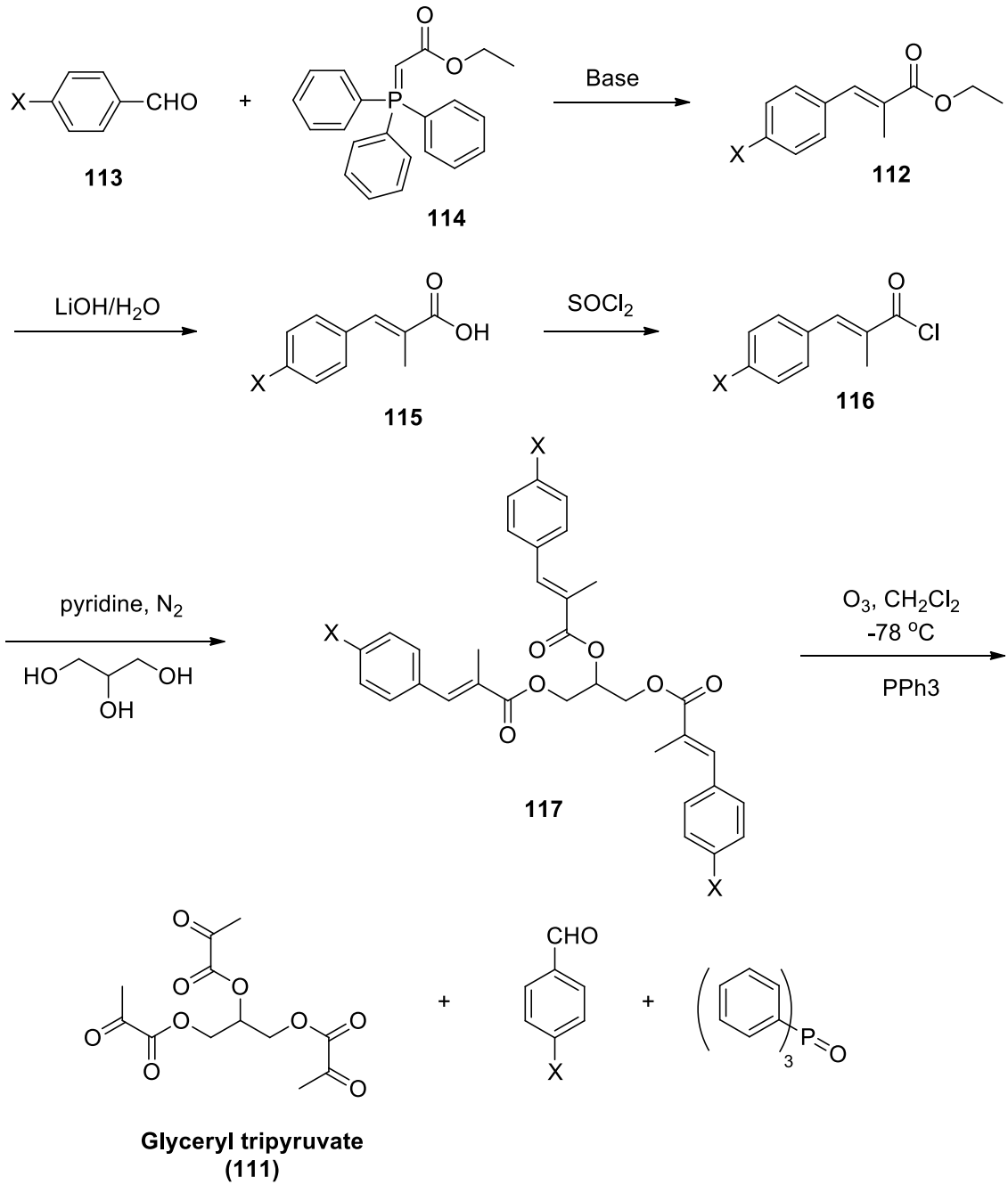
as an experimental therapeutic that effectively protects animals from ischemia/reperfusion-induced tissue injury [166] [162]. Ethyl pyruvate administration significantly improved survival in standard models of lethal hemorrhagic shock [167]. The authors reported that ethyl pyruvate rescued animals from lethal sepsis caused by peritonitis, even when dosing began 24 h after cecal puncture. It inhibited the release of tumor necrosis factor and HMGB1 from endotoxin stimulated RAW 264.7 murine cell lines and attenuated activation of both the p38 mitogen-activated protein kinase (MAPK) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathways. Ethyl pyruvate treatment of septic mice decreased circulating levels of HMGB1, indicating that delayed administration of ethyl pyruvate protects against lethal sepsis.

In 2010, Muller and coworkers reported that ethyl pyruvate elicits a potent immune-based antitumor response through inhibition of indoleamine 2,3-dioxygenase (IDO), a key tolerogenic enzyme for many human tumors [168]. Consistent with its reported ability to interfere with NF-κB function, ethyl pyruvate blocks IDO induction both *in vitro* and *in vivo*. The findings that IDO is effectively blocked by ethyl pyruvate treatment deepens emerging links between IDO and inflammatory processes. Furthermore, these findings rationalize oncologic applications for this agent by providing a compelling basis to reposition ethyl pyruvate as a low-cost immunochemotherapy for clinical evaluation in cancer patients.

3.4 Project rationale and design strategy

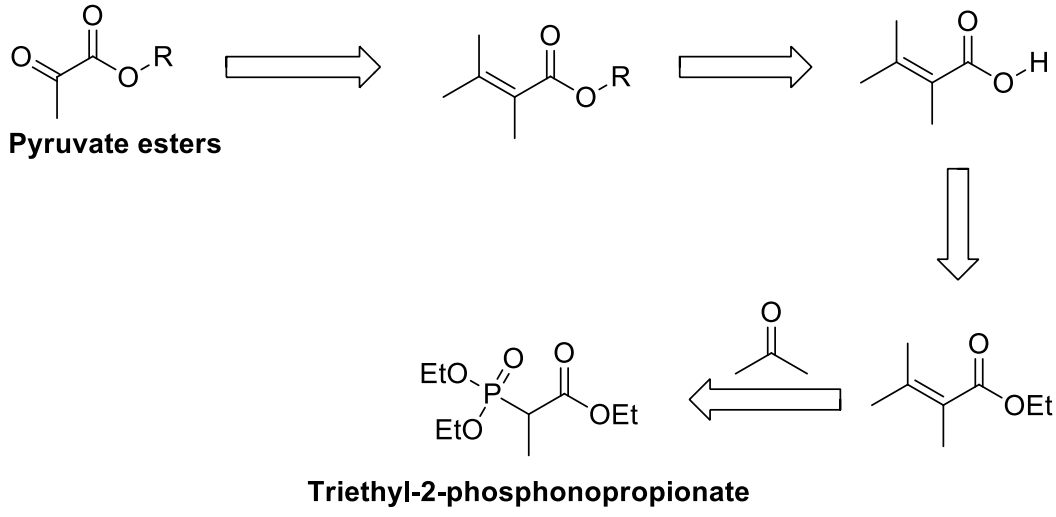
As previously described, pyruvate esters have been increasingly of interest to the pharmaceutical industry. It can be used to treat various medical conditions and has proved to more effective than pyruvic acid itself. Simple esters like methyl pyruvate and ethyl pyruvate have been synthesized and studied in the past. However, the more complex esters of pyruvic acid have been difficult to develop. Therefore, it was of interest to develop a general synthesis of a series of pyruvate esters.

Our group had previously developed a synthesis for glyceryl tripyruvate (**111**). The first step involved the synthesis of ethyl-4-substituted-α-methylcinnamate (**112**) using Horner-Wadsworth-Emmons reaction [169] starting from 4-substituted-benzaldehyde (**113**) and (carbethoxyethylidene)triphenylphosphorane (**114**). The ethyl cinnamate **112** was then converted to the corresponding carboxylic acid (**115**) which was subsequently converted to 4-substituted-α-methylcinnamoyl chloride (**116**) in quantitative yield using thionyl chloride. **116** was then converted to glyceryl tris(4-substituted-α-methylcinnamate) (**117**) in 61% yield by reacting **116** with glycerol in pyridine. The triester **117** was converted to the tripyruvate (**118**) using ozonolysis chemistry in 89% yield. The tripyruvate **118** was isolated from the byproducts by liquid/liquid extraction. The tripyruvate (**118**) water solution was then freeze-dried to yield **118** in pure form as clear oil. The synthetic scheme is illustrated in **Scheme 71**.



Scheme 71. Synthesis of glyceryl tripyruvate

Using similar synthetic procedures, a synthetic route for pyruvate esters was developed. The retrosynthetic route is illustrated in **Scheme 72**. Using Horner-Wadsworth-Emmons reaction, synthesis of ethyl 2,3-dimethylbut-2-enoate could be achieved from ethyl-2-(diethoxyphospryl)propanoate and acetone. The ethyl ester can be hydrolyzed to the corresponding acid which can be converted to a series of esters using a coupling reagent like N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDCI). The esters can then undergo ozonolysis to give the corresponding pyruvates.

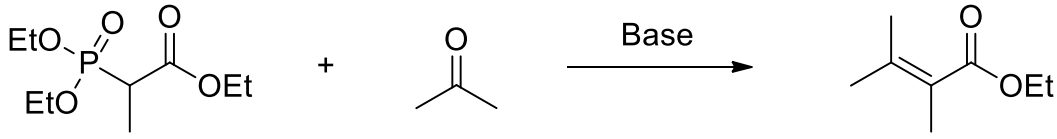


Scheme 72. Retrosynthetic route for a series of pyruvate esters

RESULTS AND DISCUSSION

3.5 Synthesis of ethyl 2,3-dimethyl-2-butenoate

Ethyl 2,3-dimethyl-2-butenoate was prepared using Horner-Wadsworth-Emmons reaction. This method is used to synthesize olefins from phosphorus ylides (phosphonates) and aldehydes/ketones [170]. Ethyl 2,3-dimethyl-2-butenoate (**118**) was synthesized from triethyl-2-phosphonopropionate (**119**) and acetone in the presence of a base. A general reaction scheme is illustrated in **Scheme 73**.

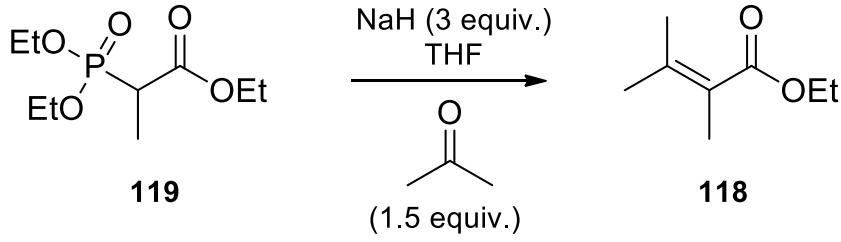


119 **118**

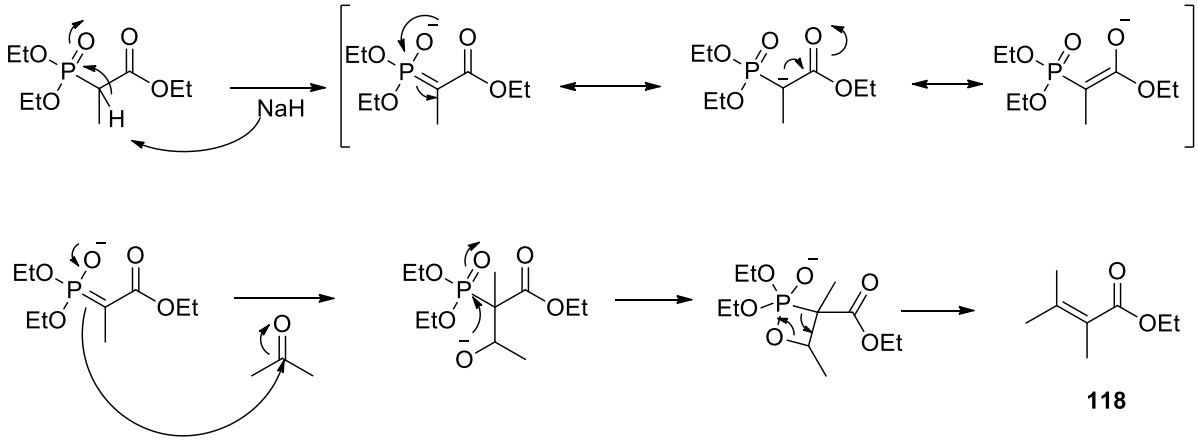
Scheme 73. General scheme for synthesis of ethyl 2,3-dimethyl-2-butenoate

Bases like sodium hydride (NaH) and sodium ethoxide (NaOEt) were found to be suitable bases for this reaction. First, sodium metal was used to prepare NaOEt *in situ* with ethanol as the solvent. The reaction was run at room temperature under an inert atmosphere to give **118** as a colorless oil. The reaction conditions were optimized by varying the number of equivalents of sodium metal. However, the highest yield was only 32%. The reaction was repeated with purchased anhydrous NaOEt in various amounts which gave **118** in 21-32% yield.

Next, NaH was used as the base in THF. Varying the amount of acetone also affected the yield of the reaction. It was determined that using 3 equivalents of NaH and 1.5 equivalents of acetone were the best conditions for the reaction and gave **118** in 75% yield.



Scheme 74. Optimized reaction scheme for synthesis of **118**



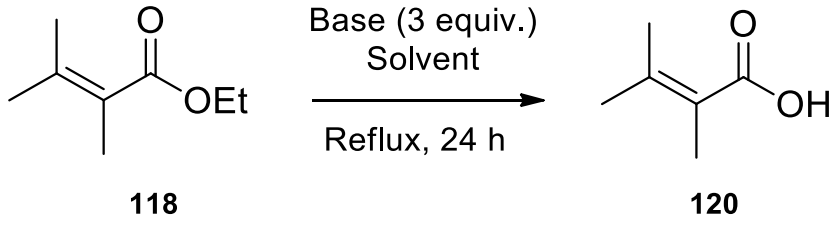
Scheme 75. Mechanism for synthesis of **118**

Table 16. Optimization of reaction conditions for synthesis of **118**

Entry	Base	Equivalents	Solvent	Acetone (equiv.)	Yield of 118 (%)
1	NaOEt	1	THF	1	21
2	NaOEt	1.5	EtOH	1	23
3	NaOEt	2	EtOH	1	23
4	NaOEt	3	EtOH	1	30
5	NaOEt	5	EtOH	1	32
6	NaOEt	5	EtOH	3	30
7	NaH	1	THF	1	56
8	NaH	1.5	THF	1	55
9	NaH	3	THF	1	60
10	NaH	3	THF	1.5	75

3.6 Synthesis of 2,3-dimethyl-2-butenoic acid

The next step in the synthesis of pyruvate esters was to convert **118** to its corresponding carboxylic acid (**120**). This was achieved by base hydrolysis of **118**. First, 3 equivalents of lithium hydroxide (LiOH) was used as the base (**Scheme 76**). The reaction was stirred at room temperature but there was no visible product formation by TLC. The reaction was then heated to 50 ℃ which led to the formation of **120** in 52% yield.



118 **120**

Scheme 76. Synthesis of **120** using LiOH

The reaction was repeated with 3 equivalents of sodium hydroxide (NaOH) as the base and EtOH as the solvent. NaOH was dissolved in minimum amount of water and was then added to the reaction mixture. The reaction was stirred overnight to give **120** in 57% yield. These conditions were optimized (**Table 17**) by heating the reaction mixture to reflux temperature. This led to the formation of the acid **120** as a light-yellow solid in 99% yield.

Table 17. Reaction conditions for synthesis of **120**

Entry	Base	Solvent	Temperature (°C)	Yield of 120 (%)
1	LiOH	THF:H_2O (1:1)	25	0
2	LiOH	THF:H_2O (1:1)	50	52
3	NaOH	EtOH/H_2O	25	57
4	NaOH	EtOH/H_2O	Reflux	99

3.7 Synthesis of esters of 2,3-dimethyl-2-butenoic acid

With the acid in hand, a series of esters were prepared using Steglich esterification [171]. The acid **120** was treated with benzyl alcohols (**121a**), 4-bromobenzyl alcohol (**121b**), ethylene glycol (**121c**) and neopentyl glycol (**121d**) (**Fig. 47**) in the presence of a carbodiimide coupling reagent (EDCI) and a suitable organic base N, N-dimethyl aminopyridine (DMAP) or N,N-diisopropyl ethylamine (DIPEA). The reaction conditions were optimized with benzyl alcohol (**121a**) (**Scheme 77**, **Table 18**). It was noted that using 3 equivalents of the alcohol and 5 equivalents of DIPEA were required in order to prepare **122a** in 86% yield.

