Journal of Steroid Biochemistry & Molecular Biology 134 (2013) 51–58

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

Pharmacological characterization of an imidazolopyrazole as novel selective androgen receptor modulator

Xuqing Zhang*, George F. Allan, Pamela Tannenbaum, Tifanie Sbriscia, Olivia Linton, Muh-Tsann Lai, Donna Haynes-Johnson, Sheela Bhattacharjee, Scott G. Lundeen, Zhihua Sui

Janssen Research and Development LLC, Welsh&McKean Roads, Spring House, PA 19477, USA

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 8 October 2012
Accepted 11 October 2012

Keywords:
Selective androgen receptor modulator
Prostate
Levator ani
Sexual behavior

ABSTRACT

Selective androgen receptor modulators (SARMs) are androgens with tissue-selective activity. SARMs that have anabolic activity on muscle while having minimal stimulatory activity on prostate are classified as SARM agonists. They can be used to prevent the loss of lean body mass that is associated with cancer, immunodeficiency, renal disease and aging. They may also have anabolic activity on bone; thus, unlike estrogens, they may reverse the loss of bone strength associated with aging or hypogonadism. Our in-house effort on SARM program discovers a nonsteroidal androgen receptor ligand with a unique imidazolopyrazole moiety in its structure. In vitro, this compound is a weak androgen receptor binder and a weak androgen agonist. Despite this, in orchidectomized mature rats it is an effective SARM agonist, with an ED_{50} on levator ani muscle of 3.3 mg/kg and an ED_{50} on ventral prostate of >30 mg/kg. It has its maximal effect on muscle at the dose of 10 mg/kg. In addition, this compound has mixed agonistic and antagonistic activities on prostate, reducing the weight of that tissue in intact rats by 22% at 10 mg/kg. The compound does not have significant effect on gonadotropin levels or testosterone levels in both orchidectomized and intact male rats. It does not have notable progestin, estrogen or glucocorticoid agonistic or antagonistic activity in rats. In a female sexual behavior model, it improves the sexual desire of ovariectomized female rats for sexually mature intact males over nonsexually ovariectomized females. Overall, the imidazolopyrazole is a potent prostate-sparing candidate for development as a SARM agonist with an appropriate pharmacological profile for clinical benefit in muscle-wasting conditions and female sexual function disorders.

1. Introduction

The androgens testosterone (T) and the more active endogenous metabolite 5-α-dihydrotesterone (DHT) are important in male physiology for their essential roles in male sexual differentiation, male puberty changes, maintenance of muscle and bone mass, prostate growth, and spermatogenesis in adults [1]. Androgens display anabolic effect in prostate, bone, muscle, and hair follicles of the scalp and skin [2]. T levels gradually decline after the 3rd decade of life and through the geriatric years [3]. Although secondary sexual traits such as facial hair are maintained, lean mass declines. Low T levels are associated with a greater risk for several age-related diseases [4]. An inevitable consequence of aging is the loss of bone and muscle mass, which is partially resulted from a steady decline in anabolic and anti-catabolic hormone signaling [5]. These lean mass and bone deficits confer higher risk for osteoporotic fractures, age related sarcopenia and loss of independence. T therapy in older men has been applied in multiple clinical trials and indicated favorable impact on body composition and strength, bone density, mood, sexual function and quality of life [2]. However, T therapy also leads to potential risks including increased hematocrit levels and possibly accelerating prostate cancer or other latent diseases [6]. Androgens may be employed in women beyond men as long as tissue selectivity can be achieved. Some recent studies clearly outlined the potential benefits of androgen therapy in women. Tissue selective androgens fortifying bone and muscle without virilizing effects on the skin, hair follicles, or vocal cords or cardiovascular side effect could be effective treatments for bone fractures in women [7]. In addition, a tissue-selective, non-virilizing androgen might be able to enhance or stimulate libido and those parameters of sexuality that androgens can influence alone or in combination with estrogen replacement [8]. However, application of androgen therapy to women's health has been hampered by some adverse factors, such as hirsutism, acne, deepening of the voice, and reproductive tissue changes. Moreover, providing women with T may increase the risk for breast cancer and

* Corresponding author at: Janssen Research and Development LLC, Welsh&McKean Roads, P.O. Box 776, Spring House, PA 19477, USA.
Tel.: +1 215 628 7877; fax: +1 215 628 4985.
E-mail address: xzhang5@its.jnj.com (X. Zhang).

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2012.10.015

other health problems due to its metabolic conversion to estradiol [9]. Given these concerns, tissue-selective androgen receptor modulators (SARMs) that restore healthy bone and muscle but do not cause uterine, prostate, or sebaceous gland hypertrophy would be attractive therapies. There has been significant progress toward discovery of chemically distinct steroidal and nonsteroidal SARMs with prostate-sparing agonistic activity [10–23]. The representative compounds are aryl propionamides (S-4 and S-22), S-40503, LGD-2226, LGD-2941, LGD-3303, BMS-564929, JNJ-28330835, JNJ-37654032, AC-262536, ACP-105, RAD140 and MK-0773. All of these SARMs demonstrated selective anabolic activity on muscle over androgenic activity on prostate in rodent models. Among them, some showed beneficial effect on bone and sexual behavior. Both bone mineral density and bone strength were improved in orchidectomized males or ovariectomized female rats. In addition, sexual functions such as the number of mounts, emissions and ejaculations were enhanced in the presence of responsive females following treatment in orchidectomized rats. Among these compounds, the aryl propionamide derivative S-23 [24], LGD-3303 [25] and JNJ-28330835 [18] have been reported in female rat models for treating sexual desire disorder with encouraging results, suggesting a novel potential indication for nonsteroidal SARMs.

