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Preclinical pharmacology of FL442, a novel nonsteroidal androgen receptor modulator

Pekka K. Poutiainen [a], Tuulia Huhtala [b,c,1], Tiina Jääskeläinen [d,e], Aleksanteri Petsalo [a,f], Jenni Küblbeck [a], Sanna Kaikkonen [d], Jorma J. Palvimo [d], Hannu Raunio [a], Ale Närvänen [a,c], Mikael Peräkylä [d,2], Risto O. Juvonen [a], Paavo Honkakoski [a], Reino Laatikainen [a], Juha T. Pulkkinen [a,*]

[a] School of Pharmacy, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[b] A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[c] Biocenter Kuopio, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[d] Institute of Biomedicine, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[e] Institute of Dentistry, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[f] Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Oulu, P.O. Box 5000, FI-90014, Finland

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ABSTRACT

The preclinical profiles of two most potent compounds of our recently published cycloalkane[d]isoxazole pharmacophore-based androgen receptor (AR) modulators, FL442 (4-(3a,4,5,6,7,7a-hexahydro-benzo[*d*]isoxazol-3-yl)-2-(trifluoromethyl)benzonitrile) and its nitro analog FL425 (3-(4-nitro-3-(trifluoromethyl)phenyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]isoxazole), were explored to evaluate their druggability for the treatment of AR dependent prostate cancer. The studies revealed that both compounds are selective to AR over other closely related steroid hormone receptors and that FL442 exhibits equal inhibition efficiency towards the androgen-responsive LNCaP prostate cancer cell line as the most widely used antiandrogen bicalutamide and the more recently discovered enzalutamide. Notably, FL442 maintains antiandrogenic activity with enzalutamide-activated AR mutant F876L. In contrast to bicalutamide, FL442 does not stimulate the VCaP prostate cancer cells which express elevated levels of the AR. Distribution analyses showed that [^{14}CN]FL442 accumulates strongly in the mouse prostate. In spite of its low plasma concentration obtained by intraperitoneal administration, FL442 significantly inhibited LNCaP xenograft tumor growth. These findings provide a preclinical proof for FL442 as a promising AR targeted candidate for a further optimization.

1. Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in Western countries (Jemal et al., 2011). Besides its normal functions, the androgen receptor (AR) is a key factor in development and progression of PCa (Gao et al., 2005; Mooradian et al., 1987; Heinlein and Chang, 2004). Usually PCa is treatable by androgen deprivation therapy (ADT), during which endogenous androgens are depleted by GnRH agonists or antagonist and AR activation by residual ligands testosterone and dihydrotestosterone (Fig. 1), is prevented by antiandrogens (Scher et al., 2010; Labrie et al., 1982; Klotz, 2008; Haendler and Cleve, 2011). Pharmacological activity of the antiandrogens is determined by their binding properties to AR's ligand-binding site in the ligand-binding domain (LBD) Tamura et al., 2006. Agonist binding to the LBD induces a conformational change in C-terminal helix 12, which allows the formation of activation function 2 (AF2) in the

Abbreviations: ADT, androgen-deprivation therapy; AF2, activation function 2; AhR, aryl hydrocarbon receptor; AR, androgen receptor; Bic, bicalutamide; CAR, constitutive androstane receptor; COS-1, African green monkey kidney cells; CRPC, castration resistant prostate cancer; DHT, 5α-dihydrotestosterone; DMEM, Dulbecco's modified Eagle medium; ER, estrogen receptor; FBS, fetal bovine serum; FLuc, firefly luciferase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GR, glucocorticoid receptor; hAR, human androgen receptor; LNCaP, lymph node carcinoma of the prostate; N, number; N.D., not detected; NR, nuclear receptor; NSB, nonspecific binding; p, probability value of statistical significance; R1881, 17β-17-hydroxy-17-methyl-estra-4,9,11-trien-3-one; PCa, prostate cancer; PR, progesterone receptor; PXR, pregnane X receptor; RBI, relative binding inhibition; SCID, severe combined immunodeficiency; SD, standard deviation; SEM, standard error of mean; SHR, steroid hormone receptor; VCaP, vertebral cancer of the prostate.

* Corresponding author. Tel.: +358 40 3553247.
E-mail address: juha.t.pulkkinen@uef.fi (J.T. Pulkkinen).

[1] Present address: Charles River Laboratories International Inc., Discovery Research Services Finland, Microkatu 1, FI-70210 Kuopio, Finland.

[2] Present address: School of Pharmacy, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland.

