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Screening baccharin analogs as selective inhibitors against type 5 17β-hydroxysteroid dehydrogenase (AKR1C3)

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ABSTRACT

Aldo–keto reductase 1C3 (AKR1C3), also known as type 5 17β-hydroxysteroid dehydrogenase, is a downstream steroidogenic enzyme and converts androgen precursors to the potent androgen receptor ligands: testosterone and 5α-dihydrotestosterone. Studies have shown that AKR1C3 is involved in the development of castration resistant prostate cancer (CRPC) and that it is a rational drug target for the treatment of CRPC. Baccharin, a component of Brazilian propolis, has been observed to exhibit a high inhibitory potency and selectivity for AKR1C3 over other AKR1C isoforms and is a promising lead compound for developing more potent and selective inhibitors. Here, we report the screening of fifteen baccharin analogs as selective inhibitors against AKR1C3 versus AKR1C2 (type 3 3α-hydroxysteroid dehydrogenase). Among these analogs, the inhibitory activity and selectivity of thirteen compounds were evaluated for the first time. The substitution of the 4-dihydrocinnamoyloxy group of baccharin by an acetate group displayed nanomolar inhibitory potency (IC_{50}: 440 nM) and a 102-fold selectivity over AKR1C2. By contrast, when the cinnamic acid group of baccharin was esterified, there was a dramatic decrease in potency and selectivity for AKR1C3 in comparison to baccharin. Low or sub-micromolar inhibition was observed when the 3-prenyl group of baccharin was removed, and the selectivity over AKR1C2 was low. Although unsubstituted baccharin was still the most potent (IC_{50}: 100 nM) and selective inhibitor for AKR1C3, these data provide structure–activity relationships required for the optimization of new baccharin analogs. They suggest that the carboxylate group on cinnamic acid, the prenyl group, and either retention of 4-dihydrocinnamoyloxy group or acetate substituent on cinnamic acid are important to maintain the high potency and selectivity for AKR1C3.

1. Introduction

Prostate cancer (CaP) is the most commonly diagnosed cancer and the second leading cause of cancer death in males in the United States [1]. The development of prostate cancer is androgen-dependent, and androgen deprivation therapy using surgical or chemical castration has been a main treatment for locally advanced or metastatic disease [2,3]. However, within 2–3 years, the recurrence of CaP, also called castration resistant prostate cancer (CRPC), occurs despite castrate levels of circulating androgens (e.g. testosterone (T)), and has the potential to become more metastatic [4,5]. Pathophysiological studies have shown that CRPC remains mainly androgen-driven, and can arise when the transcriptional activity of the androgen receptor (AR) is reactivated by the intratumoral conversion of weak adrenal androgens (e.g. dehydroepiandrosterone (DHEA) and 4-androstene-3,17-dione) into the potent AR ligands: T and 5α-dihydrotestosterone (DHT), and/or by androgen receptor amplification [5–7]. Thus, new therapeutics targeting AR signaling (either intratumoral androgen biosynthetic enzymes or AR) for the treatment of CRPC are being developed and tested in clinical trials [6,7].

Recently, abiraterone acetate which inhibits the activities of cytochrome P450c 17 (17α-hydroxylase/17,20 lyase, Fig. 1) blocks the formation of adrenal DHEA, improves overall survival in CRPC

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patients and has been approved by the FDA [8,9]. However, P450c 17 catalyzes an early-step in steroidogenesis, and the inhibition of its hydroxylase activity leads to a loss in cortisol production and over stimulation of the gland by ACTH. This can lead to an increase in levels of mineralocorticoids which can cause serious adverse side effects such as hypertension. Consequently, patients taking abiraterone must be co-administered prednisone [10]. In addition, resistance to abiraterone has been reported due to an elevated expression level of *CYP17A1* [11,12]. Therefore, new molecular targets in the AR signaling pathway have been investigated to discover superior therapeutic agents [6,13].