We have identified several distinct scaffolds of nonsteroidal SARMs [27–36]. An orchidectomized immature male rat model was used to screen all compounds in vivo. These compounds displayed either partially androgenic agonism or fully androgenic antagonism according to the degree of prostate hypertrophy observed in the absence or presence of exogenous testosterone. Herein we describe the detailed in vitro and in vivo pharmacology of a novel imidazolopyrazole SARM identified by phenotypic screening in our laboratory.

2. Materials and methods

2.1. In vitro studies

The whole-cell androgen receptor (AR) binding assays were conducted using the protocols reported previously [26]. Recombinant receptors, fluorescence-labeled ligands, and assay buffers were obtained from Invitrogen (Carlsbad, CA) to run fluorescence polarization binding assays for human PR, GR, ERα, and ERβ. Each receptor with its fluorescent ligand or test compound was incubated for 1 h in the dark at room temperature using Microflour 2 Black plates (Dynex, Chantilly, VA). After the assay was completed, it was analyzed on an LJL Analyst fluorescence polarization plate reader (Molecular Devices, Sunnyvale, CA). Percentages of inhibition relative to no-competitor and maximum-competitor controls were attained from the raw binding data (counts per minute or fluorescence polarization units). IC_{50}s were calculated from 50% of inhibition at the nonlinear regression curve. A K_i value was established from the corresponding IC_{50} using the Cheng and Prusoff formula [37].

L929 cell functional assays of androgen agonism and antagonism [26], LNCaP-PSA cell assay of androgen agonism [38], T47D cell assays of progesterone agonism and antagonism [39], A549 cell assays of glucocorticoid agonism and antagonism [40] and Ishikawa human endometrium cell assays of estrogen agonism and antagonism [18] were conducted following the protocols reported previously. Raw functional data were transformed into percentages of stimulation in agonist format relative to vehicle and maximum-stimulation controls, or to percentages of inhibition in antagonist format relative to no-inhibition and maximum-inhibition controls. All EC_{50}s and IC_{50}s were calculated as 50% of stimulation and inhibition at the nonlinear regression curves, respectively.

2.2. Mature rat tissue weight and hormone assays

All rodents in our studies were manipulated following the regulations governed by the local animal care and use committee. Mature (two-month-old) orchidectomized Sprague–Dawley rats were available from Charles River (Wilmington, MA). Animals were administrated with test compound once daily by gavage (p.o.) for two weeks using 20% (w/v) hydroxypropyl-β-D-cyclodextrin (HPBCD) from Cargil (Cedar Rapids, IA) as vehicle. Control animals were dosed either p.o. with HPBCD alone or s.c. with 0.4 mg/kg testosterone propionate (TP) formulated in sesame oil from Sigma (St. Louis, MO). For the mature intact rat model, mature (two-month-old) testis-intact male Sprague–Dawley rats from Charles River (Wilmington, MA) were orally administrated once daily for six weeks with test compound formulated in 20% (w/v) HPBCD. Control animals were dosed p.o. with HPBCD alone or with 30 mg/kg flutamide from Sigma or bicalutamide (purified in-house from 50-mg Casodex® pills from Astra Zeneca at Wilmington, DE). 20% (w/v) HPBCD was used as the dosing formulations for both flutamide and bicalutamide. Animals were weighed, anesthetized and sacrificed by asphyxiation in carbon dioxide on the day following the final dose of the studies. Blood was drawn by cardiac puncture using Monovette collection tubes from Sarstedt (Newton, NC). Test organs such as ventral prostates and levator ani muscle were dissected, cleared of extraneous tissue and weighed.

All organ wet weights (in milligrams) were normalized to total body weight (in grams) and compared. For the agonist format, normalized tissue weights were converted to percent stimulation relative to the vehicle control (0% stimulation) and to the known tissue weight ratios in age-matched intact rats ("100% stimulation"). For the antagonist format, normalized tissue weights were converted to percent inhibition relative to the intact vehicle control (0% inhibition) and vehicle-treated orchidectomized rats ("100% inhibition"). For the 100% stimulation and 100% inhibition controls, mean tissue weight ratios from numerous previous experiments were used for calculation. All ED_{50}s were determined as 50% of stimulation or inhibition at the nonlinear regression curve. The maximally efficacious dose (ED_{max}) was defined as the dose maximally stimulating levator ani weight to 100% level in orchidectomized rats that was equivalent to the level in intact rats.

Rat blood was collected at necropsy after the studies and plasma samples were prepared by a centrifuge for hormone level analysis. Serum T, LH, or FSH levels were measured using enzyme immunoassay (EIA) kits from American Laboratory Products Co. (ALPCO; Salem, NH) following the manufacturer's directions or by Laboratory Corporation of America [LabCorp] (Research Triangle Park, NC).

2.3. Female rat sexuality models

Sexual motivation was evaluated according to test compound-treated ovariectomized adult Long-Evans rats toward a sexually intact male or a nonsexually ovariectomized female in a partner preference paradigm. Ovariectomized female rats were obtained from the vendor and were ready for test after a minimum of three weeks. Prior to the first day of behavior testing and on the test day itself, female rats were administrated with the vehicle (20% HPBCD, p.o.), TP (suspended in sesame oil, s.c.), or test compound (suspended in 20% HPBCD, p.o.) for seven days. The male rats were kept as usual without any treatment. All test female rats were primed with progesterone at 0.1 mg/kg s.c. 4 h before the test and 2 h after the last oral or TP dose to facilitate the full repertoire of sexual motivation in ovariectomized rats [41]. Under such circumstances, progesterone treatment in the absence of estrogen or TP does not lead to any sexual behavior enhancement or male preference for female rats.