DESIGN, SYNTHESIS OF NEW C(14)-ANDROGRAPHOLIDE ANALOGUES AND THEIR CYTOTOXIC ACTIVITY

Narendra Sing Chauhan,¹ Venkat.R. P,¹ Virohit Patil,¹ Ravindra Patil¹,*

¹Department of Pharmaceutical Sciences, Allana College of Pharmacy, Pune, Maharashtra, India, 411038
 Corresponding author: E-mail: patilravindra118@gmail.com

ABSTRACT: A new series of andrographolide analogues were synthesized from andrographolide, the cytotoxic constituent of the plant Andrographis paniculata. The derived analogs (4a-4e) were evaluated for their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. Most of the analogues display significant cytotoxic activity against tested cell lines. The allyl derivative 4c had higher activity than parent compound andrographolide 1, and standard drug cisplatin against tested cell lines.

Key words: Andrographolide, Andrographis paniculata, cytotoxic activity, Andrographolide analogues.

INTRODUCTION

Natural products play an important role in drug discovery, serving as either a source or motivation for approximately half of all approved small-molecule drugs (Newman et al., 2012). Although a large number of these drugs are naturally occurring substances, derivatives of natural products are often essential to improve pharmacokinetic properties. These derivatives have traditionally been accessed through total synthesis and mutasynthesis (Wender et al., 2002). In cases where the natural product is readily available from the natural source, semi-synthesis is an attractive approach. Due to the large and often complex scaffolds nature develops, semi-synthesis requires highly selective transformations. Perhaps the best example of natural product derivatives that have been developed into drugs for the treatment of plethora of biological activities is andrographolide. Andrographolide (1) is a labdane diterpenoid, isolated from the whole plant of Andrographis paniculata (family Acanthaceae), it is extensively used in the traditional system of medicine in south east Asia since antiquity (Chakravarti et al., 1951). Extracts of plants and their phytochemical constituents together with andrographolide (1) have been reported to display a broad range of biological activities of therapeutic importance that include antimalarial (Najib et al., 1999, Li et al., 2007), antibacterial (Li et al., 2007), anti-inflammatory (Shen et al., 2002, Madav et al., 1995, Shen et al., 2000, Reddy et al., 2008, Salaga et al., 2014), hepatoprotective (Handa et al., 1990), antithrombotic (Li et al., 2007), immune stimulant (Kumar et al., 2004), antidepressive (White et al., 2014), antiallergic (Gupta et al., 1998), central nervous system disorders (White et al., 2014, Fajemiroye et al., 2014, Polepally et al., 2013, Prabhakar et al., 2014, Zjawiony et al., 2011), anti HIV (Li et al., 2007, Raju et al., 2008), and anticancer (Kumar et al., 2004, Nanduri et al., 2004). Andrographolide (1) has also been widely used in clinics for the treatment of fever, cold, inflammation, diarrhea and infectious diseases, so it has aroused the interest of pharmacologists. Since its discovery of plethora of activities, a large number of andrographolide (1) analogs have been prepared by semi-synthesis for the modification of the biological activities which are available in the literature (He et al., 2003, Li et al., 2006, Nanduri et al., 2004). Presuming that incorporation of alkoxy at C-14 in andrographolide might generate some bioactive molecules, herein, we report the synthesis of a new series of alkoxy andrographolide derivatives and their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines.

Chemistry

Andrographolide (1) was isolated in high yields from the plant of Andrographis paniculata and used as the starting material for the preparation of the C (14)-modified alkoxy analogue library 4a-4e (Scheme 1). Initially, Andrographolide 1 was treated with 2, 2-dimethoxy propane in the presence of pyridinium p-toluenesulfonate (PPTS) in CH₂Cl₂ at 40°C to yield 87% of compound 2.
Scheme 1. Synthesis of alkoxy-type andrographolide analogs (4a-4e). Reagents and conditions: (a) 2,2-dimethoxypropane, PPTS, DCM, reflux at 40°C, 1h; (b) appropriate alkyl halide, Et$_3$N, CBr$_4$, dry DCM, N$_2$, r.t, 3-4 h; (c) Acetic acid, H$_2$O, r.t, 30 min.

Compound 2 was treated with appropriate acid halides in the presence of diisopropylethyl amine base in DCM to give compounds 3a-3e. Derivatives 4a-4e were prepared in yields of 69-73% by reacting compounds 3a-3e with acetic acid in water to remove isopropylidene (Scheme 1).

