Bioorganic & Medicinal Chemistry 26 (2018) 1481–1487

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis, and evaluation of the antiproliferative activity of hydantoin-derived antiandrogen-genistein conjugates

Alex George [a,e], Idris Raji [a,e], Bekir Cinar [d], Omer Kucuk [b,*], Adegboyega K. Oyelere [a,c,*]

[a] School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[b] Winship Cancer Institute of Emory University, Atlanta, GA 30322, USA
[c] Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[d] Department of Biological Sciences, The Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA 30314, USA

ARTICLE INFO

Article history:
Received 8 November 2017
Revised 4 January 2018
Accepted 12 January 2018
Available online 16 February 2018

Keywords:
Prostate cancer
Genistein
Androgen receptor
Antiandrogen
LNCaP
DU145

ABSTRACT

Androgen receptor (AR) signaling is vital to the viability of all forms of prostate cancer (PCa). With the goal of investigating the effect of simultaneous inhibition and depletion of AR on viability of PCa cells, we designed, synthesized and characterized the bioactivities of bifunctional agents which incorporate the independent cancer killing properties of an antiandrogen and genistein, and the AR downregulation effect of genistein within a single molecular template. We observed that a representative conjugate, **9b**, is much more cytotoxic to both LNCaP and DU145 cells relative to the antiandrogen and genistein building blocks as single agents or their combination. Moreover, conjugate **9b** more effectively down regulates cellular AR protein levels relative to genistein and induces S phase cell cycle arrest. The promising bioactivities of these conjugates warrant further investigation.

Published by Elsevier Ltd.

1. Introduction

Among American men, prostate cancer (PCa) is the second most leading cause of cancer-related death and accounts for nearly 240,000 new cases each year.[1,2] Androgen receptor (AR) expression has been shown by numerous studies to be vital in PCa progression.[3] However, AR is also involved in the normal function of the prostate and other tissues.[4] In its unbound state, AR is a steroid hormone receptor found in the cytoplasm associated with heat shock proteins (HSP-90), cytoskeletal proteins, and other chaperones.[5] After binding to one of its natural ligands (dihydrotestosterone (DHT) or testosterone), AR undergoes a conformational change which results in homodimerization, followed by nuclear translocation, DNA binding, and the transcription of AR regulated genes.[6]

For patients with advanced hormone-sensitive PCa, androgen deprivation therapy (ADT) is often employed as a complementary therapy, in the form of antiandrogens (Fig. 1A), to luteinizing hormone-releasing hormone (LHRH) agonists. While most patients respond well to ADT, many of these patients will become refractory to treatment and develop castration resistant PCa (CRPC).[7] The importance of AR expression in CRPC progression is clear as AR expression is nearly 6-fold higher in CRPC compared to hormone-sensitive PCa.[8] As such, second line therapies such as enzalutamide and abiraterone are employed to treat CRPC by targeting AR regulation/signaling and androgen synthesis respectively.[9–11]

Genistein (Fig. 1B), a natural soy isoflavone, is among the most potent phytoestrogens to have shown beneficial antitumor activity.[12] Genistein has the potential to become a powerful therapeutic against PCa due to many properties that work in concert to exhibit anti-proliferative activity in cancer cells.[12] Several studies have shown that genistein elicits pleiotropic effects, inhibiting and/or downregulating several cancer-relevant targets within the cell. Among the targets whose inhibition and/or downregulation has been implicated in the anti-proliferative activities of genistein include: tyrosine receptor kinases (TRKs), the TRK signal transduction pathway (TRK → Raf → MEK → ERK/p38), mitogen activated protein kinase signaling pathways (MAPKs)[13,14], NF-kB[15,16], Akt signaling[16], human telomerase reverse transcriptase (hTERT)[17], vascular endothelial growth factor (VEGF), platelet-derived growth factor, matrix metalloprotease-2 and -9 (MMP 2 and 9)[18], and angiogenesis. Furthermore, genistein increases the expression of several histone acetyltransferases (HATs) in LNCaP and DuPro

* Corresponding authors at: School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA (A.K. Oyelere).
E-mail addresses: omer.kucuk@emory.edu (O. Kucuk), aoyelere@gatech.edu (A.K. Oyelere).
[e] These authors contributed equally to the manuscript.

https://doi.org/10.1016/j.bmc.2018.01.009
0968-0896/Published by Elsevier Ltd.

