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Design, Synthesis and Discovery of andrographolide derivatives against Zika virus infection

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Abstract

The Zika endemic established by imported and local transmission is of significant concern and effective anti-ZIKV drugs remain an urgent unmet need. As andrographolide was identified to be an inhibitor of DENV and CHIKV and the importance of quinoline structure against infectious diseases was considered, we are interested in studying its andrographolide derivatives with quinoline moiety against Zika virus infection. In addition to screening eight in-house derivatives of andrographolide, sixteen new derivatives were designed, synthesized and tested against Zika virus infection. Among these compounds, two most potent anti-Zika compounds of 19-acetylated 14α-(5',7'-dichloro-8'-quinolyloxy) derivative **17b** and 14β-(8'-quinolyloxy)-3,19-diol derivative **3** with the highest selectivity were discovered. The SAR analysis indicates that rational and optimal combined modification/s at 3-, 14-, or 19-positions can make derivatives less toxic and more potent against Zika infection, and both of **3** and **17b** are suitable as leads for designing new generation of andrographolide derivatives with quinoline or its structure- and property-related moieties against Zika virus and other arboviruses.

Graphical Abstract

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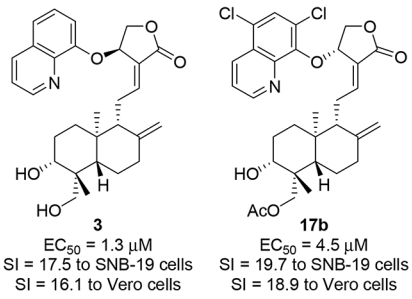
Appendix A. Supplementary data

Supplementary data of NMR spectra of new compounds to this article can be found online at http://dx.doi.Org/10.1016/j.ejmech

Declaration of interests

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.



Keywords

Zika virus; antiviral; andrographolide and derivative; quinolyloxy; 14α-(8'-quinolyloxy)

1. Introduction

Zika virus (ZIKV), one of arboviruses and belonging to *Flaviviridae* family, first emerged more than 70 years ago [1] but ZIKV infection at first was not considered as a threatening infectious disease, instead it was originally taken to be a mild self-resolving infection and frequently in that era misdiagnosed and treated as dengue virus (DENV) and chikungunya virus (CHIKV) infection due to clinical similarity and serological cross-reactivity with closely related viruses [2–5]. ZIKV, like other related arboviruses, has a monopartite, linear, positive single-stranded RNA genome with about 10794 nucleotides encoding 3419 amino acids [6] as a single polyprotein with multiple transmembrane domains that is cleaved into structural and non-structural proteins (NS) by both host and viral proteases. ZIKV is mainly spread via the bite of mosquitoes of the *Aedes* type, can also be sexually transmitted and potentially spread by blood transfusions, and infections in pregnant women can spread to the baby [7–9]. World Health Organization (WHO) declared a Public Health Emergency of International Concern in February 2016 [10] for the recent epidemic in the Americas in 2016 and at last in 84 countries as of March 2017. It is now recognized that ZIKV can cause significant neurological defects in both neonates as pediatric microcephaly[11–12] and adults as Guillain–Barré syndrome [13], which make ZIKV quite different from other arboviruses. So far, great efforts have been input in research of prevention and therapy development, but currently there is still no approved vaccine or specific antiviral against ZIKV [13–16], while clinical treatment of ZIKV-infected patients consists of only supportive care while the therapeutic approach to infected pregnant women is limited to the use of antipyretics and analgesics [17]. Avoiding mosquito bites is the best way to cut off the primary spreading of ZIKV and to prevent ZIKV infection. However, the international air travel is increased in the contemporary world by which a virus could be imported from one country into other countries in the tropical and subtropical regions [18]. Thus, the endemic established by imported and local transmission is of significant concern and mosquito transmitted diseases remain an ongoing global threat [19–21]. To search for effective antivirals to ZIKV is very urgent and of great medical significance.

Andrographis paniculata [Burm. F.] Nees is an herb known as a "natural antibiotic". It and its compositions are commonly used in China, India and Southeast Asia for the treatment of

a large variety of illnesses by especially reducing inflammation and “heat-clearing and detoxifying” defined in Chinese medicines [22–27]. Andrographolide (**1**, Fig. 1) is a bicyclic diterpenoid lactone and one of major components isolated from *Andrographis paniculata*. Importantly, some active derivatives of andrographolide of “Chuanhuning”, “Yanhuning”, and “Lianbizhi” have been used in China to treat bacterial and viral infections for many years [28–31]. In addition, andrographolide was revealed to possess many activities against microbial infection [28, 32–34], e.g. *Candida albicans* [35], *Pseudomonas aeruginosa* [36–37], *Enterococcus faecali* [38], HIV [39], herpes simplex virus type 1 [40], HBV [41], HCV [42–43], influenza virus [44–45]. Recently, andrographolide was reported to be an anti-viral agent against DENV [46–47] and (CHIKV) [48] but does not have a direct virucidal activity [46–47]. Attracted by its pharmacological activity, numerous andrographolide derivatives have been reported from time to time to improve its physiochemical properties and pharmaceutical features [28, 49–53]. We previously discovered that some 14-aryloxy analogues of andrographolide work as immunosuppressant agents [54] and antibacterial with immunosuppressant activity [38]. In this study, we pursued potent anti-Zika virus agents derived from the “natural antibiotic” lead of andrographolide, and envisioned that modification of 14-substituents can reduce the cytotoxicities of analogues and make some modified 14-aryloxy analogues be active against ZIKV infection.

2, Results and discussion

2.1. Design

As andrographolide can work as an inhibitor of DENV and CHIKV [46–48] and the importance of quinoline moiety against infectious diseases [38, 55–57] was also considered, we were interested in testing anti-Zika activity of eight derivatives of andrographolide from our in-house library with 14-quinolinyloxy group and related 14-pyridinyloxy group (Fig. 1), which were previously discovered as antibacterial agents [38]. It was discovered in this study one active anti-Zika 14-(8’-quinolyloxy) compound **3** (EC_{50} value is 1.3 μM) but its cytotoxicity (CC_{50} values are 22.7 μM in SNB-19 cell line and 20.9 μM in Vero cell line) is relatively high. In order to improve the antiviral activity and reduce the toxicity, modification of 14-(8’-quinolyloxy) group was explored that sixteen modified derivatives by the introduction of methyl group at 2-position or chloro group at 5,7-positions into 8-quinolinyloxy moiety were subsequently designed, synthesized and some more selective anti-Zika compounds were discovered.

2.2. Synthesis of titled compounds

Compounds of **2** to **9** (Fig. 1) were synthesized previously from **10a** and **10b** [38]. Synthesis of sixteen new compounds of **11a** to **18a** and **11b** to **18b** was conducted according to the previously reported synthetic method [38], which started from **10a** (14α-OH) and **10b** (14β-OH), respectively, as shown in Scheme 1. Briefly, Mitsunobu reactions of **10a** or **10b** with 2-methyl-8-quinolinol (**19**) (Scheme 1) or 5,7-dichloro-8-quinolinol (**20**) (Scheme 1) were carried out in general from 0 °C to room temperature in anhydrous THF to afford corresponding 14β-(2’-methyl-quinolyl-8’-oxy) andrographolide **11a** and 14β(5’,7’-dichloro-quinolyl-8’-oxy) andrographolide **15a**, or 14α-(2’-methyl-quinolyl-8’-oxy) andrographolide **11b** and 14a-(5’,7’-dichloro-quinolyl-8’-oxy) andrographolide **15b**,

respectively, in mild to moderate isolated yields. Deprotection of 3,19-acetonylidene from **11a**, **11b**, **15a**, and **15b** afforded the corresponding 3,19-diols of **12a**, **12b**, **16a**, and **16b**. Furthermore, mono-acetylation at 19-positions of **12a**, **12b**, **16a**, and **16b** produced corresponding **13a**, **13b**, **17a**, and **17b**, which were subsequently oxidized into 3-ketones of **14a**, **14b**, **18a**, and **18b** by Dess-Martin periodinane (DMP).

2.3. Anti-Zika activity and SAR analysis

The inhibitory activity against Zika virus infection was conducted by a ZIKV titer assay (in Vero cells for 48 h) [58–59] (Supplementary data) to measure ZIKV production in compound-treated human glioblastoma cells (SNB-19 cell line) (incubation for 24 h) infected by PRVABC59 strain at multiplicity of infection (MOI) of 1. Niclosamide, one of our previously identified active compounds against Zika virus infection [58–59], was used as a positive and quality control in each antiviral assay. The cytotoxicity of each compound in this study was assessed to SNB-19 cells for 24 h and Vero cells for 48 h corresponding to the incubation time in anti-Zika screening and listed in Table S1 (Supplementary data).

In our screening streamline, eight previously reported 14-aryloxy andrographolide derivatives (Fig. 1) of four 8’-quinolyloxy compounds of **2** to **5**, two 4’-quinolyloxy compounds of **6** and **7**, and two 2’-nitro-pyridinyl compounds of **8** and **9** were first tested. The results of active derivatives of **3**, **7** and **9** are listed in Fig. 2 and all results were included in Table S1. As shown in Fig. 2 and Table S1, compounds of **2** to **9** are relatively cytotoxic and CC_{50} values of 8’-quinolyloxy derivatives (**2** to **5**) are around 20 μM. It was found that 14β-(8’-quinolyloxy) 3,19-diol analog **3** is very effective against Zika virus infection with EC_{50} value of 1.3 μM and its safety/selectivity index (SI) >16 (CC_{50} values of 22.7 and 20.9 μM in SNB-19 and Vero cell lines, respectively). However, the antiviral activity and cytotoxicity of 14β-(8’-quinolyloxy) 3,19-acetonylidene compound **2** are similar in values (CC_{50} = 21.1 μM in SNB-19 cells and 22.0 μM in Vero cells but EC_{50} = 25.9 μM), and 14β-8’-quinolyloxy 19-acetyl compounds of 3-alcohol **4** and 3-keto **5** are not active. These results imply that more polar 14β-(8’-quinolyloxy) anadrographolide derivative **3** is more active and selective against Zika virus infection than its more hydrophobic derivatives of **2**, **4** and **5**. Among two 14-(4’-quinolyloxy)-3,19-diol andrographolide derivatives **6** and **7**, only does 14α-isomer of **7** show mild anti-Zika activity (EC_{50} = 11.0 μM, CC_{50} = 60.1 μM in SNB-19 cells and CC_{50} = 22.3 μM in Vero cells) but 14β-isomer of **6** is inactive. Anti-Zika activity difference between two 3,19-diol andrographolide derivatives of **3** bearing 14β-(8’-quinolyloxy) and **6** bearing 14β-(4’-quinolyloxy) suggests here that 14-(8’-quinolyloxy) pharmacophore is superior to 14-(4’-quinolyloxy) pharmacophore. Meanwhile, 14α-(2’-nitro-pyridinyl-3’-oxy)-3,19-diol andrographolide derivative **9** was discovered to be a mild anti-Zika agent (EC_{50} = 12.5 μM, CC_{50} = 67.7 μM in SNB-19 cells and CC_{50} = 37.7 μM in Vero cells) while 14α-(2’-nitro-pyridinyl-3’-oxy)-3,19-acetonylidene andrographolide (**8**) is not active. From the current data for eight compounds of **2** to **9** (Figs. 1 and 2), it is concluded that both of 14α- and 14β-isomers, and modifications at 3-, 19-, or 3,19-positions could contribute to the anti-Zika activity. 14β-(8’-quinolyloxy) andrographolide compound **3** is the most potent antiviral derivative among these compounds and its SI values are 17.5 to SNB-19 cell line and 16.1 to Vero cell line, respectively, but its cytotoxicity is not ideal.

