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Short Note

Synthesis, Characterization and Antileucemic Activity of 7-Hydroxy-8-acetylcoumarin Benzoylhydrazone

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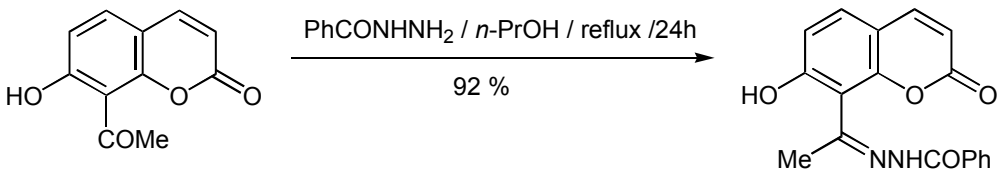
As part of a research programme targeting novel molecules derived from nitrogen derivatives of *o*-hydroxyaryl ketones [1] we synthesised 7-hydroxy-8-acetylcoumarin benzoylhydrazone. Coumarins are very well known for their biological activity [2]. Moreover hydrazone moiety has been reported to possess anticancer activity [3]. Thus, it is not unreasonable to assume that a molecule that possesses both coumarin and hydrazone features will possibly show interesting combined biological activity.



7-Hydroxy-8-acetylcoumarin was prepared according to the literature method [4] whereas commercially available benzoic hydrazide was supplied by Aldrich.

1. Method of Preparation 7-hydroxy-8-acetylcoumarin benzoylhydrazone

Benzoic hydrazide (0.61 g, 4.5 mmol) was added to a solution of 7-hydroxy-8-acetylcoumarin (1 g, 4.5 mmol) in propanol-1 (10 mL). The reaction mixture was refluxed for 24 hours. It was then allowed to cool at room temperature. Subsequently, it was stored in the refrigerator overnight. Filtration of the precipitate, which was formed, afforded (1.38 g, 92 %) of the desired *N*-benzoylhydrazone as white crystals. The product was identified by its ^{1}H NMR, ^{13}C NMR and MS and it was subjected to elemental analysis without further purification.

M.p. 248.5-249.5 °C.
^{1}H NMR (400 MHz, DMSO-d_6): 2.28 (s, 3H), 6.25-6.25 (d, *J*=8.6Hz, 1H), 6.93-6.95 (d, *J*=8.6Hz, 1H), 7.41-8.08 (m, 4H), 8.57-8.80 (m, 3H), 11.24 (s, 1H), 12.37 (s, 1H).
^{13}C NMR (100 MHz, DMSO-d_6): 19.6, 111.9, 112.0, 112.45, 114.4, 120.0, 123.8, 129.1, 130.7, 132.55, 133.9, 137.9, 145.6, 154.0, 154.9, 160.5, 160.9, 165.2.
MS *m/z* (ES+): 345 $[M+Na]^+$, 323 $[M+1]^+$.
Anal. Calc. for $C_{18}H_{14}N_2O_4$: C 67.08, H 4.38, N 8.69; found: C 66.99, H 4.30, N, 8.61.

2. Material and Methods

2.1. Mice

Male and female mice DBA/2 and BDF_1 (C57Bl6 x DBA/2) mice that were 4-6 weeks of age and weighted 20-25 g were used for toxicity and antitumor evaluation experiments. Mice, provided by the Experimental Animal production Laboratory of Theagenion Cancer Hospital, were under conditions of constant temperature and humidity, with sterile beckling, water and food.

2.2. Tumor

Transplantation of lymphocytic P388 Leucemia was carried out by withdrawing peritoneal fluid from donor DBA/2 mice with 7-day growth. The suspension was centrifuged for 2 min (2000 g). The supernatant peritoneal fluid was decanted and a fold dilution with 0.9% NaCl solution was made. The cell number was determined. The resulting cell suspension of 0.1 ml, containing 106 cells, was injected intraperitoneally into each animal.

2.3. Compounds

In all experiments, 7-hydroxy-8-acetylcoumarin benzoylhydrazone was administrated by IP injection. It was dissolved in 10% DMSO and suspended in corn oil. Stock solutions of the compound were prepared immediately before administration.

Percentage of deaths due to the toxicity of each dose is plotted on the ordinate, while the administered doses are plotted on the abscisae on semilogarithmic paper. The point of the line corresponding to 50% and 10% mortalities gives the L_{50} and L_{10} respectively [5].

3. Chemotherapy Evaluation

For the survival experiments, the antitumor activity of 7-hydroxy-8-acetylcoumarin benzoylhydrazone against P388 murine lymphocytic leukaemia was assessed from the oncostatic parameter T/C% : the mean of median survival time of the drug-treated animals (T) excluding long-term survivors, versus corn-oil treated controls (C), expressed as percentage. The minimum criterion for activity is T/C≥125%, according to the experimental evaluation of antitumor drugs in National Cancer Institute in USA [6].

4. Acute Toxicity

All experiments were consisted of six mice in each drug treatment group and eight mice in the tumor control group. Experiments were initiated by implanting mice with tumor cells. Drug treatments were given as single, or intraperitoneally injections, using LD_{40} as a therapeutic dose. Experiments were terminated when no mice remained alive [5].

5. Results

The results for the antileucemic activity of 7-hydroxy-8-acetylcoumarin benzoylhydrazone are presented in Table I.

From the Table I it can be seen that the antitumor activity of 7-hydroxy-8-acetylcoumarin benzoylhydrazone is interesting producing T/C rates of 147 and 138% when the single and intermitted (days 1,4,7) treatment schedules, respectively, were used. This activity is above the borderline activity (T/C≥125%), which is the minimum criterion of activity of drugs [5].

Table 1. Antitumer Activity of 7-hydroxy-8-acetylcoumarin benzoylhydrazone in murine P388 leukemia.

Treatment Schedule	Dosage(mg/kg)	MST[a](days)	T/C%[b]
200	Day 1	13.25 (9.0)[c]	147
100	Days 1,4,7	14.5 (10.5)[c]	138
100	Days 1-9	10.4 (9.0)[c]	116

[a]MST: survival time

[b]T/C: survival time of drug-treated animals (T) versus corn-oil control animals (C)

[c]MST of control animals

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