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# Synthesis of uracil–coumarin conjugates as potential inhibitors of virus replication

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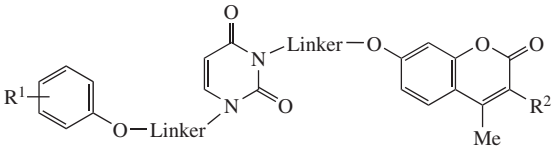
**A series of 1-[(bromophenoxy)alkyl]uracil–coumarin conjugates has been obtained through the preparation of starting 1-[(bromophenoxy)alkyl]uracil derivatives, followed by their treatment with 7-(ω-bromoalkoxy)-4-methyl-2*H*-chromen-2-ones. Two of the synthesized uracil–coumarin conjugates demonstrated a pronounced inhibitory activity against HCMV and VZV replication *in vitro*.**



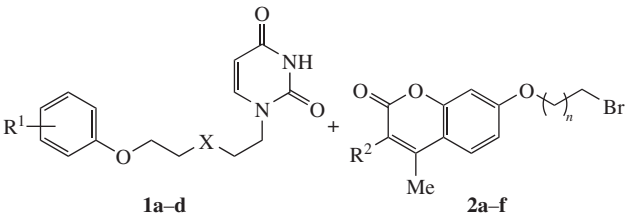

Anti-HCMV and anti-VZV activity

Coumarins are a large family of heterocyclic compounds found in higher plants and localized in their roots, bark and fruits. Natural and synthetic coumarin derivatives are known as antidepressants,[1] antimicrobials,[2] antioxidants,[3] as well as anti-inflammatory,[4] anti-asthmatic[5] and antitumor[6] agents. Coumarins were also found to exhibit inhibitory activity against HIV-1,[7] HCV,[8] and herpesvirus.[9] As well, antiviral effect was shown for the conjugates of coumarin with nucleic bases[10] and nucleosides.[11]

As an example of antiviral nucleic base conjugates, we synthesized 1-[ω-(aryloxy)alkyl]-3-[(4-phenoxyphenyl)aminocarbonylmethyl]uracil derivatives and demonstrated their inhibitory effect towards HCMV, VZV[12,13] and HCV[14] replication. In this work, we synthesized and tested a series of new uracil conjugates bearing coumarin moiety.

The synthesis of starting 1-[5-(4-bromophenoxy)pentyl]uracil **1a** was described.[15] 1-[5-(3-Bromophenoxy)pentyl]uracil **1b** and 1-[5-(2-bromophenoxy)pentyl]uracil **1c** precursors were obtained in 82 and 70% yields, respectively, by condensation of 2,4-bis(trimethylsilyloxy)pyrimidine with 1-bromo-3-[(5-bromopentyl)oxy]-benzene or 1-bromo-2-[(5-bromopentyl)oxy]benzene in equimolar amounts by heating without solvent under dry conditions as described.[15,16] 1-{2-[2-(4-Bromophenoxy)ethoxy]ethyl}uracil **1d** could not be obtained by this way, because bromotrimethylsilane, released during interaction of 2,4-bis(trimethylsilyloxy)pyrimidine with 1-bromo-4-[2-(2-bromoethoxy)ethoxy]benzene, cleaved the dialkyl ether moiety under the conditions employed. For this reason, the synthesis of uracil derivative **1d** was carried out by treatment of the latter with a fourfold molar excess of uracil in DMF in the presence of potassium carbonate. This alternative process led to 1-substituted uracil **1d** in 56% yield. Details of the synthesis and properties for precursors **1b–d** are given in Online Supplementary Materials.

Preparation of 7-(ω-bromoalkoxy)-4-methyl-2*H*-chromen-2-ones **2a–e** is well documented,[17–19] they were obtained by reaction of 7-hydroxy-4-methyl-2*H*-chromen-2-one with an excess of the corresponding α,ω-dibromoalkane in acetone in the presence of $K_2CO_3$. 7-(2-Bromoethoxy)-3,4-dimethyl-2*H*-chromen-2-one **2f** was synthesized in 72.5% yield by treatment of 7-hydroxy-3,4-dimethyl-2*H*-chromen-2-one with a fourfold molar excess of 1,2-dibromoethane under similar conditions.

