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Parthenolide derivatives as potential leukaemia drugs

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Abstract

Chronic lymphocytic leukaemia (CLL) is a common type of leukaemia and it is frequently diagnosed in Western countries. Current therapy to cure this frequently diagnosed leukaemia is unsatisfactory. Therefore, the search for potential drugs has become an important research topic in recent years.

Parthenolide, an active component from the plant Feverfew, is a sesquiterpene lactone which includes an α-methylene-γ-butyrolactone moiety. Parthenolide (PTL) displays a variety of biological activities in drug studies while exhibiting good anti-leukaemic activity against primary AML cells *in vitro*. The poor aqueous solubility and non-selective reactivity of PTL are two major issues that limit its clinical study as a potential drug. Therefore, a series of PTL derivatives have been synthesised through a Michael addition reaction in this project and try to conquer these problems.

The biologically active component, parthenolide, was extracted, isolated and purified from a few different types of medicinal herb Feverfew by recombining extraction procedures developed in this project. Before commencing the study of PTL, the α-methylene-γ-butyrolactone, tulipane, will be studied as a model substrate to test both the derivatisation chemistry and the biological screens prior to using the more precious parthenolide.

All derivatives have been preliminarily tested by Lipinski rules; they passed the tests and proved almost all the compounds are increasing the water solubility by estimated the LogP values used three different ways.

Eight PTL derivatives have been evaluated for their anti-cancer activity. Five compounds, **14a-d** and **14f**, showed similar anti-cancer activity against CLL cell lines *in vitro* compared with parent compound, with LD_{50} values in a range of 5-10 μM. The five active compounds have been taken forward to further *in vitro* and *in vivo* studies. Also, in future biological work, 10 unexamined remaining PTL derivatives (**15a-g**) should also be tested against CLL cell lines. Therefore, a structure-activity relationship will be able to be probed and developed.

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Abbreviations

AML	Acute Myeloid Leukaemia
CLL	Chronic Lymphocytic Leukaemia
DCM	Dichloromethane
DMAPT	Dimethylaminoparthenolide
GF	Golden Feverfew
HPLC	High Performance Liquid Chromatography
IR	Infrared
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PTL	Parthenolide
PI	Propidium Iodide
RT	Room Temperature
ROS	Reactive Oxygen Species
SM	Starting Material
SD	Standard derivation
TLC	Thin Layer Chromatography

Chapter 1: Introduction and Literature Review

1.1 Introduction

Parthenolide (PTL) is a sesquiterpene lactone which includes an α-methylene-γ-butyrolactone moiety. It displays a variety of biological activities in drug studies, including anti-carcinogenic and anti-inflammatory properties. This biologically active component was extracted, isolated, and purified from three different types of the medicinal herb feverfew in this project.[1,2]

This report presents the progresses on the researches of both literature and lab works about parthenolide derivatives as potential leukaemia drugs. After the first step of extracting PTL from feverfew, the next stage is the design and synthesis of PTL derivatives. Within this stage, various types of amines have been used to modify the core biological active structure of PTL to undergo a Michael addition reaction.

Then this report will conclude with advanced biological findings of synthesised derivatives. The aim of this stage is to examine the anti-cancer activity of PTL and derivatives in three different types of chronic lymphocytic leukaemia (CLL) cell lines *in vitro*. Hence, amino derivatives (**14a**–**d** and **14f**) with similar anti-cancer activity to the parent compound PTL will be reported at the end of this stage of research.

1.2 Aims of the Report

The main objective of this report is to build comprehensive knowledge in the area of the organic synthesis and medicinal chemistry of the sesquiterpene lactone (parthenolide). To achieve the main objective, there are some subsequent aims of this report:

1. Through isolating PTL from feverfew by recombining procedures developed in this project to improve isolation time, cost efficiency and obtain a good yield; to find out the best type of plant and the best harvest season for the plant containing the maximum amounts of PTL.

2. To find out the drug-like properties of PTL by modifying the α,β-unsaturated ester part of the molecule through Michael-like addition reactions.
3. To find out whether α-methylene-γ-butyrolactone (tulipane) is a suitable model for pre-screening prior to parthenolide modifications; and identify any possible relationships between PTL and tulipane derivatives.
4. Through examining anti-cancer activity of all derivatives against CLL cell lines, find out whether any derivative has better solubility than PTL whilst retaining the same activity in cell-based assays.

The following actions have been taken to achieve the above-mentioned aims. First of all, this project begins from extraction, isolation and purification of parthenolide (PTL) from the medicinal herb feverfew. Then it moves onto its development in organic synthesis. Various types of amines were used to modify the core biological active structure of PTL to undergo a Michael addition reaction to improve the aqueous solubility and retained the biological activity. Also, due to tulipane (**6)**, the α-methylene-γ-butyrolactone was studied as a model substrate to both test the derivatisation chemistry and the biological screens prior to using the more precious parthenolide.

An advanced stage of biological studies of PTL derivatives was treatment in three different types of CLL cell lines *in* vitro to examine their anti-cancer activity. Therefore, the primary objective of this project is to build comprehensive knowledge in the area of the organic synthesis of the sesquiterpene lactone (parthenolide) and application in medicinal chemistry can be established.

1.3 Cancer

Normally, the division and proliferation mechanisms of trillions of living cells in our body are controllable. If cells start to divide uncontrollably they may become cancerous.[3] Cells become cancerous because the DNA is damaged during cell division, which may inhibit the normal actions of these cells. Cells containing damaged DNA either repair the damage or die; however, if the damaged DNA persists and the cell survives, cell proliferation could lead to cancerous mutations and contribute to tumourigenesis.[3-5]

The vast majority of cases show that cancer cells form a tumour, and there are only a few cancers, e.g. leukaemia, which do not form tumours. In contrast, this kind of cancer infiltrates the blood, hematopoietic organs, and other organs and tissues.[3-6]

1.4 Chronic Lymphocytic Leukaemia (CLL)

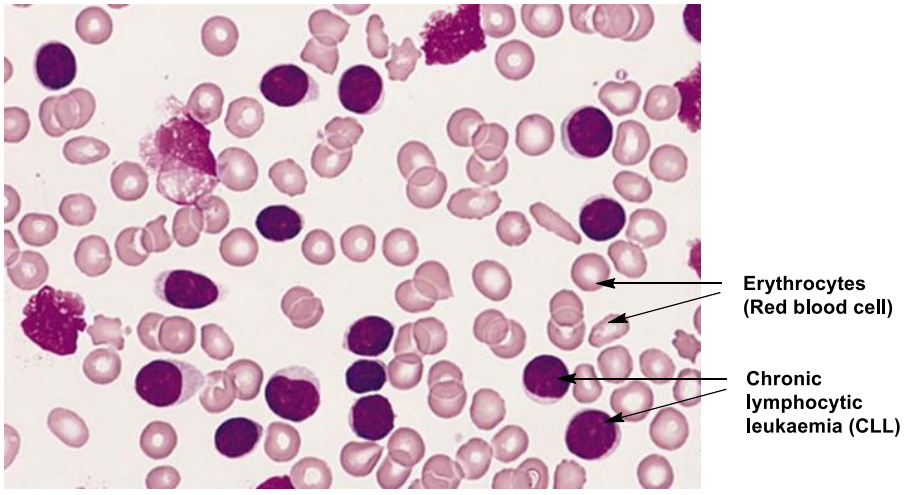

Figure 1: B-cell chronic lymphocytic leukaemia.[7]

The most frequently diagnosed leukaemic, chronic lymphocytic leukaemia (CLL), is a B-lymphocyte proliferative disease within the hematopoietic system. The hematopoietic system supplies all cellular blood components and forms new blood cells daily in the human body. CLL is cancer of the abnormal lymphocytes (a kind of white blood cells) that can be diagnosed from inspection of peripheral blood, bone marrow, the liver, the spleen, and lymph nodes where small round lymphoid aggregates are observed (**Figure 1**).[8,9]

This type of leukaemia is very common in Western countries.[9] The incidence rate is 6.15 in 10 million people, accounting for about 25% of all cases of leukaemia. CLL in Asian countries is relatively rare; the ratio does not exceed 5% of all cases of leukaemia in Japan, China and India.[8,9] CLL can occur in people at any age, but is most prevalent in people over the age of 60. The age of onset has a mean value of 65, where incidence increases with age and a higher proportion of males than females are affected, showing a ratio of approximately 2:1.[3,8-10]

So far, there is still no definite explanation for the causes and pathogenesis of CLL. CLL is most likely to be caused by both internal and external factors. For example, family history will be a major internal risk factor for CLL, while chemical substances, such as formaldehyde and benzene, will be a clear case of which an external factor contributes to CLL. These factors may be effective independently or jointly to cause cancer.[9,10]

There are no standard curative programs. The only treatment available remains palliative care. Patients with early diagnosis or stable conditions may not require the use of anti-tumour therapies, because these treatments do not generally prolong patient survival.[9-12] Also, cancer therapies such as oral alkylating agents will likely shorten the period of the patient's life rather than extend it.[8,9,11,12]

Overall, the recent CLL treatment programme mainly uses a single agent or combination of chemotherapy in late-stage patients, depending on the severity of the patient's symptoms and the degree of tolerance to chemotherapy which is dependent on age or comorbidities.[8,10,11] Therefore, there is an unmet need for less genotoxic or cytotoxic therapies for the management of this disease.

1.5 Feverfew

Feverfew (*Tanacetum parthenium* L.) (Asteraceae) is a medicinal plant which is traditionally used for the treatment of fevers and migraine.[13]

Figure 2: Tanacetum parthenium (feverfew)

From medicinal herbs such as feverfew, a variety of medicinal components can be extracted.[13] One of these components is parthenolide. Parthenolide (PTL) has shown

activity against a few tumour cells *in vitro*, inhibiting the proliferation of colon cancer, liver cancer, cholangiocarcinoma, acute and chronic leukaemia, multiple myeloma and other tumour cells.[13-15]

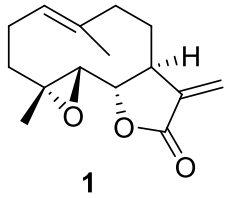

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Figure 3: Structure of Parthenolide

1.5.1 Extraction of Parthenolide

In recent years, there have been a variety of extraction procedures of parthenolide reported. There are three main differences between the extraction solvent system, extraction procedures and analysis methods used.[16-18] Yoshioka *et al.* chose chloroform or petroleum ether as the solvent to extract sesquiterpene lactones, especially PTL, and identified it with NMR spectroscopy.[19] Marchand *et al.* also used chloroform as a solvent, but a high-performance liquid chromatographic (HPLC) analysis method was first used to identify PTL.[17,19] Two different extraction methods have been published at almost the same time: Groenewegen *et al.* used a chloroform stirring extraction method, and the petroleum ether Soxhlet extraction procedure were reported by Awang *et al.*.[16] In the more recent publications, more solvents, such as acetone, acetonitrile, methanol, and organic/aqueous solvent systems were tested; and combining with the HPLC analysis, the common method to identify PTL has been determined.[19]

Only a few researches focused on the solvent effect of the extraction procedure. Zhou *et al.* examined different solvents on same amounts of feverfew with both stirring and Soxhlet extraction. The extraction results showed the highest percentage of PTL was extracted by the stirring method with acetonitrile and water mixture (90:10; v/v. 0.32%); ethanol, acetone and chloroform showed similar extraction capabilities (between 0.26% to 0.28%). However, petroleum ether showed a lower percentage of PTL (0.16%) and proved that it is not a good solvent system for extraction.[19]

When the solvent system is mixed with different amounts of water respectively, excellent results have been shown; for example, in ethanol with 10% water, acetonitrile with 10% water, and acetone with 20%, 30% water, higher than 0.3% of PTL was always extracted.[19] As water plays an important role in extraction process, a conclusion can be drawn: the reason for water's important role in extraction process is that the aqueous phase will attract the hydrophilic components of feverfew and lead to the PTL dissolving more easily into the organic phase.

Zhou's group also reported that Soxhlet extraction using ethanol, acetonitrile, acetone, chloroform, and petroleum ether, which is based on the order of decreasing solvent polarity, was adopted and the results of the percentage of PTL showed a steady decreased from ethanol to petroleum ether (0.31%, 0.3%, 0.28%, 0.27% and 0.12%). By a more polar solvent system, the efficiency of extract PTL appeared to be better, and a polar compound can better dissolve in a polar solvent system. But the nonpolar solvent petroleum ether is not a good solvent to extract PTL from feverfew, and has shown significantly poor efficiency.[19]

Therefore, stirring extraction showed a better efficiency than Soxhlet, as stirring extraction only takes a short time and room temperature, comparing to Soxhlet extraction which requires reflux for 24 hours. In addition, for the stirring extraction method, the maximum amount of PTL was extracted from feverfew within 10 minutes in the acetonitrile and water solvent system (see **Figure 4**).[19]

Furthermore, Marete *et al.* reported that PTL could be extracted into water and with the increase in temperature through an aqueous stirring extraction, the content of PTL and a number of phenolic compounds increased; also the maximum concentration of PTL was obtained at 75–80 ℃ as observed by extracts (see **Figure 5**). A higher concentration of protein was obtained at 20–70 ℃ as the color of water extracts is brownish.[20]

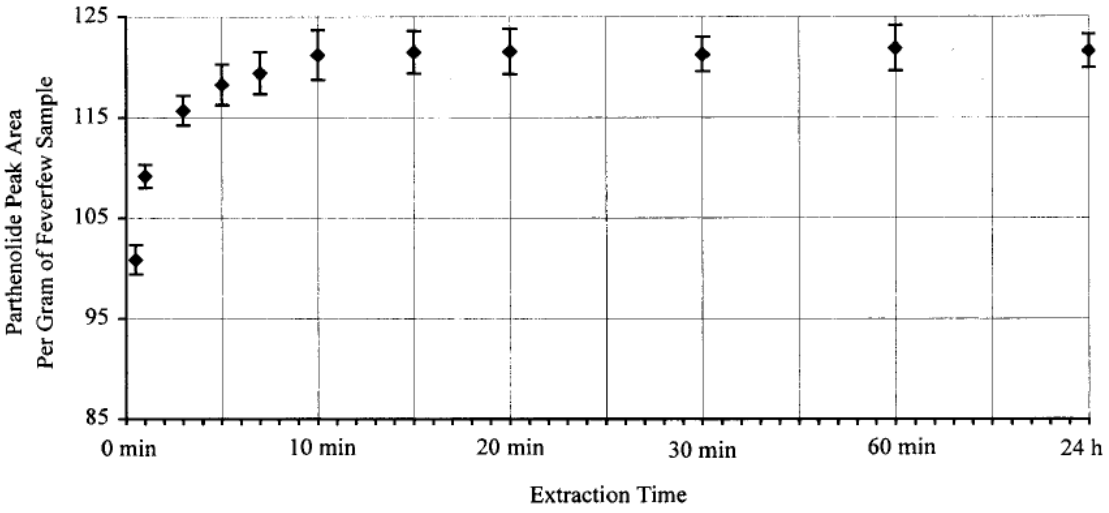


Figure 4: Maximum amount of PTL can be extracted after 10 minutes using the bottle stirring method.[19](Permission to use from copyright of American Chemical Society, 1999)

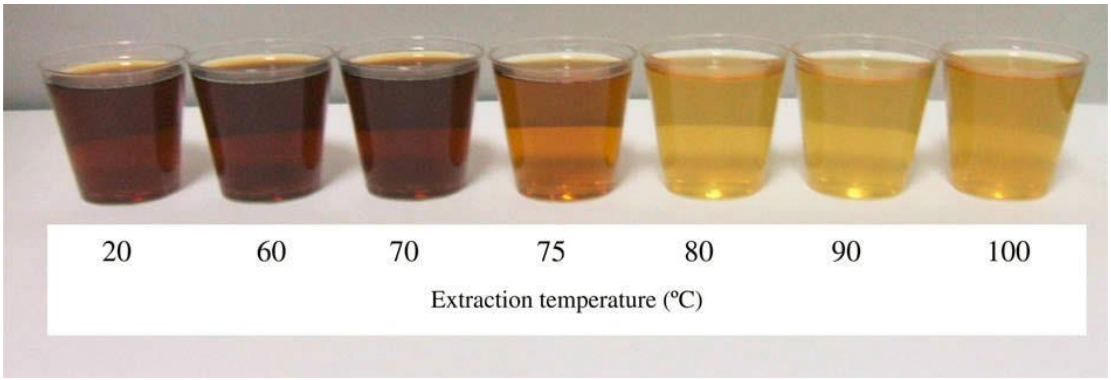


Figure 5: Feverfew samples extracted in water at different temperatures. [20] (Permission to use from copyright of Elsevier Ltd, 2009)

1.5.2 Origin and Composition of PTL

Feverfew is generally grown in Europe and North America.[18,21] Some studies have suggested that PTL was only found in Europe– and United States–grown feverfew, not in Mexican or Yugoslavian.[18] Also Awang *et al.* reported that the percentage of PTL varies in different parts of feverfew (**Table 1**)[18].

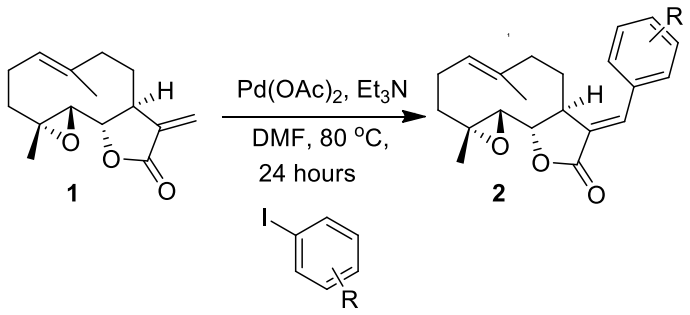
Table 1: Content of Parthenolide extracts by HPLC and NMR spectroscopy procedures.[18]

Entry	Origin	Composition	% of Dry Wt	
			HPLC	^{1}H NMR
1	UK	Leaf	**0.83**	**0.85**
2		Seed	**1.52**	**1.82**
3	USA	Mixed leaf and flowering top	**0.18**	**0.21**
4		Leaf	**0.09**	**0.13**
5		Flowering top	**0.27**	**0.35**
6	Canada	Leaf, (pre-flowering)	**0.33**	**0.64**
7		Leaf (post-flowering)	**1.27**	**1.62**
8		Flowering top	**0.46**	**1.01**

A conclusion can be drawn from the data presented in **Table 1** that seeds contain much more PTL than the other parts of feverfew such as the leaf. Extraction of seed (entry 2) is twice rich in PTL than leaf (entry 1), as well as entry 7 (mixture of leaf and seeds), has shown much higher yield obtained than leaf or flower only (entry 6 and entry 8, respectively). European feverfew is currently confirmed to contain the highest percentage of PTL (entry 1), whereas feverfew originating from the USA contains much lower percentages yield (entry 3 to entry 5).

1.6 Parthenolide

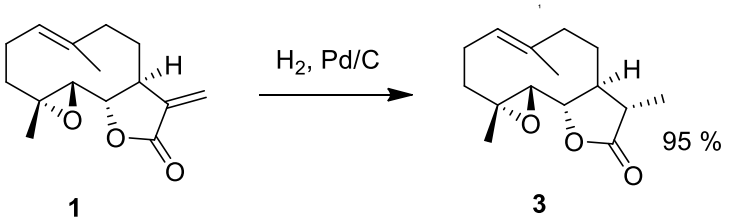
Parthenolide can be extracted from the medicinal herb feverfew. In the last few years, PTL has shown exceptional anti-cancer and anti-inflammatory properties in drug studies and development.[1,2,22] However, the poor aqueous solubility of PTL has limited its clinical study as a potential drug. Another problem presented is that of the reactivity of PTL. PTL, as a good Michael acceptor, can be bound with many intracellular thiol groups *in vitro* due to the loss of biological activity in vain. Therefore, many research groups have hypothesised a new pro-drug approach to conquer these problems.



Entry	R	Position	Yield (%)
1	Cl	Para	81
2	CF_3	Para	73
3	Ethyl	Para	57
4	NH_2	Meta	85
5	CF_3	Meta	80
6	F	Ortho	85
7	CF_3	Ortho	58

Scheme 1: Palladium-catalysed arylation of PTL.[23]

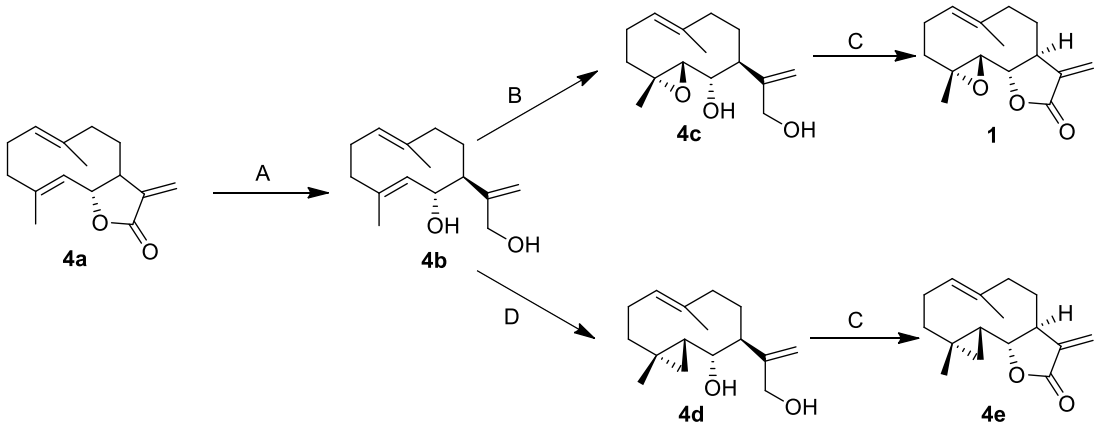
To understand the reactivity of α-methylene-γ-butyrolactone moiety of PTL, Han *et al.* reported a palladium-catalysed arylation reaction of PTL to afford **2** (**Scheme 1**). They used a series of aryl iodides undergoes in a Heck reaction and only a single *exo*-cyclic olefin product (*E*-olefin geometry) was detected. Derivatives that contained an electron-withdrawing substituent at meta or para position have shown good activity against HeLa cells.[23] Also, Hwang and co-workers modified PTL by reducing the enone olefin of α-methylene-γ-butyrolactone functional group to afford compound **3** (**Scheme 2**).[24] However, this compound has showed no anti-leukemic activity against primary acute myeloid leukaemia (AML) cells *in vivo*.[25]



Scheme 2: The enone olefin of PTL has been reduced by palladium.[24]

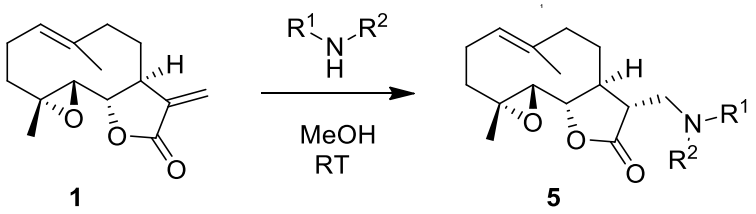
A recent study has suggested that the epoxide moiety of PTL may affect its structural stability and this is a possible reason why PTL has a short half-life in mouse plasma (only 0.34 hours).[26] Jing Long *et al*. reported a cyclopropyl analogue (**4e**) that replaces the epoxide moiety with a cyclopropyl group in three steps from **4a** (Costunolide) in September 2013. The new analogue has shown a longer half-life in mouse plasma (13.9 hours) compared with the parent compound (only 0.34 hours). This PTL derivative has been confirmed that it has a significantly more stable structure than PTL. Also this compound has shown similar biological activities compared with PTL. This may suggest that the epoxide functional group is unlikely to

affect the anti-cancer activity of PTL and **4e** may be a promising prodrug for further study.[26]



Scheme 3: A novel cyclopropyl analogue of PTL was synthesised in a three-step reaction by Jing Long *et al.*. Conditions: A) DIBAL, toluene, RT, 4 hours; B) Ti(O-^{i}-Pr)$_4$, D-(-)-DIPT, TBHP, CH_2Cl_2, -20 ℃, 20 hours; C) TEMPO, PhI(OAc)$_2$, CH_2Cl_2, RT, 10 hours; D) Et_2Zn, CH_2I_2, CH_2Cl_2, -10 ℃, 1 hour.[26]

The Michael addition reaction of nucleophiles to electron-deficient α,β-unsaturated lactones is one of the most valuable organic synthesis reactions to form a new C-C bond or C-N bond. It is also frequently used to synthesise natural products or drugs. The most important part of this class of reaction is the creation of a new C-N bond resulting in products that contain β-amino ester functionality. This C-N bond is a polar bond in which the nitrogen atom has more electronegativity than oxygen. As a result, the structure that contains a C-N bond is more soluble in water. Amines are good nucleophiles and the lone pair of nitrogen atoms will always attack an electrophile in the Michael addition mechanism. Also, some amine derivatives often have biological activities and are useful intermediates in organic chemistry.[22,24] In addition, some aminoparthenolide derivatives have been synthesised in a one-step process by Neelakantan *et al.*.[25] This is a thermodynamically controlled process under the standard conditions of the addition reaction (**Scheme 4**). When an amine attacks an α,β-unsaturated lactone moiety, the protonation of intermediate enolate is *exo* selective. The reason is that the intermediate enolate from conjugate addition on the *endo* face undergoes a 1,3-diaxial interaction of hydrogen on C-6, axial hydrogen on C-8 and two hydrogen on C-13. This means only the (*R*) configuration is displayed at carbon 11 in the Michael-type addition of amines to parthenolide (**Figure 6**)[24,26]



Scheme 4: Michael addition of PTL.[27]

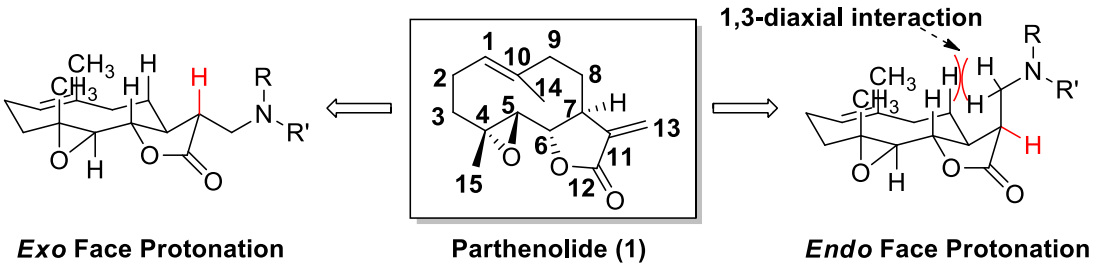


Figure 6: Structure of Parthenolide and Structure of *exo* and *endo* face protonation product[25,27]

In the past few years, Nasim's research group has reported that a series of parthenolide analogues have been synthesised and demonstrated that PTL derivatives have more water-solubility than parent compound. This result would have direct impact on their ability to become potential drugs because better solubility in water can increase the bioavailability.[27] The aminoparthenolide analogues have not only improved drugability and bioavailability, but also kept the anti-leukemic activity *in vitro* and *in vivo*.[21-25] To better understand the structure activity relationship of PTL and amino derivatives, Neelakantan *et al*. used a series of primary and secondary amines with PTL utilising a Michael-type addition to synthesise aminoparthenolide derivatives, such as DMAPT or LC-1 (compound **5a)**, which has higher bioavailability and similar durability (**Scheme 4**).[27] Compounds **5a-j** were tested for their anti-cancer properties on acute myeloid leukaemia (AML) and the results showed that amine derivatives can maintain the biological activity of PTL after chemical modifications (a portion of these derivatives can be seen in **Table 2**).[25]

Table 2: PTL and amino parthenolide derivatives against primary AML cell.[25]

Entry	Compound No.	R^1	R^2	% Cell Death(10 μM)	LD_{50}[B]
1	**1**			84	1.4
2	**5a**[A]	Methyl	Methyl	93	1.7
3	**5b**	Methyl	Ethyl	80	2.5
4	**5c**	Methyl	Propyl	95	3.2
5	**5d**	Methyl	Butyl	68	2.8
6	**5e**	Methyl	Pentyl	46	4.6
7	**5f**	Methyl	*N*-2-Hydroxyethyl	86	1.8

A: This compound is DMAPT B: LD_{50} (Median Lethal does) a commonly indicator that used for description of toxic substances or radiation toxicity

The anti-cancer activity of those aminoparthenolide derivatives against primary AML cancer cells *in vitro* has been shown in **Table 2**. PTL (entry 1) has obtained 84% cell death against primary AML cells. Derivative **5a-c** (entries 2-4) and **5f** (entry 7) showed significant levels of killing in primary AML cells (80–93% cell death); especially **5a**, **5b**, **5c** and **5f** showed a higher anti-leukaemic activity than the parent compound. Derivative **5c** (entry 4) and **5e** (entry 6) showed loss of the anti-leukaemic activity and displayed relatively higher LD_{50} values comparing with other derivatives against primary AML cells (2.8 μM and 3.2 μM respectively). Dimethylaminoparthenolide (DMAPT, **5a,** entry 2) is one of the best aminoparthenolide derivatives as it causes 93% cell death at 10 μM probe assay when the LD_{50} value is 1.7 μM. On the other hand, DMAPT has an aqueous solubility, which is 1000-fold more soluble in water than PTL, and it maintains a good anti-leukemic activity.[25,27]

Finally, in recent years, some fluorinated aminoparthenolides were designed and synthesised by Woods *et al.*.[28] They reported that a reversible Michael addition process can happen with *in vitro* tests at the presence of glutathione (cellular antioxidant) and with further derivatives be degraded to free PTL and amines (**Figure 7**).

