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Synthesis of Tetracycline Analogues and Test of Their Affinity on Synthetic Hydroxyapatite

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Abstact: Eight of new 5-oxo-[1]benzopyrano[2,3-b]pyridines as the analogues of tetracycline, were designed and synthesized, and their affinity to the hydroxyapatite — the inorganic composition of bone were tested.

Keywords: Tetracycline, affinity, hydroxyapatite, benzopyran-4-one.

To develop bone target drugs is an attractive field for increasing the potency and selectivity of these drugs in the treatment of osteoporosis and Paget's disease *etc.* As known, after systematic administration tetracycline can be distributed in bone and bind to hydroxyapatite with high affinity[1]. Thus we tried to use tetracycline as a bone targeted carrier. However, tetracycline is less stable, and its complex structure bring much difficulty in the coupling reaction and purification of the product. Hence, we tried to design and synthesize more stable and more structural analogues of tetracycline based on the previous research work for bone-targeted drugs of our group.

In view of the fact that the hydroxyl, ketocarbonyl, and carbonyl group attached on some positions of tetracycline, are responsible for affinity of tetracycline to bone as pointed out by Perrin[2] and Misra[3]. And, some compounds with benzopyran-4-one unit have been studied for treatrment of osteoporosis with inhibition of bone-absorption[4], so we have designed and synthesized some benzopyran-4-one derivatives bearing appropriate 1, 5-dicarbonyl or 1, 3-phenolic ketone groups, similar to tetracycline.

The synthetic route was outlined in **Scheme 1**. The starting materials *o*-hydroxyacetophenone **2** were easily prepared by Fries rearrangement. The reaction of **2** with Vilsmeier reagent[5] prepared from $POCl_3$ and DMF *in situ* gave the substituted 4-oxo-4*H*-1-benzopyran-3-carboxyaldehyde **3** in 41.8% (R_1=6-COOMe), 64% (R_1=5-OH) and 58.3% (R_1=6-OMe) yield respectively. **4a** was obtained by reacting **3a** with $NH_2OH \cdot HCl$, to generate the oxime which was subsequently dehydrolyzed by refluxing with acetic anhydride (yield 40%). Whereas **4b** and **4c** were synthesized by treating **3b**

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and **3c** with NH_2OH HCl and concentrated HCl in ethanol in one-pot (yield 48.3% and 55.8% respectively).

Scheme 1

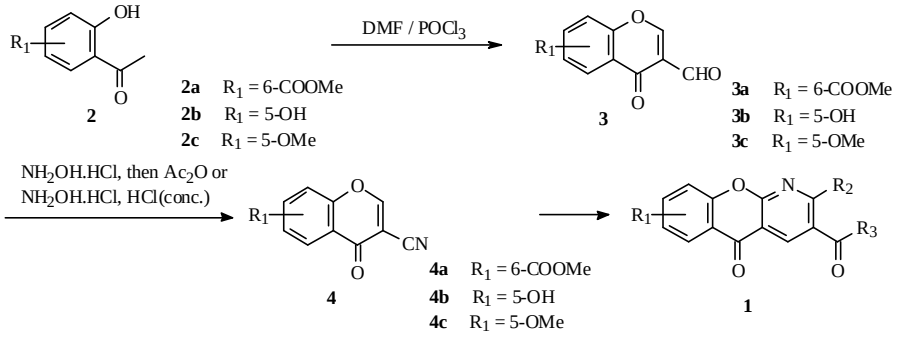

Table 1 5-Oxo-5*H*-[1]benzopyrano[2, 3-b]pyridine derivatives **1**

Compound	Substitutes	mp (°C)	yield (%)
1a[A]	R_1 = 7-COOMe, R_2 = CH_3, R_3 = CH_3	230~230.5	81
1b[A]	R_1 = 7-COOMe, R_2 = CH_3, R_3 = OEt	201~202	74
1c[A]	R_1 = 7-COOMe, R_2 = NH_2, R_3 = OEt	246~248	81.9
1d[B]	R_1 = 6-OH, R_2 = CH_3, R_3 = CH_3	185~186	52
1e[B]	R_1 = 6-OH, R_2 = CH_3, R_3 = OEt	160~162	46
1f[A]	R_1 = 6-OMe, R_2 = CH_3, R_3 =CH_3	240~242	61
1g[A]	R_1 = 6-OMe, R_2 = CH_3, R_3 = OEt	216~218	65
1h[B]	R_1 = 6-OMe, R_2 = NH_2, R_3 = OEt	246~248	60

A. Prepared by Method A, B.Prepared by method B.

Finally, treatment of **4a**, **b**, **c** with substituted acetylacetone or ethyl acetoacetate with piperidine as catalysis in refluxing anhydrous ethanol gave the aimed compoumds **1a~h**[6,7]. The structures of **1a~h** were confirmed by ^{1}H-NMR, MS and IR spectra. The structures of all analogues were listed in **Table 1**.

The primary activity test according to procedure developed by Misra[3]. In the same solvent, the change of concentration before and after absorption on hydroxyapatite was measured by dual-wavelength spectrophotometry. The results showed that the

affinity of **1c** to synthetic hydroxyapatite was comparable to tetracycline, and the affinity of other compound was poor. The study is proceeding further.

