Benzyldiene 2-Aminoimidazolones Derivatives: Synthesis and in Vitro Evaluation of Anti-tumor Carcinoma Activity

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Received May 4, 2013; accepted July 6, 2013

A series of benzyldiene 2-aminoimidazolones derivatives were synthesized. Most compounds displayed strong inhibitory activity on the proliferation of human HepG2 cells in vitro. The active compounds were further evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against five human cancer cell lines in vitro. Compound 2b exhibited the strongest antitumor activities with IC_{50} values ranging from 12.87–17.10 µM which were nearly 1–3.5 fold less than that of 5-FU (IC_{50}=18.39–56.12 µM) in vitro. Furthermore, compound 2b could induce SMMC-7721 cell apoptosis in a dose-dependent manner. Therefore, our novel findings may provide a new framework for the design of new benzyldiene 2-aminoimidazolones derivatives for the treatment of cancer.

Key words synthesis; 2-aminoimidazolone derivative; anti-tumor agent; inhibitory activity; cell apoptosis

Cancer has been reported to become the leading cause of mortality worldwide in 2010 and cancer cases are expected to double by 2020.1 Resourceful novel and structurally diverse metabolites from marine organisms, in particular those coming from sponges, have proved to display a wide variety of biological activities including antihistamine activity,2 anti-inflammation,3 neuroprotective activity,4,5 antitumor,6,7 anti-oxidant,7 etc. For example (see Fig. 1), marine natural product Dispacamide, which was isolated from Carribian Agelas sponges, shows a potent antihistamine activity,2 and Leucetamine B, from the sponge Leucetta microraphis (alcarea class) of the Argulpelu Reef in Palau, exhibits to play a role as mediator of inflammation.3 Polyandrocarpamines A, (alcarea class) of the Argulpelu Reef in Palau, exhibits to possess anticancer activities.13,14 However, their antitumor activity should be of great significance.

For the further improvement of their antitumor activities, different substituted alkane amines and alcohol amines were identified that is 2-aminoimidazolone core. Both natural and synthetic marine natural products share a common skeletal structure ranging from 12.87–17.10 µM which were nearly 1–3.5 fold less than that of 5-FU (IC_{50}=18.39–56.12 µM) in vitro. Furthermore, compound 2b could induce SMMC-7721 cell apoptosis in a dose-dependent manner. Therefore, our novel findings may provide a new framework for the design of new benzyldiene 2-aminoimidazolones derivatives for the treatment of cancer.

For the further improvement of their antitumor activities, different substituted alkane amines and alcohol amines were selected to make further modifications on the 2-aminoimidazolone core by ammonification. Furthermore, with the consideration whether the methoxy group on benzene ring could confer to pharmacological activity, benzyldiene 2-aminoimidazolones derivatives were designed via the conjugation of 2-aminoimidazolone core with vanillin or 4-hydroxybenzaldehyde. Herein, 22 target compounds 1a–k, 2a–k were synthesized and their biological evaluation for inhibitory activities on the proliferation of human cancer cell lines and cell apoptosis effect were carried out in vitro with expectation to find better antitumor agents.

Results and Discussion

Chemistry The synthetic route of 2-aminoimidazolone derivatives 1a–k, 2a–k was outlined in Chart 1. The starting material glycine 3 was treated with NH_{4}SCN at the present of acetic anhydride (Ac_{2}O) to give 1-acetyl-2-thioxoimidazolidin-4-one 4 by condensation according to the literature.6,8 Compounds 6a,b was oxidized by tert-butyldihydroperoxide (TBHP) to obtain the important intermediate 7a,b. Then corresponding amine, alkane amines or alcohol amines were introduced onto the sulfonic group of compounds 7a,b to obtain the targeted compounds 1a–k, 2a–k. The structures of the target compounds were shown in Chart 1 and confirmed by different techniques such as mass spectrometry, IR, 1H-NMR spectra and elemental analysis (EA).

In Vitro Biological Assessment The inhibitory activity of target compounds 1a–k, 2a–k on HepG2 which was incubated with 50 µM of each test compound for 48h, was primarily screened and evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-

Fig. 1. The Structures of Marine Natural Products

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diphenyltetrazolium bromide (MTT) assay in vitro, and using 5-fluorouracil (5-FU) as positive control. The results showed in Fig. 2 illustrated the inhibitory effects of target compounds on the proliferation of Hep G2. Twelve out of the twenty two tested compounds exhibited inhibitory effects that were similar or superior to 5-FU. Especially, compounds 1b, 1j, 2b and 2k (percent inhibition over 90%) displayed more potent inhibition than 5-FU. Therefore, ten compounds (list in Table 1) possessing strong inhibition on the proliferation of human Hep G2 cells were selected for the further investigation of the inhibitory activity against five human cancer cell lines in vitro, such as SMMC-7721, HepG2, MCF-7, SW480 and SGC-7901. Their IC₅₀ values are presented in Table 1. All the tested compounds showed remarkable antitumor activities in a low micromolar range (except 2a) with IC₅₀ values ranging from 12.87–39.51 µM. In addition, the antitumor activities of each active compound against SMMC-7721 and SGC-7901 cells showed much more potent compared to that against the other tumor cells. Especially, compounds 2b exhibited the strongest antitumor activities with IC₅₀ values ranging from 12.87–17.10 µM which was 1–3.5 fold less than that of 5-FU (IC₅₀=18.39–56.12 µM).