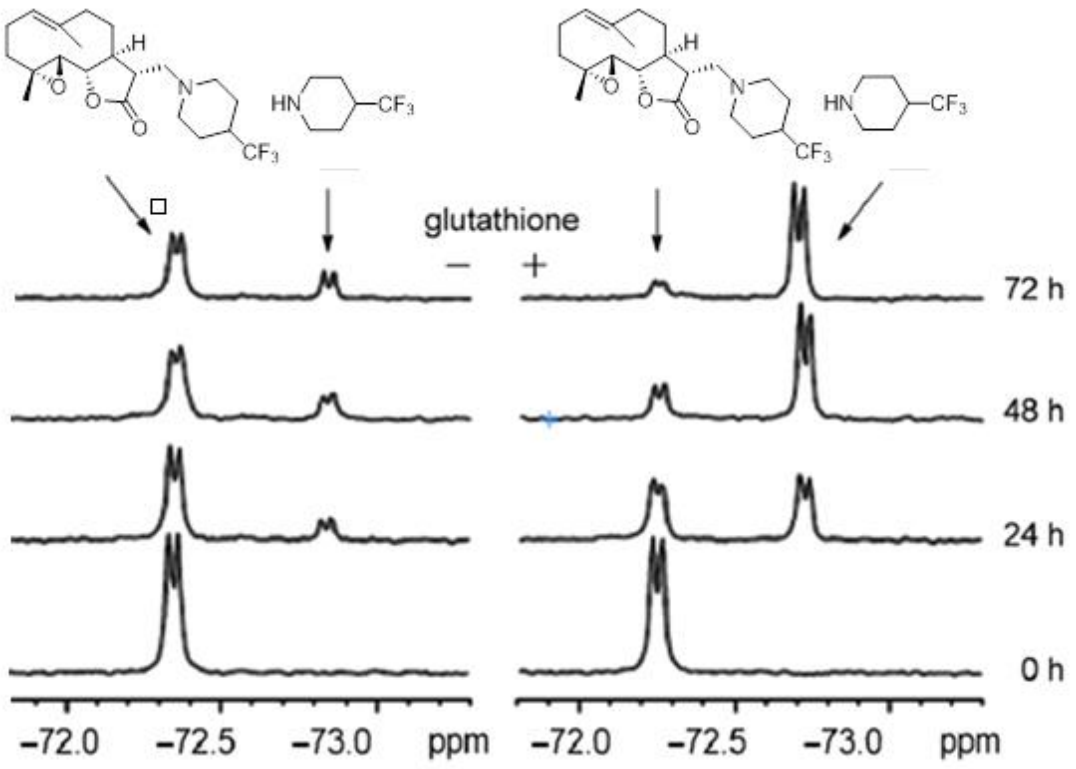
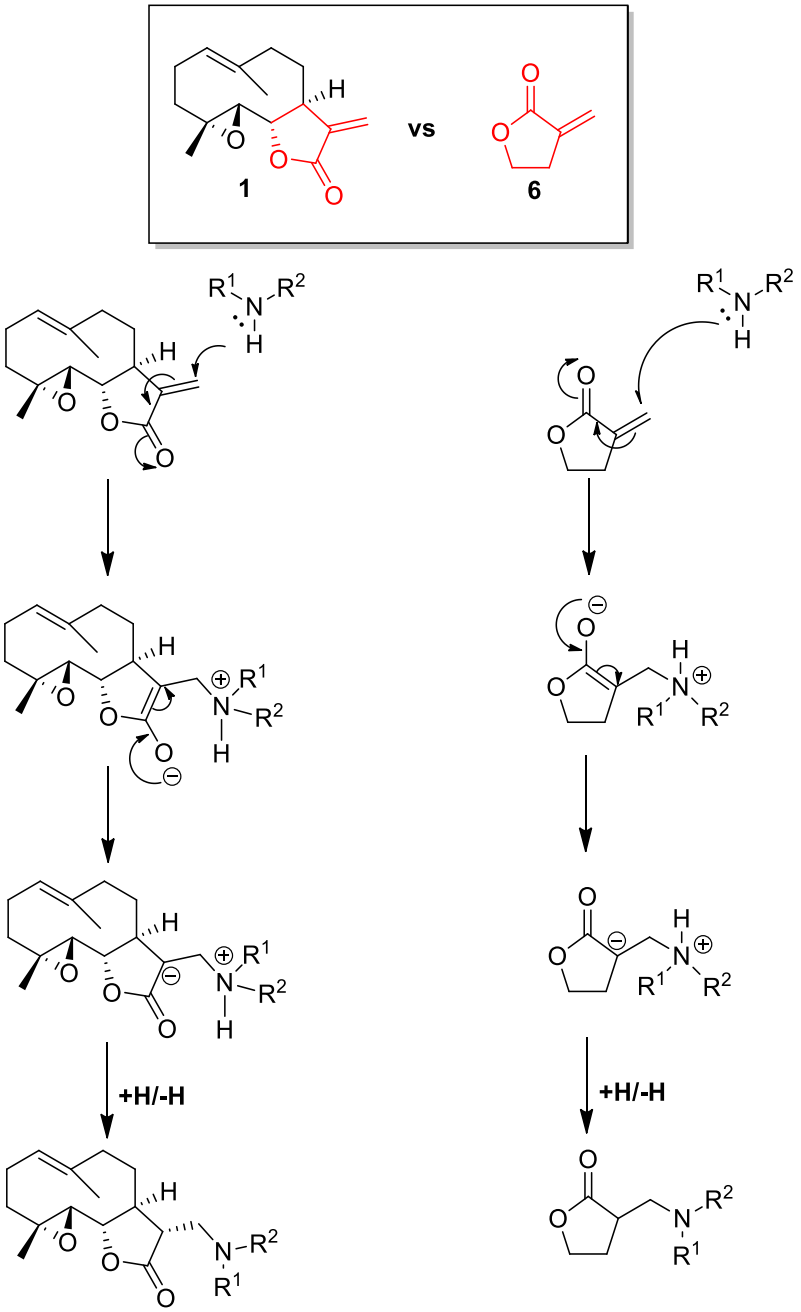


Figure 7: ^{19}F NMR spectroscopy showed prodrug convert back to SM.[22,28] (Permission to use from copyright of American Chemical Society, 2011)

As illustrated in **Figure 7**, PTL derivative can eliminate amine in the presence of biological thiol (glutathione) more easily and ^{19}F NMR data shows that the concentration of 4-trifluoromethylpiperidine is almost converted back from PTL derivative. Furthermore, the data gave a hint that free PTL would be preferentially trapped by a Michael addition of glutathione rather than recombining with 4-trifluoromethylpiperidine. On the other hand, it can be easier to understand the biological studies of PTL and derivatives under the reversible reaction *in vitro* or *in vivo*. The biological activity of PTL has been found to be related to the nucleophilic reaction of the α-methylene-γ-lactone ring (as the Michael acceptor) with intracellular thiol groups like glutathione. During the familiar Michael addition reaction of PTL and glutathione, a variety of targets will be activated or inhibited, thereby acting as a biological switch for signalling downstream events (biological study of PTL and glutathione will be outlined in detail in chapter 1.7).



Scheme 5: Michael addition reaction of Tulipane and PTL

The α-methylene-γ-butyrolactone (**6**) has a similar structural relationship to sesquiterpene lactones PTL (**1**) and additionally is a good Michael acceptor. Conjugate addition mechanisms of both tulipane (**6**) and parthenolide (**1**) are shown in **Scheme 5**. Amines are good nucleophiles. The non-bonding pair of electrons of nitrogen atom will readily attack the electrophile double bond of tulipane in the Michael addition reaction as same as PTL.

The only difference is that a single (*R*) stereocentre is obtained at C-13 for Michael addition reactions of parthenolide with amines. Stereoselectivity is observed to a selective *exo* face protonation of the enolate intermediate due to the steric effects of the substrate outline in **Figure 6**.

Therefore, due to the similar reactivity of tulipane with parthenolide and the ease of access to this readily available inexpensive lactone, tulipane can be considered a suitable pre-screening substrate prior to parthenolide modifications.

Overall, the Michael addition reaction is frequently used to synthesis natural products or drugs. The most important part of this class of reaction is the creation of a new C-N bond and the C-N polar bond of compound could be shown more soluble in water. As in previous study, Hewaman *et al*. and Neelakantan *et al.* have successfully synthesised a series of aminoparthenolide analogues undergoes Michael addition reaction. Some amino derivatives obtained promising results *in vitro* against acute myeloid leukaemia (AML) and the implication of these previous studies are that it could be a logical step to treatment aminoparthenolide derivative on CLL. Therefore, this MSc research project has used the simple and highly efficient method for the synthesis of β-amino carbonyl compounds from aliphatic, aromatic, and heterocyclic amines and α,β-unsaturated Michael acceptors under catalyst free conditions.

1.7 Biological Activity of Parthenolide

The α-methylene-γ-butyrolactone functionality appears to be at the centre of most of the biological activities connected with feverfew. The α-methylene-γ-butyrolactone moiety of PTL plays an important role for biological activities when one considers the exceptional ability of this functionality to act as a Michael acceptor in biological systems. A conjugate addition reaction presents *in vitro* or *in vivo*, the nucleophile very often appears as a thiol group and this result was firstly confirmed by Kupchan's group.[29] Recently published reports have shown that PTL displays anti-leukaemic activity and data suggests that its effects involve the induction of oxidative stress and inhibition of NF-κB signalling.[30]

1.7.1 Redox homeostasis

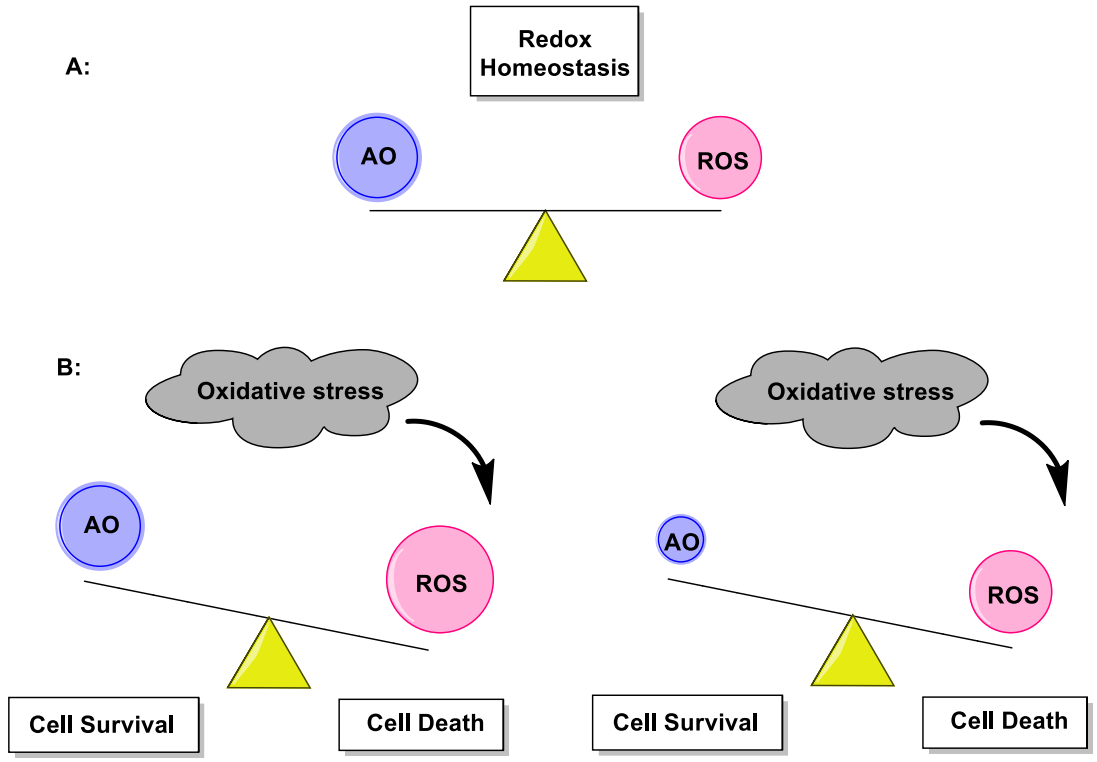


Figure 8: Part A) The balance between reactive oxygen species (ROS) and antioxidants (AO). Part B) Either increased reactive oxygen species or reduced antioxidants will disrupt redox homeostasis and eventually lead to cell death.

Redox homeostasis can be defined as the way to maintain a constant redox state between reactive oxygen species (ROS) and antioxidants in the body (**Figure 8**: Part A). [31]

Oxidative stress results from either enhanced intracellular ROS production or decreased cellular antioxidant defences. In addition, the level of oxidative stress in normal cells and tumour cells are different, with tumour cells having an especially higher level of oxidative stress.[15,32] This means that tumour cells may be more susceptible to therapies that induce more oxidative stress.

An imbalance of redox homeostasis leads to oxidative stress results in oxidative damage to cellular components such as DNA and protein. This can induce downstream cellular events that eventually lead to cell death (**Figure 8**: Part B).[33,34] An important and abundant cellular antioxidant is glutathione.

1.7.1.1 Glutathione

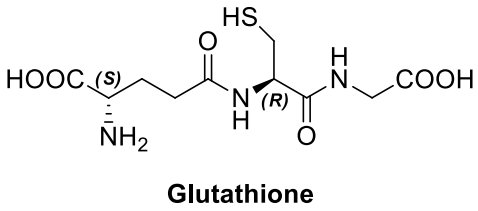


Figure 9: Structure of Glutathione

Glutathione, an endogenous tripeptide (**Figure 9**), exists in two different forms: one is GSH which is a reduced state, and the other is GSSG which is an oxidised state.[34]

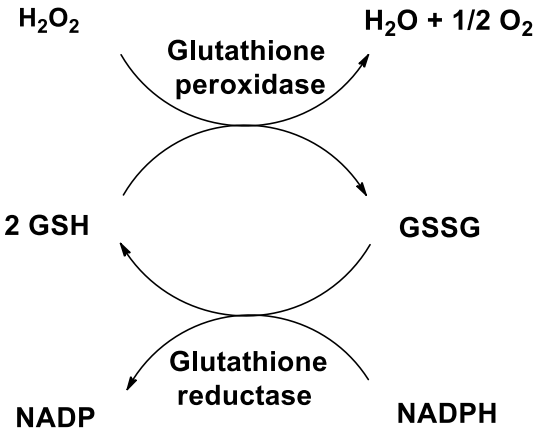


Figure 10: The Glutathione system.[34]

Glutathione converts to a reactive species which donates an electron to ROS (H_2O_2 in **Figure 10**) and reacts with another reactive glutathione rapidly to form GSSG. GSH is regenerated from GSSG using the enzymatic activity of glutathione reductase (GSR) and the NADPH cofactor. (**Figure 10**).[34]

In general, most glutathione exists in a reduced state of GSH, while less than 10% of glutathione exists as GSSG. The rising volume of GSSG regenerating to GSH can increase the oxidative stress. Indeed, Glutathione (GSH) and other protein thiol groups play an important role in cell survival. Many studies have shown that disrupting the accurate redox state by depleting GSH or other intracellular thiol groups will induce downstream cellular events that eventually lead to cell death.[34]

One of the ways that PTL is thought to induce oxidative stress is by reacting with glutathione, thereby reducing the levels of this antioxidant and exposing the cell to oxidative damage from endogenous ROS.[15,31,34]

1.7.2 NF-kB

Sen *et al.* discovered nucleoprotein factors to enhance the combination of sub B locus and immunoglobulin K light chain genes from the nuclear extracts of B lymphocytes, so that the transcription of the K light chain can be regulated. Such a nuclear transcription factor was named κB.[35]

NF-κB plays an important role in the survival and proliferation of normal and neoplastic B cells. In CLL, NF-κB has been found to be activated to a variable degree, regardless of disease stage or treatment status. NF-κB is vital for the regulation of many genes that prolong cell survival time and for the regulation of many processes in the development of cancer, including clonal expansion, growth, differentiation, angiogenesis, adhesion, and extracellular matrix degradation. Therefore, NF-κB has been suggested to be a promising target for therapy in CLL.[36-41]

1.7.2.1 Parthenolide inhibits NF-κB

NF-κB is sequestered in the cytoplasm in a non-activated form by complexing with the inhibitory protein, IκB. Phosphorylation of IκB by IκB kinase (IKK) results in the proteosomal degradation of IκB and the translocation of NF-κB into the nucleus where it can transactivate gene expression. NF-κB and IκB-α are involved jointly in the regulation of inflammation and immune responses, proliferation and apoptosis of cells.[42]

In vitro studies have shown that parthenolide inhibits the activity of NF-κB. PTL directly binds and inhibits IKK (IκB kinase), blocking the phosphorylation and degradation of IκB, The result is inhibition of NF-kB activity through stabilisation of IκB.[18,40-44] Consequently, PTL and its derivatives use IκB phosphorylation as drug targets to inhibit NF-κB, **Figure 11**.[40,41]

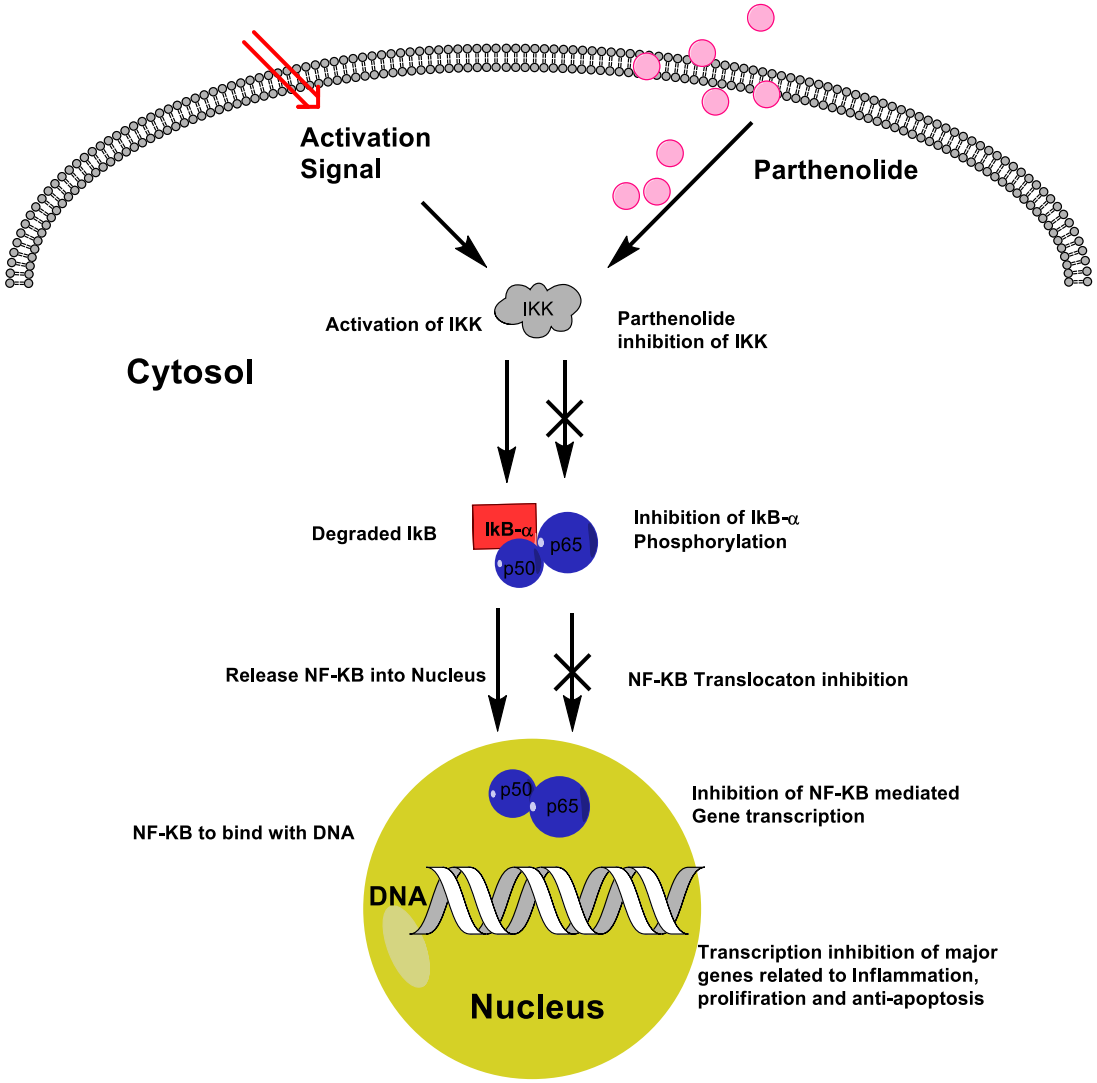


Figure 11: Left side: Basic mechanism of NF-κB action. Right side: 1) Activation of IKK. 2) Phosphorylation of IκB-α and degradation of IκB to induce the activation of NF-κB. 3) Release NF-κB into nucleus. 4) NF-κB to bind with DNA. Right side: Basic pathway of parthenolide mediated anti-cancer and anti-inflammatory activities: 1) PTL inhibition of IKK. 2) Inhibition of phosphorylation of IκB-α and degradation of IκB to induce the activation of NF-κB. 3) Inhibition of NF-κB mediated gene transcription.[16,40,41]

Chapter 2: Results and Discussions

There are two factors, origin and composition, that affect the amount of parthenolide (PTL) in feverfew. Several different types of feverfew were cultivated and then tested for the maximum content of PTL in this project. Around one hundred samples of three different types of seeds, golden feverfew, feverfew and tansy, were tested; all of them were cultivated by Winterbourne Botanical Garden at the University of Birmingham. Plants were all grown in greenhouses in winter 2012 to avoid the influence of fluctuating seasonal conditions.

2.1 Extraction of the PTL using Developed Extraction Procedure

Some literature reports suggested to use different extraction procedures as discussed in chapter 1.5.1. A recent report indicates the possibility of extracting PTL into water with HPLC (High Performance Liquid Chromatography) procedure to purify and isolate PTL.[20] However, HPLC is a time-consuming and expensive method and may not be appropriate for extraction of large quantities. So if a non-HPLC method can be developed in this project to improve isolation time, cost efficiency and obtain a good yield, it is a better method.