Aldo–keto reductase 1C3 (AKR1C3) also known as type 5 17β-hydroxysteroid dehydrogenase in the prostate, converts 4-androstene-3,17-dione and 5α-androstane-3,17-dione to T and DHT respectively which are potent ligands for the AR (Fig. 1) [14,15]. AKR1C3 is overexpressed at both the mRNA and protein levels in prostate tumors from CRPC patients [16–19]. Reduction of AKR1C3 expression levels in CaP cells or inhibition of AKR1C3 activity significantly decreases the levels of T and DHT and androgen dependent gene expression e.g. prostate specific antigen (PSA). *In vivo* inhibition of AKR1C3 leads to a reduction in growth of xenograft models of CRPC [11,19–21]. More recently, AKR1C3 was found to act as an AR coactivator which would provide an alternative mechanism by which it may promote the growth of prostate cancer cells and CRPC xenografts [21]. These findings have made AKR1C3 a promising therapeutic target for both androgen-dependent CaP and CRPC [13,15,19]. It has been proposed that inhibition of AKR1C3 might not be therapeutically efficacious based on insignificant changes in T levels in CaP cells after the treatment with the AKR1C3 inhibitor SN33638 [22]. However, data to support this notion may be circumspect because of the specificity of antibodies used in Western blot analysis, the reliance of ELISA measurements to quantitate androgens, and the maintenance of cancer cell lines in a fetal bovine serum (FBS) or fetal calf serum (FCS) media containing androgens which will suppress AKR1C3 expression [17,18].

Significant efforts have been made by our group and others to discover and develop different classes of AKR1C3 inhibitors, including steroidal based compounds (e.g. medroxyprogesterone acetate) and repurposed nonsteroidal anti-inflammatory drugs (NSAIDs) which no longer inhibit COX-1 and COX-2 [15,23–27]. One of the most important considerations in inhibitor development is to ensure that they do not inhibit other AKR1C isoforms (AKR1C1 and AKR1C2 in Fig. 1). AKR1C1 and AKR1C2 share >86% sequence identity to AKR1C3 but inactivate DHT. Thus, screening for AKR1C3-selective inhibitors is imperative [6,28,29].

Baccharin (**1**, Table 1), a naturally occurring phenolic compound extracted from Brazilian propolis which is a resinous gum collected by bees from the plant exudate, has been commonly used in medicine and nutritional products and exhibits anti-tumor and anti-inflammatory activities [30–36]. Its ability to inhibit AKR1C3 with an IC_{50} value of 110 nM and without affecting other AKR1C isoforms has been reported [37]. In order to discover more potent and selective inhibitors and better understand the structure–activity relationship of how baccharin analogs inhibit AKR1C3, we tested the inhibitory activities of 15 analogs, thirteen of which represent new analogs for screening.

2. Materials and methods

2.1. Reagents

Reagents were of ACS grade or higher and were purchased from Fisher Scientific (Pittsburgh, PA) and used without further purification. (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol (*S*-tetralol) was purchased from Sigma–Aldrich (St. Louis, MO). Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were purchased from Roche Diagnostics (Indianapolis, IN). Homogeneous recombinant enzymes AKR1C3 and AKR1C2 were prepared and purified as previously described [28]. The specific activities of AKR1C3 and AKR1C2 for the NAD^+ dependent oxidation of *S*-tetralol were 2.0 and 1.5 μmol min^{-1} mg^{-1}, respectively.