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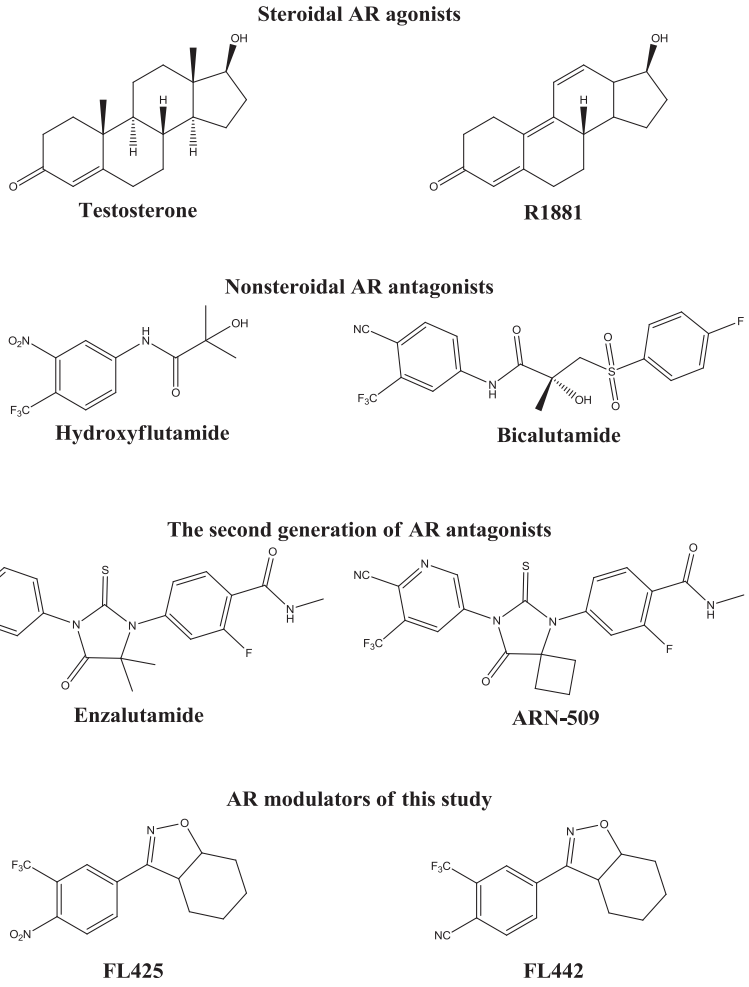

Fig. 1. Structures of androgen receptor modulators.

LBD, leading to its interaction with the AR N-terminal transactivation domain (Langley et al., 1995). In addition, the AF2 site conformation is essential for coactivator binding and thereby to the ability of the receptor to activate its target genes (Gao et al., 2005; van de Wijngaart et al., 2012; Hur et al., 2004). The first generation (relatively low affinity) antiandrogens, such as bicalutamide, appear to inhibit the AR activity by inducing partial unfolding of the AR. Moreover, bicalutamide can disturb coactivator binding to the AR (Bohl et al., 2005; Karvonen et al., 2002).

Unfortunately, anti-androgens such as bicalutamide and hydroxyflutamide, tend to lose their ability to inhibit the AR during hormone deprivation therapy, and this is associated with intracrine androgen synthesis (castration resistant prostate cancer, CRPC) (Chang, 2012). This may be associated with mutations in the AR LBD and the AR gene amplification, causing an antagonist-to-agonist switch in the receptor function, to which increased mitogen-activated protein kinase signaling may contribute (Bohl et al., 2005; Karvonen et al., 2002; Chang, 2012; Chen et al., 2004, 2009; Buchanan et al., 2001; Wilding and Chen, 1989; Taplin, 2007; Tannock et al., 2004; Petrylak et al., 2004). Therefore, new antiandrogens with improved drug properties are needed for improved treatment and better overall survival of PCa patients (Gao et al., 2005; Labrie et al., 1982; Klotz, 2008; Chen et al., 2009). New structures may offer alternative solutions to target the AR, since the variety of chemical scaffolds seen in previous antiandrogenic drug candidates have been quite limited (Haendler and Cleve, 2011). We have recently reported the synthesis, *in vitro* binding properties and inhibition efficacy on wild-type AR, as well as on its W741L and T877A mutants by novel competitive AR modulators containing a cycloalkene[*d*]isoxazole scaffold (Poutiainen et al., 2012) which represents a new type of AR pharmacophore.

In this paper, we report further *in vitro* and *in vivo* evaluation of the druggability of 4-(3a,4,5,6,7,7a-hexahydrobenzo[*d*]isoxazol-3-yl)-2-(trifluoromethyl)benzonitrile (FL442) as an AR modulator. First, we characterized the selectivity of FL442 and its nitroanalog FL425 towards other closely related steroid hormone receptors (SHRs), including estrogen receptor (ER), glucocorticoid receptor (GR) and progesterone receptor (PR). The activation of xenosensor receptors was studied to predict unwanted drug–drug interactions. In addition, the ability of the lead compound to prevent PCa cell proliferation and the AR target gene expression was studied *in vitro*. Second, the metabolic stability and the main metabolites of the most promising compound (FL442) were assessed. Finally, *in vivo* distribution experiments (using the radioligand [^{14}CN]FL442 synthesized for this purpose) and tumor growth suppression experiments were performed in mice in order to evaluate the druggability of the lead compound as a new PCa drug.

2. Materials and methods

2.1. Preparation of the compounds

Both compounds were synthesized, purified and characterized according to our previous publication (Poutiainen et al., 2012). For FL425, the method was modified in such a way that the