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Synthesis and biological activities of nucleoside–estradiol conjugates

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Abstract—Nucleosides were coupled to estradiol via a 17α-ethynyl spacer group using Pd(II) as a catalyst. The conjugates were evaluated in vitro for estrogen receptor (ER) binding affinity and cytotoxicity against cell lines with and without ER. The highest receptor binding affinities (RBA $\simeq$ 3) were observed with conjugates coupled via a relative long spacer group, while none of the conjugates exhibited cytotoxicity against either cell lines.

The clinical use of most anticancer chemotherapeutics is limited by their lack of specificity and associated undesirable side effects. To stop proliferation of all tumor cells, drugs must be given at high concentrations resulting in toxicity against fast-growing cells of both tumor and healthy tissues. Reducing the side effects requires the development of anti-tumor agents that are effective at relative low doses. Several attempts have been made to improve target selectivity of cytotoxic agents through coupling to steroid hormones that bind to receptors overexpressed in endocrine tumors such as those of the breast and prostate.[1] Such agents include steroids functionalized with groups that contain alkylating moieties such as N-mustard,[2] nitrosoureas[3] or aziridines[4] (i.e., estramustine and ninestramide) and intercalating agents such as ellipticine[5] and daunorubicin[6] derivatives. Cytotoxic metal chelates, used in the treatment of different types of cancers and known to interact with DNA, were also coupled to estrogen derivatives to target breast tumors.[7] An estrogenic-enediyne hybrid molecule has been advanced as a temperature- and concentration-dependent drug that interacts with estrogen receptors (ER) via α-cyclization.[8] Taxol, a well-known anti-tumor drug, was also coupled to estradiol through an ester linkage.[9] Steroidal nitroimidazoles were prepared as potential site-selective radiosensitizers,[10] while steroids coupled to phthalocyanines[11] and porphyrins[12] have been advanced as potential ER-binding photosensitizers.

Nucleoside analogs have emerged as important therapeutic agents for the development of antiviral and anti-tumor drugs.[13] In particular, uracil derivatives substituted at C-5 or modified at the furanose ring exhibit strong biological activities.[14] Previously, we showed that steroid–nucleoside conjugates exhibit anti-tumor activity.[15] An estrogen-bridged adenine derivative has also been shown to be cytotoxic against murine leukemia and andriamycin-resistant cells.[16] Some adenine and adenosine methylene-bridged estrogens were developed as inhibitors of the estrogen sulfotransferase.[17]

Using the palladium catalyzed cross-coupling reaction,[18] a number of estrogen-based hybrid molecules were prepared via the coupling of terminal alkyne–estrogen derivatives and halo-nucleosides, and tested for selected biological properties.

The 17α-ethynylestradiol (**1**) was dissolved in DMF/THF and treated with 5-iodo-uracil (**2a**) (room temperature, 2–4 h) in triethylamine containing copper(I) iodide and a catalytic amount of bis(triphenylphosphine)Pd(II) chloride. The product **3a** gave a molecular ion at *m*/*z* 406 corresponding to the coupled conjugate. The ^{1}H NMR spectrum of **3a** shows a singlet at δ 5.31 corresponding to the C-6′ proton of the uracil ring and other characteristic peaks between δ 6.44 and 7.05 corresponding to the C-1, C-2, and C-4 protons of the A-ring of estradiol. Using the same reaction condition we coupled the 17α-