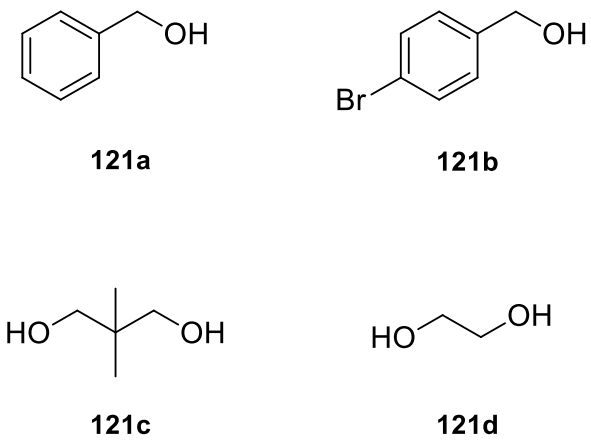
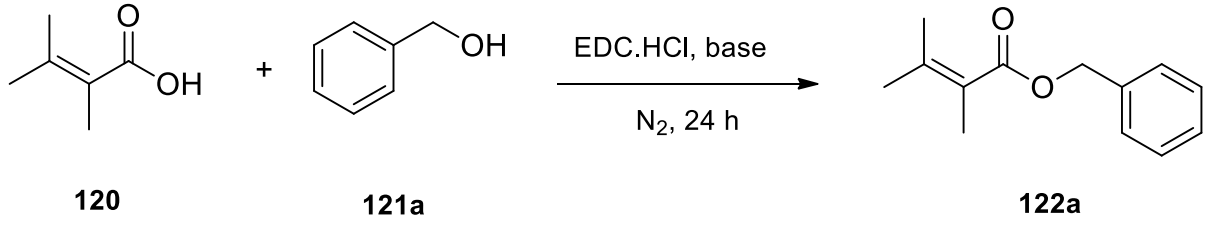


Fig. 47. Alcohols used to prepare the esters of **120**

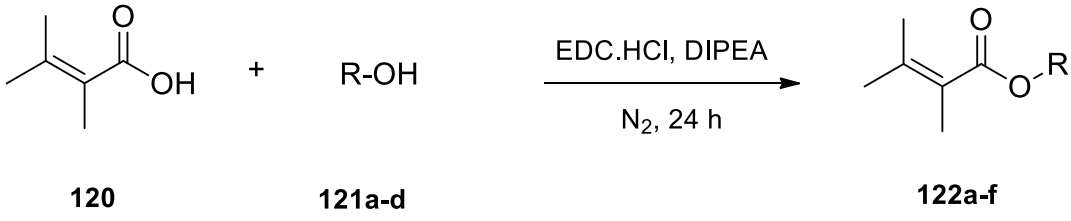


Scheme 77. Synthesis of benzyl ester **125**

Table 18. Reaction condition optimization for synthesis of **122a**

Entry	Equivalents of 121a	Base	Equivalents of base	Yield of 122a (%)
1	1	DMAP	1	46
2	1.5	DMAP	2	45
3	3	DMAP	3	50
4	3	DIPEA	3	75
5	3	DIPEA	5	86

The optimized conditions were then used to prepare esters **122a-f** in 75-89% yield (**Scheme 78**). It was observed that dialcohols like ethylene glycol and neopentyl alcohol produced the monoesters **122c** and **122d**. The monoesters were then used to repeat the esterification with the acid **120** to produce the diesters **122e** and **122f** (**Fig. 48**).



Scheme 78. Synthesis of esters **122a-f**

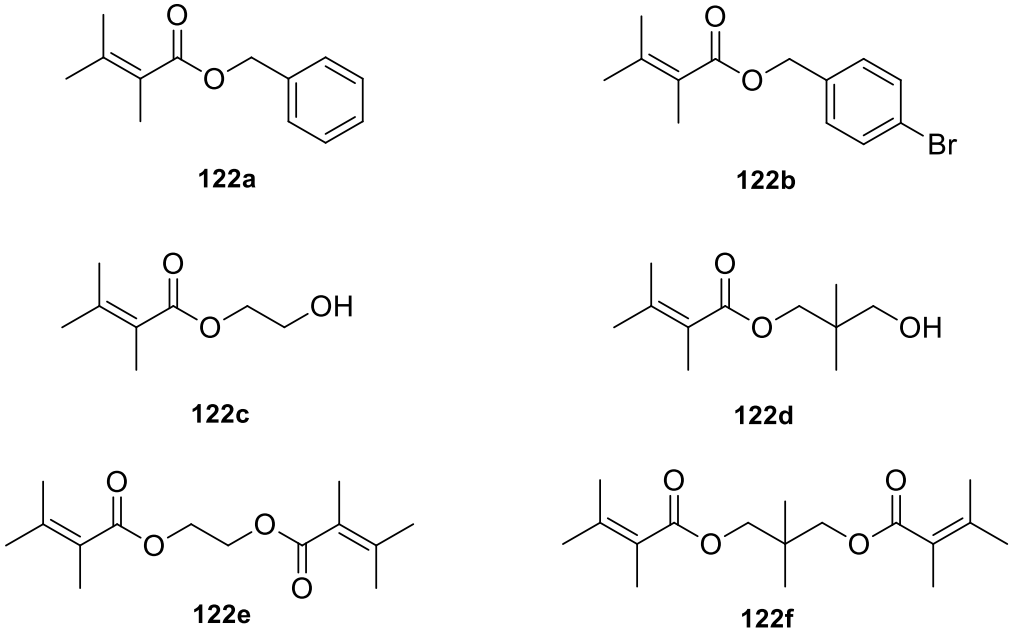
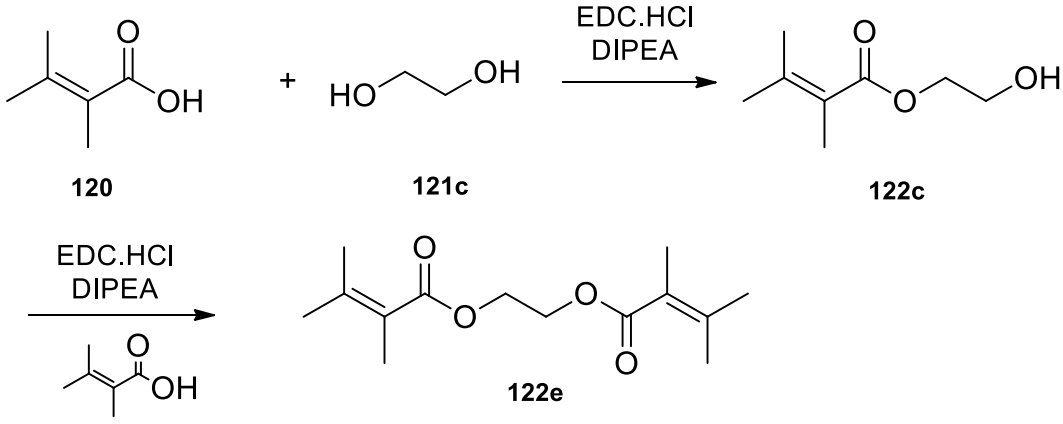
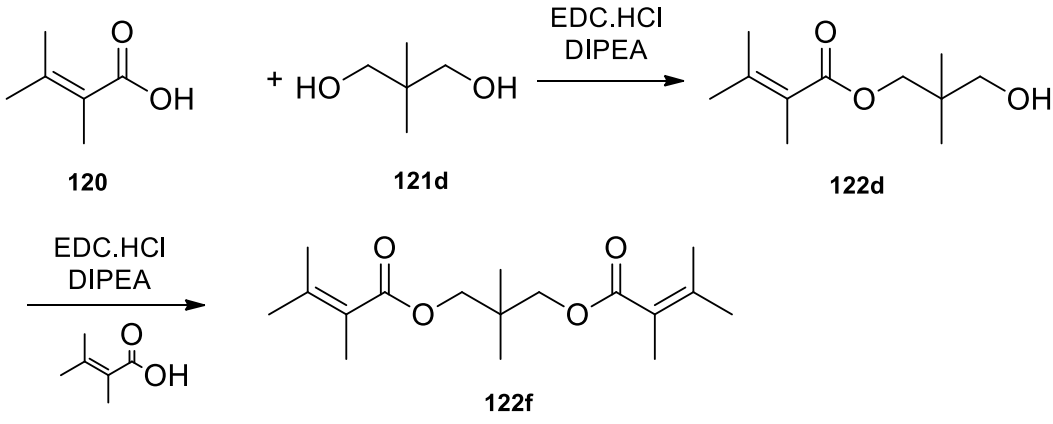


Fig. 48. Structures of synthesized esters **122a-f**

Synthesis of diesters **122e** and **122f** is illustrated in **scheme 79 and 80** below.



Scheme 79. Synthesis of diester **122e**



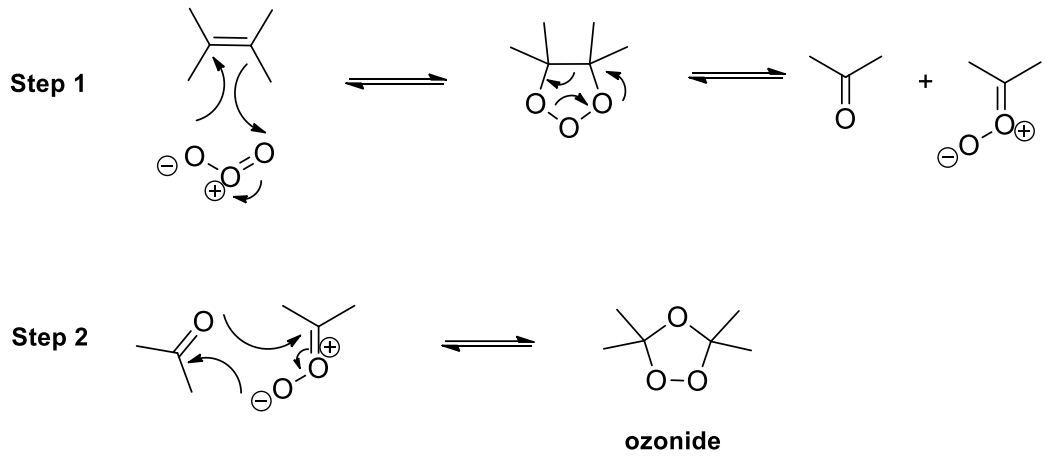
Scheme 80. Synthesis of diester **122f**

Table 19. Optimized yields of esters **122a-f**

Entry	ROH	Yield of 122 (%)
a	**121a**	86
b	**121b**	89
c	**121c**	75
d	**121d**	78
e	**122c**	76
f	**122d**	78

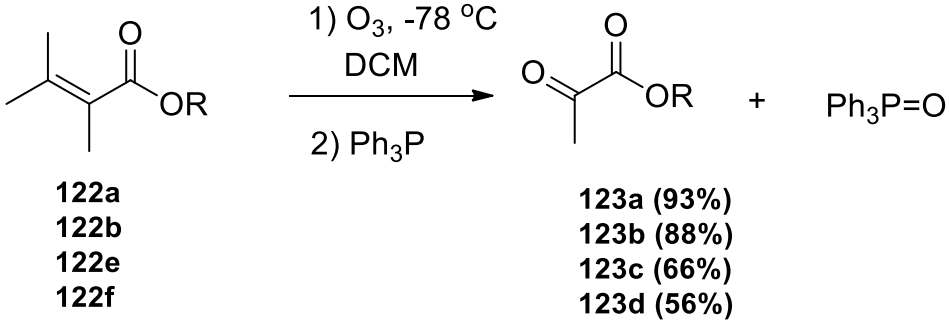
3.8 Synthesis pf pyruvate esters

Pyruvate esters were prepared by ozonolysis of the synthesized esters **122a-d**. Ozolnolysis is a method to oxidatively cleave alkenes or alkynes with ozone (O_3). The carbon-carbon double bond is replaced by a double bond with oxygen. The intermediate formed is an ozonide molecule which is further reduced to carbonyl products.



Scheme 81. Ozonide formation during ozonolysis

The ozonide intermediate is reduced with either triphenyl phosphine (PPh_3) or dimethyl sulfide (DMS) to give the carbonyl compounds. Using this method, the esters **122a-b** and **122e-f** were treated with ozone at -78 ℃ in dichloromethane and then subsequently reduced with PPh_3 to give the corresponding pyruvate esters **123a-d** (**Fig. 49**) in moderate to high yields (56-93%) (**Scheme 82**). The pyruvate esters were separated from the byproduct triphenyl phosphine oxide by column chromatography and obtained as colorless oils.



Scheme 82. Synthesis of pyruvate esters **123a-d** via ozonolysis

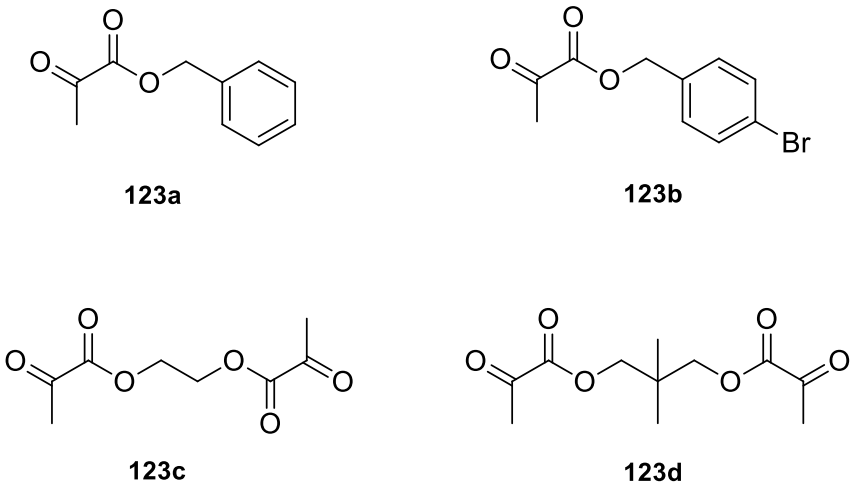


Fig. 49. Structures of synthesized pyruvate esters **123a-d**

CONCLUSION

In summary, a general synthesis for the preparation of pyruvate esters was developed. A series of alkyl pyruvate esters were synthesized in moderate to high yield in four steps. Horner-Wadsworth-Emmons reaction was used to synthesize the alkene precursor which was converted to the corresponding ester and subsequently converted to pyruvate esters via ozonolysis. The reaction conditions can be applied to prepare both mono and diesters. Mono esters were isolated in higher yields compared to diesters. The pyruvate esters were found to be stable and did not undergo decomposition.

EXPERIMENTAL

General information

All reactions were carried out in oven-dried glassware under a N_2 atmosphere unless otherwise noted. All chemicals were purchased from Alfa Aesar, Sigma Aldrich, and VWR and were used as received without further purification. Chromatography refers to flash silica gel column chromatography (0.060–0.200mm (60 Å) silica gel from Sorbent Technology was used as the stationary phase). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature in DMSO-d_6 on a Bruker 300 MHz instrument. ^{1}H chemical shifts were referenced to the DMSO solvent signal (2.48 ppm). ^{13}C chemical shifts were referenced to the DMSO solvent signal (40.00 ppm).

Synthesis of ethyl 2,3-dimethyl-2-butenoate (118)

Sodium hydride (6.05 g, 0.252 mol) was added to anhydrous THF (50 mL). The mixture was stirred at room temperature for 30 minutes under inert atmosphere. Triethyl-2-phosphonopropionate (20.0 mL, 0.0840 mol) was added to the solution and stirred for an additional 30 minutes. Anhydrous acetone (9.26 mL, 0.126 mol) was then added to the reaction mixture and the reaction was stirred for 24 hours at room temperature. The reaction was diluted with water (50 mL) extracted with diethyl ether (3 x 50 mL). The organic layers were combined and washed with brine (100 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was filtered and the solvent was removed under vacuum to give **118** as a colorless oil (9.00 g, 75% yield).

^{1}HNMR (DMSO-d_6, 300 MHz) δ: 4.08 (q, *J* = 7.0 Hz, 2H), 1.92 (s, 3H), 1.79 – 1.72 (m, 6H), 1.19 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.5, 142.2, 137.6, 122.0, 59.5, 22.4, 22.0, 15.4, 14.1.

Synthesis of 2,3-dimethyl-2-butenoic acid (120)

Ethyl 2,3-dimethyl-2-butenoate (10.0 mL, 0.0703 mol) was dissolved in ethanol (30.0 mL). NaOH (8.44 g, 0.211 mol) was dissolved separately in water (20.0 mL) was added to the reaction mixture. The mixture was heated at reflux for 24 h. The reaction mixture was concentrated under vacuum and diluted with water (50 mL). The solution was made acidic to pH 2 by adding 3M HCl. The aqueous solution then was extracted with ethyl acetate (3 x 15 mL). The organic layers were combined and washed with brine (30 mL) and dried over anhydrous sodium sulfate. The sodium sulfate was filtered and the solvent was removed under vacuum to give a light-yellow oil. The oil was cooled to give light yellow crystals (8.00 g, 99% yield). mp 52.3-54.0 ℃

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.07 (s, 1H), 1.92 (s, 3H), 1.74 (d, *J* = 3.6 Hz, 7H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 170.4, 141.3, 122.8, 22.5, 22.1, 15.6.

Synthesis of 2,3-dimethyl-2-butenoate esters (122a-f)

General procedure A

2,3-dimethyl-2-butenoic acid (1 equivalent), alcohol (3 equivalents), and EDC•HCl (3 equivalents) were dissolved in anhydrous DMF (20.0 mL). DIPEA (5 equivalents) was added to the reaction mixture and stirred under inert atmosphere for 24 h. The reaction was diluted with

water (100 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and washed with water (5 x 20 mL), brine (50 mL) and the dried over anhydrous sodium sulfate. The sodium sulfate was filtered and the solvent was removed under vacuum. The residue was purified by column chromatography (30% EtOAc:Hex) to give the esters **122a-d** as colorless oils.

