Synthesis and biological evaluation of novel bile acid-nucleoside conjugates

published results and work in progress

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synthesis and characterization of modified nucleosides

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- synthesis and characterization of modified bile acids
  - click-chemistry

biological evaluation
Synthesis of: 3α-azido-bile acids and 8-alkynyllic-2’-deoxyadenosines
Conjugate synthesis via click-chemistry

\[ \text{CuSO}_4 \cdot 5 \text{H}_2\text{O}, \text{sodium ascorbate, THF–tBuOH–H}_2\text{O (1.5 : 1 : 1), 25 °C, 18 h, 70%; or microwave 80 °C, 30 min, 73%}. \]
Synthesis of 8-alkynylated-2’-deoxyadenosines

This reaction can be smoothly performed in water and leads to a simple purification of the thioalkynylated nucleoside that only requires the extraction of the compound with warm EtOAc from the aqueous crude mixture. M. L. Capobianco and M. L. Navacchia PCT Int. Appl. WO 2012164484 A1 2012

This reaction can be smoothly performed and leads to a simple purification of the alkynylated nucleoside that requires a filtration on florisil.
Biological evaluation

*in vitro* cytotoxicity toward human fibroblast cells and anti-proliferative activity against four human cancer cell lines: Leukemic T Jurkat and K562, colon carcinoma HCT116 and ovarian cancer A2780
Cytotoxic activity of bile acid-based conjugates and alkynyl deoxyadenosines dA-A, HdA-A, and SdA-A on human cancer cell lines and human fibroblast cells\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $P^b$</th>
<th>K562 IC$_{50}$ (µM)</th>
<th>Jurkat IC$_{50}$ (µM)</th>
<th>HCT116 IC$_{50}$ (µM)</th>
<th>A2780 IC$_{50}$ (µM)</th>
<th>Human fibroblast IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dA-C</td>
<td>2.97</td>
<td>141.79 ± 2.49</td>
<td>168.97 ± 1.99</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>HdA-C</td>
<td>4.77</td>
<td>131.90 ± 9.29</td>
<td>86.18 ± 7.32</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>SdA-C</td>
<td>4.62</td>
<td>172.36 ± 9.60</td>
<td>35.86 ± 11.60</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>HM-C</td>
<td>2.35</td>
<td>51.06 ± 4.24</td>
<td>77.80 ± 0.54</td>
<td>96.93 ± 9.00</td>
<td>84.47 ± 3.90</td>
<td>128.79 ± 19.09</td>
</tr>
<tr>
<td>dA-CDC</td>
<td>4.28</td>
<td>23.25 ± 4.32</td>
<td>21.88 ± 1.16</td>
<td>146.32 ± 7.34</td>
<td>&gt;200</td>
<td>155.2 ± 3.15</td>
</tr>
<tr>
<td>HdA-CDC</td>
<td>6.08</td>
<td>8.51 ± 4.05</td>
<td>10.47 ± 2.64</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>SdA-CDC</td>
<td>5.94</td>
<td>22.05 ± 0.61</td>
<td>16.76 ± 3.06</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>HM-CDC</td>
<td>3.66</td>
<td>38.84 ± 2.50</td>
<td>51.43 ± 7.57</td>
<td>75.49 ± 11.70</td>
<td>69.88 ± 13.85</td>
<td>79.01 ± 3.90</td>
</tr>
<tr>
<td>dA-UDC</td>
<td>4.28</td>
<td>35.65 ± 2.23</td>
<td>36.17 ± 1.51</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>36.36 ± 1.21</td>
</tr>
<tr>
<td>HdA-UDC</td>
<td>6.08</td>
<td>102.35 ± 2.05</td>
<td>24.57 ± 2.31</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>SdA-UDC</td>
<td>5.94</td>
<td>125.33 ± 26.87</td>
<td>23.93 ± 0.98</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>HM-UDC</td>
<td>3.66</td>
<td>47.23 ± 0.52</td>
<td>36.44 ± 18.07</td>
<td>65.37 ± 26.02</td>
<td>84.61 ± 1.39</td>
<td>80.18 ± 1.47</td>
</tr>
<tr>
<td>dA-A</td>
<td>-0.19</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>HdA-A</td>
<td>2.24</td>
<td>156.63 ± 14.76</td>
<td>123.48 ± 19.09</td>
<td>112.58 ± 5.75</td>
<td>107.01 ± 1.27</td>
<td>&gt;200</td>
</tr>
<tr>
<td>SdA-A</td>
<td>2.09</td>
<td>174.87 ± 15.06</td>
<td>183.35 ± 17.06</td>
<td>110.69 ± 3.44</td>
<td>131.67 ± 1.94</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Cisplatin\textsuperscript{c}</td>
<td></td>
<td>5.35 ± 1.01</td>
<td>2.21 ± 1.51</td>
<td>8.47 ± 1.20</td>
<td>0.95 ± 0.35</td>
<td>25.38 ± 3.51</td>
</tr>
</tbody>
</table>

\textsuperscript{a} IC$_{50}$ values were determined from the dose–response curves using MTT assay. Results are expressed as the mean of three independent experiments ± SD. \textsuperscript{b} log $P$ was determined using the MarvinSketch program. \textsuperscript{c} Used as a reference compound.
Anti-proliferative activity of conjugated compounds against the K562 cell line
Percentage of apoptotic K562 cells determined after 24 h treatment with HdA-CDC, SdA-CDC and HM-CDC (50–10 mM) by annexin V staining.
Conclusion

• Best activity was shown by CDC-based derivatives and could be correlated to the lipophilicity and to the 7α-OH group orientation;

• furthermore, except dA-UDC and HM-bile acid series, all new conjugates did not show any significant cytotoxicity towards the human fibroblast cells whereas some of them strongly and selectively inhibited cell proliferation and induced apoptosis in leukemic K562 cells;

• therefore, these derivatives constitute a starting lot of candidate drugs.
...however many questions are still open

Do other nucleobases work?
Does the ribo form work?
How much important is the triazole ring?
H-A-A  
H-dG-A, H-G-A  
H-dU-A, H-U-A

[Chemical structures and reactions]
A new lot of 27 bioconjugates is waiting for the biological assay.
References

- Adenosine Or Deoxyadenosine Derivatives Modified At Position 8 And A Method Of Synthesis Thereof;

- Labeling Deoxyadenosine for the Preparation of Functional Conjugated Oligonucleotides;
  Massimo L. Capobianco,* Elena Marchesi, Daniela Perrone and Maria Luisa Navacchia Bioconj., 2013, 24, 1398-1407.

- Synthesis and in vitro cytotoxicity of deoxyadenosine-bile acid conjugates linked with 1,2,3-triazole;