European Journal of Medicinal Chemistry 44 (2009) 2434–2446

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Highly twisted adamantyl arotinoids: Synthesis, antiproliferative effects and RXR transactivation profiles

Santiago Pérez-Rodríguez [a], Maria A. Ortiz [b], Raquel Pereira [a], Fátima Rodríguez-Barrios [a], Ángel R. de Lera [a,*], F. Javier Piedrafita [b,**]

[a] Departamento de Química Orgánica, Universidade de Vigo, Lagoas-Marcosende, 36310 Vigo, Spain
[b] Sidney Kimmel Cancer Center, 10905 Road to the Cure, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 18 July 2008
Received in revised form
20 December 2008
Accepted 9 January 2009
Available online 20 January 2009

Keywords:
Adamantyl arotinoids
Retinoid-related molecules (RRMs)
RXR
Apoptosis
Synthesis

ABSTRACT

Retinoid-related molecules with an adamantyl group (adamantyl arotinoids) have been described with selective activities towards the retinoid receptors as agonists for NR1B2 and NR1B3 (RARβ,γ) (CD437, MX3350-1) or RAR antagonists (MX781) that induce growth arrest and apoptosis in cancer cells. Since these molecules induce apoptosis independently of RAR transactivation, we set up to synthesize novel analogs with impaired RAR binding. Here we describe adamantyl arotinoids with 2,2′-disubstituted biaryl rings prepared using the Suzuki coupling of the corresponding fragments. Those with cinnamic and naphthoic acid end groups showed significant antiproliferative activity in several cancer cell lines, and this effect correlated with the induction of apoptosis as measured by caspase activity. Strikingly, some of these compounds, whereas devoid of RAR binding capacity, were able to activate RXR.

1. Introduction

Vitamin A (retinol) and its derivatives, the retinoids, are essential regulators of many biological events including cell growth and differentiation, development, homeostasis and carcinogenesis. Because of their antiproliferative activity, retinoids have been proposed as cancer preventive and chemotherapeutic agents [1]. Retinoid signals are mediated by the retinoid receptors RARα, β, γ (NR1B1, 2, 3) and RXRα, β, γ (NR2B1, 2, 3) [2,3], which belong to the superfamily of nuclear hormone receptors [4]. These are ligand-activated transcription factors that bind to specific DNA sequences in the regulatory regions of target genes. In the absence of ligand, RAR/RXR heterodimers interact with co-repressors and bind DNA to inhibit transcription. Ligand binding induces a profound conformational change in the ligand binding domain of the receptors causing the dissociation of the co-repressor complex and the re-positioning of the activation function-2 located in helix H12. This exposes a LXXLL motif necessary for the recruitment of chromatin-modifying co-activators and the RNA polymerase II transcriptional machinery to initiate transcription (see [5,6] and references therein).

Despite their promising activity *in vitro*, most natural and synthetic retinoid analogs exhibit significant toxicity that has limited their oncological therapeutic use [7]. All-*trans*-retinoic acid (atRA) is used as a differentiation agent against acute promyelocytic leukaemia [8], whereas the synthetic RXR agonist LGD1069 (Targretin®) has been approved for the treatment of cutaneous T cell lymphoma [9] and is being clinically evaluated against lung and breast cancers [10]. Other retinoid analogs are currently under clinical evaluation with mixed results [1]. The development of novel retinoids with selectivity towards RXRs or the different RAR subtypes and isoforms has led to the intriguing discovery of a novel class of derivatives with strong pro-apoptotic activity [11]. Because they often exert their anticancer activity independently of the retinoid receptors, they are also known as atypical retinoids or retinoid-related molecules (RRMs). In particular CD437, a RARβ/γ-selective agonist and several analogs that contain an adamantyl group are classified as adamantyl arotinoids (AdArs). A small number of RRMs are known to induce apoptosis in cancer cells. These include anhydroretinol [12,13], 4-hydroxyphenylretinamide

Abbreviations: 9cRA, 9-*cis*-retinoic acid; AdAr, adamantyl arotinoid; atRA, all-*trans*-retinoic acid; IKK, IκB kinase; JNK, cJun N-terminal kinase; LBD, ligand binding domain; MAPK, mitogen activated protein kinase; NFκB, nuclear factor-κB; RAR, retinoic acid receptor; RRM, retinoid-related molecule; RXR, retinoid X receptor; SHP, short heterodimer partner.