Benzyl 2,3-dimethyl-2-butenoate (122a)

General procedure A. Ester **122a** was prepared using **120** (1.00 g, 8.76 mmol), alcohol **121a** (2.72 mL, 26.3 mmol), EDC•HCl (4.08 26.3 mmol) and DIPEA (7.63 mL, 4.37 mmol) and was obtained as a colorless oil (1.60 g, 89% yield).

^{1}H NMR (DMSO-d_6, 300MHz) δ: 7.35-7.30 (m, 5H), 5.12 (s, 2H), 1.93 (s, 3H), 1.80 (s, 3H), 1.75 (s, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.3, 143.5, 136.5, 128.4, 127.9, 121.7, 112.5, 65.3, 46.0, 22.6, 15.4.

4-bromobenzyl 2,3-dimethyl-2-butenoate (122b)

General procedure A. Ester **122b** was prepared using **120** (1.00 g, 8.76 mmol), alcohol **121a** (2.72 mL, 26.3 mmol), EDC•HCl (4.08 26.3 mmol) and DIPEA (7.63 mL, 4.37 mmol) and was obtained as a colorless oil (2.21 g, 89% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.55 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 5.09 (s, 2H), 1.93 (s, 3H), 1.78 (d, J = 11.0 Hz, 6H).

^{13}CNMR (DMSO-d_6, 75 MHz) δ: 168.3, 143.5, 136.5, 128.4, 127.9, 121.7, 112.5, 65.3, 22.6, 15.4.

2-hydroxyethyl 2,3-dimethylbut-2-enoate (122c)

General procedure A. Mono ester **122e** was prepared using **120** (1.00 g, 8.76 mmol), alcohol **121a** (2.72 mL, 26.3 mmol), EDC•HCl (4.08 26.3 mmol) and DIPEA (7.63 mL, 4.37 mmol) and was obtained as a colorless oil (1.04 g, 75% yield)

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.37 (s, 1H), 4.27 (t, J = 7.3 Hz, 2H), 3.8 (t, J = 7.3 Hz, 2H), 2.37 (s, 3H), 2.21 (s, 3H), 1.92 (s, 3H).

3-hydroxy-2,2-dimethylpropyl 2,3-dimethylbut-2-enoate (122d)

General procedure A. Mono ester **122d** was prepared using **120** (1.00 g, 8.76 mmol), alcohol **121a** (2.72 mL, 26.3 mmol), EDC•HCl (4.08 26.3 mmol) and DIPEA (7.63 mL, 4.37 mmol) and was obtained as a colorless oil (1.75 g, 78% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.60 (t, *J* = 5.4 Hz, 1H), 3.80 (s, 2H), 3.17 (d, *J* = 5.4 Hz, 2H), 1.95 (d, *J* = 6.6 Hz, 3H), 1.83 – 1.72 (m, 6H), 0.83 (s, 6H).

Ethane-1,2-diyl bis(2,3-dimethylbut-2-enoate) (122e)

General procedure A. Diester **122e** was prepared from **120** (0.120 g, 1.05 mmol), **122 c** (0.500 g, 3.16 mmol), EDC•HCl (0.490 g, 3.16 mmol) and DIPEA (1.00 mL, 5.25 mmol) and was obtained as a colorless oil (0.208g, 76% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.28 (s, 4H), 1.92 (s, 6H), 1.75 (s, 12H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 168.7, 144.0, 122.0, 112.9, 62.1, 23.0, 15.4.

2,2-dimethylpropane-1,3-diyl bis(2,3-dimethylbut-2-enoate) (122f)

General procedure A. Diester **122f** was prepared from **120** (0.200 g, 1.67 mmol), **122d** (1.00 g, 5.00 mmol), EDC•HCl (0.777 g, 5.00 mmol) and DIPEA (1.45 mL, 8.35 mmol) and was obtained as a colorless oil (0.390 g, 78% yield)

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.86 (s, 4H), 1.93 (s, 6H), 1.78-1.76 (m, 12H), 0.94 (s, 6H).

Synthesis of pyruvate esters (123a-d)

General procedure B

The esters **122a-b** and **122e-f** were dissolved in anhydrous DCM (20.0 mL) and the reaction was cooled to -78 ℃. Ozone was bubbled through the solution until blue color persisted. Oxygen was then bubbled through until the blue color disappeared and solution turned colorless. Triphenyl phosphine (2 equivalents) was added to the reaction mixture and stirred at room temperature overnight. Water (20 mL) was added to the reaction mixture and the organic and aqueous layers were separated. The aqueous layer was extracted with DCM (3 x 15 mL). The organic layers were combined and washed with brine (20 mL) the organic layer was then dried over anhydrous sodium sulfate, filtered and the solvent was removed under vacuum. The residue was purified by column chromatography (50% EtOAc/Hex) to give the pyruvate esters **123a-d** as colorless oils.

Benzyl 2-oxopropanoate (123a)

General procedure B. The pyruvate ester **123a** was obtained from **122a** (0.500 g, 2.45 mmol) as a colorless oil (0.406 g, 93% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.41-7.35 (m, 5H), 5.22 (s, 2H), 2.38 (s, 3H).

^{13}C NMR (DMSO -d_6, 75 MHz) δ: 191.6, 168.3, 143.5, 136.5, 128.4, 127.9, 65.3, 15.4.

4-Bromobenzyl 2-oxopropanoate (123b)

General procedure B. The pyruvate ester **123b** was obtained from **122b** (0.500 g, 1.76 mmol) as a colorless oil (0.400 g, 88% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.57 (d, J = 9Hz, 2H), 7.38 (d, J = 9Hz, 2H), 5.21 (s, 2H), 2.39 (s, 3H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 191.6, 168.3, 136.5, 128.4, 127.9, 121.7, 112.6, 65.3, 22.6.

Ethane-1,2-diyl bis(2-oxopropanoate) (123c)

General procedure B. The pyruvate ester **123c** was obtained from **122e** (0.100 g, 0.400 mmol) as a colorless oil (0.0453 g, 56% yield).

$_{1}$H NMR (DMSO-d_6, 300 MHz) δ: 4.44 (s, 4H), 2.36 (s, 6H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 191.6, 160.3, 63.6, 27.0.

2,2-Dimethylpropane-1,3-diyl bis(2-oxopropanoate) (123d)

General procedure B. The pyruvate ester **123d** was obtained from **122f** (0.100 g, 0.337 mmol) as a colorless oil (0.0543 g, 66% yield).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.02 (s, 4H), 2.37 (s, 6H), 1.00 (s, 6H).

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 191.7, 160.3, 70.4, 27.2, 21.6.

REFERENCES

[1] H. Tsukahara, T. Ishida and M. Mayumi, "Nitric Oxide," *Biol. Chem.,* vol. 3, p. 191, 1999.

[2] P. G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai and A. J. Janczuk, "Nitric Oxide Donors: Chemical Activities and Biological Applications," *Chem. Rev.,* vol. 102, pp. 1091-1134, 2002.

[3] F. Murad, "Discovery of Some of the Biological Effects of Nitric Oxide and it Role in Cell Signalling (Nobel Lecture)," *Angew. Chem. Int. Ed,* vol. 38, pp. 1856-1868, 1999.

[4] R. F. Furchgott, "Endothelium-Derived Relaxing Factor: Discovery, Early Studies, and Identifcation as Nitric Oxide (Nobel Lecture)," *Angew. Chem. Int. Ed.,* vol. 38, pp. 1870-1880, 1999.

[5] L. Ignarro, "Nitric Oxide: A Unique Endogenous Signaling Molecule in Vascular Biology (Nobel Lecture)," *Angew. Chem. Int. Ed.,* vol. 38, pp. 1882-1892, 1999.

[6] H. AL-SA'DONI and A. FERRO, "S-Nitrosothiols: a class of nitric oxide-donor drugs," *Clin. Sci.,* vol. 98, no. 5, pp. 507-520, 2000.

[7] R. Radil, J. S. Beckman, K. M. Bush and B. A. Freeman, "Peroxynitrite Oxidation of Sulfhydryls. THE CYTOTOXIC POTENTIAL OF SUPEROXIDE AND NITRIC OXIDE," *J. Biol. Chem.,* vol. 266, pp. 4244-4250, 1991.

[8] I. L. Megson, "Nitric Oxide Donor Drugs," *Drugs Future,* vol. 25, p. 701, 2000.

[9] J. Honeyman and J. W. Morgan, "Sugar Nitrates," *Adv. Carbohyd. Chem.,* vol. 12, pp. 117-135, 1957.

[10] C. Capellos, W. J. Fisco, C. Ribaudo, V. D. Hogan and Campisi, "Basic hydrolysis of glyceryl nitrate esters. II. 1,2-glyceryl and 1, 3-glyceryl dinitrate esters," *Int. J. Chem. Kinet. ,* vol. 16, p. 1009, 1984.

[11] G. R. J. Thatcher and H. Weldon, "NO problem for nitroglycerin: organic nitrate chemistry and therapy," *Chem. Soc. Rev.,* vol. 27, pp. 331-37, 1998.

[12] L. J. Ignarro and C. A. Gruetter, "Requirement of thiols for activation of coronary arterial guanylate cyclase by glyceryl trinitrate and sodium nitrite possible involvement of S-nitrosothiols," *Biochim. Biophys. Acta,* vol. 631, no. 2, pp. 221-231, 1980.

[13] J. D. Horowitz, E. M. Antman, B. H. Lorell and W. H. Barry, "Potentiation of the cardiovascular effects of nitroglycerin by N-acetylcysteine," *Circulation,* vol. 68, pp. 1247-1253, 1983.

[14] P. Seth and H. L. Fung, "Biochemical characterization of a membrane-bound enzyme responsible for generating nitric oxide from nitroglycerin in vascular smooth muscle cells," *Biochem. Pharmacol.,* vol. 46, no. 8, pp. 1481-1486, 1993.

[15] B. I. Jugdutt, "Role of nitrates after acute myocardial infarction," *Am. J. Cardiol.,* vol. 70, p. 82, 1992.

[16] C. V. Leier, D. Bambach, M. J. Thompson and S. M. Cattaneo, "Central and regional hemodynamic effects of intravenous isosorbide dinitrate, nitroglycerin and nitroprusside in patients with congestive heart failure," *Am. J. Cardiol.,* vol. 48, no. 6, pp. 1115-1123, 1981.

[17] B. Tander, A. Guven, S. Demirbag, Y. Ozkan and H. Ozturk, "A Prospective, Randomized, Double-Blind, Placebo-Controlled Trial of Glyceryl-Trinitrate Ointment in the Treatment of Children With Anal Fissure," *J. Pediatr. Surg.,* vol. 34, no. 12, pp. 1810-1812, 1999.

[18] C. de Mey, "Opportunities for the Treatment of Erectile Dysfunction by Modulation of the NO Axis - Alternatives to Sildenafil Citrate," *Curr. Med. Res. Opin.,* vol. 14, no. 4, pp. 187-202, 1998.

[19] J. Rhode.Patent WO Patent 99/16436, 1999.

[20] M. Nakatsuka, K. Tada, Y. Kimura, K. Asagiri and Y. Kamada, "Clinical Experience of Long-Term Transdermal Treatment with Nitric Oxide Donor for Women with Preeclampsia," *Gynecol. Obstet. Invest.,* vol. 47, pp. 13-19, 1999.

[21] M. T. Osinski, B. H. Rauch and K. Schror, "Antimitogenic Actions of Organic Nitrates Are Potentiated by Sildenafil and Mediated Via Activation of Protein Kinase A," *Mol. Pharmacol.,* vol. 59, no. 5, pp. 1044-1050, 2001.

[22] S. M. Yu, M. M. Hung and C. Lin, "cGMP-Elevating Agents Suppress Proliferation of Vascular Smooth Muscle Cells by Inhibiting the Activation of Epidermal Growth Factor Signaling Pathway," *Circulation,* vol. 95, pp. 1269-1277, 1997.

[23] T. L. Cornwell, E. Arnold, N. J. Boerth and T. M. Lincoln, "Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP," *Am. J. Physiol.,* pp. 267, C1405, 1994.

[24] M. Struehr and D. Marletta, "Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide.," *Proc. Natl. Acad. Sci. USA,* vol. 82, pp. 7738-7742, 1985.

[25] J. B. Hibbs Jr, R. R. Taintor and Z. Vavrin, "Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite.," *Science,* vol. 235, pp. 473-476, 1987.

[26] J. B. Hibs Jr, R. R. Taintor, Z. Vavrin and E. M. Rachlin, "Nitric oxide: a cytotoxic activated macrophage effector molecule.," *Biochem. Biophys. Res. Commun.,* vol. 157, pp. 87-94, 1988.

[27] E. C. Chinje and I. J. Stratford, "Role of nitric oxide in growth of solid tumours: a balancing act," *Essays Biochem.,* vol. 32, pp. 61-72, 1997.

[28] P. K. Lala and C. Chakraborty, "Role of nitric oxide in carcinogenesis and tumour progression," *Lancet. Oncol.,* vol. 2, pp. 149-156, 2001.

[29] D. C. Jenkins, I. G. Charles, L. L. Thomsen and et al, "Roles of nitric oxide in tumor growth," *Proc. Natl. Acad. Sci. USA,* vol. 92, pp. 4392-4396, 1995.

[30] K. Xie and S. Huang, "Contribution of nitric oxide-mediated apoptosis to cancer metastasis inefficiency," *Free Radic. Biol. Med.,* vol. 34, pp. 969-986, 2003.

[31] A. T. Chan, S. Ogino and C. S. Fuchs, "Aspirin and the risk of colorectal cancer in relation to the expression of COX-2.," *N. Engl. J. Med.,* vol. 356, pp. 2131-2142, 2007.

[32] G. R. Thatcher, A. C. Nicolescu, B. M. Bennett and V. Toader, "Nirates and NO release: contemporary aspects in biological and medicinal chemistry," *Free Radic. Biol. Med.,* vol. 37, pp. 1122-1143, 2004.

[33] O. A. Al-Swayeh, L. E. Futter, R. H. Clifford and P. K. Moore, "Nitroparacetamol exhibits anti-in¯ammatory and anti-nociceptive activity," *Br. J. Pharmacol.,* vol. 130, pp. 1453-1456, 2000.

[34] D. J. Bjorkman, "The effect of aspirin and nonsteroidal anti-inflammatory drugs on prostaglandins," *Am. J. Med.,* vol. 105, no. 1, pp. 85-125, 1998.

[35] A. Beaumont and J. Hughes, "Biology of Opiod Peptides," *Ann. Rev. Pharmacol. Toxicol.,* vol. 19, pp. 245-267, 1979.

[36] C. N. Sang, "NMDA-Receptor Antagonists in Neuropathic Pain: Experimental Methods to Clinical Trials," *J Pain Symptom Manag,* vol. 19, no. 1, pp. 21-25, 2000.

[37] R. E. Harris, J. Beebe-Donk, H. Doss and D. B. Doss, "Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: A critical review of non-selective COX-2 blockade (Review)," *Oncol. Rep.,* vol. 13, no. 4, pp. 559-583, 2005.

[38] S. A. Schug, F. Ffpmanzca, W. R. Garrett and G. Gillespie, "Opioid and non-opioid analgesics," *Best Practice & Research Clinical Anaesthesiology,* vol. 17, no. 1, pp. 91-110, 2003.

[39] N. G. Bazan, "SYNERGISTIC COMBINATIONS INCLUDING N-ACYLATED 4-HYDROXYPHENYLAMINE DERIVATIVES". United States of America Patent US 6,864,271 B2, 8 March 2005.

[40] P. Watkins, N. Kaplowitz and J. Slattery, "Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily," *J. Am. Med. Assoc.,* vol. 296, pp. 87-93, 2006.

[41] C. Saito, C. Zwingmann and H. Jaeschke, "Novel mechanism of protection against acetaminophen hepatoxicity in mice by glutathione and N-acetylcysteine," *Hepatology,* vol. 51, pp. 246-254, 2010.

[42] M. McGill, H. Yan, A. Ramachandran, G. Murray, D. Rollins and H. Jaeschke, "HepaRG cells: A human model to study mechanisms of acetaminophen hepatotoxicity," *Hepatology,* vol. 53, pp. 974-982, 2011.

[43] W. Lee, "Drug-Induced Hepatotoxicity," *N. Engl. J. Med.,* vol. 349, pp. 474-485, 2003.

[44] F. V. Schiodt, F. A. Rochling, D. L. Casey and W. M. Lee, "Acetaminophen Toxicity in an Urban County Hospital," *N. Engl. J. Med.,* vol. 337, pp. 1112-1118, 1997.

[45] N. G. Bazan and J. A. Gomez, "N-ACYLATED 4-HYDROXPHENYLAMINE DERIVATIVES WITH ANALGESIC PROPERTIES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM". United States Patent Patent 5,554,636, 10 September 1996.

[46] L. Miao, L. Xu, K. W. Narducy and M. L. Trudell, "First Multigram Preparation of SCP-123, A Novel Water-Soluble Analgesic," *Org. Process Res. Dev.,* vol. 13, pp. 820-822, 2009.

[47] E. E. Meyer, K. J. Rosenberg and J. Israelachvili, "Recent progress in understanding hydrophobic interactions," *Proc. Nat. Acad. Sci. USA,* vol. 103, no. 43, p. 15739–15746, 2006.

[48] G. Thomas, Fundamentals of Medicinal Chemistry, John Wiley & Sons, Inc., 2003.

