# รายงานวิจัยฉบับสมบูรณ์

**โครงการวิจัยเรื่อง**

**การพัฒนาสารสำคัญตัวที่สองจากฟ้าทะลายโจร “14-deoxy-11,12-didehydro andrographolide” สู่การเป็นสารต้านมะเร็ง**

Development of the second major component from *andrographis paniculata* “14-deoxy-11,12-didehydro andrographolide” as potential anticancer agents

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โครงการวิจัย “การพัฒนาสารสำคัญตัวที่สองจากฟ้าทะลายโจร “14-deoxy-11,12-didehydro andrographolide” สู่การเป็นสารต้านมะเร็ง” ได้รับการสนับสนุนทุนการวิจัยงบประมาณแผ่นดินประจำปีงบประมาณโครงการต่อเนื่องปี 2557 มหาวิทยาลัยบูรพา รายงานการวิจัยฉบับนี้เสนอรายละเอียดของการวิจัยซึ่งประกอบด้วย บทนำ งานวิจัยที่เกี่ยวข้อง วิธีทำการทดลอง รายละเอียดของข้อมูลการวิเคราะห์ผลิตภัณฑ์ ผลการทดลองวิจัย การอภิปรายและการสรุปผล

การวิจัย “การพัฒนาสารสำคัญตัวที่สองจากฟ้าทะลายโจร “14-deoxy-11,12-didehydro andrographolide” สำเร็จลุล่วงไปด้วยดี โดยผู้วิจัยต้องขอขอบคุณทีมวิจัยซึ่งประกอบด้วยที่ปรึกษาโครงการ ศ.ดร. อภิชาต สุขสำราญ มหาวิทยาลัยรามคำแหง และทีมตรวจสอบฤทธิ์ทางชีวภาพ ศ.ดร. ภาวิณี ปิยะจตุรวัฒน์ และคุณกนกเนตร สุขเสน มหาวิทยาลัยมหิดล ผู้ร่วมโครงการการสังเคราะห์สาร ซึ่งประกอบด้วย อ. ดร. อุทัยวรรณ ศิริอ่อน อ. ดร. อนันต์ อธิพรชัย และนิสิตปริญญาโทภาควิชาเคมี นางสาวสุดารัตน์ สมบุตร งานวิจัยนี้ได้รับการสนับสนุนจากภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพาและศูนย์นวัตกรรมความเป็นเลิศทางเคมี PERCH-CIC

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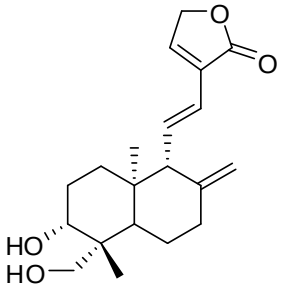
# Abstract

*Andrographis paniculata* Nees. (Acanthaceae), a Thai medicinal plant, is used for the treatment of cold, fever, laryngitis and infection in many Asian countries. The extract of the plant is a rich source of labdane diterpenoids and flavonoids. In continuation of our drug discovery program on *A. paniculata*, we isolated the second diterpenoid component, 14-deoxy-11,12-didehydroandrographolide (**2**) from the aerial part of this plant and modified the structure by chemical reactions. Twenty-one derivatives were obtained in good to excellent yields *via* silylation reaction at C-19 hydroxyl group, acetylation at C-3 hydroxyl group and epoxidation at C-8 alkene of (**2**) and thirty-seven derivatives of (**2**) were prepared *via* esterification at C-19 hydroxyl group, acetylation at C-3 in moderate to excellent yields. A number of the 14-deoxy-11,12-didehydroandrographolide analogues showed much higher cytotoxic activity than that of the parent compound (**2**) on cancer cells including P-388, KB, HT29, MCF-7, LU-1, ASK, KKU M-213, HUCCA-1 and KKU-100. SAR studies of the synthetic analogues indicated that the introduction of silyl ether or triphenyl methyl ether group in to C-19 of the parent compound led to the increasing in cytoxicity against the cancer cell. Compounds (**5a**) and (**5b**) were identified as the most potent with $ED_{50}$ values of 3.37 and 3.08 μM in KKU M-213 cell line and 2.93 and 3.27μM in K-100 cell line respectively, than the potent anti-cancer drug ellipticine. There analogues may serve as a potential structure lead for the development of new anticancer drugs.


14-deoxy-11,12-didehydro-andrographolide (2)

# CHAPTER 1
# INTRODUCTION

*Andrographis paniculata* Nees (Acanthaceae) is a medicinal plant widely cultivated in tropical regions in Asia. Traditionally, it is used for the treatment of cold, fever, laryngitis and infection in many Asian countries.

Extract of the plant, a rich source for flavonoids and labdane diterpenoids. Extracts of the plant and their constituents have been reported to exhibit a wide spectrum of biological activities of therapeutic importance including anti-bacterial (Mishra et al., 2013), anti-inflammatory (Chandrasekaran, Thiyagarajan, Deepak, & Agarwal, 2011), anti-cancer and immunostimulatory (Ajaya Kumar, Sridevi, Vijaya Kumar, Nanduri, & Rajagopal, 2004).

The active constituents of *A. paniculata* are diterpene lactone including androgarpholide (**1**), 14-deoxy-11,12-didehydroandrographolide (**2**), neoandrographo- lide (**3**) and 14-deoxyandrographolide (**4**) (Figure 1.1).

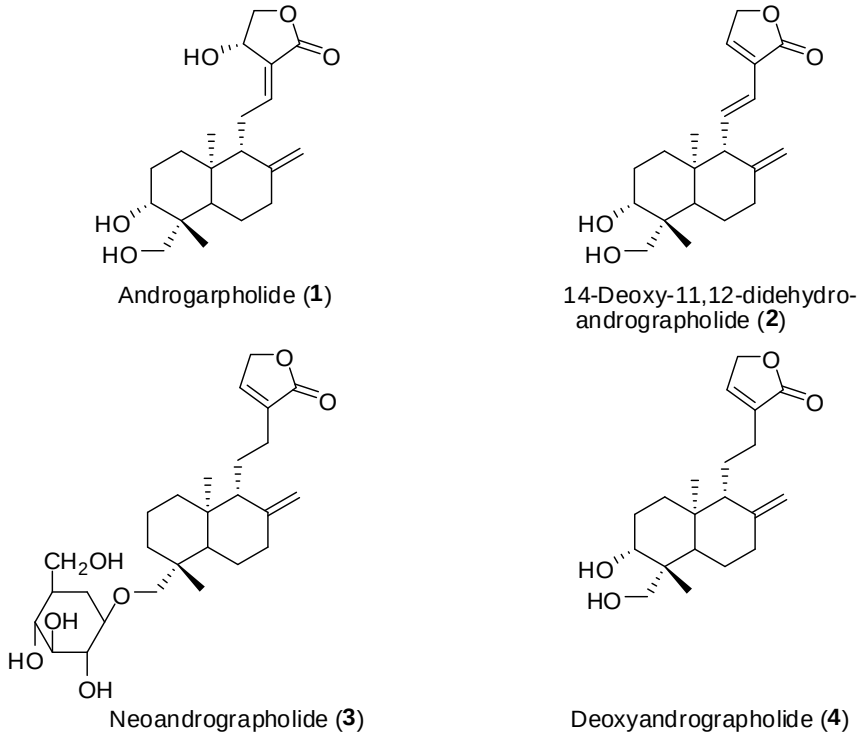



Figure 1.1 The structures of active constituents of *A. paniculata*.

14-Deoxy-11,12-didehydroandrographolide (**2**), $C_{20}H_{28}O_4$, is one of diter-penoid isolated from *A. paniculata*. It is a colorless needle crystal (from methanol) with the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene groups in its chemical structure as shown in Figure 1.2 (Matsuda, Sugiyama, Umehara, & Ueno, 1994).

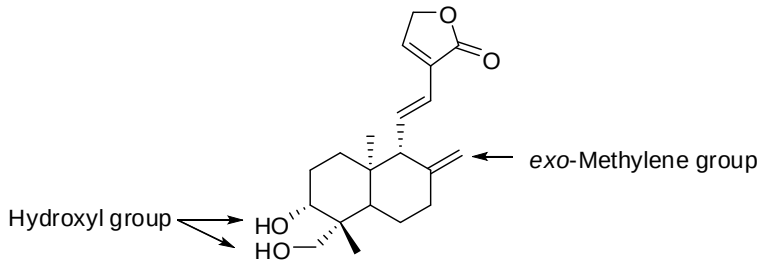



Figure 1.2 Structure of 14-deoxy-11,12-didehydroandrographolide (**2**).

Compound (**2**) show some degree of anti-malarial, anti-inflamatory (Guan, Kong, Cheng, Lim, & Wong, 2011; Zhang & Tan, 1999), anti-cardiovascular disease (Zhang, Kuroyangi, & Tan, 1998), anti-diabetic (Lee et al., 2010) and anti-cancer (Ooi, Kuroyanagi, Sulaiman, Muhammad, & Tan, 2011).

In this research, we have been interested on new analogues of 14-deoxy-11,12-didehydroandrographolide (**2**) for studying the structure-activity relationship (SAR) by modifying the two hydroxyl groups as silyl, alkyl ethers and acetyl, cinnamoyl, benzoyl esters (part A) and the *exo*-methylene group as epoxide (part B) as shown in Figure 1.3. All new synthetic analogues have been studied for anti-cancer activity.

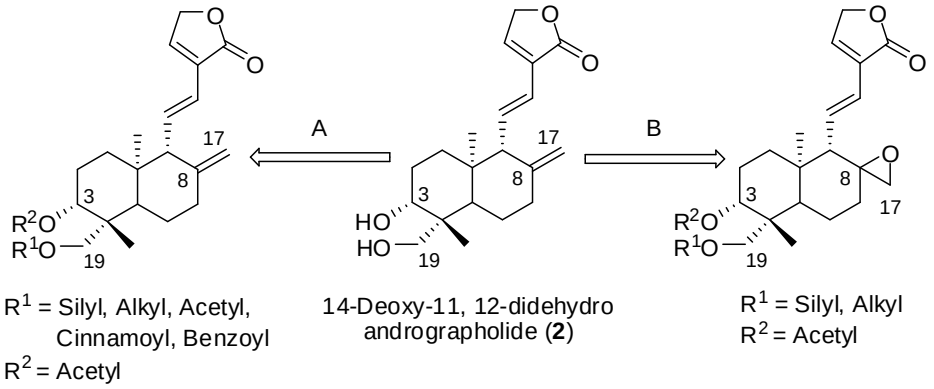



Fig.1.3 Strategies of structures modification.

# CHAPTER 2
# LITERATURE REVIEWS

*Andrographis paniculata* (Acanthaceae) is a medicinal plant used in many countries. The herb contains diterpenoids, flavonoids and polyphenols as the major bioactive components. The main diterpenoids are andrographolide (**1**), 14-deoxy-11,12-didehydroandrographide (**2**), neoandrographolide (**3**), 14-deoxyandrographo-lide (**4**) and isoandrographolide (**5**) show multiple pharmacological properties such as anti-malarial, anti-inflamatory, anti-cardiovascular disease (Chen, Song, Lu, & Xue, 2013; Zhang et al., 1998), anti-diabetic (Lee et al., 2010) and anti-cancer (Ooi et al., 2011).

## 2.1 Selected examples of the constituenets of *A. paniculata* Nees and their cytotoxic activity

The first isolation of diterpenoids from *A. paniculata* Nees was reported by Kleipool (Kleipool, 1952). The main components are diterpenoids which include andrographolide (**1**), 14-deoxy-11,12-didehydroandrographolide (**2**) and neoandro-grapholide (**3**) as shown in Figure 2.1.

Fujita et al. (1984) determined the absolute configuration at C-14 of andro-grapholide (**1**) and isolated three new *ent*-labdane diterpenoids which are androgra- phanin (**6**), andropanoside (**7**) and 14-deoxy-12-methoxyandrographolide (**8**) as shown in Figure 2.1.

Siripong et al. (1992) reported the cytotoxic activity of diterpenoid constituents from the methanolic extract of the leaves of *A. paniculata* Nees. Andrographolide (**1**) showed more cytotoxicity than 14-deoxy-11,12-didehydro-andrographolide (**2**), neoandrographolide (**3**), tetraacetateneoandrographolide (**9**) and stigmasterol (**10**) on the KB and P-388 tumor cells as shown in Table 2.1.

Table 2.1 Cytotoxicity against KB and P-388 tumor cell lines of *A. paniculata* Nees.

| **Compounds** | $ED_{50}$ (μg/ml)[a] | |
|---|---|---|
| | KB | P-388 |
| Crude MeOH extract | 5.3 | 3.1 |
| Andrographolide (**1**) | 1.5 | 1.0 |
| 14-Deoxy-11,12-didehydroandrographolide (**2**) | 20 | 9.2 |
| Neoandrographolide (**3**) | >25 | >40 |
| Tetraacetateneoandrographolide (**9**) | >25 | >40 |
| Stigmasterol (**10**) | >25 | >25 |

[a] For significant activity of the crude extract and pure compounds, $ED_{50}$ < 30 μM/mL and < 4 μM/mL respectively are required.

Ajaya Kumar, Sridevi, Vijaya Kumar and Rajagopal (2004) investigated the anti-cancer and immunostimulatory compounds from the methanolic extract of *A. paniculata*. The methanolic extract was fractionated into dichloromethane, petroleum ether and aqueous extracts and all the extracts were screened for bioactivities. The screening results indicated that the dichloromethane fraction of the methanolic extract retained the active compounds contributing to both the anti-cancer and immunostimulatory activities. Dichloromethane fraction significantly inhibited the proliferation of HT-29 (colon cancer) cells and augmented the proliferation of human peripheral blood lymphocytes (HPBLs) at low concentrations. On further fractionation of the dichloromethane extract, three diterpene compounds including andrographolide (**1**), 14-deoxy-11,12-didehydroandrographolide (**2**) and 14-deoxy-andrographolide (**4**) were isolated. Andrographolide (**1**) showed anti-cancer activity on diverse cancer cells representing different types of human cancers whereas all the three molecules showed increases in proliferation and interleukin-2 (IL-2) induction in HPBLs.

Wen and Bi (2010) reported the bioactive properties of several derivatives. Andrographolide analogues and 14-deoxy-11,12-didehydroandrographolide (**2**) showed immunostimulatory, anti-infective and anti-atherosclerotic. Neoandro- grapholide (**3**) showed anti-inflammatory, anti-infective and anti-hepatotoxic activities. 14-Deoxyandrographolide (**4**) displayed immunomodulatory and anti-atherosclerotic. Among the less abundant compounds from *A. paniculata*, andrograpanin (**6**) showed both anti-inflammatory and anti-infective activities. 14-Deoxy-14,15-dehydroandrographolide (**11**) exhibited anti-inflammatory activities. The structures are shown in Figure 2.1.

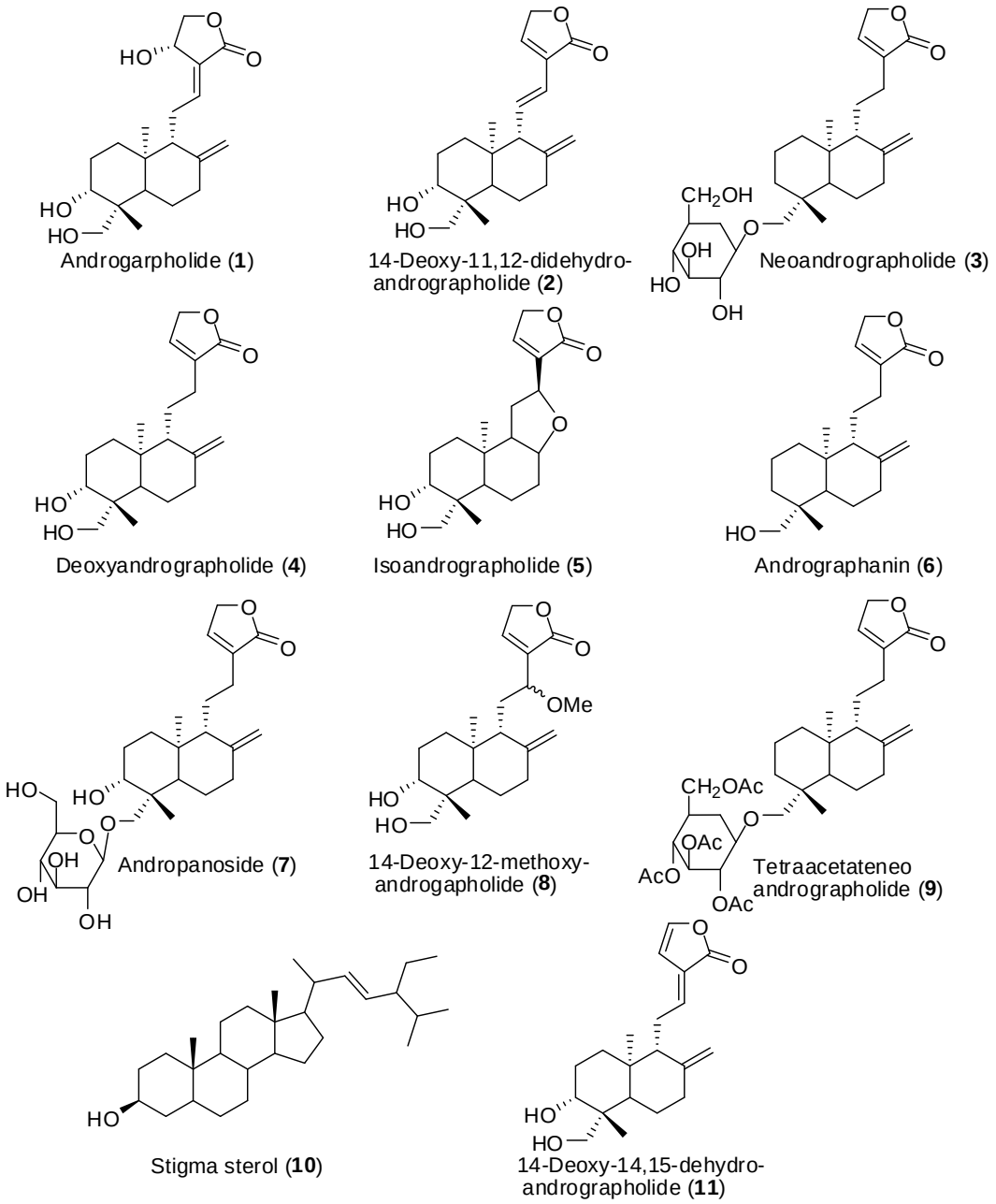
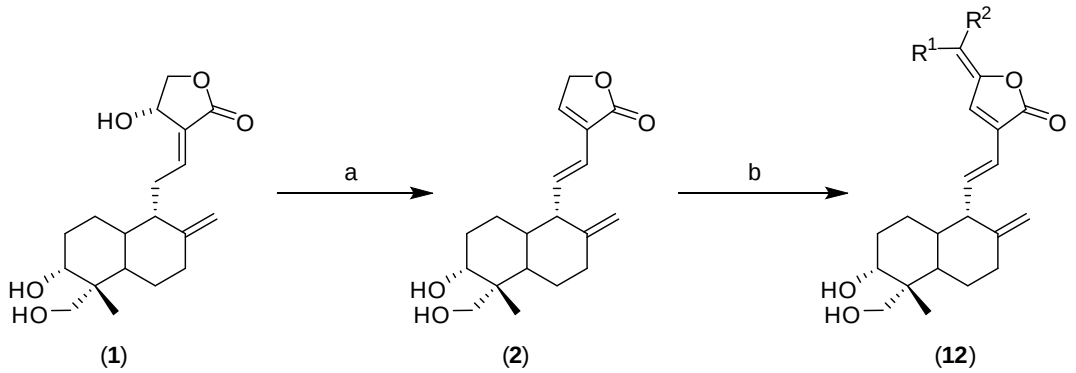



Figure 2.1 The structures of all derivatives from *A. paniculata*.

Moreover, Renugadevi, Ramanathan, Shanmuga and Thirunavukkarasu (2013) studied the effects of *A. paniculata* and *A. lineata* (Family: Acanthaceae) crude extracts against two mosquitoes *Culex quinquefasciatus* (Cx. quinquefasciatus) (Say.) and *Aedes aegypti* (Ae. aegypti) (Linn.). The results showed that maximum larvicidal activity was observed in the aqueous extract of *A. paniculata* and *A. lineata* followed by petroleum ether extracts. The combined activity of both extracts is more effective than the individual activity in all two types, the mortality rate increased considerably when the extracts were combined in aqueous extracts.

## 2.2 Selected examples of the synthesis of 14-deoxy-11,12-didehydro andrographolide analogues and biological evaluation

Dai, Xu, Wang, Liu and Liu (2006) reported the synthesis of 14-deoxy-11,12-didehydroandrographolide derivatives and studied the α-glucosidase inhibitory activity. 14-Deoxy-11,12-didehydroandrographolide (**2**) was prepared from natural andrographolide (**1**) and was modified to give product (**12**) by aldol condensation reaction at position C-15 as shown in Scheme 2.1.




| | $R^1$ | $R^2$ |
|---|---|---|
| (**12a**) | H | $CH_3$ |
| (**12b**) | $CH_3$ | $CH_3$ |
| (**12c**) | H | $(CH_2)_2CH_3$ |
| (**12d**) | H | $CH=C(CH_3)(CH_2)_2CHC(CH_3)_2$ |
| (**12e**) | H | 4-F-$C_6H_4$ |
| (**12f**) | H | 4-Cl-$C_6H_4$ |
| (**12g**) | $CH_3$ | 4-Br-$C_6H_4$ |
| (**12h**) | H | 4-MeO-$C_6H_4$ |
| (**12i**) | H | $C_6H_5CH=CH$ |

Scheme 2.1 Synthesis of 15-*ene*-substituted derivatives; Reagents and conditions aldehydes, methanol, $Na_2CO_3$, refluxing, 5-24 h, yield 50-90%.

The new analogues 15-*ene*-substituted derivatives (**12**) were screened for α-glucosidase inhibitory (Table 2.2). The results showed that increasing the carbon chain of C-15-substituted group to suitable chain length (**12a**-**12d**) could enhance the inhibitory activity against α-glucosidase. Different aromatic substitution derivatives gave inconsistent bioactivity results.

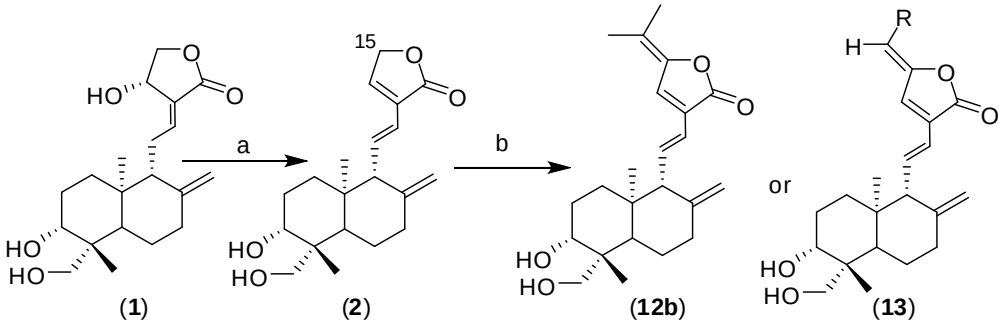
4-Fluoro and 4-chlorobenzylidene substitutions (**12e**-**12f**) caused losses in the inhibitory activity, while the activity was retained in 3-bromobenzylidene derivative (**12g**). The α-glucosidase inhibitory activity of the 4-methoxyphenylidene derivative (**12h**) and the phenylvinylidene derivative (**12i**) were much stronger than that of parent compound (**2**) with $IC_{50}$ values are 16 μM and 58 μM, respectively as shown in Table 2.2.

Table 2.2 The glucosidase inhibitory activity of analogues of 14-deoxy-11,12-dide-hydroandrographolide (**2**).

| **Compound** | % Inhibition activty[a] ($IC_{50}$, μM) | |
|---|---|---|
| | α-Glucosidase | β-Glucosidase |
| (**2**) | 16.5 | ni[b] |
| (**12a**) | 15.1 | ni |
| (**12b**) | 17.1 | ni |
| (**12c**) | 43.5 (110) | ni |
| (**12d**) | ni | nd[c] |
| (**12e**) | ni | nd |
| (**12f**) | ni | nd |
| (**12g**) | 16.7 | nd |
| (**12h**) | 100 (16) | nd |
| (**12i**) | 84.3 (58) | nd |

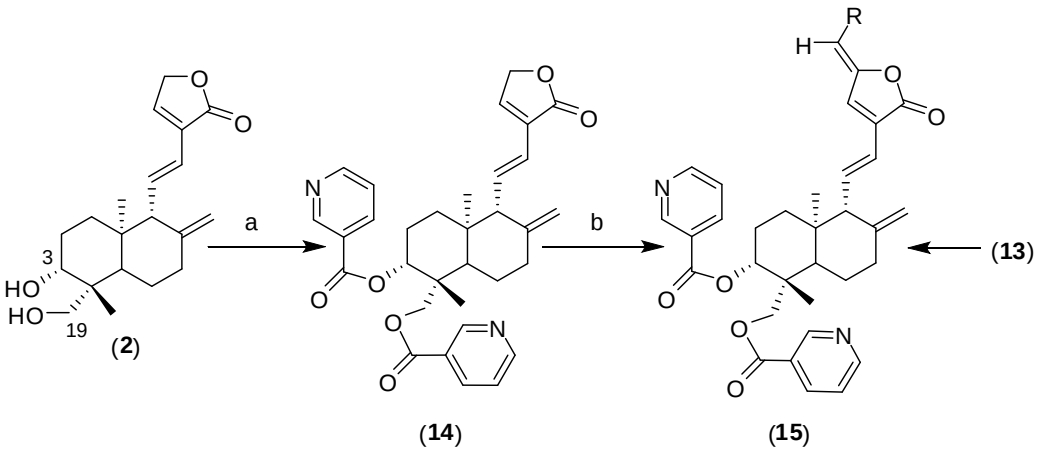
[a] Inhibition (%) determined at 100 μM concentation of compound.
[b] No inhibition at 100 μM.
[c] Not determind.

Xu, Dai, Liu, Wang, and Liu (2007) developed the synthesis of novel 14-deoxy-11,12-didehydroandrographolide analogues (**13**) and (**15**) from compound (**2**), which synthesized using andrographolide (**1**). All compounds were evaluated for α-glucosidase inhibitory activity. The reaction involved aldol condensation reaction at C-15 of compound (**2**) to give compounds (**12b**) and (**13**) in high yields and esterification at C-3 and C-19 by nicotination of compound (**2**) following aldol condensation to give the corresponding 15-alkylidene derivatives (**15**) as shown in Scheme 2.2 and 2.3.




(**13a**) R = $CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$
(**13b**) R = $C_6H_5$
(**13c**) R = 4-F-$C_6H_4$
(**13d**) R = 2,4,5-triMeO-$C_6H_4$
(**13e**) R = 4-Cl-$C_6H_4$
(**13f**) R = 3-Br-$C_6H_4$
(**13g**) R = 4-(*N*,*N*-dimethyl)-$C_6H_4$
(**13h**) R = 4-MeO-$C_6H_4$
(**13i**) R = benzo[1,3]dioxole-5-methanyl
(**13j**) R = furoyl

Scheme 2.2 Synthesis of compounds (**12b**) and (**13**); Reagents and conditions: (a) xylene, pyridine, $Al_2O_3$, reflux, 6-10 h, yield 91%; (b) acetone or aldehydes, $Na_2CO_3$, methanol, refluxing, 3-5 h, yield 60-93%.




(**15a**) R = $CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$
(**15b**) R = $C_6H_5$
(**15c**) R = 4-Cl-$C_6H_4$
(**15d**) R = 3-Br-$C_6H_4$
(**15e**) R = 4-(*N*,*N*-dimethyl)-$C_6H_4$
(**15f**) R = furoyl
(**15g**) R = 4-MeO-$C_6H_4$

Scheme 2.3 Synthesis of compounds (**14**) and (**15**); Reagents and conditions: (a) $CHCl_3$, nicotinic chloride, $Et_3N$, refluxing, 3 h, yield 92%; (b) aldehydes, $Na_2CO_3$, methanol, refluxing, 3-6 h, yield 53-80%.

The results showed that arylidene derivatives (**13**) were selective α-glucosidase inhibitor. The substitution at C-15 could enhance the inhibitory activity of α-glucosidase. The biological activity of (**15a-g**) ($IC_{50}$ = 28, 16, 6, 14, 25, 36 and 11 µM, respectively) was better than that of their corresponding compounds (**13a-b**), (**13e-h**) and (**13j**). However, the nicotinate derivative (**15c**) ($IC_{50}$ = 6 µM) of compound (**13e**) (no inhibition) showed the best bioactivity among all compounds. These results suggested that the nicotinate of hydroxyls at C-3 and C-19 was favorable to the activity as shown in Table 2.3.

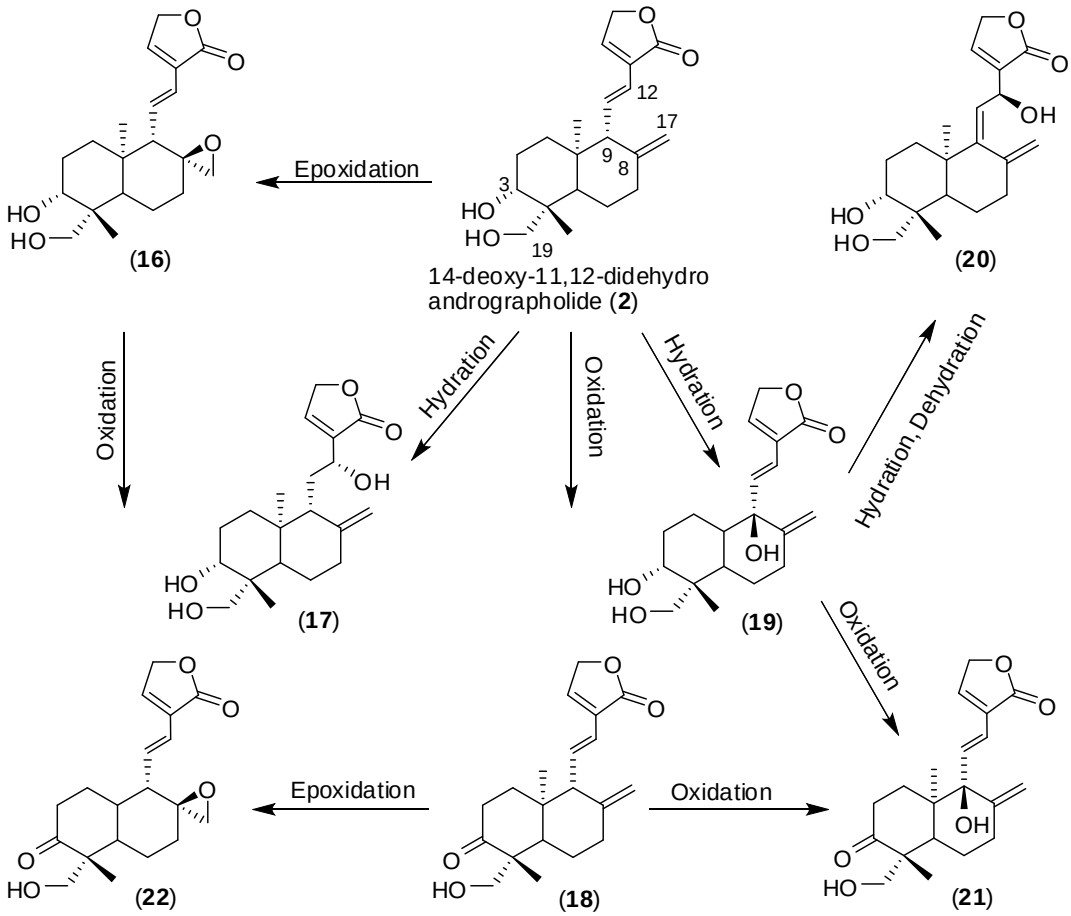
Table 2.3 α-Glucosidase inhibitory activity of 14-deoxy-11,12-didehydroandrographolide analogues.

| % Inhibition activity[a] ($IC_{50}$, μM) | | | |
|---|---|---|---|
| **Compound** | α-Glucosidase | **Compound** | α-Glucosidase |
| (**1**) | ni[b] | (**13h**) | 100 (16) |
| (**2**) | 116.5 | (**13i**) | 59.3 (82) |
| (**12b**) | 17.1 | (**13j**) | 49.6 (100) |
| (**13a**) | ni | (**15a**) | 68.9 (28) |
| (**13b**) | 183.3 (58) | (**15b**) | 100 (16) |
| (**13c**) | ni[b] | (**15c**) | 100 (6) |
| (**13d**) | 157.8 (84) | (**15d**) | 100 (14) |
| (**13e**) | ni | (**15e**) | 74.8 (25) |
| (**13f**) | 16.7 | (**15f**) | 95.9 (36) |
| (**13g**) | 60.6 | (**15g**) | 100 (11) |

Acarbose was taken as positive control. The inhibition percentage of 1 M acarbose was 56.5%
[a] Inhibition (%) determined at 100 μM concentration of compounds.
[b] No inhibition at 100 μM.

