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COMMUNICATIONS

6,8-Di-t-butyl-4-oxo-4H-1-benzopyran-2-carboxylic acid: a chromone derivative with anti-allergic, anti-inflammatory and uricosuric activity

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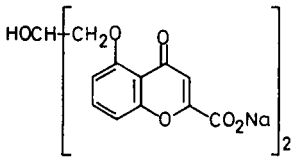
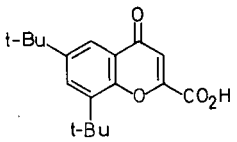
During the course of investigations aimed at producing an orally active anti-asthma drug, chromone derivatives related to sodium cromoglycate (Altounyan, 1967) were synthesized and investigated for their anti-allergic activity. One of the compounds prepared, 6,8-di-t-butyl-4-oxo-4H-1-benzopyran-2-carboxylic acid (FPL 52791), in addition to anti-allergic properties in rats, showed moderate analgesic and anti-inflammatory activity in conventional tests in mice and rats, both by the parenteral and oral routes.



Sodium cromoglycate



FPL 52791

The anti-allergic activity of FPL 52791 was demonstrated in the passive cutaneous anaphylaxis (PCA) test in rats. When administered intravenously at doses of 1·0 mg kg^{-1} and above (all doses expressed in terms of the water soluble sodium salt of FPL 52791, the free acid is insoluble in water), at the time of antigen challenge, the drug completely inhibited the PCA reaction in rats passively sensitized by intradermal injection of rat anti-ovalbumin serum (Goose & Blair, 1969). In this test system, the compound was 6 times more potent than sodium cromoglycate, having an ED50 (dose required to produce 50 % inhibition of the PCA reaction) of 0·1 mg kg^{-1} (mean of 3 experiments). When given orally 7 min before antigen challenge, FPL 52791 inhibited the PCA reaction in rats passively sensitized by the intradermal injection of rat anti-*N. brasiliensis* serum (Goose & Blair, 1969), with an ED50 of 4·2 mg kg^{-1} (mean of 3 experiments) in this test system. Furthermore, when administered directly into the duodenum of anaesthetized, laparotomized rats, 7 min before antigen challenge, the ED50 was 0·9 mg kg^{-1} (mean of 6 experiments). Sodium cromoglycate was inactive in both of these gastrointestinal PCA tests.

When given intravenously at a dose of 1 mg kg^{-1}, 1 min before the intravenous administration of 0·5 mg kg^{-1} of Compound 48/80, FPL 52791 produced a maximal inhibition of mast cell degranulation of 72 %. At both higher and lower doses the inhibition was less, i.e. the dose response curve was "bell shaped" in contrast to the intravenous PCA test where a normal dose-response curve was obtained. In this respect FPL 52791 is very similar to sodium cromoglycate which also gives a 'bell-shaped' response in the 48/80 challenge test (Orr, 1973).

When anaesthetized rats were challenged intravenously with 25 mg kg^{-1} of ovalbumin, 24 h after passive sensitization by intravenous injection of rat anti-conalbumin/*B. pertussis* (*N. brasiliensis* potentiated) serum (Farmer, Richards & others, 1975), protection from anaphylactic bronchoconstriction was produced by the intravenous administration of FPL 52791. Given 1 min before antigen challenge, the compound had an ED50 of 0·3 mg kg^{-1} and was about 3 times as potent as sodium cromoglycate in this test system.

The compound had moderate anti-inflammatory activity in rats at doses which did not produce overt signs of toxicity. In adjuvant-induced arthritis in rats (Newbould, 1963), oral administration of 100 mg kg^{-1} FPL 52791, from the day before initiation of the reaction to day 16 or 17 afterwards, produced a mean inhibition of 37·1 % (s.e.m. ± 2·4 %) of secondary swelling of the injected foot (16 experiments, total of 85 animals). When FPL 52791 was given orally, at the same dose, from day 10 to day 16 after initiation of the reaction, a greater mean inhibition of 57·4 % (± 7·6 %) was obtained (8 experiments, total of 40 animals). FPL 52791 had no effect on the disease when dosing was restricted to the period of sensitization (days −1 to +6), indicating a lack of immunosuppressant activity. The potency ratio of FPL 52791 compared to aspirin when both drugs were administered from one

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day before initiation of the disease to 16 days afterwards, was 1·86 (95% confidence limits 1·13–2·90).