[49] K. T. Savjani, A. K. Gajjar and J. K. Savjani, "Drug Solubility: Importance and Enhancement Techniques," *ISRN Pharm.,* vol. 2012, p. 195727, 2012.

[50] B. G. Davis and M. A. Robinson, "Drug delivery systems based on sugar-macromolecule conjugates," *Curr. Opin. Drug Discov. Devel.,* vol. 5, no. 2, pp. 279-288, 2002.

[51] R. Murashige, Y. Hayashi, S. Ohmori and e. al, "Comparisons of O-acylation and Friedel-Crafts acylation of phenols and acyl chlorides and Fries rearrangement of phenyl esters in trifluoromethanesulfonic acid: Effective synthesis of optically active homotyrosines," *Tetrahedron,* vol. 67, pp. 641-649, 2011.

[52] M. Das, S. Bhattacharjee, F. R. Fronczek and N. G. Bazan, "Synthesis, hepatotoxic evaluation and antipyretic activity of nitrate ester analogs of the acetaminophen derivative SCP-1," *Bioorg. Med. Chem. Lett.,* vol. 28, pp. 3798-3801, 2018.

[53] T. H. Bembry and G. Powell, "The synthesis of phenolic glycosides," *J. Am. Chem. Soc.,* vol. 64, no. 10, pp. 2419-2420, 1942.

[54] R. R. Schmidt and M. Hoffmann, "C-glycosides from O-glycosyl trichloroacetimidates," *Tetrahedron Lett.,* vol. 23, no. 4, pp. 409-412, 1982.

[55] "Chemdraw," PerkinElmer Inc., Version 12.0.1.

[56] C. Canel, R. M. Moraes, F. E. Dyan and D. Ferreira, "Podophyllotoxin," *Phytochemistry,* vol. 54, pp. 115-120, 2000.

[57] C. Tsutsui, Y. Yamada, M. Ando, D. Toyama, J. L. Wu, L. Wang, S. Taketani and T. Kataoka, "Peperomins as anti-inflammatory agents that inhibit the NF-κB signaling pathway," *Biiorg. Med. Chem. Lett.,* vol. 19, no. 15, pp. 4084-4087, 2009.

[58] A. C. Diokno, R. A. Appell, P. K. Sand, R. R. Dmochowski, B. M. Gburek, I. W. Klimberg and S. H. Kell, "Prospective, Randomized, Double-Blind Study of the Efficacy and Tolerability of the

Extended-Release Formulations of Oxybutynin and Tolterodine for Overactive Bladder: Results of the OPERA Trial," *Mayo CLin. Proc.,* vol. 78, pp. 687-695, 2003.

[59] L. Gennari, D. Merlotti, G. Martini and R. Nuti, "Lasofoxifene: a third-generation selective estrogen receptor modulator for the prevention and treatment of osteoporosis," *Opin. Invest. Drugs,* vol. 15, no. 9, p. 1091, 2006.

[60] S. Fukuzawa, T. Tsuchimoto and T. Hiyama, "Superacid-Catalyzed Reductive Friedel−Crafts Reaction of Arenes Using Arenecarbaldehyde Acetals," *J. Org. Chem.,* vol. 62, no. 1, pp. 151-156, 1997.

[61] H. Sun, B. Li, S. Chen, J. Li and R. Hua, "An efficient synthesis of unsymmetrical diarylmethanes from the dehydration of arenes with benzyl alcohols using InCl3·4H2O/acetylacetone catalyst system," *Tetrahedron,* vol. 63, no. 41, pp. 10185-10188, 2007.

[62] V. Y. Sosnovkikh and R. A. Irgashev, "Uncatalyzed addition of indoles and N-methylpyrrole to 3-formylchromones: synthesis of (chromon-3-yl)bis(indol-3-yl)methanes and E-2-hydroxy-3-(1-methylpyrrol-2-ylmethylene)chroman-4-ones under solvent-free conditions," *Tetragedron Lett.,* vol. 48, no. 42, pp. 7436-7439, 2007.

[63] M. M. Rudolf and B. Myrnoh, *Indian Chem. Eng. Sect. B,* vol. 48, p. 146, 2009.

[64] O. Mendoza, G. Rossey and L. Ghosez, "Bronsted acid-catalyzed synthesis of diarylmethanes under non-genotoxic conditions," *Tetrahedron Lett.,* vol. 52, no. 17, pp. 2235-2239, 2011.

[65] J. Barluenga, M. Tomas-Gamasa, F. Aznar and C. Valdes, "Metal-free carbon-carbon bond-forming reductive coupling between boronic acids and tosylhydrazones," *Nat. Chem.,* vol. 1, no. 6, pp. 494-499, 2009.

[66] M. M. Khodaei and E. Nazari, "Synthesis of diarylmethanes via a Friedel–Crafts benzylation using arenes and benzyl alcohols in the presence of triphenylphosphine ditriflate," *Tetrahedron Lett.,* vol. 53, no. 38, pp. 5131-5135, 2012.

[67] Y. Y. Ku, R. R. Patel and D. P. Sawick, "A general, convenient and highly efficient synthesis of diarylmethanes by copper-catalyzed reaction," *Tetrahedron Lett.,* vol. 37, no. 12, pp. 1949-1952, 1996.

[68] J. Srogl, G. D. Allred and L. S. Leibeskind, "Sulfonium Salts. Participants par Excellence in Metal-Catalyzed Carbon−Carbon Bond-Forming Reactions," *J. Am. Chem. Soc.,* vol. 119, no. 50, pp. 12376-12377, 1997.

[69] S. Zhang, D. Marshall and L. S. Leibeskind, "Efficient Pd-Catalyzed Heterobenzylic Cross-Coupling Using Sulfonium Salts as Substrates and (PhO)3P as a Supporting Ligand," *J. Org. Chem.,* vol. 64, no. 8, pp. 2796-2804, 1999.

[70] S. Chowdhury and P. E. Georghiou, "Palladium catalyzed cross-coupling between phenyl- or naphthylboronic acids and benzylic bromides," *Tetrahedron Lett.,* vol. 40, pp. 7599-7603, 1999.

[71] G. A. Molander and M. D. Elia, "Suzuki−Miyaura Cross-Coupling Reactions of Benzyl Halides with Potassium Aryltrifluoroborates," *J. Org. Chem.,* vol. 71, no. 24, pp. 9198-9202, 2006.

[72] T. Z. Nichele and A. L. Monteiro, "Synthesis of diarylmethane derivatives from Stille cross-coupling reactions of benzylic halides," *Tetrahedron Lett.,* vol. 48, no. 42, pp. 7472-7475, 2007.

[73] R. Ghosh and A. Sarkar, "Bidentate P, N−P Ligand for Nickel-Catalyzed Cross-Coupling of Aryl or Benzyl Chlorides with ArMgX," *J. Org. Chem.,* vol. 75, no. 23, pp. 8283-8286, 2010.

[74] D. Srimani, A. Bej and A. Sarkar, "Palladium Nanoparticle Catalyzed Hiyama Coupling Reaction of Benzyl Halides," *J. Org. Chem.,* vol. 75, no. 12, pp. 4296-4299, 2010.

[75] C. Duplais, A. Krasovskiy, A. Watterberg and B. H. Lipshutz, "Cross-couplings between benzylic and aryl halides “on water”: synthesis of diarylmethanes," *Chem. Comm.,* vol. 46, pp. 562-564, 2010.

[76] B. Q. Wang, S. K. Xiang, Z. P. Sun, B. T. Guan, P. Hu, K. Q. Zhao and Z. J. Shi, "Benzylation of arenes through FeCl3-catalyzed Friedel–Crafts reaction via C–O activation of benzyl ether," *Tetrahedron Lett.,* vol. 49, no. 27, pp. 4310-4312, 2008.

[77] G. A. Kraus and D. Chaudhary, "Conversion of substituted benzyl ethers to diarylmethanes. A direct synthesis of diarylbenzofurans," *Tetrahedron Lett.,* vol. 53, no. 52, pp. 7072-7074, 2012.

[78] D. F. Duxbury, "The photochemistry and photophysics of triphenylmethane dyes in solid and liquid media," *Chem. Rev.,* vol. 93, no. 1, pp. 381-433, 1993.

[79] P. Rys and H. Zollinger, "Fundamentals of the chemistry and application of dyes," New Jersey, John WIley and Sons, 1972.

[80] M. S. Shchepinov and V. A. Korshun, "Recent applications of bifunctional trityl groups," *Chem. Soc. Rev.,* vol. 32, no. 3, pp. 170-180, 2003.

[81] T. W. Greene and P. G. Wuts, Protective groups in organic synthesis, Michigan: John Wiley and Sons, 1999.

[82] K. Sumoto, N. Mibu, K. Yokomizo and M. Uyeda, "Synthesis of 2,2’-Dihydroxybisphenols and Antiviral Activity of Some Bisphenol Derivatives," *Chem. Pharm. Bull.,* vol. 50, no. 2, pp. 298-300, 2002.

[83] M. Senturk, I. Gulcin, A. Dastan, O. I. Kufrevioglu and C. T. Supuran, "Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols," *Bioorg. Med. Chem.,* vol. 17, no. 8, pp. 3207-3211, 2009.

[84] R. Rosowsky and H. Chen, "ethods for synthesis of diarylmethanes". USA Patent US 7.256,197 B2, 14 Aug 2007.

[85] F. Perez-Ruiz, E. Marimo and S. P. Chinchilla, "Hyperuricaemia with deposition: Latest evidence and therapeutic approach," *Ther. Adv. Musculoskel Dis.,* vol. 7, pp. 225-233, 2015.

[86] E. Pascual, L. Addadi, M. Andres and F. SIvera, "Mechanisms of crystal formation in gout–a structural approach," *Nat. Rev. Rheumatol.,* vol. 11, pp. 725-730, 2015.

[87] R. D. Taylor, M. MacCoss and A. D. G. Lawson, "Rings in Drugs," *J. Med. Chem.,* vol. 57, pp. 5845-5859, 2014.

[88] J. Jampilek, "Heterocycles in Medicinal Chemistry," *Molecules,* vol. 24, p. 3839, 2019.

[89] E. Lioudaki, E. S. Ganotakis, D. Mikhalidis and P. Ezetimibe, "More Than a Low Density Lipoprotein Cholesterol Lowering Drug? An Update after 4 Years," *Curr. Vasc. Pharmacol.,* vol. 9, pp. 62-86, 2011.

[90] P. M. Wright, I. B. Seiple and A. G. Myers, "The Evolving Role of Chemical Synthesis in Antibacterial Drug Discovery," *Chem., Int. Ed.,* vol. 53, pp. 8840-8869, 2014.

[91] M. L. Penningd and D. N. Reinhoudt, "Chemistry of four-membered cyclic nitrones. 5. Synthesis and oxidation of 1-hydroxyazetidines," *J. Org. Chem.,* vol. 48, no. 22, pp. 4043-4048, 1983.

[92] Y. Ju and R. S. Varma, "Aqueous N-Heterocyclization of Primary Amines and Hydrazines with Dihalides: Microwave-Assisted Syntheses of N-Azacycloalkanes, Isoindole, Pyrazole, Pyrazolidine, and Phthalazine Derivatives," *J. Org. Chem.,* vol. 71, no. 1, pp. 135-141, 2006.

[93] F. J. Sayago, M. A. Pradera, C. Gasch and J. Fuentes, "Expeditious synthesis of sulfoazetidine spiro-C-glycosides from ketose acetals," *Tetrahedron,* vol. 62, no. 5, pp. 915-921, 2006.

[94] E. Juaristi and D. Madrigal, "Use of hexamethylphosphoramide chmpa in the alkylation of aromatic amines: synthesis of azetidines, pyrrolidines, piperidines and hexahydroazepines," *Tetrahedron,* vol. 45, no. 3, pp. 629-636, 1989.

[95] J. M. Concellon, P. L. Bernad and J. A. Perez-Andrez, "Tetrahedron Lett.," *Nucleophilic ring closure and opening of aminoiodohydrins,* vol. 41, no. 8, pp. 1231-1234, 2000.

[96] M. K. Ghorai, A. Kumar and S. Halder, "Regioselective addition of 1,3-dicarbonyl dianion to N-sulfonyl aldimines: an expedient route to N-sulfonyl piperidines and N-sulfonyl azetidines," *Tetrahedron,* vol. 63, no. 22, pp. 4779-4787, 2007.

[97] P. Huszthy, J. S. Bradshaw, K. E. Krakowiak, T. Wang and N. K. Dalley, "Efficient synthesis of azetidine through N-trityl- or N-dimethoxytritylazetidines starting from 3-amino-l-propanol or 3-halopropylamine hydrohalides," *J. Heterocycl. Chem.,* vol. 30, no. 5, pp. 1197-1207, 1993.

[98] T. Axenrod, C. Watnick, H. Yazdekhasti and P. R. Dave, "Synthesis of 1,3,3-Trinitroazetidine via the Oxidative Nitrolysis of N-p-Tosyl-3-azetidinone Oxime," *J. Org. Chem.,* vol. 60, no. 7, pp. 1959-1964, 1995.

[99] M. C. Hillier and C. Cheng-yi, "A One-Pot Preparation of 1,3-Disubstituted Azetidines," *J. Org. Chem.,* vol. 71, no. 20, pp. 7885-7887, 2006.

[100] W. Aelterman, N. De Kimpe and J.-P. Declercq, "A Convenient Synthesis of 3,3-Dichloroazetidines, a New Class of Azetidines," *J. Org. Chem.,* vol. 63, no. 1, pp. 6-11, 1998.

[101] W. Van Brabandt, G. Verniest, D. De Smaele, G. Duvey and N. De Kimpe, "Synthesis of 3-Fluoroazetidines," *J. Org. Chem,* vol. 71, no. 18, pp. 7100-7102, 2006.

[102] A. Brandi, S. Cicchi and F. M. Cordero, "Novel Syntheses of Azetidines and Azetidinones," *Chem. Rev.,* vol. 108, no. 9, pp. 3988-4035, 2008.

[103] Y. Yagil, M. Miyamoto, L. Frasier, K. Oizumi and H. Koike, "Effects of CS-905, a novel dihydropyridine calcium channel blocker, on arterial pressure, renal excretory function, and inner medullary blood flow in the rat.," *Am. J. Hypertens.,* vol. 7, pp. 637-646, 1994.

[104] K. D. Rice, N. Aay, N. K. Anand, C. M. Blazey, O. J. Bowles, J. Bussenius, S. Costanzo, J. K. Curtis, S. C. Defina, L. Dubenko, S. Engst, A. A. Joshi, A. R. Kennedy, A. I. Kim, E. S. Koltun, J. C. Lougheed, J. C. L. Manalo, J. F. Martini, J. M. Nuss, C. J. Peto, T. H. Tsang, P. Yu and S. Johnston, "Novel Carboxamide-Based Allosteric MEK Inhibitors: Discovery and Optimization Efforts toward XL518 (GDC-0973)," *ACS Med. Chem. Lett.,* vol. 3, no. 5, pp. 416-421, 2012.

[105] B. I. Ericksson, S. Carlsson, M. Halvarsson, B. Risberg and C. Mattsson, *Thromb. Haemostasis,* vol. 78, pp. 1404-1407, 1997.

[106] C. R. Butler, E. M. Beck, A. Harris, Z. Huang, L. A. McAllister, C. W. am Ende, K. Fennell, T. L. Foley, K. Fonseca, S. J. Hawrylik and e. al., "Azetidine and Piperidine Carbamates as Efficient, Covalent Inhibitors of Monoacylglycerol Lipase," *J. Med. Chem.,* vol. 60, no. 23, pp. 9860-9873, 2017.

[107] T. Hart, A. T. Macias, K. Benwell, T. Brooks, J. D'Alessandro, P. Dokurno, G. Francis, B. Gibbons, T. Haymes, G. Kennett, S. Lightowler, H. Mansell, N. Matassova, A. Misra, A. Padfield, R. Parsons, R. Pratt, A. Robertson, S. Walls, M. Wong and S. Roughley, "Fatty acid amide hydrolase inhibitors. Surprising selectivity of chiral azetidine ureas," *Bioorg. Med. Chem. Lett.,* vol. 19, no. 15, pp. 4241-4244, 2009.

[108] M. Soth, J. C. Hermann, C. Yee, M. Alam, J. W. Barnett, P. Berry, M. F. Browner, K. Frank, S. Frauchiger, S. Harris and e. al, "3-Amido Pyrrolopyrazine JAK Kinase Inhibitors: Development of a JAK3 vs JAK1 Selective Inhibitor and Evaluation in Cellular and in Vivo Models," *J. Med. Chem.,* vol. 56, no. 1, pp. 345-356, 2013.

[109] A. J. Boddy, D. P. Affron, C. J. Cordier, E. L. Rivers, A. C. Spivey and J. A. Bull, "Rapid Assembly of Saturated Nitrogen Heterocycles in One-Pot: Diazo-Heterocycle “Stitching” by N–H Insertion and Cyclization," *Angew. Chem. Int. Ed.,* vol. 131, no. 5, pp. 1472-1476, 2019.

[110] S. J. Woodhead, R. Downham, C. Hamlett, S. Howard , H. F. Sore, M. L. Verdonk, D. W. Walker and R. W. A. Luke, "Arylalkylamines and heteroaryl-alkylamines as protein kinase inhibitors". Patent PCT Int. Appl. WO2006136830A1, 26 Dec 2006.