In pursuit of improving the antiviral activity and reduce the cytotoxicity of lead compound **3**, the first exploration is to modify 14-(8'-quinolyloxy) group. Considering that the cytotoxicity of **3** may derive from 1'-*N* and 8'-*O* of 14-(8'-quinolyloxy) group, it is envisaged that introduction of steric hindrance or electrostatic group/s at 2'-position or 7'-position could block or restrict some side interactions with 1'-*N* or 8'-*O* to reduce the cytotoxicity and possibly improve anti-Zika activity. Based on the available starting materials of 2-methyl-8-quinolinol (**19**, Scheme 1) and 5,7-dichloro-8-quinolinol (**20**, Scheme 1), eight 14-(2'-methyl 8'-quinolyloxy) derivatives (four 14β-siomers of **11a** to **14a** and four 14α-siomers of **11b** to **14b**) and eight 14-(5',7'-dichloro 8'-quinolyloxy) derivatives (four 14β-siomers of **15a** to **18a** and four 14α-siomers of **15b** to **18b**) were designed, synthesized and assayed against Zika virus infection.

With compounds in hand, 2'-methyl-8'-quinolinol derivatives were first assayed and the results are shown in Fig. 2 and Table S1. As expected, eight 14-(2'-methyl 8'-quinolyloxy) andrographolide derivatives are generally less cytotoxic than 14-(8'-quinolyloxy) andrographolide derivatives. Both **11a** (14β) and **11b** (14α) exhibit lower cytotoxicities (CC_{50} values are 78.4 and 66.5 μM in SNB-19 cell line, 55.9 and 65.3 μM in Vero cell line, respectively) than compound **3** but their anti-Zika activities (EC_{50} values are 8.5 and 16.6 μM, respectively) and SIs (9.2 and 6.6 to SNB-19 cell line, 4.0 and 3.9 to Vero cell line, respectively) are much lower than those of **3**. Compound **12a** (14β) is much less active (EC_{50} = 25.8 μM) against Zika virus infection with lower cytotoxicities (CC_{50} > 100 μM to SNB-19 cells or 99.8 μM to Vero cells) than compounds of **3**, **11a** and **11b**; whereas, compounds of **13a** (14β), **14a** (14β), and **12b** (14α) to **14b** (14α) are not active against Zika virus infection. The above results indicated that 14-(2'-methyl-8'-quinolyloxy) group can reduce the cytotoxicity but does not improve the antiviral activity; modifications at 3- or 19-position affect the cytotoxicity and antiviral activity. Compound **11a** possesses the most potent anti-Zika activity and the highest SI values among (2'-methyl-8'-quinolyloxy) derivatives. In addition, inactive compounds of 2'-methyl-8'-quinolinol derivatives are also less cytotoxic (Table S1) than 8'-quinolyloxy compounds (**2** to **5**). Overall, introduction of 2'-methyl to 14-(8'-quinolyloxy) reduces the cytotoxicity but also obviously decreases the anti-Zika activity.

Then, 5',7'-dichloro-8'-quinolinol derivatives were assayed (Fig. 2 and Table S1). As shown in Table S1, all of them possess relatively less cytotoxicities than 8'-quinolyloxy compounds (**2** to **5**). Two 3,19-acetonylidene-protected 5',7'-dichloro-8'-quinolinol andrographolide derivatives **15a** (14β) and **15b** (14α) do not show anti-Zika activity even though their corresponding 3,19-acetonylidene-protected 2'-methyl-8'-quinolinol derivatives of **11a** and **11b** are active. Whereas, both of 3,19-diols of **16a** (14β) and **16b** (14α) are active against Zika virus infection that **16a** (EC_{50} = 13.3 μM) is less active than **16b** (EC50 = 7.8 μM) but **16a** (CC_{50} values are higher than 100 μM in both cell lines) is much less toxic than **16b** (CC_{50}s = 85.2 and 58.6 μM in SNB-19 and Vero cell lines, respectively), indicating that the stereochemistry of 14α and 14β affects the inhibitory activity and also the cytotoxicity. 19-Acetylated 14β-derivative **17a** is not active but 19-acetylated 14α-derivative **17b** is the second most potent anti-Zika agent (SIs are 19.7 to SNB-19 cell line and 18.9 to Vero cell line, respectively) with EC_{50} value of 4.5 μM and CC_{50} values of 88.7 μM in SNB-19 cells

and 85.0 μM in Vero cells. In contrast, 19-acetylated 3-keto-14β-derivative **18a** is a weak inhibitor to Zika virus infection (EC_{50} = 24.6 μM, CC_{50}s = 72.0 and 57.4 μM in SNB-19 and Vero cell lines, respectively) while 19-acetylated 3-keto-14α-derivative **18b** is not active against Zika virus infection.

As a whole, anti-Zika activity of 14α-isomers of **16b**, **17b** and **18b** or 14β-isomers of **16a**, **17a** and **18a** varies with distinct modifications at 3-position or/and 19-position and the lipophilicity formed by these modifications (the upward order of lipophilicity from 3,19-diol, 3-alcohol with 19-actylation, to 3,19-acetonylidene or 3-keto with 19-acetlation). 3,19-Diol **16b** is active against Zika virus infection and 19-acetylation makes **17b** (3-OH-19-OAc) be more active against Zika virus and less toxic to SNB-19 and Vero cell lines than **16b**; meanwhile 3-keto-19-acetylated product **18b** is not active. These observations suggest that 3-alcohol with 19-acetylation and 14α in **17b** are slightly synergistic to the antiviral activity, which may be partly derived from the increase of lipophilicity compared to **16b**; however, too hydrophobic property of **18b** with 19-acetlation and 3-keto makes **18b** lost antiviral activity compared to **16b** and **17b**. On the other hand, 3,19-diol **16a** is active against Zika virus infection with much less cytotoxicities to SNB-19 and Vero cell lines but 19-acetylated **17a** (3-OH-19-OAc) becomes inactive and 3-keto-19-acetylated product **18a** regains part of anti-Zika activity and the cytotoxicity is slightly higher than **16a**. These results suggest that 3-alcohol, 19-acetylation and 14β in **17a** are not a good combination for the antiviral activity while the combination of 14β, 19-acetylation and 3-keto in **18a** exhibits better antiviral activity than that of 19-acetylation and 14β in **17a** but less than that of 3,19-diol and 14β in **16a**. These combined data indicate that modification of 14-(8'-quinolyloxy) by 5',7'-dichloro reduces the cytotoxicity and some derivatives (e.g. **16b** and **17b**) possess comparable anti-Zika activity to **3**.

Collectively, 14-(2'-methyl 8'-quinolyloxy) and 14-(5',7'-dicloro 8'-quinolyloxy) derivatives are less cytotoxic than 14-(8'-quinolyloxy) derivatives; and in general, 14-(5',7'-dichloro-8'-quinolyloxy) derivatives are more potent against Zika infection than 14-(2'-methyl-8'-quinolyloxy) derivatives. Two configurations of 14α and 14β could make distinct contribution to the anti-Zika activity, and optimal combination of modifications at 2'- or 5', 7'-positions of 14-(8'-quinolyloxy) and 3- or/and 19-positions improves the antiviral activity and the selectivity. Altogether, current results at least partially imply that optimal lipophilicity (hydrophobicity) by ideal combination of modifications is important for the action of a drug. It is noted that compound **17b** possesses comparable anti-Zika activity to and less toxicity than compound **3**.

3. Conclusions

In summary, eight previously reported and sixteen new synthesized derivatives of andrographolide were tested for anti-Zika virus activity. As a result, andrographolide scaffold mounted with quinoline moiety is a potential anti-Zika strategy. By combining these data, we found that rational/optimal modification/s at 3-, 14-, or 19-positions increase derivative selectivity via lowering cell toxicity and increasing antiviral potency. In general, andrographolide derivatives of 14-(2'-methyl 8'-quinolyloxy) and 14-(5',7'-dicloro 8'-quinolyloxy) are relatively less cytotoxic than those of 14-(8'-quinolyloxy) and interestingly,

5',7'-dichloro derivatives are more effective than 2'-methyl derivatives against Zika infection from the current data in this study. It is noteworthy that with their distinctive pharmacological property including rational lipophilicity (hydrophobicity), 19-acetylated 14α-(5'7'-dichloro-8'-quinolyloxy) derivative **17b** and 14β-(8'-quinolyloxy)-3,19-diol derivative **3** are the most potent compounds against Zika virus infection and possess the similar SI values but the cytotoxicity of **17b** is less than that of **3**. Therefore, both of **3** and **17b** are suitably taken as leads to design new generation of andrographolide derivatives against Zika virus and other arboviruses (for example, DENV, CHIKV, West Nile virus (WNV) and Japanese encephalitis virus (JEV)), we can envisage that it is promising to discover more potent and less toxic andrographolide derivatives with quinoline or its structure- and property-related moieties to effectively treat the infection by Zika virus and other arboviruses.

