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Design, Synthesis and Investigation of Bone Affinity of the Conjugates of 5-Fluorouracil and Hydroxybisphosphonate

Liu Hua XU, Hu ZHENG, Ling Ling WENG*

West China School of Pharmacy, Sichuan University, Chengdu 610041

Abstract: A series of novel bone-targeting antitumor agents--the conjugates of 5-fluorouracil and hydroxybisphosphonate were designed and synthesized, their structures were confirmed by ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, MS, and element analysis. The tests of bone-targeting activity show that these compounds have an obvious affinity for bone.

Keywords: 5-Fluorouracial, hydroxybisphosphonate, bone-targeting, conjugate, hydroxyapatide.

Bone tumor, as a common disease, is treated by surgical resection, radiotherapy and chemotherapy. Chemotherapy is one of the most important treatment, however, a major problem of chemotherapy is lack of selectivity of cytotoxic drugs. Although many attempts have been made to increase the selectivity of therapeutic drugs for the bone diseases, such as osteoporosis, paget's, hypercalcemia and bone metastases by conjugating them with targeting carriers[1-4], there are still no bone-targeting agents available for the treatment of bone tumor.

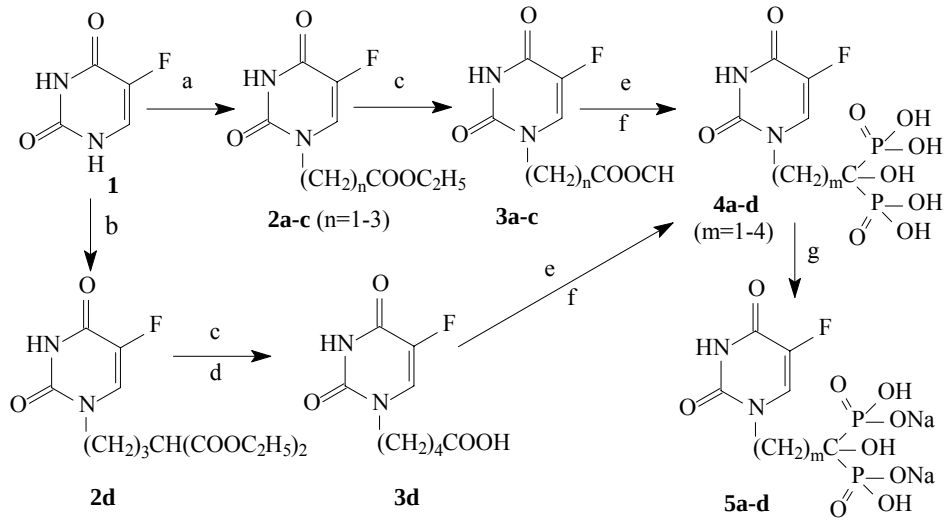
Bisphosphonates (BPs), as organic analogues of pyrophosphate, have the high affinity for bone mineral[5,6], and can target to bone mineral surface rapidly and selectivity. The affinity of BPs for hydroxyapatide (HA, the main composition of bone mineral) is a property of the P-C-P motif, which can chelate calcium ions by bidentate coordination through the oxygen atoms of phosphonate groups. The affinity of BP for calcium is even great when hydrogen is replaced by hydroxyl group[7], because this allows tridentate coordination to calcium ions. In this paper, we designed and synthesized a series of novel bone-targeting antitumor agents-the conjugates of 5-fluorouracil (5-Fu) and HBP as a prodrug, hoping they could selectively target to bone tissue.

We have tried to use ethyl bisphosphonic acid as a bone-targeting drugs carrier[8,9], but the result showed that their affinity for bone was low. Here, as HBP has the strongest affinity for bone in BP family, we chosed it to conjugate with 5-Fu. To enhance the stability of conjugates, 5-Fu and HBP are connected with alkyl chain in different length.

The synthesis of N_1-alkanoic acid substituted 5-Fu (**2a-c**) was conducted by the reaction of 5-Fu with correspounding chloro-acid ester in DMF using K_2CO_3 as a catalyst.