Chen et al. (2011) studied the microbial transformations of 14-deoxy-11,12-didehydroandrographolide (**2**) which was performed by *Cunninghamella blakesleana* to give new derivatives as shown in Scheme 2.4. Seven metabolites were obtained and evaluated for inhibitory effects on nitric oxide production in lipopolysaccharide activated macrophages

Metabolite compound (**17**) of 14-deoxy-11,12-didehydroandrographolide (**2**) showed stronger inhibitory effect on nitric oxide production induced by LPS in macrophages than the substrate and the positive drug, hydrocortisone (Table 2.4).




Scheme 2.4 Structures of biotransformed products of 14-deoxy-11,12-didehydro-andrographolide (**2**).

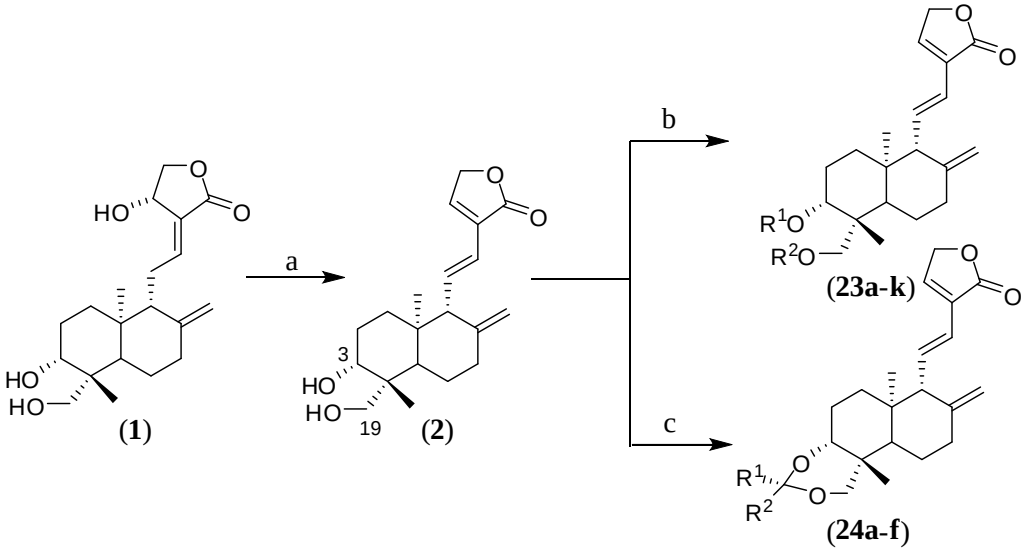
Table 2.4 Inhibitory effects of compounds (**2**) and (**16**-**22**) on NO production induced by LPS in macrophages.[a]

| **Compound** | $IC_{50}$±SD (μM) | **Compound** | $IC_{50}$±SD (μM) |
|---|---|---|---|
| (**2**) | 94.12±4.79 | (**19**) | > 100 |
| (**16**) | > 100 | (**20**) | 19.87±0.88 |
| (**17**) | 5.43±0.65 | (**21**) | 79.66±5.88 |
| (**18**) | 92.14±5.37 | (**22**) | > 100 |
| Hydrocortisone[b] | 40.64±3.22 | | |

[a] NO concentration of control group: 3.96±0.29 μM.
NO concentration of LPS-treated group: 34.78±2.54 μM.
[b] Hydrocortisone was used as positive control.

Recently, Wei et al. (2013) synthesized newly seventeen derivatives of 14-dehydroxy-11,12-didehydroandrographolide (**2**) *via* esterification and etherification. Most derivatives demonstrated significant inhibition against tumor cell growth. 14-Dehydroxy-11,12-didehydroandrographolide (**2**) was prepared from compound (**1**) in high yield.

Compounds (**23a-k**) were obtained by esterification of the two hydroxyl groups at C-3 and C-19 with either aromatic or aliphatic acids. Structurally diverse groups and hetero-atoms were incorporated into the side chains to investigate their effects on cytotoxicity. Additionally, both mono- and di-esters were prepared to further explore the SAR. Compounds (**24a-f**) were prepared *via* acid-catalyzed etherification of compound (**2**) with appropriate aromatic aldehydes. Phenyl rings with electron-withdrawing or electron-donating substituents as well as a furan ring were incorporated in the side chain of the resulting cyclic ether to further probe the structural requirements for derivatives of compound (**2**). The cell growth inhibitory activity data listed in Table 2.5.




Scheme 2.5 Synthesis of compounds (**23**) and (**24**); Reagents and conditions: (a) pyridine, $Al_2O_3$, reflux, 8-10 h, yield 68%; (b) EDCI, DMAP, DCM, rt, yield 20-60; (c) $H_2SO_4$ (0.5%), THF, reflux, 2-5 h, yield 45-85%.

Table 2.5 Cytotoxic activities of compounds (**23**) and (**24**) against tumor cell growth.

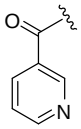
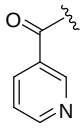
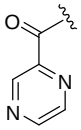
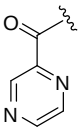
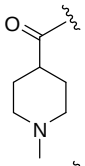
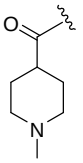
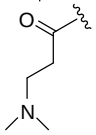
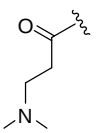
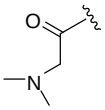
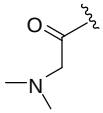
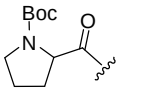
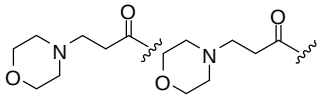
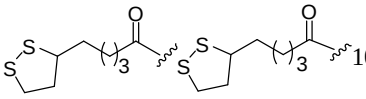
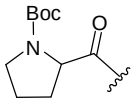
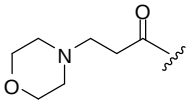
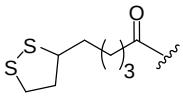
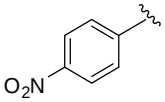
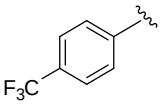
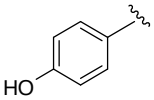
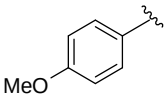
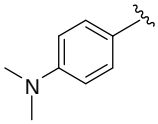
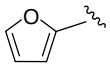
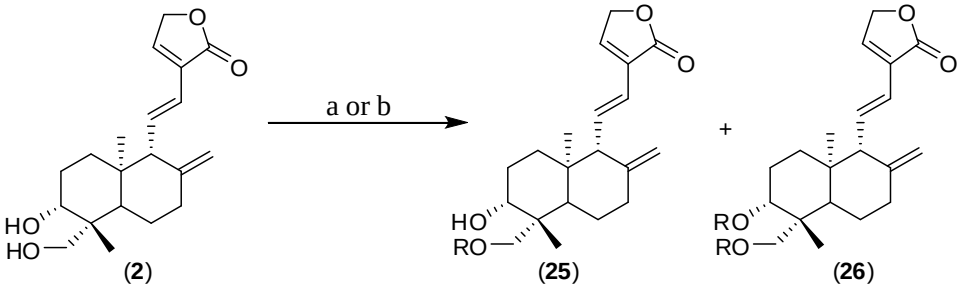
| **Compound** | $R^1$ | $R^2$ | $GI_{50}$ (μM) | | | |
|---|---|---|---|---|---|---|
| | | | A-549 | DU-145 | KB | KB-Vin |
| (**1**) | - | - | 13.37 ± 2.27 | 15.99 ± 2.35 | 13.18 ± 2.06 | 13.82 ± 2.12 |
| (**23a**) |  |  | > 30 | > 30 | >30 | > 30 |
| (**23b**) |  |  | 4.87 ± 0.99 | 8.63 ± 0.83 | 8.24 ± 0.60 | 9.19 ± 0.55 |
| (**23c**) |  |  | 1.46 ± 0.25 | 2.27 ± 0.49 | 2.55 ± 0.27 | 3.00 ± 0.16 |
| (**23d**) |  |  | 31.96 ± 5.91 | 25.29 ± 3.02 | >30 | 31.70 ± 5.22 |
| (**23e**) |  |  | > 30 | > 30 | >30 | > 30 |
| (**23f**) |  | | 10.21 ± 0.26 | 11.07 ± 1.03 | 12.50 ± 2.27 | 11.11 ± 1.50 |
| (**23g**) |  | | > 30 | > 30 | > 30 | > 30 |
| (**23h**) |  | | 10.21 ± 1.18 | 11.81 ± 1.35 | 11.74 ± 1.25 | 12.90 ± 1.81 |
| (**23i**) | H |  | 18.81 ± 0.48 | 18.82 ± 2.59 | 20.11 ± 3.29 | 17.88 ± 2.97 |
| (**23j**) | H |  | >30 | 30.33 ± 5.47 | > 30 | 29.98 ± 4.87 |
| (**23k**) | H |  | >30 | > 30 | > 30 | > 30 |

Table 2.5 Cytotoxic activities of compounds (**23**) and (**24**) against tumor cell growth (continued).

| **Compound** | $R^1$ | $R^2$ | $GI_{50}$ (μM) | | | |
|---|---|---|---|---|---|---|
| | | | A-549 | DU-145 | KB | KB-Vin |
| (**24a**) | H |  | 30.76 ± 4.76 | > 30 | > 30 | > 30 |
| (**24b**) | H |  | 16.86 ± 1.65 | 17.18 ± 4.07 | 23.45 ± 3.24 | 18.31 ± 2.48 |
| (**24c**) | H |  | 22.83 ± 3.55 | 19.11 ± 1.89 | 23.66 ± 2.67 | 22.60 ± 4.14 |
| (**24d**) | H |  | 26.90 ± 1.19 | 32.07 ± 5.84 | 32.89 ± 1.26 | > 30 |
| (**24e**) | H |  | 32.98 ± 4.60 | > 30 | > 30 | > 30 |
| (**24f**) | H |  | 33.98 ± 2.00 | 32.86 ± 0.86 | 36.35 ± 4.82 | 32.71 ± 3.49 |

Compared with the ether derivatives, the ester derivatives exhibited substantially divergent potencies. Most of the new derivatives inhibited the growth of tumor cells, and the most active compounds (**23b**) and (**23c**) showed the best $GI_{50}$ values at the micro molar level against A-549, DU-145, KB and KB-Vin tumor cells.

Recent reports from Chen et al. (2014), Structure-activity relationships and biological evaluation were studied on novel anti-hepatitis B virus agents of 14-deoxy-11,12-didehydroandrographolide and andrographolide derivatives. They synthesized by modifying on rings A, B and C to increase their anti-HBV activity and decrease cytotoxicity. Compound (**2**) was esterified with acids in the presence of 4-dimethylaminopyridine (DMAP) and *N′*,*N′*-dicyclohexylcarbodiimide (DCC) in anhydrous dichloromethane (Scheme 2.6). Generally, 19-*O*-subsituted derivatives were the main products and 3,19-*O*-disubsituted derivatives (**26**) were obtained with low yields at the same time.

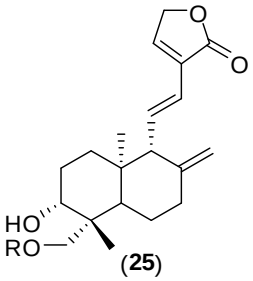



Scheme 2.6 Esterification of compound (**2**); Reagents and conditions: (a) corresponding acids, DMAP, DCC, $CH_2Cl_2$, rt, yield 47-62% for (**25a**-**25i**), yield 26-28% for (**26a**-**26c)**; (b) anhydride, DMAP, anhydrous pyridine, reflux, yield 40% for (**25j**), yield 55-57% for (**26d**-**26g**).

Other acylation products (**25j**), (**26d**-**26g**) were achieved with anhydrides and catalytic amount of DMAP in anhydrous pyridine (Scheme 2.6). Derivatives of 14-deoxy-11,12-didedehydroandrographolide (**2**) were evaluated for their anti-HBV activity, namely inhibiting the secretions of HBsAg, HBeAg, and HBV DNA replication on HepG 2.2.15 cells *in vitro* with tenofovir as the positive control. The anti-HBV activity and cytotoxicity were summarized in Table 2.6.

19-*O*-Cinnamoyl analogue (**25a**) showed high activity against HBV DNA replication with $IC_{50}$ value of 14.6 μM and 50% cytotoxicity concentration ($CC_{50}$) value of 183 μM. After the introduction of methoxyl group into cinnamoyl group at the C-19 position, the cytotoxicity of analogues (**25b**) and (**25c**) decreased obviously ($CC_{50}$ > 1970 and 1706 μM) compared with compound (**25a**) indicating that the methoxyl group play a crucial role in reducing cytotoxicity. The above conclusion was further supported by 19-*O*-(methoxy)nicotinyl analogue (**25f**) with lower cytotoxicity than 19-*O*-nicotinyl analogue (**25e**). Compound (**25c**) with 3,4,5-trimethoxycinnamic group at C-19 possessed noticeable inhibition on HBV DNA replication with the $IC_{50}$ value of 10.3 μM and lower cytotoxicity resulting in a SI value higher than 165.1, demonstrating that both 3-methoxyl and 4-methoxyl groups could enhance activity against HBV DNA replication. However, 19-*O*-(3-chloro) cinnamoyl analogue (**25d**) exhibited higher cytotoxicity and weaker activity due to the introduction of halogen into the substituent. The heteroatomic rings at C-19 position play an important role in suppressing HBV DNA replication and secretions of HBsAg and HBeAg in agreement with the high activities of compounds (**25e**), (**25g**) and (**25h**) with the $IC_{50}$ values of 22.1, 9.3 and 22.1 μM. In contrast to 19-*O*-substituted analogues, the 3,19-*O*-disubstituted compounds (**26a**-**26g**) showed dramatic decrease of anti-HBV activity resulting from the disubstituents at C-3 and C-19.

Table 2.6 Anti-HBV activity and cytotoxicity of 14-deoxy-11,12-didehydroandrographolide derivatives *in vitro*[a].



(**25**)

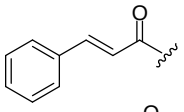
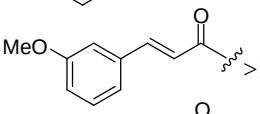
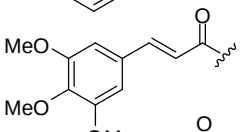
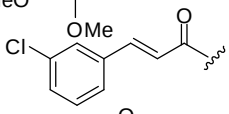
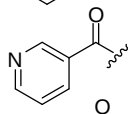
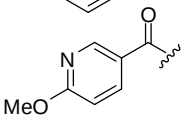
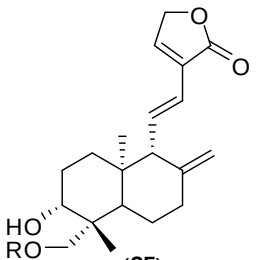
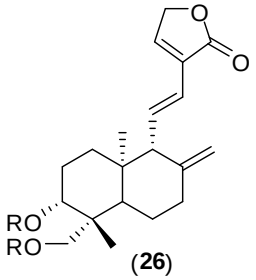
| **Compound** | R | $CC_{50}$[b] | HBsAg[c] | | HBeAg[d] | | DNA replication | |
|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$[e] (μM) | SI[f] | $IC_{50}$[e] (μM) | SI[f] | $IC_{50}$[e] (μM) | SI[f] |
| (**1**) | | 197 | -[g] | - | - | - | 22.6 | 8.7 |
| (**2**) | | 198 | - | - | - | - | 54.1 | 3.7 |
| (**25a**) |  | 183 | 18.0 | 10.2 | 28.9 | 6.3 | 14.6 | 15.5 |
| (**25b**) |  | >1970 | >1970 | - | 1476 | - | >513 | - |
| (**25c**) |  | >1706 | - | - | 201 | >8.5 | 10.3 | >165.1 |
| (**25d**) |  | >147 | 207 | - | 59.8 | 2.5 | 136 | 5.5 |
| (**25e**) |  | 113 | 40.0 | 2.8 | 63.5 | 1.8 | 22.1 | 5.1 |
| (**25f**) |  | 1339 | >2313 | - | 217 | 6.2 | >584 | - |

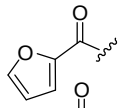
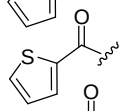
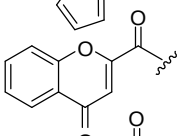
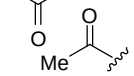
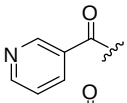
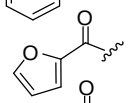
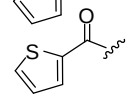
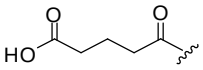
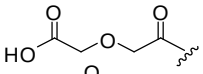
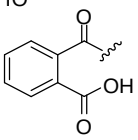
Table 2.6 Anti-HBV activity and cytotoxicity of 14-deoxy-11,12-didehydroandrogra- pholide derivatives *in vitro*[a] (continued).



(**25**)

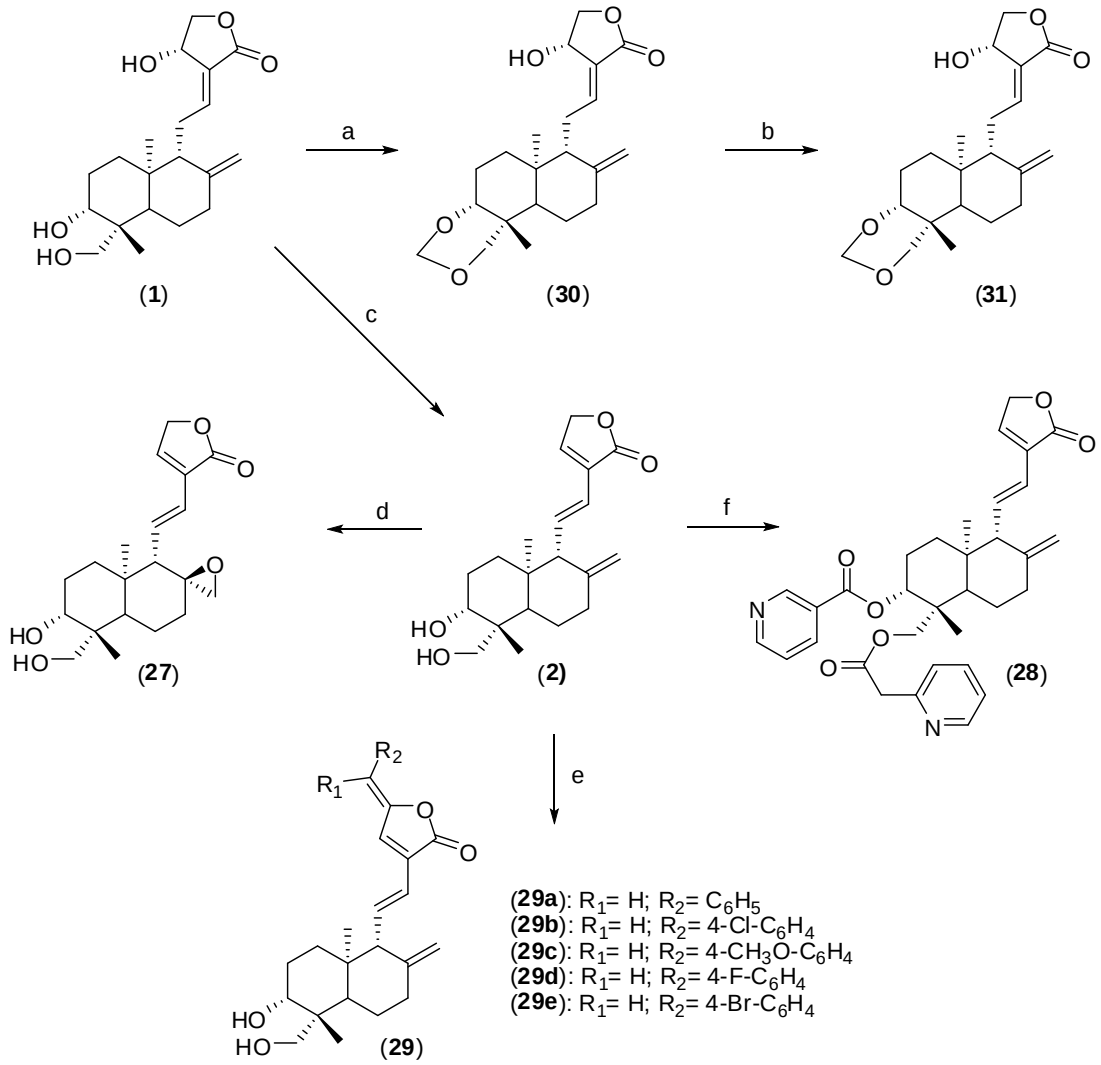


(**26**)

| **Compound** | R | $CC_{50}$[b] | HBsAg[c] | | HBeAg[d] | | DNA replication | |
|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$[e] (μM) | SI[f] | $IC_{50}$[e] (μM) | SI[f] | $IC_{50}$[e] (μM) | SI[f] |
| (**25g**) |  | 92 | 19.2 | 4.6 | 77.5 | 1.2 | 9.3 | 9.9 |
| (**25h**) |  | 557 | 162 | 3.4 | 29.2 | 19.1 | 22.1 | 25.2 |
| (**25i**) |  | 171 | - | - | 177 | 1.0 | 36.6 | 4.7 |
| (**25j**) |  | 225 | 565 | - | 2233 | - | 69.5 | 3.2 |
| (**26a**) |  | >1919 | >1919 | - | >1919 | - | >664 | - |
| (**26b**) |  | 1112 | 1407 | - | 1864 | - | 242 | 4.6 |
| (**26c**) |  | >1537 | 1448 | >1.0 | 822 | >1.8 | 185 | >8.3 |
| (**26d**) |  | 757 | 1010 | - | 1390 | - | 455 | - |
| (**26e**) |  | >1714 | >1714 | - | >1714 | - | >429 | - |
| (**26f**) |  | >1933 | >1933 | - | >1933 | - | >483 | - |
| (**26g**) |  | >1624 | - | - | - | - | 104 | >15.6 |

[a] Values are means of two independent experiments.
[b] $CC_{50}$ is 50% cytotoxicity concentration in HepG 2.2.15 cells.
[c] HBsAg: hepatitis B surface antigen.
[d] HBeAg, hepatitis B e antigen.
[e] $IC_{50}$ is 50% inhibitory concentration.
[f] SI (selectivity index) = $CC_{50}/IC_{50}$.
[g] No SI can be obtained.

Another interesting research from Wu et al. (2013) to identify potent inhibitors against tumor-cell migration and invasion was reported for the structure-activity relationships of andrographolide (**1**) and its derivatives shown in Scheme 2.7.



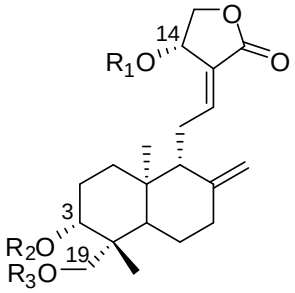

Scheme 2.7 Synthesis of andrographolide derivatives; Reagents and conditions: (a) THF, $H_2SO_4$, paraform, refluxing, 1 h; (b) *m*-CPBA, $CHCl_3$, refluxing, 2 h; (c) pyridine, $Al_2O_3$, refluxing, 6 h; (d) (i) acetone, *p*-TsOH, 2,2-dimethoxy propane, refluxing, 10 h, (ii) *m*-CPBA, $CHCl_3$, refluxing, 2 h; (e) aldehydes, $Na_2CO_3$, methanol, refluxing, 3-5 h; (f) $CHCl_3$, nicotinic chloride, $Et_3N$, refluxing, 3 h.

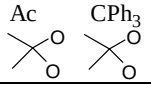
The results showed that andrographolide (**1**) exhibited moderate inhibitory activity against cell migration in A-549, reduced SGC-7901-cell migration and invasion. The inhibitory activities of 15-benzylidene substitution derivatives (**29a**-**29d**) against migration of various tumor cells were greatly enhanced. Compounds (**29a**-**29d**) were more effective against all tested cell lines compared to androgra- pholide (**1**) and 14-deoxy-11,12-didehydroandrographolide (**2**). Analyses of structure-activity relationships suggested that

unfavorable biological activity against migration results when the hydroxyl groups at C-3 and C-19 of compound (**1**) or compound (**2**) were coupled or converted to esters or the C-8 and C-17 double-bond was converted to an epoxide. On the contrary, benzylidene substitution at C-15 of compound (**2**) was an advantage.

In 2012, Sirion et al. (2012) reported the synthesis and *in vitro* cytotoxicity of andrographolide derivatives. They investigated the structure-activity relationships (SARs) of C-19 andrographolide analogues (**30**-**48**) which were synthesized by modification of the three hydroxyl groups in andrographolide (**1**) to a series of acetyl, silyl and triphenylmethyl ether groups at the C-19 position, and acetyl groups at the C-3 and C-14 positions. A number of the andrographolide analogues showed much higher cytotoxic activities than that of the parent compound on cancer cells including P-388, KB, COL-2, MCF-7, LU-1 and ASK cells. SAR studies of the synthetic analogues indicated that the introduction of silyl ether or triphenylmethyl ether group into C-19 of the parent compound led to increase in toxicity against the cancer cells. The 19-*O*-triphenylmethyl ether analogue (**46**) showed higher cytotoxic activity than the potent anti-cancer drug ellipticine, and this analogue may serve as a potential structure lead for the development of new anticancer drugs as shown in Table 2.7.

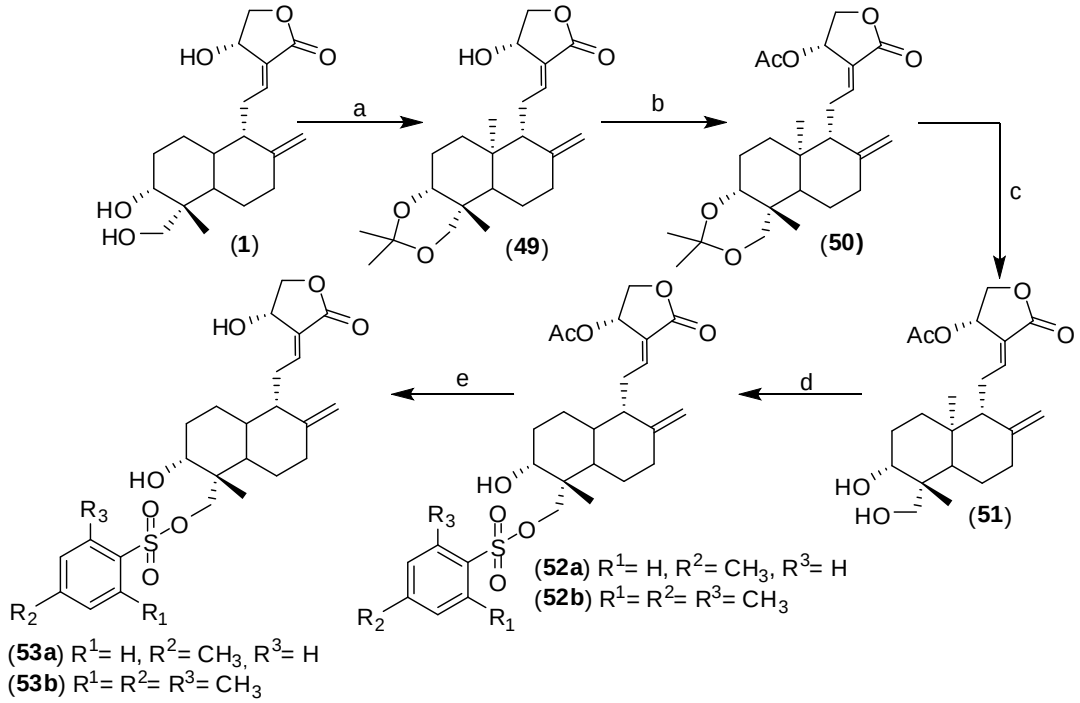
Table 2.7 Cytotoxic activity against six human cancer cells




| Compound | | | | ED$_{50}$ (μM)[a] (SRB assay) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R$^1$ | R$^2$ | R$^3$ | P-388 | KB | COL-2 | MCF-7 | LU-1 | ASK |
| (**1**) | H | H | H | 2.25 | 27.37 | 13.60 | 15.40 | 12.98 | 16.18 |
| (**30**) | H | H | TBS | 8.39 | 40.71 | 17.10 | 46.96 | 48.81 | 15.51 |
| (**31**) | H | Ac | TBS | 1.71 | 11.15 | 11.31 | 9.79 | 9.62 | 8.17 |
| (**32**) | Ac | H | TBS | 1.03 | 10.74 | 8.94 | 10.44 | 10.44 | 3.41 |
| (**33**) | Ac | Ac | TBS | 0.34 | 3.62 | 2.23 | 2.84 | 2.94 | 1.22 |
| (**34**) | H | H | TIPS | 0.88 | 2.73 | 2.73 | 2.88 | 3.01 | 2.85 |
| (**35**) | Ac | Ac | TIPS | 1.41 | 7.39 | 13.59 | 13.95 | 18.79 | 7.56 |
| (**36**) | H | H | TBDPS | 0.68 | 11.13 | 9.65 | 8.15 | 11.47 | 5.54 |
| (**37**) | H | Ac | TBDPS | 0.50 | 9.86 | 3.80 | 7.53 | 10.93 | 10.47 |
| (**38**) | Ac | H | TBDPS | 0.33 | 10.54 | 10.70 | 9.63 | 11.86 | 11.96 |
| (**39**) | Ac | Ac | TBDPS | 1.67 | 29.04 | 12.28 | 45.70 | 52.92 | 32.81 |
| (**40**) | H | Ac | H | 4.78 | 35.65 | 27.21 | 26.43 | 30.59 | 58.13 |
| (**41**) | H | H | Ac | 2.04 | 15.26 | 12.94 | 18.25 | 13.99 | 16.71 |
| (**42**) | H | Ac | Ac | 2.65 | 35.22 | 15.26 | 19.69 | 34.72 | 22.39 |
| (**43**) | Ac | H | Ac | 1.62 | 26.38 | 15.23 | 18.47 | 19.77 | 16.31 |
| (**44**) | Ac | Ac | Ac | 1.40 | 11.62 | 10.82 | 10.02 | 12.41 | 11.41 |
| (**45**) | Ac | Ac | H | 0.69 | 11.66 | 10.16 | 10.95 | 8.75 | 11.48 |
| (**46**) | H | H | CPh$_3$ | 0.45 | 2.42 | 2.73 | 2.72 | 0.88 | 2.86 |
| (**47**) | Ac | Ac | CPh$_3$ | 1.92 | 9.77 | 22.79 | 22.68 | 8.37 | 15.29 |
| (**48**) | H |  | | 3.78 | 66.45 | 39.80 | 42.80 | 52.98 | 52.22 |
| Ellipticine | | | | 2.44 | 2.46 | 2.72 | 2.71 | 1.62 | 3.56 |

[a] Cell lines used are P-388 (murine leukaemia cell line); KB (human epidermoid carcinoma of the mouth); COL-2 (human colon cancer); MCF-7 (human breast cancer); LU-1(human lung cancer); and ASK (rat glioma). Ellipticine (Ellipt) was used as a positive control. The results were expressed as ED$_{50}$ values (drug concentration causing 50% growth inhibition) in μM.

Pandeti, Sonkar, Shukla, Bhatia and Tadigoppula (2013) reported the synthesis of new andrographolide derivatives (**49-61**) and evaluated for their anti-dyslipidemic, LDL-oxidation and anti-oxidant activities. Initially, the hydroxyl groups at C-3 and C-19 of compound (**1**) were protected as an isopropylidene to give compound (**49**) and acetylation of hydroxyl at C-14 gave compound (**50**). Hydrolysis of compound (**50**) obtained 14-acetylandrographolide (**51**) using 1N HCl followed by sulfonylation reaction of compound (**51**) to generate derivatives (**52a**) and (**52b**) and subsequently deacetylation with NaOMe gave compounds (**53a**) and (**53b**) respectively (Scheme 2.8).