4. Experimental

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$, CD_3OD, $(CD_3)_2SO$ and C_6D_6 as indicated. Coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to the solvent. The high resolution of ESI-MS was recorded on an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer, respectively. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Anhydrous tetrahydrofuran (THF) was distilled from sodium-treated THF under nitrogen atmosphere and anhydrous dichloromethane (DCM) was distilled from calcium hydride-treated DCM under nitrogen atmosphere. Melting points were measured using an YRT-3 melting point apparatus (Shanghai, China) and were uncorrected. HPLC analysis condition: Sunfire C18 column (4.6 × 250 mm, 5 μm), elution by 90% methanol with 10% purified water (for compounds of **2, 3, 5, 8, 11a, 11b, 14a, 14b, 15a, 15b, 18a, 18b**) or 80% methanol with 20% purified water (for compounds of **4, 6, 7, 9, 12a, 12b, 13a, 13b, 16a, 16b, 17a, 17b**), rate = 1.0 ml/min, detection wavelength of 230 nm. The purity of all compounds by HPLC analysis is not lower than 95% (Supplementary data).

4.1.1. Preparation of 14β-isomers of 11a and 15a from 10a (14α), and 14α-isomers of 11b and 15b from 10b (14β).—

The synthesis is conducted according to ref [38].

(14β)-(Quinolyl-2'-methyl-8'-oxy)-3,19-acetonylidene andrographolide (**11a**): white solid; m.p. 146.1–148.4 °C; 75% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.26 (d, J = 8.4 Hz, 1H, 4'-*H*), 7.57 (dd, J = 8.2, 1.2 Hz, 1H, 5'-*H*), 7.51 – 7.43 (m, 2H, 6'-*H* and 7'-*H*), 7.24 – 7.11 (m, 2H, 3'-*H* and 12'-*H*), 6.05 (d, J = 5.4 Hz, 1H ,14-*H*), 4.87 (s, 1H ,17-*H*), 4.81 (m, 1H, 15-*H*), 4.69 (s, 1H ,17-*H*), 4.46 (m, 1H, 15-*H*), 3.67 (d, J = 11.6 Hz, 1H, 19-*H*), 2.94 (d, J = 11.6 Hz, 1H, 19-*H*), 2.67 (s, 3H, 2'-C*H*$_3$), 2.65 – 2.61 (m, 1H, 3-*H*), 2.39 – 2.24 (m, 3H, 7-C*H*$_2$ and 11-*H*), 2.16 (d, J = 10.2 Hz, 1H, 6-*H*), 2.00 (m, 1H, 9-*H*), 1.55 (d, J = 12.7 Hz, 1H, 11-*H*), 1.28 (m, 1H, 6-*H*), 1.19 (s, 3H, acetonylidene–C*H*$_3$), 1.14 (m, 5H, acetonylidene-C*H*$_3$, 2-*H* and 1-*H*), 0.78 (s, 3H, 20-C*H*$_3$), 0.75 (s, 1H, 2-*H*), 0.66 (s, 3H, 18-C*H*$_3$), 0.61 (m,

2H, 1-*H*, 5-*H*); ^{13}C NMR (101 MHz, DMSO-d6) δ 169.6 (16-*C*), 157.8 (8'-*C*), 151.8 (2'-*C*), 150.18 (12-*C*), 148.0 (8-*C*), 139.6 (9'-*C*), 136.4 (4'-*C*), 127.9 (13-*C*), 126.7 (10'-*C*), 125.9 (6'-*C*), 122.9 (3'-*C*), 121.0 (5'-*C*), 111.8 (acetonylidene-*C*), 108.1 (17-*C*), 98.3 (7'-*C*), 75.4 (3-*C*), 71.7 (15-*C*), 71.5 (14-*C*), 62.8 (19-*C*), 56.1 (9-*C*), 50.5 (5-*C*), 38.2 (4-*C*), 37.1 (10-*C*), 37.1 (7-*C*), 33.0 (1-*C*), 27.2 (2-*C*), 25.3[1] (6-*C*), 25.2[6] (2C, acetonylidene-2*C*), 25.2 (quinolyl-2'-*C*H$_3$), 24.1 (11-*C*), 22.8 (20-*C*), 15.7 (18-*C*); ESI-HRMS: *m/z* found 532.3060, [M+H]$^+$, calcd for $C_{33}H_{42}NO_5$, 532.3063.

(14α)-(Quinolyl-2'-methyl-8'-oxy)-3,19-acetonylidene andrographolide (**11b**): white solid; m.p. 105.1-107.7 °C; 78% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.24 (d, *J* = 8.4 Hz, 1H, 4'-*H*), 7.61 (dd, *J* = 8.2, 1.2 Hz, 1H, 5'-*H*), 7.44 (m, 2H, 6'-*H* and 7'-*H*), 7.18 (dd, *J* = 7.7, 1.2 Hz, 1H, 3'-*H*), 6.98 – 6.73 (m, 1H, 12-*H*), 6.04 (d, *J* = 5.5 Hz, 1H, 14*H*), 4.80 (s, 1H, 17-*H*), 4.76 (m, 1H, 15-*H*), 4.58 (s, 1H, 17-*H*), 4.53 (m, 1H, 15-*H*), 3.74 (d, *J* = 11.8 Hz, 1H, 19-*H*), 3.23 (d, *J* = 13.4 Hz, 1H, 19-*H*), 3.01 (d, *J* = 11.6 Hz, 1H, 3-*H*), 2.65 (s, 3H, 2'-C*H*$_3$), 2.26 (d, *J* = 12.5 Hz, 1H, 7-*H*), 2.20 (m, 2H, 7-*H* and 11-*H*), 1.90 – 1.80 (m, 2H, 6-*H* and 9-*H*), 1.76 – 1.64 (m, 1H, 11-*H*), 1.57 (d, *J* = 10.7 Hz, 1H, 6-*H*), 1.45 (m, 1H, 2-*H*), 1.25 (s, 3H, acetonylidene-C*H*$_3$), 1.20 (s, 3H, acetonylidene-C*H*$_3$), 1.17 – 1.06 (m, 3H, 2-*H* and 1-C*H*$_2$), 1.03 (s, 3H, 20-C*H*$_3$), 0.97 (m, 1H, 5-*H*), 0.53 (s, 3H, 20-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) δ 169.7 (16-*C*), 158.0 (8'-*C*), 151.9 (2'-*C*), 149.9 (12-*C*), 147.5 (8-*C*), 140.3 (9'-*C*), 136.5 (4'-*C*), 127.9 (13-*C*), 125.9 (10'-*C*), 125.7 (6'-*C*), 122.8 (3-*C*), 122.3 (5'-*C*), 115.7 (acetonylidene-*C*), 109.0 (17-*C*), 98.2 (7'-*C*), 76.1 (3-*C*), 73.7 (15-*C*), 71.7 (4-*C*), 62.8 (19-*C*), 54.8 (9-*C*), 51.6 (5-*C*), 37.9 (4-*C*), 37.1 (10-*C*), 37.1 (7-*C*), 33.9 (1-*C*), 27.8 (2-*C*), 25.9 (6-*C*), 25.4 (acetonylidene-*C*H$_3$), 25.2 (acetonylidene-*C*H$_3$), 25.0 (quinolyl-2'-*C*H$_3$), 24.9 (11-*C*), 22.8 (20-*C*), 15.4 (18-*C*); ESI-HRMS: *m/z* found 532.3052, [M+H]$^+$, calcd for $C_{33}H_{42}NO_5$, 532.3063.

(14β)-(Quinolyl-5',7'-dichloro-8'-oxy)-3,19-acetonylidene andrographolide (**15a**): white solid; m.p. 80.5-82.5 °C; 73% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.12 (dd, *J* = 4.2, 1.6 Hz, 1H, 2'-*H*), 8.63 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.05 (s, 1H, 6'-*H*), 7.84 (dd, *J* = 8.6, 4.2 Hz, 1H, 3'-*H*), 6.71 – 6.51 (m, 1H, 12-*H*), 6.44 (d, *J* = 4.5 Hz, 1H, 14-*H*), 4.75 – 4.66 (m, 2H, 17-*H* and 15-*H*), 4.61 (m, 1H, 15-*H*), 4.18 (s, 1H, 17-*H*), 3.75 (d, *J* = 11.6 Hz, 1H, 19-*H*), 3.04 (d, *J* = 11.5 Hz, 1H, 19-*H*), 2.22 (d, *J* = 11.7 Hz, 1H, 3-*H*), 1.79 (m, 3H, 3-*H* and 7-C*H*$_2$), 1.66 (d, *J* = 11.5 Hz, 1H, 6-*H*), 1.62 – 1.47 (m, 2H, 9-*H* and 11-*H*), 1.31 (s, 3H, acetonylidene–C*H*$_3$), 1.24 (s, 3H, acetonylidene –C*H*$_3$), 1.19 (d, *J* = 6.2 Hz, 1H, 6-*H*), 1.08 (m, 5H, 20-C*H*$_3$, 2-*H* and 1-*H*), 0.93 – 0.77 (m, 2H, 2-*H* and 1-*H*), 0.72 (m, 1H, 5-*H*), 0.38 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) δ 169.4 (16-*C*), 151.5 (8'-*C*), 149.1 (2'-*C*)), 147.4 (12-*C*), 147.2 (8-*C*), 143.0 (9'-*C*), 133.3 (4'-*C*), 127.6 (13-*C*), 127.2 (6'-*C*), 126.5 (10'-*C*), 125.8[7] (3'-*C*) 125.8[9] (5'-*C*), 123.4 (acetonylidene-*C*), 108.3 (7'-*C*), 98.0 (17-*C*), 77.0 (3-*C*), 75.8 (15-*C*), 71.7 (14-*C*), 62.6 (19-*C*), 54.3 (9-*C*), 51.4 (5-*C*), 37.6 (4-*C*), 36.9 (10-*C*), 36.8 (7-*C*), 33.5 (1-*C*), 27.6 (2-*C*), 25.8 (6-*C*), 25.4 (acetonylidene-*C*H$_3$), 25.1 (acetonylidene-*C*H$_3$), 24.9 (11-*C*), 22.6 (20-*C*), 15.1 (18-*C*); ESI-HRMS: *m/z* found 586.2120, [M+H]$^+$, calcd for $C_{32}H_{38}Cl_2NO_5$, 586.2127.