In order to use simplified purification methods such as column chromatography and recrystallisation, an effective extraction was required and only contains the minimal number of components. As mentioned before, PTL has shown lower water solubility, but it can be seen as an advantage if the water extraction procedure is applied. Because water will absorb all the hydrophilic compounds, PTL is more easily extracted by an organic solvent. Therefore, after the crude aqueous solution extraction, PTL can be extracted with a further organic solvent (e.g. chloroform) extraction from the water extracts. In fact, a recombining extraction and purification procedure was developed by Fossey's group in which a minimum number of components were extracted after the whole extraction procedure, compared to previous methods. Their purification method only uses traditional purification procedures such as column

chromatography and recrystallization, which is removing the HPLC procedure to obtain the pure target product (PTL).

2.2 Results from Extraction of Different Plants

Only fresh plant matter was used in this project and the plants were not dried before extraction. The extraction yields in this project and the yields from literature are not comparable because they used dried plants. Fresh golden feverfew, feverfew and tansy were extracted by this novel extraction procedure. The amount of extracted of PTL was shown in **Table 3**.

Table 3: Plants were extracted by novel procedure

Entry	Type of feverfew used	Amount of plant matter used	Crude extract of PTL	Amount of PTL (recrystallisation)	Yield (Wt. %)
1	Fresh golden feverfew	844 g	5 g	1.3 g	0.15
2	Fresh feverfew	798 g	3 g	0.1 g	0.01
3	Fresh tansy (flowering)	2.56 kg	6 g	1.4 g	0.05
4	Fresh tansy (seeds)	2.242 kg	8 g	2.9 g	0.13

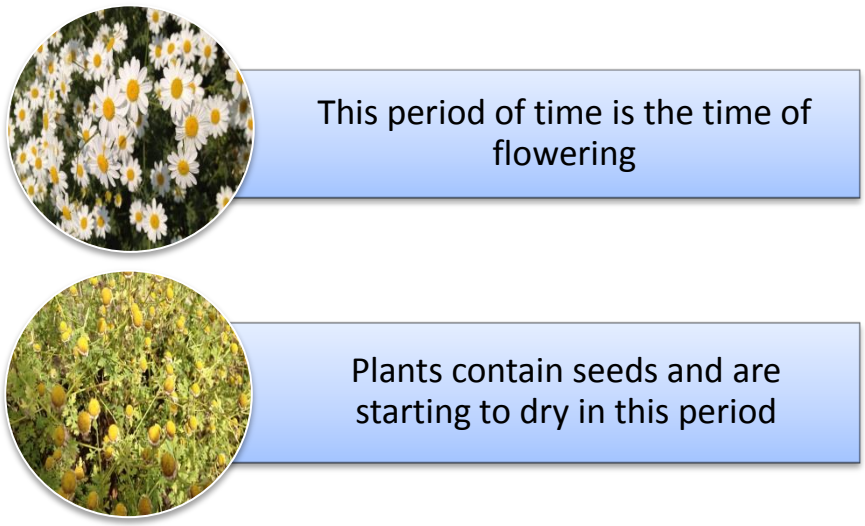


Figure 12: The different periods of tansy

When comparing entry 1 and 2 in **Table 3**, the obtained yield of PTL from golden feverfew is fifteen times more than feverfew when using similar weight of plant matter. Entries 3 and 4 are both extraction of tansy; the yield of PTL for tansy is also less than golden feverfew. However, when extracting similar amounts of tansy matter (Entries 3 and 4 in **Table 3**), Tansy matter containing seeds has threefold more parthenolide compared to the flowering plant. Consequently, the results may indicate the relative concentrations of PTL in different species type. Golden feverfew was the best plant containing the maximum amount of PTL. Also, this result confirms seeds contain much more PTL than the other parts of feverfew.

PTL was fully characterised by melting point, mass spectrometry, proton NMR, carbon NMR and IR. As the formation of PTL is colourless crystal, it has also confirmed by single crystal X-ray analysis (**Figure 13**). The absolute structure of PTL has been shown in **Figure 14** and it indicated the same stereochemical features compared with literature structure.[45]

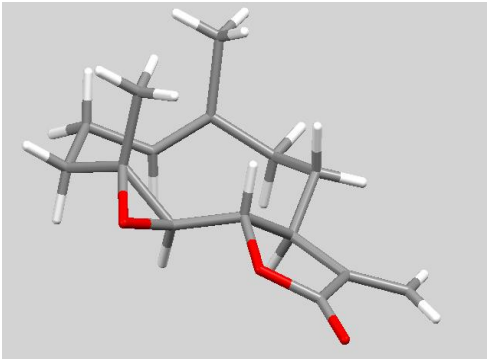


Figure 13: Single crystal structure of PTL

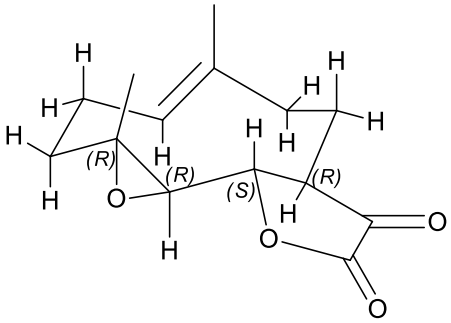


Figure 14: The absolute structure of PTL

2.3 Tulipane (6) Modification

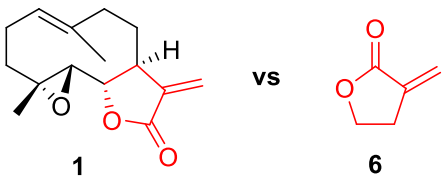


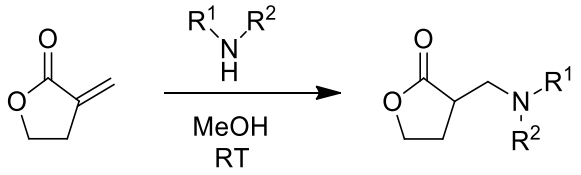
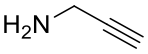
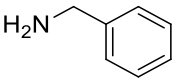
Figure 15: Tulipane has a similar structural relationship to PTL.

Tulipane (**6**), α-methylene-γ-butyrolactone, has a similar structural relationship with PTL. It is a good Michael acceptor and it shows similar reactivity to PTL in organic chemistry. Both Michael addition mechanisms of PTL and tulipane were given in **Scheme 5** and they were proceeded *via* the conjugate addition to the *exo*cyclic double

bond on the α,β-unsaturated lactone. In addition, tulipane is a simple, readily available chemical reagent with a lower price than PTL.

Due to the similar reactivity of tulipane with PTL and the ease of access to this readily available inexpensive lactone, tulipane was used as a model substrate to test both derivatisation chemistry and the biological screens prior to using the more precious parthenolide. Therefore, the simply and highly efficient method of Michael addition reaction was used to synthesis β-amino carbonyl compounds from aliphatic, aromatic, heterocyclic amines and α,β-unsaturated Michael acceptors (PTL and tulipane) under catalyst-free conditions.

Table 4: Tulipane derivatives have synthesised by primary amines.

Entry No.	**Target No.**	**Amine**	**R^1**	**R^2**	**Yield obtained (%)**
					
1	**6a**		Hydrogen	Prop-1-yne	60
2	**6b**		Hydrogen	Benzyl	61

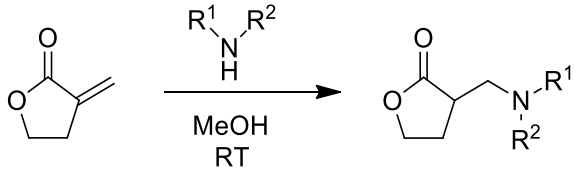
Primary amines were attempted first in this project. Two tulipane derivatives were obtained from the Michael reaction of primary amines with tulipane. Propargylamine, a primary alkyne amine, contains a carbon-carbon triple bond. One advantage of propargylamine is that it can be used as an intermediate to increase the diversity of its derivative undergoing 'click chemistry' in future, such as to introduce a triazole derivative. Propargylamine was reacted with α,β-unsaturated lactone into a stirred methanol solution at room temperature for 24 hours. Reaction progress was monitored

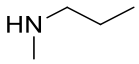
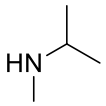
by Thin-layer chromatography (TLC) and target product **6a** was isolated using the general experimental procedure (Method A can be found in experimental section).

Target compound **6b** was synthesised using benzylamine, a primary aromatic amine, as the starting marital reacted with tulipane through the general procedure A. Both primary amine derivatives **6a** and **6b** have shown similar reaction yields, 60 % and 61 % respectively. For target compound **6b** and **9e** (**Table 7**, entry 5), both contain aromatic groups, and the different number of aromatic groups may give rise to different biological results.

However, previous work by Fossey's group suggests that when reactions used excess amounts of primary amines, they caused a major by-product. These reactions have undergone the 1,4-conjugate addition firstly, then were followed with a 1,2-addition of excess primary amine. The by-product occurred after the double addition in which the lactone ring was opened while amide and primary alcohol functional groups formed. Also, Fossey's group reported the second addition of primary amine only occurred after 1,4-conjugate addition completely. Therefore, only one equivalent primary amine was added into each reaction to avoid a second addition to form undesired product. Nevertheless, even reaction use excess amounts of secondary amines, only the desired product is formed, due to only 1,4-conjugate addition occurred in reaction.[4]

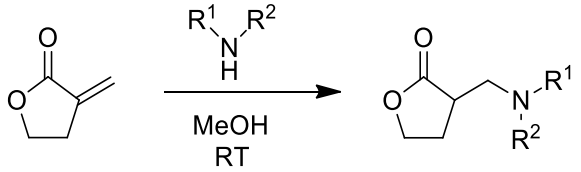
Table 5: Tulipane derivatives have synthesised by acyclic aliphatic secondary amine.

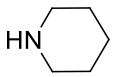
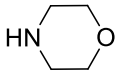
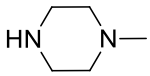
Entry No.	Target No.	Amine	R^1	R^2	Yield obtained (%)
					

1	**7a**		Methyl	N-propyl	78
2	**7b**		Methyl	Isopropyl	32

N-Methylpropylamine and *N*-Isopropylmethylamine, two acyclic aliphatic secondary amines, are structural isomers which have the same chemical formula ($C_4H_{11}N$). The difference between these two amines is that *N*-Isopropylmethylamine has a methyl group attached to the secondary carbon and this attached group gives it a side chain which is slightly bulky. When compared to each other, both compounds may found different medical properties of the pair of isomers. They were reacted with tulipane to undergo the addition reaction to afford target product **7a** and **7b**. Additionally, the yields between them were so different that **7a** is twice as high as **7b**. To explain the difference, one possible reason is that structural isomers process different chemical reactivity such as the effect of steric hindrance of the bulky side chain of *N*-Isopropylmethylamine.

Table 6: Tulipane derivatives have synthesised by cyclic aliphatic amine.

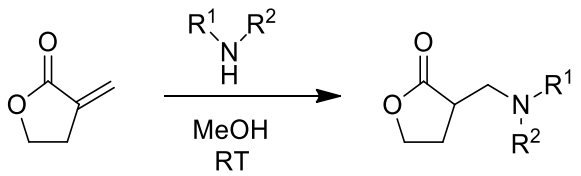
Entry No.	**Target No.**	**Structure of Amine**	**Amine**	**Yield obtained (%)**
				
1	**8a**		Pyrrolidine	86

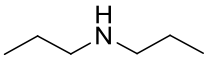
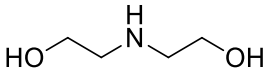
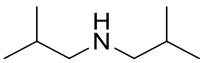
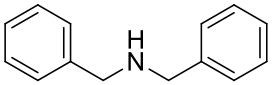
2	**8b**		Piperidine	91
3	**8c**		Morpholine	81
4	**8d**		1-Methylpiperazine	61

Four cyclic aliphatic amines, namely Pyrrolidine, Piperidine, Morpholine and 1-Methylpiperazine, were synthesised with **6** to obtain target **8** (**a-d**) by general method A. As compound **8a** has a five-membered ring and **8b** has a six-membered ring. Comparing both products to make a better understanding of the biological activity of ring size is one goal of this project.

Target **8c** and **8d** also contain a six-membered ring, but **8c** adds an oxygen atom into the six-membered ring and a nitrogen atom was replaced by a carbon while attached an *N*-methyl group to form **8d**. Therefore, we can see the different chemical properties and biological activity between the conditions; namely that the six-membered ring structure contains a heteroatom and does not have a heteroatom. In addition, only compound **8d** has a relatively lower yield (61%) compared with other three cyclic amine derivatives (the yields were in the range 81-91%).

Table 7: Tulipane derivatives synthesised by symmetrical secondary aliphatic amines

Entry No.	**Target No.**	**Amine**	**R^1**	**R^2**	**Yield obtained (%)**
					

1	**9a**		Methyl	Methyl	70
2	**9b**		N-propyl	N-propyl	35
3	**9c**		N-2-hydroxyethyl	N-2-hydroxyethyl	
4	**9d**		Isopropyl	Isopropyl	
5	**9e**		Benzyl	Benzyl	

In this symmetrical secondary aliphatic amine series (**Table 7**), only two target compounds (**9a** and **9b**) were successfully synthesised using the general method (Method A). The yield of dimethyl tulipane derivative **9a** is twice than **9b.** With **9b** increasing the chain length in the propyl group, different chain length of the alkyl moiety of **9a** and **9b** may also affect their biological activity.

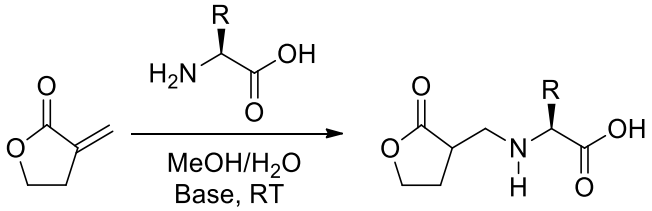
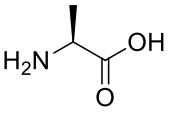
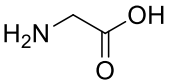
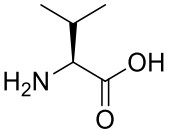
However, for compound **9c**, no product was observed after 24 hours by TLC-scanning. Hence, a suitable base (2 equivalents potassium carbonate) was added into the reaction to deprotonates amines and makes it more nucleophilic; this process also makes the whole reaction faster and more effective. After 12 hours, the TLC plate showed a new spot and reaction mixture was purified by column chromatography on silica with a dichloromethane and methanol eluent system. The proton NMR spectrum was quite complex and did not exactly match what we expected which products observed turned out to be disappointing. One possible reason is that the expected product might have been decomposed during the process of column chromatography.

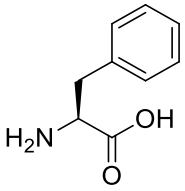
For target **9d** and **9e**, a similar situation occurred as the amines both contain the bulky side chain. This seems a possible reason to understand why the reactions were not complete after a longer period (more than 30 hours) by TLC-scanning. Both reaction

mixtures were purified by column chromatography on silica with a dichloromethane and methanol solvent system. However, no expected product was present in either ^{1}H NMR spectrums respectively. Both reactions have been repeated under the same previous conditions to exam if any better results can be obtained. However, both repeated reactions failed again as a result of disordered ^{1}H NMR spectrums obtained.

The possible reasons for the failure of these reactions are the general space issues in chemical reactivity. In other words, if the nitrogen in amines has large substituents attached, it may hinder the ability to generate a new C-N bond. Also the steric effects of bulky substituents of amines may destabilise the compound and hence lead to the unstable and rapid decomposition of two targets **9d** and **9e.**

Table 8: Tulipane derivatives synthesised by amino acid

Entry No.	Target No.	Amino acid	Structure of Amino acid	R	Yield obtained (%)
					
1	**10a**	L-Alanine		Methyl	33
2	**10b**	Glycine		Hydrogen	25
3	**10c**	L-Valine		Isopropyl	20

4	**10d**	L-Phenylalanine		Benzyl	13

Compound **10a, 10b, 10c** and **10d** have used the amino acid as the starting material(SM). A carboxylic functional group of the synthesised compounds might play an important role of *in vivo* testing. For example, the amino acid reacts with another amino group to generate peptide chains. Also the different R group of amino acids, such as hydrogen, methyl, isopropyl and benzyl side chain, can give a better understanding of whether the effects contained a smaller or larger size substituent in organic chemistry and biochemistry.

However, further purification of those compounds became difficult due to greater water solubility. A new method (Method B) was developed in which one equivalent amino acid and excess tulipane were used in reaction. Product formation was judged by TLC and reaction mixture reduced to dryness *in vacuo*. The residue was washed with ethanol three times to afford a colourless crystalline solid.

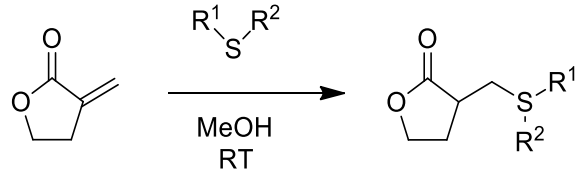
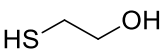
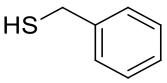
Compound **10a** and **10b** have been successfully synthesised by implementing the new reaction procedure. The purity of both compounds is assessed by mass spectrometry, NMR (proton and carbon nuclear magnet resonance spectrometry) and IR. Compound **10c** also used the new method to synthesise with **10a** and **10b**. The crude product of **10d** was washed by using numerous common organic solvents that are supplied in the lab to obtain pure product. However the results of the proton NMR were similar to the crude product, as the ^{1}H NMR spectrum still showed few impurity peaks. Carbon C-18 column on CombiFlash Chromatography System was used to purify this crude product later (water and acetonitrile mixture as solvent system), but **10d** cannot show a better purity. Even so, the compound **10c** proves successful synthesised by Proton NMR, Carbon NMR, IR and mass spectrometry. This situation is not ideal but **10d** can still be used initially to detect whether it has anti-cancer activity or not.

Compound **10c** was successfully synthesised by this new method and judged by Proton NMR, Carbon NMR, IR and mass spectrometry. However, purification of this

compound is also difficult. The product showed very low solubility in most common solvents and was only soluble in water. So, compound **10c** cannot be purified by normal column chromatography. Even after being purified later by using a recrystallization method, the product purity was still not improved. Then, attempts using C-18 Column for further purification yielded a clear separation that was achieved by using water and acetonitrile mixture solvent systems.

Therefore, the reaction yields of amino acid derivatives were in a lower range of 13–33%. The yield of **10c** and **10d** especially have been reduced during the difficult purification process.

Table 9: Tulipane derivatives synthesised by thiol group

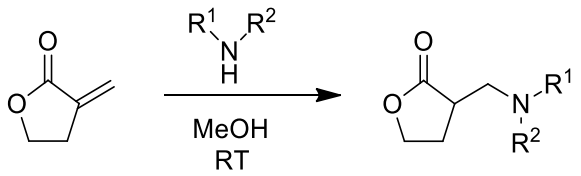
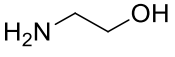
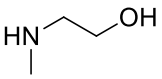
Entry No.	**Target No.**	**Thiol**	**R^1**	**R^2**	**Yield obtained (%)**
					
1	**11a**		Hydrogen	N-2-hydroxyethyl	78
2	**11b**		Hydrogen	Benzyl	85

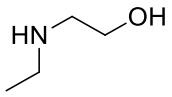
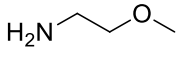
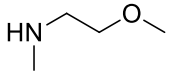
Two thiols, 2-mercaptoethanol and benzylmercapton, were reacted with tulipane undergoing the Michael addition reaction to afford target product **11a** and **11b** respectively. Compound **11b** is a sulphur derivative of **6b** in which a nitrogen atom was replaced by a sulphur atom. Thiol is an organosulfur compound that contains an SH functional group; it is the same as amine which is also a common 'soft' nucleophile. As the β carbon of α,β-unsaturated lactone is soft electrophile, both soft

nucleophile and electrophile are favoured to react with each other, and to and form a new C-S bond undergoes 1,4-conjugate reaction. Thiol groups play a crucially important role in biochemistry study, the compounds have worked through the conjugate addition reaction with intercellular thiol groups. As mentioned before, compared with amine derivatives, it is more difficult for the thiol derivatives to undergo an irreversible Michael addition reaction *in vitro*, during which the intercellular thiol groups cannot degenerate this thiol derivative to form free α,β-unsaturated lactone very easily. Therefore, compound **11a** and **11b** can be used as an indicator, an inactive compound, to judge the biological activity of other tulipane derivatives in *in vitro* studies.

Compound **11a** and **11b** showed no product formation after 24 hours by TLC. One possible reason is that the thiol group shows a relatively lower nucleophilicity compared to amine. Therefore, a suitable base, triethylamine (TEA), was added into the reaction to increase the nucleophile of thiol group. After further 12 hours, two targets were obtained by TLC-scanning and isolated using the general experimental procedure (Method A). The purity of both compounds was assessed by mass spectrometry, NMR and IR. Because the additional base was used, both compounds **11a** and **11b** obtained a relatively high yield (78% and 85% respectively).

Table 10: Tulipane derivatives synthesised by aliphatic amines

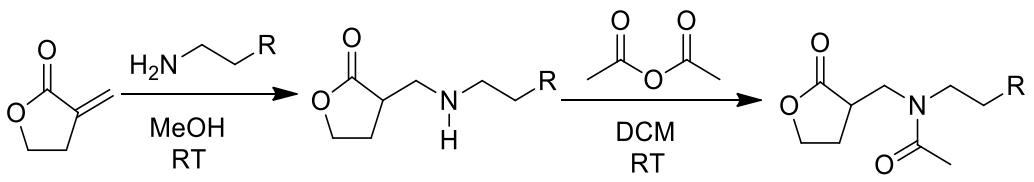
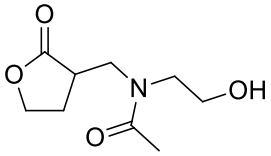
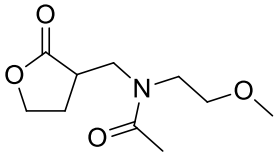
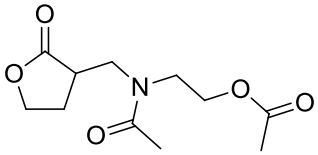
Entry No.	**Target No.**	**Amine**	**R^1**	**R^2**	**Yield obtained (%)**
					
1	**12a**		Hydrogen	*N*-2-hydroxyethyl	71
2	**12b**		Methyl	*N*-2-hydroxyethyl	91

3	**12c**		Ethyl	*N*-2-hydroxyethyl	61
4	**12d**		Hydrogen	*N*-2-methoxyethyl	82
5	**12e**		Methyl	*N*-2-methoxyethyl	48

Two secondary aliphatic amines derivatives (**7a** and **7b**) have been discussed in **Table 5** (entry 1 and 2). Compared with **7a** and **12b**, the relationship between both targets is that the propyl chain of **7a** has been converted to an ethanol to form **12b**. Compound **12a** and **12c,** also contained an alcohol functional group, have the same R^2 substitute *N*-2-methoxyethyl side chain compared with **12b**. Compare the three amino alcohol derivatives, **12a**, **12b** and **12c,** the only difference is that the compounds contain different alkyl moiety which increased the chain length of R^1. Therefore, to compare the structure-activity relationship and water solubility of the compound including OH group or not are one of the goals of this experiment. The reaction yield of **12a** and **12c** were in the range 61–71%. Target **12b** obtained the highest yield (91%) in **Table 10**. Also, the purity of those compounds was assessed by mass spectrometry, proton NMR, carbon NMR and IR.

In this project, two amino ether tulipane derivatives (**12d** and **12e**) have also been synthesized by the general experiment method (**Table 10**, entry 4 and 5). The difference between compound **12a** and **12d** is that an *N*-methyl group has replaced the hydrogen to attach the oxygen atom and converted to an ether functional group as same as target **12b** and **12e**. Therefore, comparing those targets which contain an alcohol or ether functional group to find out which one has obtained the higher aqueous solubility or better biological activity is another goal to achieve in this project.

Table 11: Novel amide tulipane derivatives were synthesised in two steps reaction.

