Synthesis and Bone Resorption Effect of Alkoxy-Substituted Xanthones

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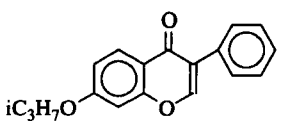
Summary

A topological modification of ipriflavone **1**, a recent anti-osteoporotic drug, is described. The flavone moiety of **1** has been replaced by a xanthone one. Among the new derivatives, the 3,6-diisopropoxyxanthone (**2a**) has shown significant bone resorption inhibition in *in vitro* and *in vivo* tests.

Introduction

Osteoporosis is the pathological term used to describe skeletal abnormalities that arise from a loss of a bone mass[1,2]. Osteoporosis is common in elderly women and is asymptomatic until it is complicated by a fracture or a permanent bone remodelling deformity in the vertebral bodies. Since these abnormalities are caused by an imbalance between bone resorption and formation, therapeutic regimens aim to control, at cellular level, the osteoclastic and osteoblastic remodelling activities. Most drugs used in therapy belong to the antiresorptive class, while those that increase bone formation are rarely used due to possible toxic effects.

Among derivatives of the first class such as estrogens, calcium, calcitonin, and bisphosphonates, we singled out ipriflavone **1** (7-isopropoxyisoflavone)[3,4] because it belongs to the large family of flavonoids that has interested us for some time [5]. In particular, we wished to verify in this field of medicinal chemistry the validity of the conversion of a biologically active flavone into a xanthone. This transformation, which may be regarded as a quasi-isomerization, had been successfully undertaken amongst analeptics[6], adrenergics[7], and cytotoxic agents[8]. In the present case the result of the above transformation stays the same even when the parent compound is an isoflavone.



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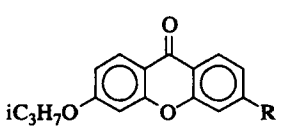

2a, R = OiC_3H_7

2b, R = H

With this aim we have prepared two xanthone derivatives **2a** and **2b**, where compound **2a** may be viewed as a Siamese twin drug [9].

Results and Discussion

On the basis of a preliminary screening on human osteoblastic sarcoma cells proliferation assay[10], compound **2a** was selected and evaluated through *in vitro* and *in vivo* tests.

Its effect on bone resorption was studied *in vitro* according to Zambonin-Zallone *et al.*[11] and measured according to Blair *et al.* [12] using ^{3}H-proline prelabeled rat bone; the results are shown in Table 1.

Table 1. Effect of compound **2a** on bone resorption of hen osteoclasts.

Compound	Conc. mg/ml	Bone resorption (mg)	
		24 h	48 h
Controls (DMSO)	–	44 ± 7.5	72.7 ± 9.6
1	25	25.5 ± 1	22.4 ± 1.0*
2a	10	32.1 ± 3	50.2 ± 6.3
	25	16.0 ± 1.0*	21.0 ± 1.0*

$n = 4$; * $p < 0.01$.

At concentrations of 10 and 25 mg/ml, **2a** showed a dose dependent activity. Bone resorption inhibition was in fact 60% higher than in unexposed controls, both at 24 and at 48 h. Significance was evaluated by Student's test versus the group treated with just the solvent (control).

In vivo, the antiosteoporotic activity of **2a**, administered orally, was evaluated on suckling rats placed on a hypocalcic diet according to the slightly modified method disclosed by Lozupane *et al.* [13].

Table 2. The *in vivo* activity of compound **2a**.

Compd	Dose (mg/kg/oral)	Compacta thickness			
		Diaphysis mm ± S.D.	%	Methaphysis mm ± S.D.	%
Control (Methocel 0.5%)	–	0.55 ± 0.04	–	0.31 ± 0.08	–
2a	250	0.71 ± 0.03*	+29	0.040 ± 0.12*	+29

$n = 3$; * $p < 0.05$.

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The results are reported in Table 2. Compound **2a** showed significant antiosteoporotic activity: the diaphysis and methaphysis both revealed a thickness that was 29% higher than that of the control group.

The acute toxicity of **2a** was evaluated in the rat after oral and intraperitoneal administration, and the LD_{50} was calculated with probit methods[14]. Compound **2a** has an LD_{50}>4000 mg/kg (LD_0) after oral administration and an LD_{50}>1000 mg/kg (LD_{20}) through the intraperitoneal route.

Therefore, we believe that our working hypothesis is confirmed and that **2a** represents a significant improvement of the antiosteoporotic activity of ipriflavone suggesting further structural modifications of the parent compound.

Experimental

Melting points were determined on a Büchi apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained for $CDCl_3$ solutions on a Gemini 300 spectrometer. Elemental analysis were within ± 0.4% of the theoretical value. Mass spectra were recorded on a V.G. 7070 E spectrometer.

3,6-Di(isopropoxy)xanthen-9-one (**2a**)

A mixture of 27.5g (0.121 mol) of 3,6-dihydroxyxanth-9-one[15], 54.6 ml (0.546 mol) of isopropyl bromide and 27.5 g of *N*-benzyltriethylammonium chloride in 1 l toluene and 580 ml of 50% aqueous sodium hydroxide was refluxed under stirring for 5 h. The mixture was cooled to room temperature and the organic phase was separated, washed with water to neutrality, dried on sodium sulphate and evaporated. The residue was crystallized from ligroin to yield 31 g (84%) of **2a** , mp 135–36 °C. Anal. ($C_{19}H_{20}O_4$). ^1H NMR δ: 1.44 (d, 12H), 4.7 (m, 2H), 6.9 (m, 4H), 8.22 (d, 2H). ^{13}C NMR δ: 22.76; 71.55; 102.47; 114.71; 116.40; 129.02; 159.93; 163.97; 176.36. MS: *m/z* (relative abundance): 312 (M^+, 20.18), 229 (13.33), 228 (100), 200 (7.35), 199 (7.46), 57 (5.50), 43 (22.60), 41 (16.69), 39 (6.23), 32 (44.78).

3-Isopropoxyxanthen-9-one (**2b**)

Using the same procedure and starting from 3-hydroxyxanthen-9-one[16] (21.2 g, 0.1 mol), 22.8 g (90%) of **2b**, mp 89–90 °C (ligroin) [lit.[17] mp 90–92 °C]. Anal. ($C_{16}H_{14}O_3$). ^1H NMR δ: 1.42 (d, 6H), 4.7 (m, 1H), 6.8–8.4 (m, 7H). ^{13}C NMR δ: 22.56; 71.44; 102.17; 114.93; 116.11; 118.35; 122.62; 124.44; 127.25; 128.89; 134.86; 156.84; 158.73; 164.23; 176.83. MS: *m/z* (relative abundance): 254 (M^+, 25.40), 213 (13.83), 212 (100), 184 (10.82), 155 (6.58), 128 (6.64), 127 (6.39), 70 (6.54), 63 (6.99), 57 (8.43), 43 (9.17), 41 (9.44), 32 (23.02).

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