Life Sciences 86 (2010) 819–824

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Antidepressant-like profile and MAO-A inhibitory activity of 4-propyl-2H-benzo[h]-chromen-2-one

Nadezdha E. Vergel [a], José L. López [b], Francisco Orallo [c,1], Dolores Viña [c], Diana M. Buitrago [a], Esther del Olmo [b], Juan A. Mico [d], Mario F. Guerrero [a,*]

[a] Pharmacy Department, School of Sciences, National University of Colombia, Colombia
[b] Pharmaceutical Chemistry Department, School of Pharmacy, Salamanca University, Spain
[c] Pharmacology Department, School of Pharmacy, University of Santiago de Compostela, Spain
[d] Department of Neuroscience, Pharmacology and Psychiaty, Ciber of Mental Health, CIBERSAM School of Medicine, University of Cadiz, Spain

ARTICLE INFO

Article history:
Received 2 December 2009
Accepted 31 March 2010

Keywords:
Antidepressant
Coumarin
Drug design
MAO-A
Monoamine oxidase

ABSTRACT

Aims: To evaluate the central nervous pharmacological profile of 4-propyl-2H-benzo[h]-chromen-2-one (FCS-304) in ICR mice and its monoamine oxidase inhibitor activity.
Main methods: FCS-304 was evaluated in screening test of central nervous system in ICR mice and against MAO activity.
Key findings: FCS-304 (25–200 mg/Kg, p.o.) significantly reduced immobility time during the forced swimming test (FST) and tail suspension test (TST), but did not show effects in the rota-rod tests, maximal electroshock seizures (MES), pentylenetetrazole seizures, light–dark box, barbiturate sleeping time and cold plate tests. Furthermore, FCS-304 inhibited monoamine oxidase A (MAO-A) with IC_{50}: 2.28 ± 0.15 μM, but not MAO-B.
Significance: These results suggest that FCS-304 could elicit antidepressant effects related to MAO-A inhibitory activity.

Introduction

Coumarin (cromen-2-ona) is an active compound found in medicinal species such as *Peucedanum palustre*, *Ruta graveolens* (Ojala 2001) and *Hygrophylla tytha* (Ariza et al. 2006, 2007) which are used in folk medicine as tranquilizers. Coumarins are a well-known natural product group with a broad profile of pharmacological actions such as anticoagulation and antioxidation (Payá et al. 1992). They have anti-inflammatory (Fylaktakidou et al. 2004), immuno-modulatory (NTP 1993), anti-infective (Rehman et al. 2005) and photosensitive (Bisagni 1992) properties. In addition they have possible sedative (Apseloff et al. 1991), tranquilizing (Barreiro-Arcos et al. 2006), analgesic (Meotti et al. 2006), antidepressant (Chen et al. 2005), anticonvulsant (Ariza et al. 2007) and neuro-protective (Kang et al. 2005; Howes et al. 2003) effects on the central nervous system. Many synthetic analogues derivatives have been also prepared in the laboratory.

The structural modification of active compounds isolated from species with medicinal uses is one of the strategies used in the search for new drug options for treating disorders with high public health impact, including chronic neurological diseases (Pieters and Vlietinck 2005). Some coumarin compounds isolated from plant species exert inhibitory effects on the enzyme monoamine oxidase (MAO) and appropriate modifications to its structure have led to obtain compounds with greater pharmacological interest (Thull and Testa 1994; Yun et al. 2001; Rendenbach-Müller et al. 1994; Gnerre et al. 2000). Monoamine oxidase A (MAO-A), an enzyme that selectively catalyzes serotonin and norepinephrine neurotransmitters, is a pharmacological target at searching useful agents for the treatment of major depression. This is due in part to its favorable safety profile with respect to nonselective and irreversible MAO and tricyclic antidepressants, and its effectiveness, particularly in patients with refractory depression (Bonnet 2003).

This study describes the neuropharmacological profile in ICR mice of 4-propyl-2H-benzo[h]-chromen-2-one (FCS-304), a coumarin analogue prototype with a potential antidepressant-like effect associated with the selective inhibition of MAO-A. No previous biological assays have been ever been published for this compound.

Materials and methods

Animals

Male 7–9 week-old ICR albino mice, weighing between 20 and 30 g obtained from the Department of Pharmacy, National University

* Corresponding author. AK 30 # 45-03, Departamento de Farmacia 450-311. Bogotá, Colombia. Tel.: +57 1 3165000x14631; fax: +57 1 3165060.
E-mail address: mfguerrerop@unal.edu.co (M.F. Guerrero).

[1] We wish to dedicate this paper to Dr. Francisco Orallo, who died during the course of this work.

0024-3205/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2010.04.001

of Colombia were used in this study. They were held in conditions of controlled temperature and humidity, photoperiod of 12 h light/darkness and free access to food and water, except on the test day. The animals were subjected to a fasting period of no more than 6 h during the test day in order to not affect the convulsive threshold (Swinyard et al. 1989). 8–10 animals per each treatment and per each dose were used except in the preliminary observation Irwin test, where three animals per group were used. To examine the behavior of each animal in the absence of the experimenter a digital video camera was used. All procedures were conducted following the care principles for the management of laboratory animals. The Ethics Committee of the Faculty of Science at the National University of Colombia endorsed this study.