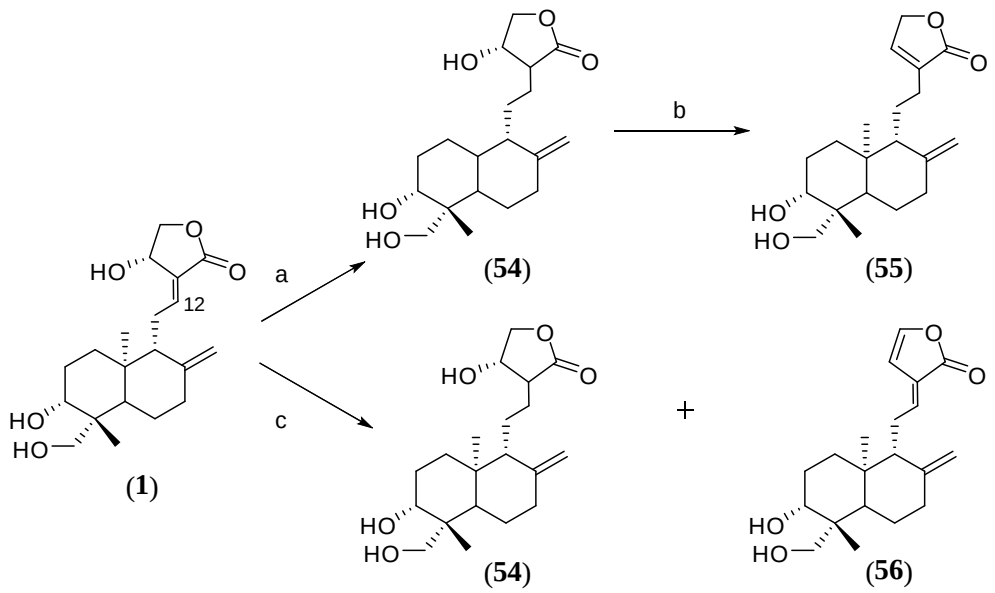



Scheme 2.8 Synthesis of sulfonyl derivatives (**49**-**53**) of andrographolide (**1**); Reagents and conditions: (a) $I_2$, acetone, rt, 2 h, yield 90%; (b) $Ac_2O$, pyridine, rt, 1 h, yield 98%; (c) 1N HCl, THF, T = 20 $^oC$, 2 h, yield 95%; (d) Tosyl chloride/2-mesitylene sulfonyl chloride, pyridine, 60-70 $^oC$, 4 h, yield 70%; (e) NaOMe, MeOH, rt, 1 h, yield 85%.

Reduction of alkene moiety at C-12 followed by dehydration of allylic hydroxyl at C-14 of andrographolide (**1**) gave compounds (**54**) and (**55**) respectively. Compound (**56**) was synthesized from compound (**1**) using $NaBH_4$ and Amberlyst-15 as shown in Scheme 2.9.

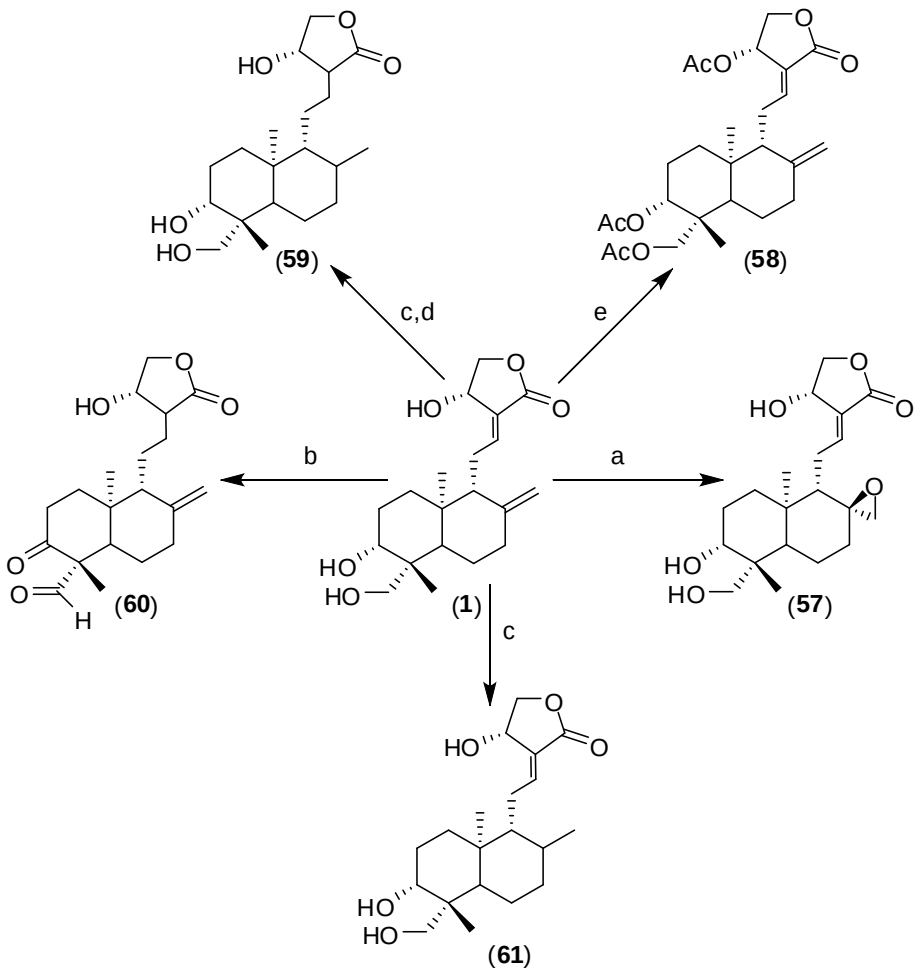
Selective epoxidation of the exocyclic double bond of compound (**1**) with *m*-CPBA led to compound (**57**). Oxidation of the C-3 and C-19 hydroxyl groups of compound (**1**) with DMP yielded dicarbonyl compound (**60**). To understand the role of the two olefin bonds of compound (**1**), compounds (**59**) and compound (**61**) were prepared by selectively reducing the double bond in Scheme 2.10.

The parent compound, andrographolide (**1**) is causing a decrease in plasma levels of TC, PL and TG by 27%, 26% and 28% respectively followed 16% increase in PHLA level as compared to triton induced rats. Among these derivatives (**49**-**61**), the sulfonyl derivative (**53a**) turned out to be most potent lipid lowering agent, causing a decrease in plasma levels.



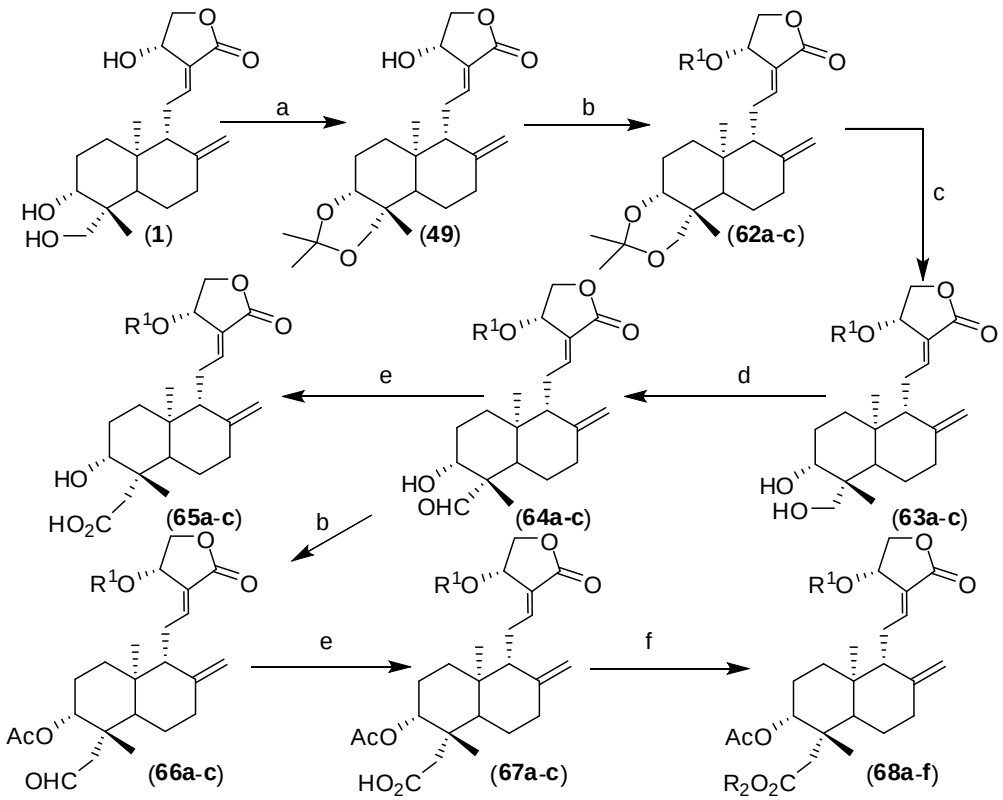

Scheme 2.9 Hydrogenation of andrographolide (**1**); Reagents and conditions: (a) $NaBH_4$, $NiCl_2.6H_2O$, MeOH, rt, 30 min, yield 90%; (b) $Na_2CO_3$, MeOH, 50 $^oC$, 2 h, yield 80%; (c) $NaBH_4$, Amberlyst-15, THF, rt, 2 h, yield 75:25.

Chen, Song, Lu and Xue (2013) reported the synthesis of twelve andrographolide-19-oic acid analogues (**62-68**) and evaluation for their *in vitro* anti-tumor activities against human cancer cell lines HCT-116 and MCF-7. C-19-Hydroxyl group of andrographolide (**1**) was oxidized to various carboxyl groups (Scheme 2.11).




Scheme 2.10 Acetylation, hydrogenation, oxidation and epoxidation of andrographolide (**1**); Reagents and conditions: (a) *m*-CPBA, MeOH, rt, 12 h, yield 80%; (b) DMP, DCM, 0 $^{o}$C, 30 min, yield 70%; (c) 10% Pd/C, dry MeOH, rt, 5 h, yield 90%; (d) $NaBH_4$, $NiCl_2.6H_2O$, MeOH, rt, 30 min, yield 90% (e) $Ac_2O$, pyridine, 60-70 $^{o}$C, 4 h, yield 98%.

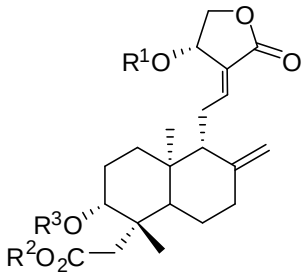
The preliminary results indicated that the oxidation of C-19-hydroxyl of the parent compound (**1**) to carboxylic acid group, especially the subsequent etherification of the formed carboxylic acid led to a big increase in activity against the tested cancer cells.




Scheme 2.11 Synthesis of andrographolide-19-oic acid analogues. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH (cat.), Tol/DMSO, 80 $^{o}$C, 1.5 h, yield 93%; (b) for (**62a**): $Ac_2O$, reflux 1.5 h; for (**62b)**: BzCl, TEA, DMAP, $CH_2Cl_2$, rt, 4 h; for (**62c**): *p*-methoxycinnamic acid/$Tf_2O$, TEA,$CH_2Cl_2$, 0 $^{o}$C; (c) HOAc:$H_2O$ (7:3), rt, yield 42-85% for two-steps; (d) TEMPO, TBAI, NCS, $CH_2Cl_2$, $K_2CO_3$-$KHCO_3$, buffer, 0 $^{o}$C, 9 h, yield 83-91%; (e) $NaClO_2$-$NaH_2PO_4$, isopantene, *t*-BuOH, DMSO, 0 $^{o}$C to rt, 48 h, yield 42-59%, (f) for (**68a**), (**68c**) and (**68e**): MeI, $K_2CO_3$, DMF, rt, 1 h, yield 60-62%; for (**68b**), (**68d**) and (**68f**): BnBr, $K_2CO_3$, DMF, rt, 1 h, yield 49-55%.

Most compounds were found to exhibit significant cytotoxicity, better than andrographolide (**1**), compounds (**68d**) and (**68b**) were identified as the most potent with $IC_{50}$ values of 1.18 and 6.28 µM against HCT-116 and MCF-7 cell lines, respectively. The compound (**68d**) showed 11-folds higher cytotoxic activity against HCT-116 than the anti-cancer drug *cis*-platin.

Table 2.8 Evaluation of *in vitro* cell growth inhibitory effects of andrographolide analogues against two cell lines.

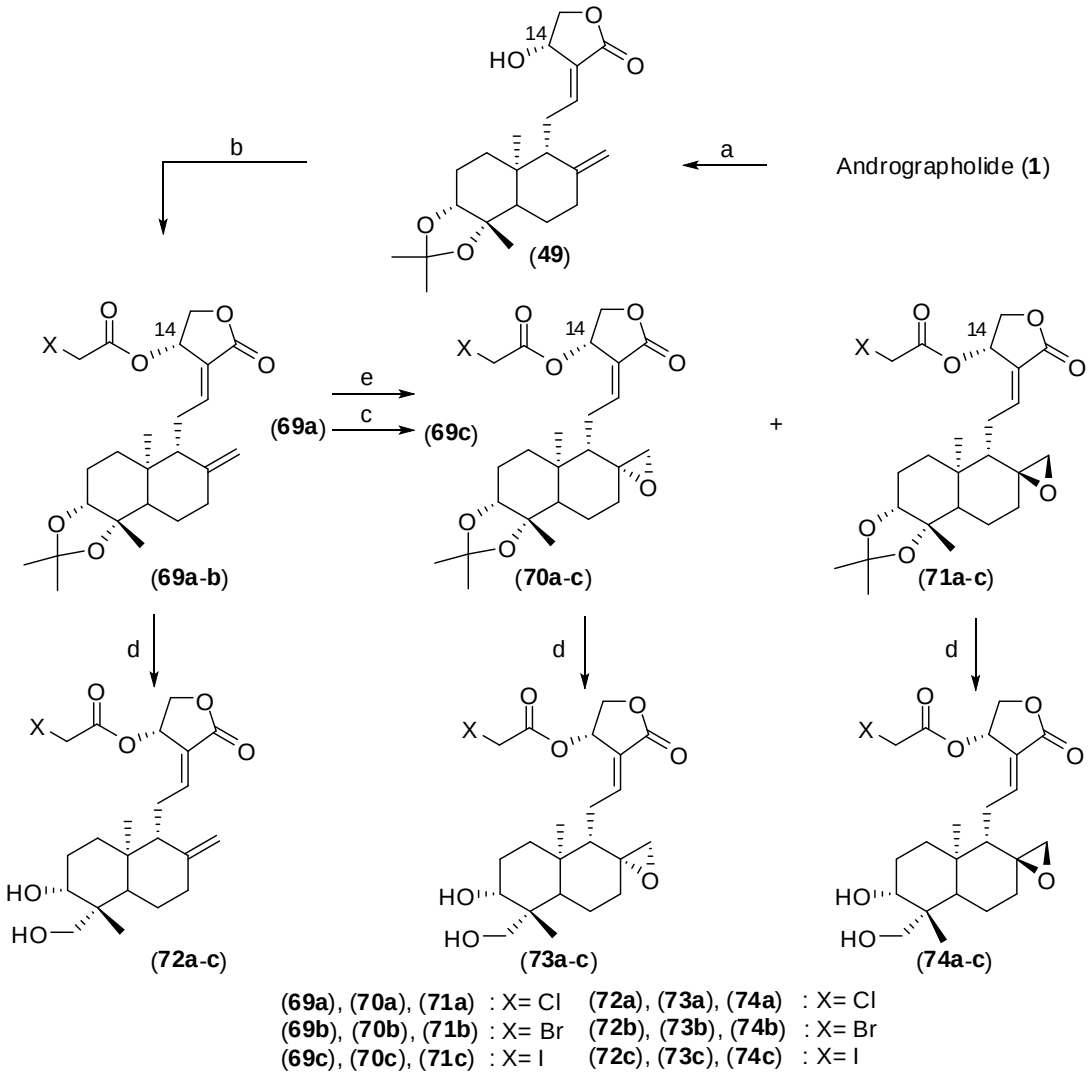



| Compound | Cell growth inhibition in terms of $IC_{50}$ (μM)[a] | | | | |
|---|---|---|---|---|---|
| | $R^1$ | $R^2$ | $R^3$ | HCT-116 | MCF-7 |
| (**65a**) | Ac | H | H | 29.24 | 35.66 |
| (**65b**) | Bz | H | H | 36.85 | 40.20 |
| (**65c**) | *p*-MC[b] | H | H | 32.39 | 95.00 |
| (**67a**) | Ac | H | Ac | 20.90 | >100 |
| (**67b**) | Bz | H | Ac | 17.81 | 44.57 |
| (**67c**) | *p*-MC | H | Ac | 9.73 | 43.31 |
| (**68a**) | Ac | Me | Ac | 2.29 | 11.80 |
| (**68b**) | Ac | Bn | Ac | 2.48 | 6.28 |
| (**68c**) | Bz | Me | Ac | 3.01 | 10.62 |
| (**68d**) | Bz | Bn | Ac | 1.18 | 8.37 |
| (**68e**) | *p*-MC | Me | Ac | 3.01 | 19.73 |
| (**68f**) | *p*-MC | Bn | Ac | 3.00 | 8.92 |
| (**1**) | | | | 24.91 | >100 |
| *cis*-Platin[c] | | | | 13.36 | 89.83 |

[a] $IC_{50}$ was expressed as an average value of two experiments.
[b] *p*-MC = *p*-methoxycinnamoyl.
[c] Positive control.

In later years, Preet et al. (2014) reported the synthesis and biological of andrographolide analogues (**69-74**) as anti-cancer agent. A new family of C-14-ester analogs of andrographolide and their α and β diastereomeric epoxy derivatives were synthesized (Scheme 2.11) and screened *in vitro* against kidney (HEK-293) and breast cancer cells (MCF-7) and compared with the results in corresponding normal (VERO and MCF-10A) cells.

The anti-cancer effects of the active analogues in Scheme 2.11; compounds (**73b**), (**73c**) and (**75c**) were determined by multiple cell based assays such as MTT, immunostaining, FACS, western blotting and transcriptional inhibition of NF-*k*B activity. Importantly, these compounds were found to possess higher anti-cancer potency than andrographolide (**1**) and low toxicity to normal (VERO and MCF-10A) cells.




Scheme 2.12 Synthesis of andrographolide analogues (**69-74**). Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH (cat.), acetone, reflux, 2 h, yield 70%; (b) chloroacetyl chloride or bromoacetyl bromide, pyridine, DMAP(cat.), dry THF, 0 $^{o}$C, 30 min, yield 66% for (**69a**), yield 63% for (**69b**); (c) NaI, acetone, rt, 3 h, yield 65%; (d) acetic acid:water (7:3), rt, 30 min, yield 67-75%; (e) *m*-CPBA, 0 $^{o}$C, DCM, 3 h, yield 51-54%.

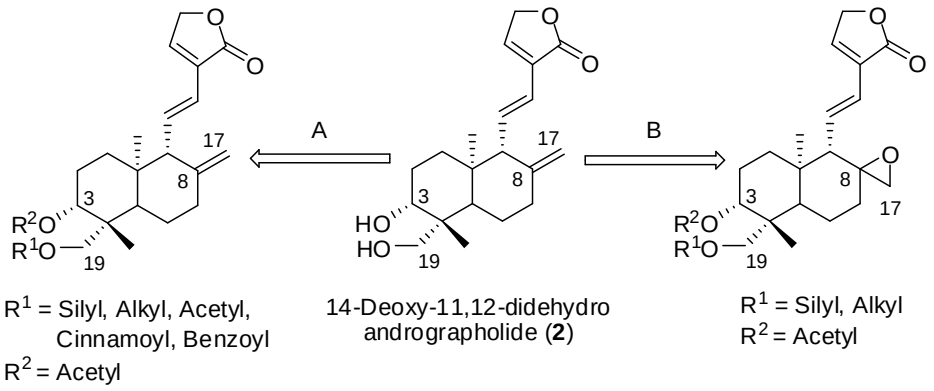
# CHAPTER 3
# RESEARCH METHODOLOGY

## 3.1 General Methods

All chemicals were purchased from commercial sources and used without further purification. Proton NMR spectra were recorded on a BRUKER AVANCE (400 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as an internal standard. Data are reported as follows; chemical shift (multiplicity, integrate intensity or assignment, coupling constants in Hz, assignment). Carbon NMR spectra were recorded on a BRUKER AVANCE (100 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to $CDCl_3$ (δ 77.0) as internal standard. High-resolution mass spectra (HRMS) data were obtained with a Finnigan MAT 95. Infrared spectra (IR) were determined on a PERKIN ELMER FT/IR-2000S spectrophotometer and are reported in wave number ($cm^{-1}$). Analytical thin-layer chromatrography (TLC) was conducted on precoated TLC plates; silica gel 60F-254 (E. Merck, Darmstadt, Germany). Silica gel columns for open-column chromatrography utilized silica gel 60 (Grade 7729, < 230 mesh, 7734, 70-230 mesh and 9385, 230-400 mesh, E. Merck, Darmstadt, Germany). Melting points were recorded using GALLENKAMP Melting point apparatus Griffin.

## 3.2 Methodology for modification of 14-deoxy-11,12-didehydroandrographolide (2)

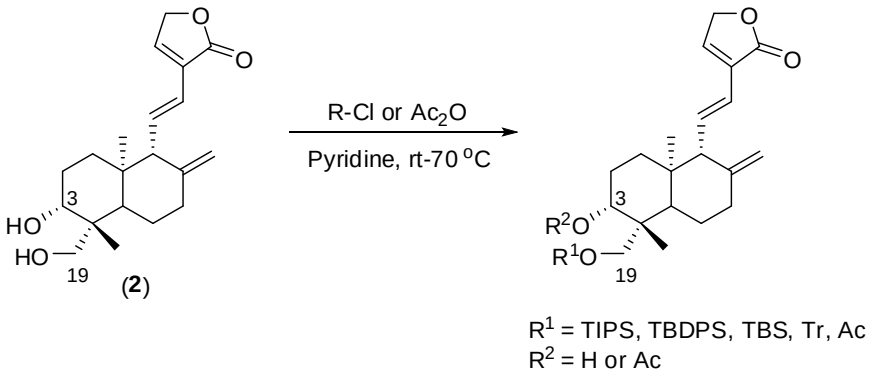
In this research, we synthesized the new analogues of 14-deoxy-11,12-didehydrographolide (**2**) and screened for their cytotoxic activity *in vitro*. Initially, the structural modification was desired by conversion of the two hydroxyl groups of compound (**2**) at C-3 and C-19 with various silyl, alkyl ethers and acetyl, cinnamoyl, benzoyl esters (part A). The *exo*-methylene moieties were also modified by epoxidation at C-8 using facile route to obtain the new derivatives (part B) as shown in Scheme 3.1.




Scheme 3.1 The synthetic plan for modification of 14-deoxy-11,12-didehydroandrographolide (**2**).

### 3.2.1 Modification of two hydroxyl groups at C-3 and C-19 of 14-deoxy-11,12-didehydroandrographolide

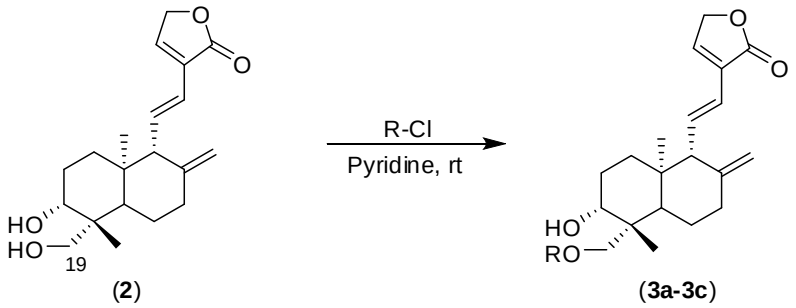
The structure of 14-deoxy-11,12-didehydroandrographolide (**2**) was modified by conversion of the hydroxyl group at C-19 position with silylation or alkylation and acetylation at C-3 position to obtain the new derivatives of 14-deoxy-11,12-didehydroandrographolide (**2**) as shown in Scheme 3.2.




Scheme 3.2 The synthesis of C-19 and C-3 substituted of 14-deoxy-11,12-didehydroandrographolide (**2**).

### 3.2.1.1 Silylation reactions of 14-deoxy-11,12-didehydroandrogra- pholide (2)

**General procedure A**




(**2**) (**3a-3c**)

R = TIPS, TBDPS, TBS

To a stirred solution of 14-deoxy-11,12-didehydroandrographolide (**2**) in pyridine was added trialkyl-silylchloride (TIPS-Cl, TBDPS-Cl or TBS-Cl) at room temperature. After the stirring was continued at room temperature until the TLC showed complete conversion, the reaction mixture was diluted with EtOAc and quenched with satd. $NH_4Cl$ and the mixture was extracted with EtOAc (x3). The combined organic layer was washed with brine (10 mL), dried over $Na_2SO_4$ anhydrous and then concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) to give (**3a**-**3c**).

#### 3.2.1.1.1 Synthesis of 19-*O*-TIPS-14-deoxy-11,12-didehydroandro-grapholide (3a)

Following the **general procedure A**, a mixture of 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1007 g, 0.303 mmol) in pyridine was added triisopropylsilyl chloride (TIPS-Cl) (0.30 mL, 1.400 mmol) at room temperature. After stirring was continued for 4.0 h, the residue was purified by column chromatography to afford 19-*O*-TIPS-14-deoxy-11,12-didehydroandrographolide (**3a**) in 63% yield (0.0932 g) as a pale yellow oil.

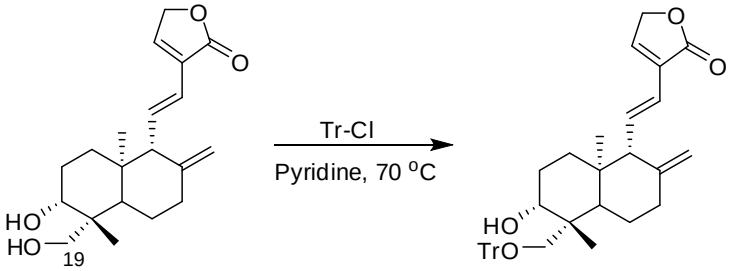
#### 3.2.1.1.2 Synthesis of 19-*O*-TBDPS-14-deoxy-11,12-didehydro-andrographolide (3b)

Following the **general procedure A**, a mixture of 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1010 g, 0.303 mmol) in pyridine was added *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) (0.40 mL, 1.560 mmol) at room temperature. After stirring was continued for 1.0 h, the residue was purified by column chromatography to afford 19-*O*-TBDPS-14-deoxy-11,12-didehydroandrographolide (**3b**) in 70% yield (0.1212 g) as a white solid.

#### 3.2.1.1.3 Synthesis of 19-*O*-TBS-14-deoxy-11,12-didehydroandro-grapholide (3c)

Following the **general procedure A**, a mixture of 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1036 g, 0.312 mmol) in pyridine was added *tert*-butyldimethylsilyl (TBS-Cl) (0.2378 g, 1.578 mmol) at room temperature. After stirring was continued at for 1.0 h, the residue was purified by column chromatography to afford 19-*O*-TBS-14-deoxy-11,12-didehydroandrographolide (**3c**) in 79% yield (0.1099 g) as a white solid.

### 3.2.1.2 Alkylation reaction of 14-deoxy-11,12-didehydroandrogra- pholide (2) with triphenylmethyl chloride

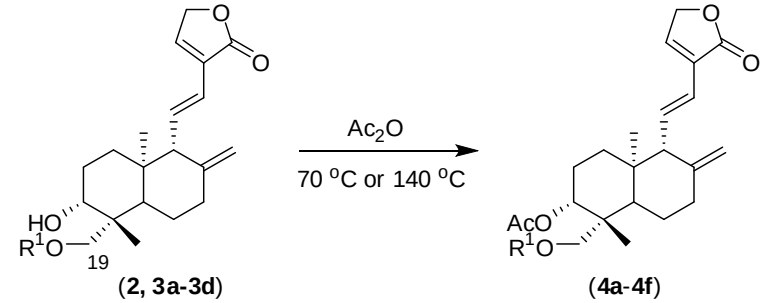



(**2**) (**3d**)

To a stirred solution of 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1018 g, 0.306 mmol) in pyridine was added triphenylmethyl chloride (Tr-Cl) (0.3032 g, 1.088 mmol), then the reaction mixture was heated to 70 °C and continued stirring at 70 °C for 1.0 h. The reaction mixture was quenched by addition to EtOAc and satd. $NH_4Cl$. The reaction mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography to give 19-*O*-Tr-14-deoxy-11,12-didehydroandrographolide (**3d**) in 99% yield (0.1748 g) as a white solid.

### 3.2.1.3 Acetylation reaction of 14-deoxy-11,12-didehydroandrogra- pholide derivatives

**General procedure B**




(**2, 3a-3d**) (**4a-4f**)

$R^1$= H, TIPS, TBDPS, TBS, Tr

$R^1$ = TIPS, TBDPS, TBS, Tr, Ac
$R^2$ = H, Ac

A stirred solution of 14-deoxy-11,12-didehydroandrographolide (**2**) or analogues (**3a**-**3d**) in acetic anhydride was heated to 70 $^{o}$C or 140 $^{o}$C. After the TLC showed completed reaction, the mixture was diluted with EtOAc and quenched with satd. $NaHCO_3$. The mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) to afford the corresponding products (**4a**-**4f**).

**3.2.1.3.1 Acetylation reaction of 19-*O*-substituted-14-deoxy-11,12-didehydroandrographolide (3a-3d)**

**3.2.1.3.1.1 Acetylation reaction of 19-*O*-TIPS-14-deoxy-11,12-didehydroandrographolide (3a)**

Following the **general procedure B**, a stirred solution of compound (**3a**) (0.0504 g, 0.103 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TIPS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**4a**) in 64% yield (0.0350 g) as a yellow oil.

**3.2.1.3.1.2 Acetylation reaction of 19-*O*-TBDPS-14-deoxy-11,12-didehydroandrographolide (3b)**

Following the **general procedure B**, a stirred solution of compound (**3b**) (0.0490 g, 0.086 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TBDPS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**4b**) in 95% yield (0.0502 g) as a white solid.

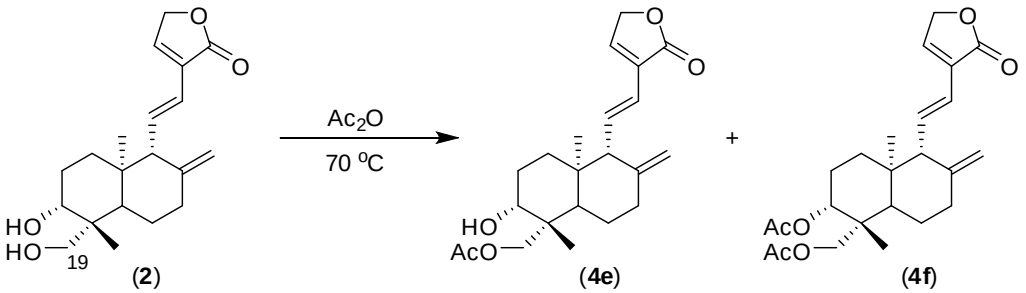
**3.2.1.3.1.3 Acetylation reaction of 19-*O*-TBS-14-deoxy-11,12-didehydroandrographolide (3c)**

Following the **general procedure B**, a stirred solution of compound (**3c**) (0.0418 g, 0.094 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TBS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**4c**) in 76% yield (0.0346 g) as a white solid.

**3.2.1.3.1.4 Acetylation reaction of 19-*O*-Tr-14-deoxy-11,12-dide-hydroandrographolide (3d)**

Following the **general procedure B**, a stirred solution of compound (**3d**) (0.0600 g, 0.104 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-Tr-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**4d**) in 75% yield (0.0486 g) as a white solid.