* E-mail: l.l.weng@263.net

Scheme 1 The synthetic route of the title compounds



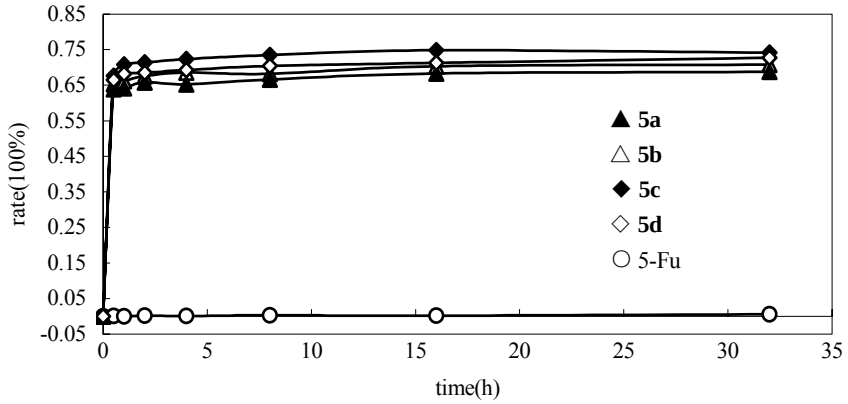
a) $Cl(CH_2)_nCO_2C_2H_5/DMSO/K_2CO_3/75℃$; b) $Cl(CH_2)_3CH(CO_2C_2H_5)_2/DMSO/K_2CO_3/75℃$; c) conc. HCl/reflux; d) 140℃; e) $H_3PO_3/PCl_3/100℃$; f) 5 mol/L HCl/reflux; g) NaOH.

The obtained esters were then hydroyzed in 5 mol/L HCl to give N_1-alkanoic acid substituted 5-Fu (**3a-c**). But for N_1 -valeric acid (**3d**), diethyl 3-chloropropyl malonate was used instead. Finally, these N_1-alkonoic acid (**3a-d**) were treated with PCl_3 and H_3PO_3 at 100℃ to give the target compounds (**4a-d**). The synthetic route is shown in **Scheme 1.**

In the synthesis of the compounds **4a-d**, the reaction has extremely poor physical characteristics, at the begining the reaction mixture was as a two-phase melt, it gradually thickened and then turned into semisolid. We did not find out the suitable solvent to make it homogeneous. To solve this problem, the reaction was carried out under the condition of over-amount of phosphorous acid (about 5-10 fold) to remain as a melt. After hydrolysis, the aq. HCl was evaporated and the over-amount of phosphorous acid was removed through reflux extraction with cyclohexane/acetone (1:2 V:V) twice, acetone twice and acetone/ethanol (1:1 V:V) in turn. The residues were dissolved in hot EtOH or MeOH and gave a visous oil compounds **4a-d** (about 90-95% HPLC purity) after nature cooling, which were dried *in vacuo* at 60℃ to give a moisture-sensitive foaming solid, then compounds **4a-d** were transformed into their disodium salt, recrystallized from H_2O / MeOH (2:1 V:V) to yield 32-41% of a white solid **5a-d** (>98.5% HPLC purity).

The bone-targeting activities of the title compounds were tested through the adsorption experiments of HA. The procedure was as follows: In 2 mL teflon tube, 5 mg HA was added to 1 mL aq. solution of examined compounds (1 μ mol/mL), followed by supersonic shake for 1 minute. One tube was taken and centrifuged in 0.5 h, 1 h, 2 h, 4 h respectively, the supernatants were chromatographed. The adsorption amounts at various times were determined by HPLC which were expressed as percent of the initial

Figure 1 Time-adsorption amount courses for compounds **5a-d** and 5-Fu



amount. **Figure 1** is the time *vs* adsorption of compounds **5a-d** and 5-Fu. It showed that adsorption of compounds **5a-d** increased with time, after one hour more than 90% of compounds were adsorbed.

In summary, we designed and prepared the conjugates of 5-Fu and HBP connecting with alkyl chain in different length and tested their adsorption ability to bone. The adsorption experiment of HA showed that the title compounds exhibited obvious affinity for bone. Their antitumor activities are currently under test.

Acknowledgments

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References and Notes

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10. Selected spectroscopic data for **5c**:
    ^{1}H-NMR (D_2O, δ ppm): 7.65 ($J_{H\text{-}F}$=4.8 Hz), 3.94, 2.05; ^{13}C-NMR (D_2O, δ ppm): 162.5(d, $J_{C\text{-}F}$=100.4 Hz), 153, 142.8(d, $J_{C\text{-}F}$=919.4 Hz), 133.7(d, $J_{C\text{-}F}$=126.4 Hz), 76.0(t, $J_{C\text{-}P}$=537.6, 537.8 Hz), 51.9, 33.0, 25.7(t, $J_{C\text{-}P}$=24.6, 24.6 Hz); MS (ESI), *m/z* 361(M^+-1); ^{31}P-NMR (D_2O, δ ppm): 18.68; Anal. Calcd. for $C_8H_{11}N_2O_9P_2Na_2$•$2H_2O$: C, 21.72, H, 3.39, N, 6.33; found: C, 21.84, H, 3.31, N, 6.26.

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