Biological activity:

Andrographolide (1) and its dicarboxylic ester type analogs (4a-4e) were evaluated for their in vitro cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. The in vitro cytotoxic activity assays were conducted using classical MTT method (Anne et al., 1991). The cytotoxicity data of 1 and its analogs are collated in Table 1. For comparison purpose, IC$_{50}$ values of positive control, cisplatin against cell lines are included in the Table 1. Most of the synthesized alkoxy andrographolide derivatives showed appreciable cytotoxic activity compared to the parent compound Andrographolide 1 against tested cell lines. Analogs 4c had shown potent activity than the standard cisplatin and parent compound Andrographolide 1.

As demonstrated in table 1, among all derivatives allyloxy derivative 4c had significant cytotoxic activity against tested cell lines. The allyl derivative 4c had higher activity than parent compound andrographolide 1 (IC$_{50}$ = 4.35 vs 17.85 µM against H522; 3.98 vs 16.15 µM against K562; 10.23 vs 13.82 µM against MCF-7; 5.50 vs 8.17 µM against DU145 respectively), and significant activity than standard drug cisplatin against tested cell lines (IC$_{50}$ = 4.35 vs 4.74 µM against H522; 3.98 vs 3.76 µM against K562; 10.23 vs 9.55 µM against MCF-7; 5.50 vs 5.54 µM against DU145 respectively) (Table 1). The methoxy derivative 4a had higher activity than parent compound andrographolide against H522, K562 and MCF-7 cell lines (IC$_{50}$ = 7.56 vs 17.85 µM; 9.55 vs 16.15 µM; 8.30 vs 13.82 µM respectively) (Table 1), and reduced activity than cisplatin.

Similarly, the ethoxy derivative 4b also had higher activity than parent compound andrographolide against H522, K562 and MCF-7 cell lines (IC$_{50}$ = 7.56 vs 17.85 µM; 9.55 vs 16.15 µM; 8.30 vs 13.82 µM respectively) (Table 1), and reduced activity than cisplatin (Table 1). Compounds 4e and 4f have reduced activity than standard cisplatin, but still show appreciable cytotoxicity compared to the parent andrographolide 1 (Table 1); this reducing activity against cell lines may be due to presence of bulkier phenyl ring in their structures at C-14 position.

Compounds 4a-4e were prepared in yields of 69-73% by reacting compounds 3a-3e with acetic acid in water to remove isopropylidene (Scheme 1).
In summary, a series of new dicarboxylic ester-type analogs of andrographolide were synthesized in an effort to explore the cytotoxic effects of C-14 substitution against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. All the synthesized analogs showed significant cytotoxic activity against tested cell lines compared to the parent andrographolide. Analogs allyl derivative 4e had higher activity than parent compound andrographolide and standard cisplatin against H522, K562, MCF-7 and DU145 cell lines.

Table 1. Cytotoxicity effects of C(14)- alkoxy derived andrographolide analogues (4a-4e) against cancer cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>H522</th>
<th>K562</th>
<th>MCF-7/ADR</th>
<th>DU145</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.85±3.50</td>
<td>16.15±3.35</td>
<td>13.82±2.56</td>
<td>8.17±1.15</td>
</tr>
<tr>
<td>4a</td>
<td>7.56±2.14b</td>
<td>9.55±2.95</td>
<td>8.30±2.75</td>
<td>10.56±2.75</td>
</tr>
<tr>
<td>4b</td>
<td>9.85±2.45</td>
<td>11.98±2.85</td>
<td>10.65±3.65</td>
<td>17.50±2.89</td>
</tr>
<tr>
<td>4c</td>
<td>4.35±1.45</td>
<td>3.98±2.12</td>
<td>10.23±2.65</td>
<td>5.50±2.75</td>
</tr>
<tr>
<td>4d</td>
<td>20.15±3.30</td>
<td>15.90±3.55</td>
<td>23.85±5.45</td>
<td>10.96±2.85</td>
</tr>
<tr>
<td>4e</td>
<td>16.20±4.30</td>
<td>15.76±5.36</td>
<td>29.74±4.94</td>
<td>8.95±2.73</td>
</tr>
</tbody>
</table>

cisplatinc 4.74±0.50 3.76±0.85 9.55±1.25 5.54±1.35

*Concentration of compound required to inhibit cell growth by 50% as determined by MTT assay; data are expressed as mean-standard deviation; cisplatin was used as positive control; NA- not active; NT- not tested; NA- not active; NT- not tested;