(14α)-(Quinolyl-5',7'-dichloro-8'-oxy)-3,19-acetonylidene andrographolide (**15b**): white solid; m.p. 83.5-85.3 °C; 77% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.08 (dd, *J* = 4.2, 1.6 Hz, 1H, 2'-*H*), 8.61 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.00 (s, 1H, 6'-*H*), 7.81 (dd, *J* = 8.6, 4.2

Hz, 1H, 3'-*H*), 6.70 (dd, *J* = 4.5 Hz, 1H, 12-*H*), 6.32 (d, *J* = 4.5 Hz, 1H, 14-*H*), 4.72 – 4.63 (m, 2H, 17-*H* and 15-*H*), 4.54 (m, 1H, 15-*H*), 4.10 (s, 1H, 17-*H*), 3.74 (d, *J* = 11.7 Hz, 1H, 19-*H*), 3.28 (m, 1H, 19-*H*), 3.01 (d, *J* = 11.6 Hz, 1H, 3-*H*), 2.22 (m, 1H, 7-*H*), 1.92 – 1.67 (m, 3H, 7-*H*, 11-*H* and 6-*H*), 1.62 (d, *J* = 11.5 Hz, 1H, 9-*H*), 1.52 (m, 3H, 11-H, 6-*H* and 2-*H*), 1.30 (s, 3H, acetonylidene–CH_3), 1.22 (m, 4H, acetonylidene–CH_3), 1.17 (d, *J* = 6.2 Hz, 1H, 1-*H*), 1.07 (m, 2H, 2-*H* and 1-*H*), 1.04 (s, 3H, 20–CH_3), 0.89 (m, 1H, 5-*H*), 0.43 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 169.8 (16-*C*), 151.7 (8'-*C*), 148.7 (2'-*C*), 147.5 (12-*C*), 147.4 (8-*C*), 143.1 (9'-*C*), 133.6 (4'-*C*), 127.7 (13-*C*), 127.4 (6'-*C*), 126.6 (10'-*C*), 126.2 (3'-*C*), 126.1 (5'-*C*), 123.6 (acetonylidene-*C*), 108.1 (7'-*C*), 98.3 (17-*C*), 77.0 (3-*C*), 75.9 (15-*C*), 71.8 (14-*C*), 62.8 (19-*C*), 54.5 (9-*C*), 51.5 (5-*C*), 37.9 (4-*C*), 37.2 (10-*C*), 37.0 (7-*C*), 33.9 (1-*C*), 27.7 (2-*C*), 25.9 (acetonylidene-*C*H_3), 25.5 (acetonylidene-*C*H_3), 24.9 (16-*C*), 24.8 (11-*C*), 22.8 (20-*C*), 15.3 (8-*C*); ESI-HRMS: *m/z* found 586.2127, $[M+H]^+$, calcd for $C_{32}H_{38}Cl_2NO_5$, 586.2127.

4.1.2. Preparation of 14β-isomers of 12a and 16a, and 14α-isomers of 12b and 16b.—

The synthesis is conducted according to ref [38].

(14β)-(Quinolyl-2'-methyl-8'-oxy) andrographolide (**12a**): white solid; m.p. 98.0–101.0 °C; 92% yield; ^{1}H NMR (400 MHz,DMSO-d6) *δ* 8.24 (d, *J* = 8.4 Hz, 1H, 4'-*H*), 7.57 (dd, *J* = 8.3, 1.1 Hz, 1H, 5'-*H*), 7.46 (dd, *J* = 8.3, 6.9 Hz, 2H, 6'-*H* and 7'-*H*), 7.15 (dd, *J* = 7.7, 1.2 Hz, 1H, 3'-*H*), 7.10 (m, 1H, 12-*H*), 6.05 (d, *J* = 5.3 Hz, 1H, 14-*H*), 4.81 (s, 1H, 17-*H*), 4.77 (m, 2H, 3-O*H* and 15-*H*), 4.58 (s, 1H, 17-*H*), 4.46 (m, 1H, 15-*H*), 4.03 (d, *J* = 7.1 Hz, 1H, 19-O*H*), 3.64 (m, 1H, 19-*H*), 3.08 (m, 1H, 19-*H*), 2.67 (s, 3H, 2'-CH_3), 2.32 – 2.15 (m, 4H, 3-*H*, 7-CH_2 and 11-*H*), 2.04 (d, *J* = 10.8 Hz, 1H, 6-*H*), 1.94 (m, 1H, 9-H), 1.61 (d, *J* = 13.1 Hz, 2H, 11-*H* and 6-*H*), 1.33 – 1.22 (m, 2H, 2-*H* and 1-*H*), 1.10 – 0.96 (m, 2H, 2-*H* and 1-*H*), 0.82 (s, 3H, 20-CH_3), 0.72 (m, 1H, 5-*H*), 0.41 (s, 3H, 19- CH_3); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 169.5 (16-*C*), 157.7 (8'-*C*), 151.8 (2'-*C*), 150.2 (12-*C*), 147.9 (8-*C*), 139.6 (9'-*C*), 136.3 (4'-*C*), 127.8 (13-*C*), 126.5 (10'-*C*), 125.8 (6'-*C*), 122.8 (3'-*C*), 121.1 (5'-*C*), 112.3 (17-*C*), 107.6 (7'-*C*), 78.1 (3-*C*), 72.0 (15-*C*), 71.4 (14-*C*), 62.5 (19-*C*), 56.0 (9-*C*), 53.5 (15-*C*), 41.9 (4-*C*), 38.7 (10-*C*), 37.4 (7-*C*), 35.6 (1-*C*), 27.7 (2-*C*), 25.2 (6-*C*), 25.0 (quinolyl-2'-*C*H_3), 23.9 (11-*C*), 22.7 (20-*C*), 14.5 (18-*C*); ESI-HRMS: *m/z* found 492.2746, $[M+H]^+$, calcd for $C_{30}H_{38}NO_5$, 492.2750.

(14α)-(Quinolyl-2'-methyl-8'-oxy) andrographolide (**12b**): white solid; m.p. 106.2–107.8 °C; 90% yield; ^{1}H NMR (400 MHz,DMSO-d6) *δ* 8.25 (d, *J* = 8.5 Hz, 1H, 4'-*H*), 7.64 – 7.56 (m, 1H, 5'-*H*), 7.45 (m, *J* = 8.2 Hz, 2H, 6'-*H* and 7'-*H*), 7.18 (dd, *J* = 7.7, 1.2 Hz, 1H, 3'-*H*), 6.89 – 6.79 (m, 1H, 12-*H*), 6.05 (d, *J* = 5.5 Hz, 1H, 14-*H*), 4.99 (d, *J* = 4.8 Hz, 1H, 3-O*H*), 4.77 (m, 2H, 17-*H* and 15-*H*), 4.59 – 4.52 (m, 2H, 17-*H* and 15-*H*), 4.11 – 4.03 (m, 1H, 19-O*H*), 3.71 (d, *J* = 10.9 Hz, 1H, 19-*H*), 3.18 – 3.12 (m, 1H, 19-*H*), 3.06 (m, 1H, 3-*H*), 2.66 (s, 3H, 2'-CH_3), 2.24 (m, 1H, 7-*H*), 2.19 – 2.09 (m, 2H, 7-*H* and 11-*H*), 1.82 (m, 3H, 6-*H*, 9-*H* and 11-*H*), 1.65 (d, *J* = 17.7 Hz, 2H, 6-*H* and 2-*H*), 1.39 (d, *J* = 4.7 Hz, 1H, 1-*H*), 1.21 – 1.18 (m, 2H, 2-*H* and 1-*H*), 1.00 (s, 3H, 20-CH_3), 0.96 – 0.89 (m, 1H, 5-*H*), 0.33 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 169.5 (16-*C*), 157.8 (8'-*C*), 151.8 (2'-*C*), 149.7 (12-*C*), 147.3 (8-*C*), 140.2 (9'-*C*), 136.4 (4'-*C*), 127.8 (13-*C*), 125.7 (10'-*C*), 125.6 (6'-*C*), 122.6 (3'-*C*), 122.2 (5'-*C*), 115.8 (17-*C*), 108.4 (7'-*C*), 78.3 (3-*C*), 73.6 (15-*C*), 71.6 (14-*C*), 62.5 (19-*C*), 54.9 (9-*C*), 54.2 (5-*C*), 42.2 (4-*C*), 38.3 (10-*C*), 37.3 (7-*C*), 36.1 (1-*C*),

27.8 (2-*C*), 25.1 (6-*C*), 24.6 (quinolyl-2'-*C*H_3), 23.9 (11-*C*), 23.0 (20-*C*), 14.4 (18-*C*); ESI-HRMS: *m/z* found 492.2745, $[M+H]^+$, calcd for $C_{30}H_{38}NO_5$, 492.2750.

(14β)-(Quinolyl-5',7'-dichloro-8'-oxy)andrographolide(**16a**): white solid; m.p. 161.5–163.0 °C; 89% yield; ^{1}H NMR (400 MHz,DMSO-d6) *δ* 9.11 (dd, *J* = 4.2, 1.6 Hz, 1H, 2'-*H*), 8.62 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.04 (s, 1H, 6'-*H*), 7.83 (m, 1H, 3'-*H*), 6.58 (m, 1H, 12'-*H*), 6.52 – 6.33 (m, 1H, 14-*H*), 5.03 (d, *J* = 4.8 Hz, 1H, 3-O*H*), 4.71 (d, *J* = 10.9 Hz, 1H, 17-*H*), 4.67 – 4.54 (m, 2H, 15-*H* and 17-*H*), 4.07 (m, 2H, 15-*H* and 19-O*H*), 3.68 (m, 1H, 19-*H*), 3.10 (m, 2H, 19-*H* and 3-*H*), 2.21 – 2.11 (m, 1H, 7-*H*), 1.76 (m, 1H, 7-*H*), 1.65 – 1.52 (m, 3H, 11-*H*, 6-*H* and 9-*H*), 1.52 – 1.36 (m, 2H, 11-*H* and 6-*H*), 1.24 – 1.17 (m, 1H, 2-*H*), 1.14 – 1.06 (m, 1H, 1-*H*), 1.00 (s, 3H, 20-C*H*$_3$), 0.96 (m, 1H, 2-*H*), 0.72 – 0.57 (m, 2H, 1-*H* and 5-*H*), 0.10 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 169.4 (16-*C*), 151.6 (8'-*C*), 149.2 (2'-*C*), 147.5 (12-*C*), 147.2 (8-*C*), 143.1 (9'-*C*), 133.4 (4'-*C*), 127.6 (13-*C*), 127.4 (6'-*C*), 126.6 (10'-*C*), 125.9 (3'-*C*), 125.8 (5'-*C*), 123.5 (7'-*C*), 107.9 (17-*C*), 78.1 (3-*C*), 77.0 (15-*C*), 71.7 (14-*C*), 62.5 (19-*C*), 54.4 (9-*C*), 54.0 (5-*C*), 42.1 (4-*C*), 38.0 (10-*C*), 37.2 (7-*C*), 35.9 (1-*C*), 27.8 (2-*C*), 25.1 (6-*C*), 23.9 (11-*C*), 23.0 (20-*C*), 14.2 (18-*C*); ESI-HRMS: *m/z* found 546.1809, $[M+H]^+$, calcd for $C_{29}H_{34}Cl_2NO_5$, 546.1814.