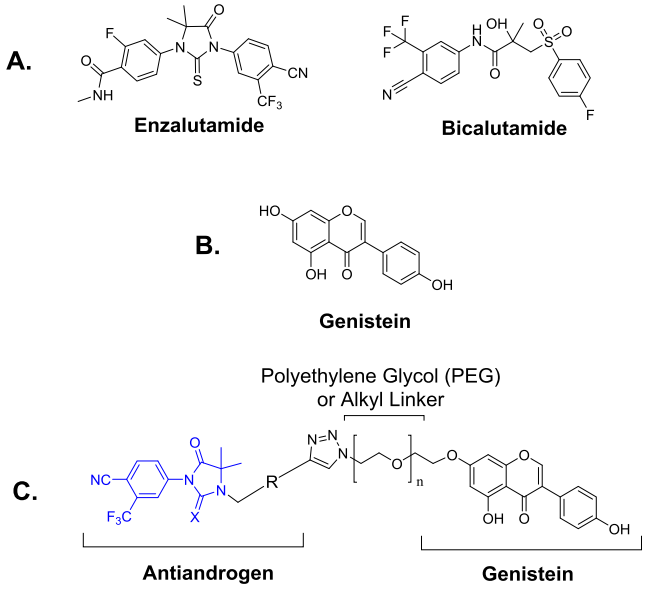

Fig. 1. (A) Structures of representative FDA approved antiandrogens, (B) Structure of genistein, (C) Design of thio/hydantoin -derived antiandrogen-genistein conjugates (X = O or S).

PCa cell lines as well as normal prostate epithelial cells.[19] The increased expression of HATs allows for the increased acetylation of histones H3 and H4 which increases the transcription of p21 and p16, genes that induce cell cycle arrest and apoptosis.[19] Also, increased H3K9 acetylation by genistein caused the re-expression of important tumor suppressor genes such as PTEN, p53, CYLD and FOXO3a.[20]

Genistein also exerts antiestrogenic and AR modulation activities. In PCa cells, genistein has been shown to decrease AR protein levels and AR-mediated transcriptional activation of prostate specific antigen (PSA) in androgen-dependent cells.[21,22] Mechanistically, it has been shown that the cellular reduction in AR protein induced by genistein in PCa cell is due to the estrogenic activity of genistein which causes downregulation of HDAC6 and a concomitant inhibition of the chaperone function of HSP90, an activity that is essential for the stability of AR protein.[23] The impressive bioactivities of genistein strongly support epidemiological studies which have demonstrated a close association between the intake of soy products rich in genistein and daidzein and the reduction in prostate cancer risk.[24] However, other reports suggest that the effect of genistein is highly dependent on the mutational status of the AR. Genistein elicits a biphasic effect in LNCaP cells expressing T877A mutant AR, stimulating cell proliferation, AR expression, and transcriptional activity at physiologically attainable concentrations while inducing antiproliferative activities at higher doses.[25,26]

Since AR signaling is vital to the viability of all forms of PCa including CRPC, we postulate that simultaneous inhibition and depletion of AR could prove a beneficial therapeutic strategy for all stage of AR dependent PCa. In this study, we disclose bifunctional agents designed to incorporate the independent cancer killing properties of an antiandrogen and genistein, and the AR downregulation effect of genistein within a single molecular template. We observed that a representative bifunctional agent **9b** is much more cytotoxic to LNCaP and DU145 cells relative to the antiandrogen and genistein building blocks as single agents or their combination. Moreover, **9b** more effectively down regulates AR protein cellular levels relative to genistein and induces S phase cell cycle arrest.

