European Journal of Medicinal Chemistry 118 (2016) 230–243

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design and synthesis of novel bicalutamide and enzalutamide derivatives as antiproliferative agents for the treatment of prostate cancer

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form
20 April 2016
Accepted 21 April 2016
Available online 22 April 2016
This work is dedicated to the memory of Prof. Chris McGuigan, a great colleague and scientist, invaluable source of inspiration and love for research.

Keywords:
Bicalutamide
Enzalutamide
Prostate cancer
Perfluoroalkyl
Androgen receptor
Antiproliferative activity

ABSTRACT

Prostate cancer (PC) is one of the major causes of male death worldwide and the development of new and more potent anti-PC compounds is a constant requirement. Among the current treatments, (*R*)-bicalutamide and enzalutamide are non-steroidal androgen receptor antagonist drugs approved also in the case of castration-resistant forms. Both these drugs present a moderate antiproliferative activity and their use is limited due to the development of resistant mutants of their biological target.

Insertion of fluorinated and perfluorinated groups in biologically active compounds is a current trend in medicinal chemistry, applied to improve their efficacy and stability profiles. As a means to obtain such effects, different modifications with perfluoro groups were rationally designed on the bicalutamide and enzalutamide structures, leading to the synthesis of a series of new antiproliferative compounds. Several new analogues displayed improved *in vitro* activity towards four different prostate cancer cell lines, while maintaining full AR antagonism and therefore representing promising leads for further development.

Furthermore, a series of molecular modelling studies were performed on the AR antagonist conformation, providing useful insights on potential protein-ligand interactions.

1. Introduction

Prostate cancer (PC) is a leading cause of male death worldwide and it is the most frequently diagnosed cancer among men aged 65–74 [1]. The prognosis varies greatly, being highly dependent on a number of factors such as stage of diagnosis, race and age. Currently, PC treatment includes androgen deprivation, surgery, radiation, endocrine therapy and radical prostatectomy.

PC cell growth is strongly dependent on androgens, therefore blocking their effect can be beneficial to the patient's health. Such outcomes can be achieved by antagonism of the androgen receptor (AR) using anti-androgen drugs, which have been extensively explored either alone or in combination with castration [2]. Flutamide (Eulexin®) (**1**) (in its active form as hydroxyflutamide (**2**)), bicalutamide (Casodex®) (**3**), nilutamide (Niladron®) (**4**) and enzalutamide (previously called MDV3100) (Xtandi®) (**5**) are all non-steroidal androgen receptor antagonists approved for the treatment of PC (Fig. 1). In many cases, after extended treatment over several years, these anti-androgens become ineffective and the disease may progress to a more aggressive and lethal form, known as castration resistant prostate cancer (CRPC). The major cause of this progressive disease is the emergence of different mutations on the AR, which cause the anti-androgen compounds to function as agonists, making them tumour-stimulating agents [3].

Among the drugs used for the treatment of PC, bicalutamide and enzalutamide selectively block the action of androgens while presenting fewer side effects in comparison with other AR antagonists [4–6]. The structure of these molecules is characterised by the presence of a trifluoromethyl substituted anilide, which appears to be critical for biological activity (Fig. 1). As a means to improve the anti-proliferative activity of these compounds, and in order to exploit the well established potential of the fluorine atom in enhancing the pharmacological properties and drug-like

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http://dx.doi.org/10.1016/j.ejmech.2016.04.052

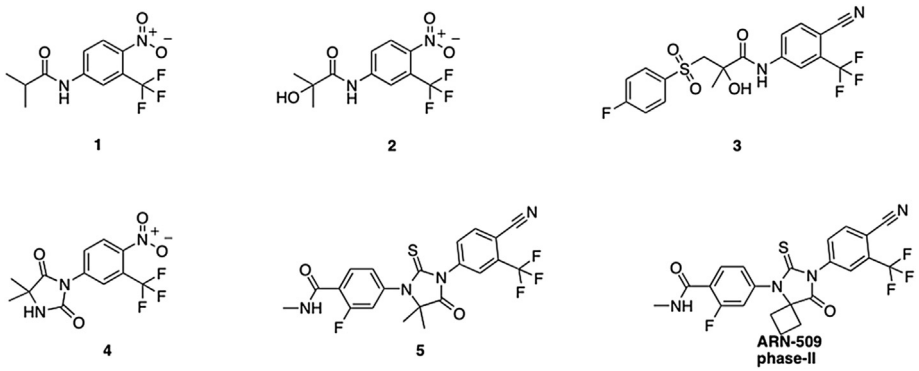

Fig. 1. Structure of anti-androgen small molecules approved by FDA or in clinical development for the treatment of PC.

physicochemical characteristics of candidate compounds [7–9], a wide array of diverse new structures has been rationally designed and synthesised, through the introduction of fluoro-, trifluoromethyl- and trifluoromethoxy groups in diverse positions of both aromatic rings of the parent scaffolds. Our modifications resulted in a marked improvement of *in vitro* anti-proliferative activities on a range of human PC cell lines (VCap, LNCaP, DU-145 and 22RV1). In addition, we probed full versus partial AR antagonism for our new compounds.