Biological tests

Irwin test

This test was used for assessing preliminary drug effect on the behavioral and physiological state of mice. Animals were dosed with 25, 50, 100, 200 and 500 mg/kg, p.o. of FCS-304 and a vehicle (cyclodextrin: 10%, as a control). The animals were observed at 15, 30, 60, 120 and 180 min, and 24 h after administration, recording the presence or absence of: mortality, seizure, erection of the tail (Straub sign), sedation, excitation, abnormal gait, jumps, motor incoordination, abdominal torsion, piloerection, stereotypy, ticks, and the increase or decrease in respiration (Roux et al. 2003).

Maximum electroshock seizure (MES)

It is a test to identify potentially effective agents in preventing tonic clonic seizures. Groups of 10 animals were dosed with FCS-304 (100 mg/kg, p.o.), sodium phenytoin (positive control, 20 mg/kg, p.o.) or vehicle (negative control: cyclodextrin 10% by volume of 0.01 ml/g, p.o.) 1 h prior to exposure to an electric shock of 60 v, 100 Hz and 0.1 s through corneal electrodes (Ugo Basile® stimulator). A protective effect was assumed when the drug prevented the tonic extension of hind legs at an angle greater than 45° (Swinyard et al. 1989).

Seizures induced by pentylenetetrazole (PTZ)

A test was done to detect potentially effective agents to prevent absence seizures. Groups of 10 animals dosed 1 h prior with FCS-304 (100 mg/kg, p.o.), clonazepam (positive control, 0.5 mg/kg, p.o.) or vehicle were administered PTZ (GABA antagonist, 85 mg/kg, s.c.). An animal that did not show clonic seizures in its head, back or limbs for more than 5 s over 30 min of observation after PTZ administration was considered to be protected (Swinyard et al. 1989).

Light–dark box

It is a test to detect potentially effective anxiolytic agents. Animals divided into groups of 8–10 dosed 1 h previously with FCS-304 (100 mg/kg, p.o.), diazepam (positive control, 0.5 mg/kg, i.p.) or vehicle were placed one by one into the lit compartment (100 W, 20×30x×30 cm) of an acrylic box, opposite the hole (10×10 cm) of a contiguous dark area (40×30×30 cm). The total time spent in the lit zone was recorded for 5 min (Costall et al. 1989).

Barbiturate sleeping time

It is a useful test for detecting agents with sedative effects. Animals distributed into groups of 8–10 and dosed previously with FCS-304 (100 mg/kg, p.o.), diazepam (0.5 mg/kg, i.p.) or vehicle were treated with pentobarbitone (50 mg/kg, i.p.). Sleeping time was recorded from the moment the animal completely lost its gait until the moment it recovered it (Lapa et al. 2002).

Rota-rod

It is a test to detect potentially neurotoxic agents. Two groups of 8–10 animals were trained to maintain their balance on an axis of 3 cm in diameter powered by a small engine at 10 rpm. Mice were previously trained on the rota-rod for 3 min at a speed of 10 rpm. For testing, the animals were placed on the rota-rod 1 h after the administration of FCS-304 (100 mg/kg, p.o.) or vehicle. Then, the speed was set at 10 rpm for 60 s and subsequently accelerated to 60 rpm. The time and speed taken for mice to fall after the beginning of the acceleration was recorded (Jones and Roberts 1968).

Cold plate

It is a useful test to identify potentially antineuralgic agents. Groups of 8–10 animals were subjected to sciatic nerve ligation under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). The nerve was exposed after an incision at the thigh and ligated with four points of suture (thread Chrome 5.0) without compromising the epineural circulation, before suturing the skin and muscle layers. After a week of postoperative recovery, animals were placed one by one, on a cold metal surface (cold plate, 4 °C, Ugo Basile®) to record pain, expressed by the number of licks of the hind legs in basal conditions, and then 30 and 60 min after administration of FCS-304 (100 mg/kg, p.o.) or vehicle (Bennett and Xie 1988).

Forced swimming (FST)

It is a test to detect potential antidepressant agents. Animals divided into seven groups of 8–10 and treated 1 h previously with FCS-304 (25, 50, 100 and 200 mg/kg, p.o), imipramine (positive control of tricyclic antidepressant class, 32 mg/kg, p.o.), fluoxetine (positive control of selective serotonin reuptake inhibitor class, 50 mg/kg, p.o.) or vehicle, were placed one by one into a plastic cylinder (35×24 cm) containing water (20 °C) to a height of 13.5 cm. The total immobility time, defined as movements only necessary for the animal to stay afloat, was recorded over 5 min (Porsolt et al. 1977).

Tail suspension (TST)

It is also a useful test for detecting potentially antidepressant agents. Animals assigned to seven groups of 8–10 and dosed with FCS-304 (25, 50, 100 and 200 mg/kg, p.o.), imipramine (32 mg/kg. p.o), fluoxetine (50 mg/kg, p.o) or vehicle, were suspended by the tail through a metal loop on [in] the inner surface of a 20 cm high acrylic chamber. Immobility time was recorded over 6 min (Steru et al. 1985).

Determination of human monoamine oxidase (hMAO) isoform activity

The potential effects of the test drugs on human hMAO activity were investigated by measuring their effects on the production of hydrogen peroxide (H_2O_2) from p-tyramine, using the Amplex® Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, USA) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B (Sigma-Aldrich Química S.A., Alcobendas, Spain).

The production of H_2O_2 catalyzed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red reagent), a non-fluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. In this study hMAO activity was evaluated using the above method following the general procedure previously described by us (Santana et al. 2008).

Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the test drugs (new compounds or reference inhibitors) in various concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity, i.e., to oxidize (in the control group) the same concentration of substrate: 165 pmol of p-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of p-tyramine oxidized to p-hydroxyphenylacetaldehyde/