Simultaneous oral administration of FPL 52791 (100 mg kg^{-1}) and prednisolone (10 mg kg^{-1}), to rats with adjuvant-induced arthritis, neither potentiated nor inhibited the effect of either drug. Acute administration of FPL 52791 inhibited carrageenan-induced oedema in rat hindfeet (Winter, Risley & Nuss, 1962). The mean inhibition of foot swelling produced by FPL 52791 was 21·1 % (± 4·4 %) at an oral dose of 200 mg kg^{-1} (6 experiments, total of 58 animals), at which dose the compound was equipotent with aspirin, and 39·1 % (± 2·4 %) at an intraperitoneal dose of 30 mg kg^{-1} (27 experiments, total of 268 animals).

Gastric retention of the drug after acute oral dosing in rats was very substantial and may explain the different potencies observed between oral and intraperitoneal administration. This gastric retention was overcome by chronic dosing of the compound, thus explaining its greater oral activity in screens such as the adjuvant-induced arthritis test.

Chronic dosing with FPL 52791 neither increased adrenal weight nor decreased adrenal ascorbic acid and, in acute studies, carrageenan induced-oedema in adrenalectomized rats was inhibited by the compound, suggesting that its anti-inflammatory action is not a consequence of adrenal stimulation. FPL 52791 inhibited an isolated microsomal enzyme preparation of pig lung prostaglandin (PG) synthetase at 10–100 μg ml^{-1}, and produced 20–84 % inhibition of PG production and/or release from isolated segments of rabbit duodenum at 0·1–10 μg ml^{-1}.

Since FPL 52791 possessed inhibitory activity against PG synthetase *in vitro*, its analgesic activity in tests in which PG synthetase inhibitors such as aspirin are active was investigated (Vane, 1971). In the acetic acid induced writhing test in mice (Whittle, 1964) it had an oral ED 50 of 48 mg kg^{-1} and was approximately 8 times as active as aspirin. It also inhibited the increase in capillary permeability produced by the acetic acid, with an oral ED 50 of 160 mg kg^{-1}, and was 6 times less active than aspirin in this test. Further evidence of its non-narcotic analgesic activity was its ability to reduce the pain response in rats to pressure on an inflamed foot (ED 50 = 649 mg kg^{-1}), without modifying the response to pressure on a normal foot (Randall & Selitto, 1957). In this respect it was equipotent with aspirin.

FPL 52791 also had antipyretic activity in yeast-induced pyrexia in rats (Boreus & Sandberg, 1953) at 30–100 mg kg^{-1}, and was equipotent in this test to aspirin.

This new chromone is strongly acidic, pKa 1·6, and is very soluble in most organic solvents, but insoluble in water. It is a stable colourless solid, m.p. 231–235°, which is prepared via a three stage process from 2,4-di-t-butylphenol. The initial reaction involves a Michael condensation of the phenol with dimethyl acetylene dicarboxylate and is followed by the hydrolysis of the resulting phenoxyfumarate ester to the corresponding fumaric acid. The final stage consists of the dehydrative cyclization of the fumaric acid to give the benzopyran-2-carboxylic acid. The structure of the compound was confirmed by standard physico-chemical techniques.

Because of its interesting combination of anti-allergic and anti-inflammatory properties, FPL 52791 was progressed for further evaluation in man. During the course of the pharmacological activity study of FPL 52791 in normal volunteers it was noted that, after single doses of the compound, plasma urate concentrations fell, in a dose related fashion (dose range 50–400 mg), and an increase in urinary excretion of urate was also observed. Full details of these studies will be reported separately.

Currently, clinical studies are in progress to evaluate the potential of this compound in the clinical management of both the acute and chronic hyperuricaemic state, and to investigate the compound's potential anti-inflammatory, analgesic and anti-allergic activity in man.

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