Entry No.	Target No.	Structure of Target	R	Yield obtained (%)
				
1	**13a**		Hydroxyl	51
2	**13b**		Methoxy	48
3	**13c**			*NB*

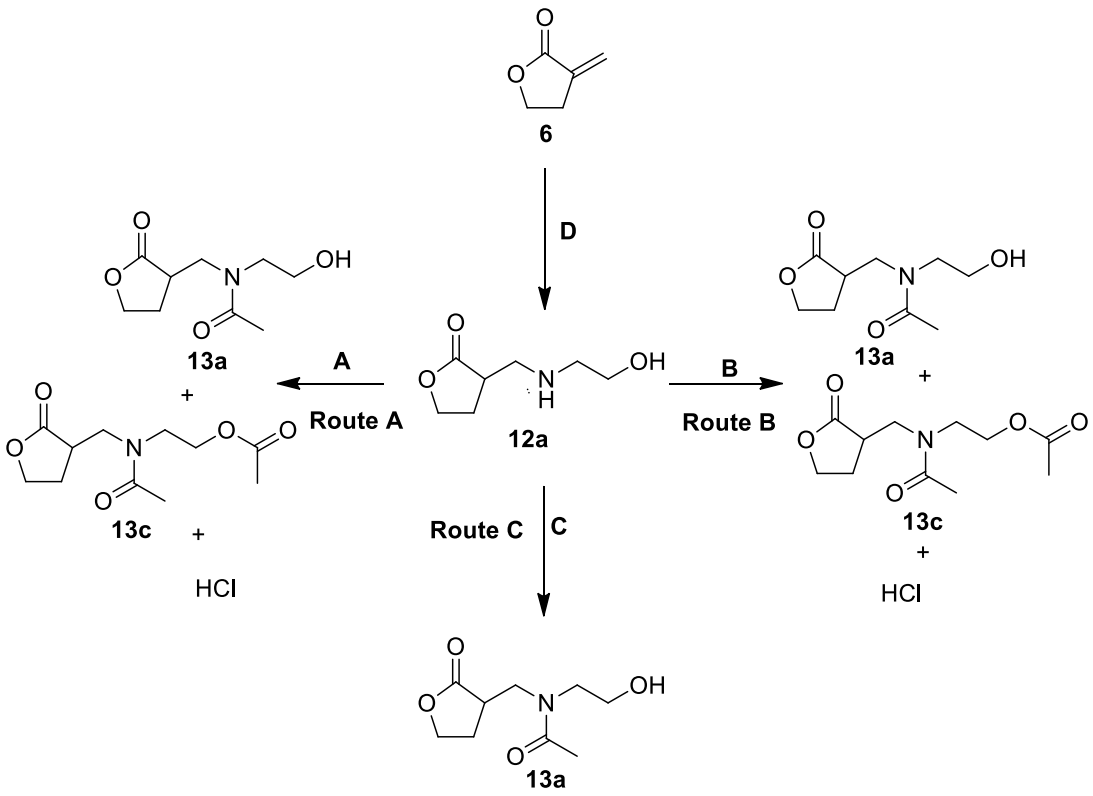
NB: Compound **13c** is a major by-product of compound **13a** when use acyl chloride as SM, and this issue will be discussed in chapter 2.4

Compound **12a** and **12d**, as secondary amine analogues, are available to undergo a further reaction. Therefore, two amide tulipane derivatives (**13a** and **13b**) have been synthesised in **Table 11**, which is judged by mass spectrometry, proton NMR, carbon NMR and IR. Derivative **13a** and **13b** have undergone a two-step reaction. The first step has been used the general method (Method A) to synthesise the secondary amino-tulipane derivatives (**12a** and **12d** respectively). Later, the amide derivatives were formed by an alkylation reaction (**Scheme 7**). The reaction yield of amide tulipane derivatives **13a** and **13b** were in the range of 48–51%. A possible benefit is that the

enzymes which live in the biological system may slowly decompose the amide and allow the compound to stay in inactive state longer. Therefore, derivatives could be breaking down to free α,β-unsaturated lactone slowly while losing biological activity by being substituted by biological nucleophiles quickly before the target was found. In addition， derivative **13c** (entry 3) is a major by-product of compound **13a**. This issue will be discussed in chapter 2.4.

2.4 By-Product Study

As mentioned above, the secondary amine derivative undergoes a second modification to produce a novel amide derivative. Firstly, an excess amount of acetyl chloride was reacted with compound **12a** (secondary amine derivative) in which a by-product was obtained (Route A).



Scheme 6: Compound **13a** was synthesised by using two different starting materials. Conditions: A) Excess Acetyl Chloride; B) 1 equiv Acetyl Chloride; C) Excess (1.2 equiv) Acetic anhydride; D) 1 equiv Ethanolamine

One possible reason the by-product is generated is due to the nitrogen atom having a more reactive lone pair than oxygen (nitrogen being less electronegative than oxygen), which makes it more nucleophilic to attack the carbonyl in aryl chloride. However, excess of acetyl chloride will push the reaction forward, because it is reacting with the amino group to form an amide and then also reacting with alcohols to form esters (Route A). Therefore, if only one equivalent of acetyl chloride is used in the reaction, the desired product can be isolated preferentially. In fact, one equivalent of acetyl chloride was used and both the desired and undesired products have been obtained in parallel. The most likely reason is that acetyl chlorides reacts with the amino group and alcohol to form amide and ester respectively and both reactions occurred at a similar rate. (Route B).

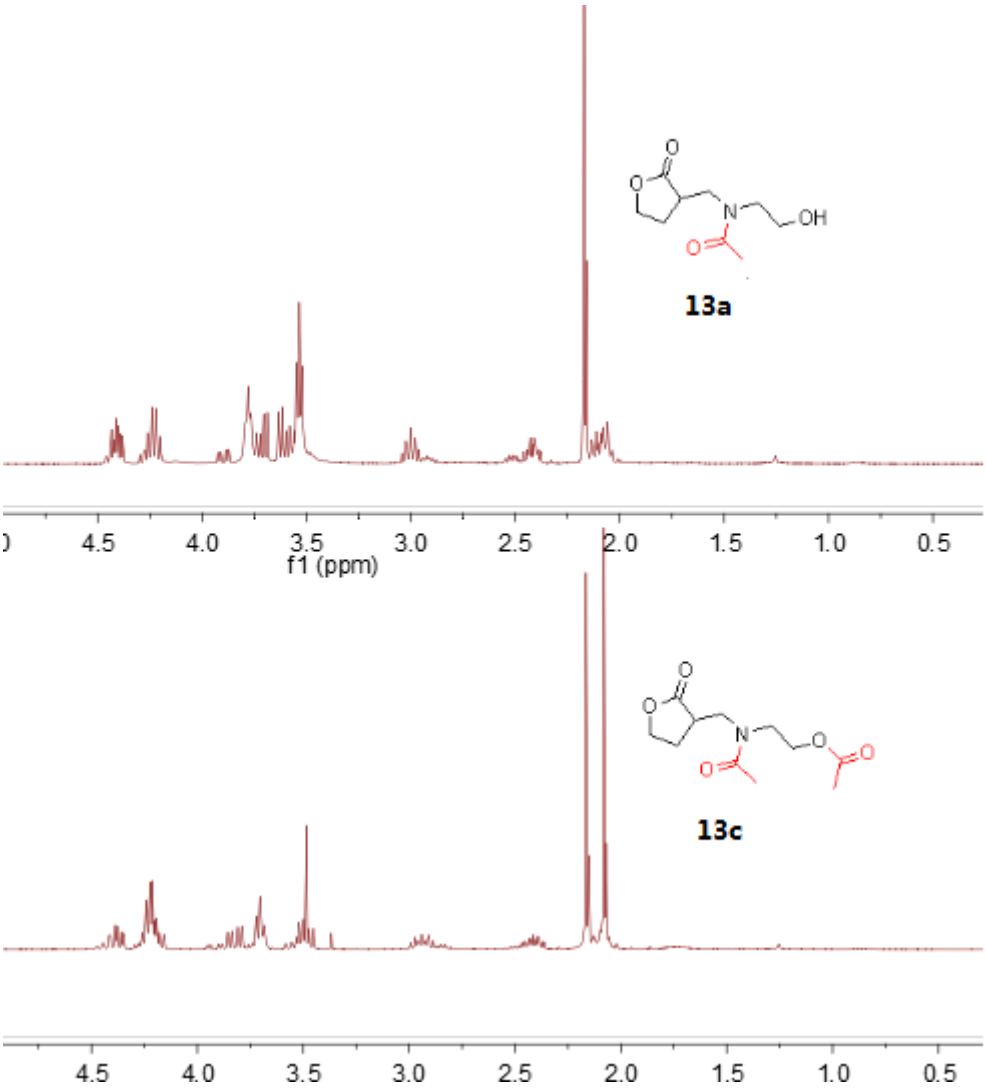
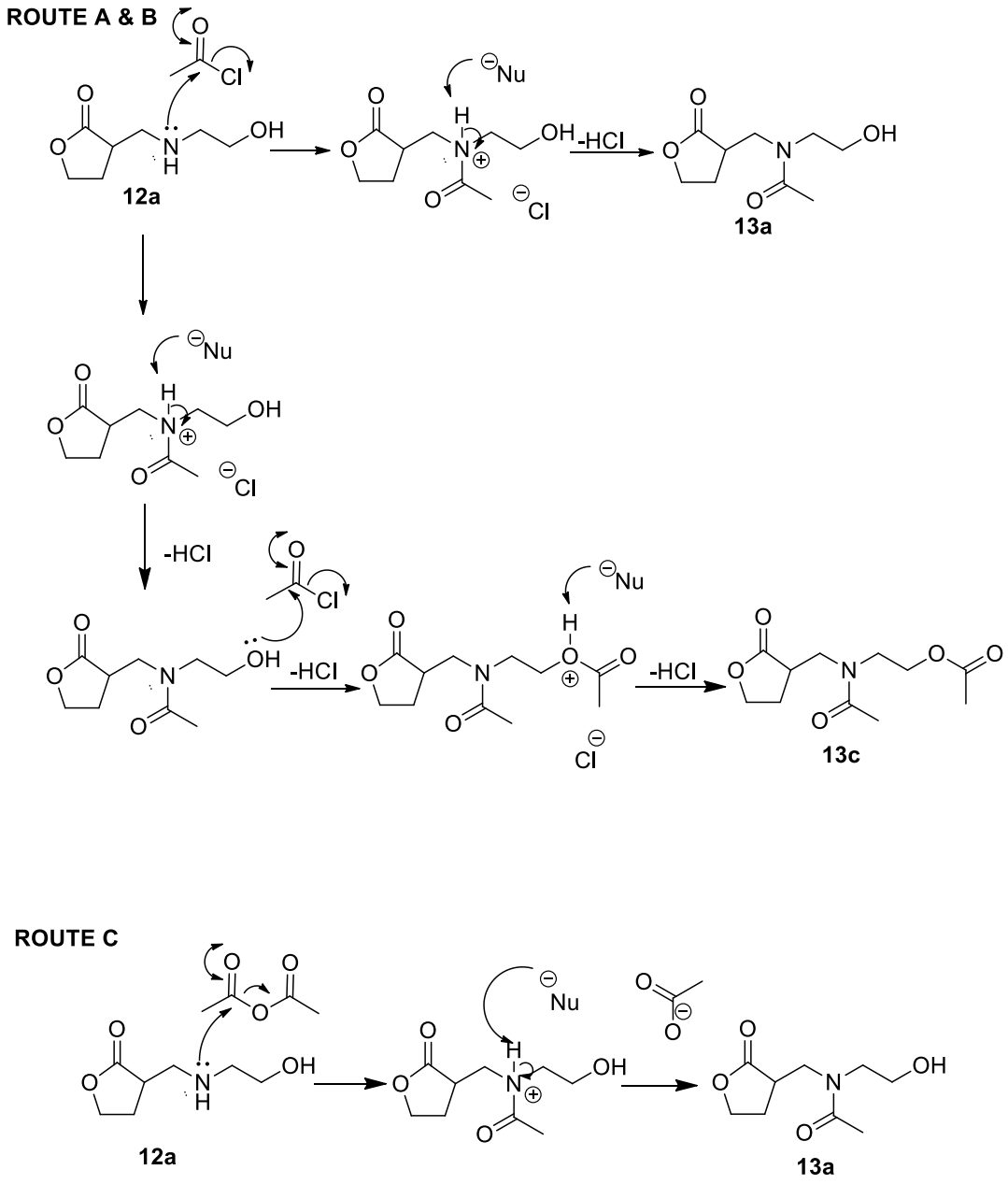


Figure 16: Proton NMRs of compound **13a** and **13c**.



Scheme 7: Mechanism for formation of the compounds **13a** and **13c**

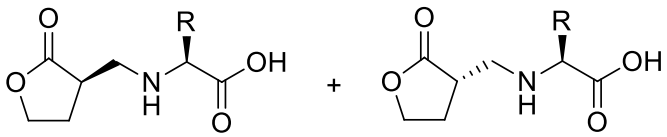
Amide and ester function group of the by-product were identified (**13c**) via Infrared, Proton and Carbon NMR spectroscopy. IR (Infrared) shows three C=O groups stretches in the range of 1770-1640 cm^{-1} (amide, ester and lactone function group).^{1}H NMR showed two singlets between 2 to 2.2 ppm whose peaks belong to each of the two methyl groups of compound **13c.** Also, ^{13}C NMR showed 11 peaks with 11 different carbon environments which two methyl groups at 21.7 and 21.0 ppm respectively. All information above has suggested a by-product synthesised during the Route A and Route B (see **Scheme 6** and **Scheme 7**). Therefore, acetic anhydride was

used to replace the acetyl chloride to react with tulipane derivative (**12a**) and this reaction forms the desired product after 24 hours (Route C).

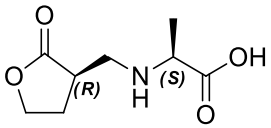
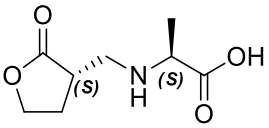
2.5 Synthesis of Diastereoisomers

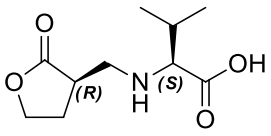
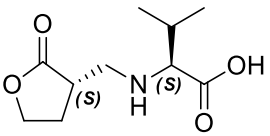
Both amino acid derivatives **10a** and **10c** constitute a pair of diastereoisomers respectively. The amino acid as a starting material has contributed to a stereogenic centre while another stereogenic centre was generated undergoing the Michael addition reaction. In general, the chemical environments of pair of diastereoisomers are different, which has been shown in the different symmetry. Therefore, the diastereotopic protons, such as proton in *N*-methyl group of **10a** and *N*-isopropyl group of **10c**, can have different chemical shifts and coupling constants. Hence, proton NMR spectroscopy can be used to differentiate the pair of diastereoisomers and determine the diastereomeric ratio.

Table 12: A pair of diastereoisomers of amino acid derivatives



Syn Anti

Compound No.	**Amino acid used**	**Syn(1)**	**Anti(2)**
10a	L-Alanine	 **10a-1**	 **10a-2**

10c	L-Valine	 **10c-1**	 **10c-2**

2.5.1 L-Valine Derivatives (10c-1 and 10c-2)

By analysing the structure of L-Valine derivatives, the proton in isopropyl group coupled with diastereotopic proton next to it give two doublets in the spectrum. According to the ^{1}H NMR spectrum of the material isolated from the reaction, the pair of doublet peaks can be easily identified and it was found out the CH groups shift from 3.35 ppm to 3.55 ppm. When analysing the coupling tree of CH groups there is a coupling constant of 4 Hz (J_b) within the doublet and also the two doublets are separated in 0.2 ppm.

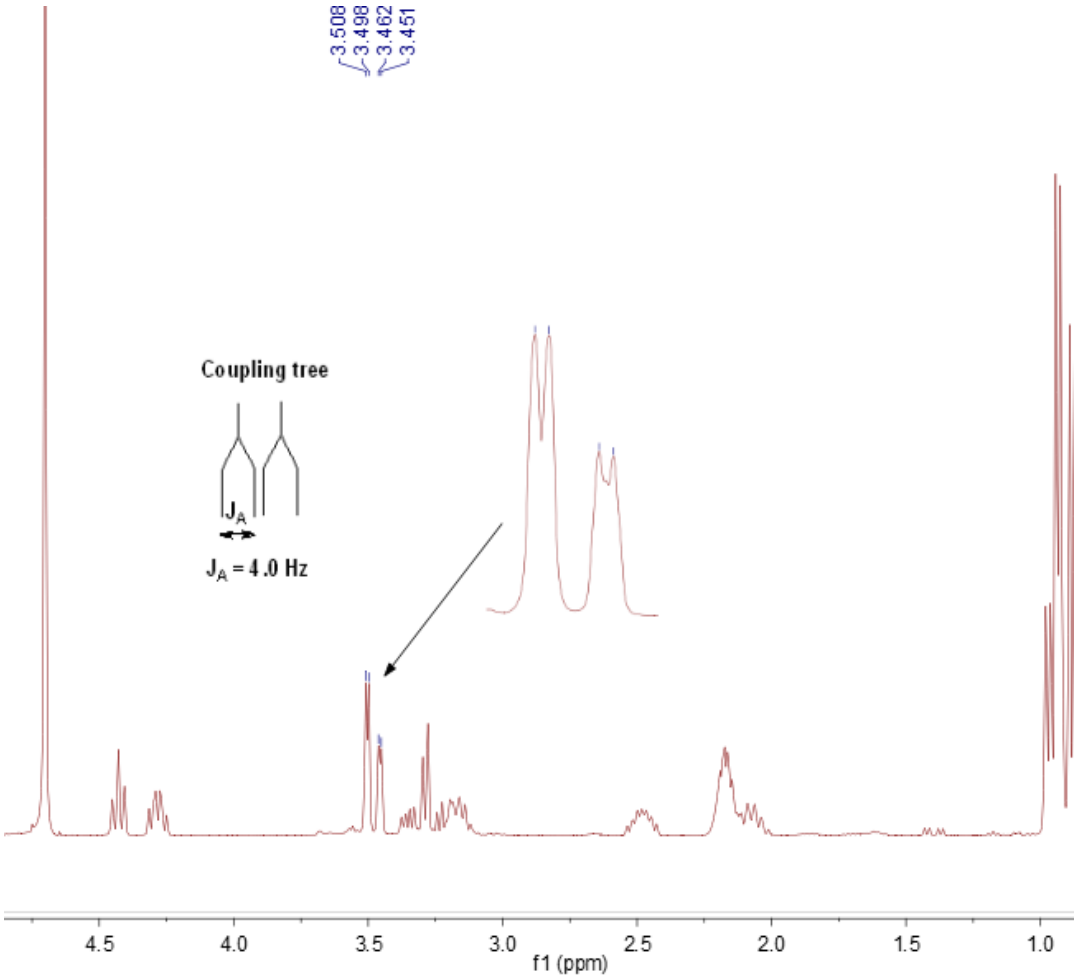


Figure 17: Proton NMR of compound **10c-1** and **10c-2** and coupling constants between the alpha proton and a proton of isopropyl group next it

Figure 17 shows diastereoisomers represented by the doublet peaks. Now it can be understood more easily about which peaks respond to each doublet. The ratio of pair of diastereoisomers is determined by calculating the integration of each doublet peak. Therefore, crude analysis of the diastereomeric ratio of two diastereoisomers **10c-1** and **10c-2** with ^{1}H NMR was estimated around 37:63 or 63:37.

2.5.2 L-Alanine Derivatives (10a-1 and10a-2)

For proton NMR spectrum of the material isolated from the L-Alanine reaction, we have defined a ratio of pair of diastereoisomers by analysing the coupling between the *N*-methyl group and the alpha proton next to it.

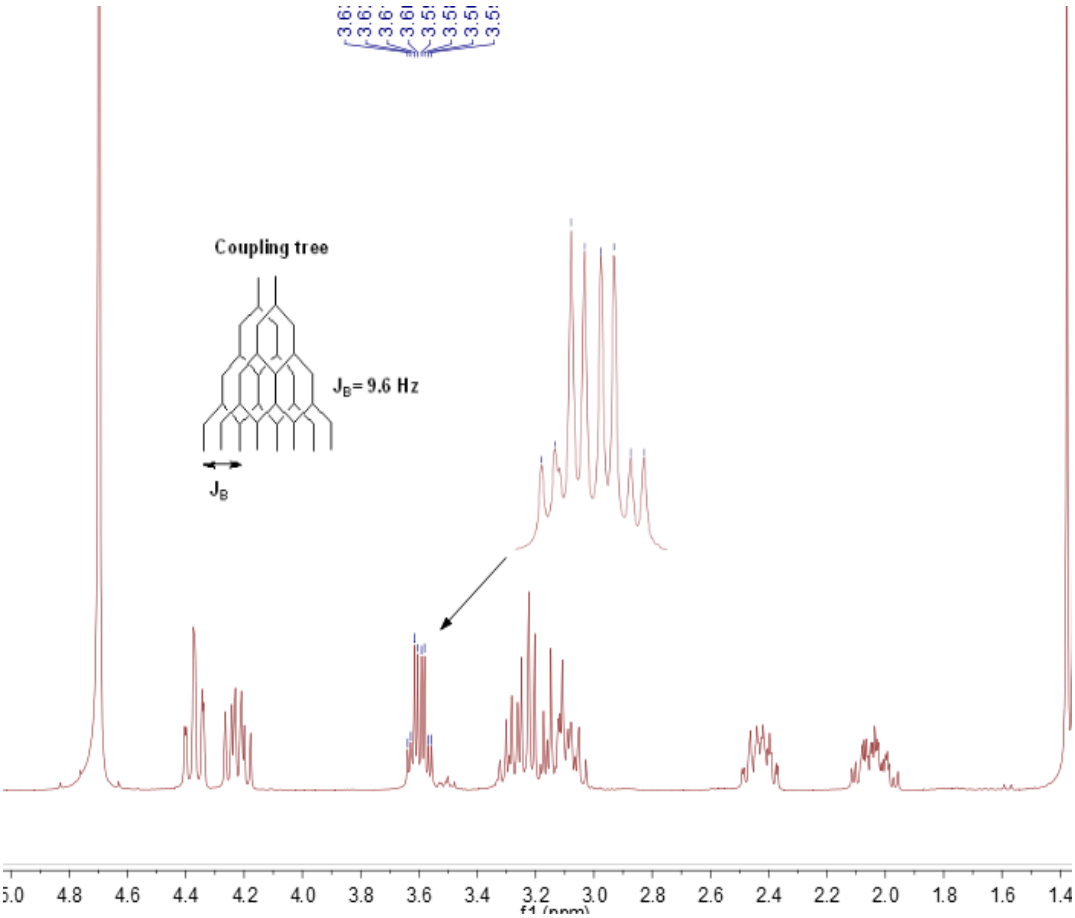


Figure 18: ^{1}H NMR of compound **10c-1** and**10c-2** and coupling constants between two methyl and beta protons

The alpha hydrogen shows two quartets at 3.6 ppm and these two quartets are overlapping each other. However, both quartets can be easily recognised with a coupling constant of 9.6 Hz (J_B). Therefore, the peaks of each quartet were identified and the integrating of each quartet peak has obtained the diastereomeric ratio of two diastereoisomers around 50:50.

2.6 Parthenolide Modification

For clinical drug studies discussed above, there is a major issue that PTL has very poor water solubility. In the past few years, a series of PTL analogues have been synthesised by Michael addition reaction which had higher water-solubility than PTL (**Scheme 4**). One advantage of this type of reaction is that only a single desired product is formed and the stereospecificity of the Michael addition reaction is

established. The reason is that the intermediate enolate of the conjugate addition from the *endo* face undergoes a 1,3-diaxial interaction of hydrogen on carbon 6, axial hydrogen on carbon 8 and two hydrogen on carbon 13.

The rationale of PTL derivatives is same as tulipane derivatives in which tulipane have successfully passed the preliminary chemical reactivity test (as discussed in chapter 2.3). It has proven that tulipane complies with the PTL theory.

The eighteen PTL derivatives, **14a-h** and **15a-j**, that have been successful synthesised and the reaction yield of each derivative can be found in **Table 14**; almost all the derivatives were synthesised using the general experimental method as outlined above (**Scheme 4**). Only the amino acid derivatives were synthesised using a developed procedure in which methanol and water mixture were used as solvents and an appropriate base (potassium carbonate) added in the reaction. PTL was reacted with 1.2 equivalent different types of amines and amino acids, further with the purification of the reaction mixture by column chromatography (silica, ethyl acetate and hexane as the solvent system). The purity of PTL derivatives was assessed by mass spectrometry, proton NMR, carbon NMR and IR. In addition, all aminoparthenolide derivatives prove that only *R* configuration was displayed at carbon 11 during the Michael addition reaction as judged by Specific rotation (this can be found in the experimental section). Only one PTL derivative (**14h**) has the *S* configuration at carbon 11; the thiol group undergoes the Michael addition on the enone olefin of PTL and obtained the (*S*) stereocentre in which the specific optical rotation of this compound was shown a positive value. A final compound library of tulipane and PTL derivatives can be seen in **Table 13** and **Table 14**.