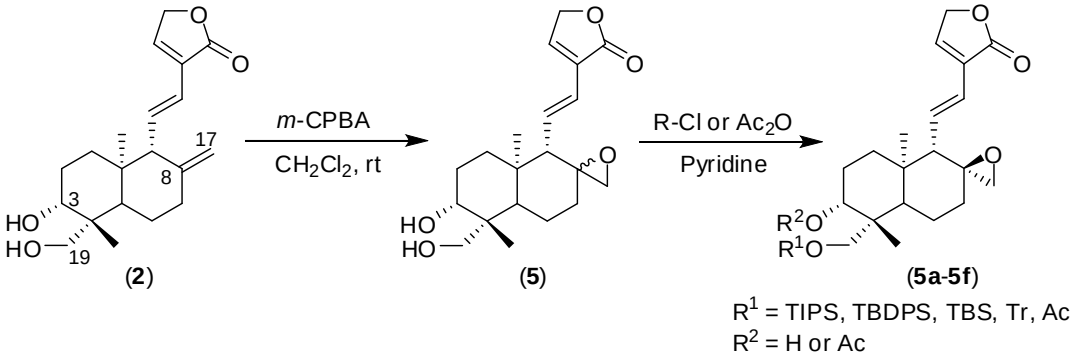
### 3.2.1.3.2 Acetylation reaction of 14-deoxy-11,12-didehydroandro-grapholide (2)




Following the **general procedure B**, 14-deoxy-11,12-didehydroandro- grapholide (**2**) (0.1608 g, 0.484 mmol) in acetic anhydride was heated at 70 $^{o}$C with stirring for 4.5 h. The residue was purified by column chromatography to give 19-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**4e**) in 59% yield (0.1076 g) as a pale yellow oil and 3,19-*O*-diacetyl-14-deoxy-11,12-didehydroandrographolide (**4f**) in 34% yield (0.0692 g) as a white solid.

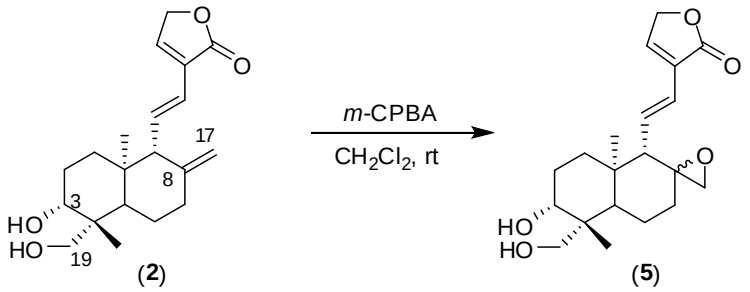
### 3.2.2 Modification of the *exo*-alkene group at C-8 of 14-deoxy-11,12-didehydroandrographolide (2)

The structure of 14-deoxy-11,12-didehydroandrographolide (**2**) was modified by conversion of the *exo*-alkene at C-8 to epoxide followed by modification of two hydroxyl groups at C-3 and C-19 to silyl ether, ether and acetyl in Scheme 3.3.




Scheme 3.3 The synthesis of *exo*-alkene group at C-8 and C-19 silyl ether or ether or ester 14-deoxy-11,12-didehydroandrographolide (**5a**-**5f**).

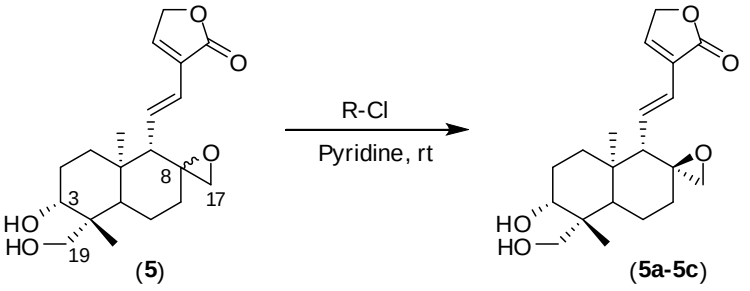
### 3.2.2.1 Epoxidation the *exo*-alkene group at C-8 of 14-deoxy-11,12-didehydroandrographolide (2)




To a stirred solution of 14-deoxy-11,12-didehydroandrographolide (**2**) (0.3137 g, 0.944 mmol) in $CH_2Cl_2$ was added *meta*-chloroperoxybenzoic acid (*m*-CPBA) (0.3257 g, 1.887 mmol) at room temperature. After stirring was continued for 1.0 h, the reaction mixture was diluted with EtOAc and quenched with satd. $NaHCO_3$. The mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous and then concentrated under reduced pressure. The residue was purified by column chromatography to give a mixture of isomer (**5**) in 99 % yield (0.5188 g) as white solid (the ratio of α- and β- isomer was not identified).

### 3.2.2.2 Silylation reaction of 8,17-epoxy-14-deoxy-11,12-didehydro-andrographolide (5)

**General procedure C**




To a stirred solution of 8,17-epoxy-14-deoxy-11,12-didehydroandro- grapholide (**5**) in pyridine was added trialkyl-silylchloride (TIPS-Cl, TBDPS-Cl or TBS-Cl) at room temperature. After the stirring was continued until the TLC showed complete conversion, the reaction mixture was diluted with EtOAc and quenched with satd. $NH_4Cl$ and the mixture was extracted with EtOAc (x3). The combined organic layer was washed with brine, dried over $Na_2SO_4$ anhydrous and then concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) to give (**5a**-**5c**).

**3.2.2.2.1 Reaction of 19-*O*-TIPS-8,17-epoxy-14-deoxy-11,12-dide-hydroandrographolide (5a)**

Following the **general procedure C**, a mixture of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5**) (0.0845 g, 0.243 mmol) in pyridine was added triisopropylsilyl chloride (TIPS-Cl) (0.30 mL, 1.400 mmol) at room temperature. After stirring was continued for 4.0 h, the residue was purified by column chromatography to give 19-*O*-TIPS-epoxy-14-deoxy-11,12-didehydroandrographolide (**5a**) in 62% yield (0.0759 g) as a yellow oil.

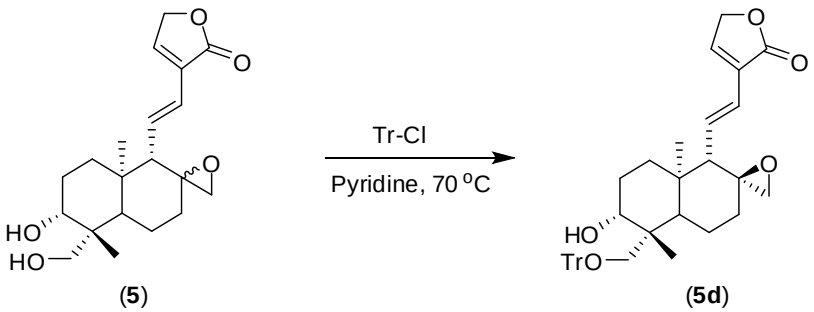
**3.2.2.2.2 Reaction of 19-*O*-TBDPS-8,17-epoxy-14-deoxy-11,12-dide hydroandrographolide (5b)**

Following the **general procedure C**, a mixture of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5**) (0.0812 g, 0.233 mmol) in pyridine (0.50 mL) was added *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) (0.30 mL, 1.153 mmol). After stirring was continued for 1.0 h, the residue was purified by column chromatography to give 19-*O*-TBDPS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5b**) in 74% yield (0.1011 g) as a white solid.

**3.2.2.2.3 Reaction of 19-*O*-TBS-8,17-epoxy-14-deoxy-11,12-dide-hydroandrographolide (5c)**

Following the **general procedure C**, a mixture of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5**) (0.0827 g, 0.237 mmol) in pyridine was added *tert*-butyldimethylsilyl (TBS-Cl) (0.2241 g, 1.487 mmol) at room temperature. After stirring was continued for 1.0 h, the residue was purified by column chromatography to give 19-TBS-8,17-epoxy-14-deoxy-11,12-didehydroandro grapholide (**5c**) in 70% yield (0.0768 g) as a white solid.

**3.2.2.3 Alkylation reaction of 8,17-epoxy-14-deoxy-11,12-didehydro-andrographolide (5)**



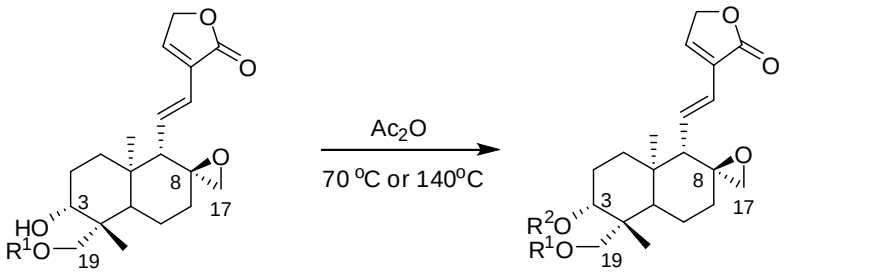

(**5**) (**5d**)

To a stirred solution of 8,17-epoxy-14-deoxy-11,12-didehydroandro- grapholide (**5**) (0.0980 g, 0.281 mmol) in pyridine was added triphenyl-methyl chloride (Tr-Cl) (0.3720

g, 1.334 mmol), then the reaction mixture was heated to 70 °C and continued at 70 °C for 1.0 h. The reaction mixture was quenched by addition to EtOAc and satd. $NH_4Cl$. The reaction mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography to give 19-*O*-Tr-8,17-epoxy-14-deoxy-11,12-didehydro andrographolide (**5d**) in 63% yield (0.1046 g) as a white solid.

**3.2.2.4 Acetylation reaction of 8,17-epoxy-14-deoxy-11,12-didehydro-andrographolide derivatives**

**General procedure D**




(**5**, **5a**-**d**)

$R^1$ = H, TIPS, TBDPS, TBS, Tr

**(6a**-**6f)**

$R^1$ = TIPS, TBDPS, TBS, Tr, Ac

$R^2$ = H, Ac

A stirred solution of 8,17-epoxy-14-deoxy-11,12-didehydroandrographo lide (**5**) or analogues (**5a**-**5d**) in acetic anhydride was heated to 70 °C or 140 °C. After the TLC showed completed reaction, the mixture was diluted with EtOAc and quenched with satd. $NaHCO_3$. The mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) to afford the corresponding products (**6a**-**6f**).

**3.2.2.4.1 Acetylation reaction of silylether or ether of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide derivatives (5a-5d)**

**3.2.2.4.1.1 Acetylation reaction 19-*O*-TIPS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5a)**

Following the **general procedure D**, a stirred solution of compound (**5a**) (0.0290 g, 0.0575 mmol) in acetic anhydride was heated at 140 °C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TIPS-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**6a**) in 69% yield (0.0217 g) as a yellow oil.

**3.2.2.4.1.2 Acetylation reaction of 19-*O*-TBDPS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5b)**

Following the **general procedure D**, a stirred solution of compound (**5b**) (0.0406 g, 0.069 mmol) in acetic anhydride (1.50 mL) was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TBDPS-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**6b**) in 70% yield (0.0305 g) as a white solid.

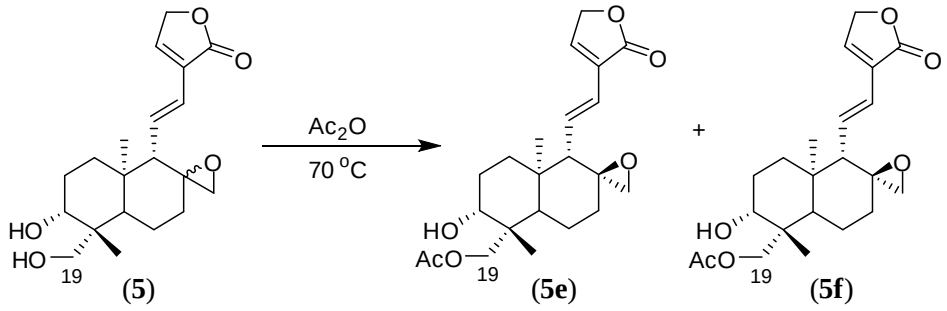
**3.2.2.4.1.3 Acetylation reaction of 19-*O*-TBS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5c)**

Following the **general procedure D**, a stirred solution of compound (**5c**) (0.0415 g, 0.089 mmol) in acetic anhydride (1.50 mL) was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-TBS-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**6c**) in 76% yield (0.0344 g) as a white solid.

**3.2.2.4.1.4 Acetylation reaction of 19-*O*-Tr-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5d)**

Following the **general procedure D**, a stirred solution of compound (**5d**) (0.0465 g, 0.140 mmol) in acetic anhydride (1.50 mL) was heated at 140 $^{o}$C with stirring for 1.5 h. The residue was purified by column chromatography to give 19-*O*-Tr-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**6d**) in 75% yield (0.0373 g) as a yellow oil.

**3.2.2.4.2 Acetylation reaction of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5)**

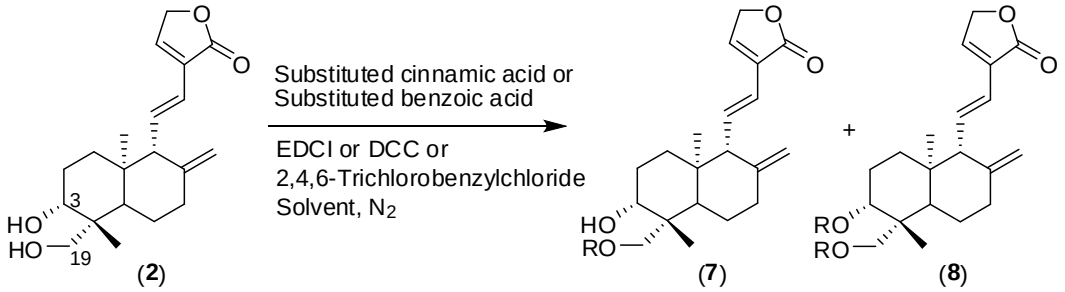



(**5**) (**5e**) (**5f**)

Following the **general procedure D**, 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5**) (0.1537 g, 0.441 mmol) in acetic anhydride was heated at 70 $^{o}$C with stirring for 4.5 h. The residue was purified by column chromatography to give 19-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5e**) in 60% yield (0.1033 g) as a pale yellow oil and 3,19-*O*-diacetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (**5f**) in 30% yield (0.0572 g) as a yellow solid.

### 3.2.3 Modification of the two hydroxyl groups at C-3, C-19 of 14-deoxy-11,12-didehydroandrographolide (2) *via* esterification

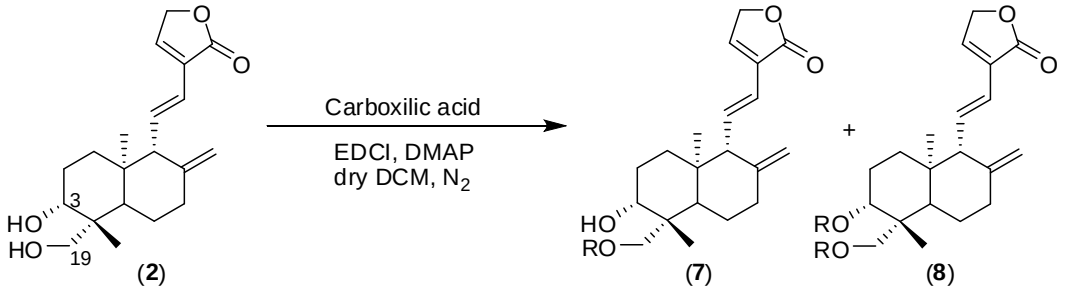
The structure of 14-deoxy-11,12-didehydroandrographolide (**2**) was modified by esterification reaction of the hydroxyl group at position C-19 with derivatives of carboxylic acid to obtain the new derivatives of 14-deoxy-11,12-didehydroandrographolide (**2**).




Scheme 3.4 The synthesis of C-19 and C-3 substituted of compound (**2**) *via* esterification.

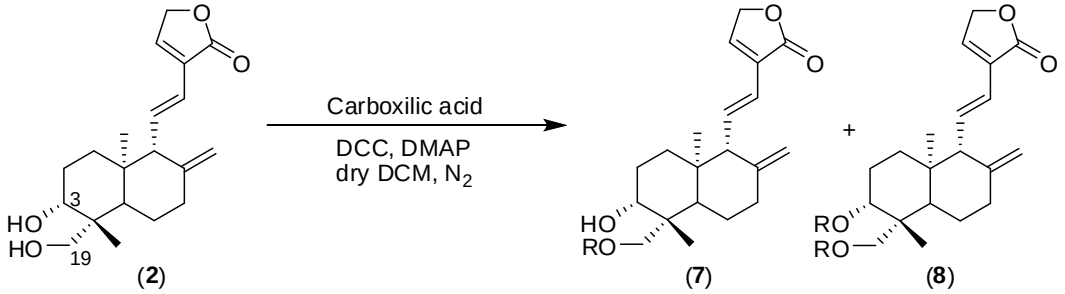
#### 3.2.3.1 Synthesis of 14-deoxy-11,12-didehydroandrographolide derivatives by esterification at C-19 with carboxylic acid

**General procedure E**



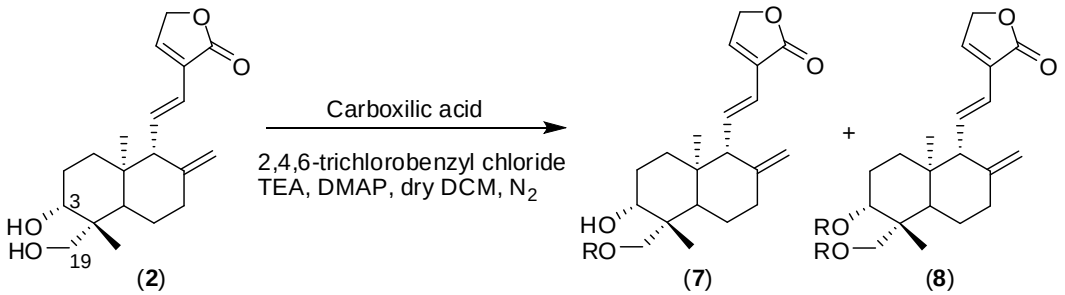

14-Deoxy-11,12-didehydroandrographolide (**2**) and appropriate carboxylic acid (1.2 equiv.) were dissolved in dry dichloromethane (dry DCM). EDCI (1.2 equiv.) and catalytic amount of DMAP were added to the reaction mixture under $N_2$ gas and stirred at room temperature until the starting material was vanished by TLC. The reaction mixture was filtered and extracted with DCM. Then, the DCM solution was washed with $H_2O$. The combined organic layer was washed with brine, dried over $Na_2SO_4$ anhydrous and then concentrated to dryness rotary under reduced pressure. The residue was purified by column chromatography (DCM:EtOAc) over the silica gel to yield the target compounds.

**General procedure F**



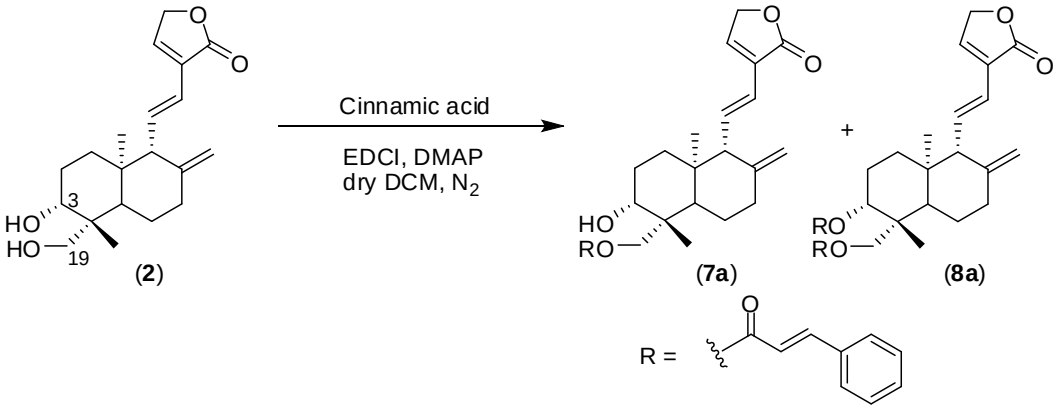

14-Deoxy-11,12-didehydroandrographolide (**2**) and appropriate carboxylic acid (1.2 equiv) were dissolved in dry dichloromethane (dry DCM). DCC (1.2 equiv.) and a catalytic amount of DMAP were added to the reaction mixture under $N_2$ gas and stirred at room temperature until the starting material was vanished by TLC. The reaction mixture was filtered and extracted with DCM. Then, the DCM solution was washed with satd. $NaHCO_3$ and $H_2O$. The combined organic layer was washed with brine, dried over $Na_2SO_4$ anhydrous and then concentrated to dryness rotary under reduced pressure. The residue was purified by column chromatography over the silica gel to yield the target compound.

**General procedure G**



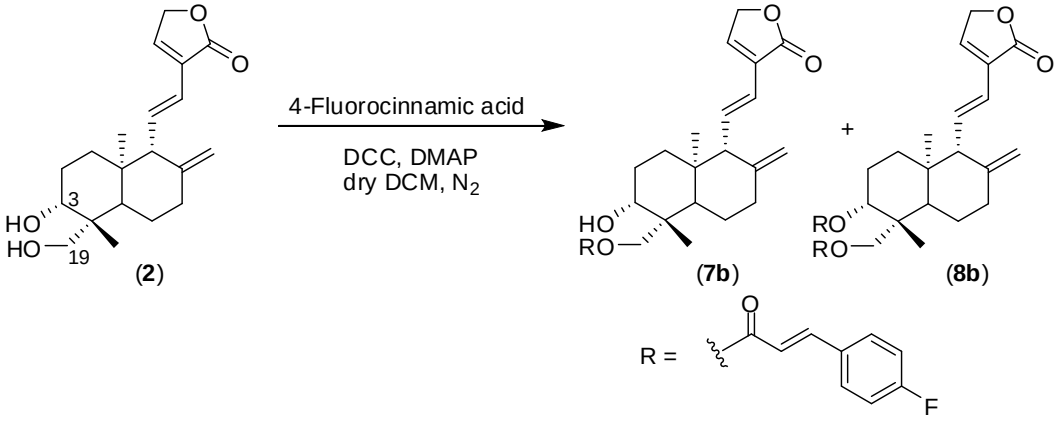

14-Deoxy-11,12-didehydroandrographolide (**2**) and appropriate carboxylic acid (1.3 equiv.) were dissolved in dry dichloromethane (dry DCM). 2,4,6-trichlorobenzyl chloride (1.3 equiv.), TEA (1.2 equiv.) and DMAP (1.2 equiv.) were added to the reaction mixture and stirred at room temperature under $N_2$ until the starting material was vanished by TLC. The reaction mixture was diluted with DCM and quenched with satd. $NH_4Cl$ and $H_2O$. The combined organic layer was washed with brine, dried over $Na_2SO_4$ anhydrous and then concentrated to dryness rotary under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) over the silica gel to yield the target compound.

### 3.4.3.1.1 Synthesis of 19-*O*-cinnamoyl-14-deoxy-11,12-dehydro-andrographolide (7a)



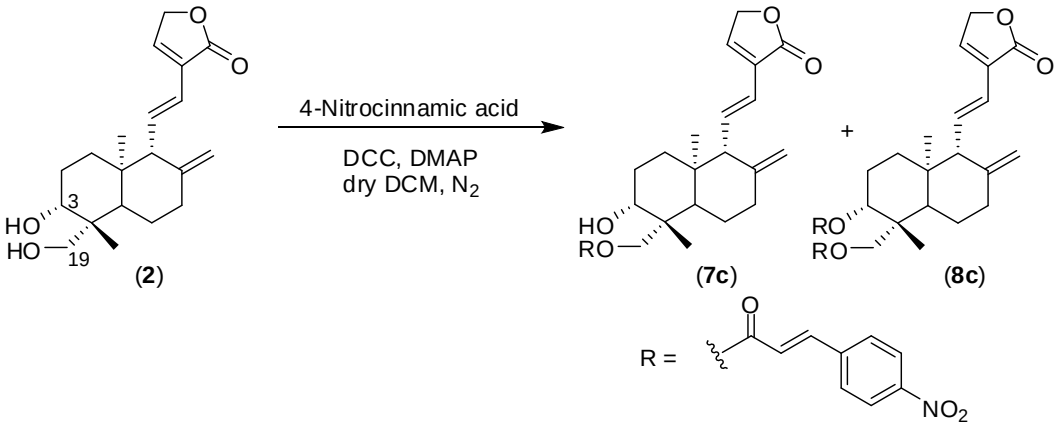

Following the **general procedure E**, 14-deoxy-11,12-didehydroandro-grapholide (**2**) (0.1078 g, 0.261 mmol) and cinnamic acid (0.0881 g, 0.595 mmol) were dissolved in dry dichloromethane (dry DCM). EDCI and DMAP were added to the reaction mixture and stirred for 5.0 h. The residue was purified by column chromatography to afford (**7a**) in 28% yield (0.0338 g) as a white solid base on recovered starting material and (**8a**) in 16% yield (0.0255 g) as a yellow solid base on recovered starting material (0.0209 g of starting material remain).

### 3.2.3.1.2 Synthesis of 19-*O*-(4′-fluoro)cinnamoyl-14-deoxy-11,12-dehydroandrographolide (7b)



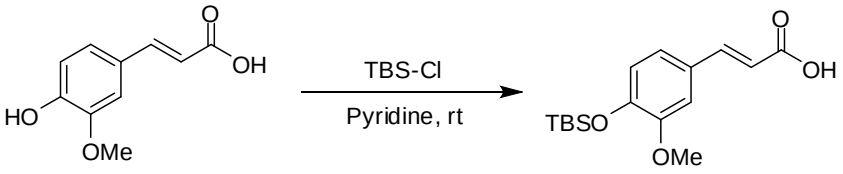

Following the **general procedure F**, 14-deoxy-11,12-didehydroandro-grapholide (**2**) (0.2584 g, 0.777 mmol) and 4-fluorocinnamic acid (0.2578 g, 1.552 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 1.5 h. The residue was purified by column chromatography to afford 19-*O*-(4′-fluoro)cinnamoyl-14-deoxy-11,12-didehydroandrographo- lide (**7b**) in 48% yield (0.1787 g) as a white solid and 3,19-*O*-di(4′-fluoro) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**8b**) in 12% yield (0.0626 g) as a white solid.

### 3.2.3.1.3 Synthesis of 19-*O*-(4′-nitro)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7c)



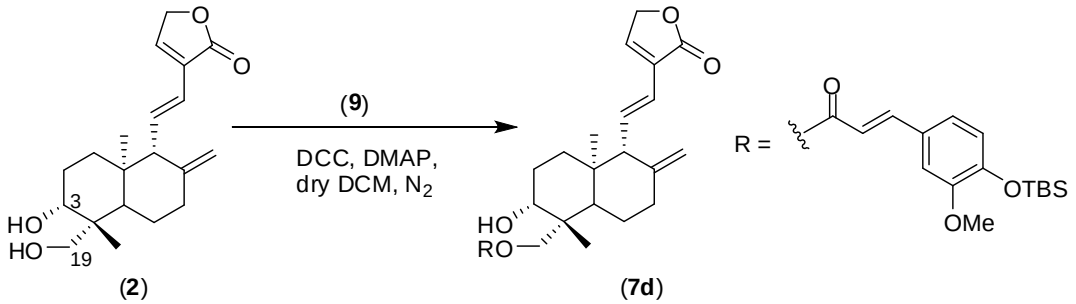

Following the **general procedure F**, 14-deoxy-11,12-didehydroandro grapholide (**2**) (0.1158 g, 0.348 mmol) and 4-nitrocinnamic acid (0.1339 g, 0.693 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 1.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-nitro)cinnamoyl-14-deoxy-11,12-didehydroandro-grapholide (**7c**) in 44% yield (0.0782 g) as a white solid and 3,19-*O*-di(4′-nitro)cinna- moyl-14-deoxy-11,12-didehydroandrographolide (**8c**) in 34% yield (0.0802 g) as a white solid.

### 3.2.3.1.4 Synthesis of 19-*O*-(3′-methoxyl-4′-TBS)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7d)



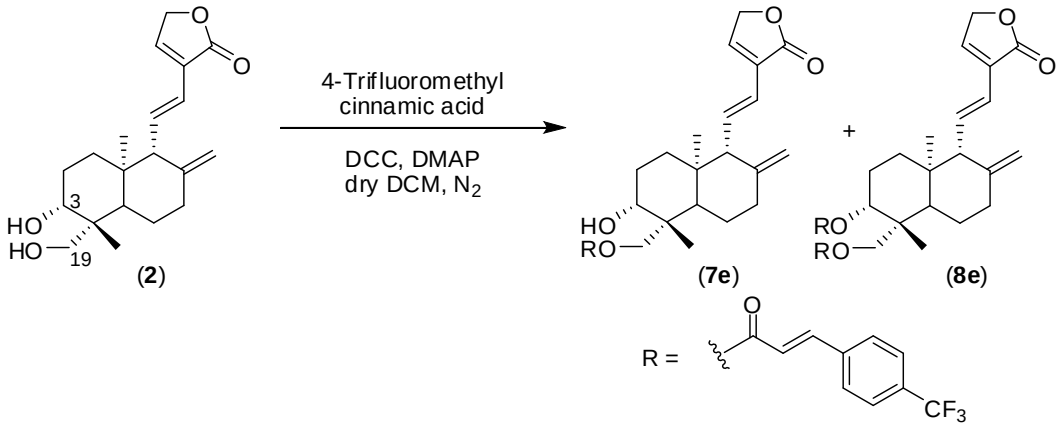

**Ferulic acid** (**9**)

To a stirred solution of ferulic acid (1.0873 g, 5.599 mmol) in pyridine (6.00 mL) was added *tert*-butyldimethylsilyl chloride (TBS-Cl) (2.6582 g, 17.637 mmol) at room temperature. After stirring was continued at room temperature for 2.5 h, the reaction mixture was diluted with EtOAc and quenched with satd. $NH_4Cl$ and the mixture was extracted with EtOAc (x3). The combined organic layer was washed with brine, dried over $Na_2SO_4$ anhydrous and then concentrated under reduced pressure. The residue was purified by column chromatography to afford product (**9**) in 71% yield (1.2171 g) as a white solid.



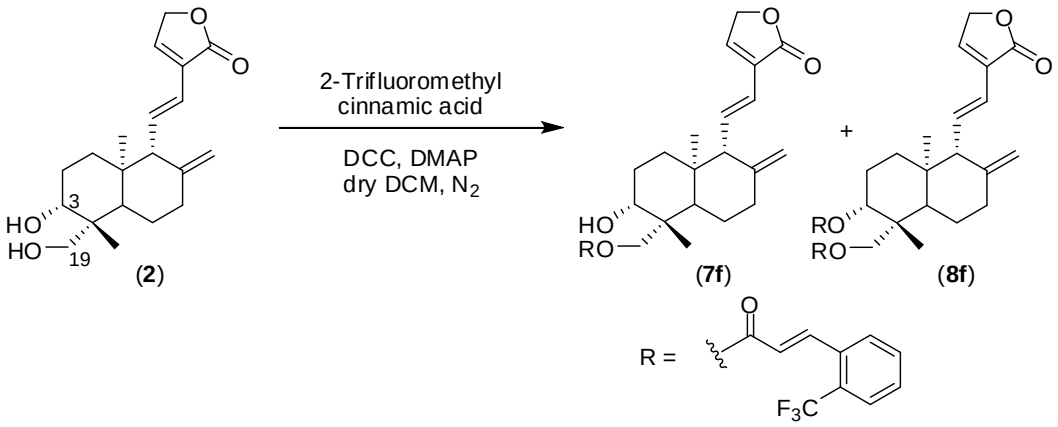

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.0500 g, 0.150 mmol) and compound (**9**) (0.1270 g, 0.412 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 11.0 h. The residue was purified by column chromatography to afford 19-*O*-(3′-methoxyl-4′-TBS)cinnamoyl-14-deoxy-11,12-didehydroandrogra-pholide (**7d**) as a white solid in 36% yield (0.0260 g) base on recovered starting material (0.0109 g of starting material remain).

### 3.2.3.1.5 Synthesis of 19-*O*-(4′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7e)



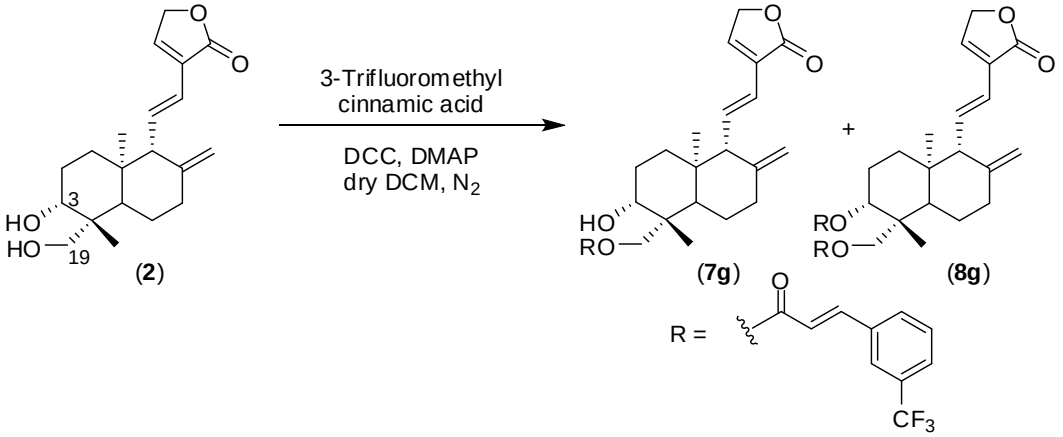

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.2704 g, 0.816 mmol) and 4-trifluoromethylcinnamic acid (0.2113 g, 0.978 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**7e**) as a white solid in 61% yield (0.1706 g) base on recovered starting material and 3,19-*O*-di(4′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**8e**) as a white solid in 49% yield (0.1908 g) base on recovered starting material (0.0945 g of starting material remain).