(14α)-(Quinolyl-5',7'-dichloro-8'-oxy)andrographolide(**16b**): white solid; m.p. 120.8–121.9 °C; 86% yield; ^{1}H NMR (400 MHz, DMSO-d6) *δ*9.10 (dd, *J* = 4.1, 1.6 Hz, 1H, 2'-*H*), 8.62 (dd, *J* = 8.7, 1.6 Hz, 1H, 4'-*H*), 8.02 (s, 1H, 6'-*H*), 7.83 (dd, *J* = 8.6, 4.2 Hz, 1H, 3'-*H*), 6.73 (m, 1H, 12-*H*), 6.32 (d, *J* = 4.5 Hz, 1H, 14-*H*), 5.00 (d, *J* = 4.9 Hz, 1H, 3-O*H*), 4.67 (d, *J* = 10.3 Hz, 2H, 17-*H* and 15-*H*), 4.56 (m, 1H, 19-O*H*), 4.10 (m, 2H, 17-*H* and 15-*H*), 3.71 (m, 1H, 19-*H*), 3.17 (m, 1H, 19-*H*), 3.07 (m, 1H, 3-*H*), 2.26 – 2.16 (m, 1H, 7-*H*), 1.93 – 1.72 (m, 2H, 7-*H* and 11-*H*), 1.61 (m, 2H, 6-*H* and 9-*H*), 1.48 (m, 3H, 11-H, 6-*H* and 2-*H*), 1.30 – 1.12 (m, 3H, 1-*H*, 2-*H* and 1-*H*), 1.00 (s, 3H, 20-C*H*$_3$), 0.82 (m, 1H, 5-*H*), 0.25 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 169.5 (16-*C*), 151.5 (8'-*C*), 148.5 (2'-*C*), 147.4 (12-*C*), 147.3 (8-*C*), 142.9 (9'-*C*), 133.5 (4'-*C*), 127.6 (13-*C*), 127.2 (6'-*C*), 126.4 (10'-*C*), 126.1 (3'-*C*), 125.9 (5'-*C*), 123.4 (7'-*C*), 107.4 (17-*C*), 78.2 (3-*C*), 76.9 (15-*C*), 71.6 (14-*C*), 62.5 (19-*C*), 54.5 (9-*C*), 54.1 (5-*C*), 42.2 (4-*C*), 38.3 (10-*C*), 37.3 (7-*C*), 36.2 (1-*C*), 27.7 (2-*C*), 24.5 (6'-*C*), 23.9 (11-*C*), 23.0 (20-*C*), 14.2 (18-*C*); ESI-HRMS: *m/z* found 546.1810, $[M+H]^+$, calcd for $C_{29}H_{34}Cl_2NO_5$, 546.1814.

4.1.3. Preparation of 14β-isomers of 13a and 17a, and 14α-isomers of 13b and 17b.—

The synthesis is conducted according to ref [38].

(14β)-(Quinolyl-2'-methyl-8'-oxy)-19-acetoxyandrographolide (**13a**): white solid; m. p. 79.9–81.5 °C; 83% yield; ^{1}H NMR (400 MHz, DMSO-d6) *δ*8.24 (d, *J* = 8.5 Hz, 1H, 4'-*H*), 7.57 (d, *J* = 8.1 Hz, 1H, 3'-*H*), 7.46 (m, 2H, 6'-*H* and 7'-*H*), 7.15 (d, *J* = 7.6 Hz, 1H, 3'-*H*), 7.11 (m, 1H, 12-*H*), 6.06 (d, *J* = 5.3 Hz, 1H, 14-*H*), 4.83 (s, 1H, 17-*H*), 4.79 (m, 2H, 3-O*H* and 15-*H*), 4.59 (s, 1H, 17-*H*), 4.46 (d, *J* = 10.9 Hz, 1H, 15-*H*), 4.40 (d, *J* = 4.6 Hz, 1H, 19-*H*), 4.09 (m, 1H, 19-*H*), 3.14 (m, 1H, 3-*H*), 2.67 (s, 3H, 2'-C*H*$_3$), 2.34 – 2.28 (m, 2H, 7-C*H*$_2$), 2.20 (m, 2H, 11-*H* and 6-*H*), 2.10 – 2.02 (m, 1H, 9-*H*), 1.95 (s, 3H, 19-acetoxy-C*H*$_3$), 1.80 – 1.63 (m, 2H, 11-*H* and 6-*H*), 1.46 (m, 2H, 2-*H* and 1-*H*), 1.01 (d, *J* = 15.2 Hz, 1H, 2-*H*,), 0.96 – 0.88 (m, 1H, 1-*H*), 0.77 (s, 3H, 20-C*H*$_3$), 0.54 (m, 1H, 5-*H*), 0.43 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) *δ* 170.8 (19-acetoxy-*C*), 169.8 (16-*C*), 158.0 (8'-*C*),

152.2 (2'-*C*), 150.5 (12-*C*), 148.3 (8-*C*), 140.1 (9'-*C*), 136.8 (4'-*C*), 128.2 (13-*C*), 126.9 (10'-*C*), 126.2 (6'-*C*), 123.2 (3'-*C*), 121.6 (5'-*C*), 112.9 (17-*C*), 108.0 (7'-*C*), 76.6 (3-*C*), 72.5 (15-*C*), 71.8 (14-*C*), 65.4 (19-*C*), 56.4 (9-*C*), 53.5 (5-*C*), 41.7 (4-*C*), 39.2 (10-*C*), 37.9 (7-*C*), 36.1 (1-*C*), 27.7 (2-*C*), 25.6 (6-*C*), 25.3 (2'-*C*H_3), 25.2 (11-*C*), 22.9 (20-*C*), 21.4 (19-acetoxy-*C*H_3), 14.2 (18-*C*); ESI-HRMS: *m/z* found 534.2851, $[M+H]^+$, calcd for $C_{32}H_{40}NO_6$, 534.2855.

(14α)-(Quinolyl-2'-methyl-8'-oxy)-19-acetoxyandrographolide (**13b**): white solid; m. p. 76.8–77.9 °C; 80% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.25 (d, *J* = 8.5 Hz, 1H, 4'-*H*), 7.62 (dd, *J* = 8.2, 1.2 Hz, 1H, 3'-*H*), 7.45 (m, 2H, 6'-*H* and 7'-*H*), 7.17 (dd, *J* = 7.7, 1.2 Hz, 1H, 3'-*H*), 6.85 (m, 1H, 12-*H*), 6.06 (d, *J* = 5.7 Hz, 1H, 14-*H*), 4.79 – 4.75 (m, 2H, 3'-O*H* and 17-*H*), 4.67 (d, *J* = 4.7 Hz, 1H, 15-*H*), 4.56 – 4.51 (m, 2H, 15-*H* and 17-*H*), 4.06 – 3.99 (m, 2H, 19-*H*), 3.00 (m, 1H, 3-*H*), 2.66 (s, 3H, 2'-C*H*$_3$), 2.26 (m, 2H, 7-C*H*$_2$), 2.21 – 2.10 (m, 2H, 11-*H* and 6-*H*), 1.95 (m, 1H, 9-*H*), 1.91 (s, 3H, 19-acetoxy-C*H*$_3$), 1.83 (m, 2H, 9-*H* and 11-*H*), 1.71 (m, 2H, 6-*H* and 2-*H*), 1.34 (m, 2H, 1-*H* and 2-*H*), 1.06 – 0.99 (m, 2H, 1-*H* and 5-*H*), 0.94 (s, 3H, 20-C*H*$_3$), 0.34 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) δ 170.4 (19-acetoxy-*C*), 169.5 (16-*C*), 157.8 (8'-*C*), 151.8 (2'-*C*), 149.7 (12-*C*), 147.3 (8-*C*), 140.3 (9'-*C*), 136.4 (4'-*C*), 127.8 (13-*C*), 125.7 (10'-*C*), 125.7 (6'-*C*), 122.6 (3'-*C*), 122.2 (5'-*C*), 116.0 (17-*C*), 108.4 (7'-*C*), 76.4 (3-*C*), 73.6 (15-*C*), 71.6 (14-*C*), 65.0 (19-*C*), 54.9 (9-*C*), 53.8 (5-*C*), 41.6 (4-*C*), 38.5 (10-*C*), 37.5 (7-*C*), 36.2 (1-*C*), 27.4 (2-*C*), 25.1 (6-*C*), 24.7 (2'-*C*H_3), 24.5 (11-*C*), 22.7 (20-*C*), 21.0 (19-acetoxy-*C*H_3), 13.6 (18-*C*); ESI-HRMS: *m/z* found 534.2856, $[M+H]^+$, calcd for $C_{32}H_{40}NO_6$, 534.2855.

