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Design and synthesis of alepterolic acid and 5-fluorouracil conjugates as potential anticancer agents

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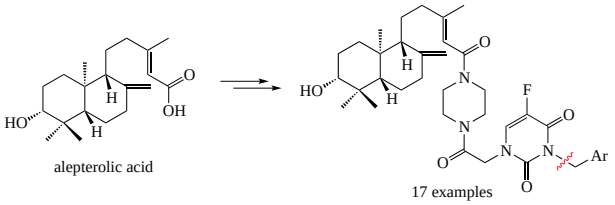
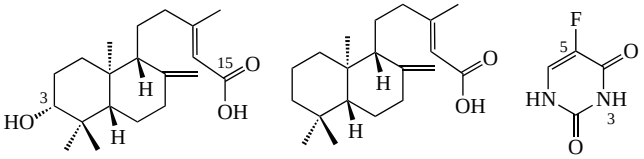
Conjugates of alepterolic acid with 5-fluorouracil derivatives have been synthesized in 70–90% yields. The cytotoxic evaluation against two human cancer cell lines, *viz*. MCF-7 (breast) and A549 (lung), using MTT assay showed anticancer activities of the obtained compounds.



Keywords: alepterolic acid, 5-fluorouracil, piperazine, conjugate, anticancer, amination, coupling.

Alepterolic acid **1** firstly isolated from *Aleuritopteris argentea*, also known as *Cheilanthes argentea* in 1962, is a kind of diterpeniod compound with potential biological activities.[1] This fern, widely distributed in Russia, China, Japan and the Korean Peninsula, has been extensively applied in ethnic medicine.[2] From this plant, another diterpene *ent*-copalic acid **1′** with the structure similar to that of alepterolic acid has been isolated. Idippily reported synthesis of derivatives of *ent*-copalic acid and their increased anticancer activity against LNCaP cell line.[3] We carried out structural modifications on the alepterolic acid leading to a series of diterpenoid analogs with improved antitumor activity.[4] Hence, we aim to develop further means to derivatize alepterolic acid for screening as anticancer prodrugs.



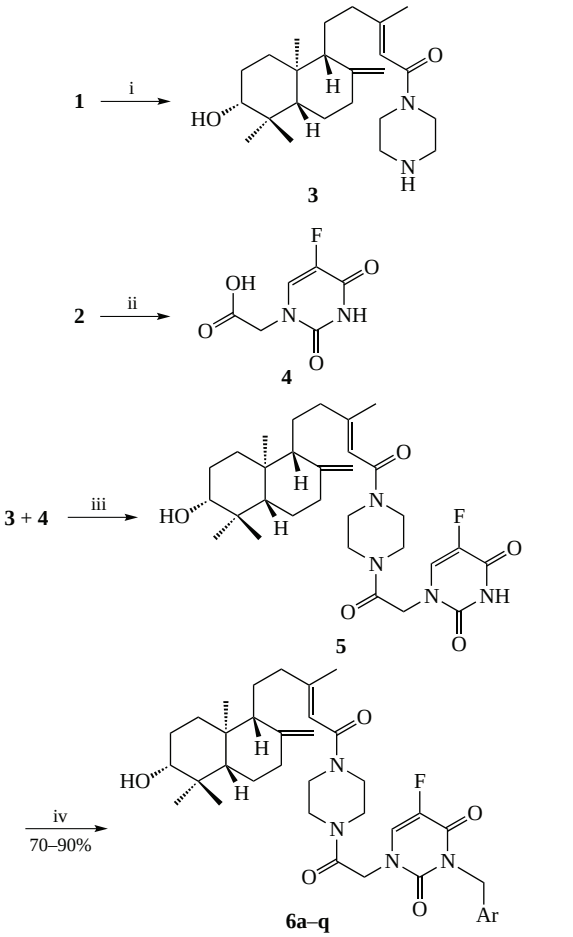
Alepterolic acid **1** | *ent*-Copalic acid **1′** | 5-Fluorouracil **2**

5-Fluorouracil (FU, **2**) is an antimetabolic drug of pyrimidine type widely used for the treatment of cancer, particularly for colorectal cancer.[5] However, the short plasma half-life, poor tumor affinity, bone marrow suppression and strong intestinal toxicity of FU are the reason for its multifaceted clinical application limitations.[6] Therefore, much research has been focused on pursuing suitable carrier-linked prodrugs combining FU with a wide range of carriers, including glucose,[7] polysaccharides,[8] peptides,[9] *etc*. Recently, Wu reported the synthesis of fluoropyrimidinyl-2,4-dihydroxy-5-isopropylbenzamides consisting of FU substituted at 3-*N*-position by large groups containing benzyl moiety, as potential agents and a new strategy for development of novel HSP-90 inhibitors for the treatment of cancer.[10]

Molecular combination strategy is to reasonably design new chemical entities based on the structure of two or more bioactive molecules. Conjugates are usually produced by the connection of two molecules through a chemically stable linker. In general, conjugates possess several obvious advantages, such as improved affinity and efficacy, increased solubility, multiple targets in a molecule, or counteract known side effects.[11] The selection of two chemical entities for fusion is usually based on observed synergistic or additive pharmacological activities.[12] The combination of FU with other bioactive drug molecules such as natural products is predictable to produce hybrid molecules with better bioactive properties.[13]