2. Results and discussion

2.1. Conjugate design and synthesis

We have shown that the linkage of aryl hydantoin- and aryl thiohydantoin-based antiandrogens through a short PEG linker to plasmonic gold nanoparticles resulted in nanoconjugates with enhanced antiandrogen activities.[27] We envisioned that a similar linkage of the antiandrogen to the *O*-7 position of genistein, a moiety whose modification is compatible with the bioactivity of genistein, will furnish conjugates (Fig. 1C) possessing AR inhibition and depletion activities.[28–30]

The synthesis of the requisite antiandrogen-genistein conjugates was achieved using a flexible synthetic route (Scheme 1) which enabled variation of the linker lengths and antiandrogen templates in order test the effects of these changes on the bioactivities of the conjugates. The precursors for the reaction – tetrabutylammonium salt of genistein **1**, 2-azidoethyl 4-methylbenzenesulfonate **2,** PEG-Tosyl-Azide **4** and thio/hydantoin **6–8** – were prepared as previously reported in the literature.[27,31–35] Genistein-alkyl-azide **3** and genistein-PEG-azide **5** intermediates were made via the condensation of **1** with **2** and **4,** respectively. Subsequent Cu(I) catalyzed Huisgen cycloaddition reactions[36] between the azides **(3** and **5)** and the thio/hydantoin **6–8** yielded the desired conjugates **9a-b**, **10a-b**, and **11a-b** (Scheme 1).

2.2. In vitro anti-proliferative activity study

The antiproliferative potential of the synthesized compounds was first evaluated in representative androgen dependent LNCaP and androgen independent DU145 PCa cells lines. A screen of our compounds in LNCaP and DU145 identified **9b** as the most potent among the conjugates synthesized while others do not show appreciable activity (data not shown). Importantly, **9b** was more potent than the antiandrogen and genistein building blocks, either as single agents or their combination. Specifically, genistein exhibited an IC_{50} of 24.0 ± 0.9 μM in LNCaP cells and a combination of enzalutamide and genistein did not significantly improve upon the IC_{50} of genistein, showing IC_{50} of 31.7 ± 0.8 μM (Fig. 2a and b). The inhibition profile obtained for genistein in LNCaP is within the range of IC_{50} values (10–40 μM) reported in the literature.[37,38] In contrast, conjugate (**9b)** was about 16-fold more potent than genistein, inhibiting the proliferation of LNCaP with an IC_{50} of 1.4 ± 0.9 μM (Fig. 2c). While genistein and its combination with enzalutamide showed a dose-dependent activity in DU145 cells, 50% inhibition was not obtained within the tested concentration range and we could not calculate the IC_{50} values (Fig. 2d and e). Gratifyingly, conjugate **9b** still exhibited antiproliferative activity against DU145 cells with IC_{50} of 3.4 ± 0.9 μM (Fig. 2f).

We observed that the percent of LNCaP cell survival for the combination of genistein and enzalutamide hovered around 40% even at concentrations as high as 100 μM. Interestingly, conjugate **9b** induced a similar effect in both LNCaP and DU145 cells at the low concentration range that enabled IC_{50} measurement. We reasoned that exposure to higher concentration of **9b** will further reduce cell viability below 40%. However, we noticed that higher concentrations of **9b** (>20 μM) resulted in aggregate formation and precipitation of the compound out of the culture media in both LNCaP and DU145. This solubility problem seems to paradoxically blunt the antiproliferative effects of **9b** against LNCaP at much higher concentrations while this effect was less pronounced in DU145 cells (Fig. S1).

Subsequently, we tested the effects of genistein, enzalutamide and compound **9b** on other PCa cell lines – PC3, C4-2 and 22Rv1