(14β)-(Quinolyl-5',7'-dichloro-8'-oxy)-19-acetoxyandrographolide (**17a**): white solid; m. p. 86.1–87.4 °C; 78% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.12 (dd, *J* = 4.3, 1.6 Hz, 1H, 2'-*H*), 8.63 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-H), 8.05 (s, 1H, 6'-H), 7.84 (dd, *J* = 8.6, 4.2 Hz, 1H, 3'-H), 6.60 (d, *J* = 8.1 Hz, 1H, 12-*H*), 6.44 (d, *J* = 4.5 Hz, 1H, 14-*H*), 4.74 – 4.65 (m, 3H, 3-O*H* and 17-*H*$_2$), 4.61 (m, 1H, 15-*H*), 4.10 – 4.03 (m, 2H, 15-*H* and 19-*H*), 3.94 (d, *J* = 11.7 Hz, 1H, 19-*H*), 3.03 (m, 1H, 3-*H*), 2.19 (d, *J* = 12.6 Hz, 1H, 7-*H*), 1.92 (s, 3H, 19-acetoxy-C*H*$_3$), 1.74 (m, 2H, 7-*H* and 11-*H*), 1.63 – 1.53 (m, 2H, 6-*H* and 9-*H*), 1.44 – 1.22 (m, 3H, 11-H, 6-*H* and 2-*H*), 1.14 – 1.06 (m, 1H, 1-*H*), 1.05 – 0.98 (m, 1H, 2-*H*), 0.96 (s, 3H, 20-C*H*$_3$), 0.71 (m, 2H, 1-*H* and 5-*H*), 0.12 (s, 3H, 18-C*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d6) δ 170.5 (19-acetoxy-*C*), 169.5 (16-*C*), 151.6 (8'-*C*), 149.3 (2'-*C*), 147.5 (12-*C*), 147.2 (8-*C*), 143.1 (9'-*C*), 133.5 (4'-*C*), 127.6 (13-*C*), 127.5 (10'-*C*), 126.7 (6'-*C*), 126.0 (3'-*C*), 125.9 (5'-*C*), 123.5 (17-*C*), 107.9 (7'-*C*), 77.1 (3-*C*), 76.3 (15-*C*), 71.7 (14-*C*), 65.0 (19-*C*), 54.4 (9-*C*), 53.6 (5-*C*), 41.5 (4-*C*), 38.2 (10-*C*), 37.4 (7-*C*), 36.0 (1-*C*), 27.5 (2-*C*), 25.0 (6-*C*), 24.6 (11-*C*), 22.7 (20-*C*), 20.9 (19-acetoxy-*C*H_3), 13.6 (18-*C*); *m/z* found 588.1913, $[M+H]^+$, calcd for $C^{31}H^{36}Cl^2NO^6$, 588.1920.

(14α)-(Quinolyl-5',7'-dichloro-8'-oxy)-19-acetoxyandrographolide (**17b**): white solid; m.p. 84.9–96.1 °C; 81% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.10 (dd, *J* = 4.2, 1.6 Hz, 1H, 2'-*H*), 8.63 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.03 (s, 1H, 6'-*H*), 7.85 – 7.80 (m, 1H, 3'-*H*), 6.72 (dd, *J* = 8.7, 5.1 Hz, 1H, 12-*H*), 6.33 (d, *J* = 4.6 Hz, 1H, 14-*H*), 4.71 – 4.65 (m, 3H, 3-O*H* and 17–2*H*), 4.55 (m, 1H, 15-*H*), 4.12 – 4.05 (m, 2H, 15-*H* and 19-*H*), 3.96 (d, *J* = 11.7 Hz, 1H, 19-*H*), 3.03 (m, 1H, 3-*H*), 2.26 – 2.19 (m, 1H, 7-*H*), 1.93 (s, 3H, 19-acetoxy-C*H*$_3$), 1.75 (m, 2H, 7-*H* and 11-*H*), 1.62 (d, *J* = 11.5 Hz, 1H, 6-*H*), 1.51 (m, J = 16.1, 2H, 9-*H* and

11-H), 1.40 – 1.31 (m, 2H, 6-H and 2-H), 1.08 – 0.99 (m, 2H, 1-H and 2-H), 0.96 (s, 3H, 20-CH_3), 0.93 – 0.82 (m, 2H, 1-H and 5-H), 0.27 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) δ 170.3 (19-acetoxy-C), 169.5 (16-C), 151.5 (8'-C), 148.4 (2'-C), 147.3 (12-C), 147.3 (8-C), 142.9 (9'-C), 133.5 (4'-C), 127.6 (13-C), 127.1 (10'-C), 126.4 (6'-C), 126.1 (3'-C), 125.9 (5'-C), 123.3 (17-C), 107.4 (7'-C), 76.8 (3-C), 76.3 (15-C), 71.6 (14-C), 64.9 (19-C), 54.5 (9-C), 53.7 (5-C), 41.5 (4-C), 38.5 (10-C), 37.4 (7-C), 36.2 (1-C), 27.4 (2-C), 24.6 (6-C), 24.4 (11-C), 22.7 (20-C), 20.9 (19-acetoxy-CH_3), 13.4 (18-C); *m/z* found 588.1922, $[M+H]^+$, calcd for $C_{31}H_{36}Cl_2NO_6$, 588.1920.

4.1.4. Preparation of 14β-isomers of 14a and 18a, and 14α-isomers of 14b and 18b.—

The synthesis is conducted according to ref [38].

(14β)-(Quinolyl-2'-methyl-8'-oxy)-3-keto-19-acetoxy andrographolide (**14a**): white solid; m.p. 72.6–73.1 °C; 90% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.23 (d, *J* = 8.4 Hz, 1H, 4'-H), 7.63 – 7.53 (m, 1H, 5'-H), 7.45 (dd, *J* = 8.3, 7.0 Hz, 2H, 6'-H and 7'-H), 7.26 – 7.10 (m, 1H, 3'-H), 7.03 (t, *J* = 7.1 Hz, 1H, 12-H), 6.11 (d, *J* = 5.5 Hz, 1H, 14-H), 4.90 (s, 1H, 17-H), 4.79 (m,, 1H, 15-H), 4.63 (s, 1H, 17-H), 4.47 (d, *J* = 11.3 Hz, 1H, 15-H), 4.33 (d, *J* = 11.3 Hz, 1H, 19-H), 3.78 (d, *J* = 11.4 Hz, 1H, 19-H), 2.64 (s, 3H, 2'-CH_3), 2.32 (d, *J* = 11.0 Hz, 3H, 7-CH_2 and 11-H), 2.11 (d, *J* = 10.1 Hz, 1H, 6-H), 2.03 – 1.89 (m, 2H, 9-H and 11-H), 1.86 (s, 3H, 19-acetoxy-CH_3), 1.63 (m, 2H, 6-H and 2-H), 1.52 (m, 1H, 1-H), 1.18 (m, 1H, 2-H), 1.07 – 0.90 (m, 2H, 1-H and 5-H), 0.83 (s, 3H, 20-CH_3), 0.66 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) δ 211.2 (3-C), 170.0 (19-acetoxy-C), 169.3 (16-C), 157.7 (8'-C), 151.6 (2'-C), 149.5 (12-C), 146.9 (8-C), 139.6 (9'-C), 136.4 (4'-C), 127.8 (13-C), 126.5 (10'-C), 125.7 (6'-C), 122.7 (3'-C), 121.5 (5'-C), 113.3 (17'-C), 108.6 (7'-C), 72.4 (15-C), 71.4 (14-C), 65.3 (19-C), 55.0 (9-C), 54.6 (5-C), 51.1 (4-C), 38.6 (10-C), 36.8 (7-C), 36.4 (1-C), 34.5 (2-C), 25.2 (6-C), 25.1 (2'-CH_3), 24.2 (11-C), 20.5 (20-C), 20.1 (19-acetoxy-CH_3), 13.8 (18-C); *m/z* found532.2695, $[M+H]^+$, calcd for $C_{32}H_{38}NO_6$, 532.2699.

(14α)-(Quinolyl-2'-methyl-8'-oxy)-3-keto-19-acetoxy andrographolide (**14b**): white solid; m.p. 62.8–63.6 °C; 86% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.25 (d, *J* = 8.4 Hz, 1H, 4'-H), 7.62 (d, *J* = 8.1 Hz, 1H, 5'-H), 7.45 (t, *J* = 7.7 Hz, 2H, 6'-H and 7'-H), 7.19 (d, *J* = 7.6 Hz, 1H, 3'-H), 6.87 (t, *J* = 6.5 Hz, 1H, 12-H), 6.06 (d, *J* = 5.5 Hz, 1H, 14-H), 4.87 (s, 1H, 17-H), 4.78 (m, 1H, 15-H), 4.69 (s, 1H, 17-H), 4.55 (m, 1H, 15-H), 4.39 (d, *J* = 11.3 Hz, 1H, 19-H), 3.82 (d, *J* = 11.4 Hz, 1H, 19-H), 2.66 (s, 3H, 2'-CH_3), 2.59 (m, 1H, 7-H), 2.36 – 2.22 (m, 3H, 7-H, 11-H and 6-H), 1.88 (s, 3H, 19-acetoxy-CH_3), 1.70 – 1.63 (m, 1H, 9-H), 1.58 (m, 1H, 11-H), 1.52 – 1.44 (m, 1H, 6-H), 1.39 (m, 3H, 2-CH_2 and 1-H), 1.32 (m, 1H, 1-H), 0.98 (s, 3H, 20-CH_3), 0.85 (d, *J* = 4.9 Hz, 1H, 5-H), 0.60 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) δ 211.8 (3-C), 170.1 (19-acetoxy-C), 169.4 (16-C), 157.8 (8'-C), 151.9 (2'-C), 149.4 (12-C), 146.5 (8-C), 140.2 (9'-C), 136.4 (4'-C), 127.8 (13-C), 125.8 (10'-C), 125.7 (6'-C), 122.7 (3'-C), 122.1 (5'-C), 115.6 (17'-C), 109.5 (7'-C), 73.6 (15-C), 71.5 (14-C), 65.4 (19-C), 55.4 (9-C), 53.9 (5-C), 51.3 (4-C), 38.3 (10-C), 36.8 (7-C), 36.7 (1-C), 34.8 (2-C), 25.1 (6-C), 24.7 (2'-CH_3), 24.2 (11-C), 20.5 (20-C), 20.3 (19-acetoxy-CH_3), 13.8(18-C); *m/z* found532.2693, $[M+H]^+$, calcd for $C_{32}H_{38}NO_6$, 532.2699.