[111] D. K. Donald, A. S. R. Jennings, N. C. Ray, F. Roussel, J. M. Sutton, P. Tisselli and M. Wilson, "Cyclic amine derivatives having beta2 adrenergic receptor agonist and muscarinic receptor antagonist activity". Patent PCT Int. Appl. WO2011048409A1, 28 Apr 2011.

[112] C. Dennis, M. A. Dubois, A. S. Voisin-Chiret, R. Bureau, C. Choi, J. J. Mousseau and J. A. Bull, "Synthesis of 3,3-Diarylazetidines by Calcium(II)-Catalyzed Friedel–Crafts Reaction of Azetidinols with Unexpected Cbz Enhanced Reactivity," *Org. Lett.,* vol. 21, pp. 300-304, 2019.

[113] A. Thaxton, "University of New Orleans Theses and Dissertations," 2013. [Online]. Available: http://scholarworks.uno.edu/td/1764.

[114] F. D. Klingler and W. Ebertz, "Oxocarboxylic acids," in *Ullmann's Encyclopedia of industrial chemistry*, Wiley-VCH Verlag GmbH & Co., 2005.

[115] D. M. Nelson and M. M. Cox, Lehninger, Princliples of Biochemistry 3rd Ed., New York: Worth Publishing, 2000.

[116] C. Chinopolous, "Which way does the citric acid cycle turn during hypoxia? The critical role of α-ketoglutarate dehydrogenase complex," *J. Neurosci. Res.,* vol. 91, no. 8, pp. 1030-1043, 2013.

[117] J. M. Lowenstein, Methods in enzymology, Volume 13: Citric acid cycle, Boston: Academic Press, 1969.

[118] J. Kay and P. D. Weitzman, Krebs' citric acid cycle: half a century and still turning, London: Biochemical Society, 1987.

[119] K. L. Waters, "The a-Keto acids," *Chem. Rev.,* vol. 41, no. 3, pp. 585-598, 1947.

[120] F. Adickes and G. Andresen, "Series of normal aliphatic b-hydroxy and a-keto acids," *Justus Liebigs Ann. Chem,* vol. 41, p. 555, 1943.

[121] H. Goldfine, "The formation of γ-hydroxy-γ-methylglutamic acid from a common impurity in pyruvic acid," *Biochim. Biophys.. Act.,* vol. 40, pp. 557-559, 1960.

[122] A. Schellenberger and G. Oehme, "Studies on the theory of α - keto acids. VII. Structure and reaction behavior of dimeric α - keto acids," *J. Prakt. Chem.,* vol. 24, pp. 231-238, 1964.

[123] K. Brunner, *Monatsh. Chem.,* vol. 15, p. 751, 1894.

[124] D. R. Sanadi, "The Enzymes," New York, Academic press, 1963, p. 307.

[125] J. D. Lu, "Studies on the metabolism of pyruvic acid in normal and vitamin B1-deficient states," *Biochem. J.,* vol. 33, no. 2, pp. 249-254, 1939.

[126] A. Meister, " Preparation and enzymatic reactions of the keto analogues of asparagine and glutamine," *J. Biol. Chem.,* vol. 200, p. 571, 1953.

[127] P. Karrer and F. Haab, *Chem. Act.,* vol. 32, p. 950, 1949.

[128] W. H. Hatcher and A. C. Hill, *Trans. R. Soc. Can.,* vol. 22, no. 23, pp. 211-213, 1928.

[129] E. Baer, "Oxidative Cleavage of α-Keto Acids and α-Keto Alcohols by Means of Lead Tetra-acetate," *J. Am. Chem. Soc.,* vol. 62, no. 6, pp. 1597-1606, 1940.

[130] B. Siegel and J. Lanphear, "Iron-catalyzed oxidative decarboxylation of benzoylformic acid," *J. Am. Chem. Soc.,* vol. 101, no. 8, pp. 2221-2222, 1979.

[131] A. Meister, "Reduction of α, γ-diektoand α-keto acids catalyzed by muscle preparations and by crystalline lactic dehydrogenase," *J. Bio. Chem.,* vol. 184, pp. 117-130, 1950.

[132] T. R. Evans and P. A. Leermakers, "Photochemistry of .alpha.-keto acids and .alpha.-keto esters VI. V. The Norrish type II process in .alpha.-keto acids. Photolysis of .alpha.-ketodecanoic acid in benzene," *J. Am. Chem. Soc.,* vol. 90, no. 7, pp. 1840-1842, 1968.

[133] J. J. Berzelius, "About the distillation products of grape," *Ann. Phys. (Leipzig),* vol. 112, no. 9, pp. 1-29, 1835.

[134] H. H. Wasserman and W.-B. Ho, "(Cyanomethylene)phosphoranes as Novel Carbonyl 1,1-Dipole Synthons: An Efficient Synthesis of a-Keto Acids, Esters, and Amides," *J. Org. Chem.,* vol. 59, pp. 4364-4366, 1994.

[135] D. Crich and Y. Zhou, "Catalytic Oxidation Adjacent to Carbonyl Groups and at Benzylic Positions with a Fluorous Seleninic Acid in the Presence of Iodoxybenzene," *J. Org. Chem.,* vol. 70, pp. 3309-3311, 2005.

[136] K. Furukawa, H. Inada, M. Shibuya and Y. Yamamoto, "Chemoselective Conversion from α-Hydroxy Acids to α-Keto Acids Enabled by Nitroxyl-Radical-Catalyzed Aerobic Oxidation," *Org. Lett.,* vol. 18, pp. 4230-4233, 2016.

[137] Q. Ke, E. Ferrara, F. Radicchi and A. Flammini, "Defining and Identifying Sleeping Beauties in Science," *Proc. Natl. Acad. Sci. U. S. A.,* vol. 112, pp. 7426-7431, 2015.

[138] L. J. Gooβen, F. Rudolphi, C. Oppel and N. Rodríguez, "Synthesis of Ketones from α-Oxocarboxylates and Aryl Bromides by Bu/ crosscoupling of potassium aryltrifluoroborates with Cross-Coupling," *Angew. Chem. Int. Ed.,* vol. 47, pp. 3043-3045, 2008.

[139] H. Huang, G. Zhang and Chen Y, "Dual Hypervalent Iodine(III) Reagents and Photoredox Catalysis Enable Decarboxylative Ynonylation Under Mild Conditions," *Angew. Chem. Int. Ed.,* vol. 54, pp. 7872-7876, 2015.

[140] G. Jaeschke, J. G. Wettstein, R. E. Nordquist and W. Spooren, "mGlu5 Receptor Antagonists and Their Therapeutic Potential," *Expert Opin. Ther. Pat.,* vol. 18, pp. 123-142, 2008.

[141] R. T. Stanko, D. L. Tietze and J. E. Arch, "Body composition, energy utilization, and nitrogen metabolism with a 4.25-MJ/d low-energy diet supplemented with pyruvate," *Am. J. Clin. Nutr.,* vol. 56, pp. 630-635, 1992.

[142] R. T. Stank, R. J. Robertson, R. W. Galbreath, J. J. Reilly, K. D. Greenawalt and F. L. Gross, "Enhanced leg exercise endurance with a high-carbohydrate diet and dihydroxyacetone and pyruvate," *J. Appl. Physiol.,* vol. 69, pp. 1651-1656, 1990.

[143] R. T. Stanko, H. R. Reynolds, R. Hoyson, J. E. Janosky and R. Wolf, "Pyruvate supplementation of a low-cholesterol, low-fat diet: effects on plasma lipid concentrations and body composition in hyperlipidemic patients," *Am. J. Clin. Nutr.,* vol. 59, pp. 423-427, 1994.

[144] L. W. V. DeBoer, P. A. Bekx, L. Han and L. Steinke, "Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation," *J. Appl. Physiol.,* vol. 265, pp. 1571-1576, 1993.

[145] A. Borle and R. T. Stanko, "Pyruvate reduces anoxic injury and free radical formation in perfused rat hepatocytes," *J. Appl. Physiol.,* vol. 270, pp. 535-540, 1996.

[146] J. W. Howard and W. A. Fraser, "Preparation of pyruvic acid," *Org. Synth. Coll.,* vol. 1, pp. 475-480, 1932.

[147] R. Miyata, H. Tsutsui and T. Yonehara, "Manufacture of pyruvic acid with Tirulopsis species". Japan Patent JP patent 0155185, 2000.

[148] T. Yonehara, R. Miyata, H. Matsuno, M. Goto and S. Yahanda, "Development of fermentative production of pyruvate by metabolic control," *Seibutsu Kogaku Kaishi,* vol. 78, pp. 56-62, 2000.

[149] B. Wang, G. Miller and S. Janagani, "Pyruvate derivatives". USA Patent 6,900,218 B2, 31 May 2005.

[150] R. T. Mallet, "Pyruvate: Metabolic Protector of Cardiac Performance," *Proc. Soc. Exp. Biol. Med,,* vol. 2223, no. 2, pp. 136-148, 2000.

[151] R. T. Stanko, "Method for treating a medical patient for cardiac trauma". USA Patent 5,294,641, 15 March 1994.

[152] H. Brunengraber, C. Bomont, F. David, P. T. Hallowell, K. D. Cooper and T. Kasoumov, "Medical uses of pyruvates". USA Patent 6,086,789, 11 July 2000.

[153] H. Brunengraber, C. Bomont, F. David and P. T. Hallowell, "Pyruvate compounds and methods of use thereof". USA Patent 5,968,727, 19 October 1999.

[154] R. H. Miller, N. L. Parlet, K. L. Cipollo, M. K. Dhaon, J. K. Houbion and E. O. Lundell, "Method for reducing insulin in mammals". USA Patent 5,283,260, 1 Feb 1994.

[155] R. Yang, D. J. Gallo, J. J. Baust, T. Uchiyama, S. K. Watkins and M. P. Fink, "Ethyl pyruvate modulates inflammatory gene expression in mice subjected to hemorrhagic shock," *Am. J. Physiol. Gastrointest. Liver Physiol.,* vol. 283, no. 1, pp. G212-G221, 2002.

[156] R. Yang, T. Uchiyama, S. M. Alber, X. Han, S. K. Watkins, R. L. Delude and M. P. Fink, "Ethyl pyruvate ameliorates distant organ injury in a murine model of acute necrotizing pancreatitis," *Crit. Care Med.,* vol. 32, no. 7, pp. 1453-1459, 2004.

[157] Y. M. Yu, J. B. Kim, K. W. Lee, S. Y. Kim, P. L. Han and J. K. Lee, "Inhibition of the Cerebral Ischemic Injury by Ethyl Pyruvate With a Wide Therapeutic Window," *Stroke,* vol. 36, no. 10, pp. 2238-2243, 2005.

[158] L. Ulloa, M. Ochani, H. Yang, M. Tanovic, D. Halperin, R. Yang, C. J. Czura, M. P. Fink and K. J. Tracey, "Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation," *Proc. Natl. Acad. Sci. USA,* vol. 99, no. 19, pp. 12351-12356, 2002.

[159] S. Sugiyama, S. Fukunaga, K. Ito, S. Ohigashi and H. Hayashi, "Catalysts for vapor-phase dehydration of ethylene glycol and their application to pyruvic acid synthesis," *J. Catal.,* vol. 129, no. 1, pp. 12-18, 1991.

[160] T. Lu, J. Zou, Y. Zhan, X. Yang, Y. Wen, X. Wang, L. Zhou and J. Xu, "Highly Efficient Oxidation of Ethyl Lactate to Ethyl Pyruvate Catalyzed by TS-1 Under Mild Conditions," *ACS Catal.,* vol. 8, pp. 1287-1296, 2018.

[161] W. Zhang, G. Innocenti, P. Oulego, V. Gitis, H. Wu, B. Ensing, F. Cavani, G. Rothenberg and N. R. Shiju, "Highly Selective Oxidation of Ethyl Lactate to Ethyl Pyruvate Catalyzed by Mesoporous Vanadia−Titania," *ACS Catal.,* vol. 8, pp. 2365-2374, 2018.

[162] C. A. Sims, S. Wattanasirichaigoon, M. J. Menconi, A. M. Ajami and M. P. Fink, "Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats," *Crit. Care Med.,* vol. 29, no. 8, pp. 1513-1518, 2001.

[163] S. D. Varma, P. S. Devamanoharan and A. H. Ali, "Prevention of intracellular oxidative stress to lens by pyruvate and its ester," *Free Rad. Res.,* vol. 28, no. 2, pp. 131-135, 1998.

[164] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care," *Crit. Care Med.,* vol. 29, no. 7, pp. 1303-1310, 2001.

[165] J. C. Marshall, "Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome," *Crit. Care Med.,* vol. 29, no. 7, pp. S99-S106, 2001.

[166] M. P. Fink, "Reactive oxygen species as mediators of organ dysfunction caused by sepsis, acute respiratory distress syndrome, or hemorrhagic shock: potential benefits of resuscitation with Ringer's ethyl pyruvate solution," *Curr. Opin. Clin. Nutr. Metab. Care,* vol. 5, no. 2, pp. 167-174, 2002.

[167] Z. Tawadrous, R. L. Delude and M. P. Fink, "Resuscitation from Hemorrhagic Shock with Ringer's Ethyl Pyruvate Solution Improves Survival And Ameliorates Intestinal Mucosal Hyperpermeability in Rats," *Shock,* vol. 17, no. 6, pp. 473-477, 2002.

[168] A. J. Muller, J. B. DuHadaway, D. Jaller, P. Curtis, R. Metz and G. C. Prendergast, "Immunotherapeutic Suppression of Indoleamine 2,3-Dioxygenase and Tumor Growth with Ethyl Pyruvate," *Caner Res.,* vol. 70, no. 5, pp. 1845-1853, 2010.

[169] W. J. Gensler and E. Berman, "Decarboxylative Condensation. α-Alkylcinnamic Acids from Aromatic Aldehydes and Alkylmalonic Acids," *J. Am. Chem. Soc.,* vol. 80, no. 18, pp. 4949-4954, 1958.

[170] W. J. Stec, "Wadsworth-Emmons reaction revisited," *Acc. Chem. Res.,* vol. 16, no. 11, pp. 411-417, 1983.

[171] B. Neises and D. W. Steglich, "Simple Method for the Esterification of Carboxylic Acids," *Angew. Chem. Int. Ed,* vol. 17, no. 7, pp. 522-524, 1978.

[172] N. G. Bazan and J. Gomez, "Process for preparing N-acylated 4-hydroxyphenylamine derivatives with analgesic properties". United States Patent 5,621,110, 15 April 1997.

APPENDIX

Crystal data for compound 23b

Empirical formula	$C_{18}H_{15}Cl_2O_6S$
Formula weight	422.83 g mol^{-1}
Temperature	90 K
Cu Kα radiation, λ	1.54184 Å
Crystal system	Orthorhombic, $P2_12_12_1$
Unit cell dimensions	a = 4.793 (2) Å
	b = 10.7214(5) Å
	c = 35.1362 (15) Å
Volume	1807.19 (14) $Å^3$
Z	4
F (000)	872
Density, D_x	1.554 Mg m^{-3}
Absorption coefficient, μ	3.32 mm^{-1}
Glancing angle, θ	2.5–69.2°
Crystal size	0.38 x 0.04 x 0.02 mm
Physical appearance	Needle, colorless
Reflections	7594

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($Å^2$)

	X	y	z	Uiso*/Ueq
Cl1	0.1699 (2)	0.18375 (15)	0.66169 (3)	0.0475 (4)
S1	0.41916 (18)	0.93409 (8)	0.33766 (2)	0.0171 (2)
O1	0.2404 (7)	0.5987 (3)	0.32286 (9)	0.0348 (8)
O2	0.5969 (6)	0.9658 (3)	0.36869 (7)	0.0223 (6)
O3	0.2181 (6)	1.0249 (3)	0.32606 (7)	0.0219 (6)
O4	0.4988 (5)	0.6870 (3)	0.40902 (8)	0.0220 (6)
O5	0.4861 (5)	0.4160 (2)	0.56660 (7)	0.0162 (5)
O6	0.1861 (6)	0.2545 (3)	0.56434 (7)	0.0220 (6)
N1	0.2633 (6)	0.7966 (3)	0.34577 (8)	0.0181 (7)
N2	0.0749 (6)	0.6470 (3)	0.43556 (8)	0.0143 (6)
H2N	-0.092 (5)	0.663 (4)	0.4328 (12)	0.017*
C1	0.3330 (8)	0.6971 (4)	0.32104 (10)	0.0221 (9)
C2	0.5372 (7)	0.7492 (4)	0.29226 (10)	0.0178 (7)
C3	0.5967 (7)	0.8740 (3)	0.29797 (10)	0.0156 (7)
C4	0.7692 (7)	0.9412 (4)	0.27388 (10)	0.0178 (7)
H4	0.8083	1.0269	0.2782	0.021*
C5	0.8834 (8)	0.8772 (4)	0.24288 (10)	0.0219 (8)
H5	1.0025	0.9200	0.2257	0.026*
C6	0.8250 (9)	0.7530 (4)	0.23700 (10)	0.0236 (8)
H6	0.9042	0.7113	0.2157	0.028*
C7	0.6522 (8)	0.6875 (4)	0.26170 (11)	0.0228 (8)
H7	0.6141	0.6016	0.2575	0.027*
C8	0.0922 (8)	0.7755 (4)	0.37926 (10)	0.0212 (8)
H8A	-0.0812	0.7321	0.3716	0.025*
H8B	0.0397	0.8569	0.3905	0.025*
C9	0.2446 (7)	0.6973 (4)	0.40930 (10)	0.0154 (7)
C10	0.1705 (7)	0.5830 (3)	0.46866 (9)	0.0135 (7)
C11	0.0632 (8)	0.6143 (3)	0.50398 (10)	0.0174 (7)
H11	-0.0777	0.6763	0.5058	0.021*
C12	0.1598 (8)	0.5558 (4)	0.53683 (10)	0.0172 (7)
H12	0.0855	0.5767	0.5611	0.021*
C13	0.3664 (7)	0.4667 (3)	0.53331 (9)	0.0147 (7)
C14	0.4713 (7)	0.4314 (4)	0.49841 (10)	0.0181 (7)
H14	0.6092	0.3681	0.4967	0.022*
C15	0.3729 (8)	0.4897 (3)	0.46556 (10)	0.0167 (7)
H15	0.4428	0.4663	0.4413	0.020*
C16	0.3845 (8)	0.3036 (3)	0.57833 (9)	0.0147 (7)
C17	0.5638 (8)	0.2532 (4)	0.60987 (10)	0.0191 (8)
H17A	0.7490	0.2314	0.5994	0.023*
H17B	0.5914	0.3194	0.6291	0.023*
C18	0.4432 (9)	0.1403 (4)	0.62901 (12)	0.0280 (9)
H18A	0.5921	0.0958	0.6430	0.034*
H18B	0.3672	0.0830	0.6095	0.034*