### 3.2.3.1.6 Synthesis of 19-*O*-(2′-trifluormeththyl)cinnamoyl-14- deoxy-11,12-didehydroandrographolide (7f)




Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.2534 g, 0.762 mmol) and 2-trifluoromethylcinnamic acid (0.1977 g, 0.915 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(2′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**7f**) as a pale yellow oil in 44% yield (0.1262 g) base on recovered starting material and 3,19-*O*-di(2′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**8f**) as a white solid in 46% yield (0.1735 g) base on recovered starting material (0.0730 g of starting material remain).

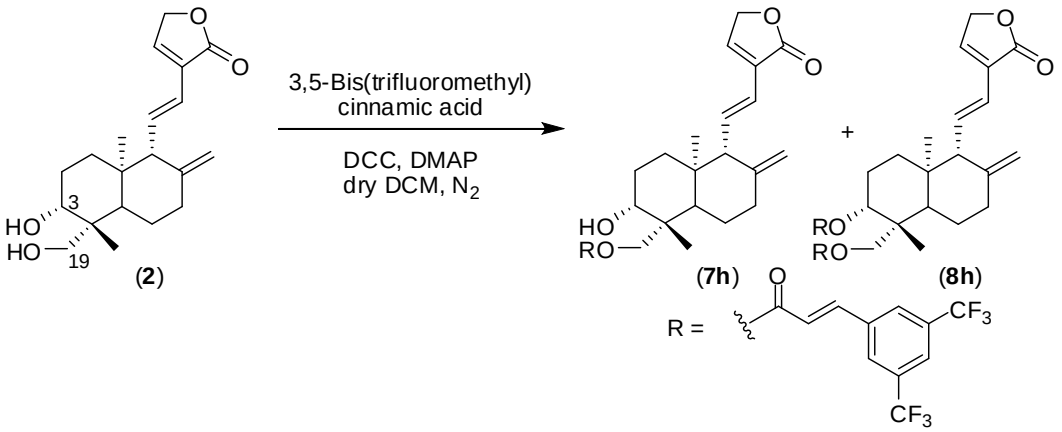
### 3.2.3.1.7 Synthesis of 19-*O*-(3′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7g)




Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.2531 g, 0.761 mmol) and 3-trifluoromethylcinnamic acid (0.1982 g, 0.917 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column

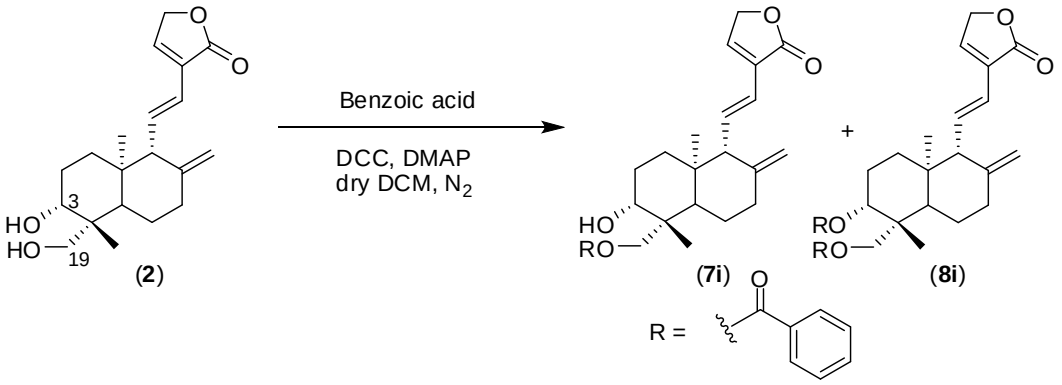
chromatography to afford 19-*O*-(3′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**7g**) as a white solid in 55% yield (0.1636 g) base on recovered starting material and 3,19-*O*-di(3′-trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (**8g**) as a white solid in 49% yield (0.2010 g) base on recovered starting material (0.0670 g of starting material remain).

**3.2.3.1.8 Synthesis of 19-*O*-3,5-bis(trifluormethylcinnamoyl)-14-deoxy-11,12-didehydroandrographolide (7h)**



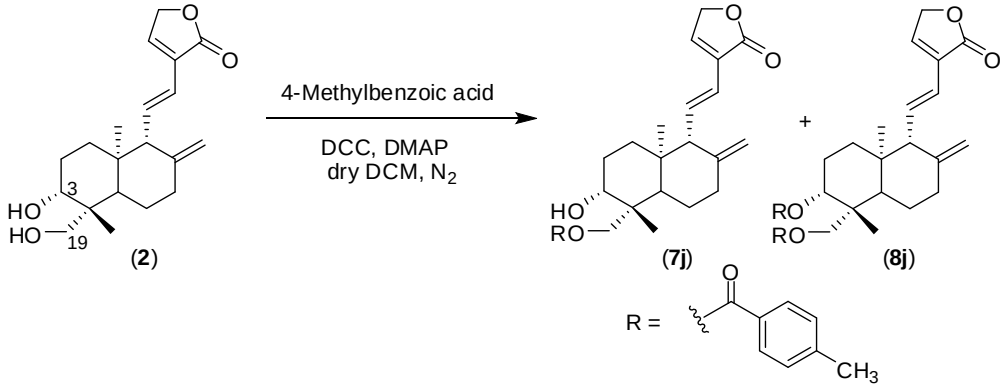

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.2496 g, 0.751 mmol) and 3,5-bis(trifluoromethyl)cinnamic acid (0.2560 g, 0.900 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(3′,5′-bis(trifluormeththyl))cinna- moyl-14-deoxy-11,12-didehydroandrographolide (**7h**) as a pale yellow oil in 48% yield (0.1604 g) base on recovered starting material and 3,19-*O*-di(3′,5′-bis(trifluoromethyl))cinnamoyl-14-deoxy-11,12-didehydroandro-grapholide (**8h**) as a white solid 17% yield (0.0819 g) base on recovered starting material (0.0636 g of starting material remain).

### 3.2.3.1.9 Synthesis of 19-*O*-benzoyl-14-deoxy-11,12-didehydro-andrographolide (7i)



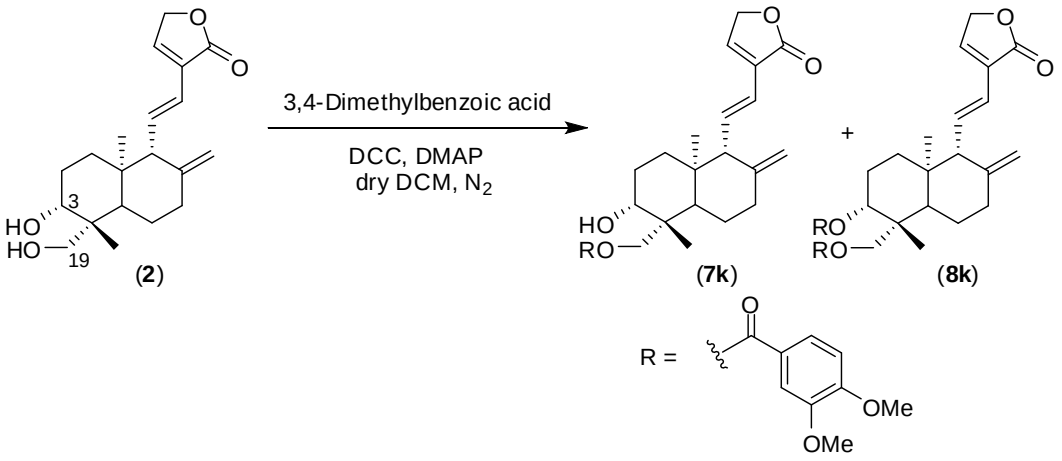

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1553 g, 0.467 mmol) and benzoic acid (0.0683 g, 0.559 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-benzoyl-14-deoxy-11,12-didehydroandrographolide (**7i**) as a white solid in 65% yield (0.1115 g) base on recovered starting material and 3,19-*O*-di(benzoyl)-14-deoxy-11,12-didehydroandrographolide (**8i**) as a white solid in 31% yield (0.0670 g) base on recovered starting material (0.1115 g of starting material remain).

### 3.2.3.1.10 Synthesis of 19-*O*-(4′-methyl)benzoyl-14-deoxy-11,12-di-dehydroandrographolide (7j)



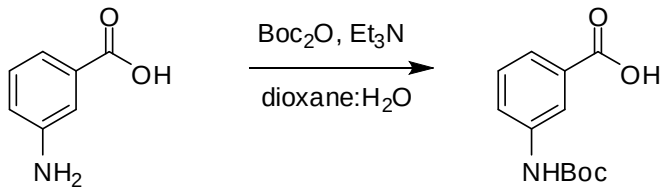

Following the **general procedure E**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1999 g, 0.599 mmol) and 4-methylbenzoic acid (0.1691 g, 1.242 mmol) were dissolved in dry dichloromethane (dry DCM). EDCI and DMAP were added to the reaction mixture and stirred for 5.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-methyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (**7j**) as a white solid in 48% yield (0.1179 g) base on recovered starting material and 3,19-*O*-di(4′-methyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (**8j**) as a white solid in 4% yield (0.0110 g) base on recovered starting material (0.0191 g of starting material remain).

### 3.2.3.1.11 Synthesis of 19-*O*-(3′, 4′-dimethoxy)benzoyl-14-deoxy-11,12-didehydroandrographolide (7k)

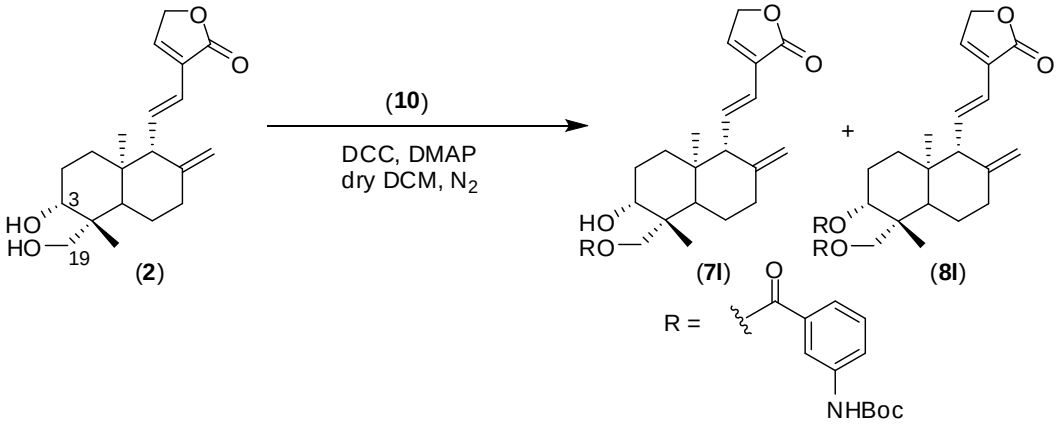



Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.0838 g, 0.252 mmol) and 3,4-dimethoxybenzoic acid (0.0911 g, 0.500 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(3′,4′-dimethoxy)benzoyl-14-deoxy-11,12-didehydroandrographolide (**7k**) in 52% yield (0.0641 g) as a pale yellow oil and 3,19-*O*-bis (3′,4′-dimethoxy)benzoyl-14-deoxy-11,12-didehydroandrographolide (**8k**) as a white solid in 40% yield (0.0545 g of starting material remain).

### 3.2.3.1.12 Synthesis of 19-*O*-(3′-*tert*-butoxycarbonylamino)benzoyl-14-deoxy-11,12-didehydroandrographolide (7l)

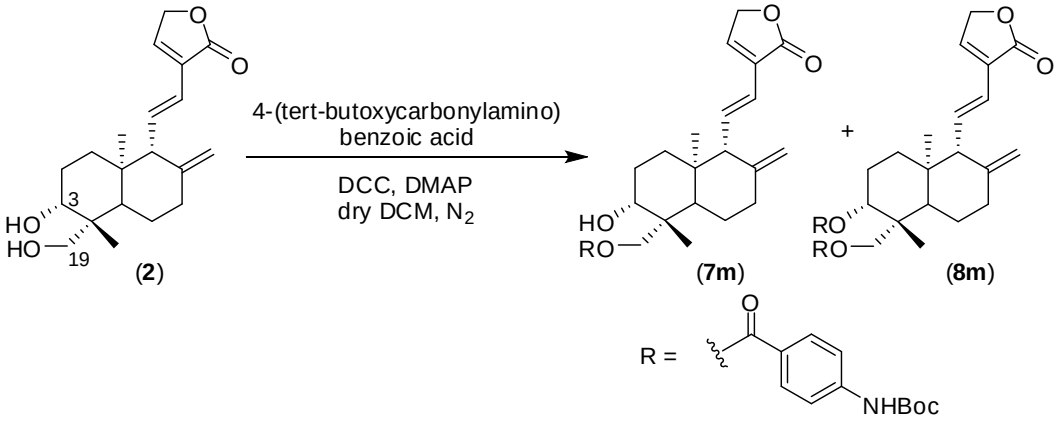



A solution of 3-aminobenzoic acid (1.0448 g, 7.618 mmol) in water (2.0 mL) and dioxane (5.0 mL) was added triethylamine (1.3 mL) followed by a solution of $Boc_2O$ (1.9950 g, 9.140 mmol) in dioxane. The reaction mixture was stirred at room temperature for 0.5 h. The residue was purified by column chromatography (1:1 DCM:EtOAc) to give product (**10**) in 42% yield (0.5455 g) as a white solid.



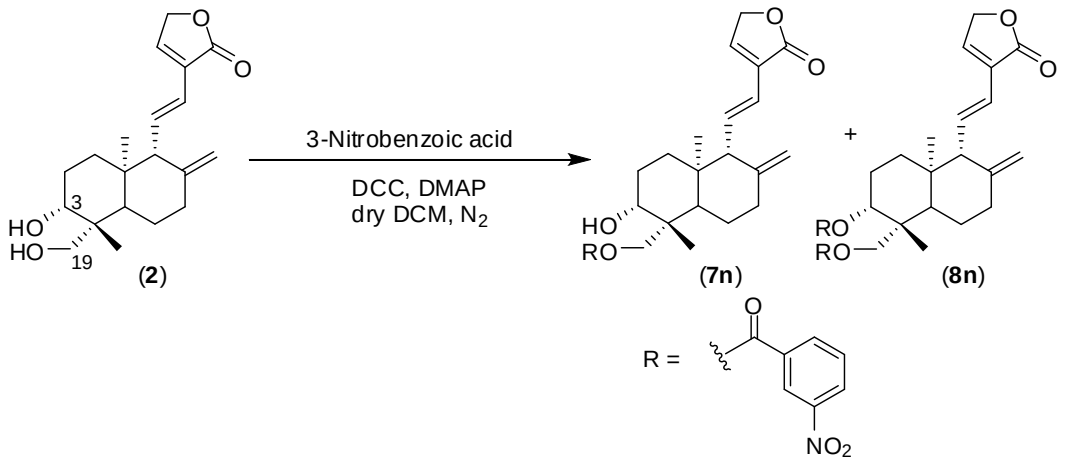

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1434 g, 0.432 mmol) and compound (**10**) (0.2047 g, 0.863 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.5 h. The residue was purified by column chromatography to afford 19-*O*-(3′-*tert*-butoxycarbonylamino)benzoyl-14-deoxy-11,12-didehydroandrographolide (**7l**) in 62% yield (0.1480 g) as a white solid base on recovered starting material (0.0447 g of starting material remain).

**3.2.3.1.13 Synthesis of 19-*O*-(4′-*tert*-butoxycarbonylamino)benzoyl-14-deoxy-11,12didehydroandrographolide (7m)**



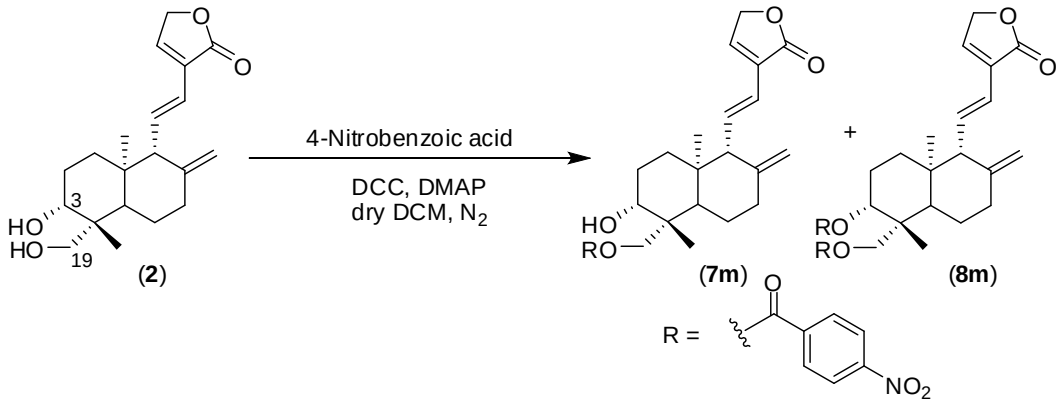

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.2447 g, 0.736 mmol) and 4-(*tert*-butoxycarbonylamino)benzoic acid (0.2698 g, 1.137 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-*tert*-butoxycarbonylamino) benzoyl-14-deoxy-11,12-didehydroandrographolide (**7m**) in 66% yield (0.1480 g) as a white solid and 19-*O*-di(4′-*tert*-butoxycarbonylamino)benzoyl-14-deoxy-11,12-didehydroandrographolide (**8m**) in 34% yield (0.1924 g) as a white solid.

**3.2.3.1.14 Synthesis of 19-*O*-(3′-nitro)benzoyl-14-deoxy-11,12-dide hydroandrographolide (7n)**



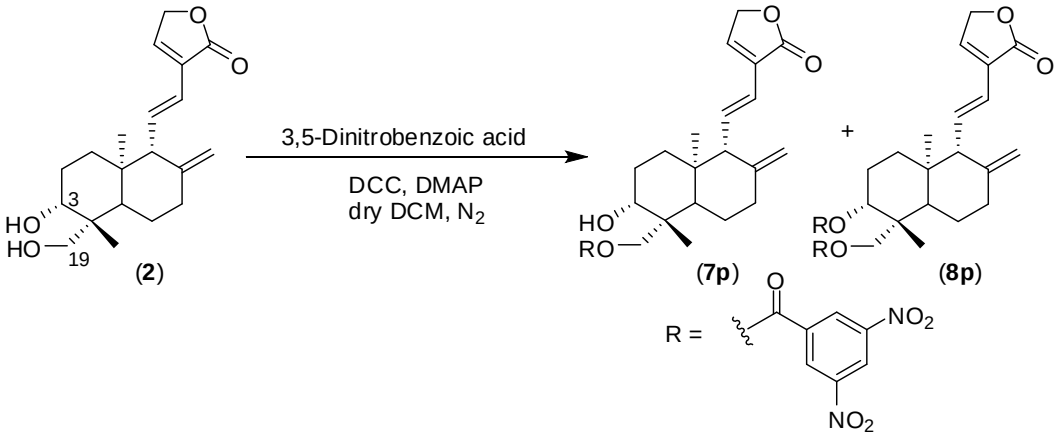

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1301 g, 0.391 mmol) and 3-nitrobenzoic acid (0.0794 g, 0.469 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford (**7n**) as a white solid in 60% yield (0.0785 g) base on recovered starting material and (**8n**) in 30% yield (0.0510 g) as a white solid base on recovered starting material (0.0294 of starting material remain).

**3.2.3.1.15 Synthesis of 19-*O*-(4′-nitro)benzoyl-14-deoxy-11,12-dide-hydroandrographolide (7m)**



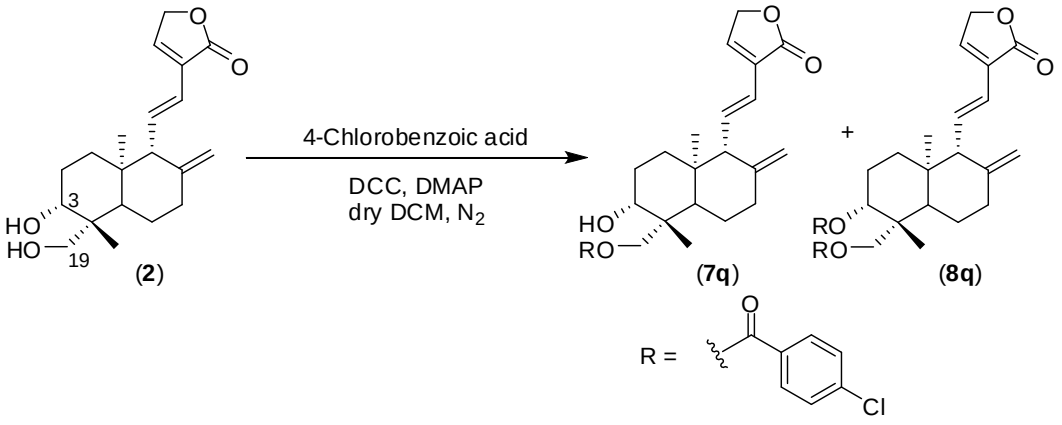

Following the **general procedure F**, 14-deoxy-11,12-didehydroandrographolide (**2**) (0.1074 g, 0.323 mmol) and 4-nitrobenzoic acid (0.0653 g, 0.391 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford (**7o**) in 50% yield (0.0694 g) as a white solid base on recovered starting material and (**8o**) in 30% yield (0.0538 g) as a white solid base on recovered starting material (0.0120 g of starting material remain).

### 3.2.3.1.16 Synthesis of 19-*O*-(3′,5′-dinitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7p)




Following the **general procedure F**, 14-deoxy-11,12-didehydroandro grapholide (**2**) (0.2266 g, 0.682 mmol) and 3,5-nitrobenzoic acid (0.1866 g, 0.799 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford (**7p**) in 50% yield (0.1406 g) as a white solid base on recovered starting material and (**8p**) in 47% yield (0.1796 g) as a white solid base on recovered starting material (0.0500 g of starting material remain).

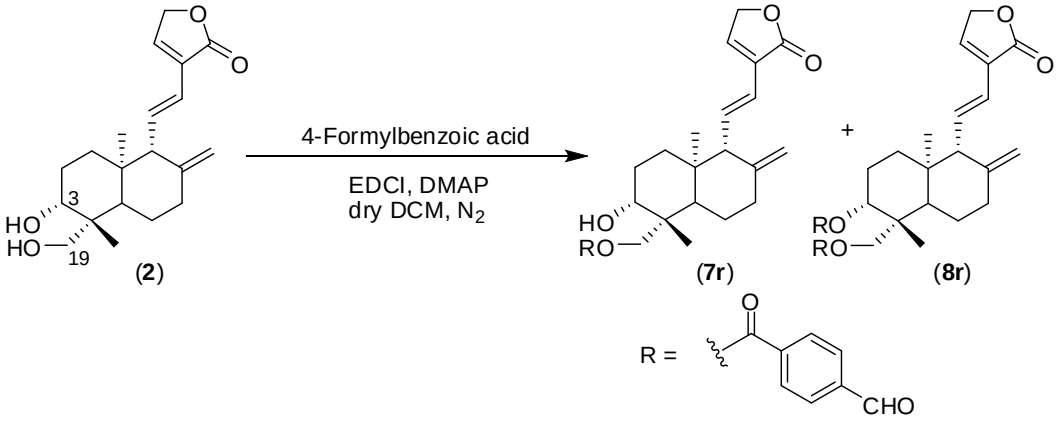
### 3.2.3.1.17 Synthesis of 19-*O*-(4′-chloro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7q)




Following the **general procedure F**, 14-deoxy-11,12-didehydroandro-grapholide (**2**) (0.1121 g, 0.34 mmol) and 4-chlorobenzoic acid (0.0640 g, 0.409 mmol) were dissolved in dry dichloromethane (dry DCM). DCC and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-chloro)benzoyl-14-deoxy-11,12-didehydroandrographolide (**7q**) in 65% yield (0.0798 g) as a white solid base on recovered starting material and 3,19-*O*-di(4′-

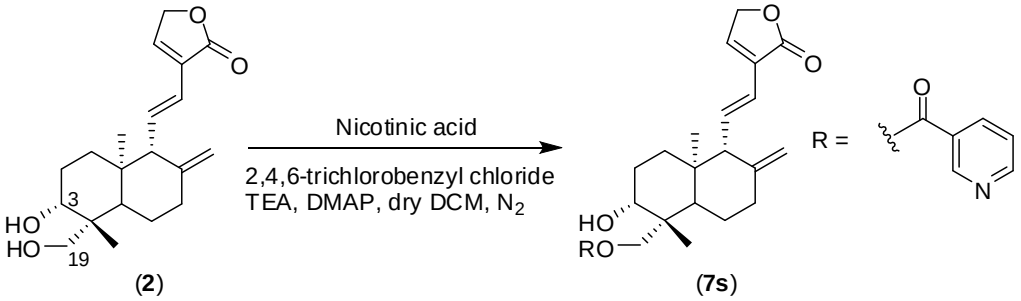
chloro)benzoyl-14-deoxy-11,12-didehydroandrographolide (**8q**) in 35% yield (0.0507 g) as a white solid base on recovered starting material (0.0254 g of starting material remain).

### 3.2.3.1.18 Synthesis of 19-*O*-(4′-formyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (7r)




Following the **general procedure E**, 14-deoxy-11,12-didehydroandro grapholide (**2**) (0.2047 g, 0.616 mmol) and 4-formylbenzoic acid (0.1197 g, 0.797 mmol) were dissolved in dry dichloromethane (dry DCM). EDCI and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-(4′-formyl)benzoyl-14-deoxy-11,12-didehydroandro- grapholide (**7r**) in 30% yield (0.0587 g) as a white solid base on recovered starting material. 3,19-*O*-di(4′-formyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (**8r**) in 9% yield (0.0220 g) as a white solid base on recovered starting material (0.0628 g).

### 3.2.3.1.19 Synthesis of 19-*O*-nicotinonyl-14-deoxy-11,12-dide-hydroandrographolide (7s)

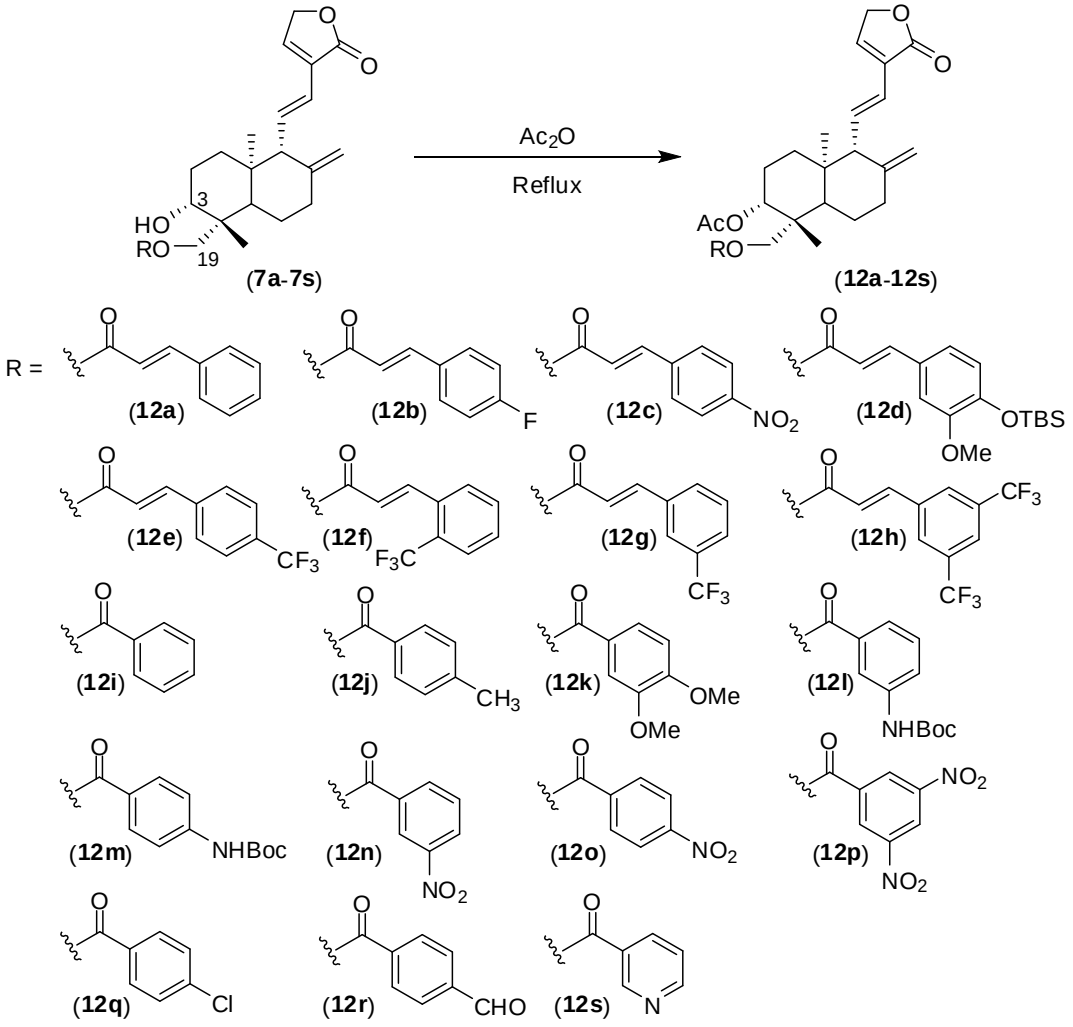



Following the **general procedure G**, 14-deoxy-11,12-didehydroandro grapholide (**2**) (0.1000 g, 0.300 mmol) and nicotinic acid (0.0480 g, 0.390 mmol) were dissolved in dry dichloromethane (DCM). 2,4,6-trichlorobenzyl chloride, TEA and DMAP were added to the reaction mixture and stirred for 2.0 h. The residue was purified by column chromatography to afford 19-*O*-nicotinonyl-14-deoxy-11,12-didehydroandrographolide (**7s**) in 72% yield as a yellow solid (0.0567 g).

### 3.2.3.2 Acetylation reaction of C-19-substituted-14-deoxy-11,12-didehydroandrographolide derivatives (7a-7s)

**General procedure H**

A stirred solution of 14-deoxy-11,12-didehydroandrographolide derivatives (**7a-7s**) in acetic anhydride was reflux at 140 $^{o}$C. After the TLC showed completed reaction, the mixture was diluted with EtOAc and quenched with satd. $NaHCO_3$. The mixture was extracted with EtOAc (x3). The combined organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$ anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc) to afford the corresponding products (**12a-12s**).




#### 3.2.3.2.1 Acetylation reaction of 19-*O*-cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7a)

Following the **general procedure H**, a stirred solution of compound (**7a**) (0.0300 g, 0.065 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The

residue was purified by column chromatography to give 19-*O*-cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12a**) in 62% yield (0.0203 g) as a white solid.

**3.2.3.2.2 Acetylation reaction of 19-*O*-(4′-fluoro)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7b)**

Following the **general procedure H**, a stirred solution of compound (**7b**) (0.0554 g, 0.113 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-fluoro)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12b**) in 97% yield (0.0585 g) as a pale yellow oil.

**3.2.3.2.3 Acetylation reaction of 19-*O*-(4′-nitro)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7c)**

Following the **general procedure H**, a stirred solution of compound (**7c**) (0.0460 g, 0.091 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-Nitro)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12c**) in 75% yield (0.0373 g) as a pale yellow oil.

**3.2.3.2.4 Acetylation reaction of 19-*O*-(3′-methoxyl-4′-TBS) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7d)**

Following the **general procedure H**, a stirred solution of compound (**7d**) (0.1000 g, 0.161 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′-methoxyl-4′-TBS)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12d**) in 27% yield (0.0290 g) as a white solid.