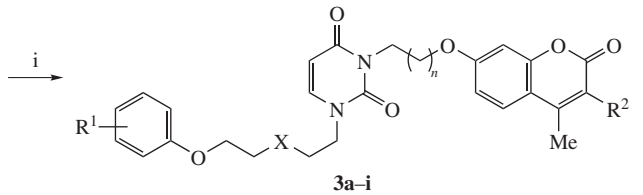


**1a–d**

**a** $R^1$ = 4-Br, X = $CH_2$
**b** $R^1$ = 3-Br, X = $CH_2$
**c** $R^1$ = 2-Br, X = $CH_2$
**d** $R^1$ = 4-Br, X = O

**2a–f**

**a** $R^2$ = H, $n$ = 1
**b** $R^2$ = H, $n$ = 2
**c** $R^2$ = H, $n$ = 3
**d** $R^2$ = H, $n$ = 4
**e** $R^2$ = H, $n$ = 5
**f** $R^2$ = Me, $n$ = 1




**3a–i**

**a** $R^1$ = 4-Br, $R^2$ = H, X = $CH_2$, $n$ = 1
**b** $R^1$ = 4-Br, $R^2$ = H, X = $CH_2$, $n$ = 2
**c** $R^1$ = 4-Br, $R^2$ = H, X = $CH_2$, $n$ = 3
**d** $R^1$ = 4-Br, $R^2$ = H, X = $CH_2$, $n$ = 4
**e** $R^1$ = 4-Br, $R^2$ = H, X = $CH_2$, $n$ = 5
**f** $R^1$ = 3-Br, $R^2$ = Me, X = $CH_2$, $n$ = 1
**g** $R^1$ = 2-Br, $R^2$ = Me, X = $CH_2$, $n$ = 1
**h** $R^1$ = 4-Br, $R^2$ = Me, X = $CH_2$, $n$ = 1
**i** $R^1$ = 4-Br, $R^2$ = H, X = O, $n$ = 1

**Scheme 1** *Reagents and conditions*: i, DMF, $K_2CO_3$, 80 °C, 18 h.

**Table 1** Anti-HCMV and anti-VZV activity of uracil–coumarin conjugates **3a–i** in HEL cell culture.

| Compound | Antiviral activity, $EC_{50}$/μM[a] | | | | Cytotoxicity | |
|---|---|---|---|---|---|---|
| | HCMV AD-169 | HCMV Davis | VZV Oka ($TK^+$) | VZV 07-1 ($TK^-$) | Cell morphology MCC/μM[b] | Cell growth $CC_{50}$/μM[c] |
| **3a** | >20 | >20 | >20 | >20 | 100 | –[d] |
| **3b** | 0.39 | 0.51 | 0.52 | 0.6 | 4 | – |
| **3c** | >0.8 | >0.8 | 1.37 | >0.8 | 4 | – |
| **3d** | >4 | >4 | >4 | >4 | 20 | – |
| **3e** | 0.44 | 0.44 | 0.16 | 0.47 | 4 | 4.95 |
| **3f** | >100 | >100 | >100 | >100 | >100 | – |
| **3g** | >4 | 4 | >100 | >100 | 20 | – |
| **3h** | >20 | >20 | >100 | >100 | 100 | – |
| **3i** | >0.8 | >0.8 | 11.7 | 5.72 | <0.8 | – |
| Ganciclovir | 2.4 | 2.01 | – | – | 350 | 196.41 |
| Cidofovir | 0.38 | 0.38 | – | – | 300 | 129.43 |
| Acyclovir | – | – | 0.44 | 2.89 | >100 | >100 |
| Brivudine | – | – | 0.022 | 12.01 | 100 | >100 |

[a] Concentration required to reduce virus plaque formation by 50%; virus load was 100 plaque forming units (PFU). [b] Concentration that caused a microscopically detectable alteration of cell morphology. [c] Concentration required to reduce cell growth by 50%. [d] Not determined.

The synthesis of the target uracil–coumarin conjugates was carried out in 74–86% yields by the treatment of 1-substituted uracil derivatives **1a–d** with an equimolar amount of bromides **2a–f** in DMF in the presence of $K_2CO_3$ (Scheme 1). Details of the synthesis and properties for the resulting compounds **3a–i** are given in Online Supplementary Materials.

Activity of the obtained uracil–coumarin conjugates against HCMV strains AD-169 and Davis as well as against VZV strains Oka and 07-1 was tested in HEL cell culture (Table 1). Compounds **3b** and **3e** have been found to inhibit HCMV replication with $EC_{50}$ 0.39–0.51 μM, which is more effective than ganciclovir and comparable with cidofovir, though the test compounds are much more cytotoxic than both ganciclovir and cidofovir. The uracil–coumarin conjugates **3b** and **3e** have also been found to effectively block VZV replication with $EC_{50}$ 0.16–0.52 μM. Note that, unlike acyclovir or brivudine, these two compounds demonstrate a similar inhibitory effect on both VZV strains tested, namely the wild type Oka strain and the thymidine kinase-deficient ($TK^-$) mutant 07-1 strain. This allows us to suggest, that conjugates **3b** and **3e** do not interact with viral thymidine kinase, and the mechanism of their anti-VZV effect differs from that for acyclovir or brivudine.

In summary, we have synthesized a number of new uracil–coumarin conjugates, and some of them have demonstrated activity against HCMV and VZV *in vitro*. These results open a possibility for a further search for potent antiviral agents based on 1-[ω-(aryloxy)alkyl]uracil structure.

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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.2019.11.010.

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