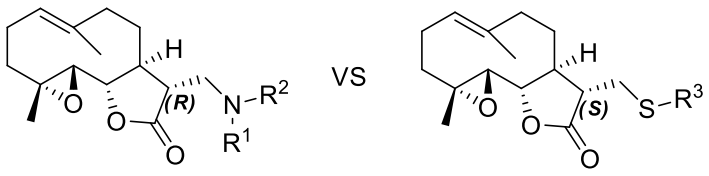
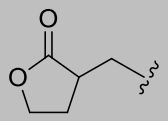
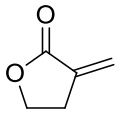
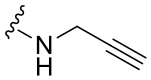
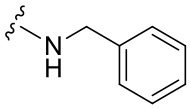
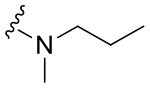
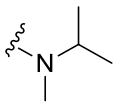
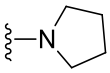
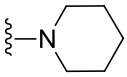
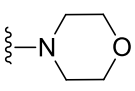
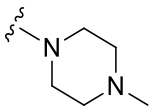
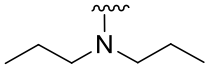
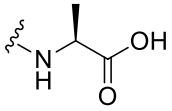
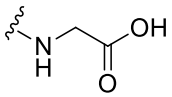
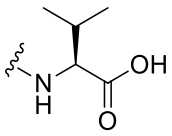
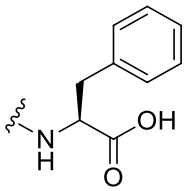
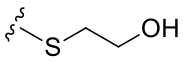
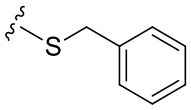
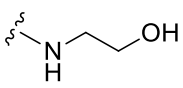


Figure 19: Amino derivative and Thiol derivative have different stereocentres at C-11

Table 13: Tulipane derivatives library

Entry No.	Target No.	Structure of derivatives	Yield (%)	Log P			
				OSIRIS Property Explorer	ChemBio Draw	logP Calculator	SD
1	**6**			0.500	0.480	0.009	0.28
2	**6a**		60	-0.610	-0.040	-0.006	0.34
3	**6b**		61	0.860	1.480	1.448	0.35
4	**7a**		78	0.610	0.950	0.758	0.17
5	**7b**		32	0.550	0.780	0.860	0.16
6	**8a**		86	0.520	0.440	0.483	0.04
7	**8b**		91	0.84	0.86	0.841	0.01
8	**8c**		81	-0.370	-0.280	-0.423	0.07

9	**8d**		61	-0.250	-0.120	-0.380	0.13
10	**9a**		70	-0.290	0.120	-0.023	0.21
11	**9b**		35	1.510	1.770	1.539	0.14
12	**10a**		33	-1.020	-0.500	-0.319	0.36
13	**10b**		25	-1.420	-0.990	-0.845	0.30
14	**10c**		20	-0.220	0.390	0.336	0.34
15	**10d**		13	0.250	1.180	1.126	0.52
16	**11a**		78	0.190	0.170	-0.604	0.45
17	**11b**		85	2.140	2.420	1.815	0.30
18	**12a**		71	-1.020	-0.770	-0.971	0.13

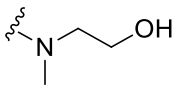
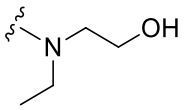
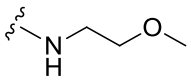
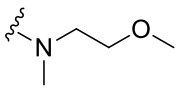
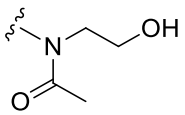
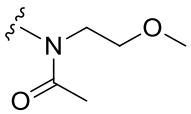
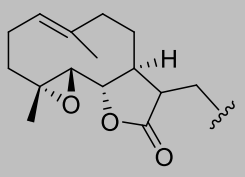
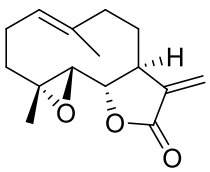
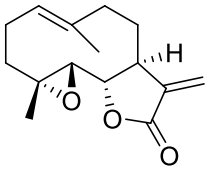
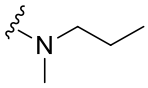
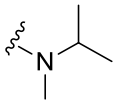
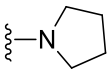
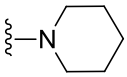
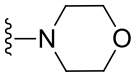
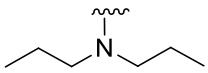
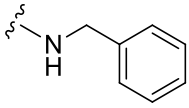
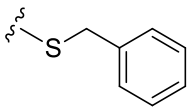
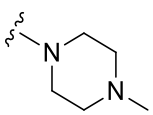
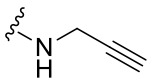
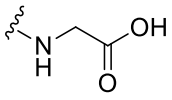
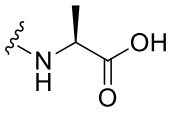
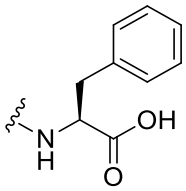
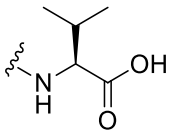
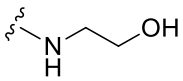
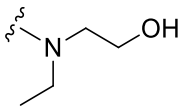
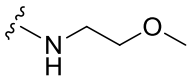
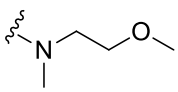
19	**12b**		91	-0.390	-0.732	-0.800	0.22
20	**12c**		61	-0.370	-0.060	-0.309	0.16
21	**12d**		82	-0.570	-0.410	-0.452	0.08
22	**12e**		48	-0.350	-0.030	-0.213	0.16
23	**13a**		51	-0.770	-1.120	-0.908	0.18
24	**13b**		48	-0.570	-0.410	-0.452	0.08

Table 14: Parthenolide derivatives library

Entry No.	**Target No.**	**Structure of derivatives**	**Yield (%)**	**Log P**			
				OSIRIS Property Explorer	ChemBio Draw	logP Calculator	SD
 SM → **Product** Condition: A) MeOH, RT B) MeOH/H_2O, Base, RT							

1	**1**			2.770	2.070	1.280	0.75
2	**14a**		79	2.890	2.540	2.033	0.43
3	**14b**		36	2.820	2.370	2.135	0.35
4	**14c**		83	2.800	2.030	1.758	0.54
5	**14d**		92	3.110	2.450	2.116	0.51
6	**14e**		84	1.910	1.320	0.852	0.53
7	**14f**		43	3.790	3.360	2.814	0.49
8	**14g**		75	3.140	3.070	2.723	0.22
9	**14h**		56	4.410	4.010	3.090	0.68
10	**15a**		37	2.030	1.470	0.895	0.58

11	**15b**		36	1.660	1.550	1.281	0.19
12	**15c**		28	0.850	0.600	0.430	0.21
13	**15d**		36	1.250	1.100	0.956	0.14
14	**15e**		34	2.530	2.770	2.401	0.19
15	**15f**		26	2.060	1.980	1.611	0.24
16	**15g**		21	1.250	0.820	0.304	0.47
17	**15h**		77	1.910	1.540	0.966	0.48
18	**15i**		57	1.920	1.560	1.062	0.43
19	**15j**		23	1.700	1.180	0.823	0.44

2.7 Application of Lipinski's rule to derivatives

Christopher A. Lipinski, a senior medicinal chemist, proposed five basic rules (Lipinski rules) to quickly screen potential prodrugs in 1997. If a compound complies with all the requirements of Lipinski rules, it will usually appear to have better pharmacokinetic properties, higher bioavailability *in vivo* and thus be more likely to become a suitable oral medication. Therefore, Lipinski rules have been used to primarily screen all derivatives to examine compounds that are suitable for further biological studies. The main Lipinski rules are:

1. No more than 5 hydrogen bond donors
2. No more than 10 hydrogen bond acceptors
3. A molecular mass less than 500 daltons (g/mol)
4. A value of log *P* no greater than 5

Using these conditions to analyse tulipane derivatives and PTL derivative, the results show that none of them have contained either more than 5 hydrogen bond donors or 10 hydrogen bond acceptors. Molecular weight of each compound was less than 500 g/mol and as presented in **Table 13** and **Table 14**, the value of logP of each compound is not greater than 5. Therefore, all compounds comply with the requirements of the primary drug testing. The result suggested that the derivatives are suitable for evaluating their anti-cancer activities using three different types of chronic lymphocytic leukaemia (CLL) cell lines *in vitro*.

2.8 Predicting logP of derivatives using different software

Water solubility is a common issue in drug studies because it affects the biological activity as well as toxicity. To better understand the water solubility of tulipane derivatives and PTL derivatives, the octanol-water partition coefficient (logP) of each compound was calculated and a rational level of molecular hydrophobicity was measured. As logP presents the effects on biological activity when increasing water solubility; thus logP can be used to identify whether the drug effectiveness of PTL is limited by water solubility or not. The simple and reliable experimental method of log

P measurement is the shake-flask technique. However, the measurement of log P using shake-flask technique is a time-consuming and material-consuming method. Therefore, in this project, a faster and easier theoretical calculation method was used to predict logP values that quickly screen compound as to whether they comply with the requirement of Lipinski rules or not. Each compound were calculated with ChemBioDraw software and two molecular property website (**Table 13** and **Table 14**) and all software were predicting log P value by chemical structure of each compound . There were two different calculation methods used: atomic-based prediction and fragment-based prediction.

In summary, the structure solubility data of tulipane and PTL derivatives are shown in **Table 13** and **Table 14**. The derivatives which contained aromatic groups exhibit significantly less aqueous solubility compared to those derivatives from aliphatic amines. Comparing the cyclic aliphatic derivatives, such as **8a** to **8b** and **14c** to **14d**, ring size played an important role in water solubility, where an increase in the ring size reduced the solubility. However, if the six-membered rings contained a heteroatom the solubility was significant increased such as in **8b-8d** and**14d-14e**.

Amino acid derivatives exhibit distinct water solubility. Due to the variable R groups found in amino acids, such as hydrogen, methyl, isopropyl and benzyl, the bigger the side chain, the greater the reduction in solubility (**10a-d** and **15c-f**).

Almost all tulipane and PTL derivatives had increased aqueous solubility compared with both parent compounds (**1** and **5**), especially those derivatives containing alcohol, ether and amide functional groups. The solubility of those compounds was much better in water than tulipane or PTL.

The standard deviation (SD) of predicted log P data for derivatives was shown in **Table 13** and **Table 14**. The general SD value of derivative was quite high, which was not only caused by software using different computation mode, atomic-based prediction or fragment-based prediction calculation method; also, based on the same fragment-based prediction calculation method, the molecular structure could be broken down in various ways and the contribution of different fragments will produce various values of log P. Moreover, the reliability of calculated log P values are still

limited. Even the aim of this theoretical calculation study was not to work out the exact log P value for each compound, but to highlight the reliability of pesticide log P values in a series of experimental measurements is indispensable work in future. Furthermore, the experiment data should be compared with the calculated Log P values, to find which calculation method is closer to reality.

2.9 Biological Activity Studies

Three chronic lymphocytic leukaemia (CLL) cell lines (MEC1, CII and PGA) were obtained from the American Type Culture Collection (ATCC). This is the first study of anti-cancer activity of PTL and tulipane using PGA and Mec1 cell line. All cell lines were previously established from peripheral blood; refer to **Table 15**.

Table 15: Details of PGA, CII and MEC1 cell lines.[46,47]

Cell line	From	Established	Information
PGA	man	From peripheral blood	Epstein-Barr Virus (EBV)-immortalized CLL cells.
CII	47-year-old woman	From peripheral blood	Cell line includes a type-A glucose-6-phosphate dehydrogenase (G6PD) pattern identical with the *in vivo* CLL clone and a μ^+ and λ^+ phenotype.
MEC1	58-year-old man	From peripheral blood	Shows the IGHV4-59 rearrangement with patient peripheral leukemic clone.

2.9.1 Parthenolide and Derivatives

2.9.1.1 Determining the effective concentration range and treatment time for PTL.

Parthenolide and eight PTL derivatives (**14a-h**) were tested for their cytotoxic activity on CLL cell lines. First of all, PTL was tested on the CLL cell lines CII and PGA. Propidium iodide (PI) was used for the measurement of cytotoxic activity. PI is excluded from a viable cell by the intact plasma membrane. In a dead cell, however, the plasma membrane is compromised thus allowing PI to enter and intercalate with nucleic acids enabling the detection and quantification of dead cells by flow cytometry. There are two factors to examine in the first biological experiment:

1. Effect of concentration range of PTL against PGA and CII cell lines.
2. Effect of time.

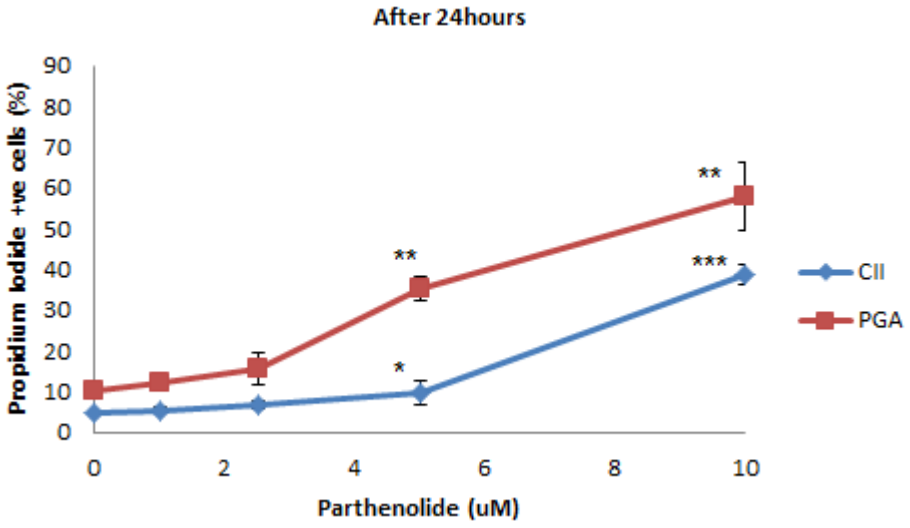


Figure 20: Dose response curve showing effect of increasing concentrations of parthenolide on CLL cell lines CII and PGA after 24hours. Student's test was used to compare treated cells with untreated cells.

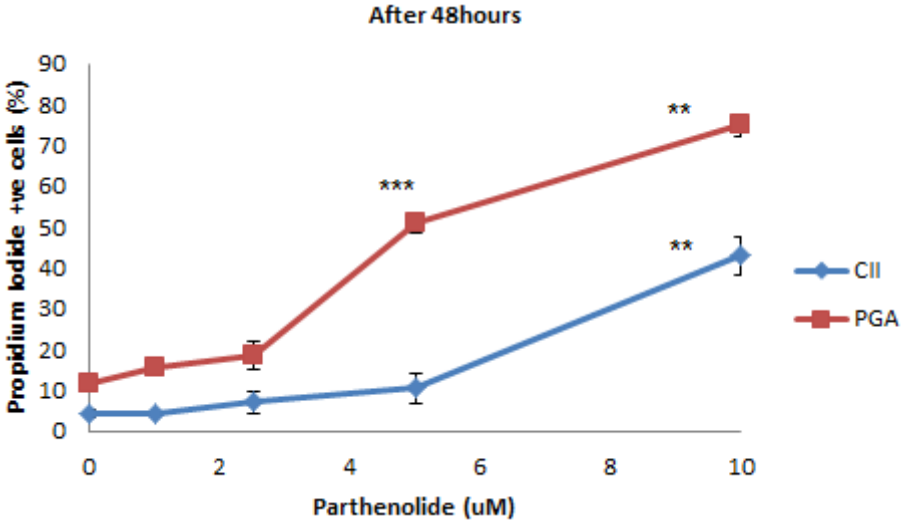


Figure 21: Dose response curve showing effect of increasing concentrations of parthenolide on CLL cell lines CII and PGA after 48hours. Student's Ttest was used to compare treated cells with untreated cells.

Treatment of PGA and CII cells with increasing dose of PTL for 24 hours resulted in an significant increase in cell death at 5 and 10 µM PTL; see **Figure 20**. This effect was enhanced with increased treatment time; see **Figure 21**.

For the concentration ranging from 0 to 10 µM, **Figure 20** and **Figure 21** have showed the PGA cell line was more sensitive than CII cell line. The amount of cell death of both cell lines was increasing by about 10% with longer incubation. However, with both time points the PGA cell line was more sensitive than CII. Therefore, PTL was effective over the concentration range of 0-10 µM following 24 hours' treatment. These concentrations were used in subsequent experiments.

2.9.1.2. Comparison of PTL and PTL derivatives at 10 µM.

The next step of this study was to compare the effectiveness of PTL and PTL derivatives in CII, PGA and MEC1 cells.

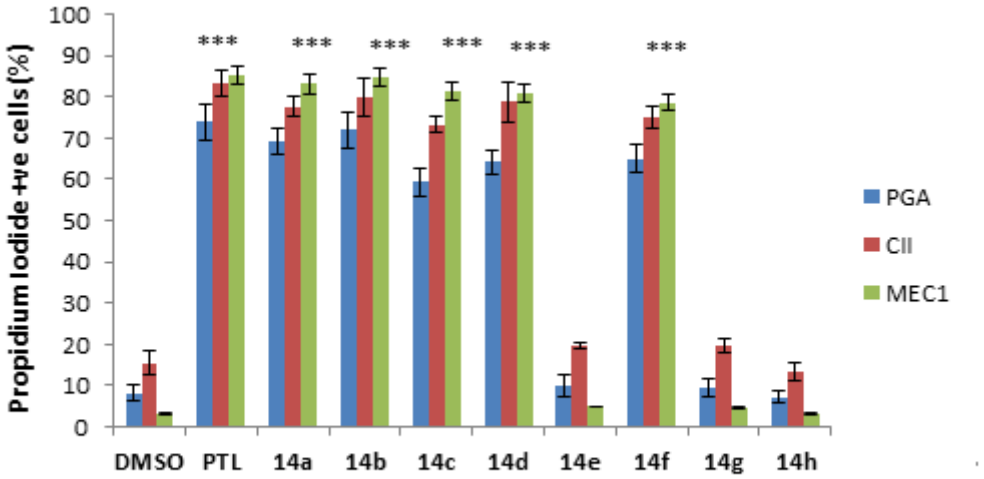


Figure 22: PTL and **14a-h** against three CLL cell lines at the same time. Student's test was used to compare treated cells with untreated cells

In initial experiments, PTL and the corresponding derivatives **14a-h** were tested on PGA, CII and MEC1 cell lines using a single concentration of 10 μM. The data shown is the mean of three independent assays set up in triplicate, **Figure 22**. Compared to control cells, five of eight compounds showed significant levels of killing in all cell lines, **Figure 22**. The anti-cancer activity of all derivatives was generally reduced compared with PTL. When compared to PTL, compounds **14a**, **14b**, **14c**, **14d** and **14f** did not show significant reduction in killing activity. Compound **14e** and **14g** did not show any killing activity, indicating that amino modification blocks the anti-leukaemic activity of PTL as well as the benzylmercapton adduct (**14h**).

2.9.1.3. Determination of LD_{50} of biologically active PTL derivatives.

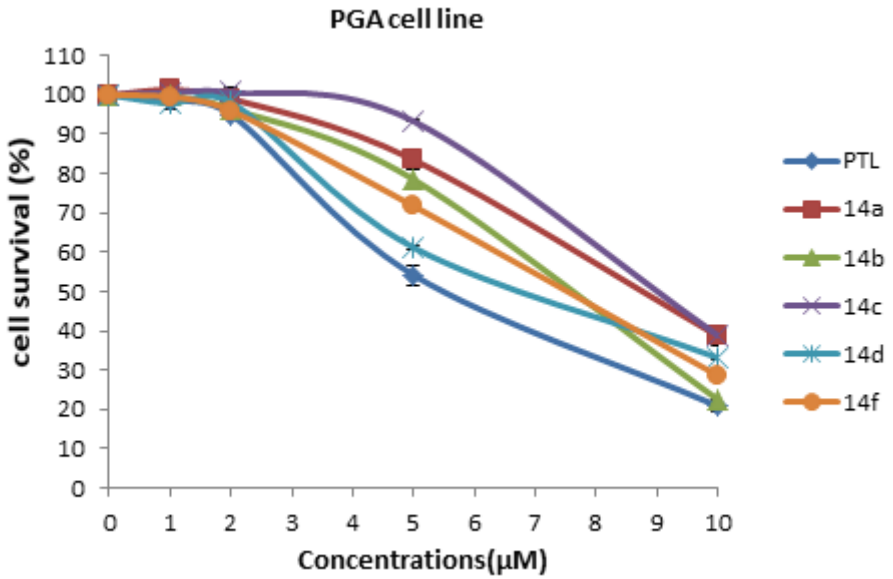


Figure 23: Dose response curve showing effect of increasing concentrations of PTL and derivatives on PGA cell line after 24 hours

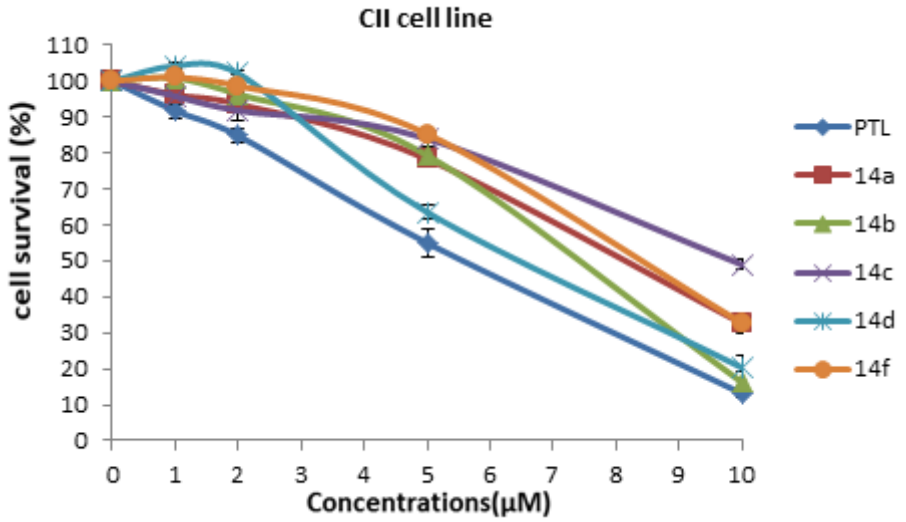


Figure 24: Dose response curve showing effect of increasing concentrations of PTL and derivatives on CII cell line after 24 hours

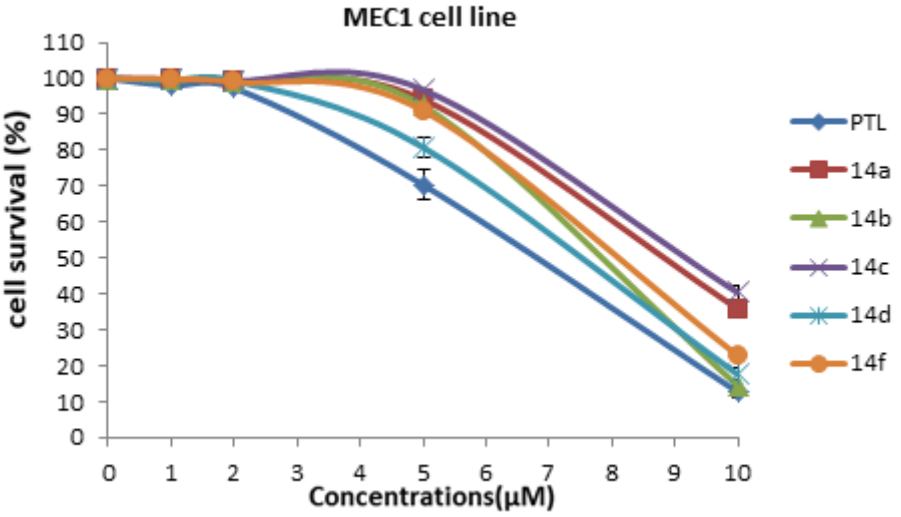
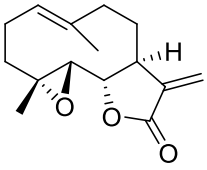
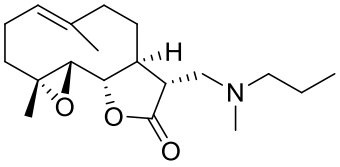
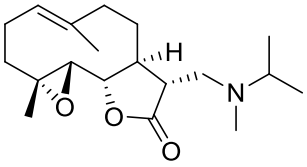
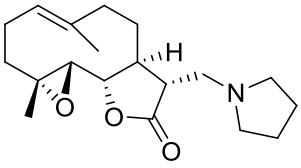
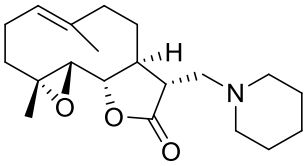
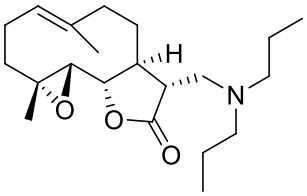


Figure 25: Dose response curve showing effect of increasing concentrations of PTL and derivatives on MEC1 cell line after 24 hours

A series of dose response assays were used to determine the LD_{50} of each of the biologically active PTL derivatives in CII, PGA and MEC1 cells. The LD_{50} were calculated using the dose response curves shown in **Figure 23**, **Figure 24** and **Figure 25**. PTL anti-leukaemic activity has been shown to be dependent in the α-methylene-γ-butyrolactone, as shown in **Table 16**. A possible reason for LD_{50} of all derivatives being higher than PTL is due to the hampered nucleophilicity when amine was added to the Michael accepter, α-methylene-γ-butyrolactone moiety. All compounds were shown to have an LD_{50} in the low µM range in all CLL cell lines tested.

Table 16: The LD_{50} values of PTL and five derivatives which against three CLL cell lines

Entry	Target No.	Structure	LD_{50} (µM)		
			(PGA)	(CII)	(MEC1)
1	**1**		5.4	5.6	6.8

2	**14a**		8.7	8.2	8.8
3	**14b**		7.5	7.6	7.9
4	**14c**		9	9.8	9.2
5	**14d**		6.7	6.6	7.4
6	**14f**		7.4	8.5	8.1

2.9.2 Tulipane and Derivatives

2.9.2.1. Determining effective concentration range for tulipane

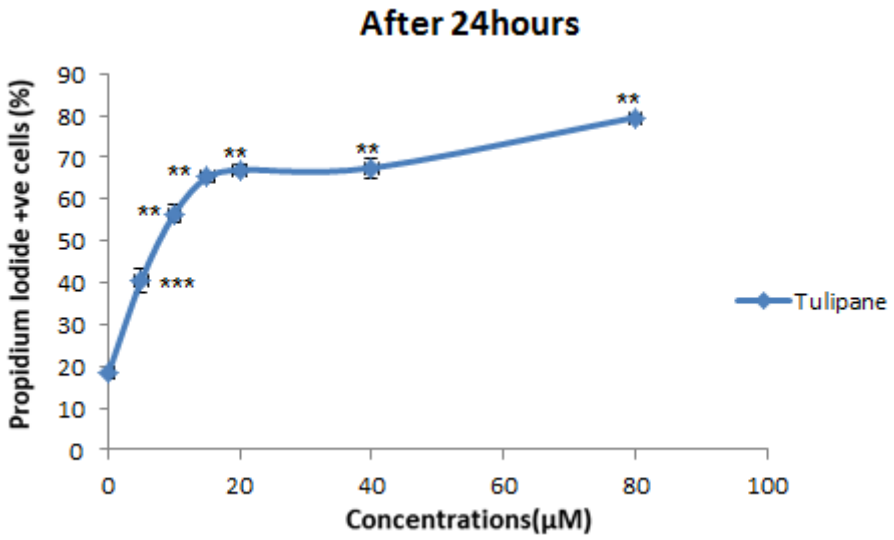


Figure 26: Dose response curve showing effect of increasing concentrations of tulipane on PGA cell lines after 24 hours. Student's test was used to compare treated cells with untreated cells

Compared to PTL, the structurally similar molecule, tulipane, was used to optimise the derivatisation of PTL. This resulted in the generation of a panel of tulipane derivatives with the same additional groups as in the PTL panel. Therefore, we have investigated the effect of these modifications on the biological activity of tulipane. The effective concentration range of tulipane was determined in **Figure 26**. Tulipane induced significant levels of cell deaths at 5 µM and above.