For determining whether the most potent compound 2b was due to cell apoptosis, apoptosis assay employing human hepatocellular carcinoma cells (SMMC-7721) was further assessed to determine the effect of 2b on cell apoptosis. The SMMC-7721 cells were incubated with vehicle alone or with 2b at 6.25, 12.5 or 25 µM final concentrations. The percentages of apoptotic cells were determined by flow cytometry analysis. As shown in Fig. 3, in the untreated group, the frequency of SMMC-7721 cell apoptosis was unobvious. In contrast, the frequency of the cell apoptosis showed an upward trend in 2b-treated SMMC-7721 cell with the dose increased. Low concentration (6.25 µM) of 2b only induced 25.9% apoptosis activity on SMMC-7721 cells. Following treatment with 25 µM

Reagents and conditions: (a) NH₄SCN, Ac₂O, AcOH, reflux, 2h. (b) NaOAc, AcOH, reflux, 5h. (c) TBHP, MeOH, rt, 2h. (d) Alkane amines or alcohol amines, MeOH, rt, 10h.

Chart 1

Fig. 2. The Inhibitory Activity of Target Compounds 1a–k, 2a–k (50 µM) on Proliferation of Hep G2 Cells
induced over 80% of SMMC-7721 cell apoptosis, which was significantly higher frequency of the cell apoptosis compared to the control cells, and it revealed that the antitumor activity of 2b appeared to be concentration-dependent. However, the precise mechanisms underlying the apoptosis effect of these compounds selectively to tumor cells remain to be further investigated.

**Structure–Activity Relationships (SARs)**

Analysis of structure–activity relationships revealed that in series of target compounds 1a–e or 2a–e with different unsubstituted or substituted alkane amines, the compound 1b or 2b linked with methylamine possessed better anticancer activity compared to compound 1a or 2a linked with unsubstituted amine. However, increasing the length of alkane did not contribute to their activities in vitro while 1b or 2b showed the strongest anticancer activity among their respective series in vitro. In the other hand, the compounds 1f–k or 2f–k linked with alcohol amine substituents displayed slightly stronger anticancer activity compared to that with alkane amine substituents, and the anticancer activity was similarly decreased with increasing the chain length of alcohol amines in vitro. Interestingly, the compounds 2j and 2k linked with propylene glycol side chain displayed strong anticancer activity. In addition, the active compounds displayed more potent inhibitory activity on SMMC-7721 cells than the other four cancer cells. However, the precise SAR of these compounds with potent anticancer activity should be further investigated.

**Conclusion**

In summary, 20 benzylidene 2-aminoimidazolones derivatives with various substituted alkane amines or alcohol amines were synthesized. Their inhibitory activity against tumor cell lines of synthetic compounds was evaluated in vitro. Most compounds displayed strong anticancer activity. Particularly compounds 2b and 2k had a great potency superior to 5-FU in these cancer cells. Furthermore, compound 2b with the strongest antitumor activity could induce cancer cell apoptosis in a dose-dependent manner. We are interested in further investigating the mechanisms of these 2-aminoimidazolone derivatives underlying the action of these compounds in inhibition of human tumorigenesis.

**Table 1. The Inhibitory Activity (IC_{50}, \mu M) of 1a–v against Five Human Cancer Cell Lines**

<table>
<thead>
<tr>
<th>Compound</th>
<th>SMMC-7721</th>
<th>MCF-7</th>
<th>Hep G2</th>
<th>SW480</th>
<th>SGC-7901</th>
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<tr>
<td>5-FU</td>
<td>37.84</td>
<td>18.39</td>
<td>35.67</td>
<td>23.11</td>
<td>56.12</td>
</tr>
<tr>
<td>1b</td>
<td>17.76</td>
<td>25.03</td>
<td>21.52</td>
<td>22.87</td>
<td>20.97</td>
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<tr>
<td>1j</td>
<td>19.62</td>
<td>22.18</td>
<td>19.87</td>
<td>23.79</td>
<td>27.29</td>
</tr>
<tr>
<td>1k</td>
<td>28.06</td>
<td>35.02</td>
<td>25.96</td>
<td>33.05</td>
<td>29.65</td>
</tr>
<tr>
<td>2a</td>
<td>33.48</td>
<td>&gt;50</td>
<td>39.51</td>
<td>34.75</td>
<td>35.61</td>
</tr>
<tr>
<td>2b</td>
<td>12.87</td>
<td>15.16</td>
<td>14.93</td>
<td>17.10</td>
<td>15.53</td>
</tr>
<tr>
<td>2c</td>
<td>27.46</td>
<td>30.24</td>
<td>32.39</td>
<td>29.65</td>
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</tr>
<tr>
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<td>19.98</td>
<td>20.53</td>
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<td>22.87</td>
</tr>
<tr>
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<td>24.86</td>
<td>20.25</td>
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<tr>
<td>2k</td>
<td>14.12</td>
<td>20.74</td>
<td>18.05</td>
<td>21.17</td>
<td>16.92</td>
</tr>
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</table>

Fig. 3. Analysis of 2b-Induced Cell Apoptosis in SMMC-7721 Cells

The SMMC-7721 cells were treated with 6.25, 12.5 or 25 \mu M 2b for 48h. (A) Apoptosis was determined by Annexin V-FITC/PI staining in the SMMC-7721 cells by flow cytometry. (B) Quantitative analysis of apoptotic cells. Data are representative flow cytometry charts that are illustrated as a mean±S.E.M. of the percentages of apoptotic cells from three duplicate experiments.
**Experimental**

General chemistry methods, synthesis procedures, spectral data, and bioassay methods are given in Supplemental information.

**Acknowledgments** The work was supported by Applied Research Projects of Nantong City (BK2012085) and the Priority Academic Programs Development of Jiangsu Higher Education Institutions (PAPD).

**References**