Atomic displacement parameters ($Å^2$)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
Cl1	0.0232 (5)	0.0896 (10)	0.0297 (5)	-0.0022 (6)	-0.0033 (4)	0.0278 (6)
S1	0.0176 (4)	0.0203 (4)	0.0133 (4)	0.0019 (4)	-0.0004 (3)	0.0023 (4)

O1	0.0450 (19)	0.0235 (17)	0.0360 (17)	-0.0023 (15)	0.0029 (15)	0.0060 (13)
O2	0.0233 (13)	0.0289 (15)	0.0147 (12)	0.0032 (12)	-0.0029 (11)	-0.0020 (10)
O3	0.0238 (14)	0.0206 (14)	0.0215 (13)	0.0067 (11)	-0.0025 (11)	0.0007 (10)
O4	0.0107 (13)	0.0298 (15)	0.0256 (13)	0.0024 (11)	0.0002 (10)	0.0100 (12)
O5	0.0197 (12)	0.0161 (13)	0.0129 (11)	-0.0005 (10)	-0.0035 (9)	0.0038 (10)
O6	0.0260 (14)	0.0201 (14)	0.0199 (13)	-0.0068 (12)	-0.0079 (11)	0.0039 (11)
N1	0.0192 (14)	0.0203 (16)	0.0147 (15)	-0.0016 (12)	0.0010 (12)	0.0055 (12)
N2	0.0101 (13)	0.0184 (15)	0.0145 (14)	0.0020 (12)	-0.0020 (12)	0.0027 (12)
C1	0.0134 (17)	0.040 (3)	0.0127 (16)	-0.0007 (17)	-0.0042 (14)	0.0001 (16)
C2	0.0179 (17)	0.0188 (18)	0.0169 (16)	0.0000 (15)	-0.0043 (14)	0.0039 (15)
C3	0.0165 (16)	0.0179 (18)	0.0125 (15)	0.0027 (15)	-0.0020 (14)	0.0028 (13)
C4	0.0204 (17)	0.0153 (17)	0.0179 (17)	0.0001 (15)	-0.0026 (14)	0.0006 (15)
C5	0.0210 (18)	0.030 (2)	0.0148 (17)	0.0024 (17)	0.0005 (15)	0.0031 (15)
C6	0.0246 (19)	0.030 (2)	0.0161 (17)	0.0054 (18)	0.0011 (15)	-0.0071 (16)
C7	0.025 (2)	0.0182 (19)	0.0255 (19)	-0.0003 (17)	-0.0049 (16)	-0.0052 (16)
C8	0.0148 (17)	0.029 (2)	0.0199 (17)	0.0041 (16)	0.0033 (15)	0.0109 (16)
C9	0.0157 (17)	0.0174 (18)	0.0132 (16)	-0.0003 (14)	-0.0009 (13)	0.0050 (14)
C10	0.0117 (15)	0.0147 (17)	0.0142 (16)	-0.0004 (13)	-0.0008 (13)	0.0020 (13)
C11	0.0182 (17)	0.0171 (17)	0.0169 (17)	0.0036 (15)	0.0009 (15)	0.0029 (14)
C12	0.0200 (16)	0.0196 (18)	0.0119 (15)	0.0010 (15)	0.0029 (13)	-0.0004 (14)
C13	0.0164 (16)	0.0158 (18)	0.0120 (16)	-0.0041 (13)	-0.0043 (13)	0.0039 (13)
C14	0.0197 (17)	0.0178 (18)	0.0167 (16)	0.0067 (15)	-0.0011 (14)	0.0005 (15)
C15	0.0208 (18)	0.0180 (18)	0.0115 (16)	0.0028 (15)	0.0020 (14)	-0.0004 (13)
C16	0.0197 (18)	0.0114 (16)	0.0131 (15)	0.0027 (15)	0.0018 (14)	-0.0014 (13)
C17	0.0190 (17)	0.0205 (19)	0.0180 (17)	0.0023 (17)	-0.0041 (14)	0.0034 (15)
C18	0.029 (2)	0.027 (2)	0.028 (2)	-0.0016 (19)	-0.0092 (18)	0.0103 (17)

Geometric parameters (Å, º)

Cl1—C18	1.804 (5)	C6—C7	1.390 (6)
S1—O2	1.425 (3)	C6—H6	0.9500
S1—O3	1.430 (3)	C7—H7	0.9500
S1—N1	1.677 (3)	C8—C9	1.534 (5)
S1—C3	1.756 (4)	C8—H8A	0.9900
O1—C1	1.146 (5)	C8—H8B	0.9900
O4—C9	1.225 (4)	C10—C11	1.384 (5)
O5—C16	1.363 (4)	C10—C15	1.398 (5)
O5—C13	1.412 (4)	C11—C12	1.393 (5)
O6—C16	1.194 (5)	C11—H11	0.9500
N1—C1	1.416 (5)	C12—C13	1.383 (5)
N1—C8	1.452 (4)	C12—H12	0.9500
N2—C9	1.344 (5)	C13—C14	1.378 (5)
N2—C10	1.426 (4)	C14—C15	1.395 (5)
N2—H2N	0.82 (2)	C14—H14	0.9500
C1—C2	1.514 (5)	C15—H15	0.9500
C2—C7	1.376 (5)	C16—C17	1.503 (5)
C2—C3	1.384 (5)	C17—C18	1.501 (6)
C3—C4	1.386 (5)	C17—H17A	0.9900
C4—C5	1.399 (5)	C17—H17B	0.9900
C4—H4	0.9500	C18—H18A	0.9900
C5—C6	1.377 (6)	C18—H18B	0.9900
C5—H5	0.9500		

O2—S1—O3	117.34 (17)	C9—C8—H8B	109.2
O2—S1—N1	110.26 (16)	H8A—C8—H8B	107.9
O3—S1—N1	110.25 (17)	O4—C9—N2	124.9 (3)
O2—S1—C3	113.88 (17)	O4—C9—C8	121.2 (3)
O3—S1—C3	110.51 (16)	N2—C9—C8	113.8 (3)
N1—S1—C3	91.65 (16)	C11—C10—C15	120.1 (3)
C16—O5—C13	116.5 (3)	C11—C10—N2	119.7 (3)
C1—N1—C8	120.9 (3)	C15—C10—N2	120.2 (3)
C1—N1—S1	116.9 (3)	C10—C11—C12	120.7 (3)
C8—N1—S1	121.8 (3)	C10—C11—H11	119.7
C9—N2—C10	123.9 (3)	C12—C11—H11	119.7
C9—N2—H2N	115 (3)	C13—C12—C11	118.4 (3)
C10—N2—H2N	120 (3)	C13—C12—H12	120.8
O1—C1—N1	124.6 (4)	C11—C12—H12	120.8
O1—C1—C2	128.8 (4)	C14—C13—C12	122.1 (3)
N1—C1—C2	106.5 (3)	C14—C13—O5	118.9 (3)
C7—C2—C3	119.7 (4)	C12—C13—O5	118.9 (3)
C7—C2—C1	127.1 (4)	C13—C14—C15	119.3 (3)
C3—C2—C1	113.2 (3)	C13—C14—H14	120.3
C2—C3—C4	122.5 (3)	C15—C14—H14	120.3
C2—C3—S1	111.7 (3)	C14—C15—C10	119.4 (3)
C4—C3—S1	125.7 (3)	C14—C15—H15	120.3
C3—C4—C5	117.0 (4)	C10—C15—H15	120.3
C3—C4—H4	121.5	O6—C16—O5	123.4 (3)
C5—C4—H4	121.5	O6—C16—C17	127.0 (3)
C6—C5—C4	120.8 (4)	O5—C16—C17	109.6 (3)
C6—C5—H5	119.6	C18—C17—C16	113.5 (3)
C4—C5—H5	119.6	C18—C17—H17A	108.9
C5—C6—C7	121.1 (4)	C16—C17—H17A	108.9
C5—C6—H6	119.5	C18—C17—H17B	108.9
C7—C6—H6	119.5	C16—C17—H17B	108.9
C2—C7—C6	118.9 (4)	H17A—C17—H17B	107.7
C2—C7—H7	120.5	C17—C18—Cl1	110.9 (3)
C6—C7—H7	120.5	C17—C18—H18A	109.5
N1—C8—C9	111.9 (3)	Cl1—C18—H18A	109.5
N1—C8—H8A	109.2	C17—C18—H18B	109.5
C9—C8—H8A	109.2	Cl1—C18—H18B	109.5
N1—C8—H8B	109.2	H18A—C18—H18B	108.0

O2—S1—N1—C1	-114.8 (3)	C3—C2—C7—C6	-0.4 (5)
O3—S1—N1—C1	114.0 (3)	C1—C2—C7—C6	176.5 (3)
C3—S1—N1—C1	1.4 (3)	C5—C6—C7—C2	0.5 (6)
O2—S1—N1—C8	57.6 (3)	C1—N1—C8—C9	68.3 (4)
O3—S1—N1—C8	-73.5 (3)	S1—N1—C8—C9	-103.8 (3)
C3—S1—N1—C8	173.9 (3)	C10—N2—C9—O4	5.5 (6)
C8—N1—C1—O1	8.5 (6)	C10—N2—C9—C8	-172.4 (3)
S1—N1—C1—O1	-179.0 (3)	N1—C8—C9—O4	19.2 (6)
C8—N1—C1—C2	-173.7 (3)	N1—C8—C9—N2	-162.7 (3)
S1—N1—C1—C2	-1.2 (4)	C9—N2—C10—C11	130.4 (4)
O1—C1—C2—C7	0.7 (7)	C9—N2—C10—C15	-49.2 (5)
N1—C1—C2—C7	-176.9 (4)	C15—C10—C11—C12	1.6 (6)

O1—C1—C2—C3	177.8 (4)	N2—C10—C11—C12	-178.0 (3)
N1—C1—C2—C3	0.1 (4)	C10—C11—C12—C13	0.5 (6)
C7—C2—C3—C4	0.2 (5)	C11—C12—C13—C14	-2.3 (6)
C1—C2—C3—C4	-177.2 (3)	C11—C12—C13—O5	173.6 (3)
C7—C2—C3—S1	178.2 (3)	C16—O5—C13—C14	-85.8 (4)
C1—C2—C3—S1	0.8 (4)	C16—O5—C13—C12	98.2 (4)
O2—S1—C3—C2	111.8 (3)	C12—C13—C14—C15	2.0 (6)
O3—S1—C3—C2	-113.6 (3)	O5—C13—C14—C15	-173.9 (3)
N1—S1—C3—C2	-1.3 (3)	C13—C14—C15—C10	0.2 (6)
O2—S1—C3—C4	-70.3 (4)	C11—C10—C15—C14	-1.9 (6)
O3—S1—C3—C4	64.3 (4)	N2—C10—C15—C14	177.6 (3)
N1—S1—C3—C4	176.7 (3)	C13—O5—C16—O6	-7.8 (5)
C2—C3—C4—C5	0.1 (5)	C13—O5—C16—C17	170.7 (3)
S1—C3—C4—C5	-177.6 (3)	O6—C16—C17—C18	-11.0 (6)
C3—C4—C5—C6	-0.1 (5)	O5—C16—C17—C18	170.6 (3)
C4—C5—C6—C7	-0.2 (6)	C16—C17—C18—Cl1	-77.7 (4)

Hydrogen-bond geometry (Å, º)

D—H...*A*	*D*—H	H...*A*	*D*...*A*	*D*—H...*A*
N2—H2*N*...O4^{i}	0.82 (2)	2.15 (3)	2.948 (4)	163 (4)

Symmetry code: (i) *x*−1, *y*, *z*.

Crystal structure data for compound 29

Empirical formula	$C_{22}H_{26}ClNO_{11}$
Formula weight	515.89 g mol^{-1}
Crystal system	Triclinic, P1
Unit cell dimensions	a = 5.6938 (2) Å
	b = 9.5493 (3) Å
	c = 23.0194 (10) Å
	α = 80.255 (3)°
	β = 84.336 (3)°
	γ = 74.482 (3)°
Volume	1186.04 (8) $Å^3$
Z	2
F(000)	540
Density, D_x	1.445 Mg m^3
Reflections	4969
Cu Kα radiation, γ	1.54184 Å
Absorption coefficient, μ	1.98 mm^{-1}
Glancing angle, θ	3.9–67.9°
Temperature	100 K
Crystal appearance	Lath, colorless
Crystal dimensions	0.31 x 0.12 x 0.01 mm

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($Å^2$)

	x	*y*	*z*	U_{iso}*/U_{eq}
Cl1	-0.7062 (2)	1.00327 (13)	0.50527 (6)	0.0312 (3)
O1	0.4826 (6)	0.6306 (4)	0.77619 (15)	0.0263 (8)
O2	0.6996 (6)	0.3340 (4)	0.81977 (15)	0.0244 (7)
O3	0.7625 (6)	0.2967 (4)	0.94607 (16)	0.0255 (7)
O4	0.2796 (6)	0.4222 (4)	1.00594 (15)	0.0255 (7)
O5	0.2860 (6)	0.6612 (4)	0.86546 (15)	0.0261 (8)
O6	-0.1918 (7)	0.7809 (4)	0.92324 (16)	0.0310 (8)
O7	1.0924 (7)	0.3215 (4)	0.83128 (19)	0.0372 (9)
O8	0.5915 (7)	0.1134 (4)	0.98913 (18)	0.0365 (9)
O9	0.5555 (8)	0.4408 (5)	1.06609 (18)	0.0418 (10)
O10	-0.0614 (7)	0.9829 (4)	0.88502 (18)	0.0355 (9)
O11	-0.3347 (7)	0.8003 (4)	0.57867 (16)	0.0314 (8)
N1	-0.1986 (8)	0.9709 (4)	0.61503 (19)	0.0238 (9)
H1N	-0.190 (11)	1.059 (5)	0.612 (2)	0.029*
C1	0.3965 (9)	0.5551 (5)	0.8286 (2)	0.0232 (10)
H1	0.2763	0.5031	0.8198	0.028*
C2	0.6153 (9)	0.4463 (5)	0.8574 (2)	0.0233 (10)
H2	0.7466	0.4963	0.8600	0.028*
C3	0.5412 (9)	0.3774 (5)	0.9182 (2)	0.0232 (10)
H3	0.4418	0.3079	0.9142	0.028*
C4	0.3936 (9)	0.4927 (5)	0.9549 (2)	0.0234 (10)
H4	0.5002	0.5495	0.9669	0.028*
C5	0.1851 (9)	0.5953 (5)	0.9195 (2)	0.0256 (11)
H5	0.0763	0.5366	0.9100	0.031*
C6	0.0363 (10)	0.7155 (6)	0.9522 (2)	0.0334 (13)
H6A	0.0029	0.6747	0.9938	0.040*
H6B	0.1268	0.7910	0.9517	0.040*
C7	0.9415 (10)	0.2832 (5)	0.8091 (2)	0.0285 (11)
C8	0.9947 (11)	0.1763 (6)	0.7659 (3)	0.0342 (12)
H8A	1.0625	0.2201	0.7285	0.051*
H8B	0.8434	0.1528	0.7590	0.051*
H8C	1.1129	0.0861	0.7819	0.051*
C9	0.7644 (10)	0.1653 (6)	0.9804 (2)	0.0273 (11)
C10	1.0094 (11)	0.0966 (6)	1.0043 (3)	0.0378 (13)
H10A	1.0060	0.0045	1.0305	0.057*
H10B	1.0525	0.1640	1.0266	0.057*
H10C	1.1311	0.0761	0.9716	0.057*
C11	0.3745 (10)	0.4047 (5)	1.0592 (2)	0.0282 (11)
C12	0.2280 (11)	0.3298 (6)	1.1066 (2)	0.0350 (13)
H12A	0.3028	0.2236	1.1126	0.053*
H12B	0.0607	0.3487	1.0945	0.053*
H12C	0.2252	0.3683	1.1436	0.053*
C13	-0.2142 (10)	0.9146 (6)	0.8899 (2)	0.0313 (12)
C14	-0.4503 (11)	0.9640 (6)	0.8605 (3)	0.0393 (14)
H14A	-0.5842	0.9550	0.8899	0.059*
H14B	-0.4471	0.9026	0.8303	0.059*
H14C	-0.4750	1.0669	0.8419	0.059*
C15	0.3047 (9)	0.7108 (5)	0.7371 (2)	0.0240 (10)
C16	0.1213 (10)	0.6551 (5)	0.7224 (2)	0.0285 (11)