**3.2.3.2.5 Acetylation reaction of 19-*O*-(4′-trifluormeththyl) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7e)**

Following the **general procedure H**, a stirred solution of compound (**7e**) (0.0414 g, 0.078 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12e**) in 53% yield (0.0238 g) as a pale yellow oil.

**3.2.3.2.6 Acetylation reaction of 19-*O*-(2′-trifluormeththyl) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7f)**

Following the **general procedure H**, a stirred solution of compound (**7f**) (0.0358 g, 0.068 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(2′-

trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12f**) in 50% yield (0.0190 g) as a yellow solid.

**3.2.3.2.7 Acetylation reaction of 19-*O*-(3′-trifluormeththyl) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7g)**

Following the **general procedure H**, a stirred solution of compound (**7g**) (0.0308 g, 0.058 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(2′-trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12g**) in 52% yield (0.0174 g) as a white solid.

**3.2.3.2.8 Acetylation reaction of 19-*O*-(3′,5′-bis(trifluormeththyl)) cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7h)**

Following the **general procedure H**, a stirred solution of compound (**7h**) (0.0400 g, 0.067 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′,5′-trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12h**) in 93% yield (0.0399 g) as a white solid.

**3.2.3.2.9 Acetylation reaction of 19-*O*-benzoyl-14-deoxy-11,12-didehydroandrographolide (7i)**

Following the **general procedure H**, a stirred solution of compound (**7i**) (0.0335 g, 0.077 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12i**) in 78% yield (0.0285 g) as a white solid.

**3.2.3.2.10 Acetylation reaction of 19-*O*-(4′-methyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (7j)**

Following the **general procedure H**, a stirred solution of compound (**7j**) (0.0298 g, 0.066 mmol) in acetic anhydride was heated at 120 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-methyl)benzoyl-3-*O*-acetyl-14-deoxy-11,12-dide-hydroandrographolide (**12j**) 75% yield (0.0245 g) as a white solid.

**3.2.3.2.11 Acetylation reaction of 19-*O*-(3′,4′-dimethoxy)benzoyl-14-deoxy-11,12-didehydroandrographolide (7k)**

Following the **general procedure H**, a stirred solution of compound (**7k**) (0.0300 g, 0.060 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′,4′-dimethoxy)benzoyl-3-*O*-

acetyl-14-deoxy-11,12-didehydroandrographolide (**12k**) in 43% yield (0.0138 g) as a pale yellow oil.

**3.2.3.2.12 Acetylation reaction of 19-*O*-(3′-*tert*-butoxycarbonyl amino)-14-deoxy-11,12-didehydroandrographolide (7l)**

Following the **general procedure H**, a stirred solution of compound (**7l**) (0.040 g, 0.072 mmol) in acetic anhydride was heated at 120 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′-*tert*-butoxycarbonylamino)-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12l**) in 91% yield (0.0391 g) as a pale yellow oil.

**3.2.3.2.13 Acetylation reaction of 19-*O*-(4′-*tert*-butoxycarbonyl amino)-14-deoxy-11,12-didehydroandrographolide (7m)**

Following the **general procedure H**, a stirred solution of compound (**7m**) (0.2500 g, 0.450 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-*tert*-butoxycarbonylamino)-3-*O*-acetyl-14-deoxy-11, 12-didehydroandrographolide (**12m**) in 73% yield (0.1955 g) as a white solid.

**3.2.3.2.14 Acetylation reaction of 19-*O*-(3′-nitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7n)**

Following the **general procedure H**, a stirred solution of compound (**7n**) (0.0308 g, 0.064 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′-nitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehy-droandrographolide (**12n**) in 50% yield (0.0167 g) as a white solid.

**3.2.3.2.15 Acetylation reaction of 19-*O*-(4′-nitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7o)**

Following the **general procedure H**, a stirred solution of compound (**7o**) (0.0301 g, 0.062 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-nitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehy-droandrographolide (**12o**) in 77% yield (0.0167 g) as a white solid.

**3.2.3.2.16 Acetylation reaction of 19-*O*-(3′,5′-dinitro)benzoyl-14- deoxy-11,12-didehydroandrographolide (7p)**

Following the **general procedure H**, a stirred solution of compound (**7p**) (0.0347 g, 0.072 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(3′,5′-dinitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12p**) in 43% yield (0.0161 g) as a white solid.

**3.2.3.2.17 Acetylation reaction of 19-*O*-(4′-chloro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7q)**

Following the **general procedure H**, a stirred solution of compound (**7q**) (0.0300 g, 0.063 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-chloro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12q**) in 60% yield (0.0195 g) as a white solid.

**3.2.3.2.18 Acetylation reaction of 19-*O*-(4′-formyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (7r)**

Following the **general procedure H**, a stirred solution of compound (**7r**) (0.0300 g, 0.065 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-(4′-formyl)benzenyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12r**) in 38% yield (0.0124 g) as a white solid.

**3.2.3.2.19 Acetylation reaction of 19-*O*-nicotinic-14-deoxy-11,12-didehydroandrographolide (7s)**

Following the **general procedure H**, a stirred solution of compound (**7s**) (0.0280 g, 0.064 mmol) in acetic anhydride was heated at 140 $^{o}$C with stirring for 1.0 h. The residue was purified by column chromatography to give 19-*O*-nicotinic-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (**12s**) in 50% yield (0.0159 g) as a white solid.

# CHAPTER 4
# RESULTS, DISCUSSION & CONCLUSION

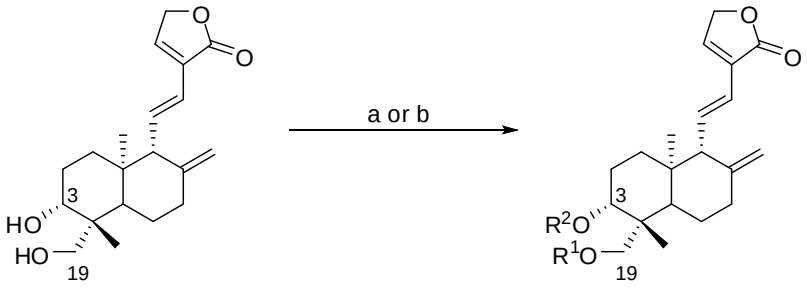
In this research, synthesis and cytotoxicity of 14-deoxy-11,12-didehydroandrographolide (**2**) have been demonstrated. The structure of compound (**2**) was modified to improve their cytotoxic activity in many approaches by silylation, etheration, acetylation and epoxidation. The synthetic analogues were tested for cytotoxic activities in nine cancer cell lines including P-388 (murine leukaemia cell line), KB (human epidermoid carcinoma of the mouth), HT-29 (human colorectal Adenocarcinoma Cell Line), MCF-7 (human breast cancer), LU-1 (human lung cancer), ASK (rat glioma), KKU M-213 (adenosquamous cell carcinoma), HUCCA-1 (human cholangio carcinoma cell line), K-100 (poorly differentiate adnocarcinoma) and HEK-293 (vero cells).

## 4.1 Modification of the two hydroxyl groups at C-3 and C-19 of 14-deoxy-11,12-didehydroandrographolide

The structure of 14-deoxy-11,12-didehydroandrographolide (**2**) was modified by conversion of hydroxyl group at C-19 position *via* silylation or alkylation or acetylation and followed by acetylation at C-3 position to obtain the new derivatives of 14-deoxy-11,12-didehydroandrographolide (**2**) as shown in Table 4.1 and 4.2.

The modification was first studied by silylation at C-19 of (**2**) to obtain 19-*O*-TIPS-14-deoxy-11,12-didehydroandrographolide (**3a**) in moderate yield (63%) as long as 4.0 hours due to the steric effect of the three isopropyl groups (entry 1, Table 4.1). Products (**3b**) and (**3c**) were afforded in good yields when carrying out the reaction of (**2**) and TBDPS-Cl or TBS-Cl in the presence of pyridine as base (entry 2 and 3). 19-*O*-Trityl derivative was prepared by reaction of (**2**) with Tr-Cl at 70 $^{o}$C, the product was obtained in excellent yield (99%) (entry 4). Acetylation of compound (**2**) provided a mixture of two compounds (**4e**) and (**4f**) in 93% yield of total yield which could be easily separated by column chromatography as shown in Table 4.1.

Table 4.1 The synthesis of C-19 derivatives of 14-deoxy-11,12-didehydroandrographolide (**3a**-**3d**), (**4e**) and (**4f**).



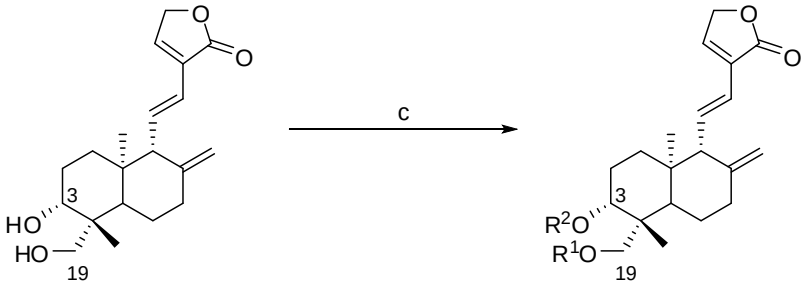

(**2**) (**3a**-**3d**, **4e**-**4f**)

| Entry | $R^1$ | $R^2$ | Condition | Temp. ($^oC$) | Time (h) | Compound | % Yield |
|---|---|---|---|---|---|---|---|
| 1 | TIPS | H | a | rt | 4.0 | **3a** | 63 |
| 2 | TBDPS | H | a | rt | 1.0 | **3b** | 70 |
| 3 | TBS | H | a | rt | 1.0 | **3c** | 79 |
| 4 | Tr | H | a | 70 | 1.0 | **3d** | 99 |
| 5 | Ac | H | b | 70 | 4.5 | **4e** | 59 |
| | Ac | Ac | | | | **4f** | 34 |

Conditions; a) R-Cl, pyridine, rt-70 $^oC$ for (**3a**-**3d**); b) $Ac_2O$, 70 $^oC$ for (**4e**-**4f**).

Acetylation of the remaining hydroxyl group at C-3 position of (**3a**), (**3b**), (**3c**) and (**3d**) was carried out to obtain products in moderate to high yield (64-95%) as shown in Table 4.2.

Table 4.2 The synthesis of compound (**4a**-**4d**) by acetylation.



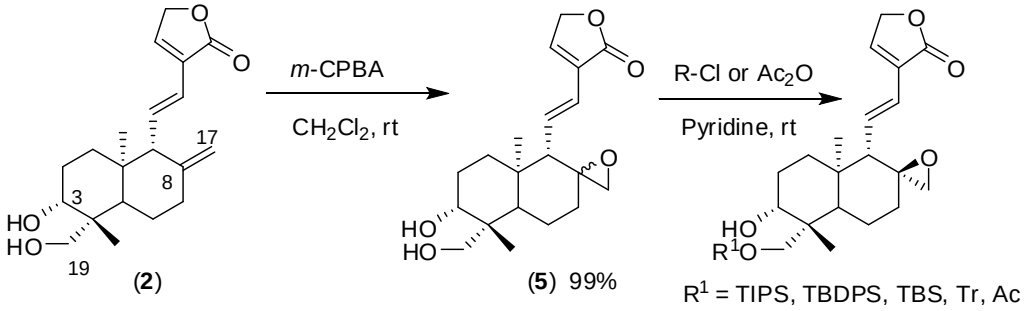

(**3a-3d**) (**4a-4d**)

| Entry | $R^1$ | $R^2$ | Condition | Time (h) | Compound | % Yield |
|---|---|---|---|---|---|---|
| 1 | TIPS | Ac | c | 1.5 | **4a** | 64 |
| 2 | TBDPS | Ac | c | 1.5 | **4b** | 95 |
| 3 | TBS | Ac | c | 1.5 | **4c** | 76 |
| 4 | Tr | Ac | c | 1.0 | **4d** | 75 |

Condition; a) $Ac_2O$, 140 $^oC$.

## 4.2 Modification of the *exo*-alkene group at C-8 of 14-deoxy-11,12-didehydroandrographolide (2)

The structure of 14-deoxy-11,12-didehydroandrographolide was modified by conversion of the *exo*-alkene at C-8 to obtain epoxide compound followed by modification of the hydroxyl group at C-19 as shown in scheme 4.1.



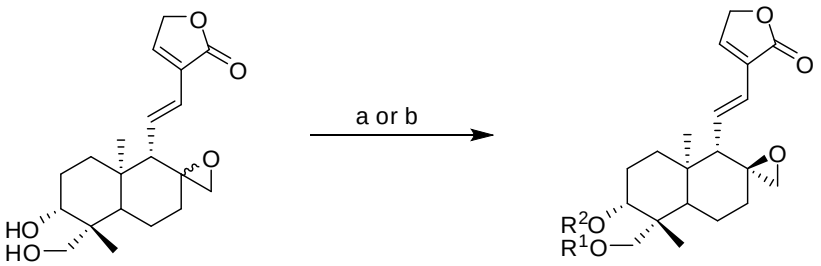

Scheme 4.1 Epoxidation of *exo*-alkene group at C-8 and modification of C-19 to silyl- ether or ether.

Epoxidation of 14-deoxy-11,12-didehydroandrographolide (**2**) with *meta*-perchlorobenzoic acid in DCM afforded the mixture isomers of 8,17-epoxy-14-deoxy-11,12-didehydroandrographolide product (**5**) in 99% yield. Then, the hydroxyl of compound (**5**) at C-19 position was modified by silylation, etheration or acetylation as shown the result in Table 4.3.

19-*O*-silyl analoges (**5a**-**5c**) were prepared by silylation of mixture isomers (**5**) with trialkyl-silyl chloride using pyridine to provide triisopropylsilyl (TIPS), *tert*-butyldiphenyl silyl (TBDPS) and *tert*-butyldimethylsilyl (TBS) ether derivatives in good yields of major isomer and trace amount of minor isomer, which was not isolated. Tritylation of compound (**5**) under basic condition in pyridine with trityl chloride at 70 $^{o}$C for 1.0 h afforded trityl ether (**5d**) in 63 %yield. Acetylation at C-19 and C-3 hydroxyl groups afforded (**5e**) and (**5f**).

Acetylation of the remaining C-3 hydroxyl groups of compounds (**5a**-**5d**) were performed by heating in acetic anhydride at 140 $^{o}$C led to products (**6a**-**6d**) in good yields as shown in Table 4.4.

Table 4.3 The synthesis of C-19 8,17-substituted epoxy-14-deoxy-11,12-didehydro-andrographolide.

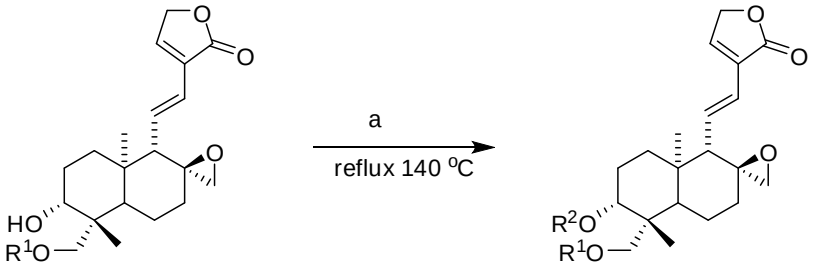



(**5**) (**5a**-**5f**)

| Entry | $R^1$ | $R^2$ | Condition | Temp. ($^oC$) | Time (h) | Compound | % Yield |
|---|---|---|---|---|---|---|---|
| 1 | TIPS | H | a | rt | 4.0 | **5a** | 59 |
| 2 | TBDPS | H | a | rt | 1.0 | **5b** | 74 |
| 3 | TBS | H | a | rt | 1.0 | **5c** | 70 |
| 4 | Tr | H | a | 70 | 1.0 | **5d** | 63 |
| 5 | Ac | H | b | 70 | 4.5 | **5e** | 60 |
| | Ac | Ac | b | | | **5f** | 30 |

Conditions; a) R-Cl, pyridine for (**5a**-**5d**); b) $Ac_2O$, 70 $^oC$ for (**5e**-**5f**).

Table 4.4 Acetylation of C-19 substituted-14-deoxy-11,12-didehydroandrographolide.

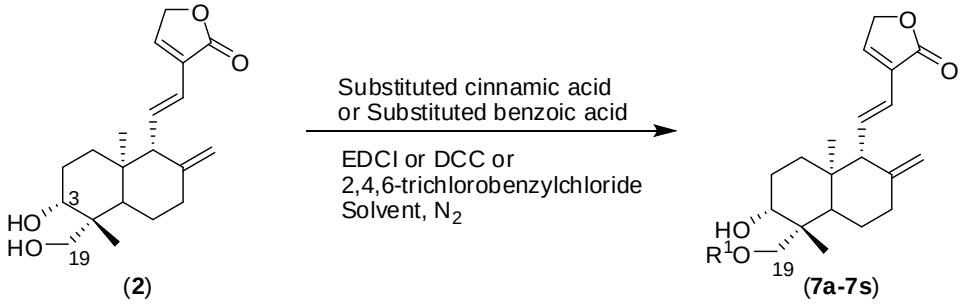



(**5a**-**5d**) (**6a**-**6d**)

| Entry | $R^1$ | $R^2$ | Condition | Time (h) | Compound | % Yield |
|---|---|---|---|---|---|---|
| 1 | TIPS | Ac | a | 1.5 | **6a** | 69 |
| 2 | TBDPS | Ac | a | 1.5 | **6b** | 70 |
| 3 | TBS | Ac | a | 1.5 | **6c** | 70 |
| 4 | Tr | Ac | a | 1.0 | **6d** | 75 |

Condition; a) $Ac_2O$, 140 $^oC$.

## 4.3 Modification of the two hydroxyl groups at C-3 and C-19 of 14-deoxy-11,12-didehydroandrographolide (2) *via* esterification




(**2**) (**7a-7s**)

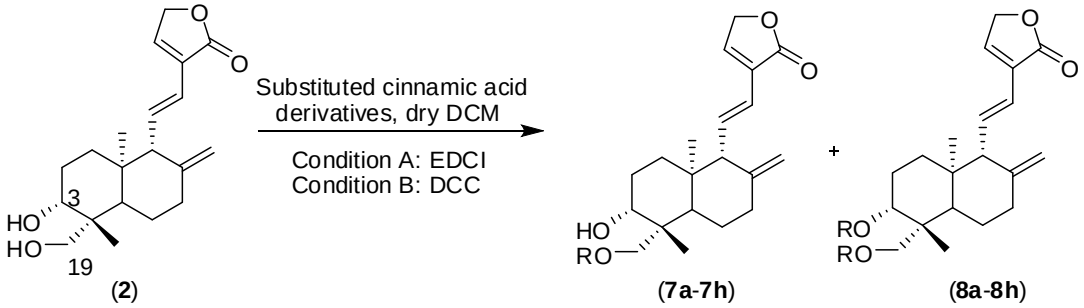
The structure of 14-deoxy-11,12-didehydroandrographolide (**2**) was modified by esterification reaction of hydroxyl group at C-19 position with various carboxylic acid such as cinnamic acid and benzoic acid derivatives to obtain the new derivatives of 14-deoxy-11,12-didehydroandrographolide (**2**).

### 4.3.1 Synthesis of cinnamoyl-14-deoxy-11,12-didehydroandrographolide derivatives by esterification at C-19 with substituted cinnamic acid

The derivatives of 14-deoxy-11,12-didehydroandrographolide (**2**) were obtained by the Steglich esterification to incorporate cinnamoyl groups at C-19 and C-3 positions. Mono-substituted cinnamoyl derivatives were obtained as the major products and disubstituted cinnamoyl derivatives was observed as minor product.

Compound (**7a**) was prepared by esterification of compound (**2**) with cinnamic acids in the presence of 4-dimethylaminopyridine (DMAP) and 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (EDCI) in dry dichloromethane showed moderate yield (28%), while the condition B (DCC) to give impure product with *N′*,*N′*-dicyclohexyl carbodiimide (DCC). The Steglich esterification of compound (**2**) with substituted cinnamoyl derivatives using condition B (DCC) in the presence of catalytic DMAP provided compounds (**7b**-**7h**) and (**8b**-**8h**) show higther than condition A in moderate yield as shown in Table 4.5.

Table 4.5 The synthesis of cinnamoyl-14-deoxy-11,12-didehydroandrographolide derivatives.




(**2**) (**7a**-**7h**) (**8a**-**8h**)

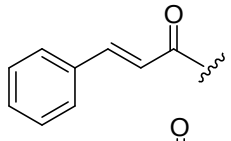
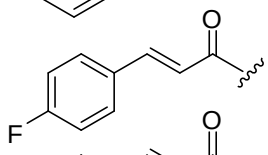
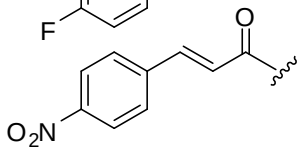
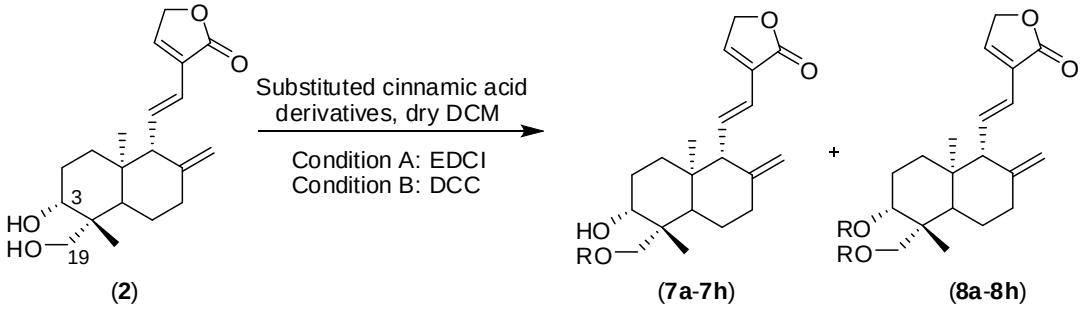
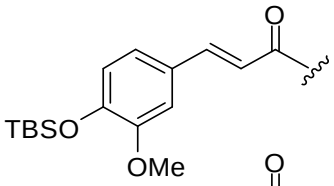
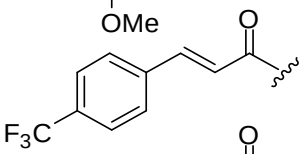
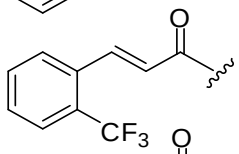
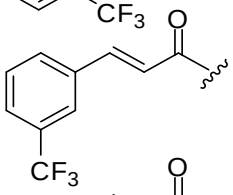
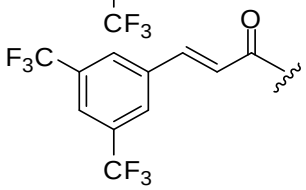
| Entry | R | Time (h) | Condition | Compound | %Yield |
|---|---|---|---|---|---|
| 1 |  | 5.0 | A | **7a** : **8a** | 28 : 16[a] |
| | | 1.0 | B | | >99[b,c] |
| 2 |  | 14.0 | A | **7b** : **8b** | 10 : 23[a] |
| | | 2.0 | B | | 48 : 12[b] |
| 3 |  | 2.0 | A | **7c** : **8c** | 22 : 4[a] |
| | | 1.0 | B | | 44 : 34[b] |

Table 4.5 The synthesis of cinnamoyl-14-deoxy-11,12-didehydroandrographolide derivatives (continue).




(**2**) (**7a**-**7h**) (**8a**-**8h**)

| Entry | R | Time (h) | Condition | Compound | %Yield |
|---|---|---|---|---|---|
| 4 |  | 11.0 | B | **7d** : **8d** | 36 : 0[a] |
| 5 |  | 2.0 | B | **7e** : **8e** | 61: 39[a] |
| 6 |  | 2.0 | B | **7f** : **8f** | 44 : 44[a] |
| 7 |  | 2.0 | B | **7g** : **8g** | 55 : 40[a] |
| 8 |  | 2.0 | B | **7h** : **8h** | 48 : 17[a] |

[a] % yield base on recovered starting material.

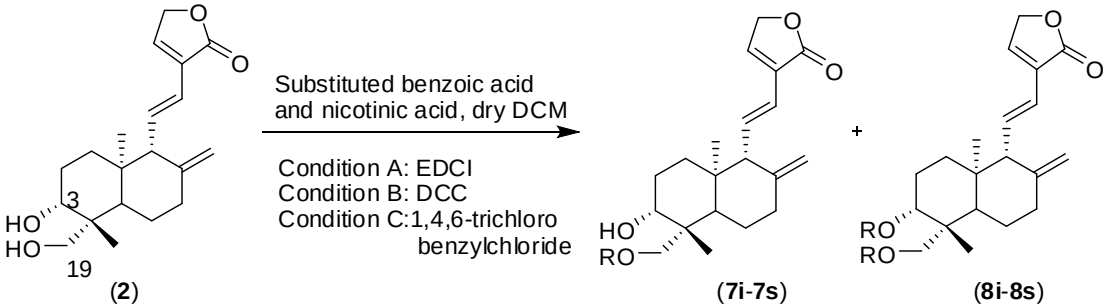
[b] % isolated yield.

[c] complex mixture with DCC.

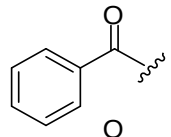
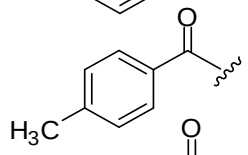
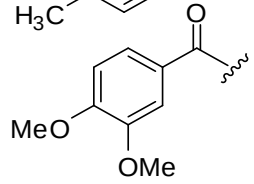
### 4.3.2 Synthesis of 14-deoxy-11,12-didehydroandrographolide derivatives by esterification at C-19 with substituted benzoyl derivatives

Benzoate derivatives of compound (**2**) were prepared by esterification with various substituted benzoic acid to obtain C-19 monobenzoate derivatives (**7i-7s**) as major product. The disubstituted C-3 and C-19 derivatives (**8i-8s**) were observed as minor compound in some cases. Compounds (**7j**) and (**7r**) were prepared by esterification with 4-methylbenzoic acid and 4-formylbenzoic acid respectively, in the presence of EDCI and catalytic DMAP in dry dichloromethane as shown in entries 2 and 10. Where as, other analogues were prepared by esterification using DCC except compound (**7s**). Nicotinyl ester analogue (**7s**) was prepared by Yamaguchi reaction using 2,4,6-trichlorobenzylchloride, TEA and DMAP to obtain product in good yield as shown in entry 11, Table 4.6.

Table 4.6 The synthesis of compound (**2**) with benzoyl derivatives.

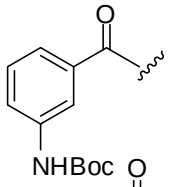
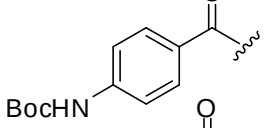
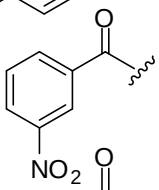
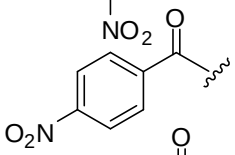
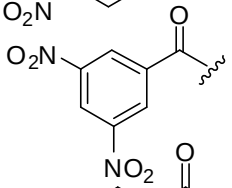
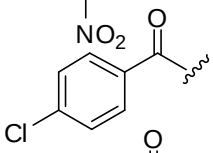
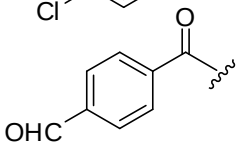
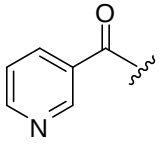



(**2**) (**7i-7s**) (**8i-8s**)

| Entry | R | Time (h) | Condition | Compound | %Yield[a] |
|---|---|---|---|---|---|
| 1 |  | 13.0<br>2.0 | A<br>B | **7i** : **8i** | 66 : 9<br>65 : 31 |
| 2 |  | 2.0<br>1.0<br>2.0 | A<br>B<br>C | **7j** : **8j** | 48 : 4<br>22 : 25<br>complex mixture |
| 3 |  | 2.0<br>2.0 | B<br>C | **7k** : **8k** | 52 : 34<br>32 : 0 |

[a] % yield base on recovered starting material.

Table 4.6 The synthesis of compound (**2**) with benzoyl derivatives (continued).

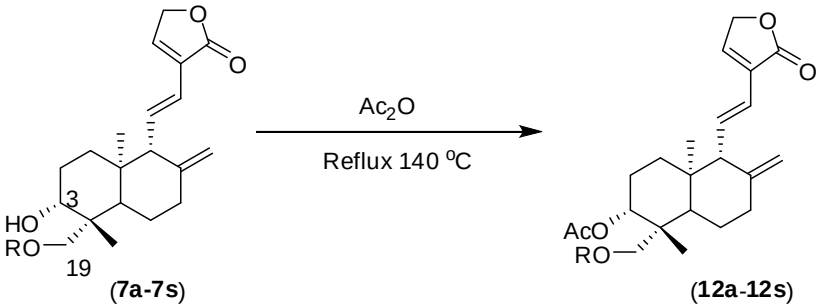
| Entry | R | Time (h) | Condition | Compound | %Yield[a] |
|---|---|---|---|---|---|
| 4 |  | 2.5 | B | **7l** : **8l** | 62 : 0 |
| 5 |  | 2.0 | B | **7m** : **8m** | 66 : 34 |
| 6 |  | 2.0 | B | **7n** : **8n** | 60 : 30 |
| 7 |  | 2.0 | B | **7o** : **8o** | 50 : 30 |
| 8 |  | 2.0 | B | **7p** : **8p** | 50 : 47 |
| | | 2.0 | C | | complex mixture |
| 9 |  | 2.0 | B | **7q** : **8q** | 65 : 35 |
| | | 2.0 | C | | complex mixture |
| 10 |  | 2.0 | A | **7r** : **8r** | 30 : 9 |
| | | 2.0 | B | | 60 : 22[c] |
| | | 2.0 | C | | complex mixture |
| 11 |  | 2.0 | C | **7s** : **8s** | 72 : 0 |

[a] % yield base on recovered starting material.
[c] complex mixture with DCC.

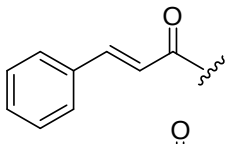
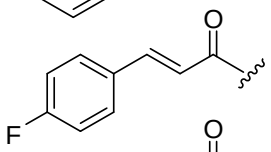
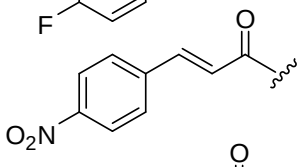
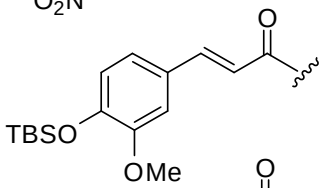
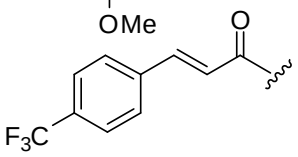
## 4.4 Acetylation reactions at C-3 position of cinnamoyl, benzoyl and nicotinyl ester of 14-deoxy-11,12-didehydroandrographolide (2)

Acetylation of hydroxyl group at C-3 of analogues (**7a**-**7s**) with $Ac_2O$ and reflux at 140 $^oC$ gave analogues (**12**) in good yields except (**12d**) and (**12k**). Esterification of (**7k**) with electron rich dissubstituted cinnamic acid gave low yield of product (entry 4 and 11).

Table 4.7 Synthesis of analogues (**12**) by acetylation at C-3 position of analogues (**7**).