(14β)-(Quinolyl-5',7'-dichloro-8'-oxy)-3-keto-19-acetoxy andrographolide (**18a**): white solid; m.p. 77.5–78.2 °C; 86% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.13 (dd, *J* = 4.2,

1.6 Hz, 1H, 2'-*H*), 8.62 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.03 (s, 1H, 6'-*H*), 7.83 (dd, *J* = 8.6, 4.2 Hz, 1H, 3'-*H*), 6.62 (m, 1H, 12-*H*), 6.45 (d, *J* = 4.5 Hz, 1H, 14-*H*), 4.76 – 4.66 (m, 2H, 15-*H* and 17-*H*), 4.60 (m, 1H, 15-*H*), 4.38 (d, *J* = 11.3 Hz, 1H, 19-*H*), 4.20 (s, 1H, 17-*H*), 3.81 (d, *J* = 11.3 Hz, 1H, 19-*H*), 2.60 (m, 1H, 7-*H*), 2.24 (m, 1H, 7-*H*), 2.15 – 2.05 (m, 1H, 11-*H*), 1.90 (s, 3H,19-acetoxy-CH_3), 1.88 – 1.81 (m, 1H, 6-*H*), 1.81 – 1.71 (m, 2H, 9-*H* and 11-*H*), 1.66 (m, 1H, 6-*H*), 1.54 (m, 1H, 2-*H*), 1.27 (m, *J* = 23.0, 13.7, 4.4 Hz, 2H, 1-*H* and 2-*H*), 1.17 – 1.03 (m, 2H, 1-*H* and 5-*H*), 0.99 (s, 3H, 20-CH_3), 0.40 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) δ 211.6 (3-*C*), 170.1 (19-acetoxy-*C*), 169.4 (16-*C*), 151.6 (8'-*C*), 149.0 (2'-*C*), 147.2 (12-*C*), 146.6 (8-*C*), 143.1 (9'-*C*), 133.4 (4'-*C*), 127.7 (13-*C*), 127.5 (10'-*C*), 126.6 (6'-*C*), 126.0 (3'-*C*), 125.9 (5'-*C*), 123.5 (17-*C*), 108.9 (7'-*C*), 77.1 (15-*C*), 71.6 (14-*C*), 65.4 (19-*C*), 55.1 (9-*C*), 53.2 (5-*C*), 51.2 (4-*C*), 38.0 (10-*C*), 36.6 (7-*C*), 36.5 (1-*C*), 34.8 (2-*C*), 25.2 (6-*C*), 24.1 (11-*C*), 20.5 (20-*C*), 20.4 (19-acetoxy-*C*H_3), 13.7 (18-*C*); *m/z* found 586.1758, $[M+H]^+$, calcd for $C_{31}H_{34}Cl_2NO_6$, 586.1763.

(14α)-(Quinolyl-5',7'-dichloro-8'-oxy)-3-keto-19-acetoxy andrographolide (**18b**): white solid; m.p. 81.2–83.0 °C; 85% yield; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.11 (dd, *J* = 4.2, 1.6 Hz, 1H, 2'-*H*), 8.64 (dd, *J* = 8.6, 1.6 Hz, 1H, 4'-*H*), 8.04 (s, 1H, 6'-*H*), 7.91 – 7.73 (m, 1H, 3'-*H*), 6.74 (m, 1H, 12-*H*), 6.36 (d, *J* = 4.5 Hz, 1H, 14-*H*), 4.77 (d, *J* = 1.6 Hz, 1H, 17-*H*), 4.66 (d, *J* = 11.0 Hz, 1H, 15-*H*), 4.53 (m, 1H, 15-*H*), 4.41 (d, *J* = 11.3 Hz, 1H, 19-*H*), 4.20 (s, 1H, 17-*H*), 3.83 (d, *J* = 11.4 Hz, 1H, 19-*H*), 2.62 (m, 1H, 7-*H*), 2.33 – 2.23 (m, 1H, 7-*H*), 2.09 (m, 1H, 11-*H*), 1.97 – 1.91 (m, 1H, 6-*H*), 1.90 (s, 3H, 19-acetoxy-CH_3), 1.86 (m, 1H, 9-*H*), 1.80 (d, *J* = 11.6 Hz, 1H, 11-*H*), 1.68 (m, 2H, 6-*H* and 2-*H*), 1.59 (m, 1H, 1-*H*), 1.36 – 1.20 (m, 3H, 2-*H*, 1-*H* and 5-*H*), 0.99 (s, 3H, 20-CH_3), 0.53 (s, 3H, 18-CH_3); ^{13}C NMR (101 MHz, DMSO-d6) δ 211.7 (3-*C*), 170.1 (19-acetoxy-*C*), 169.5 (16-*C*), 151.6 (8'-*C*), 148.3 (2'-*C*), 147.3 (12-*C*), 146.5 (8-*C*), 142.9 (9'-*C*), 133.5 (4'-*C*), 127.6 (13-*C*), 127.2 (10'-*C*), 126.4 (6'-*C*), 126.2 (3'-*C*), 125.9 (5'-*C*), 123.4 (17-*C*), 108.5 (7'-*C*), 76.9 (15-*C*), 71.6 (14-*C*), 65.4 (19-*C*), 55.2 (4-*C*), 53.4 (5-*C*), 51.3 (4-*C*), 38.3 (10-*C*), 36.8 (7-*C*), 36.7 (1-*C*), 34.7 (2-*C*), 24.7 (6-*C*), 24.2 (11-*C*), 20.5 (20-*C*), 20.3 (19-acetoxy-*C*H_3), 13.6 (18-*C*); *m/z* found 586.1761, $[M+H]^+$, calcd for $C_{31}H_{34}Cl_2NO_6$, 586.1763.

4.2. Biology

The screening procedure was adopted from the references [58–59]. All compounds were dissolved in DMSO before diluted with medium into stock solutions and the final concentration of DMSO in each well is 0.1%. Niclosamide of a previously identified active compound against Zika virus infection in Nature Medicine (2016) [59] was included in viral titer assays each time as a reference and quality control compound.

Cell culture

The glioblastoma SNB-19 cell line was maintained at 37 °C in 5% CO_2 in RPMI-1640 medium, 1× penicillin–streptomycin-amphotericin B (PSA), and 10% fetal bovine serum (FBS) (Thermo Fisher Scientific). The monkey kidney Vero cell line was maintained at 37 °C in 5% CO_2 in DMEM, 1× penicillin-streptomycin, and 10% FBS.

The *Aedes albopictus* C6/36 cell line was maintained at 28 °C in 5% CO_2 in EMEM media (ATCC), penicillin-streptomycin, and 10% FBS.

ZIKV culture, ZIKV infection of SNB-19 and drug addition

Zika virus stocks were generated in *Aedes albopictus* clone C6/36 cells from PRVABC59-ZIKV strain stocks (ATCC). To amplify viral stocks, a T-75 flask of C6/36 cells (90–95% confluency) was inoculated with 1 X 10^6 Zika virions in low volume (3 ml) for 1 hour, with rocking to disperse media every 15 minutes. After 1 hour, 17ml of media was added and C6/36 cells were maintained at 28°C in 5% CO_2 for 7 days. At day 7 and day 8 post viral inoculation, supernatants were harvested, filtered, and stored at −80°C. ZIKV titer was determined by focus forming unit assay.

SNB-19 cells were seeded into 96-well plates 1 day prior to viral infection, and then SNB-19 cells were incubated with medium (control) or gradient concentrations of each compound for 1 h prior to addition of C6/36 derived-ZIKV at MOI = 1. SNB-19 cell supernatants were then harvested at 24 hours post infection from the 96-well plates and either frozen at −80°C or used immediately for focus forming unit assay.

ZIKV titer assay

Vero cells were seeded overnight into 96-well plates to achieve a confluent monolayer the next day. Supernatant from drug-treated ZIKV-infected SNB-19 cells was diluted in sterile 96-well plates and subsequently used to inoculate the naive Vero cell plate, which was then incubated for 2 hours prior to removal of supernatant and replacement with a methyl-cellulose overlay. After 48 hours further incubation, the methyl-cellulose overlay was removed by aspiration, wells gently washed with phosphate buffered saline (PBS), and wells fixed with 100μl 4% paraformaldehyde for 15 minutes. Then, paraformaldehyde was removed, wells gently washed with 200μ1 PBS per well three times, and cells blocked with 100μl PBTG (PBS containing 0.1% Triton X-100, 10% normal goat serum, and 1% bovine serum albumin) for 1 hour at room temperature while rocking. Blocking solution was removed and wells were incubated with 50μl primary antibody solution (4G2, 1:500 in PBTG) at 4°C overnight while rocking. The next day, cells were washed gently with 200μl per well PBS three times, and then incubated with 50μl secondary antibody solution (goat anti-mouse-IgG HRP, 1:500 in PBTG) for 1 hour at room temperature. Cells were then washed with 200μl per well PBS three times, and incubated with 50μl freshly prepared DAB solution per well for 10 minutes, prior to rinsing with deionized water (DAB prepared with nickel according to manufacturer's instructions) and colonies quantified.

Cytotoxicity of testing compounds to SNB-19 cells and Vero cells.

SNB-19 cells or Vero cells were plated in a 96-well plate at a concentration of 1.0 ×10^4 cells per well overnight to allow cell attachment. Working solutions of the testing compounds, or the 0.1% DMSO vehicle as a control were dispensed appropriately into the partitioned 96-well plates, which were then incubated for another 24 h for SNB-19 cells or 48 h for Vero cells. Then, the medium was discarded and the cells were incubated for 4 h at 37 °C in MTT solution (final concentration 0.5 mg/mL). The solution was then replaced with 100 mL DMSO to dissolve the violet formazan crystals in the intact cells. Cell growth was assessed by MTT according to the manufacturer's protocol. The absorbance was measured at 570 nm as the reference wavelength. The cytotoxicity as the CC_{50} value (concentration of 50% cell

growth/viability inhibition) was calculated based on the percentage of cell viability data compared to the control group. Each concentration was repeated 3 times independently.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- 24 Analogs of 14-aryloxy andrographolide were tested for anti-Zika virus activity.
- Optimal modification/s at 3-, 14-, or 19-positions can make derivatives less toxic.
- 14β-(8'-Quinolyloxy)-3,19-diol derivative **3** exhibits the most potent activity.
- 14α-(5',7'-Dichloro-8'-quinolyloxy)-19-OAc analog **17b** is the most selective agent.