<table>
<thead>
<tr>
<th>Compounds</th>
<th>H522</th>
<th>K562</th>
<th>MCF-7/ADR</th>
<th>DU145</th>
</tr>
</thead>
</table>
| Ethyl-14-O-andrographolide (4b). White amorphous powder, 1H NMR (400 MHz, CDCl3): δ 7.04 (t, 6.8 Hz, 1H), 5.95 (d, 5.8 Hz, 1H), 4.93 (s, 1H), 4.53-4.48 (m, 2H), 4.21-4.13 (m, 2H), 3.87 (d, 11.6 Hz, 1H), 3.54-3.45 (m, 1H), 3.45 (q, 2H), 3.31 (d, 10.6 Hz, 1H), 2.51-2.31 (m, 4H), 1.97-1.92 (m, 1H), 1.80-1.69 (m, 5H), 1.21-1.13 (m, 6H), 1.11 (t, 3H), 0.72 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 173.5, 153.4, 148.7, 124.3, 109.3, 80.8, 72.8, 70.4, 63.9, 62.1, 57.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS (m/z): [M+H]+ calculated for C23H34O5, 365.29; found, 365.31.

Allyl-14-o-andrographolide (4c). White amorphous powder, 1H NMR (400 MHz, CDCl3): δ 7.03 (t, 6.8 Hz, 1H), 6.12 (m, 1H (olefin proton)), 5.91 (d, 5.8 Hz, 1H), 5.46 (d, 12.6 Hz, 1H), 5.32 (d, 6.2 Hz, 1H), 4.91 (s, 1H), 4.55-4.49 (m, 2H), 4.23-4.04 (m, 4H), 3.89 (d, 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.19 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 173.5, 153.4, 148.7, 134.8, 124.3, 117.3, 109.3, 80.8, 72.8, 72.1, 70.4, 63.9, 62.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS (m/z): [M+H]+ calculated for C23H34O5, 391.24; found, 391.19.

Benzyl-14-o-andrographolide (4d). White amorphous powder, 1H NMR (400 MHz, CDCl3): δ 7.29-7.26 (m, 5H), 7.03 (t, 6.8 Hz, 1H), 5.93 (d, 5.8 Hz, 1H), 4.92 (s, 1H), 4.63 (s, 2H), 4.55-4.49 (m, 2H), 4.23-4.14 (m, 2H), 3.89 (d, 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.69 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 173.5, 153.4, 148.7, 137.5, 129.6, 128.6, 127.5, 124.3, 109.3, 80.8, 72.8, 72.5, 70.4, 63.9, 62.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS (m/z): [M+H]+ calculated for C25H36O5, 441.26; found, 441.19.

Para-Chlorobenzyl-14-o-andrographolide (4e). White amorphous powder, 1H NMR (400 MHz, CDCl3): δ 7.39 (d, 6.8 Hz, 2H), 7.22 (d, 5.8 Hz, 2H), 7.02 (t, 6.8 Hz, 1H), 5.93 (d, 5.8 Hz, 1H), 4.69 (s, 2H), 4.92 (s, 1H), 4.55-4.49 (m, 2H), 4.23-4.14 (m, 2H), 3.89 (d, 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.19 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 173.5, 153.4, 148.7, 134.3, 128.7, 125.3, 124.3, 109.3, 80.8, 72.8, 70.4, 63.9, 62.1, 57.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS (m/z): [M+H]+ calculated for C25H35OCl, 476.21; found, 476.29.
ACKNOWLEDGEMENTS:
The authors are thankful to Head of the department of Pharmaceutical sciences, Allan College of Pharmacy. We also thankful to Invocan Pharmaceuticals, Aurangabad, Maharashtra for providing NMR and mass data for synthesized compounds, and also to Rubicon formulations for biological activity studies.

REFERENCES


Polepally, P. R. Setola, V. Vardy, E. Roth, B. L. Zjawiony, J. K. (2013). New Michael Acceptor-Type of Salvinorin A Ligands to Kappa-Opioid Receptor. Planta Med. 79(05), P41.

Polepally, P. R. White, K. Roth, B. L.; Zjawiony, J. K. (2013). Convenient Synthesis and In Vitro Pharmacological Activity of Thioesters of Salvinorin B. Planta Med. 79(05), P43.