2.9.2.2. Comparison of tulipane and tulipane derivatives at 20 µM

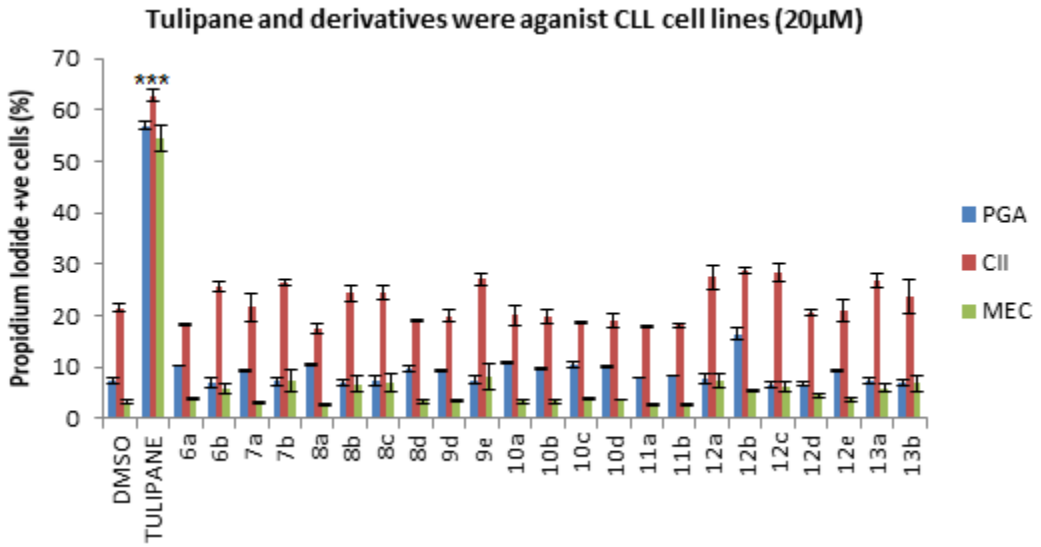


Figure 27: Tulipane and derivatives were tested on CLL cell lines after 24 hours. Student's test was used to compare treated cells with untreated cells

As an initial screen for tulipane derivatives with biological activity, CLL cell lines PGA, CII and MEC1 were treated with 20 µM of each derivative for 24 hours. In this assay, tulipane consistently demonstrated significant killing of all three cell lines. However, all killing activity was lost upon derivatisation in all cases.

In this series of experiments, the anti-leukaemic activity of PTL derivatives and the analog tulipane derivatives were determined in CLL cell lines. Five of eight derivatives, **14a**, **14b**, **14c**, **14d** and **14f**, showed a similar anti-leukaemic activity to parent compound. The LD_{50} value of PTL and five active derivatives was identified in the range of 6 µM to 10 µM in PGA, CII and MEC1 cell lines. The PGA cell line showed response to these five derivatives and the obtained LD_{50} was lower than the others, whereas any modification of tulipane resulted in loss of activity. The five active PTL derivatives **14a**, **14b**, **14c**, **14d** and **14f** will be used for further experiments *in vivo*. Therefore, the calculated logP should be compared with biological activity of derivatives and PTL; it will either be able to support or deny the hypothesis that the low water solubility of PTL is the reason it does not function well as a drug in whole organisms.

Comparing PTL and tulipane, both compounds have shown significant levels of killing activity on CLL cell lines. Furthermore, tulipane induced a lower level of cell

death than PTL at the same concentration, suggesting that the specific cyclic ten-membered ring skeleton jointing with α-methylene-γ-butyrolactone moiety enhances the anti-leukaemic activity.

Comparing the difference between PTL derivative and tulipane analog, tulipane derivatives had relatively better structural stability than PTL derivatives. Therefore, it was more difficult for the tulipane derivatives to carry out the retro Michael addition reaction and release the free *exo*cyclic double bond on the α,β-unsaturated lactone. On the other hand, the specific skeleton of PTL indicated that it may destabilise the lactone moiety of derivative and regenerate free PTL more easily.

Unlike amino PTL derivatives, thiol PTL derivative had not displayed any anti-leukaemic activity in the experiment. This may due to the chemical properties of the thiol group, which is a weaker electrophilic-leaving group compared to the amino group. Therefore, thiol derivative was more difficult to convert into free PTL undergoing the retro Michael addition reaction *in vitro*.

Combining the experimental data with literatures, PTL had obtained a significant level of killing against both AML and CLL cells, but the literature LD_{50} value of PTL against AML cell was only 1.7 μM and the LD_{50} value of PTL against CLL in this series experiment was around 6 μM. Moreover, the LD_{50} value of active aminoparthenolide analog was also identified in a relatively low concentration range against AML compared to CLL cells.

There is often a difference between the results of *in vitro* and *in vivo* studies. Because *in in vivo* studies, compounds are tested in living organisms and undergone an uncontrolled environment comparing to the conditions *in vitro* study. Without the issue of water solubility of PTL, there was still another important condition, the non-selective reactivity of PTL, which hampered its clinical translation *in vivo*. Many different thiol groups exist in living cells and result in PTL being rapidly trapped by a Michael addition of the undesired targets. This might cause low anti-cancer activity and side effects in real applications. Therefore, this modification of PTL might be able to overcome this non-selective problem of PTL. Derivatives may show better effect compared to the parent compound during *in vivo* tests.

Chapter 3: Conclusion and Future Work

In summary, the major objectives of this study have been achieved. First of all, a recombining extraction and purification procedure has been used for the isolation of PTL from feverfew. Secondly, we found that the maximum amount of PTL can be extracted from golden feverfew in the post-flowering period. For different types of feverfew, plant matter containing seeds, post-flowering season, was much richer in PTL than in pre-flowering season. In addition, the colourless crystalline solid of PTL was fully characterised by melting point, mass spectrometry, proton NMR, carbon NMR and IR and the structure has also been confirmed by single crystal X-ray analysis.

Thirdly, twenty-three tulipane derivatives have been successful synthesised. All compounds have screened and passed the preliminary test according to Lipinski rule. Most of tulipane derivatives have been tested on a CLL cell line. However, the results have shown those compounds have significantly lost the anti-cancer activity compared with the parent compound. In contrast, tulipane has successfully passed the preliminary chemical reactivity test. It has proven that tulipane complies with the PTL theory.

Eighteen PTL derivatives, after modification by the same functional group as tulipane derivatives, were synthesised successfully at the same time. Compounds were also passed the preliminary test by Lipinski rules. Eight of the PTL derivatives appeared to be effective against CLL cell line; and the preliminary biological results showed five of them to be active compounds (**14a**, **14b**, **14c**, **14d** and **14f**) which showed similar anti-cancer activity compared with parent. Those potential compounds will be tested in the next stage of biological studies *in vivo.*

Finally, the water solubility of those derivatives was evaluated and results showed that derivatives contained an aromatic group exhibiting relatively less aqueous solubility. The larger ring size of cyclic aliphatic derivatives has shown less water solubility. If a heteroatom is included in the ring structure, then the solubility was significantly increased. Amino acid derivatives exhibit distinct water solubility due to the different R group of amino acids. Finally, compounds that showed greater water

solubility than tulipane and PTL contained an alcohol, ether or amide functional group.

The researcher's aim of future work of organic chemistry will be focusing on High Performance Liquid Chromatography (HPLC) works. The two diastereomers of amino acid tulipane derivatives, which could separate via HPLC, are very crucial; and HPLC is an advanced purification method to improve the purity of compound. Lastly, the water-solubility of few compounds should be tested and the experiment data should be compared with the calculated Log P values to find which theoretical calculation method is closer to reality.

It is difficult to understand the structural-activity relationship of derivatives before all derivatives have been tested and all biological experiments have been finished. Therefore, in future biological work, unexamined remaining PTL derivatives (**15a-g**) should also be tested against CLL cell lines. By doing so, researchers will be able to expand and understand the anti-cancer activity relationship between the different structure functionality of compounds.

Moreover, a series of possible experiments aiming to look at the effect of those novel compounds on redox homeostasis and NF-kB signalling to identify mechanism of action should also be established.

Chapter 4: Experimental

4.1 General

All commercially available solvents and reagents were used without further purification. Proton NMR spectra were recorded at 300 MHz on a Bruker AVIII300 NMR spectrometer and at 400 MHz on a Bruker AVIII400 NMR spectrometer. Carbon NMR spectra were recorded at 100 MHz on a Bruker AVIII400 NMR spectrometer at room temperature and are proton decoupled. Data was processed with Mestrec version 5.2.5-4731 and Topspin 2.0 (Version of: Nov 9th 2006). Chemical shifts (δ) are reported in ppm relative to TMS (δ 0.00) for the ^{1}H NMR and to chloroform (δ 77.0) for the ^{13}C NMR measurements, coupling constant *J* are expressed in Hertz. Mass spectra were recorded with electrospray MS Waters LCT Time of Flight Mass Spectrometer and with EI (GC/MS) Waters GCT Premier Time of Flight Mass Spectrometer. Infrared Spectra were recorded on a PerkinElmer 100FT-IR spectrometer at room temperature. Melting points were measured using a SMP10 melting point apparatus. Specific rotations were recorded on an Optical PolAAr 2001 automatic polarimeter at room temperature. X-ray crystal structure was recorded on an Agilent SuperNova X-ray diffractometer with an Atlas detector using an X-ray source with wavelength 1.5418 Angstroms.

4.2 Novel Extraction

Plant matter was added into water (10 g per 100 ml) and the solution was stirred at 80 ℃ for 10 minutes. The filtrate was allowed to cool at the room temperature. Then use chloroform to wash it 3 times (3 ×500 ml), the chloroform portions were combined and reduced to dryness under reduced pressure to afford a dark green residue. The purification of the residue was done by column chromatography (EtOAc with Hexane) and then recrystallized using the same solvent system EtOAc / Hexane to afford parthenolide.

4.3 Experimental Procedures

Method A**:** The amine (Excess) and α,β-unsaturated lactone were stirred in methanol at room temperature for 24 hours. Reaction progress was monitored by Thin-layer chromatography. The solvent was removed under reduced pressure and product was isolated by column chromatography over silica gel.

Method B**:** The amino acid and α,β-unsaturated lactone (Excess) were stirred in methanol and water mixture at room temperature for 24 hours. An appropriate amount of TEA (or K_2CO_3) has been used in this method. Reaction progress was monitored by Thin-layer chromatography. Then the target compound was reduced to dryness *in vacuum* and future washed the compound with Ethanol three times to afford a colourless crystalline solid.

Method C: The Acetic anhydride and tulipane derivative **12a** or **12d** were stirred in DCM at room temperature for 24 hours. Reaction progress was monitored by Thin-layer chromatography. The solvent was removed under reduced pressure and product was isolated by column chromatography over silica gel.

All compounds were fully characterised by melting point (solid), specific rotation (PTL derivatives only) mass spectrometry, proton NMR, carbon NMR and IR.

4.4 Biological Work

4.4.1 Cell lines and cell culture

Three Chronic Lymphocytic leukaemia (CLL) cell lines (MEC1, CII and PGA) were obtained from the American Type Culture Collection (ATCC). Also PGA and Mec1 cell lines have never been used before to test the anti-cancer activity of PTL and tulipane. All of PGA, CII and MEC1 cell lines ware previously established from peripheral blood, refer to **Table 15**.

PGA, CII and MEC1 cell lines were maintained in RPMI1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and [+]L-Glutamine. Cells were always kept in

small sample flask and leaved into the conventional culture incubator in 37 ℃ and with the volume fraction of 5% CO_2.

4.4.2 Biological Experimental Work

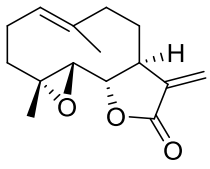
Cells were quantified using a haemocytometer. Cells were seeded onto a 96 well culture plate in triplicate and compounds dissolved in DMSO were added. Cells were plated at 10 000 cells per well and incubated with each compound for 24 hours. For each compound concentration the volume of DMSO used were adjusted so that the concentration of DMSO was the same for each drug treatment.

4.4.3 Flow cytometric analysis of cell death

Following 24 hours of treatment, PI (Propidium Iodide) was added to cell media. PI +ve cells were measured using BD Accuri C6 Flow Cytometer. Data was expressed either as percentage of PI + ve cells or as the surviving fractions (PI -ve) relative to control. Statistical analysis was done using student's Ttest: $p<0.05$(*), $p<0.01$(**) and $p<0.001$(***).

4.5 Parthenolide

(1a*R*, 7a*S*, 10a*S*, 10b*S*, *E*)-1a,5,7a-Trimethyl-8-methylene-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH):one: Compound 1



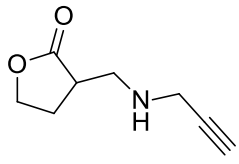
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (d, *J* = 3.7, 1H, C=C$\underline{H}_2$), 5.64 (d, *J* = 3.3, 1H, C=C$\underline{H}_2$), 5.23 (dd, *J* = 12.0, 2.4, 1H, CH_3C=C$\underline{H}$), 3.88 (t, *J* = 8.6, 1H, C$\underline{H}$OCO), 2.93 to 2.68 (m, 2H), 2.51 to 2.34 (m, 2H), 2.21 to 2.12 (m, 3H), 1.81 to 1.71 (m, 4H), 1.37 to 1.14 (m, 5H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.24 (C=O), 139.26 ($\underline{C}$=CH_2), 134.60 (CH=$\underline{C}CH_3$); 125.30 ($\underline{C}$H=CCH_3), 121.23 (C=$\underline{C}H_2$), 82.45 ($\underline{C}$HOCO), 66.41 (CH, epoxide moiety), 61.52 ($CH_3\underline{C}$O), 47.69 ($CH_2C\underline{C}$H), 41.24 ($CH_2CHC\underline{C}H_2$), 36.38 (OC$\underline{C}H_2$), 30.67 (CH_2=CCH$\underline{C}H_2$), 24.16 ($\underline{C}H_2CH_2$CO), 17.29

($\underline{C}H_3C$-O), 16.96 ($\underline{C}H_3CCHCH_2$); IR (neat) cm-1 1753 cm^{-1} (C=O); MS (EI+) *m/z* ($[M+ Na]^+$)= 271; mp= 144-146 ℃ (colourless crystalline solid).

Literature data of Parthenolide: ^1H NMR ($CDCl_3$, 300 MHz) δ 6.34 (d, *J* = 3.6, 1H, C=C$\underline{H}_2$), 5.62 (d, *J* = 3.0, 1H, C=C$\underline{H}_2$), 5.21 (dd, *J* = 2.7, 12.0, 1H, CH_3C=C$\underline{H}$), 3.86 (t, *J* = 8.4, 1H, C$\underline{H}$OCO), 2.79 (d, *J* = 9.0, 1H, CH, epoxide moiety), 2.74 to 2.82 (m, 1H, C$\underline{H}$C=CH_2), 2.32 to 2.49 (m, 2H), 2.11 to 2.21 (m, 4H), 1.72 (s, 3H, $CH_2CHCC\underline{H}_3$), 1.70 to 1.77 (m, 1H, $CH_3CCH_2C\underline{H}_2$), 1.31 (s, 3H, $C\underline{H}_3CO$), 1.20 to 1.28 (m, 1H, $C\underline{H}_2CHO$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.3 (C=O), 139.2 ($\underline{C}$=CH_2), 134.6 (CH=$\underline{C}CH_3$); 125.3 ($\underline{C}$H=CCH_3), 121.3 (C=$\underline{C}H_2$), 82.4 ($\underline{C}$HOCO), 66.4 (CH, epoxide moiety), 61.5 ($CH_3\underline{C}O$), 47.7 ($CH_2C\underline{C}H$), 41.2 ($CH_2CHC\underline{C}H_2$), 36.3 ($OC\underline{C}H_2$), 30.6 (CH_2=$CCH\underline{C}H_2$), 24.1 ($\underline{C}H_2CH_2CO$), 17.3 ($\underline{C}H_3CO$), 16.9 ($\underline{C}H_3CCHCH_2$); mp=145-146 ℃.

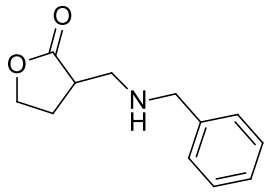
4.6 Tulipane Derivatives

3-((Prop-2-yn-1-ylamino) methyl) dihydrofuran-2(3H)-one: Compound 6a.



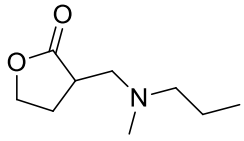
Propargylamine (2.45 mmol, 0.157 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **6a** was obtained as clear oil (60 %, 0.186 g). ^1H NMR δ_H (400 MHz, $CDCl_3$) δ4.35 (1H, td, *J* = 8.8, 2.4, C$\underline{H}_2$OC=O), 4.25 to 4.28 (1H, m, C$\underline{H}_2$OC=O), 3.50 (2H, s, NHC$\underline{H}_2$), 3.00 to 2.90 (2H, dd, *J* = 11.6, 6.0, CHC$\underline{H}_2$N), 2.70 to 2.77 (1H, m, C$\underline{H}$), 2.37 to 2.47(1H, m, $CH_2C\underline{H}_2CH$), 2.27 (1H, s, C$\underline{H}$), 2.08 to 2.12 (1H, m, $CH_2C\underline{H}_2CH$), 1.83 (1H, N$\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ26.8 ($CH_2\underline{C}H_2CH$), 38.3 ($\underline{C}H_2NH$), 39.6 (CH), 48.3 ($N\underline{C}H_2CH$), 66.9 ($\underline{C}H_2OC$=O), 71.7 ($\underline{C}$H), 81.7 ($\underline{C}$), 178.6 ($\underline{C}$=O); IR (neat) cm^{-1} 3281 (NH), 1758(C=O).; MS (EI+) *m/z* ($[M+ H]^+$)= 153.

3-((Benzylamino) methyl) dihydrofuran-2(3H)-one: Compound 6b.



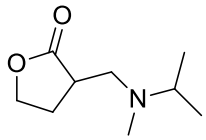
Benzylamine (2.04 mmol, 0.222 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **6b** was obtained as yellow oil (61.5 %, 0.257 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ7.30 (4H, d, *J* = 4.4, Ph), 7.22 to 7.15 (1H, m, Ph), 4.29 (1H, td, *J* = 8.8, 2.8 C$\underline{H}_2$OC=O), 4.15 (1H, td, *J* = 6.8, 9.2 C$\underline{H}_2$OC=O), 3.80 (2H, d, *J* = 2.8, Ph-C$\underline{H}_2$), 2.80 to 2.90 (2H, m, CHC$\underline{H}_2$N), 2.70 (1H, m, C$\underline{H}$), 2.25 to 2.33 (1H, m, $CH_2C\underline{H}_2CH$), 2.09 to 2.12 (1H, m, $CH_2C\underline{H}_2CH$), 1.85 (1H, N$\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ26.8 ($CH_2\underline{C}H_2CH$), 39.8 (CH), 48.9 (N$\underline{C}H_2CH$), 53.9 (NH$\underline{C}H_2Ph$), 66.9 ($\underline{C}$H2OC=O), 127.0 (Ph), 128.1 (Ph), 128.4 (Ph), 140.1 ($\underline{C}$),178.9 ($\underline{C}$=O); IR (neat) cm^{-1} 3316 (NH), 3029 (CH/Ph), 1762 (C=O).; MS (EI+) *m/z* ($[M+ H]^+$)= 205.

3-((Methyl (propyl) amino) methyl) dihydrofuran-2(3H)-one: Compound 7a



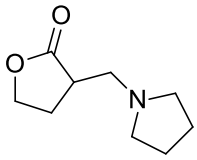
N-methylpropylamine (2.45 mmol, 0.25 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **7a** was obtained as yellow oil (78 %, 0.272 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 4.39 (td, *J* = 8.8, 3.3, 1H, , C$\underline{H}_2$-O-C=O), 4.24 (td, *J* = 9.2, 7.0, 1H, , C$\underline{H}_2$OC=O), 2.84 to 2.69 (m, 2H), 2.60 to 2.51 (m, 1H), 2.49 to 2.27 (m, 3H), 2.26 (s, 3H, C$\underline{H}_3$N), 2.16 (td, *J* = 16.5, 6.5, 1H), 1.56 to 1.41 (m, 2H, $CH_2C\underline{H}_2CH_3$), 0.90 (t, *J* = 7.4, 3H, C$\underline{H}_3$). ^{13}C NMR (100MHz, $CDCl_3$) 11.7 ($\underline{C}H_3$), 20.3 ($CH_2\underline{C}H_2CH_3$), 28.1 ($CH_2\underline{C}H_2CH$), 38.5 ($\underline{C}$H), 42.3 ($\underline{C}H_3$N), 57.8 ($CH_2OH\underline{C}H_2N$), 59.8 (N$\underline{C}H_2CH_2$), 66.8 ($\underline{C}$HOC=O), 178.8 ($\underline{C}$=O); IR (Neat) cm^{-1} 1765 (C=O).; MS (EI+) *m/z* ($[M]^+$)= 171.

3-((Isopropyl (methyl) amino) methyl) dihydrofuran-2(3H)-one: Compound 7b.



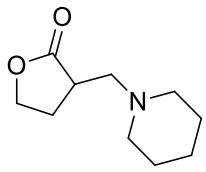
N-Isopropylmethylamine (2.45 mmol, 0.225 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **7b** was obtained as yellow oil (32 %, 0.112 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.40 (1H, td, *J* = 3.6, 8.8, C$\underline{H}_2$OC=O), 4.25 (1H, td, *J* = 7.2, 9.2, C$\underline{H}_2$OC=O), 2.80 to 2.90 (2H), 2.65 to 2.75 (1H, m), 2.55 (1H, dd, *J* = 3.2, 12.8, $CH_2OHC\underline{H}_2N$), 2.40 to 2.45 (1H, m, $CH_2C\underline{H}_2CH$), 2.25 (3H, s, C$\underline{H}_3$N), 2.1 to 2.20 (1H, m, $CH_2C\underline{H}_2CH$), 1.05 (6H, dd, *J* = 6.8, 4, CHC$\underline{H}_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0 (CH$\underline{C}H_3$), 18.5 (CH$\underline{C}H_3$), 27.7 ($CH_2\underline{C}H_2CH$), 37.3 ($\underline{C}H_3N$), 28.9 ($\underline{C}H$), ($CH_2\underline{C}H_2CH$), 38.8 (CH), 58.6 (CH2OH$\underline{C}H_2N$), 54.1 (N$\underline{C}HCH_3$), 66.9 (CH_2OC=O), 179.0 (C=O); IR (neat) cm^{-1} 1763 (C=O).; MS (EI+) *m/z* ([M+H]$^+$)=172.

3-(Pyrrolidin-1-ylmethyl) dihydrofuran-2(3H)-one: Compound 8a



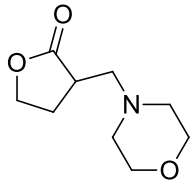
Pyrrolidine (2.45 mmol, 0.28 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **8a** was obtained as yellow oil (86 %, 0.295 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.35 (1H, td, *J* = 8.8, 3.2, C$\underline{H}_2$OC=O), 4.20 (1H, , td, *J* = 9.6, 7.2, C$\underline{H}_2$OC=O), 2.90 (dd, 2H, *J* = 11.6, 3.6, $CH_2OHC\underline{H}_2N$), 2.65 to 2.75 (m, 2 H), 2.55 to 2.62 (m, 2H), 2.31 (m, 1H), 1.76 to 1.82 (m, 4H) ^{13}C NMR(100MHz, $CDCl_3$) 23.4 ($CH_2\underline{C}H_2CH_2$), 23.6 ($CH_2\underline{C}H_2CH_2$), 28.1 ($CH_2\underline{C}H_2CH$), 39.7 (CH), 53.9 (CH_2N), 54.4 (CH_2N), 56.3 ($CH_2OH\underline{C}H_2N$), 66.8 ($\underline{C}H_2OC$=O), 178.6 ($\underline{C}$=O); IR (Neat) cm^{-1} 1763 (C=O).; MS (EI+) m/z ([M+H] $^+$)=170.

3-(Piperidin-1-ylmethyl) dihydrofuran-2(3H)-one: Compound 8b



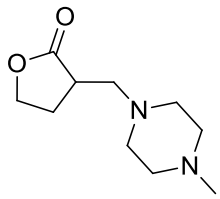
Piperidine (2.45 mmol, 0.242 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **8b** was obtained as pale yellow oil (91 %, 0.339 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 4.30 (td, *J* = 8.8, 3.3, 1H, C$\underline{H}_2$OC=O), 4.14 (td, *J* = 9.1, 6.9, 1H, C$\underline{H}_2$OC=O), 2.79 to 2.62 (m, 2H), 2.51 to 2.26 (m, 6H), 2.17 to 2.03 (m, 1H), 1.50 to 1.58 (4H, m, NCH$_2$C$\underline{H}_2$CH$_2$), 1.35 to 1.42 (2H, m, NCH$_2$CH$_2$C$\underline{H}_2$).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.2 (CH$_2$C$\underline{H}_2$CH$_2$), 26.0 (CH$_2$C$\underline{H}_2$CH$_2$), 28.2 (CH$_2$C$\underline{H}_2$CH), 38.2 (C$\underline{H}$); 54.7 (CH$_2$C$\underline{H}_2$N), 59.1 (CH$_2$OHC$\underline{H}_2$N), 66.8 (C$\underline{H}_2$OC=O), 178.8 (C$\underline{=}$O); IR (neat) cm^{-1} 1762 (C=O).; MS (EI+) m/z ([M+H]$^+$)=184.