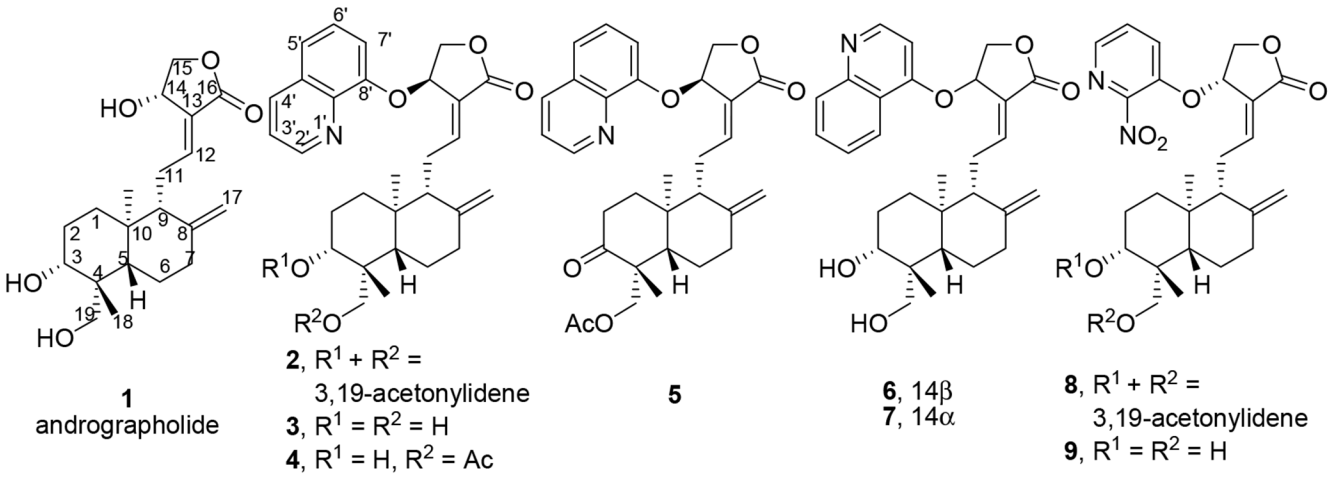
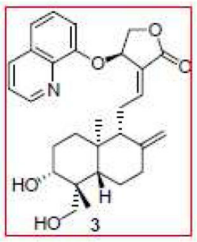
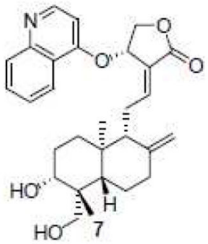
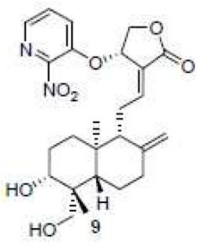
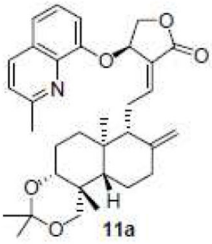
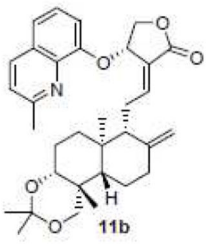


Fig. 1.

Structures of andrographolide (**1**) and its previously reported derivatives (**2** to **9**) [38] from our in-house library.

Compound		 3	 7	 9	 11a	 11b
EC_{50}		1.3 ± 0.1 μM	11.0 ± 0.2 μM	12.5 ± 0.4 μM	8.5 ± 0.4 μM	16.6 ± 0.3 μM
to SNB-19	CC_{50}	22.7 ± 1.1 μM	60.1 ± 1.1 μM	67.7 ± 1.1 μM	78.4 ± 1.0 μM	66.5 ± 1.0μM
	SI	17.5	5.5	5.4	9.2	4.0
to Vero	CC_{50}	20.8 ± 0.5 μM	22.8 ± 0.2 μM	37.7 ± 0.1 μM	55.9 ± 0.2 μM	65.3 ± 03 μM
	SI	16.1	2.1	3.0	6.6	3.9

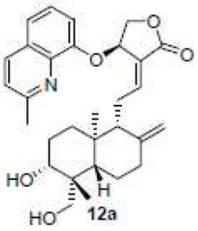
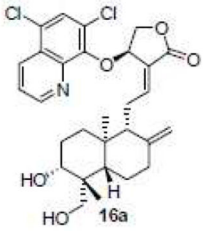
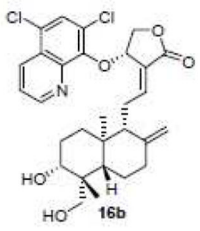
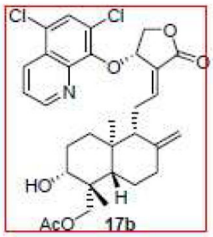
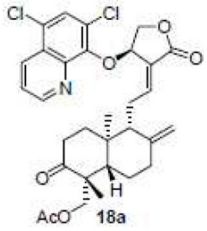
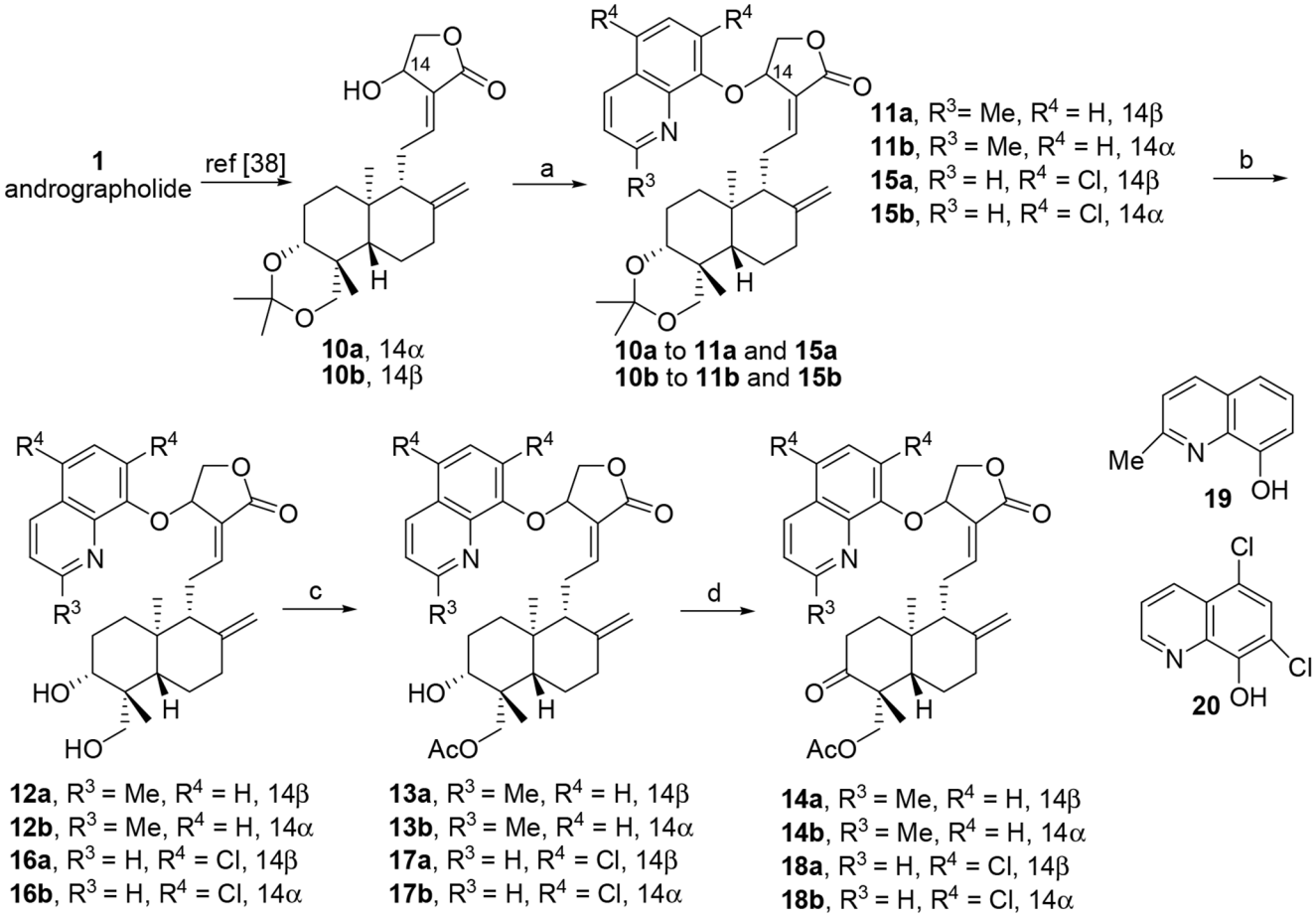
Compound		 12a	 16a	 16b	 17b	 18a
EC_{50}		25.8 ± 1.1 μM	13.3 ± 0.5 μM	7.8 ± 0.4 μM	4.5 ± 0.2 μM	24.6 ± 0.9 μM
To SNB-19	CC_{50}	>100 μM	>100 μM	85.2 ± 1.0 μM	88.7 ± 1.1 μM	72.0 ± 1.0 μM
	SI	> 3.9	>7.5	10.9	19.7	2.9
to Vero	CC_{50}	99.8 ± 0.7 μM	>100 μM	82.5 ± 2.2 μM	85.0 ± 1.6 μM	57.4 ± 0.3 μM
	SI	3.9	>7.5	7.5	18.9	2.3

Fig. 2.

Anti-Zika active derivatives of andrographolide. Compounds of **3** and **17b** (in red rectangular boxes) are the most active and selective compounds. (SNB-19: SNB-19 cell line; Vero: Vero cell line)



Scheme 1.

Reagents and conditions: (a) anhydrous THF, **10a** or **10b** (1.0 eq.), **19** or **20** (1.5 eq.), DIAD (1.5 eq.), PPh_3 (1.5 eq.), 0 °C to room temperature; (b) $MeOH/H_2O$ (4/1), $TsOH{\cdot}H_2O$, 20 °C; (c) AcCl, TEA, 0 °C; (d) DCM, Dess–Martin periodinane, rt.