* Corresponding author. Present address: Torrey Pines Institute for Molecular Studies, 3550 General Atomics Ct., San Diego, CA 92121, USA. Tel.: +34 986 812316; fax +34 986 811940.
** Corresponding author. Tel.: +1 858 597 3881; fax: +1 858 455 3804.
E-mail addresses: qolera@uvigo.es (Á.R. de Lera), jpiedrafita@tpims.org (F.J. Piedrafita).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.01.011

(4-HPR) [14], some heteroarotinoids [15], and a few AdAr (MX781 **9**, CD2325 **2** and their analogs, Fig. 1) [16].

CD437 **1** is the prototype of the RRM subfamily of RARβ/γ-selective AdAr agonists [11] that includes CD2325 **2**, MX2870-1 **3**, and MX3350-1 **4** (Fig. 1). CD437 **1** was first shown to induce growth arrest and apoptosis in breast cancer cells [17] and several AdAr were later found to inhibit growth of numerous cancer cell lines *in vitro* and in animal models (see [16] and references therein). Given that AdAr inhibit cell growth independently of RAR transactivation [18–20], great efforts have aimed at characterizing the mechanism of AdAr-induced apoptosis and, ultimately, at identifying AdAr targets that mediate their anticancer activity. Thus, CD437 **1**-induced apoptosis requires transcription/translation in a cell-type dependent manner [21–24] (reviewed in [16]), and caspases are activated via the mitochondrial pathway [22,25–27], although a role for death-receptor signalling has also been suggested with some compounds [28–30]. CD437 **1** and AdAr alike cause a strong and sustained activation of JNK and p38 stress kinases that precedes the release of cytochrome c and subsequent induction of apoptosis [31]; however, contrasting results have been reported by different laboratories using a variety of kinase inhibitors and cell lines [32–36]. In contrast to the activation of JNK/p38 MAPKs, certain apoptotic AdAr target the IKK/NFκB signalling pathway [20], which evokes survival signals [37]. MX781 **9** and CD2325 **2** significantly inhibited kinase activity of immunopurified IKK complex *in vitro* [20]; furthermore, using purified recombinant kinases we have recently proved that MX781 **9** is a selective inhibitor of IKKβ and several analogs have been prepared with enhanced anti-IKKβ and growth inhibitory activities [38]. Our findings disagree with recent reports indicating that CD437 **1** and its analog 3-Cl-AHPC **7** induced apoptosis via activation of NFκB [39,40].

Second generation AdAr have been described with improved anticancer activity [36,41–43]. Cinnamic acid derivative ST1926 (AHPC) **6** activates RARγ and induces apoptosis in various cancer cell lines with stronger potency as compared to CD437 **1** [44,45]. Derivatives of CD437 **1** lacking RAR transactivation activity, most notably 3-Cl-AHPN (MM11453) [41], 3-Cl-AHPC **7** [43,46], and 5-Cl-AHPN **5** [47], also elicit anticancer activity comparable to the parent compound, whereas derivative 3-A-AHPC **8** prevented the induction of apoptosis by CD437 **1** analogs but did not inhibit their effect on cell cycle [47]. 3-Cl-AHPC **7** is an AdAr with cinnamic acid substructure (Fig. 1) that induces cell-cycle arrest and apoptosis in several cancer cell lines. Induction of apoptosis by 3-Cl-AHPC **7** and some of its analogs was later shown to occur through binding to the nuclear receptor SHP (small heterodimer partner, NR0B2) [48].