H16	0.1104	0.5595	0.7403	0.034*
C17	-0.0459 (10)	0.7371 (5)	0.6821 (2)	0.0252 (10)
H17	-0.1717	0.6983	0.6724	0.030*
C18	-0.0291 (9)	0.8783 (5)	0.6554 (2)	0.0218 (10)
C19	0.1549 (10)	0.9332 (6)	0.6707 (2)	0.0272 (11)
H19	0.1674	1.0285	0.6528	0.033*
C20	0.3199 (9)	0.8513 (5)	0.7114 (2)	0.0261 (11)
H20	0.4435	0.8908	0.7219	0.031*
C21	-0.3351 (10)	0.9285 (5)	0.5801 (2)	0.0245 (10)
C22	-0.4925 (10)	1.0582 (5)	0.5416 (2)	0.0289 (11)
H22A	-0.3866	1.1038	0.5117	0.035*
H22B	-0.5809	1.1330	0.5663	0.035*
Cl2	-0.5533 (2)	0.31244 (14)	0.66050 (6)	0.0367 (3)
O12	0.7046 (6)	0.5089 (4)	0.40215 (15)	0.0272 (8)
O13	0.7343 (6)	0.7914 (4)	0.35463 (15)	0.0258 (8)
O14	0.8760 (6)	0.8247 (4)	0.22857 (15)	0.0258 (8)
O15	0.5657 (6)	0.6921 (4)	0.16795 (15)	0.0276 (8)
O16	0.6617 (7)	0.4608 (4)	0.31063 (15)	0.0285 (8)
O17	0.3916 (7)	0.3178 (4)	0.25014 (17)	0.0342 (9)
O18	1.1230 (7)	0.8094 (4)	0.34078 (18)	0.0356 (9)
O19	0.5747 (7)	1.0042 (4)	0.18398 (18)	0.0381 (9)
O20	0.9071 (7)	0.6781 (5)	0.10762 (17)	0.0382 (9)
O21	0.6816 (8)	0.1321 (4)	0.29495 (18)	0.0400 (10)
O22	-0.0867 (7)	0.2630 (4)	0.58902 (16)	0.0315 (9)
N2	-0.0767 (8)	0.5040 (5)	0.56905 (19)	0.0261 (9)
H2N	-0.135 (10)	0.587 (5)	0.579 (3)	0.031*
C23	0.6287 (9)	0.5742 (5)	0.3455 (2)	0.0242 (10)
H23	0.4528	0.6294	0.3478	0.029*
C24	0.7832 (10)	0.6774 (5)	0.3180 (2)	0.0247 (10)
H24	0.9603	0.6243	0.3172	0.030*
C25	0.7040 (9)	0.7436 (5)	0.2559 (2)	0.0237 (10)
H25	0.5370	0.8117	0.2579	0.028*
C26	0.7032 (10)	0.6248 (6)	0.2199 (2)	0.0251 (11)
H26	0.8734	0.5714	0.2085	0.030*
C27	0.5622 (10)	0.5181 (6)	0.2542 (2)	0.0289 (11)
H27	0.3869	0.5718	0.2599	0.035*
C28	0.5779 (11)	0.3896 (6)	0.2232 (2)	0.0355 (13)
H28A	0.5516	0.4238	0.1807	0.043*
H28B	0.7414	0.3201	0.2271	0.043*
C29	0.9255 (10)	0.8414 (6)	0.3653 (2)	0.0283 (11)
C30	0.8543 (11)	0.9361 (6)	0.4127 (3)	0.0366 (13)
H30A	0.8860	0.8750	0.4513	0.055*
H30B	0.6801	0.9863	0.4113	0.055*
H30C	0.9500	1.0094	0.4065	0.055*
C31	0.7857 (10)	0.9558 (5)	0.1942 (2)	0.0274 (11)
C32	0.9889 (11)	1.0265 (6)	0.1720 (3)	0.0368 (13)
H32A	0.9264	1.1162	0.1442	0.055*
H32B	1.1176	0.9582	0.1518	0.055*
H32C	1.0559	1.0515	0.2053	0.055*
C33	0.6862 (10)	0.7126 (6)	0.1150 (2)	0.0270 (11)
C34	0.5109 (10)	0.7885 (6)	0.0689 (2)	0.0326 (12)
H34A	0.4874	0.8951	0.0654	0.049*

H34B	0.3541	0.7642	0.0799	0.049*
H34C	0.5753	0.7564	0.0310	0.049*
C35	0.4721 (11)	0.1927 (6)	0.2877 (3)	0.0352 (13)
C36	0.2583 (11)	0.1405 (7)	0.3189 (3)	0.0394 (14)
H36A	0.3184	0.0488	0.3458	0.059*
H36B	0.1591	0.1233	0.2897	0.059*
H36C	0.1587	0.2155	0.3416	0.059*
C37	0.5096 (9)	0.5037 (5)	0.4437 (2)	0.0240 (10)
C38	0.3782 (10)	0.4004 (5)	0.4462 (2)	0.0261 (11)
H38	0.4220	0.3304	0.4197	0.031*
C39	0.1826 (9)	0.3980 (5)	0.4870 (2)	0.0271 (11)
H39	0.0902	0.3282	0.4879	0.033*
C40	0.1229 (9)	0.4987 (5)	0.5267 (2)	0.0239 (10)
C41	0.2536 (10)	0.6036 (6)	0.5240 (2)	0.0291 (11)
H41	0.2101	0.6735	0.5505	0.035*
C42	0.4495 (10)	0.6065 (6)	0.4822 (2)	0.0275 (11)
H42	0.5399	0.6777	0.4803	0.033*
C43	-0.1665 (9)	0.3916 (5)	0.5971 (2)	0.0252 (11)
C44	-0.3681 (10)	0.4395 (6)	0.6425 (3)	0.0308 (12)
H44A	-0.4713	0.5375	0.6270	0.037*
H44B	-0.2961	0.4483	0.6787	0.037*

Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
Cl1	0.0314 (7)	0.0264 (6)	0.0369 (7)	-0.0049 (5)	-0.0084 (5)	-0.0080 (5)
O1	0.0244 (19)	0.0255 (18)	0.0278 (19)	-0.0068 (15)	-0.0015 (14)	-0.0004 (14)
O2	0.0250 (18)	0.0207 (17)	0.0271 (18)	-0.0029 (14)	0.0000 (14)	-0.0080 (14)
O3	0.0206 (18)	0.0226 (17)	0.0320 (19)	-0.0024 (14)	-0.0057 (14)	-0.0029 (14)
O4	0.0256 (18)	0.0283 (18)	0.0231 (18)	-0.0073 (15)	-0.0020 (14)	-0.0041 (14)
O5	0.028 (2)	0.0201 (17)	0.0264 (19)	-0.0002 (15)	-0.0004 (14)	-0.0031 (14)
O6	0.026 (2)	0.0266 (19)	0.036 (2)	-0.0023 (16)	0.0013 (16)	-0.0025 (15)
O7	0.024 (2)	0.030 (2)	0.059 (3)	-0.0093 (17)	0.0035 (18)	-0.0127 (18)
O8	0.030 (2)	0.031 (2)	0.046 (2)	-0.0104 (17)	-0.0003 (17)	0.0034 (17)
O9	0.048 (3)	0.051 (3)	0.035 (2)	-0.029 (2)	-0.0115 (19)	0.0006 (19)
O10	0.033 (2)	0.034 (2)	0.042 (2)	-0.0132 (18)	-0.0041 (17)	-0.0035 (17)
O11	0.043 (2)	0.0182 (18)	0.035 (2)	-0.0059 (16)	-0.0099 (17)	-0.0059 (14)
N1	0.025 (2)	0.016 (2)	0.031 (2)	-0.0063 (18)	-0.0074 (18)	-0.0009 (17)
C1	0.020 (2)	0.019 (2)	0.029 (3)	-0.0005 (19)	-0.0006 (19)	-0.0077 (19)
C2	0.026 (3)	0.015 (2)	0.030 (3)	-0.004 (2)	-0.002 (2)	-0.0078 (19)
C3	0.017 (2)	0.023 (3)	0.028 (3)	-0.001 (2)	-0.0051 (19)	-0.005 (2)
C4	0.022 (3)	0.023 (2)	0.024 (3)	-0.004 (2)	-0.0004 (19)	-0.0037 (19)
C5	0.023 (3)	0.025 (3)	0.026 (3)	-0.002 (2)	0.000 (2)	-0.004 (2)
C6	0.030 (3)	0.032 (3)	0.033 (3)	0.003 (2)	-0.005 (2)	-0.006 (2)
C7	0.032 (3)	0.019 (2)	0.030 (3)	-0.006 (2)	-0.001 (2)	0.004 (2)
C8	0.035 (3)	0.031 (3)	0.035 (3)	-0.006 (2)	0.005 (2)	-0.007 (2)
C9	0.031 (3)	0.024 (3)	0.026 (3)	-0.004 (2)	-0.005 (2)	-0.004 (2)
C10	0.037 (3)	0.034 (3)	0.039 (3)	0.001 (3)	-0.016 (3)	-0.002 (2)
C11	0.037 (3)	0.021 (2)	0.028 (3)	-0.007 (2)	-0.007 (2)	-0.004 (2)
C12	0.038 (3)	0.034 (3)	0.032 (3)	-0.011 (3)	0.000 (2)	0.001 (2)
C13	0.032 (3)	0.027 (3)	0.032 (3)	-0.001 (2)	-0.001 (2)	-0.008 (2)
C14	0.029 (3)	0.032 (3)	0.057 (4)	-0.009 (3)	-0.013 (3)	0.001 (3)

C15	0.026 (3)	0.020 (2)	0.023 (3)	-0.001 (2)	-0.003 (2)	-0.0014 (19)
C16	0.033 (3)	0.018 (2)	0.034 (3)	-0.006 (2)	-0.005 (2)	-0.004 (2)
C17	0.029 (3)	0.022 (3)	0.026 (3)	-0.008 (2)	-0.003 (2)	-0.003 (2)
C18	0.022 (3)	0.018 (2)	0.024 (2)	-0.001 (2)	-0.0015 (19)	-0.0054 (18)
C19	0.030 (3)	0.023 (3)	0.031 (3)	-0.008 (2)	-0.003 (2)	-0.006 (2)
C20	0.026 (3)	0.022 (3)	0.033 (3)	-0.010 (2)	-0.001 (2)	-0.005 (2)
C21	0.031 (3)	0.019 (2)	0.022 (2)	-0.004 (2)	-0.001 (2)	-0.0038 (18)
C22	0.035 (3)	0.019 (2)	0.032 (3)	0.000 (2)	-0.012 (2)	-0.008 (2)
Cl2	0.0351 (7)	0.0325 (7)	0.0451 (8)	-0.0143 (6)	0.0095 (6)	-0.0101 (6)
O12	0.0262 (19)	0.0301 (19)	0.0247 (19)	-0.0078 (15)	-0.0007 (14)	-0.0015 (14)
O13	0.0211 (18)	0.0265 (18)	0.033 (2)	-0.0087 (15)	-0.0023 (14)	-0.0099 (15)
O14	0.0259 (19)	0.0236 (18)	0.0302 (19)	-0.0122 (15)	-0.0003 (15)	-0.0018 (14)
O15	0.0284 (19)	0.0315 (19)	0.0255 (19)	-0.0115 (16)	-0.0007 (15)	-0.0054 (15)
O16	0.038 (2)	0.0242 (18)	0.0272 (19)	-0.0135 (16)	-0.0027 (15)	-0.0048 (14)
O17	0.040 (2)	0.033 (2)	0.034 (2)	-0.0158 (18)	-0.0082 (17)	-0.0023 (16)
O18	0.026 (2)	0.029 (2)	0.054 (3)	-0.0076 (16)	-0.0023 (18)	-0.0102 (18)
O19	0.038 (2)	0.029 (2)	0.046 (2)	-0.0095 (18)	-0.0080 (18)	0.0017 (17)
O20	0.030 (2)	0.047 (2)	0.032 (2)	-0.0057 (19)	0.0036 (16)	-0.0028 (17)
O21	0.041 (3)	0.036 (2)	0.045 (2)	-0.0137 (19)	-0.0050 (19)	-0.0042 (18)
O22	0.036 (2)	0.0209 (19)	0.036 (2)	-0.0077 (16)	0.0056 (16)	-0.0044 (15)
N2	0.029 (2)	0.016 (2)	0.034 (2)	-0.0068 (18)	0.0015 (18)	-0.0061 (17)
C23	0.027 (3)	0.022 (2)	0.025 (3)	-0.007 (2)	-0.001 (2)	-0.0050 (19)
C24	0.030 (3)	0.022 (2)	0.025 (3)	-0.011 (2)	0.000 (2)	-0.005 (2)
C25	0.019 (3)	0.025 (3)	0.028 (3)	-0.008 (2)	0.001 (2)	-0.004 (2)
C26	0.029 (3)	0.029 (3)	0.020 (2)	-0.014 (2)	0.000 (2)	-0.003 (2)
C27	0.033 (3)	0.032 (3)	0.025 (3)	-0.018 (2)	0.002 (2)	-0.003 (2)
C28	0.048 (4)	0.036 (3)	0.031 (3)	-0.026 (3)	0.002 (2)	-0.005 (2)
C29	0.027 (3)	0.028 (3)	0.032 (3)	-0.011 (2)	-0.007 (2)	-0.001 (2)
C30	0.044 (4)	0.039 (3)	0.034 (3)	-0.020 (3)	-0.004 (3)	-0.009 (2)
C31	0.032 (3)	0.023 (3)	0.028 (3)	-0.008 (2)	-0.002 (2)	-0.005 (2)
C32	0.042 (3)	0.029 (3)	0.042 (3)	-0.016 (3)	0.008 (3)	-0.007 (2)
C33	0.027 (3)	0.027 (3)	0.027 (3)	-0.007 (2)	0.004 (2)	-0.008 (2)
C34	0.031 (3)	0.036 (3)	0.031 (3)	-0.009 (2)	-0.002 (2)	-0.006 (2)
C35	0.041 (4)	0.033 (3)	0.033 (3)	-0.009 (3)	-0.008 (3)	-0.005 (2)
C36	0.036 (3)	0.037 (3)	0.050 (4)	-0.020 (3)	-0.005 (3)	-0.001 (3)
C37	0.024 (3)	0.019 (2)	0.024 (3)	-0.002 (2)	-0.001 (2)	0.0025 (19)
C38	0.033 (3)	0.020 (2)	0.024 (3)	-0.005 (2)	-0.001 (2)	-0.0041 (19)
C39	0.029 (3)	0.021 (2)	0.033 (3)	-0.008 (2)	-0.002 (2)	-0.006 (2)
C40	0.026 (3)	0.014 (2)	0.028 (3)	-0.0010 (19)	-0.001 (2)	-0.0008 (18)
C41	0.035 (3)	0.024 (3)	0.029 (3)	-0.006 (2)	-0.003 (2)	-0.006 (2)
C42	0.033 (3)	0.024 (3)	0.027 (3)	-0.012 (2)	-0.001 (2)	-0.001 (2)
C43	0.030 (3)	0.018 (2)	0.028 (3)	-0.008 (2)	-0.004 (2)	-0.0040 (19)
C44	0.033 (3)	0.022 (3)	0.037 (3)	-0.008 (2)	0.003 (2)	-0.004 (2)

Geometric parameters (Å, º)

Cl1—C22	1.774 (5)	Cl2—C44	1.783 (5)
O1—C15	1.396 (6)	O12—C37	1.397 (6)
O1—C1	1.409 (6)	O12—C23	1.401 (6)
O2—C7	1.345 (6)	O13—C29	1.359 (6)
O2—C2	1.448 (5)	O13—C24	1.443 (6)
O3—C9	1.360 (6)	O14—C31	1.360 (6)

