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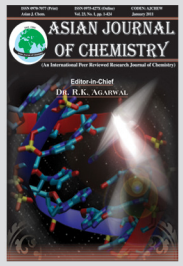

NOTE

Synthesis of Water-Soluble Prodrug of Florfenicol

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Water-soluble prodrug of florfenicol was synthesized by introducing a water-soluble small moiety to the hydroxyl of florfenicol according to the prodrug design principles. The water-solubility of the prodrug was about 500 times higher than florfenicol and the water soluble prodrug of forfenicol displays high aqueous solubility and is stable in water as well as in organic solvents.

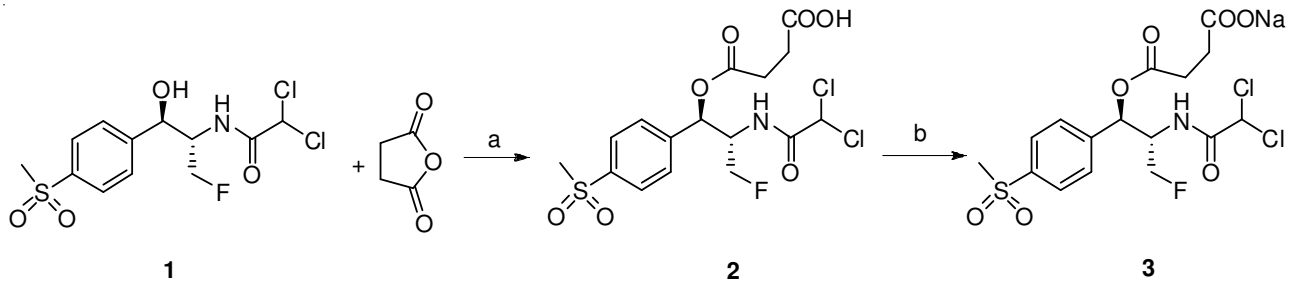
Key Words: Florfenicol, Prodrug, Synthesis, DMAP, Esterification.

Florfenicol, also known as a new fluorinated analog of thiamphenicol, firstly was developed by the Schering-Plough Animal Health Corporation, United States in 1988 as the third generation of chloramphenicol product. It is a veterinary wide-spectrum, synthetic antibacterial substance with good absorptivity and low remain and it had obvious effect on poultry caused by bacterial disease treatment significantly[1,2]. Up till now, florfenicol has been commercially available in more than 20 countries in Asia, Europe and America, mainly for prevention and treatment of bacterial infections due to susceptible pathogens in birds, reptiles, fish, shellfish and mammals[3]. However, the aqueous solubility of florfenicol is quite limited (*ca.* 1.3 mg/mL) and consequently the solubilization of florfenicol in water is slow. Usually, certain organic solvent must be added to pre-dissolve florfenicol to achieve the desired concentration of it in drinking water, in form of a concentration in a water-miscible organic solvent. The use of organic solvents results in toxicaly or higher cost and also leads to injection site irritation and tissue damage upon intramuscular administration. It was reported that sodium salt of florfenicol had been prepared, which improved the water-solubility[4]. While the expensive reagents, complexed separation method and restrict reaction conditions makes the existing synthetic procedure of sodium salt of florfenicol difficult to be industrialized. Yeramilli *et al.*[5], prepared a series of florfenicol acetates and propionic esters, whereas the solubility was only slightly changed. In present study, we described our efforts directed towards these goals, which improve solubility use a convenient process according to the prodrug design principles.

4-((1R,2S)-2-(2,2-Dichloroacetamido)-3-fluoro-1-(4-(methylsulfonyl)phenyl)propoxy)-4-oxobutanoic acid (2)[6-9]: To the solution of florfenicol (5.3 g, 0.015 mol) in anhydrous acetone (50 mL), DMAP (0.3 g, 0.0075 mol) was added. The mixture was stirred at 50 ºC for 2 h after adding succinic anhydride (2.25 g, 0.025 mol) and then kept standing overnight. Filtration of solid was followed by evaporation of the solvent, giving a viscous oil. The oil was dissolved in ethyl acetate (80 mL), washed by hydrochloric acid solution (1 mol/L, 3 × 20 mL) and water (3 × 20 mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4. Filtration of Na_2SO_4 and distillation of ethyl acetate under vacuum gave crude product. Pure florfenicol amber acid ester was obtained by recrystallization from ethanol/water (1/1) as a white solid (3.84 g, 72.5 %).