In this work, we designed and synthesized a series of conjugates of alepterolic acid **1** and FU **2** *via* chemically stable linker, followed by substitution of benzyl groups at 3-*N*-position of FU. Piperazine is widely used in pharmaceutical synthesis because of high stability, low toxicity and ability to increase the water solubility of drugs.[14] Therefore, we used piperazine as the linker in this study (Scheme 1). The intermediate **3** was synthesized with the use of alepterolic acid **1**, coupling reagent 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), diisopropylethylamine (DIPEA) and piperazine. There was almost nothing of disubstitution product in this reaction, owing to the control of HATU's dosage. The second intermediate **4** was prepared from FU according to reported procedures.[15] Next, intermediate **5** was obtained from compounds **3** and **4** under similar condensation conditions. Finally, intermediate **5** was treated with various substituted benzyl halides and potassium carbonate as base in DMF to afford desired products **6a**–**q** in good yields (see Scheme 1).[16]

The ^{1}H and ^{13}C NMR data of new compounds **6a**–**q** are consistent with the related structures. Their HRMS spectra contained peaks corresponding to the molecular weight of the



a Ar = Ph
b Ar = 2-MeC_6H_4
c Ar = 3-MeC_6H_4
d Ar = 4-MeC_6H_4
e Ar = 4-FC_6H_4
f Ar = 4-ClC_6H_4
g Ar = 4-BrC_6H_4
h Ar = 2,4-$Cl_2C_6H_3$
i Ar = 3,4-$Cl_2C_6H_3$
j Ar = 4-$MeOC_6H_4$
k Ar = 4-$MeOC(O)C_6H_4$
l Ar = 4-$F_3CC_6H_4$
m Ar = 4-$F_3COC_6H_4$
n Ar = 4-$O_2NC_6H_4$
o Ar = 4-NCC_6H_4
p Ar = 1-naphthyl
q Ar = 2-naphthyl

Scheme 1 *Reagents and conditions*: i, DIPEA, HATU, piperazine, CH_2Cl_2, room temperature; ii, $BrCH_2CO_2H$, KOH, H_2O, 60 °C; iii, DIPEA, HATU, CH_2Cl_2, room temperature; iv, $ArCH_2Br$, K_2CO_3, DMF, room temperature.

acquired structures. Detailed analysis and original NMR spectra are provided in Online Supplementary Materials.

The cytotoxic activities of the synthesized analogs (Table 1) were evaluated using human breast cancer (MCF-7) and human lung cancer (A549) cell lines and cisplain as the control. From the results of the MTT assay, most compounds show certain cytotoxicity against MCF-7 cell line. Cytotoxicity of compounds **6a**–**c**,**g**–**i** are relatively close to that of cisplatin. Similarly, the majority of the compounds demonstrated considerable cytotoxicity against A549 cell line while compounds **6c**–**e**,**g**–**i** exhibited comparable activities to the control. As for the structure–activity relationship, it is worth mentioning that the activities of compounds **6k**,**p** are significantly lower than those of other compounds. Activity reduction of **6k** indicates that the introduction of ester group would significantly suppress the cytotoxic activity of the derivatives. While **6p**, compared with **6q**, suggests that the decrease in activity may come from the increase of steric hindrance at 3-*N*-position of FU. With respect to substituents, benzyl groups with small electron-donating substituents are preferred to improve the cytotoxic activity. Among all the compounds, **6h**,**i** showed the best inhibitory activity against both MCF-7 and A549 cell lines. This proved that the additon of halogen substituents is conducive to the more potent cytotoxic activity of these compounds.

Table 1 IC_{50} determination of cytotoxicity of compounds against human cancer cell lines.

Compound	Cytotoxicity (IC_{50})/μM	
	MCF-7	A549
6a	26.59 ± 1.16	38.34 ± 0.93
6b	24.33 ± 2.55	31.03 ± 2.74
6c	30.64 ± 3.21	23.68 ± 0.81
6d	48.78 ± 3.78	21.82 ± 0.61
6e	49.42 ± 2.31	26.49 ± 1.99
6f	34.41 ± 6.40	34.83 ± 1.22
6g	34.34 ± 2.41	21.64 ± 1.27
6h	27.39 ± 0.86	27.21 ± 1.24
6i	28.82 ± 2.99	22.68 ± 1.10
6j	51.80 ± 3.25	82.15 ± 2.09
6k	>100	>100
6l	59.38 ± 0.90	54.63 ± 3.14
6m	59.29 ± 8.46	65.39 ± 6.98
6n	55.11 ± 4.43	93.215 ± 1.43
6o	41.50 ± 2.57	68.94 ± 4.62
6p	>100	>100
6q	42.29 ± 8.99	60.125 ± 5.77
Cisplain	21.64 ± 2.75	19.745 ± 1.14

In summary, seventeen new alepterolic acid–5-fluorouracil conjugates were synthesized, characterized by spectral data and evaluated for their cytotoxic activity. The reaction process is facile, efficient and fast, the conditions are easy to operate, and all products are obtained in high yields. *In vitro* cytotoxicity study clearly emerged that most compounds revealed certain cytotoxicity against MCF-7 and A549 cell lines. The inhibitory activities of four and six products against MCF-7 and A549 cell lines, respectively, were similar to those of cisplatin as control. Based on these results, it appears that conjugates as prodrugs are potential candidates for anticancer drug delivery. Further work to evaluate the biological effects of these hybrids is underway.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.05.024.

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