2. Results and discussion

Trifluoromethyl and trifluoromethoxy functions were systematically inserted on both aromatic rings of bicalutamide and enzalutamide, with the aim to explore the effect of perfluoro groups on their biological activity. As a means to further expand structure-activity relationship studies, the insertion of different linkers was also envisaged, along with the replacement of the bicalutamide methyl group with a trifluoromethyl function.

The main proposed modifications are summarised in Fig. 2.

2.1. Chemistry

Several reported methods for the synthesis of racemic bicalutamide were explored to find a rapid methodology that would allow the preparation of a wide range of new derivatives [10–12]. Modification and optimization of these procedures led to the development of the synthetic pathway shown in Scheme 1. Phenylacrylamides **12**–**16** were prepared by reacting the corresponding aniline (**7**–**11**) with methacryloyl chloride (**6**) in dimethylacetamide (DMA), modifying a reported methodology [10]. In particular, due to the presence of electron withdrawing groups (nitro, cyano, trifluoromethyl) in different positions, anilines **7**–**11** were in some cases (**9** and **10** in particular) of low reactivity towards nucleophilic displacement. Synthetic efforts were made to achieve good yields (Supplementary data). Phenylacrylamides **12**–**16** were converted into the corresponding epoxides **17**–**21** in the presence of a large excess of hydrogen peroxide and trifluoroacetic anhydride in dichloromethane [11]. Opening of the epoxide rings of **17**–**21** with commercially available phenols and thiophenols gave a series of ethers (**27**–**31**) and thioether derivatives (**22**–**26**), respectively, in good yields after purification by column chromatography [11]. Thioethers **22**–**25** were finally oxidised to the corresponding sulfones **32**–**35** using mCPBA, maintaining the temperature at 25 °C [12]. Racemic bicalutamide (**3**) was prepared as a positive control following this route.

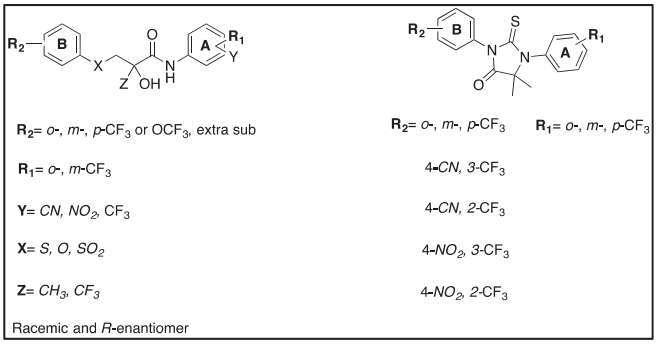

Fig. 2. Proposed modifications on bicalutamide and enzalutamide scaffolds.

Since *R*-bicalutamide is known to be the most active enantiomer [13], chiral synthesis of selected *R*-bicalutamide analogues was carried out as shown in Scheme 2. (*R*)-*N-Methacryloylproline* **37**, prepared using (*R*)-proline (**36**) and methacryloyl chloride (**6**), was reacted with N-bromosuccinimide in DMF to afford bromolactone **38** as a single enantiomer [14]. Acid hydrolysis of **38** resulted in the formation of bromohydrin acid **39**, which was then converted into the corresponding chiral anilide (**40**–**41**) [14]. Amide derivatives **40**–**41** were reacted with the sodium salt of different commercial thiophenols in tetrahydrofuran to give, after purification by silica gel chromatography, the desired (*R*)-thioethers (**42**–**44**) [15]. Reaction of amide **40** with the sodium salt of 4-cyanophenol gave the reference compound (*S*)-enobosarm (**44e**). Oxidation of thioethers **42**–**43** with mCPBA provided sulfones **45**–**46**, with the desired *R* absolute configuration [12]. Reference (*R*)-bicalutamide (**45a**) was prepared following this synthetic route.

For one of the most active compounds initially found, **23d**, replacement of the central methyl group with an extra trifluoromethyl function was planned and carried out (Scheme 3).

3-Bromo-1,1,1-trifluoroacetone (**48**) was coupled with thiophenol **47** to afford **49**, which was then converted into cyano derivative **50** using potassium cyanide and 25% sulfuric acid [16]. Intermediate **51** was obtained after refluxing **50** in concentrated HCl and glacial acetic acid. Coupling of **51** with commercially available 4-nitro-3-(trifluoromethyl)aniline **8** yielded the desired amide **52**.

Several methods have been reported for the preparation of enzalutamide, all showing as a synthetic challenge the formation of the *N*-substituted thiohydantoin ring [17–19]. The method selected in this study was a three-step synthesis involving the preparation of different isothiocyanates (**54**–**58**), obtained in quantitative yield after treating the corresponding aniline (**7**–**10**, **53**) with thiophosgene (Scheme 4) [17]. Reaction conditions were optimised for the different anilines used, with **9** and **10** requiring higher