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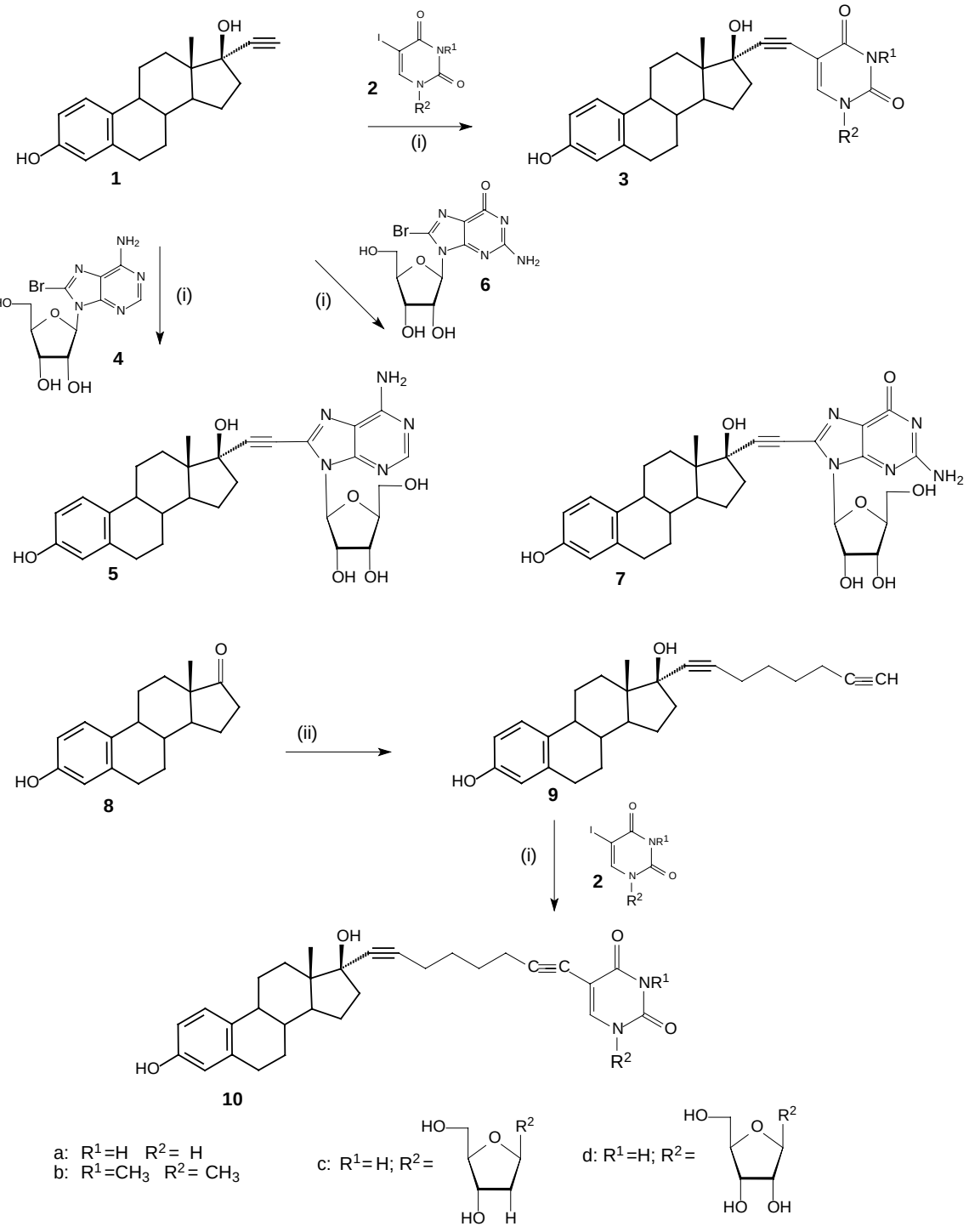
ethynylestradiol (**1**) with **2b**,**c**,**d** to yield corresponding adducts **3b**,**c**,**d**. The estrogen conjugates **5** and **7** were also prepared by coupling **1** with 8-bromoadenosine (**4**) and 8-bromoguanosine (**6**). We previously showed that attachment of a long chain at the 17α-position increases the ER binding affinity. Thus, we prepared compound **9** by reacting estrone with 1,7-di-octyne to yield a terminal alkyne that was used for further substitution. Using the same reaction conditions, compound **9** was coupled with **2** to yield conjugate **10**. All products were characterized by ^{1}H NMR and mass spectral analysis (Scheme 1).

Relative binding affinity (RBA). Binding affinities for ER were measured by a competitive [^{3}H]estradiol binding assay using Flash Plate technology, taking estradiol as unity (RBA = 100).[19] The RBA values are summarized in Table 1. RBA values increase proportionally with lengthening of the spacer chain. Changing the spac-



Scheme 1. Reagents: (i) Pd(II)Cl, CuI, TEA, DMF; (ii) *n*-BuLi, 1,7-di-octyne, THF.

Table 1. Relative binding affinity (RBA) for ER of steroid–nucleoside conjugates

Conjugate	RBA (SD)[a]
Estradiol	112 (46)
Tamoxifen	18 (5)
3a	0.07
3b	0.01
3c	0.18
5	0.02
10a	2.9 (0.6)
10b	0.5 (0.2)
10c	1.4 (0.5)
10d	1.8 (0.6)

[a] RBA values were measured by a competitive binding essay against [^{3}H]estradiol.

er chain from two (**3a**) to eight carbon atoms (**10a**) results in a substantial increase of the RBA. Further substitution of **10a** with a sugar moiety (**10c** and **10d**) results in decrease of RBA values (Student's t test: $t(4) = 2.205$, $p < 0.05$, one-tailed), suggesting a possible interference of the bulky sugar moiety nucleoside with the receptor binding process.

In vitro cytotoxicity. Cytotoxicity was evaluated in vitro against two human breast cancer cell lines. The MCF-7 cell line expressing ER and the MDA-MB-231 cell line (American Type Culture Collection) lacking ER.[20] None of the conjugates or free estradiol showed activity against the MCF-7 or MDA-MB-231 cell lines at all concentrations and all incubation times tested. Only 5-fluorouracil at 10 μM showed activity against test cell lines after 72 h incubation, that is, 40% and 67% cell survival against MCF-7 and MDA-MB-231 cells, respectively. Tamoxifen at 10 μM showed only activity against MCF-7 cells (72% cell survival after 72 h incubation). This limited biological effect likely results from its low affinity for the ER.

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20. Both cell lines were cultured at 37 °C in a 5% CO_2 atmosphere in Gibco's minimal essential medium (MEM, supplemented with 10% FBS, 0.1% sodium pyruvate, 0.02% penicillin, 0.02% streptomycin, 0.02% amphotericin, and 5% glutamine). Cells were plated in 96-well flat-bottomed microplates at a density of 2500 cells/well. The test and reference compounds (in PBS, 0.1% EtOH) were added to the well at least 24 h after plating at 0.1–10 μM concentration and incubated at 37 °C for 24–72 h. After the incubation, the growth medium and test compounds were replaced with MTT reagent (1 mg/mL, Sigma) in serum-free MEM. The cells were then incubated for 4 h before sodium dodecyl sulfate was added to solubilize the MTT–formazan complex, which is indicative of mitochondrial activity. MTT–formazan concentrations are measured at 570 nm on an ELISA reader and correlated to % cell survival.[21]
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