2.2. Chemistry

Baccharin (**1**) and compounds **2**, **3**, **4**, **6**, and **7** were synthesized using a modified literature procedure [38]. The general scheme for the synthetic procedure is shown in Scheme 1. Compounds **14** (*p*-coumaric acid), **15** (*m*-coumaric acid), and **16** (ferulic acid) were obtained from Sigma–Aldrich (Milwaukee, WI) and were used without further purification. Reaction progress was monitored by thin-layer chromatography (TLC) carried out on silica gel plates (2.5 cm × 7.5 cm, 200 μm thick, 60 F254) and visualized by using UV lamp (254 nm). Flash column chromatography was performed with silica gel (40–63 μm, 60 Å) using the mobile phase indicated in the following procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded in the indicated solvent by a Bruker 400 MHz Advance III HD spectrometer at 400 and 100 MHz for ^{1}H and ^{13}C respectively with TMS as an internal standard. Multiplicities are indicated by s (single), d (doublet), dd (doublet of doublets) t (triplet), q (quartet), m (multiplet). Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz).

4-iodo-2-(3-methylbut-2-en-1-yl)phenol (I-A): To a solution of *p*-iodophenol (2 g, 9.08 mmol) in toluene (20 mL) was added NaH (60% dispersion in mineral oil, 540 mg, 13.62 mmol). When the effervescence ceased, 3,3-dimethylallyl bromide (1.1 mL, 9.5 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was acidified to pH 1 using AcOH, washed with H_2O, extracted with DCM, dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (5–10% of Et_2O in hexane, v:v) provided the title compound as a yellow oil (1.43 g, 4.96 mmol, 55%). R_f: 0.5 (4:1 hexane:EtOAc, v:v).

^{1}H NMR (400 MHz; $CDCl_3$): δ 1.8 (6H, d, J = 8.0 Hz, CH_3), 3.33 (2H, d, J = 6.88 Hz, CH_2), 5.31 (1H, t, J = 6.6 Hz, CH), 5.46 (1H, s, —OH), 6.59 (1H, d, J = 8.2 Hz, Ar—H), 7.41 (2H, s, Ar—H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 17.8, 25.8, 29.3, 82.7, 117.9, 120.7, 129.7, 135.4, 136.1, 138.3, 154.2.

4-iodo-2-(3-methylbut-2-en-1-yl)phenyl-3-phenylpropanoate (I-B): To a solution of 4-iodo-2-(3-methylbut-2-en-1-yl)phenol (560 mg, 1.94 mmol) in DCM (4 mL) was added DMAP (25 mg, 0.2 mmol) followed by addition of 3-phenyl propionyl chloride (495 mg, 2.93 mmol) and NEt_3 (0.4 mL, 2.91 mmol). The mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ was added and the layers were separated. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and concentrated to provide the title compound without further purification (490 mg, 1.16 mmol, 60%).

^{1}H NMR (400 MHz; $CDCl_3$): δ 1.66 (3H, s, CH_3), 1.76 (3H, s, CH_3), 2.91 (2H, t, J = 7.5 Hz, CH_2) 3.09 (4H, t, J = 6.0 Hz, CH_2CH_2), 5.16 (1H, t, J = 7.1 Hz, CH), 6.69 (1H, d, J = 8.3 Hz, ArCH), 7.21–7.36 (6H, m, ArCH), 7.53 (1H, s, ArCH). ^{13}C NMR (100 MHz; $CDCl_3$): δ 17.8, 25.9, 28.3, 30.2, 30.9, 35.7, 36.4, 90.5, 120.7, 124.4, 126.7, 128.4, 128.8, 133.9, 135.9, 136.3, 138.8, 140.0, 148.6, 171.0.

4-iodo-2-(3-methylbut-2-en-1-yl)phenyl acetate (I-C): To a solution of 4-iodo-2-(3-methylbut-2-en-1-yl)phenol (332 mg, 1.15 mmol) in DCM (4 mL) was added DMAP (14 mg, 0.1 mmol) followed by addition of acetyl chloride (0.122 mL, 1.7 mmol) and NEt_3 (0.236 mL, 1.7 mmol). The mixture was stirred overnight. Saturated $NaHCO_3$ was added and the layers were separated. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated *in vacuo* to provide the title compound (350 mg, 1.06 mmol, 92%).