3-(Morpholinomethyl) dihydrofuran-2(3H)-one: Compound 8c



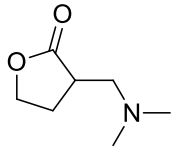
Morpholine (2.45 mmol, 0.214 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, Compound **8c** was obtained as pale yellow oil (81 %, 0.307 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 4.40 (td, *J* = 8.8, 3.2, 1H, C$\underline{H}_2$OC=O), 4.25 (td, *J* = 9.2, 7.0, 1H, C$\underline{H}_2$OC=O), 3.77 to 3.66 (m, 4H), 2.90 to 2.70 (m, 2H), 2.65 to 2.38 (m, 6H), 2.21 (ddd, *J* = 18.1, 12.9, 9.2, 1H, CH$_2$C$\underline{H}_2$CH).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9 (CH$_2$C$\underline{H}_2$CH), 38.0 (CH); 53.8 (CH$_2$C$\underline{H}_2$N), 58.6 (CH$_2$OHC$\underline{H}_2$N), 66.6 (C$\underline{H}_2$OC$\underline{H}_2$), 66.8 (C$\underline{H}_2$OC$\underline{H}_2$), 66.9 (CH$_2$OC=O), 178.8 (C=O); IR (neat) cm^{-1} 1760 (C=O).; MS (EI+) *m/z* ([M+H]$^+$)=186.

3-((4-Methylpiperazin-1-yl) methyl) dihydrofuran-2(3H)-one: Compound 8d



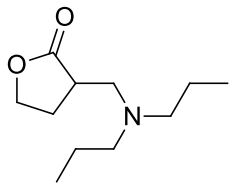
1-Methylpiperazine (2.45 mmol, 0.272 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **8d** was obtained as yellow oil (61 %, 0.245 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.38 (1H, td, *J* = 8.8, 3.2 C$\underline{H}_2$OC=O), 4.22 (1H, td, *J* = 4.3, 8.8 C$\underline{H}_2$OC=O), 2.85 (1H, dd, *J* = 12.4, 4.4, $CH_2OHC\underline{H}_2N$), 2.8 to 2.7 (1H, m, CH), 2.31 to 2.69 (10 H, m), 2.31 (3H, s, NC$\underline{H}_3$), 2.10 to 2.23 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ28.1 ($CH_2\underline{C}H_2CH$), 38.1 (CH), 46.0 (CH_3), 53.3 ($CH_2\underline{C}H_2N$), 55.1 (CH_2CH_2N), 58.3 (NCH_2CH), 66.8 (CH_2OC=O), 178.7 (C=O); IR (neat) cm^{-1} 1750 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)= 199.

3-((Dimethylamino) methyl) dihydrofuran-2(3H)-one: Compound 9a



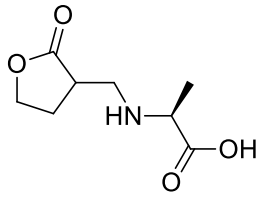
Dimethylamine (2.45 mmol, 0.225 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **9a** was obtained as yellow oil (70 %, 0.204 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.11 (1H, td, *J* = 2.4, 8.8, C$\underline{H}_2$OC=O), 3.95 (1H, td, *J* = 9.0, 6.8, C$\underline{H}_2$OC=O), 2.35 to 2.5 (2H, m), 2.10 to 2.18 (2H, m), 2.05 (6H, s, C$\underline{H}_3$N), 1.33 to 1.38(1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ27.7 ($CH_2\underline{C}H_2CH$), 38.1 (CH), 45.1 ($\underline{C}H_3$-N), 45.3 ($\underline{C}H_3$-N), 59.5 ($CH_2OH\underline{C}H_2N$), 66.5 (CH_2OC=O), 178.3 (C=O); IR (neat) cm^{-1} 1764 (C=O); MS (EI+) *m/z* ($[M+Na]^+$) =166.

3-((Dipropylamino) methyl) dihydrofuran-2(3H)-one: Compound 9b



Dipropylamine (2.45 mmol, 0.336 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **9b** was obtained as yellow oil (35 %, 0.141 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.40 (1H, td, *J* = 8.8, 3.6, C$\underline{H}_2$OC=O), 4.21 (td, *J* = 8.8, 6.8, 1H, C$\underline{H}_2$OC=O), 2.92 (1H, dd, *J* = 12.8, 4, $CH_2OHC\underline{H}_2N$), 2.70 to 2.75 (1H, m, CH), 2.55 (1H, dd, *J* = 10, 3.2, $CH_2OHC\underline{H}_2N$), 2.32 to 2.55 (5H, m), 2.18 to 2.25 (1H, m), 1.40 to 1.49 (4H, m, $CH_2C\underline{H}_2CH_3$), 0.85 (6H, t, *J* = 7.2, $CH_2CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8 ($CH_2\underline{C}H_3$), 20.2 ($CH_2\underline{C}H_2CH_3$), 28.1 ($CH_2\underline{C}H_2CH$), 38.8(CH), 54.6(CH$\underline{C}H_2N$), 56.1 ($CH_2\underline{C}H_2N$), 66.9 (CH_2OC=O), 179.0 (C=O); IR (neat) cm^{-1} 1766 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=200.

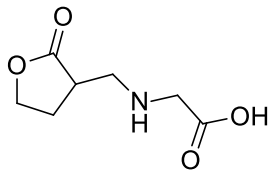
(2*S*)-2-(((2-Oxotetrahydrofuran-3-yl) methyl) amino) propanoic acid: Compound 10a.



L-Alaine (2.04 mmol, 0.182 g) was added into the stirred solution of 1.2eq tulipane (0.215 mL, 2.45 mmol) in 15 ml methanol and water mixture and using the general procedure B, mixture of diastereoisomers of **10a** was obtained as colourless crystalline solid (33 %, 0.126 g). A crude analysis of the diastereomeric ratio of two diastereoisomers: **10a-1** and **10a-2** by proton NMR, was estimated around 1:1. ^{1}H NMR δ_H (400 MHz, D_2O) δ4.42 (1H, td, *J* = 4, 8.4, C$\underline{H}_2$OC=O), 4.28 (1H, td, *J* = 6.4, 8.8, C$\underline{H}_2$OC=O), 3.55 to 3.75 (1H, m), 3.05 to 3.40 (3H, m), 2.45 to 2.51 (1 H, m, $CH_2C\underline{H}_2CH$), 2.08 to 2.15 (1H, m, $CH_2C\underline{H}_2CH$), 1.42 (3H, d, *J* = 9, C$\underline{H}_3$). ^{13}C NMR (100 MHz, D_2O) 14.8 ($\underline{C}H_3$), 14.9 ($\underline{C}H_3$), 26.5 ($CH_2\underline{C}H_2CH$), 26.7 ($CH_2\underline{C}H_2CH$), 36.9 ($CH_2CH_2\underline{C}H$), 37.1 ($CH_2CH_2\underline{C}H$), 45.7 (CH$\underline{C}H_2N$), 58.4 ($\underline{C}H$-CH_3), 59.2 ($\underline{C}HCH_3$), 66.2 ($CH_2\underline{C}H_2O$), 174.4 (C=O/Carboxylic acid), 179.4 (C=O),

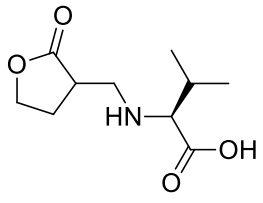
179.7 (C=O); mp=253-255 ℃; IR (neat) cm^{-1} 3350 (OH), 1765 and 1620 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)=210.

2-(((2-Oxotetrahydrofuran-3-yl) methyl) amino) acetic acid: Compound 10b



Glycine (2.04 mmol, 0.153 g) was added into the stirred solution of 1.2eq tulipane (0.215 mL, 2.45 mmol) in 15 ml methanol and water mixture and using the general procedure B, **10b** was obtained as colourless crystalline solid (20 %, 0.071 g). ^{1}H NMR δ_H (400 MHz, D_2O) δ4.45 (1H, td, *J* = 2.8, 8.8, C$\underline{H}_2$OC=O), 4.25 (1H, td, *J* = 7.2, 2.4, C$\underline{H}_2$OC=O), 3.60 (2H, s), 3.37 (1H, dd, *J* = 7.6, 12.8, CHC$\underline{H}_2$N), 3.15 to 3.30 (2H, m), 2.15 to 2.45 (2H, m, $CH_2C\underline{H}_2CH$). ^{13}C NMR (100 MHz, D_2O) 26.6 ($CH_2\underline{C}H_2CH$), 36.8 (<u>C</u>H), 46.9 (CH<u>C</u>H_2N), 49.5 (<u>C</u>H_2CO_2H), 68.5 ($CH_2\underline{C}H_2O$), 170.8 (C=O/<u>Carboxylic acid</u>), 179.6 (C=O); mp=225-227 ℃; IR (neat) cm^{-1} 3333 (OH), 1756 and 1622 (C=O).; MS (EI+) *m/z* ($[M-H]^-$)=172.

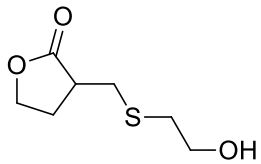
(2*S*)-3-Methyl-2-(((2-oxotetrahydrofuran-3-yl) methyl) amino) butanoic acid: Compound 10c



L-valine (2.04 mmol, 0.239 g) was added into the stirred solution of 1.2eq tulipane (0.215 mL, 2.45 mmol) in 15 ml methanol and water mixture. Using the general procedure B, mixture of diastereoisomers of **10c** was obtained as colourless crystalline solid (13 %, 0.069 g). A crude analysis of the diastereomeric ratio of two diastereoisomers **10c-1** and **10c-2** by proton NMR was estimated around 37:63 or 63:37. ^{1}H NMR δ_H (400 MHz, D_2O) δ4.45 (1H, td, *J* = 4.0, 8.4, C$\underline{H}_2$OC=O), 4.28 (1H, td, *J* = 6.4, 8.8, C$\underline{H}_2$OC=O), 3.40 to 3.45 (1H, dd, *J* = 4.0) 3.15 to 3.35 (3H,

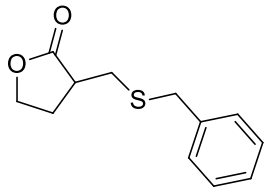
m), 2.46 to 2.52 (1H, m), 2.01 to 2.22 (3 H, m), 0.93 (6H, dd, *J* = 6.8, 14.8, $CHC\underline{H}_3$). ^{13}C NMR (100 MHz, D_2O) 17.1 (CH_3), 17.3 (CH_3), 17.9 (CH_3), 18.0 (CH_3), 26.6 ($CH_2\underline{C}H_2CH$), 26.8 ($CH_2\underline{C}H_2CH$), 29.0 ($CH\underline{C}HCH_3$), 29.2 ($CH\underline{C}HCH_3$), 47.2 ($CH_2\underline{C}H_2CH$), 47.6 ($N\underline{C}H_2CH$), 60.3 ($\underline{C}HCH_2N$), 68.6 ($CH_2\underline{C}H_2O$), 69.1 ($\underline{C}HCO_2H$), 172.2 (C=O/Carboxylic acid), 172.3 (C=O/Carboxylic acid), 179.9 (C=O), 180.0 (C=O); mp=265-266 ℃; IR (neat) cm^{-1} 3345 (OH), 1770 and 1571 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)=238.

3-(((2-Hydroxyethyl)thio)methyl)dihydrofuran-2(3H)-one: Compound 11a



2-Mercaptoethanol (2.45 mmol, 0.172 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **11a** was obtained as yellow oil (78 %, 0.28 g). ^1H NMR δ_H (400 MHz, $CDCl_3$) δ4.41 (1H, td, *J* = 2.8, 8.8, $C\underline{H}_2OC=O$), 4.25 (1H, td, *J* = 9.6, 6.8, $C\underline{H}_2OC=O$), 3.75 (2H, t, *J* = 6.2, $CH_2C\underline{H}_2S$), 3.05 (1H, OH), 2.91 (1H, dd, *J* = 13.2, 4.4, $CH_2OHC\underline{H}_2N$), 2.65 to 2.82 (3H, m), 2.25 to 2.50 (2H, m, $CH_2C\underline{H}_2CH$). ^{13}C NMR (100 MHz, $CDCl_3$) δ27.8 ($CH_2\underline{C}H_2CH$), 32.0 ($CH_2OH\underline{C}H_2N$), 35.5 ($CH_2\underline{C}H_2S$), 40.0 ($\underline{C}H$), 61.0 ($\underline{C}H_2OH$), 66.9 ($CH_2OC=O$), 178.2 (C=O); IR (neat) cm^{-1} 3423 (NH), 2917 (OH), 1755 (C=O); MS (EI+) *m/z* ($[M+Na]^+$)=199.

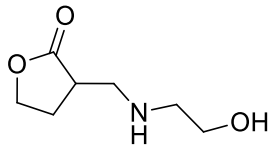
3-((Benzylthio) methyl) dihydrofuran-2(3H)-one: Compound 11b



Benzylamine (2.04 mmol, 0.222 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **11b** was obtained as yellow oil (84.8 %, 0.384 g). ^1H NMR δ_H (400 MHz, $CDCl_3$) δ7.28 (4H, d, *J* = 4.4, Ph), 7.20 (1H, m, Ph), 4.27 (1H, td, *J* = 8.8,

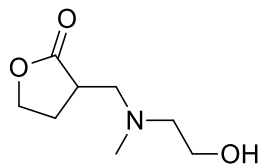
2.8, C<u>H</u>$_2$OC=O), 4.10 (1H, td, *J* = 6.8, 9.2, C<u>H</u>$_2$OC=O), 3.70 (2H, d, *J* = 2.8, PhC<u>H</u>$_2$), 2.90 (2H, dd *J* = 4.0, 13.2 CHC<u>H</u>$_2$N), 2.70 (1H, m, C<u>H</u>), 2.30 to 2.40 (1H, m), 1.97 to 2.10 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ28.0 ($CH_2\underline{C}H_2CH$), 31.8 (S<u>C</u>H_2CH), 37.0 (S<u>C</u>H_2Ph), 39.7 (CH), 66.7 (<u>C</u>H_2OC=O), 127.2 (Ph), 128.9 (Ph), 129.1 (Ph), 138.0 (<u>C</u>), 177.8 (<u>C</u>=O); IR (neat) cm^{-1} 2914 (CH/Ph), 1760 (C=O).; MS (EI+) *m/z* ([M+ H]$^+$)= 223.

3-(((2-Hydroxyethyl) amino) methyl) dihydrofuran-2(3H)-one: Compound 12a



Ethanolamine (2.45 mmol, 0.15 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **12a** was obtained as yellow oil (71 %, 0.232 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.40 (1H, td, *J* = 11.6, 2.8 C<u>H</u>$_2$OC=O), 4.31 (1H, td, *J* = 6.8, 9.2 C<u>H</u>$_2$OC=O), 3.65 (2H, t, *J* = 5.2 CH_2C<u>H</u>$_2$OH),2.95 (1H, dd, *J* = 12, 6.4, $CHCH_2N$), 2.75 to 2.92 (4H, m), 2.35 to 2.40 (1H, m), 2.05 to 2.20 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ26.9 ($CH_2\underline{C}H_2CH$), 39.6 (<u>C</u>H), 49.2 (CH<u>C</u>H_2), 51.3 ($CH_2\underline{C}H_2N$), 60.7 ($CH_2\underline{C}H_2OH$), 66.9 ($CH_2OC=O$), 179.0 (C=O); IR (neat) cm^{-1} 1763 (C=O).; MS (EI+) *m/z* ([M+Na]$^+$)=180.

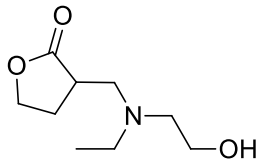
3-(((2-Hydroxyethyl) (methyl) amino) methyl) dihydrofuran-2(*3H)*-one: Compound 12b



N-Methylethanolamine (2.45 mmol, 0.19 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **12b** was obtained as pale yellow oil (91 %, 0.321 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.38 (1H, td, *J* = 8.8, 2.8, C<u>H</u>$_2$OC=O), 4.24 (1H, td, *J* = 9.6, 2.8 C<u>H</u>$_2$OC=O), 3.60 (2H, t, *J* = 5.2, CH_2OH), 2.93

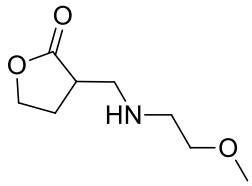
(2H, dd, *J* = 12.4, 5.2, CHC<u>H</u>$_2$N), 2.70 to 2.80 (1H, m, CH), 2. 55 to 2.65 (2H, m, C<u>H</u>$_2$CH$_2$OH), 2.41 to 2.49 (1H, m), 2.32 (3H, s, Methyl group), 2.10 to 2.15 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 28 (CH$_2$<u>C</u>H$_2$CH), 38.3 (CH$_3$), 41.9 (CH), 58.0 (CH$_2$N), 58.5 (CH$_2$OH<u>C</u>H$_2$N), 58.9 (<u>C</u>H$_2$OH), 66.7 (<u>C</u>H$_2$OC=O), 178.4 (<u>C</u>=O); IR (Neat) cm^{-1} 3421 (OH), 1758 (C=O).; MS (EI+) *m/z* ([M]$^+$)=173.

3-((Ethyl (2-hydroxyethyl) amino) methyl) dihydrofuran-2(3H)-one: Compound 12c



2-(Ethylamino) ethanol (2.45 mmol, 0.239 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **12c** was obtained as yellow oil (61 %, 0.232 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 4.37 (1H, td, *J* = 8.8, 3.6 C<u>H</u>$_2$OC=O), 4.24 (1H, td, *J* = 6.8, 9.2, C<u>H</u>$_2$OC=O), 3.57 (2H, t, *J* = 5.6 CH$_2$C<u>H</u>$_2$OH), 3.33 (1H, s, OH), 2.95 (1H, dd, *J* = 13.2, 5.2, CHCH$_2$N), 2.80 to 2.70 (1H, m, CH), 2.71 to 2.55 (5H, m), 2.50 to 2.4 0 (1H, m), 1.99 to 2.15 (1H, m), 1.05 (3H, t, *J* = 7). ^{13}C NMR (100 MHz, $CDCl_3$) δ11.4 (CH$_3$), 28.0 (CH$_2$<u>C</u>H$_2$CH), 38.3 (CH), 47.5 (CH$_3$<u>C</u>H$_2$), 53.9 (CH<u>C</u>H$_2$N), 55.0 (CH$_2$<u>C</u>H$_2$N), 58.7 (CH$_2$<u>C</u>H$_2$OH), 66.7 (CH$_2$OC=O), 178.7 (C=O); IR (neat) cm^{-1} 1760 (C=O).; MS (EI+) *m/z* ([M+Na]$^+$)= 210.

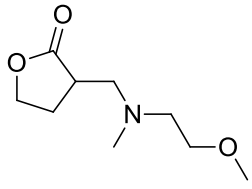
3-(((2-Methoxyethyl) amino) methyl) dihydrofuran-2(3H)-one: Compound 12d



2-Methoxyethylamine (2.45 mmol, 0.18 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol and using the general procedure A, **12d** was obtained as clear oil (81 %, 0.291 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.37 (1H, td, *J* = 8.8, 2.8 C<u>H</u>$_2$OC=O), 4.23 (1H, td, *J* =

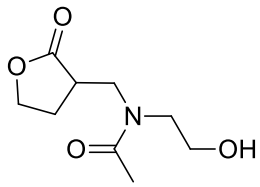
6.8, 9.6 C$\underline{H}_2$OC=O), 3.48 (2H, t, *J* = 5.2 MeOC$\underline{H}_2$), 3.34 (3H, s, CH_3), 2.95 (1H, dd, *J* = 12, 5.6, CHCH_2N), 2.72 to 2.88 (4H, m), 2.35 to 2.45 (1H, m, CH_2CH_2CH), 2.06 to 2.18 (1H, m, CH_2CH_2CH), 1.95 to 1.99 (1H, m, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ26.7 ($CH_2\underline{C}H_2$CH), 39.6 (CH), 49.1 (N$\underline{C}H_2$), 49.4 (CH$\underline{C}H_2$N), 58.5 (CH_3), 66.7 (CH_2OC=O), 71.7 ($\underline{C}H_2$OMe), 178.6 (C=O); IR (neat) cm^{-1} 1758 (C=O).; MS (EI+) *m/z* ([M+Na]$^+$)= 196.

3-(((2-Methoxyethyl)(methyl)amino)methyl)dihydrofuran-2(3H)-one: Compound 12e



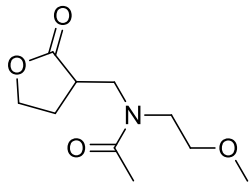
(2-Methoxyethyl) methylamine (2.45 mmol, 0.266 mL) was added into the stirred solution of tulipane (200 mg, 0.179 mL, 2.04 mmol) in 15 mL methanol, **12e** was obtained as clear oil (48 %, 0.183 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.35 (1H, td, *J* = 3.6, 8.8 C$\underline{H}_2$OC=O), 4.21 (1H, td, *J* = 9.2, 6.8, C$\underline{H}_2$OC=O), 3.45 to 3.55 (2H, m, NCH_2C$\underline{H}_2$O), 3.35 (3H, s, OC$\underline{H}_3$), 2.90 (1H, dd, *J* = 12.8, 4.4, CH_2OHC$\underline{H}_2$N), 2.65 to 2.80 (2H, m), 2.51 to 2.65 (2H, m), 2.40 to 2.45 (1H, m), 2.30 (3H, s, NC$\underline{H}_3$), 2.15 to 2.20 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ28.1 ($CH_2\underline{C}H_2$CH), 38.4 (CH), 42.9 ($\underline{C}H_3$N), 56.9 (N$\underline{C}H_2CH_2$O), 58.0 (CH_2OH$\underline{C}H_2$N), 58.8 (N$\underline{C}H_3$), 66.8 (CH_2OC=O), 70.5 (NC$H_2\underline{C}H_2$O), 178.3 (C=O); IR (neat) cm^{-1} 1763 (C=O).; MS (EI+) *m/z* ([M+Na]$^+$)= 210.

N-(2-Hydroxyethyl)-N-((2-oxotetrahydrofuran-3-yl) methyl) acetamide: Compound 13a



Acetic Anhydride (1.06 mmol 0.1 mL) was added into the stirred solution of Compound **12a** (0.141 g, 0.9 mmol) in 15 mL DCM and using the general procedure C, **13a** was obtained as yellow oil (51 %, 0.092 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 4.40 (1H, td, *J* = 8.4, 4.0, C$\underline{H}_2$OC=O), 4.25 (1H, td, *J* = 6.4, 8.8, C$\underline{H}_2$OC=O), 3.40 to 3.95 (7H, m), 2.91 to 3.05 (1H, m), 1.95 to 2.15 (1H, m), 2.20 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9 ($\underline{C}H_3$), 27.6 ($CH_2\underline{C}H_2CH$), 38.9 (CH), 46.2 ($\underline{C}H_2CH$), 52.0 ($CH_2\underline{C}H_2N$), 60.2 ($\underline{C}H_2OH$), 67.0 (CH_2OC=O), 172.4 (C=O/amide), 178.8 (C=O); IR (neat) cm^{-1} 1759 and 1617 (C=O); MS (EI+) *m/z* ($[M+Na]^+$) = 224.

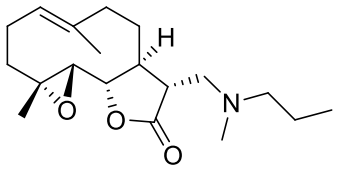
N-(2-Methoxyethyl)-N-((2-oxotetrahydrofuran-3-yl) methyl) acetamide: Compound 13b



Acetyl chloride (1.6 mmol 0.115ml) was added into the stirred solution of Compound **12d** (0.141 g, 1.3 mmol) in 15 mL DCM and using the general procedure C, **13b** was obtained as clear oil (48 %, 0.134 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ4.37 (1H, td, *J* = 8.8, 2.8, C$\underline{H}_2$OC=O), 4.23 (1H, td, *J* = 6.8, 9.6, C$\underline{H}_2$OC=O), 3.48 (2H, t, *J* = 5.2, MeOC$\underline{H}_2$), 3.34 (3H, s, CH_3), 2.95 (1H, dd, *J* = 12, 5.6, $CHCH_2N$), 2.72 to 2.88 (4H, m), 2.35 to 2.45 (1H, m, CH_2CH_2CH), 2.06 to 2.18 (1H, m, CH_2CH_2CH), 1.95 to 2.00 (1H, m, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ21.7 ($\underline{C}H_3$), 27.3 ($CH_2\underline{C}H_2CH$), 39.0 (CH), 45.7 ($\underline{C}H_2CH$), 49.2 ($CH_2\underline{C}H_2N$), 59.0 ($\underline{C}H_3O$), 66.8 (CH_2OC=O), 70.6 ($\underline{C}H_2O$), 171.8 (C=O/amide), 178.2 (C=O); IR (neat) cm^{-1} 1761 and 1636 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)= 238.