With the exception of IKKβ [20,38] and SHP [48], the cellular targets that mediate the anticancer activity of these AdAr are largely unknown, which represents a significant drawback for the drug development efforts. Existing SAR studies of the RRM family of compounds have shown the important synergistic role of the adamantyl and phenol groups on RAR binding selectivity [49]. Moreover, the bulky adamantyl group appears to be necessary for anticancer activity but not sufficient, since several other AdAr exhibit low or moderate activity. The carboxylic acid might play a role in apoptosis because its replacement by other bioisosters and related groups led to reduction or loss of activity [50].

Previous docking studies in the RARγ LBD of 5-Cl-AHPN **5**, 3-Cl-AHPC **7** and analogs have revealed that the steric clash of the substituents located *ortho* to the biaryl bond (chloro, 3-acetamidopropoxy of **8**) induce a twist of that bond that displaces the adamantyl fragment from the coplanarity with the aromatic rings of their polar termini (naphthoic acid of CD437 or cinnamic acid of AHPC) [47]. As a consequence, the position of helix H12 is not appropriate for the interaction with the co-activator [51,52], and the transactivation activities are considerably reduced [47]. Nevertheless, competition experiments with [^{3}H]-9cRA revealed that 3-Cl-AHPC **7** efficiently and selectively competed with the native ligand for binding to RARγ (83% displacement; cf. 31% RARα, 17% RARβ, 16% RXRα). Further analysis led to the suggestion that the conformational effect induced by 3-Cl-AHPC **7** was not sufficient to induce dissociation of co-repressors and association of co-activators, and therefore the ligand apparently behaved as a trans-activational antagonist for RARγ [47].

Based on this model, we considered to further increase the steric interactions on that region by incorporation of two substituents (Cl and Me) at the vicinal positions of the biaryl connection, which would further impair binding of the ligand to the RAR receptor subtypes. Moreover, to additionally increase the bulk of the analogs, we considered the incorporation of a MEM substituent on the phenol after recognizing that the combined effect of the acetal and the adamantyl group of MX781 **9** is likely associated to its RAR antagonistic properties. In addition to the naphthoic acid derivatives of CD437 **1**, other conformationally more flexible polar chains of arylacetic and cinnamic acids were also considered (Scheme 1). These AdAr were evaluated with regard to their ability to inhibit cancer cell proliferation and induce apoptosis. Interestingly, we found that while the anticancer effects are independent of RAR transactivation, some of these highly twisted analogs bind and transactivate through RXR.

2. Chemistry

The Suzuki coupling between an arylboronic acid and an aryl triflate, a strategy also utilized by Dawson et al. [47], was chosen as a key step of the synthetic sequence to generate the highly congested 2,2′-disubstituted biaryl bond (Scheme 1). The greater tolerance to steric hindrance of the Suzuki reaction relative to other palladium-catalyzed cross-coupling variants is well established [53,54].

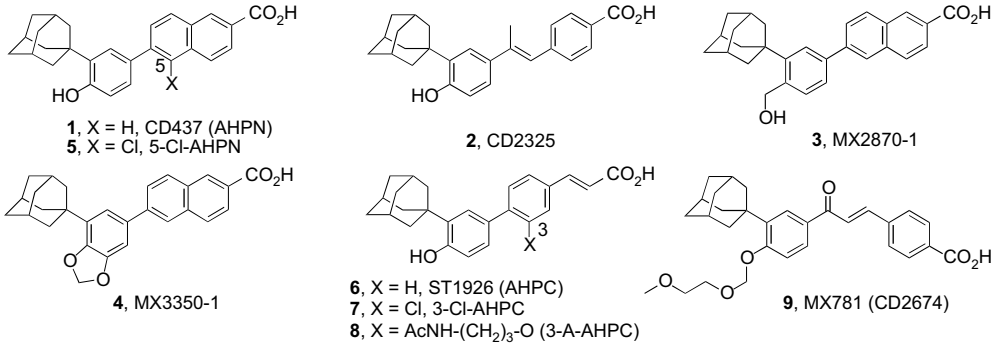

Fig. 1. Representative adamantyl arotinoids (AdAr).