Sodium-4-((1R,2S)-2-(2,2-dichloroacetamido)-3-fluoro-1-(4-(methylsulfonyl)phenyl)propoxy)-4-oxobutanoate (3): 10 % sodium hydroxide (4.0 g, 0.1 mol) solution was added to the satuated ethanol solution of **2** (45.82 g, 0.1 mol), kept stirring for 2 h. Evaporation of ethanol under reduced pressure gave sodium salt. Further recrystallization from ethanol/water (v:v = 1:1) afforded compound **3** as a white solid (39.86 g, 97 %).

Characterization of the structure of 2: The results of esterification of florfennicol to water-soluble prodrug of florfenicol by DMAP in the acetone. Yield 72.5 %, the purity by HPLC analysis is above 96.5 %. White solid; m.p. 129.6-130.1 ºC. IR (KBr, ν_{max}, cm^{-1}): 3357, 2987, 2959, 2939, 1741, 1702, 1672, 1528, 829, 803; ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.91 (1H, d, J = 8.8 Hz, NH), 7.88 (2H, d, J = 8.4 Hz), 7.80 (2H, dd, J = 8.4 Hz, Ar-H), 6.43 (1H, s, J = 1.6 Hz, $CHCl_2$), 6.01



Scheme-I: Synthesis study of water-soluble prodrug of florfenicol. Reagents and conditions: (a) DMAP, acetone, 50 ºC, 2 h, 72.5 %; (b) NaOH, EtOH, 0.5 h, 87 %

(1H, d, J = 4.0 Hz, CHOCO), 4.32-4.59 (3H, m, -CH-NH-, CH_2F), 3.19 (3H, s, $-CH_3$), 2.49-2.67 (4H, m, $-CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.2 (COOH), 171.1 (-COO-), 163.8 ($-COCH_2Cl_2$), 142.5 (Ar-C), 140.3, 127.2, 126.8, 82.7 (CH), 81.0 ($CHCl_2$), 72.5 (CHNH), 66.1 (CH_2F), 43.3 (CH_3), 28.7 (CH_2), 28.4 (CH_2); MS (m/z): 457.6 (MH), 475.0 [$M^+ + NH_4$]. Through the characterization of that IR, MS, ^{1}H and ^{13}C NMR, We confirm the structure of the compound (**2**) as 4-((1R,2S)-2-(2,2-dichloroacetamido)-3-fluoro-1-(4-(methylsulfonyl)-phenyl)propoxy)-4-oxobutanoic acid. Through the orthogonal experiment, from the results, the optimum reaction conditions are as follows: under the 60 ºC, *n*(florfenicol):*n*(succinic anhydride) is 1:1.5 in the presence of 4 % DMAP by stirring is for 4 h; under this condition the yield of compound (**2**) was 88.1 % and the purity was above 96.5 %.

***In vitro* stabilities and aqueous solubilities of compounds:** Table-1 showed that the aqueous solubility of compound **1** is 1.3 mg/mL. This is a significant (> 500-fold) improvement over the compound **1** and provides levels of solubility that could reduce or eliminate the need to use some organic media such as propylene glycol or ketopyrrolidine. Meanwhile we also found that this influence is not obviously about ester bond broken of the florfenicol sodium succinate in the water, because just only 0.52 % (the peak area of compound **1**/the peak area of compound **3**) of compound **3** have been changed in to compound **1** *in vitro* stabilities as determined by HPLC after 60 days at room temperature.

Thus, the use of succinic acid derived prodrugs **3** was initially considered a potential approach to improve solubility.

TABLE-1
IN VITRO STABILITIES AND AQUEOUS SOLUBILITIES OF COMPOUNDS

Compound	Aqueous solubility (mg/mL)	To increase multiple	The proportion of have been hydrolyzed (%)
1	1.3	–	–
2	22.5	17	0.65
3	More than 500	More than 500	0.52

In vitro stabilities as determined by HPLC after 60 days at room temperature. Thermodynamic solubilities. Values are typically means of two measurements, variation < 20 %. Detailed method in accordance with Ref. 10.

Further it was anticipated that the esters would undergo enzymatic hydrolysis *in vivo* to generate the biologically active parent along with succinic acid and those results have been proved through the rat PK profile following i.m. of 30 mg/kg in our earlier studies.

Conclusion

DMAP catalyzed the reaction of estefication of florfenicol, introducing a water-soluble small moiety to the hydroxyl of florfenicol molecule. The prodrug of florfenicol, sodium salt of esterfication product of florfenicol, exhibited 500 times as water-soluble as florfenicol (from 1.3-500 mg/mL), which solved the problem of low water-solubility of florfenicol. In particular, compound **3** displays high aqueous solubility and is stable in water as well as organic. The low cost of the starting materials, mild reaction condition and high yield of the prodrug presented the major advantages of our methods and brought a bright future to its industrialization. The research on its toxicity and the metabolism of pharmacology is underway.

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