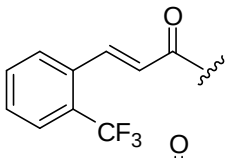
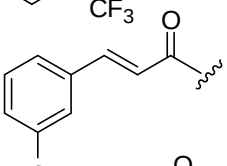
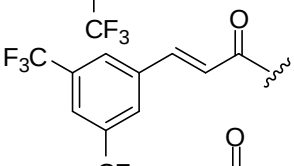
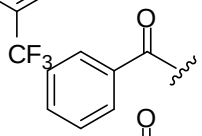
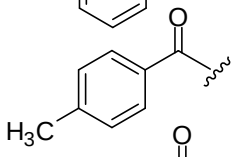
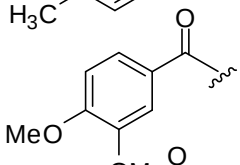
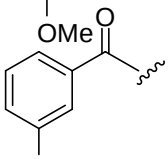
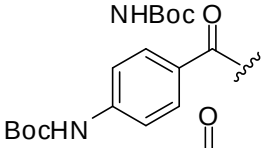
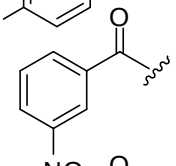
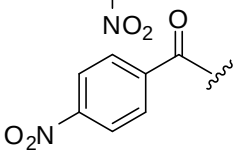



(**7a**-**7s**) (**12a**-**12s**)

| Entry | R | Time (h) | Compound | %Yield[b] |
|---|---|---|---|---|
| 1 |  | 1.0 | **12a** | 62 |
| 2 |  | 1.0 | **12b** | 97 |
| 3 |  | 1.0 | **12c** | 75 |
| 4 |  | 1.0 | **12d** | 27 |
| 5 |  | 1.0 | **12e** | 53 |

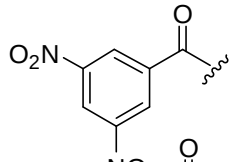
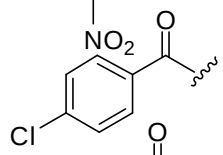
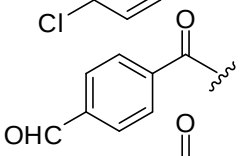
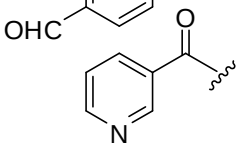
[b] % isolated yield.

Table 4.7 Synthesis of analogues (**12**) by acetylation at C-3 position of analogues (**7**). (continued).

| Entry | R | Time (h) | Compound | %Yield[b] |
|---|---|---|---|---|
| 6 |  | 1.0 | **12f** | 50 |
| 7 |  | 1.0 | **12g** | 52 |
| 8 |  | 1.0 | **12h** | 93 |
| 9 |  | 1.0 | **12i** | 78 |
| 10 |  | 1.0 | **12j** | 75 |
| 11 |  | 1.0 | **12k** | 43 |
| 12 |  | 1.0 | **12l** | 91 |
| 13 |  | 1.0 | **12m** | 73 |
| 14 |  | 1.0 | **12n** | 50 |
| 15 |  | 1.0 | **12o** | 77 |

[b] % isolated yield.

Table 4.7 Synthesis of analogues (**12**) by acetylation at C-3 position of analogues (**7**). (continued).

| Entry | R | Time (h) | Compound | %Yield[b] |
|---|---|---|---|---|
| 16 |  | 1.0 | **12p** | 43 |
| 17 |  | 1.0 | **12q** | 60 |
| 18 |  | 1.0 | **12r** | 38 |
| 19 |  | 1.0 | **12s** | 50 |

[b] % isolated yield.

## 4.5 Cytotoxic activity of 14-deoxy-11,12-didehydroandrographolide derivatives

14-Deoxy-11,12-didehydroandrographolide (2) was modified by conversion of hydroxyl group at C-19 position *via* silylation, alkylation and acetylation. Twenty one analogues were evaluated for cytotoxic activity in ten cancer cell lines as shown in table 4.8 to 4.9. The *in vitro* screening was carried out in selected nine cancer cell lines including P-388 (murine leukaemia cell line), KB (human epidermoid carcinoma of the mouth), HT-29 (human Colorectal Adenocarcinoma Cell Line), MCF-7 (human breast cancer), LU-1 (human lung cancer), ASK (rat glioma), KKU M-213 (adenosquamous cell carcinoma), HUCCA-1 (human cholangio ncarcinoma cell line), KKU-100 (poorly differentiateadnocarcinoma) and HEK-293 (vero cell), were also checked. The sulforhodamine B (SRB) assay was carried out determining the antiproliferative activities of the synthesized compounds. All tested analogues were dissolved in DMSO (0.1%). Ellipticine, potent anti-cancer agent was used as a positive control. As shown in Tables 4.8 to 4.9, the obtained results were expressed as $ED_{50}$ values (drug concentration causing 50% growth inhibition).

Eight out of twenty one synthetic analogues (**3a**-**3d**), (**4c**), (**5a**-**5b**) and (**5d**) displayed cytotoxic activity stronger than that of the parent compound (**2**) in all cancer cell lines. Comparison on the cytotoxic activities of C-19 mono-substituted analogues, (**3a**-**3d**) and (**4e**) revealed the importance of the protecting group of the hydroxyl function at C-19 on the 14-deoxy-11,12-didehydroandrographolide core.

In the silyl series, 19-*O*-TBDPS analogue (**3b**) showed higher activity than silylether analogues (**3a**) and (**3c**) in several cell line. Moreover, (**3b**) also showed better activity than trityl ether analogue (**3d**) and acetyl compound (**4e**) in seven cancer cell lines (KB, HT-29, MCF-7, A-549, KUU M-213, HUCCA-1 and K-100). TIPS-analogue (**3a**) showed the highest activity on ASK cancer cell with $ED_{50}$ value 7.35 μM, while (**3c**) show high selective and stronger cytotoxicity on K-100 cancer cell than positive control ellipticine. Trityl analogue (**3d**) exhibited highest cytotoxic activity to P-388 cell with $ED_{50}$ value of 3.87 μM.

In contrast to 19-*O*-substituted analogues, the 3,19-*O*-disubstituted compounds showed dramatic decrease of anti-cancer activity resulting from the substituents of acetate groups at C-3 except (**4c**). Compound (**4c**) exhibited increased activity after modification of the hydroxyl group to the acetate group compared to (**3c**). Analogue **4a** exhibited the potent cytotoxic activity on KKU-100 cancer cell lines with $ED_{50}$ value of 4.10 μM which were showed cytotoxicity stronger than that of the drug ellipticine (Table 4.8).

The modification of exo-alkene C-8 of 14-deoxy-11,12-didehydroandrogra-pholide to compound epoxide (**5**) led to the dramatic decrease of cytotoxicity in every cell line compared with parent (**2**). However, modification of epoxy analogue (**5**) by conversion C-19 hydroxyl to silyl-, tritry-ether and acetyl led to the increasing of cytotoxic activity. Compounds (**5a**), (**5b**) and (**5d**) exhibited cytotoxic activites stronger than that of compounds (**3a**), (**3b**) and (**3d**) on several cancer cell. Among the epoxide analogues, compounds (**5a**) and (**5b**) showed the ability to inhibit cancer cells, especially cholangiocarcinoma (KKU M-213 and KKU-100) than positive control ellipticine. Compound (**5a**) also showed highest activity against HT-29 cancer cell with $ED_{50}$ values of 2.28 μM, stronger than the positive control. Finally, acetyl analogues (**6a**-**6e**) decreased obviously in cytotoxicity compared with compounds (**5a**-**5e**) indicating that the acetate group play a crucial role in reducing cytotoxicity as showed in Table 4.9.

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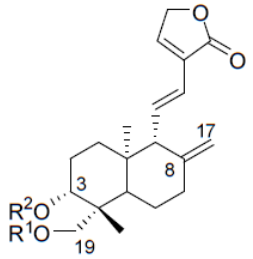
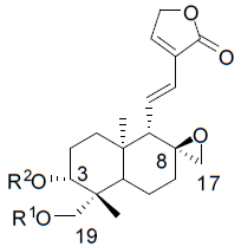
Table 4.8 Cytotoxic activity of 14-deoxy-11,12-didehydroandrographolide derivative against nine human cancer cells and one vero cell.




| Compound. | $R^1$ | $R^2$ | $ED_{50}$ (μM)[a] (SRB assay) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | P-388 | KB | HT-29 | MCF-7 | A-549 | ASK | M-213 | HUCCA-1 | K-100 | HEK-293 |
| **2** | H | H | 35.01±0.28 | 5.07±0.03 | 28.55±0.43 | 18.67±0.89 | 28.15±1.45 | > 50 | > 50 | 35.83±0.73 | 36.86±3.37 | 7.53±0.20 |
| **3a** | TIPS | H | 5.96 ±0.02 | 4.70 ±0.02 | 7.14 ±0.09 | 5.46 ±0.08 | 7.95 ±0.20 | 7.35 ±0.23 | 7.06±0.65 | 6.72±0.27 | 18.24±0.69 | 5.28 ±0.25 |
| **3b** | TBDPS | H | 5.51±0.20 | 4.45±0.03 | 6.48±0.07 | 4.44±0.25 | 6.49±0.11 | 7.72±0.58 | 5.07±0.04 | 5.53±0.20 | 14.74±0.59 | 4.40±0.03 |
| **3c** | TBS | H | 8.24±0.29 | 5.12±0.05 | 8.48±0.20 | 7.61±0.25 | 17.16±0.12 | 9.61±0.10 | 27.37±2.78 | 18.17±2.39 | 3.09±0.70 | 6.61±0.21 |
| **3d** | Tr | H | 3.87±0.16 | 4.94±0.03 | 8.38±0.09 | 4.86±0.10 | 6.79±0.10 | 7.45±0.16 | 5.42±0.17 | 7.78±0.27 | 36.24±1.16 | 4.68±0.07 |
| **4a** | TIPS | Ac | 12.14±0.41 | 7.17±0.42 | 17.91±0.25 | 7.07±0.21 | 22.02±0.54 | 29.42±0.06 | 12.62±0.78 | 10.23±-.15 | 4.10±0.21 | 8.28±0.32 |
| **4b** | TBDPS | Ac | 14.53±0.75 | 5.15±0.04 | 32.51±0.63 | 7.77±0.06 | 9.47±0.13 | >50 | 16.03±0.16 | 24.29±1.44 | >50 | 5.57±0.16 |
| **4c** | TBS | Ac | 6.56±0.05 | 4.94±0.02 | 12.82±1.24 | 6.37±0.10 | 9.18±0.01 | 16.23±1.30 | 12.08±0.82 | 15.96±0.82 | 14.02±1.57 | 6.36±0.20 |
| **4d** | Tr | Ac | 12.87±0.98 | 6.36±0.02 | 21.89±0.91 | 9.12±0.04 | 12.00±0.95 | 39.86±-.75 | 9.98±0.10 | 38.55±1.20 | >50 | 7.02±0.20 |
| **4e** | Ac | H | 27.65±1.25 | 25.66±0.54 | 45.04±0.10 | 31.07±1.39 | 32.75±1.26 | >50 | >50 | >50 | 40.25±2.70 | 27.77±0.71 |
| **4f** | Ac | Ac | 27.68±1.39 | 25.72±0.74 | 41.87±0.78 | 28.78±0.24 | 33.96±0.30 | 42.45±1.46 | 37.30±0.97 | 40.93±1.08 | >50 | 24.96±0.50 |
| **Ellipticine** | | | 2.12±0.17 | 2.34±0.03 | 2.68±0.17 | 1.66±0.09 | 2.37±0.21 | 2.17±0.23 | 4.75±0.43 | 3.46±0.83 | 4.16±0.23 | 2.27±0.10 |

[a] including P-388 (murine leukaemia cell line), KB (human epidermoid carcinoma of the mouth), HT-29 (Human Colorectal Adenocarcinoma Cell Line), MCF-7 (human breast cancer), LU-1 (human lung cancer), ASK (rat glioma), M-213 (adenosquamous cell carcinoma), HUCCA-1 (human cholangiocarcinoma cell line ), K-100 (poorly differentiate adnocarcinoma), HEK-293 (vero cell),. Ellipticine (Ellipt) was used as a positive control. The results were expressed as $ED_{50}$ values (drug concentration causing 50% growth inhibition) in μM.

Table 4.9 Cytotoxic activity of 8,17-epoxy-14deoxy-11,12-didehydroandrographolide derivative against nine human cancer cells and one vero cell.




| Compound. | $R^1$ | $R^2$ | $ED_{50}$ (μM)[a] (SRB assay) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | P-388 | KB | HT-29 | MCF-7 | A-549 | ASK | M-213 | HUCCA-1 | K-100 | HEK-293 |
| **5** | H | H | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| **5a** | TIPS | H | 3.86±0.08 | 4.59±0.03 | 2.28±0.42 | 4.98±0.12 | 6.58±0.01 | 6.60±0.09 | 3.37±0.31 | 7.35±0.13 | 2.93±0.36 | 4.04±0.23 |
| **5b** | TBDPS | H | 4.13±0.08 | 4.02±0.04 | 4.43±0.04 | 4.23±0.04 | 6.22±0.15 | 6.81±0.15 | 3.08±0.17 | 6.41±0.13 | 3.27±0.18 | 4.97±0.16 |
| **5c** | TBS | H | 15.07±2.47 | 19.24±1.01 | 7.24±0.09 | 8.18±0.11 | 9.36±0.18 | 18.97±4.58 | 8.57±0.21 | >50 | 9.25±0.10 | 6.15±0.00 |
| **5d** | Tr | H | 3.33±0.12 | 4.92±0.05 | 6.21±0.05 | 5.38±0.09 | 5.88±0.10 | 6.47±0.18 | 4.68±0.18 | 7.81±0.07 | 28.75±2.68 | 5.39±0.14 |
| **6a** | TIPS | Ac | 7.84±0.14 | 8.48±0.11 | 7.40±0.08 | 7.84±0.08 | 8.65±0.18 | 15.72±1.28 | 7.22±0.30 | 8.92±0.16 | 17.51±0.31 | 7.39±0.07 |
| **6b** | TBDPS | Ac | 5.25±0.11 | 5.19±0.10 | 5.22±0.05 | 6.38±0.12 | 8.31±0.10 | 8.96±0.33 | 5.13±0.11 | 8.08±0.02 | 4.50±0.18 | 6.01±0.05 |
| **6c** | TBS | Ac | 7.45±0.07 | 25.81±0.15 | 18.59±0.74 | 16.55±0.27 | 24.10±0.41 | 22.41±0.76 | 19.13±1.69 | 23.35±0.14 | 16.44±059 | 18.08±0.56 |
| **6d** | Tr | Ac | 5.28±0.08 | 5.12±0.01 | 6.74±0.11 | 6.84±0.05 | 8.16±0.29 | 8.29±0.40 | 10.16±2.35 | 9.74±0.09 | 14.09±0.20 | 5.99±0.05 |
| **6e** | Ac | H | 19.97±0.44 | >50 | 48.93±0.16 | 43.50±1.30 | 46.26±1.89 | >50 | 48.93±2.56 | >50 | >50 | 38.13±0.74 |
| **6f** | Ac | Ac | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| **Ellipticine** | | | 2.12±0.17 | 2.34±0.03 | 2.68±0.17 | 1.66±0.09 | 2.37±0.21 | 2.17±0.23 | 4.75±0.43 | 3.46±0.83 | 4.16±0.23 | 2.27±0.10 |

[a] including P-388 (murine leukaemia cell line), KB (human epidermoid carcinoma of the mouth), HT-29 (Human Colorectal Adenocarcinoma Cell Line), MCF-7 (human breast cancer), LU-1 (human lung cancer), ASK (rat glioma), M-213 (adenosquamous cell carcinoma), HUCCA-1 (human cholangiocarcinoma cell line ), K-100 (poorly differentiate adnocarcinoma), HEK-293 (vero cell),. Ellipticine (Ellipt) was used as a positive control. The results were expressed as $ED_{50}$ values (drug concentration causing 50% growth inhibition) in μM.

## 4.6 Compounds characterization.

The products were characterized by spectroscopic methods (NMR)

**14-Deoxy-11,12-didehydroandrographolide (2)**; White solid; yield 76%; Mp: 179-181 $^{o}$C; $R_f$ = 0.48 (3:1 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, H-20), 1.25 (3H, s, H-18), 3.35 (1H, d, *J* = 11.0 Hz, H-19b), 3.47 (1H, dd, *J* = 11.0, 4.0 Hz, H-3), 4.21 (1H, d, *J* = 11.0 Hz, H-19a), 4.52 (1H, s, H-17b), 4.77 (1H, s, H-17a), 4.81 (1H, s, H-15), 6.11 (1H, d, *J* = 15.5, H-12), 6.86 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.16 (1H, s, H-14)

**19-*O*-TIPS-14-deoxy-11,12-didehydroandrographolide (3a)**; A pale yellow oil; yield 63%; $R_f$ = 0.52 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s, H-20), 1.07 (3H, s, 3×SiC*H*$(CH_3)_2$), 1.08 (18H, s, 3×SiCH(C*H*$_3)_2$), 1.30 (3H, s, H-18), 3.33 (1H, m, H-3), 3.51 (1H, d, *J* = 10.0 Hz, H-19b), 4.35 (1H, d, *J* = 10.0 Hz, H-19a), 4.54 (1H, d, *J* = 1.5 Hz, H-17b), 4.77 (1H, d, *J* = 1.5 Hz, H-17a), 4.81 (2H, d, *J* = 2.0 Hz, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.92 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.16 (1H, t, *J* = 2.0 Hz, H-14)

**19-*O*-TBDPS-14-deoxy-11,12-didehydroandrographolide (3b)**; White solid; yield 70%; Mp: 67-68 $^{o}$C; $R_f$ = 0.37 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.56 (3H, s, H-20), 1.05 (9H, s, SiC(C*H*$_3)_3$), 1.33 (3H, s, H-18), 3.35 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 3.37 (1H, d, *J* = 10.0 Hz, H-19b), 4.20 (1H, d, *J* = 10.0 Hz, H-19a), 4.45 (1H, d, *J* = 1.0 Hz, H-17b), 4.67 (1H, d, *J* = 1.0 Hz, H-17a), 4.79 (2H, d, *J* = 1.5 Hz, H-15), 6.08 (1H, d, *J* = 15.5 Hz, H-12), 6.84 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.13 (1H, t, *J* = 1.5 Hz, H-14), 7.37-7.47 (6H, m, Ar-H), 7.63-7.69 (4H, m, Ar-H)

**19-*O*-TBS-14-deoxy-11,12-didehydroandrographolide (3c)**; White solid; yield 79%; Mp: 89-90 $^{o}$C; $R_f$ = 0.29 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.15 (6H, s, Si(C*H*$_3)_2$), 0.91 (3H, s, C-20), 0.98 (9H, s, SiC(C*H*$_3)_3$), 1.32 (3H, s, H-18), 3.39 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 3.49 (1H, d, *J* = 10.0 Hz, H-19b), 4.30 (1H, d, *J* = 10.0 Hz, H-19b), 4.62 (1H, d, *J* = 1.5 Hz, H-17b), 4.58 (1H, d, *J* = 1.5 Hz, H-17a), 4.89 (2H, d, *J* = 1.5 Hz, H-15), 6.20 (1H, d, *J* = 15.5 Hz, H-12), 6.99 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.24 (1H, t, *J* = 1.5 Hz, H-14)

**19-*O*-Tr-14-deoxy-11,12-didehydroandrographolide (3d)**; White solid; yield 99%; Mp: 176-178 $^{o}$C; $R_f$ = 0.73 (2:3 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.30 (3H, s, H-20), 1.56 (3H, s, H-18), 3.15 (1H, d, *J* = 9.0 Hz, H-19b), 3.22 (1H, m, H-3), 3.37 (1H, d, *J* = 9.0 Hz, H-19a), 4.43 (1H, s, H-17b), 4.67 (1H, s, H-17a), 4.77 (2H, s, H-15), 6.05

(1H, d, *J* = 15.5, H-12), 6.81 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.09 (1H, s, H-14), 7.24 (3H, t, *J* = 7.5 Hz, Ar-H ), 7.31 (6H, t, *J* = 7.5 Hz, Ar-H ), 7.44 (6H, d, *J* = 7.5 Hz, Ar-H)

**19-*O*-TIPS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide(4a)**; Yellow oil; yield 64 %; $R_f$ = 0.49 (7:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.01 (3H, s, H-18), 1.07 (21H, s, 3xSiC*H*($CH_3$)$_2$ ), 2.03 (3H, s, $COCH_3$), 3.81 (1H, d, *J* = 10.0 Hz, H-19b), 3.91 (1H, d, *J* = 10.0 Hz, H-19a), 4.53 (1H, s, H-17b), 4.58 (1H, m, H-3), 4.78 (1H, s, H-17a), 4.80 (2H, s, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.15 (1H, s, H-14)

**19-*O*-TBDPS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (4b)**; White solid; yield 95 %; Mp: 141-143 $^o$C; $R_f$ = 0.67 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, brs, H-20), 1.06 (9H, s, Si($CH_3$)$_3$), 1.25 (3H, s, H-18), 1.90 (3H, s, COC$H_3$), 3.73 (1H, d, *J* = 10.5 Hz, H-19b), 3.83 (1H, d, *J* = 10.5 Hz, H-19a), 4.53 (1H, brs, H-17b), 4.57 (1H, m, H-3), 4.79 (1H, brs, H-17a), 4.80 (1H, brs, H-15), 6.11 (1H, d, *J* = 15.5 Hz, H-12), 6.92 (1H, dd, *J* = 15.0, 10.0 Hz, H-11), 7.14 (1H, s, H-14), 7.34-7.48 (6H, m, Ar-H), 7.65-7.71 (4H, m, Ar-H)

**19-*O*-TBS-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (4c)**; White solid; yield 64 %; Mp: 136-138 $^o$C; $R_f$ = 0.56 (7:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.03 (Si($CH_3$)$_2$), 0.88 (Si($CH_3$)$_3$), 0.95 (3H, s, H-20), 1.25 (3H, s, H-18), 2.04 (3H, s, COC$H_3$), 3.61 (1H, d, *J* = 10.0, H-19b), 3.83 (1H, d, *J* = 10.0, H-19a), 4.52 (1H, brs, H-17b), 4.58 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 4.77 (1H, brs, H-17a), 4.81 (2H, brs, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.15 (1H, brs, H-14)

**19-*O*-Tr-3-*O*-acetyl-14-deoxy-11,12-dide-hydroandrographolide (4d)**; White solid; yield 75 %; Mp: 97-99 $^o$C; $R_f$ = 0.58 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.40 (3H, s, H-20), 1.18 (3H, s, H-18), 2.00 (3H, s, COC$H_3$), 3.14 (1H, d, *J* = 9.0 Hz, H-19b), 3.35 (1H, d, *J* = 9.0 Hz, H-19b), 4.46 (1H, s, H-17b), 4.55 (1H, m, H-3), 4.72 (1H, s, H-17a), 4.78 (2H, s, H-15), 6.07 (1H, d, *J* = 15.5 Hz, H-12), 6.84 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.11 (1H, s, H-14), 7.19-7.32 (9H, m, Ar-H), 7.47 (6H, m, Ar-H)

**19-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (4e)**; A pale yellow oil; yield 59%; $R_f$ = 0.49 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, s, H-20), 1.18 (3H, s, H-18), 2.07 (3H, s, $COCH_3$), 3.34 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 4.17 (1H, d, *J* = 11.5, H-19b), 4.36 (1H,d, *J* = 11.5 Hz, H-19a), 4.56 (1H, d, *J* = 1.0 Hz, H-17b), 4.81

(1H, d, *J* = 1.0 Hz, H-17a), 4.83 (2H, d, *J* = 1.5 Hz , H-15), 6.14 (1H, d, J = 15.5, H-12), 6.90 (1H, dd, J = 15.5, 10.0 Hz, H-11), 7.19 (1H, t, *J* = 2.0 Hz, H-14)

**3,19-*O*-diacetyl-14-deoxy-11,12-didehydroandrographolide (4f)**; White solid; yield 64 %; Mp: 89-90 $^{o}$C; $R_f$ = 0.61 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, s, H-20), 1.06 (3H, s, H-18), 2.06 (3H, s,COC$H_3$), 2.07 (3H, s,COC$H_3$), 4.17 (1H, d, *J* = 11.5, H-19b), 4.40 (1H, d, *J* = 11.5 Hz, H-19a), 4.57 (1H, d, *J* = 1.5 Hz, H-17b), 4.61 (1H, m, H-3), 4.81 (1H, d, *J* = 1.5 Hz, H-17a), 4.83 (1H, d, *J* = 2.0 Hz , H-15), 6.15 (1H, d, *J* = 16.0, H-12), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.17 (1H, t, *J* = 2.0 Hz, H-14)

**8,17-Epoxy-14-deoxy-11,12-didehydroandrographolide (5)**; White solid; yield 99 % mixisomer; Mp. 170-172 $^{o}$C; $R_f$ = (0.32, EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, H-20), 1.20 (3H, s, H-18), 2.52 (1H, d, *J* = 4.0 Hz, H-17b), 2.75 (1H, d, *J* = 4.0 Hz, H-17a), 3.31 (1H, d, *J* = 11.0 Hz, H-19b), 3.40 (1H, dd, *J* = 11.0, 4.0 Hz, H-3), 4.17 (1H, d, *J* = 11.0 Hz, H-19a), 4.75 (2H, s, H-15), 6.10 (1H, d, *J* = 15.5 Hz, H-12), 6.45 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.13 (1H, s, H-14).

**19-*O*-TIPS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5a)**; Yellow oil; yield 59 %; $R_f$ = 0.58 (2:3 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, s, H-20), 1.10 (21H, s, SiC$H$($CH_3$)$_6$), 1.32 (3H, s, H-18), 2.55 (1H, brs, H-17b), 2.81 (1H, brs, H-17a), 3.33 (1H, m, H-3), 3.55 (1H, d, *J* = 9.5 Hz, H-19b), 4.37 (1H, d, *J* = 9.5 Hz, H-19a), 4.78 (2H, s, H-15), 6.17 (1H, d, *J* = 15.5 Hz, H-12), 6.57 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.14 (1H, s, H-14)

**19-*O*-TBDPS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5b)**; Yellow oil; yield 74 %; $R_f$ = 0.50 (2:3 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-20), 1.06 (9H, s, SiC($CH_3$)$_3$), 1.36 (3H, s, H-18), 2.44 (1H, brs, H-17b), 2.71(1H, brs, H-17a), 3.35 (1H, m, H-3), 3.42 (1H, d, *J* = 10.0 Hz, H-19b), 4.21 (1H, d, *J* = 10.0 Hz, H-19a), 4.77 (2H, s, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.50 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.11 (1H, s, H-14), 7.38-7.50 (6H, m, Ar-H), 7.68 (4H, t, *J* = 7.0 Hz, Ar-H)

**19-*O*-TBS-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5c)**; White solid; yield 70 %; Mp: 173-175 $^{o}$C; $R_f$ = 0.54 (1:1 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.11(6H, s, Si($CH_3$)$_2$), 0.88 (9H, s, SiC($CH_3$)$_3$), 0.96 (3H, s, H-20), 1.24 (3H, s, H-18), 2.54 (1H, d, *J* = 4.0 Hz, H-17b), 2.80 (1H, d, *J* = 4.0 Hz, H-17a), 3.30 (1H, dd, *J* = 11.5,

4.0 Hz, H-3), 3.43 (1H,d, *J* = 10.0 Hz, H-19b), 4.22 (1H, d, *J* = 10.0 Hz, H-19a), 4.77 (2H, s, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.55 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.12 (1H, t, *J* = 1.5 Hz, H-14)

**19-*O*-Tr-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5d)**; Yellow oil; yield 75 %; $R_f$ = 0.50 (2:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.43 (3H, s, H-20), 1.59 (6H, s, H-18), 2.45 (1H, s, H-17b), 2.69 (1H, s, H-17a), 3.21 (1H, m, H-19b), 3.38 (1H, d, *J* = 8.0 Hz, H-3), 4.10 (1H, d, *J* = 8.0 Hz, H-19a), 4.75 (2H, s, H-15), 6.10 (1H, d, *J* = 15.5 Hz, H-12), 6.46 (1H, dd, *J* = 15.5, 10.5 Hz, H-11), 7.08 (1H, s, H-14), 7.28-7.36 (9H, m, Ar-H), 7.45 (6H, m, Ar-H)

**19-*O*-TIPS-3-*O*-acetyl-8,17epoxy-14-deoxy-11,12-didehydroandrogra-pholide (6a)**; Yellow oil; yield 69 %; $R_f$ = 0.57 (7:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, s, H-20), 1.08 (21H, s, Si*CH*($CH_3$)$_6$), 1.62 (3H, s, H-18), 2.03 (3H, s, COC$H_3$), 2.57 (1H, d, *J* = 4.0 Hz, H-17b), 2.82 (1H, d, *J* = 4.0 Hz, H-17a), 3.82 (1H, d, *J* = 10.5 Hz, H-19b), 3.97 (1H, d, *J* =10.5 Hz, H-19a), 4.55 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 4.78 (2H, s, H-15), 6.17 (1H, d, *J* = 15.5 Hz, H-12), 6.60 (1H, dd, *J* = 15.0, 10.0 Hz, H-11), 7.13 (1H, s, H-14)

**19-*O*-TBPS-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrogra-pholide (6b)**; Yellow solid; yield 70%; Mp: 119-111$^o$C; $R_f$ = 0.47 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, s, H-20), 1.07 (12H, s, H-18, Si($CH_3$)$_6$), 1.90 (3H, s, COC$H_3$), 2.57 (1H, s, H-17b), 2.81(1H, s, H-17a), 3.75 (1H, d, *J* = 10.5 Hz, H-19b), 3.87 (1H, d, *J* = 10.5 Hz, H-19a), 4.55 (1H, m, H-3), 4.77 (2H, s, H-15), 6.15 (1H, d, *J* = 15.0 Hz, H-12), 6.57 (1H, dd, *J* = 15.0, 10.0 Hz, H-11), 7.12 (1H, s, H-14), 7.35-7.46 (6H, m, Ar-H), 7.67 (4H, d, , *J* = 6.0 Hz, Ar-H)

**19-*O*-TBS-3-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrogra-pholide (6c)**; Yellow solid; yield 70 %; Mp: 159-161 $^o$C; $R_f$ = 0.56 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.01 (6H, s, Si($CH_3$)$_2$), 0.90 (18H, s, Si($CH_3$)$_3$), 1.11 (3H, s, H-20), 1.25 (3H, s, H-18), 2.04 (3H, s, COC$H_3$), 2.58 (1H, s, H-17b), 2.83 (1H, s, H-17a), 3.61 (1H, d, *J* = 10.5 Hz, H-19b), 3.89 (1H, d, *J* = 10.5 Hz, H-19a), 4.58 (1H, d, *J* = 11.5 Hz, H-3), 4.78 (2H, s, H-15), 6.16 (1H, m, H-12), 6.60 (1H, m, H-11), 7.13 (1H, s, H-14)

**19-*O*-Tr-3-*O*-acetyl-8,17epoxy-14-deoxy-11,12-didehydroandrographo-lide (6d)**; Yellow oil; yield 75 %; $R_f$ = 0.32 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.55 (3H, s, H-20), 1.63 (3H, s, H-18), 2.10 (3H, s, COC$H_3$), 2.49 (1H, d, *J* = 4.0 Hz, H-17a), 2.72 (1H, d, *J* = 4.0 Hz, H-17a), 3.18 (1H, d, *J* = 9.0 Hz, H-19b), 3.39 (1H, d, *J* = 9.0 Hz, H-19a), 4.55 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 4.76 (2H, s, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.48 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.10 (1H, s, H-14), 7.24 (3H, t, *J* = 7.0 Hz, Ar-H), 7.30 (6H, t, *J* = 7.0 Hz, Ar-H), 7.48 (6H, d, *J* = 7.5 Hz, H-Ph)

**19-*O*-acetyl-8,17-epoxy-14-deoxy-11,12-didehydroandrographolide (5e)**; Yellow oil; yield 60 %; $R_f$ = 0.48 (2:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.99 (3H, s, H-20), 1.17 (3H, s, H-18), 2.07 (3H, s, COC$H_3$), 2.58 (1H, d, *J* = 4.0 Hz, H-17b), 2.82 (1H, d, *J* = 4.0 Hz, H-17a), 3.34 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 4.20 (1H, d, *J* = 11.5 Hz, H-19b), 4.36 (1H, d, *J* = 11.8 Hz, H-19a), 4.79 (2H, s, H-15), 6.17 (1H, d, *J* = 15.5, H-12), 6.56 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.15 (1H, s, H-14)

**3,19-*O*-diacetyl-14-deoxy-11,12-didehydroandrographolide (5f)**; Yellow solid; yield 30 %; Mp: 119-111$^o$C; $R_f$ = 0.47 (2:3 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.05 (6H, s, H-18, 20), 2.05 (3H, s, COC$H_3$), 2.07 (3H, s, COC$H_3$), 2.59 (1H, s, H-17b), 2.83 (1H, s, H-17a), 4.20 (1H, d, *J* = 12.0 Hz, H-19b), 3.37 (1H, d, *J* = 12.0 Hz, H-19a), 4.61 (1H, t, *J* = 7.5 Hz, H-3), 4.80 (2H, s, H-15), 6.18 (1H, d, *J* = 15.5, H-12), 6.58 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.16 (1H, s, H-14)