O3—C3	1.437 (6)	O14—C25	1.439 (6)
O4—C11	1.355 (6)	O15—C33	1.349 (6)
O4—C4	1.437 (6)	O15—C26	1.451 (6)
O5—C1	1.412 (5)	O16—C23	1.420 (6)
O5—C5	1.434 (6)	O16—C27	1.430 (6)
O6—C13	1.352 (7)	O17—C35	1.347 (7)
O6—C6	1.453 (6)	O17—C28	1.446 (6)
O7—C7	1.205 (7)	O18—C29	1.195 (7)
O8—C9	1.201 (7)	O19—C31	1.197 (7)
O9—C11	1.203 (7)	O20—C33	1.214 (6)
O10—C13	1.206 (7)	O21—C35	1.193 (7)
O11—C21	1.230 (6)	O22—C43	1.230 (6)
N1—C21	1.348 (6)	N2—C43	1.348 (6)
N1—C18	1.419 (6)	N2—C40	1.419 (6)
N1—H1N	0.85 (4)	N2—H2N	0.84 (4)
C1—C2	1.518 (7)	C23—C24	1.511 (6)
C1—H1	1.0000	C23—H23	1.0000
C2—C3	1.510 (7)	C24—C25	1.520 (7)
C2—H2	1.0000	C24—H24	1.0000
C3—C4	1.523 (6)	C25—C26	1.517 (7)
C3—H3	1.0000	C25—H25	1.0000
C4—C5	1.526 (6)	C26—C27	1.530 (7)
C4—H4	1.0000	C26—H26	1.0000
C5—C6	1.501 (7)	C27—C28	1.500 (7)
C5—H5	1.0000	C27—H27	1.0000
C6—H6A	0.9900	C28—H28A	0.9900
C6—H6B	0.9900	C28—H28B	0.9900
C7—C8	1.500 (7)	C29—C30	1.493 (7)
C8—H8A	0.9800	C30—H30A	0.9800
C8—H8B	0.9800	C30—H30B	0.9800
C8—H8C	0.9800	C30—H30C	0.9800
C9—C10	1.489 (7)	C31—C32	1.496 (7)
C10—H10A	0.9800	C32—H32A	0.9800
C10—H10B	0.9800	C32—H32B	0.9800
C10—H10C	0.9800	C32—H32C	0.9800
C11—C12	1.506 (7)	C33—C34	1.484 (7)
C12—H12A	0.9800	C34—H34A	0.9800
C12—H12B	0.9800	C34—H34B	0.9800
C12—H12C	0.9800	C34—H34C	0.9800
C13—C14	1.490 (8)	C35—C36	1.508 (8)
C14—H14A	0.9800	C36—H36A	0.9800
C14—H14B	0.9800	C36—H36B	0.9800
C14—H14C	0.9800	C36—H36C	0.9800
C15—C16	1.384 (7)	C37—C38	1.379 (7)
C15—C20	1.391 (7)	C37—C42	1.389 (7)
C16—C17	1.381 (7)	C38—C39	1.387 (7)
C16—H16	0.9500	C38—H38	0.9500
C17—C18	1.406 (7)	C39—C40	1.394 (7)
C17—H17	0.9500	C39—H39	0.9500
C18—C19	1.388 (7)	C40—C41	1.389 (7)
C19—C20	1.379 (7)	C41—C42	1.402 (7)
C19—H19	0.9500	C41—H41	0.9500

C20—H20	0.9500	C42—H42	0.9500
C21—C22	1.519 (7)	C43—C44	1.505 (7)
C22—H22A	0.9900	C44—H44A	0.9900
C22—H22B	0.9900	C44—H44B	0.9900
C15—O1—C1	115.7 (4)	C37—O12—C23	112.8 (4)
C7—O2—C2	118.3 (4)	C29—O13—C24	117.5 (4)
C9—O3—C3	118.0 (4)	C31—O14—C25	117.3 (4)
C11—O4—C4	118.9 (4)	C33—O15—C26	119.4 (4)
C1—O5—C5	111.2 (4)	C23—O16—C27	110.9 (4)
C13—O6—C6	116.8 (4)	C35—O17—C28	115.3 (5)
C21—N1—C18	127.0 (4)	C43—N2—C40	128.0 (4)
C21—N1—H1N	123 (4)	C43—N2—H2N	118 (4)
C18—N1—H1N	110 (4)	C40—N2—H2N	114 (4)
O1—C1—O5	107.0 (4)	O12—C23—O16	108.0 (4)
O1—C1—C2	107.8 (4)	O12—C23—C24	109.8 (4)
O5—C1—C2	110.0 (4)	O16—C23—C24	109.0 (4)
O1—C1—H1	110.7	O12—C23—H23	110.0
O5—C1—H1	110.7	O16—C23—H23	110.0
C2—C1—H1	110.7	C24—C23—H23	110.0
O2—C2—C3	109.5 (4)	O13—C24—C23	105.7 (4)
O2—C2—C1	105.9 (4)	O13—C24—C25	109.9 (4)
C3—C2—C1	110.5 (4)	C23—C24—C25	109.2 (4)
O2—C2—H2	110.3	O13—C24—H24	110.7
C3—C2—H2	110.3	C23—C24—H24	110.7
C1—C2—H2	110.3	C25—C24—H24	110.7
O3—C3—C2	106.8 (4)	O14—C25—C26	109.5 (4)
O3—C3—C4	110.3 (4)	O14—C25—C24	107.2 (4)
C2—C3—C4	111.9 (4)	C26—C25—C24	111.2 (4)
O3—C3—H3	109.3	O14—C25—H25	109.6
C2—C3—H3	109.3	C26—C25—H25	109.6
C4—C3—H3	109.3	C24—C25—H25	109.6
O4—C4—C3	109.5 (4)	O15—C26—C25	108.7 (4)
O4—C4—C5	105.7 (4)	O15—C26—C27	104.7 (4)
C3—C4—C5	109.4 (4)	C25—C26—C27	110.4 (4)
O4—C4—H4	110.7	O15—C26—H26	110.9
C3—C4—H4	110.7	C25—C26—H26	110.9
C5—C4—H4	110.7	C27—C26—H26	110.9
O5—C5—C6	108.5 (4)	O16—C27—C28	107.4 (4)
O5—C5—C4	108.7 (4)	O16—C27—C26	109.5 (4)
C6—C5—C4	112.5 (4)	C28—C27—C26	112.4 (4)
O5—C5—H5	109.0	O16—C27—H27	109.2
C6—C5—H5	109.0	C28—C27—H27	109.2
C4—C5—H5	109.0	C26—C27—H27	109.2
O6—C6—C5	108.3 (4)	O17—C28—C27	107.9 (4)
O6—C6—H6A	110.0	O17—C28—H28A	110.1
C5—C6—H6A	110.0	C27—C28—H28A	110.1
O6—C6—H6B	110.0	O17—C28—H28B	110.1
C5—C6—H6B	110.0	C27—C28—H28B	110.1
H6A—C6—H6B	108.4	H28A—C28—H28B	108.4
O7—C7—O2	123.5 (5)	O18—C29—O13	123.7 (5)
O7—C7—C8	125.4 (5)	O18—C29—C30	125.9 (5)

O2—C7—C8	111.0 (5)	O13—C29—C30	110.4 (5)
C7—C8—H8A	109.5	C29—C30—H30A	109.5
C7—C8—H8B	109.5	C29—C30—H30B	109.5
H8A—C8—H8B	109.5	H30A—C30—H30B	109.5
C7—C8—H8C	109.5	C29—C30—H30C	109.5
H8A—C8—H8C	109.5	H30A—C30—H30C	109.5
H8B—C8—H8C	109.5	H30B—C30—H30C	109.5
O8—C9—O3	123.9 (5)	O19—C31—O14	123.9 (5)
O8—C9—C10	125.8 (5)	O19—C31—C32	126.5 (5)
O3—C9—C10	110.3 (4)	O14—C31—C32	109.6 (5)
C9—C10—H10A	109.5	C31—C32—H32A	109.5
C9—C10—H10B	109.5	C31—C32—H32B	109.5
H10A—C10—H10B	109.5	H32A—C32—H32B	109.5
C9—C10—H10C	109.5	C31—C32—H32C	109.5
H10A—C10—H10C	109.5	H32A—C32—H32C	109.5
H10B—C10—H10C	109.5	H32B—C32—H32C	109.5
O9—C11—O4	123.5 (5)	O20—C33—O15	123.8 (5)
O9—C11—C12	125.9 (5)	O20—C33—C34	126.0 (5)
O4—C11—C12	110.5 (4)	O15—C33—C34	110.2 (4)
C11—C12—H12A	109.5	C33—C34—H34A	109.5
C11—C12—H12B	109.5	C33—C34—H34B	109.5
H12A—C12—H12B	109.5	H34A—C34—H34B	109.5
C11—C12—H12C	109.5	C33—C34—H34C	109.5
H12A—C12—H12C	109.5	H34A—C34—H34C	109.5
H12B—C12—H12C	109.5	H34B—C34—H34C	109.5
O10—C13—O6	123.8 (5)	O21—C35—O17	124.9 (6)
O10—C13—C14	125.3 (5)	O21—C35—C36	125.1 (5)
O6—C13—C14	110.8 (5)	O17—C35—C36	110.0 (5)
C13—C14—H14A	109.5	C35—C36—H36A	109.5
C13—C14—H14B	109.5	C35—C36—H36B	109.5
H14A—C14—H14B	109.5	H36A—C36—H36B	109.5
C13—C14—H14C	109.5	C35—C36—H36C	109.5
H14A—C14—H14C	109.5	H36A—C36—H36C	109.5
H14B—C14—H14C	109.5	H36B—C36—H36C	109.5
C16—C15—C20	119.9 (5)	C38—C37—C42	120.6 (5)
C16—C15—O1	122.8 (4)	C38—C37—O12	121.2 (4)
C20—C15—O1	117.3 (4)	C42—C37—O12	118.2 (4)
C17—C16—C15	120.6 (5)	C37—C38—C39	120.6 (5)
C17—C16—H16	119.7	C37—C38—H38	119.7
C15—C16—H16	119.7	C39—C38—H38	119.7
C16—C17—C18	119.7 (5)	C38—C39—C40	119.4 (5)
C16—C17—H17	120.1	C38—C39—H39	120.3
C18—C17—H17	120.1	C40—C39—H39	120.3
C19—C18—C17	119.1 (5)	C41—C40—C39	120.1 (5)
C19—C18—N1	117.9 (4)	C41—C40—N2	117.2 (4)
C17—C18—N1	122.9 (4)	C39—C40—N2	122.5 (4)
C20—C19—C18	120.9 (5)	C40—C41—C42	120.1 (5)
C20—C19—H19	119.5	C40—C41—H41	119.9
C18—C19—H19	119.5	C42—C41—H41	119.9
C19—C20—C15	119.8 (5)	C37—C42—C41	119.1 (5)
C19—C20—H20	120.1	C37—C42—H42	120.5
C15—C20—H20	120.1	C41—C42—H42	120.5

O11—C21—N1	124.9 (5)	O22—C43—N2	124.5 (5)
O11—C21—C22	122.8 (4)	O22—C43—C44	123.1 (5)
N1—C21—C22	112.3 (4)	N2—C43—C44	112.3 (4)
C21—C22—Cl1	111.5 (4)	C43—C44—Cl2	111.7 (4)
C21—C22—H22A	109.3	C43—C44—H44A	109.3
Cl1—C22—H22A	109.3	Cl2—C44—H44A	109.3
C21—C22—H22B	109.3	C43—C44—H44B	109.3
Cl1—C22—H22B	109.3	Cl2—C44—H44B	109.3
H22A—C22—H22B	108.0	H44A—C44—H44B	107.9
C15—O1—C1—O5	-75.7 (5)	C37—O12—C23—O16	-104.4 (4)
C15—O1—C1—C2	166.1 (4)	C37—O12—C23—C24	136.9 (4)
C5—O5—C1—O1	178.1 (4)	C27—O16—C23—O12	173.4 (4)
C5—O5—C1—C2	-65.1 (5)	C27—O16—C23—C24	-67.3 (5)
C7—O2—C2—C3	-102.3 (5)	C29—O13—C24—C23	140.7 (4)
C7—O2—C2—C1	138.5 (4)	C29—O13—C24—C25	-101.6 (5)
O1—C1—C2—O2	-69.8 (5)	O12—C23—C24—O13	-63.7 (5)
O5—C1—C2—O2	174.0 (4)	O16—C23—C24—O13	178.3 (4)
O1—C1—C2—C3	171.7 (4)	O12—C23—C24—C25	178.2 (4)
O5—C1—C2—C3	55.5 (5)	O16—C23—C24—C25	60.1 (5)
C9—O3—C3—C2	-141.7 (4)	C31—O14—C25—C26	99.1 (5)
C9—O3—C3—C4	96.6 (5)	C31—O14—C25—C24	-140.2 (4)
O2—C2—C3—O3	73.5 (4)	O13—C24—C25—O14	72.2 (5)
C1—C2—C3—O3	-170.2 (4)	C23—C24—C25—O14	-172.4 (4)
O2—C2—C3—C4	-165.8 (4)	O13—C24—C25—C26	-168.2 (4)
C1—C2—C3—C4	-49.5 (5)	C23—C24—C25—C26	-52.7 (5)
C11—O4—C4—C3	104.1 (5)	C33—O15—C26—C25	104.0 (5)
C11—O4—C4—C5	-138.0 (4)	C33—O15—C26—C27	-138.0 (4)
O3—C3—C4—O4	-74.9 (5)	O14—C25—C26—O15	-77.1 (5)
C2—C3—C4—O4	166.4 (4)	C24—C25—C26—O15	164.6 (4)
O3—C3—C4—C5	169.6 (4)	O14—C25—C26—C27	168.6 (4)
C2—C3—C4—C5	50.9 (5)	C24—C25—C26—C27	50.3 (6)
C1—O5—C5—C6	-170.9 (4)	C23—O16—C27—C28	-173.4 (4)
C1—O5—C5—C4	66.4 (5)	C23—O16—C27—C26	64.3 (5)
O4—C4—C5—O5	-175.6 (4)	O15—C26—C27—O16	-171.7 (4)
C3—C4—C5—O5	-57.7 (5)	C25—C26—C27—O16	-54.8 (6)
O4—C4—C5—C6	64.1 (5)	O15—C26—C27—C28	69.1 (6)
C3—C4—C5—C6	-178.0 (5)	C25—C26—C27—C28	-174.1 (5)
C13—O6—C6—C5	-105.1 (5)	C35—O17—C28—C27	-102.8 (5)
O5—C5—C6—O6	75.3 (5)	O16—C27—C28—O17	74.3 (6)
C4—C5—C6—O6	-164.4 (4)	C26—C27—C28—O17	-165.2 (4)
C2—O2—C7—O7	3.3 (7)	C24—O13—C29—O18	9.2 (7)
C2—O2—C7—C8	-175.8 (4)	C24—O13—C29—C30	-169.0 (4)
C3—O3—C9—O8	-0.6 (7)	C25—O14—C31—O19	-2.6 (7)
C3—O3—C9—C10	178.7 (4)	C25—O14—C31—C32	177.6 (4)
C4—O4—C11—O9	-2.6 (7)	C26—O15—C33—O20	-0.2 (7)
C4—O4—C11—C12	179.6 (4)	C26—O15—C33—C34	-177.6 (4)
C6—O6—C13—O10	-2.9 (7)	C28—O17—C35—O21	-7.9 (8)
C6—O6—C13—C14	176.9 (4)	C28—O17—C35—C36	172.2 (4)
C1—O1—C15—C16	-45.2 (6)	C23—O12—C37—C38	77.4 (6)
C1—O1—C15—C20	136.2 (5)	C23—O12—C37—C42	-102.0 (5)
C20—C15—C16—C17	0.6 (8)	C42—C37—C38—C39	0.4 (8)

O1—C15—C16—C17	-178.0 (4)	O12—C37—C38—C39	-179.0 (5)
C15—C16—C17—C18	0.3 (8)	C37—C38—C39—C40	-1.6 (8)
C16—C17—C18—C19	-0.6 (7)	C38—C39—C40—C41	2.0 (8)
C16—C17—C18—N1	-177.9 (5)	C38—C39—C40—N2	179.1 (5)
C21—N1—C18—C19	156.5 (5)	C43—N2—C40—C41	-150.7 (5)
C21—N1—C18—C17	-26.2 (8)	C43—N2—C40—C39	32.2 (8)
C17—C18—C19—C20	0.0 (7)	C39—C40—C41—C42	-1.4 (8)
N1—C18—C19—C20	177.4 (4)	N2—C40—C41—C42	-178.6 (5)
C18—C19—C20—C15	1.0 (8)	C38—C37—C42—C41	0.2 (8)
C16—C15—C20—C19	-1.3 (8)	O12—C37—C42—C41	179.7 (4)
O1—C15—C20—C19	177.4 (4)	C40—C41—C42—C37	0.2 (8)
C18—N1—C21—O11	1.0 (9)	C40—N2—C43—O22	-1.0 (8)
C18—N1—C21—C22	-178.8 (5)	C40—N2—C43—C44	175.6 (5)
O11—C21—C22—Cl1	10.0 (7)	O22—C43—C44—Cl2	-23.5 (7)
N1—C21—C22—Cl1	-170.3 (4)	N2—C43—C44—Cl2	159.8 (4)

Hydrogen-bond geometry (Å, °)

D—H...*A*	*D*—H	H...*A*	*D*...*A*	*D*—H...*A*
N1—H1*N*...O22^{i}	0.85 (4)	2.15 (5)	2.973 (5)	165 (5)
N2—H2*N*...O11	0.84 (4)	2.05 (5)	2.854 (5)	161 (6)

Symmetry code: (i) *X*, *y*+1, *z*.

VITA

The author was born in New Delhi, India on September 22, 1991. She graduated from The Mother's International School in 2009. She received her Bachelor of Science (Hons) degree in Chemistry from Hindu College, University of Delhi in 2012. She graduated from the University of Leeds, United Kingdom in 2014 with Master of Science degree in Chemical Biology and Drug Design. In fall 2015, she joined the University of New Orleans as a graduate student and obtained a Master of Science degree in Chemistry in 2018. She continued her graduate studies and completed the requirements for the degree of Doctor of Philosophy in Organic Chemistry in May, 2020 under the supervision of Dr. Mark L Trudell.