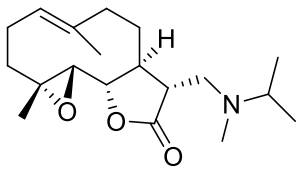
4.7 Parthenolide Derivatives

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8 ((propylamino)methyl)-2,3,6,7,7a,8,10a,10b octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14a



N-Methylpropylamine (0.48 mmol, 0.05 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14a** was obtained as colourless solid (78.6 %, 0.101 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.20 (1H, dd, *J* = 12, 2, C<u>H</u>=C), 3.82 (1H, t, *J* = 9, C<u>H</u>-O-CO), 2.65 to 2.85 (3H, m), 2.01 to 2.50 (13H, m), 1.70 (1H, s, CH=CCH_3), 1.55 to 1.65 (1H, m), 1.47 (2H, dd, *J* = 7.2, 14.8), 1.30 (3H, s), 1.20 to 1.25 (1H, m), 0.9 (3H, t, *J* = 7.4, C<u>H</u>$_3$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ11.8, 17.0, 17.2, 20.6, 24.1, 30.0, 36.7, 41.1, 42.6, 46.5, 47.9, 56.3, 60.5, 61.5, 66.6, 82.2, 124.9, 134.8, 176.7.; mp= 117-119 ℃; $[\alpha]^{18}_{D}$ =-23.2 ° (*c* =1, $CDCl_3$); IR (neat) cm^{-1} 1769 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)= 322.

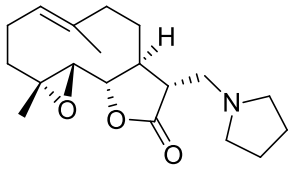
(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-((Isopropyl(methyl)amino)methyl)-1a,5-dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14b



N-Isopropylmethylamine (0.48 mmol, 0.05 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14b** was obtained as colourless solid (36 %, 0.046 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.21 (1H, dd, *J* = 12, 2, C<u>H</u>=C), 3.82 (1H, t, *J* = 8.8, C<u>H</u>OCO), 2.65 to 2.85 (4H, m), 2.21 to 2.50 (11H, m), 1.70 (3H, s, CH=CCH_3), 1.55 to 1.65 (1H, m), 1.3 (3H, s, NC<u>H</u>$_3$), 1.19 to 1.29 (2H, m,), 0.98 to 1.10 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 17.0, 17.2, 17.8, 17.9, 24.1, 30.1, 36.7, 37.3, 41.1, 46.6,

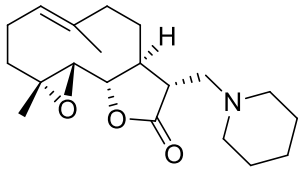
47.8, 52.0, 54.7, 61.5, 66.7, 82.2, 124.9, 134.8, 176.9.; mp= 111-113 ℃; $[\alpha]^{18}_{D}$ =-12.4 ° (*c* =1, $CDCl_3$); IR (neat) cm^{-1} 1766 (C=O); MS (EI+) *m/z* ($[M+H]^+$)=344.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8-(pyrrolidin-1-ylmethyl)-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14c



Pyrrolidine (0.48 mmol, 0.04 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14c** was obtained as colourless solid (83 %, 0.106 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 5.22 (1H, d, *J* = 12, 2, C<u>H</u>=C), 3.81 (1H, t, *J* = 9, C<u>H</u>OCO), 2.89 (2H, qd, *J* = 4.8, 13.2, 31.2, $CH_2C\underline{H}_2CH_2$), 2.74 (1H, d, *J* = 8.8, OC<u>H</u>CHOCO), 2.50 to 2.60 (4H, m), 2.02 to 2.45 (8H, m), 1.58 to 1.80 (8H, m), 1.31 (3H, s), 1.20 to 1.25 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.0, 17.2, 23.8, 24.1, 30.0, 36.7, 41.1, 47.4, 47.6, 53.7, 54.8, 61.5, 66.6, 82.1, 125.0, 134.8, 176.6.; mp= 128-129 ℃; $[\alpha]^{18}_{D}$ =-16.4 ° (*c* =1, $CDCl_3$); IR (neat) cm^{-1} 1768 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=320.

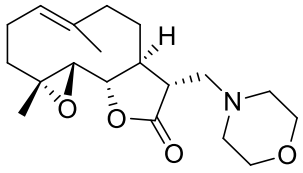
(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8-(piperidin-1-ylmethyl)-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14d



Piperidine (0.48 mmol, 0.047 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14d** was obtained as colourless solid (83 %, 0.106 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ 5.20 (1H, dd, *J* = 12.0, 2.0, C<u>H</u>=C), 3.75 (1H, t, *J* = 9, C<u>H</u>OCO), 2.75 (2H, td, *J* = 4.8, 9.2, $CH_2C\underline{H}_2CH_2$), 2.60 (1H, dd, *J* = 5.6, 13.2, $CHC\underline{H}_2N$), 2.35 to

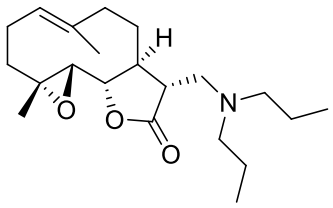
2.51 (6H, m), 2.00 to 2.30 (6H, m), 1.71 (3H, s), 1.50 to 1.68 (5H, m), 1.38 to 1.48 (2H, m), 1.32 (3H, s), 1.22 to 1.26 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.1, 17.2, 24.1, 14.2, 26.1, 30.1, 36.7, 41.2, 46.1, 48.2, 55.1, 57.5, 61.4, 66.7, 82.3, 124.7, 134.9, 176.8.; mp= 140-141 ℃; $[\alpha]^{18}_D$ =-4.8 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 1764 (C=O); MS (EI+) *m/z* ($[M+H]^+$)=334.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8-(morpholinomethyl)-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14e



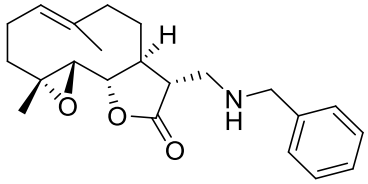
Morpholine (0.48 mmol, 0.042 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14e** was obtained as colourless solid (83.5 %, 0.112 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.20 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.85 (1H, t, *J* = 9.0, C<u>H</u>OCO), 3.67(3H, t, *J* = 4.6, $CH_2C\underline{H}_2O$), 2.68 to 2.84 (3H, m), 2.50 (3H, t, *J* = 4.2, $C\underline{H}_2CH_2O$), 2.00 to 2.48 (9H, m), 1.70 (3H, s), 1.61 to 1.68 (1H, m), 1.31 (3H, s), 1.19 to 1.27 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.0, 17.2, 24.1, 29.7, 30.0, 36.6, 41.2, 46.1, 48.0, 54.3, 56.9, 61.5, 66.6, 67.0, 82.2, 124.9, 134.7, 176.4.; mp= 172-173 ℃; $[\alpha]^{18}_D$ =-2.8 ° (c =1, $CDCl_3$); IR (neat) cm^{-1} 1768 (C=O); MS (EI+) *m/z* ($[M+H]^+$)=336.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-((Dipropylamino)methyl)-1a,5-Dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14f



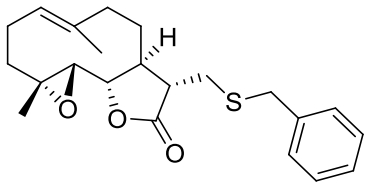
Dipropylamine (0.48 mmol, 0.066 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14f** was obtained as colourless solid (43 %, 0.06 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.21 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.85 (1H, t, *J* = 9.0, C<u>H</u>OCO), 2.71 to 2.91 (2H, m), 2.05 to 2.50 (10H, m), 1.70 (3H, s), 1.21 to 1.68 (12H, m), 0.85 to 0.95 (6H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 11.9, 17.0, 20.1, 24.1, 30.1, 33.4, 36.7, 41.2, 46.8, 48.2, 53.7, 56.3, 66.7, 82.2, 124.9, 134.8, 176.8, 207.0.; mp= 166-167 ℃; $[\alpha]^{18}_D$ =-6 ° (c =1, $CDCl_3$); IR (neat) cm^{-1} 1771 (C=O); MS (EI+) *m/z* ($[M+H]^+$)=350.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-((Benzylamino)methyl)-1a,5-Dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound14g



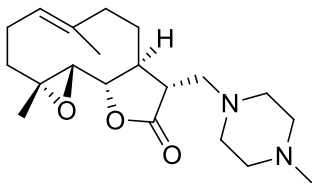
Benzylamine (0.48 mmol, 0.052 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14g** was obtained as pale yellow solid (75 %, 0.107 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ7.20 to 7.35 (5H, m), 5.15 (1H, dd, *J* = 2, 12.4, C<u>H</u>=C), 3.74 to 3.90 (3H, m), 3.01 (1H, dd, *J* = 12.0, 3.6, CHC<u>H_2</u>N), 2.70 to 2.77 (2H, m), 2.01 to 2.47 (6H, m), 1.92 to 1.99 (2H, m), 1.80 (1H, dd, *J* = 14.8, 6.4, CH_2C<u>H_2</u>C), 1.67 (3H, s), 1.55 to 1.68 (1H, m), 1.28 (3H, s), 1.16 to 1.27 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ16.9, 17.2, 24.1, 30.0, 36.6, 41.0, 46.0, 46.5, 48.2, 53.9, 61.5, 66.4, 82.6, 125.1, 127.0, 128.1, 128.4, 134.6, 140.1, 176.7. mp= 160-161 ℃; $[\alpha]^{18}_D$ =-15.2 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 1763 (C=O); MS (EI+) *m/z* ($[M+Na]^+$)= 378.

(1a*R*, 7a*S*, 8*S*, 10a*S*, 10b*S*, *E*)-8-((Benzylthio)methyl)-1a,5-Dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 14h



Benzyl Mercapton (0.48 mmol, 0.056 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **14h** was obtained as yellow semi-solid (56 %, 0.084 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ7.20 to 7.40 (5H, m), 5.15 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.74 to 3.90 (3H, m), 3.01 (1H, dd, *J* = 9.8, 3.6, CHC<u>H</u>$_2$N), 2.7 0 to 2.77 (2H, m), 2.00 to 2.47 (6H, m), 1.89 to 2.02 (2H, m), 1.83 (H, dd, *J* = 14.8, 6.4, CH_2C<u>H</u>$_2$C), 1.67 (3H, s), 1.55 to 1.68 (1H, m), 1.28 (3H, s), 1.16 to 1.28 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.1, 24.1, 29.8, 34.7, 36.8, 37.8, 40.9, 47.7, 48.0, 50.3, 61.6, 66.3, 82.6, 124.5, 125.1, 128.6, 129.2, 134.5, 140.1, 175.6.; $[\alpha]^{18}_D$ =+13.6 ° (c =1, $CDCl_3$); IR (neat) cm^{-1} 1770 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)=395.

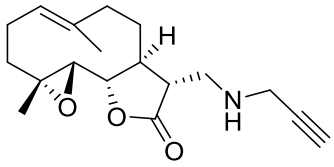
(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8-((4-methylpiperazin-1-yl)methyl)-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15a



1-Methylpiperazine (0.48 mmol, 0.050 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15a** was obtained as colourless solid (37 %, 0.051 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.21 (1H, dd, *J* = 2.2, 12.0, C<u>H</u>=C), 3.80 (1H, t, *J* = 9.2, C<u>H</u>OCO), 2.80 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.75 (2H, dd, *J* = 5.6, 14.4, CHC<u>H</u>CH_2), 2.01 to 2.60 (18H, m), 1.80 (1H, s), 1.70 (3H, s, C<u>H</u>$_3$C=CH), 1.55 to 1.68 (1H, m), 1.28 (3H, s, C<u>H</u>$_3$C), 1.16 to 1.27 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.1, 24.1, 30.1, 36.7, 41.2, 46.0, 46.3, 48.0, 53.8, 55.2, 56.4, 61.5, 66.7, 82.3, 124.9,

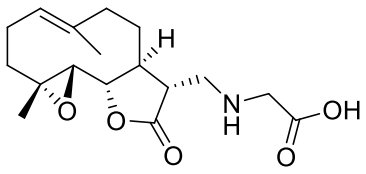
134.7, 176.5.; mp=163-164 ℃; $[\alpha]^{18}_{D}$ =-2 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 1769 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=349.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-1a,5-Dimethyl-8-((prop-2-yn-1-ylamino)methyl)-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15b



Propargylamine (0.48 mmol, 0.03 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15b** was obtained as yellow oil (36 %, 0.044 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.32 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.80 (1H, t, *J* = 9.2, C<u>H</u>OCO), 3.48 (2H, d, *J* = 2.4, NC<u>H</u>$_2$C), 3.08 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.91 (2H, dd, *J* = 5.6, 14.4, CHC<u>H</u>CH$_2$), 2.78 (1H, d, *J* = 8.8, C<u>H</u>CHCH$_2$), 1.85 to 2.55 (11H, m), 1.73 (3H, s, C<u>H</u>$_3$C=CH), 1.65 to 1.70 (1H, m), 1.31 (3H, s, C<u>H</u>$_3$C), 1.18 to 1.28 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ16.9, 17.2, 24.1, 30.0, 36.6, 38.6, 41.1, 46.1, 46.7, 48.0, 61.5, 66.3, 71.7, 81.7, 82.6, 125.2, 134.4, 176.5.; $[\alpha]^{18}_{D}$ =-34 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 3279(NH), 1762(C=O).; MS (EI+) *m/z* ($[M+H]^+$)=304.

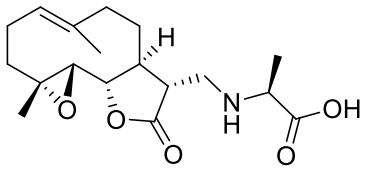
2-((((1a*R*,7a*S*,8*R*,10a*S*,10b*S*,*E*)-1a,5-Dimethyl-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-8-yl)methyl)amino)acetic acid: Compound 15c



Glycine (0.48 mmol, 0.022g) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 15ml methanol and water mixture and using the general procedure B, **15c** was obtained as a colourless crystalline solid (28 %, 0.036 g). ^{1}H NMR δ_H (400 MHz, DMSO) δ5.25 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C),

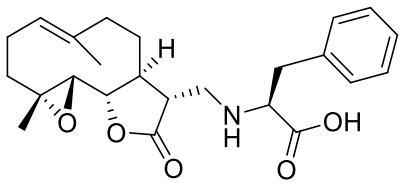
3.80 (1H, t, *J* = 9.2, CHOCO), 2.78 (4H, d, *J* = 9.2), 1.85 to 2.55 (10H, m), 1.65 (3H, s, CH_3C=CH), 1.60 to 1.70 (1H, m), 1.20 (3H, s, CH_3C), 1.05 to 1.15 (1H, m). ^{13}C NMR (100 MHz, DMSO): δ16.6, 16.8, 23.7, 28.9, 36.1, 40.5, 45.8, 47.1, 47.6, 55.0, 61.1, 65.5, 81.5, 124.4, 134.4, 173.1, 176.8.; mp= 164-165 ℃; $[\alpha]^{18}_D$ =-27.6 ° (c =1, D_2O); IR (neat) cm^{-1} 3339 (OH), 2976 (NH), 1578 and 1757 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)=346.

(*S*)-2-((((1a*R*,7a*S*,8*R*,10a*S*,10b*S*,*E*)-1a,5-Dimethyl-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-8-yl)methyl)amino)propanoic acid: Compound 15d



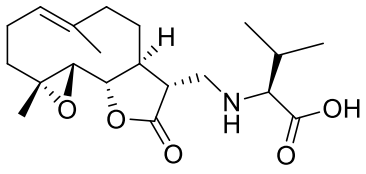
L-alanine (0.48 mmol, 0.043g) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 15ml methanol and water mixture and using the general procedure B, **15d** was obtained as a colourless crystalline solid (36 %, 0.046 g).^{1}H NMR δ_H (400 MHz, DMSO) δ5.20 (1H, dd, *J* = 2.4, 12.0, CH=C), 4.05 (1H, t, *J* = 9.1, CHOCO), 3.60 to 3.82 (2H, m), 3.18 to 3.50 (2H, m), 2.85 to 3.17 (2H, m), 1.90 to 2.80 (6H, m), 1.78 (2H, s), 1.61 (3H, s, CH_3C), 1.05 to 1.27 (6H, m). ^{13}C NMR (100 MHz, DMSO): δ14.0, 16.6, 16.8, 18.1, 20.7, 23.6, 28.8, 36.1, 45.7, 46.9, 49.8, 57.4, 59.7, 65.3, 81.8, 124.4, 134.4, 176.7.; mp= 189-191 ℃; $[\alpha]^{18}_D$ =-26.8 ° (c =1, D_2O); IR (neat) cm^{-1} 3256 (OH), 2976 (NH), 1603 and 1775 (C=O).; MS (EI+) *m/z* ($[M+Na]^+$)=360.

(*S*)-2-((((1a*R*,7a*S*,8*R*,10a*S*,10b*S*,*E*)-1a,5-Dimethyl-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-8-yl)methyl)amino)-3-phenylpropanoic acid: Compound15e



L-phenylalanine (0.48 mmol, 0.08g) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 15ml methanol and water mixture and using the general procedure B, **15e** was obtained as a colourless crystalline solid (34 %, 0.056 g). ^{1}H NMR δ_H (400 MHz, DMSO) δ7.25 (5H, m, Ph), 5.25 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.90 (1H, t, *J* = 9.2, C<u>H</u>OCO), 3.40 to 3.48 (2H, m), 2.85 to 2.91 (2H, m), 2.81 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.60 to 2.69 (2H, m), 2.00 to 2.55 (6H, m), 1.90 (1H, t, *J* = 12, CC<u>H</u>$_2$CH$_2$CH), 1.75 (1H, dd, *J* = 5.6, 14.8, CH$_3$CC<u>H</u>$_2$CH$_2$), 1.60 (3H, s, C<u>H</u>$_3$C=CH), 1.60 to 1.70 (1H, m), 1.20 (3H, s, C<u>H</u>$_3$C), 1.05 to 1.15 (1H, m). ^{13}C NMR (100 MHz, DMSO): δ16.6, 16.8, 23.6, 28.8, 36.1, 38.6, 38.9, 39.1, 44.8, 45.5, 47.4, 61.0, 62.9, 65.4, 81.6, 124.3, 126.1, 128.0, 129.2, 134.4, 138.5, 148.0, 175.1, 176.8.; mp= 150-152 ℃; $[\alpha]^{18}_{D}$ =-14 ° (c =1, D$_2$O); IR (neat) cm^{-1} 3427 (OH), 2978 (NH), 1600 and 1762 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=414.

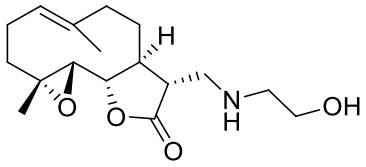
(*S*)-2-((((1a*R*,7a*S*,8*R*,10a*S*,10b*S*,*E*)-1a,5-Dimethyl-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-8-yl)methyl)amino)-3-methylbutanoic acid: Compound 15f



L-valine (0.48 mmol, 0.056g) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 15ml methanol and water mixture and using the general procedure B, **15f** was obtained as a colourless crystalline solid (26 %, 0.038 g). ^{1}H NMR δ_H (400 MHz, D$_2$O) δ7.80 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 6.78 (1H, t, *J* = 9, C<u>H</u>OCO), 5.85 to 5.95 (1H, m), 5.78 to 5.82 (2H, m), 5.52 to 5.56 (2H, m), 4.50 to 5.01 (8H, m), 4.29 to 4.33 (2H, m), 4.21 (3H, s, C<u>H</u>$_3$-C=CH), 3.80 (3H, s, C<u>H</u>$_3$-C), 3.15 to 3.25 (1H, m), 3.45 (6H, dd, *J* = 6.8, 14 CHC<u>H</u>$_3$).^{13}C NMR (100 MHz, D$_2$O): δ18.5, 18.6, 19.8, 20.7, 26.0, 30.9, 32.0, 38.0, 38.7, 42.5, 46.0, 48.2, 49.3, 67.4, 69.2, 71.4, 85.9, 127.3, 138.4, 180.7. mp= 119-120 ℃; $[\alpha]^{18}_{D}$ =-6.4 ° (c =1,

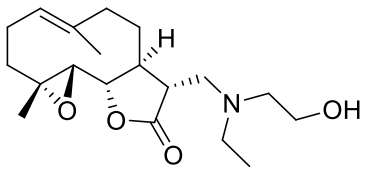
D_2O); IR (neat) cm^{-1} 3433 (OH), 2922 (NH), 1671 and 1767 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=366.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-(((2-Hydroxyethyl)amino)methyl)-1a,5-dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15g



Ethanolamine (0.48 mmol, 0.03 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15g** was obtained as yellow oil (77 %, 0.095 g). ^1H NMR δ_H (400 MHz, $CDCl_3$) δ5.35 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.90 (1H, t, *J* = 9.2, C<u>H</u>OCO), 3.65 (2H, t, *J* = 4.8, C<u>H</u>$_3$OH), 3.00 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.70 to 2.90 (4H, m), 1.85 to 2.65 (10, m), 1.70 (3H, s, C<u>H</u>$_3$C=CH), 1.65 to 1.75 (1H, m), 1.30 (3H, s, C<u>H</u>$_3$C), 1.18 to 1.28 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ16.9, 17.2, 24.1, 29.9, 36.6, 41.1, 46.8, 47.9, 51.4, 60.8, 61.7, 66.3, 82.6, 125.1, 134.5, 177.0.; $[\alpha]^{18}_D$ =-38 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 3329 (OH), 2978 (NH), 1760 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=310.

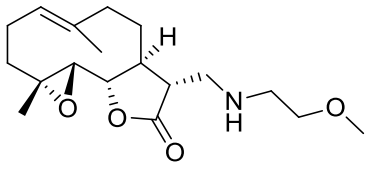
(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-((Ethyl(2-hydroxyethyl)amino)methyl)-1a,5-dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15h



2-(Ethylamino)ethanol (0.48 mmol, 0.047 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15h** was obtained as clear oil (21 %, 0.028 g). ^1H NMR δ_H (400 MHz, $CDCl_3$) δ5.20 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.85 (1H, t, *J* = 9,

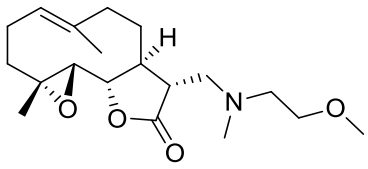
C<u>H</u>OCO), 3.60 (2H, t, *J* = 5.2, C<u>H</u>$_2$O), 2.80 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.60 to 2.75 (5H, m), 2.00 to 2.45 (10, m), 1.70 (3H, s, C<u>H</u>$_3$C=CH), 1.65 to 1.75 (1H, m), 1.30 (3H, s, C<u>H</u>$_3$C), 1.18 to 1.28 (1H, m), 1.06 (3H, t, *J* = 7.2, C<u>H</u>$_3$CH$_2$N). ^{13}C NMR (100 MHz, $CDCl_3$): δ11.4, 16.9, 17.2, 23.1, 24.1, 30.0, 36.6, 41.1, 46.5, 47.7, 48.2, 52.4, 55.4, 58.9, 61.5, 66.4, 82.1, 125.3, 134.3, 176.5.; $[\alpha]^{18}_D$ =-26 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 3418 (OH), 1763 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=338.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-(((2-Methoxyethyl)amino)methyl)-1a,5-dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15i



2-Methyoxyethylamine (0.48 mmol, 0.047 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15i** was obtained as yellow solid (57 %, 0.074 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.18 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.85 (1H, t, *J* = 9, C<u>H</u>OCO), 3.50 (2H, t, *J* = 5.2, C<u>H</u>$_2$O), 3.35 (3H, t, OC<u>H</u>$_3$), 3.00 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.70 to 2.90 (4H, m), 2.05 to 2.50 (8H, m), 1.95 (1H, dd, *J* = 6, 14.8, CH$_2$C<u>H</u>$_2$CO), 1.70 (3H, s, C<u>H</u>$_3$C=CH), 1.65 to 1.75 (1H, m), 1.30 (3H, s, C<u>H</u>$_3$C), 1.15 to 1.25 (1H, m).^{13}C NMR (100 MHz, $CDCl_3$): δ16.9, 17.2, 24.1, 30.0, 36.6, 41.1, 46.7, 46.8, 47.4, 47.9, 49.5, 58.8, 61.5, 66.3, 72.0, 82.5, 125.1, 134.5, 176.7.;mp=105-107 ℃; $[\alpha]^{18}_D$ =-49.6 °(c =1, $CDCl_3$); IR (neat) cm^{-1} 3332 (NH), 1763 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=324.

(1a*R*, 7a*S*, 8*R*, 10a*S*, 10b*S*, *E*)-8-(((2-Methoxyethyl)(methyl)amino)methyl)-1a,5-dimethyl-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-9(1aH)-one: Compound 15j



(2-Methoxyethyl)methylamine (0.48 mmol, 0.052 mL) was added into the stirred solution of Parthenolide (100 mg, 0.4 mmol) in 10 mL methanol and using the general procedure A, **15j** was obtained as yellow semi-solid (13 %, 0.02 g). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) δ5.20 (1H, dd, *J* = 2.0, 12.0, C<u>H</u>=C), 3.80 (1H, t, *J* = 9, C<u>H</u>OCO), 3.50 (2H, t, *J* = 5.2, C<u>H</u>$_2$O), 3.35 (3H, t, OC<u>H</u>$_3$), 2.85 (1H, dd, *J* = 4.8, 13.6, CHC<u>H</u>$_2$N), 2.70 to 2.80 (2H, m), 2.65 (2H, t, *J* = 5.6, CH$_2$C<u>H</u>$_2$N), 2.00 to 2.45 (11H, m), 1.70 (3H, s, C<u>H</u>$_3$C=CH), 1.65 to 1.75 (1H, m), 1.30 (3H, s, C<u>H</u>$_3$C), 1.15 to 1.25 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.0, 17.2, 24.1, 29.7, 29.9, 36.7, 41.0, 43.5, 46.7, 47.6, 56.1, 57.7, 58.8, 61.5, 66.5, 71.0, 82.2, 124.9, 134.7, 176.7.; $[\alpha]^{18}_D$ =-10 ° (c =1, $CDCl_3$); IR (neat) cm^{-1} 1768 (C=O).; MS (EI+) *m/z* ($[M+H]^+$)=338.

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