**19-*O*-Cinnamoyl-14-deoxy-11,12-didehydroandrographolide** (**7a**); White solid; yield 28 %; Mp: 170-171 $^o$C; $R_f$ = 0.39 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-20)**,** 1.23 (3H, s, H-18), 3.36 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.31 (1H, d, *J* = 11.5 Hz, H-19b), 4.49 (1H, d, *J* = 11.5 Hz, H-19a), 4.56 (1H, d, *J* = 1.5 Hz, H-17b), 4.81 (1H, d, *J* = 1.5 Hz, H-17a), 4.83 (2H, brs, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.42 (1H, d, *J* = 15.5 Hz, H-2′), 6.90 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, brs, H-14), 7.39 (3H, m, Ar-H), 7.53 (2H, m, Ar-H), 7.67 (1H, d, *J* = 15.5 Hz, H-3′)

**19-*O*-(4′-Fluoro)cinnamoyl-14-deoxy-11,12-didehydroandrographolide** (**7b**); White solid; yield 48 %; Mp: 87-88 $^o$C; $R_f$ = 0.39 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-20), 1.22 (3H, s, H-18), 3.36 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.49 (1H, d, *J* = 11.5 Hz, H-19a), 4.56 (1H, brs, H-17b), 4.81 (1H, brs, H-17a), 4.82 (2H, brs, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.35 (1H, d, *J* = 15.5

Hz, H-2′), 6.9 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.08 (2H, t, *J* = 8.5 Hz, Ar-H), 7.19 (1H, brs, H-14), 7.52 (2H, q, *J* = 8.5, 5.5 Hz, Ar-H), 7.63 (1H, d, *J* = 15.5 Hz, H-3′)

**19-*O*-(4′-Nitro)cinnamoyl-14-deoxy-11,12-didehydroandrographolide** (**7c**); White solid; yield 44 %; Mp: 147-149 $^{o}$C; $R_f$ = 0.34 (10:1 DCM:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-20) 1.23 (3H, s, H-18), 3.38 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 4.31 (1H, d, *J* = 11.5 Hz, H-19b), 4.53 (1H, d, *J* = 11.5 Hz, H-19a), 4.56 (1H, brs, H-17b), 4.81 (1H, brs, H-17a), 4.83 (2H, brs, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.55 (1H, d, *J* = 15.5 Hz, H-2′), 6.91 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, brs, H-14), 7.70 (1H, d, *J* = 15.5 Hz, H-3′), 7.69 (2H, d, *J* = 8.0 Hz, Ar-H ), 8.26 (2H, d, *J* = 8.0 Hz, Ar-H)

**19-*O*-(3′-Methoxyl-4′-TBS)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7d)**; White solid; yield 36%; Mp: 113-114 $^{o}$C; $R_f$ = 0.45 (10:1 DCM: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.18 (6H, s, Si($CH_3$)$_2$), 0.90 (3H, s, H-20), 1.00 (9H, s, SiC($CH_3$)$_3$), 1.21 (3H, s, H-18), 3.35 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 3.85 (3H, s, $OCH_3$), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.47 (1H, d, *J* = 11.5 Hz, H-19a), 4.56 (1H, brs, H-17b), 4.81 (1H, brs, H-17a), 4.82 (2H, brs, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.28 (1H, d, *J* = 15.5 Hz, H-2′), 6.84 (1H, d, *J* = 8.5 Hz ), 6.90 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.01 (1H, brs, Ar-H), 7.02 (1H, m, Ar-H), 7.19 (1H, brs, H-14), 7.60 (1H, d, *J* = 15.5 Hz, H-3′)

**19-*O*-(4′-Trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7e)**; White solid; yield 61%; Mp: 97-99 $^{o}$C; $R_f$ = 0.67 (10:1 DCM: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-20), 1.22 (3H, s, H-18), 3.36 (1H, dd, *J* = 11.5, 4.0 Hz, H-3), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.50 (1H, d, *J* = 11.5 Hz, H-19a), 4.54 (1H, brs, H-17b), 4.79 (1H, brs, H-17a), 4.82 (2H, s, H-15), 6.13 (1H, d, *J* = 15.5 Hz, H-12), 6.48 (1H, d, *J* = 15.5 Hz, H-2′), 6.89 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, s, H-14), 7.62 (4H, m, Ar-H), 7.67 (1H, d, *J* = 15.5 Hz, H-3′)

**19-*O*-(2′-Trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandrographolide (7e)**; A pale yellow oil; yield 61%; $R_f$ = 0.64 (10:1 DCM:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, s, H-20), 1.20 (3H, s, H-18), 3.35 (1H, dd, *J* = 11.0, 4.0 Hz, H-3), 4.29 (1H, d, *J* = 11.5 Hz, H-19b), 4.47 (1H, d, *J* = 11.5 Hz, H-19a), 4.51 (1H, s, H-17b), 4.76 (1H, s, H-17a), 4.79 (2H, s, H-15), 6.10 (1H, d, *J* = 15.5 Hz, H-12), 6.37 (1H, d, *J* = 15.5 Hz, H-2′), 6.86 (1H, dd, *J* = 15.5, 10.0 Hz ), 7.18 (1H, s, H-14), 7.45 (1H, t, *J* = 7.5 Hz, Ar-H), 7.54 (1H, t, *J* = 7.5 Hz, Ar-H), 7.67 (1H, d, *J* = 8.0 Hz, Ar-H), 7.69 (1H, d, *J* = 8.0 Hz, Ar-H), 8.01 (1H, d, *J* = 15.5 Hz, H-3′)

**19-*O*-(2′-Trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydroandro grapholide (7g)**; White solid; yield 55%; Mp: 87-89 $^{o}$C; $R_f$ = 0.33 (10:1 DCM: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, s, H-20), 1.21 (3H, s, H-18), 3.35 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.29 (1H, d, *J* = 11.5 Hz, H-19b), 4.48 (1H, d, *J* = 11.5 Hz, H-19a), 4.53 (1H, s, H-17b), 4.78 (1H, s, H-17a), 4.80 (2H, s, H-15), 6.12 (1H, d, *J* = 15.5 Hz, H-12), 6.47 (1H, d, *J* = 15.5 Hz, H-2′), 6.88 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, s, H-14), 7.50 (1H, t, *J* = 7.5 Hz, Ar-H), 7.61 (1H, d, *J* = 8.0 Hz, Ar-H), 7.65 (1H, d, *J* = 15.5 Hz, H-3′), 7.68 (1H, d, *J* = 8.0 Hz, Ar-H), 7.74 (1H, s, Ar-H)

**19-*O*-(3′,5′-Trifluormeththyl)cinnamoyl-14-deoxy-11,12-didehydro-andrographolide (7h)**; A pale yellow oil; yield 48%; $R_f$ = 0.74 (10:1 DCM:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, s, H-20), 1.25 (3H, s, H-18), 3.39 (1H, dd, *J* = 11.0, 4.0 Hz, H-3), 4.34 (1H, d, *J* = 11.5 Hz, H-19b), 4.54 (1H, d, *J* = 11.5 Hz, H-19a), 4.56 (1H, s, H-17b), 4.81 (1H, s, H-17a), 4.84 (2H, s, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.58 (1H, d, *J* = 15.5 Hz, H-2′), 6.91 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.23 (1H, s, H-14), 7.71 (1H, d, *J* = 15.5 Hz, H-3′), 7.88 (1H, s, Ar-H), 7.96 (2H, s, Ar-H )

**19-*O*-Benzoyl-14-deoxy-11,12-didehydroandrographolide (7h)**; White solid; yield 65 %; Mp: 156-157 $^{o}$C; $R_f$ = 0.52 (10:1 DCM:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, s, H-20), 1.27 (3H, s, H-18), 3.33 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.39 (1H, d, *J* = 12.0 Hz, H-19b), 4.54 (1H, brs, H-17b), 4.60 (1H, d, *J* = 12.0 Hz, H-19a), 4.79 (1H, brs, H-17a), 4.81 (2H, brs, H-15), 6.13 (1H, d, *J* = 15.5 Hz, H-12), 6.89 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 7.43 (2H, t, *J* = 7.5 Hz, Ar-H), 7.56 (1H, t, *J* = 7.5 Hz, Ar-H), 7.99 (2H, d, *J* = 7.5 Hz, Ar-H)

**19-*O*-(4′-Methyl)benzoyl-14-deoxy-11,12-didehydroandrographolide (7j)**; White solid; yield 48 %; Mp: 107-109 $^{o}$C; $R_f$ = 0.42 (10:1 DCM:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, s, H-20), 1.27 (3H, s, H-18), 2.41 (3H, s, $CH_3$), 3.38 (1H, dd, *J* = 11.0, 4.0 Hz, H-3), 4.38 (1H, d, *J* = 12.0 Hz, H-19b), 4.55 (1H, brs, H-17b), 4.58 (1H, d, *J* = 12.0 Hz, H-19a), 4.79 (1H, brs, H-17a), 4.82 (2H, brs, H-15), 6.13 (1H, d, *J* = 16.0 Hz, H-12), 6.90 (1H, dd, *J* = 16.0, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 7.24 (2H, d, *J* = 8.0 Hz, Ar-H), 7.88 (2H, d, *J* = 8.0 Hz, Ar-H)

**19-*O*-(3′,4′-Dimethoxy)benzoyl-14-deoxy-11,12-didehydroandrographo- lide (7k)**; A pale yellow oil; yield 52 %; $R_f$ = 0.24 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, H-20), 1.17 (3H, s, H-18), 3.31 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 3.85 (3H, s, OC$H_3$), 3.87 (3H, s, OC$H_3$), 4.31 (1H, d, *J* = 11.5 Hz, H-19b), 4.48 (1H, brs, H-17b), 4.52 (1H, d, *J* = 11.5 Hz, H-19a), 4.73 (1H, brs, H-17a), 4.75 (2H, s, H-15), 6.07 (1H, d, *J* = 15.5 Hz, H-12), 6.82 (1H, d, *J* = 8.0 Hz, Ar-H), 6.84 (1H, dd, *J* = 15.5, 10.5 Hz, H-11), 7.12 (1H, s, H-14), 7.48 (1H, brs, Ar-H), 7.57 (1H, dd, *J* = 8.0, 1.0 Hz, Ar-H)

**19-*O*-(3′-*tert*-Butoxycarbonylamino)benzoyl-14-deoxy-11,12-didehydro-andrographolide (7l)**; White solid; yield 52%; Mp: 90-92 $^o$C; $R_f$ = 0.20 (10:1 DCM: EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-20), 1.27 (3H, s, H-18), 1.52 (9H, s, C(C$H_3$)$_3$), 3.39 (1H, dd, *J* = 11.0, 4.5 Hz, H-3), 4.39 (1H, d, *J* = 11.5 Hz, H-19b), 4.55 (1H, s, H-17b), 4.60 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, s, H-17a), 4.83 (2H, s, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.86 (1H, s, Ar-H), 6.89 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.20 (1H, brs, H-14), 7.35 (1H, t, *J* = 7.5 Hz, Ar-H), 7.65 (1H, d, *J* = 7.5 Hz, Ar-H), 7.74 (1H, d, *J* = 7.5 Hz, Ar-H), 7.83 (1H, s, Ar-H)

**19-*O*-(3′-*tert*-Butoxycarbonylamino)benzoyl-14-deoxy-11,12-didehydro andrographolide (7m)**; White solid; yield 66 %; Mp: 115-117 $^o$C; $R_f$ = 0.22 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-20), 1.26 (3H, s, H-18), 1.52 (9H, s, C(C$H_3$)$_3$), 3.38 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.37 (1H, d, *J* = 11.5 Hz, H-19b), 4.55 (1H, brs, H-17b), 4.58 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, brs, H-17a), 4.82 (2H, s, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.89 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 6.92 (1H, s, N*H*), 7.20 (1H, s, H-14), 7.44 (2H, d, *J* = 8.0 Hz, Ar-H), 7.92 (2H, d, *J* = 8.0 Hz, Ar-H)

**19-*O*-(3′-Nitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7n)**; White solid; yield 60%; Mp: 147-149 $^o$C; $R_f$ = 0.48 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, H-20), 1.30 (3H, s, H-18), 3.42 (1H, dd, *J* = 10.0, 5.5 Hz, H-3), 4.41 (1H, d, *J* = 11.5 Hz, H-19b), 4.56 (1H, brs, H-17b), 4.73 (1H, d, *J* = 11.5 Hz, H-19a), 4.81 (1H, brs, H-17a), 4.84 (2H, brs, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.91 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.21 (1H, brs, H-14), 7.67 (1H, t, *J* = 8.0 Hz, Ar-H ), 8.34 (1H, d, *J* = 8.0 Hz, Ar-H), 8.42 (1H, brd, *J* = 8.0 Hz, Ar-H), 8.84 (1H, brs, Ar-H)

**19-*O*-(4′-Nitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7o)**; White solid; yield 50%; Mp: 158-159 $^o$C; $R_f$ = 0.50 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz,

$CDCl_3$): δ 0.91 (3H, s, H-20), 1.30 (3H, s, H-18), 3.42 (1H, dd, *J* = 10.5, 5.0 Hz, H-3), 4.41 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.71 (1H, d, *J* = 11.5 Hz, H-19a), 4.81 (1H, brs, H-17a), 4.83 (2H, brs, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.91 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, brs, H-14), 8.18 (2H, d, *J* = 8.5 Hz, Ar-H), 8.30 (2H, t, *J* = 10.0 Hz, Ar-H)

**19-*O*-(3′,5′-Dinitro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7p)**; White solid; yield 50 %; Mp: 182-184 $^oC$; $R_f$ = 0.40 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, s, H-20), 1.38 (3H, s, H-18), 3.50 (1H, t, *J* = 8.0 Hz, H-3), 4.46 (1H, d, *J* = 11.5 Hz, H-19b), 4.62 (1H, brs, H-17b), 4.87 (1H, brs, H-17a), 4.89 (2H, brs, H-15), 4.91 (1H, d, *J* = 11.5 Hz, H-19a), 6.20 (1H, d, *J* = 15.5 Hz, H-12), 6.96 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.33 (1H, brs, H-14), 9.19 (2H, d, *J* = 1.5 Hz, Ar-H), 9.27 (1H, t, *J* = 1.5 Hz, Ar-H)

**19-*O*-(4′-Chloro)benzoyl-14-deoxy-11,12-didehydroandrographolide (7q)**; White solid; yield 65%; Mp: 120-121$^oC$; $R_f$ = 0.50 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, H-20), 1.27 (3H, s, H-18), 3.39 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 4.38 (1H, d, *J* = 11.5 Hz, H-19b), 4.56 (1H, brs, H-17b), 4.63 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, brs, H-17a), 4.83 (2H, brs, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.90 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, brs, H-14), 7.42 (2H, d, *J* = 8.5 Hz, Ar-H), 7.94 (2H, t, *J* = 1.5 Hz, Ar-H)

**19-*O*-(4′-Formyl)benzoyl-14-deoxy-11,12-didehydroandrographolide** (**7r**); White solid; yield 30 %; Mp: 168-170 $^oC$; $R_f$ = 0.22 (10:1 DCM:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-20), 1.22 (3H, s, H-18), 3.34 (1H, dd, *J* = 11.0, 5.0 Hz, H-3), 4.34 (1H, d, *J* = 11.5 Hz, H-19b), 4.48 (1H, s, H-17b), 4.60 (1H, d, *J* = 11.5 Hz, H-19a), 4.73 (1H, s, H-17a), 4.76 (2H, s, H-15), 6.07 (1H, d, *J* = 15.5 Hz, H-12), 6.83 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.13 (1H, s, H-14), 7.88 (2H, d, *J* = 8.0 Hz, Ar-H), 8.09 (2H, d, *J* = 8.0 Hz, Ar-H), 10.02 (1H, s, C*H*O)

**19-*O*-Nicotinonyl-14-deoxy-11,12-didehydroandrographolide (7s)**; Yellow solid; yield 72%; Mp: 109-111$^oC$; $R_f$ = 0.30 (3:7 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-20), 1.21 (3H, s, H-18), 3.33 (1H, dd, *J* = 10.5, 5.0 Hz, H-3), 4.34 (1H, d, *J* = 11.5 Hz, H-19b), 4.48 (1H, brs, H-17b), 4.61 (1H, d, *J* = 11.5 Hz, H-19a), 4.72 (1H, brs, H-17a), 4.75 (2H, brs, H-15), 6.06 (1H, d, *J* = 15.5 Hz, H-12), 6.82 (1H, dd, *J* = 15.5, 10.0 Hz,

H-11), 7.12 (1H, brs, H-14), 7.35 (1H, m, Ar-H), 8.22 (1H, d, *J* = 7.5 Hz, Ar-H), 8.70 (1H, brs, Ar-H), 9.13 (1H, brs, Ar-H)

**19-*O*-Cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12a)**; White solid; yield 62%; Mp: 91-92 $^{o}$C; $R_f$ = 0.46 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.12 (3H, s, H-18), 2.03 (3H, s, Ac), 4.25 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, s, H-17b), 4.60 (1H, d, *J* = 11.5 Hz, H-19a), 4.65 (1H, m, H-3), 4.82 (3H, s, H-17a, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.43 (1H, d, *J* = 16.0 Hz, H-2′), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 7.40 (3H, m, Ar-H), 7.54 (2H, m, Ar-H), 7.68 (1H, d, *J* = 16.0 Hz, H-3′)

**19-*O*-(4′-Fluoro)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12b**); A pale yellow oil; yield 97 %; $R_f$ = 0.34 (7:3 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.11 (3H, s, H-18), 2.02 (3H, s, Ac), 4.25 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, s, H-17b), 4.62 (1H, d, *J* = 11.5 Hz, H-19a), 4.64 (1H, m, H-3), 4.81 (1H, s, H-17a), 4.82 (2H, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.35 (1H, d, *J* = 16.0 Hz, H-2'), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.10 (2H, m, Ar-H), 7.17 (1H, brs, H-14), 7.53 (2H, m, Ar-H), 7.64 (1H, d, *J* = 16.0 Hz, H-3')

**19-*O*-(4′-Nitro)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12c**); Yellow oil; yield 75 %; $R_f$ = 0.36 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.12 (3H, s, H-18), 2.03 (3H, s, Ac), 4.29 (1H, d, *J* = 11.5 Hz, H-19b), 4.58 (1H, brs, H-17b), 4.62 (1H, d, *J* = 11.5 Hz, H-19a), 4.65 (1H, m, H-3), 4.81 (1H, brs, H-17a), 4.83 (2H, brs, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.56 (1H, d, *J* = 16.0 Hz, H-2′), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.20 (1H, brs, H-14), 7.70 (2H, d, *J* = 8.5 Hz, Ar-H), 7.72 (1H, d, *J* = 8.5 Hz, H-3′), 8.26 (2H, d, *J* = 8.5 Hz, Ar-H)

**19-*O*-(3′-Methoxyl-4′-TBS)cinnamoyl-3-*O*-14-deoxy-11,12-didehydroandrographolide** (**12d**); White solid; yield 27%; Mp: 147-149 $^{o}$C; $R_f$ = 0.54 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.19 (6H, s, $Si(CH_3)_2$), 0.96 (3H, s, H-20), 1.01 (9H, s, $SiC(CH_3)_3$), 1.13 (3H, s, H-18), 2.04 (3H, s, Ac), 3.87 (3H, s, $OCH_3$), 4.27 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.59 (1H, d, *J* = 11.5 Hz, H-19a), 4.65 (1H, dd, *J* = 10.0, 6.0 Hz, H-3), 4.82 (1H, brs, H-17a), 4.84 (2H, brs, H-15), 6.16 (1H, d, *J* = 15.5 Hz, H-12), 6.29 (1H, d, *J* = 16.0 Hz, H-2'), 6.86 (1H, d, *J* = 8.0 Hz, Ar-H), 6.95 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.04 (2H, m, Ar-H), 7.19 (1H, s, H-14), 7.62 (1H, d, *J* = 16.0 Hz, H-3')

**19-*O*-(4′-Trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-dide-hydroandrographolide (12e)**; A pale yellow oil; yield 53%; $R_f$ = 0.56 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, s, H-20), 1.13 (3H, s, H-18), 2.03 (3H, s, Ac), 4.28 (1H, d, *J* = 11.5 Hz, H-19b), 4.60 (1H, d, *J* = 11.5 Hz, H-19a), 4.65 (2H, m, H-3, H-17b), 4.83 (3H, brs, H-17a, H-15), 6.16 (1H, d, *J* = 15.5 Hz, H-12), 6.52 (1H, d, *J* = 16.0 Hz, H-2'), 6.95 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, s, H-14), 7.66 (4H, m, Ar-H), 7.70 (1H, d *J* = 16.0 Hz, H-3')

**19-*O*-(2′-Trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-dide-hydroandrographolide (12f)**; Yellow solid; yield 78 %; Mp: 167-169 $^o$C; $R_f$ = 0.53 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, H-20), 1.11 (3H, s, H-18), 2.00 (3H, s, Ac), 4.26 (1H, d, *J* = 11.5 Hz, H-19b), 4.55 (1H, s, H-17b), 4.61 (1H, d, *J* = 11.5 Hz, H-19a), 4.62 (1H, m, H-3), 4.79 (1H, s, H-17a), 4.80 (2H, brs, H-15 ), 6.13 (1H, d, *J* = 15.5 Hz, H-12), 6.39 (1H, d, *J* = 15.5 Hz, H-2'), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.17 (1H, s, H-14), 7.49 (1H, t, *J* = 7.5 Hz, Ar-H), 7.58 (1H, t, *J* = 7.5 Hz, Ar-H), 7.71 (2H, m, Ar-H), 8.05 (1H, d, *J* = 15.5 Hz, H-3')

**19-*O*-(3′-Trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-dide-hydroandrographolide (12g)**; White solid; yield 52%; Mp: 150-152 $^o$C; $R_f$ = 0.60 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.12 (3H, s, H-18), 2.02 (3H, s, Ac), 4.27 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.61 (1H, d, *J* = 11.5 Hz, H-19a), 4.65 (1H, m, H-3), 4.82 (3H, brs, H-17a, H-15 ), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.49 (1H, d, *J* = 15.5 Hz, H-2′), 6.95 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, s, H-14), 7.54 (1H, t, *J* = 8.0 Hz, Ar-H), 7.67 (2H, m, Ar-H), 7.70 (1H, d, *J* = 16.0 Hz, H-3′), 7.77 (1H, s, Ar-H)

**19-*O*-(3′,5′-Trifluormeththyl)cinnamoyl-3-*O*-acetyl-14-deoxy-11,12-di-dehydroandrographolide (12h)**; White solid; yield 93 %; Mp: 134-136 $^o$C; $R_f$ = 0.57 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, H-20), 1.11 (3H, s, H-18), 2.01 (3H, s, Ac), 4.28 (1H, d, *J* = 11.5 Hz, H-19b), 4.56 (1H, brs, H-17b), 4.61 (1H, d, *J* = 11.5 Hz, H-19a), 4.63 (1H, m, H-3), 4.80 (1H, brs, H-17a), 4.81 (2H, s, H-15 ), 6.13 (1H, d, *J* = 15.5 Hz, H-12), 6.55 (1H, d, *J* = 16.0 Hz, H-2′), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.17 (1H, s, H-14), 7.70 (1H, d, *J* = 16.0 Hz, H-3′), 7.87 (1H, s, H-Ar), 7.94 (2H, s, H-Ar)

**19-*O*-Benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12i)**;White solid; yield 78 %; Mp: 165-166 $^{o}$C; $R_f$ = 0.46 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.18 (3H, s, H-18), 1.87 (3H, s, Ac), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.56 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.0, 5.5 Hz, H-3), 4.77 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, s, H-17a), 4.82 (2H, s, H-15 ), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, s, H-14), 7.45 (1H, t, *J* = 7.0 Hz, Ar-H), 7.56 (1H, t, *J* = 7.0 Hz, Ar-H), 8.03 (2H, d, *J* = 7.0 Hz, Ar-H)

**19-*O*-(4′-Methyl)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12j)**; White solid; yield 75%; Mp: 147-149 $^{o}$C; $R_f$ = 0.54 (3:2 *n*-hexane: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.18 (3H, s, H-18), 1.89 (3H, s, Ac), 2.41 (3H, s, $CH_3$), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.75 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, s, H-17a), 4.82 (2H, s, H-15 ), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.19 (1H, s, H-14), 7.25 (2H, d, *J* = 8.0 Hz, Ar-H), 7.92 (2H, d, *J* = 8.0 Hz, Ar-H)

**19-*O*-(3′,4′-Dimethoxyl)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12k)**; A pale yellow oil; yield 43 %; $R_f$ = 0.49 (3:2 *n*-hexane: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, s, H-20), 1.17 (3H, s, H-18), 1.90 (3H, s, Ac), 3.93 (3H, s, OC$H_3$), 3.95 (3H, s, OC$H_3$), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.74 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, s, H-17a), 4.82 (2H, s, H-15 ), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.90 (1H, d, *J* = 8.5 Hz, H-Ar), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.17 (1H, s, H-14), 7.56 (1H, d, *J* = 1.5 Hz, Ar-H), 7.66 (1H, dd, *J* = 8.5, 1.5 Hz, Ar-H)

**19-*O*-(3′-*tert*-Butoxycarbonylamino)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12l)**; A pale yellow oil; yield 91%; $R_f$ = 0.32 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, s, H-20), 1.18 (3H, s, H-18), 1.53 (9H, s, C(C$H_3$)$_3$), 1.90 (9H, s, Ac), 4.31 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.0, 6.0 Hz, H-3), 4.76 (1H, d, *J* = 11.5 Hz, H-19a), 4.81 (1H, s, H-17a), 4.82 (2H, s, H-15), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.59 (1H, s, NH), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, s, H-14), 7.37 (1H, t, *J* = 7.5 Hz, Ar-H), 7.68 (2H, m, Ar-H), 7.96 (1H, s, Ar-H)

**19-*O*-(4′-*tert*-Butoxycarbonylamino)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12m)**; White solid; yield 73 %; Mp: 147-149 $^{o}$C; $R_f$ = 0.54 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, H-20), 1.08 (3H, s, H-18), 1.42 (9H, s, C($CH_3$)$_3$), 1.96 (9H, s, Ac), 4.20 (1H, d, *J* = 11.5 Hz, H-19b), 4.47 (1H, brs, H-17b), 4.55 (1H, dd, *J* = 10.5, 6.0 Hz, H-3), 4.66 (1H, d, *J* = 11.5 Hz, H-19a), 4.71 (1H, s, H-17a), 4.74 (2H, s, H-15 ), 6.06 (1H, d, *J* = 15.5 Hz, H-12), 6.83 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.15 (1H, s, H-14), 7.42 (2H, d, *J* = 8.5 Hz, Ar-H), 7.87 (1H, d, *J* = 8.5 Hz, Ar-H)

**19-*O*-(3′-Nitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrogra- pholide (12n)**; White solid; yield 50 %; Mp: 166-167 $^{o}$C; $R_f$ = 0.46 (3:2 *n*-hexane: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$) : δ 0.90 (3H, s, H-20), 1.17 (3H, s, H-18), 1.93 (3H, s, Ac), 4.42 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.66 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.76 (1H, d, *J* = 11.5 Hz, H-19a), 4.80 (1H, s, H-17a), 4.82 (2H, s, H-15 ), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.93 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, s, H-14), 7.68 (1H, t, *J* = 8.0 Hz, Ar-H), 8.36 (1H, d, *J* = 7.5 Hz, Ar-H), 8.43 (1H, dd, *J* = 7.5, 1.0 Hz, Ar-H), 8.84 (1H, brs, Ar-H)

**19-*O*-(4′-Nitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12o**); White solid; yield 77%; Mp: 161-162 $^{o}$C; $R_f$ = 0.38 (3:2 *n*-hexane:EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, H-20), 1.18 (3H, s, H-18), 1.87 (3H, s, Ac), 4.35 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.79-4.85 (4H,m, H-19a, 17a, 15 ), 6.14 (1H, d, *J* = 15.5 Hz, H-12), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 8.19 (2H, d, *J* = 8.5 Hz, Ar-H), 8.31 (2H, d, *J* = 8.5 Hz, Ar-H)

**19-*O*-(3′,5′-Dinitro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12p**); White solid; yield 43 %; Mp: 165-166 $^{o}$C; $R_f$ = 2.8 (3:2 *n*-hexane: EtOAc); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.18 (3H, s, H-18), 2.00 (3H, s, Ac), 4.56 (1H, d, *J* = 11.5 Hz, H-19b), 4.59 (1H, brs, H-17b), 4.68 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.77 (1H, d, *J* = 11.5 Hz, H-19a), 4.83 (3H, brs, H-17a, H-15 ), 6.16 (1H, d, *J* = 15.5 Hz, H-12), 6.95 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 9.15 (2H, brs, Ar-H), 9.25 (1H, brs, Ar-H)

**19-*O*-(4′-Chloro)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12q**); White solid; yield 60%; Mp: 167-169 $^{o}$C; $R_f$ = 0.54 (3:2 *n*-hexane:

EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, s, H-20), 1.17 (3H, s, H-18), 1.88 (3H, s, Ac), 4.30 (1H, d, *J* = 11.5 Hz, H-19b), 4.57 (1H, brs, H-17b), 4.65 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.77 (1H, d, *J* = 11.5 Hz, H-19a), 4.81 (1H, s, H-17a), 4.83 (2H, brs, H-15), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 6.94 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 7.43 (2H, d, *J* = 8.5 Hz, H-Ar), 7.97 (2H, d, *J* = 8.5 Hz, H-Ar)

**19-*O*-(4′-Formyl)benzoyl-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide** (**12r**); White solid; yield 38%; Mp: 91-93 °C; $R_f$ = 0.41 (3:2 *n*-hexane: EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, s, H-20), 1.21 (3H, s, H-18), 1.90 (3H, s, Ac), 4.36 (1H, d, *J* = 11.5 Hz, H-19b), 4.59 (1H, brs, H-17b), 4.68 (1H, m, H-3), 4.84 (4H, s, H-19a, H-17a, H-15), 6.17 (1H, d, *J* = 15.5 Hz, H-12), 6.96 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.20 (1H, s, H-14), 7.99 (2H, d, *J* = 7.0 Hz, Ar-H), 8.21 (2H, d, *J* = 7.0 Hz, Ar-H), 10.13 (1H, s, Ar-H)

**19-*O*-Nicotinic-3-*O*-acetyl-14-deoxy-11,12-didehydroandrographolide (12s)**; White solid; yield 50 %; Mp: 179-180 °C; $R_f$ = 0.54 (3:2 *n*-hexane:EtOAc); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, s, H-20), 1.18 (3H, s, H-18), 1.91 (3H, s, Ac), 4.37 (1H, d, *J* = 11.5 Hz, H-19b), 4.58 (1H, brs, H-17b), 4.66 (1H, dd, *J* = 10.5, 5.5 Hz, H-3), 4.77-4.86 (4H, m, H-19a, H-17a, H-115), 6.16 (1H, d, *J* = 15.5 Hz, H-12), 6.95 (1H, dd, *J* = 15.5, 10.0 Hz, H-11), 7.18 (1H, brs, H-14), 7.45 (1H, dd, *J* = 8.5, 5.0 Hz, H-Ar), 8.33 (1H, d, *J* = 7.5 Hz, H-Ar), 8.81 (1H, brs, H-Ar), 9.24 (1H, brs, H-Ar)

## 4.7 Conclusion

In conclusion, we have successfully modified the hydroxyl groups at C-3 and C-19 of 14-deoxy-11,12-didehydroandrographolide (**2**) to fifty eight newly analogues. All fifty eight analogues were simply prepared with moderate to excellent yields using 14-deoxy-11,12-didehydroandrographolide (**2**) which was synthesized from natural andrographolide. Twenty one derivatives were screened for *in vitro* cytotoxic activity. More than nine analogues of the 14-deoxy-11,12-didehydro- andrographolide (**2**) showed much higher cytotoxic activity than that of the parent compound (**2**) on cancer cell including P-388, KB, HT-29, MCF-7, LU-1, ASK, KKU M-213, HUCCA-1 and KKU-100. Structure activity relationship studies of the synthetic analogues indicated that the introduction of silyl ether or triphenylmethyl ether group in to C-19 of the parent compound led to the increasing in cytotoxicity against the cancer cells. It was found that C-19 TBS-analog (**3c**) showed potent cytotoxic activity over drug ellipticin on K-100 cancer cells. Moreover epoxy analogues (**5a**) and (**5b**) were identified as the most potent with $ED_{50}$ values of 3.37 and 3.08 μM on KKU

M-213 cell lines and 2.93 and 3.27μM on K-100 cell lines respectively. They also exhibited cytotoxicity potent than anticancer drug ellipticine. These analogues may serve as a potential structure lead for the development of new anticancer drugs.

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