Acknowledgment

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References and Notes

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6. Method A: for example **1a**, the solution of **4a** (0.44 mmol), acetylacetone (0.44 mmol) and 2 drops of piperidine in 1.5 mL of anhydrous ethanol was refluxed with stirring for 8 hr, cooled then filtrated, the solid was recrystallized from DMF/EtOH to give white crystal. Method B: for example **1e**. The solution of **4b** (0.44 mmol), ethyl acetoacetate (0.44 mmol), and 3 drops of piperidine in 1.5 mL of ethanol was refluxed with stirring for 8 hr. After being cooled, filtration and filter liquor was concentrated in vacuo, the residue was purified by flash column chromatography to give yellowish-green crystal.
7. Data of analogues: **1a.** ^{1}HNMR ($CDCl_3$, δ ppm): 9.01 (s, 1H, H-4), 8.98 (d, 1H, J=2.2Hz, H-6), 8.48 (dd, 1H, J=8.8, 2.2Hz, H-8), 7.67 (d, 1H, J=8.8Hz, H-9), 3.98 (s, 3H, 7-$COOCH_3$), 2.92 (s, 3H, 2-CH_3), 2.72 (s, 3H, 3-$COCH_3$); IR (KBr, cm^{-1}): 1724, 1697; EI-MS, *m/z* 311 (M^+); **1b.** ^{1}HNMR ($CDCl_3$, δ ppm): 9.20 (s, 1H, H-4), 9.01 (d, 1H, J=2.1Hz, H-4), 8.42 (dd, 1H, J=8.8, 2.1Hz, H-8), 7.71 (d, 1H, J=8.8Hz, H-9), 4.40 (q, 2H, J=7.1Hz, 3-$COOCH_2$–), 3.98 (s, 3H, 7-$COOCH_3$), 2.99 (s, 3H, 2-CH_3), 1.42 (t, 3H, J=7.1Hz, –CH_2CH_3); IR (KBr, cm^{-1}): 1722, 1671; EI-MS, *m/z* 341 (M^+); **1c.** ^{1}HNMR ($CDCl_3$, δ ppm): 9.14 (s, 1H, H-4), 8.92 (d, 1H, J=2.2Hz, H-6), 8.47 (b, 1H, NH), 8.35 (dd, 1H, J=8.8, 2.2Hz, H-8), 7.54 (d, 1H, J=8.8Hz, H-9), 6.16 (b, 1H, NH), 4.40 (q, 2H, J=7.1Hz, –$COOCH_2$-), 3.96 (s, 3H, –$COOCH_3$), 1.43 (t, 3H, J=7.1Hz, –CH_2CH_3); IR (KBr, cm^{-1}): 3442, 3333, 1694, 1661; EI-MS, *m/z* 342 (M^+); **1d.** ^{1}HNMR ($CDCl_3$, δ ppm): 12.14 (s, 1H, 6-OH), 8.93 (s, 1H, H-4), 7.70 (t, 1H, J=8.3Hz, H-8), 7.05 (d, 1H, J=8.3Hz, H-7), 6.90 (d, 1H, J=8.3Hz, H-9), 2.91 (s, 3H, 2-CH_3), 2.71 (s, 3H, 3-$COCH_3$); IR (KBr, cm^{-1}): 3471, 1688, 1641; EI-MS, *m/z* 269 (M^+); **1e.** ^{1}HNMR ($CDCl_3$, δ ppm): 12.20 (s, 1H, 6-OH), 9.14 (s, 1H, H-4), 7.65 (t, 1H, J-8.3Hz, H-8), 7.05 (t, 1H, J=8.3Hz, H-7), 6.88 (d, 1H, J=8.3Hz, H-9), 4.21 (q, 2H, J=7.1Hz, 3-$COOCH_2$–), 2.98 (s, 3H, 2-CH_3), 1.44 (t, 3H, J=7.1Hz, -CH_2CH_3); IR (KBr, cm^{-1}): 2981, 1726, 1649; EI-MS, *m/z* 299 (M^+); **1f.** ^{1}HNMR ($CDCl_3$, δ ppm): 8.96 (s, 1H, H-4), 7.65 (t, 1H, J=8.4Hz, H-8), 7.2 (d, 1H, J=8.4Hz, H-7), 6.90 (d, 1H, J=8.4Hz, H-9), 4.03 (s, 3H, –OCH_3), 2.88 (s, 3H, 2-CH_3), 2.70 (s, 3H, –$COCH_3$); EI-MS, *m/z* 283 (M^+); **1g.** ^{1}HNMR ($CDCl_3$, δ ppm): 9.13 (s, 1H, H-4), 7.86 (t, 1H, J=8.3Hz, H-8), 7.17 (d, 1H, J=8.3Hz, H-7), 6.86 (d, 1H, J=8.3Hz, H-9), 4.4 (q, 2H, J=7.1Hz), 4.02 (s, 3H, –OCH_3), 2.96 (s, 3H, 2-CH_3), 1.42 (t, 3H, J=7.1Hz, –CH_2CH_3); EI-MS, *m/z* 313 (M^+); **1h.** ^{1}HNMR ($CDCl_3$, δ ppm): 9.10 (s, 1H, H-4), 8.4 (b,

1H, NH), 7.6 (t, 1H, J=8.4Hz, H-8), 7.1 (d, 1H, J=8.4Hz, H-7), 6.84 (d, 1H, J=8.4Hz, H-9), 6.23 (br., 1H, NH), 4.4 (q, 2H, J=7.1Hz, $-CH_2CH_3$), 4.0 (s, 3H, $-OCH_3$), 1.4 (t, 3H, J=7.1Hz, $-CH_2CH_3$). EI-MS, *m/z* 314 (M^+).

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