Bioorganic & Medicinal Chemistry Letters 28 (2018) 2442–2445

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Development of biotin-retinoid conjugates as chemical probes for analysis of retinoid function

Shinya Fujii[a,b,*], Shuichi Mori[b], Hiroyuki Kagechika[b], Marco Antonio Mendoza Parra[c,*], Hinrich Gronemeyer[c]

[a] Institute for Quantitative Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
[b] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[c] Department of Functional Genomics and Cancer, Institute of Genetics and Molecular and Cellular Biology, (IGBMC), Centre National de la Recherche Scientifique UMR7104, Institut National de la Santé et de la Recherche Médicale U964, Université de Strasbourg, BP 10142, Illkirch Cedex 67404, France

ARTICLE INFO

Keywords:
Retinoid
Biotin conjugate
P19 cells
Neural differentiation
Chem-seq

ABSTRACT

Herein, we report the rational design, synthesis and biological evaluation of conjugates consisting of the synthetic retinoid Am580 and biotin connected via a linker moiety. We found that the linking substructure between the retinoid part and the biotin part is critical for retaining the biological activity. Conjugate **4** with a shorter linker showed similar potency to endogenous retinoid ATRA (**1**) and the parent compound Am580 (**2**) for neural differentiation of mouse embryotic carcinoma P19 cells, and showed the same pattern of induction of gene expression. It is expected to be useful as a probe for investigations of retinoid function. The design rationale and structure-activity relationship of the linker moiety are expected to be helpful for developing biotin conjugates of other nuclear receptor ligands.

The tight interaction between biotin and streptavidin has been widely used in biochemistry and chemical biology,[1] and many kinds of biotin conjugates have been developed for target identification, labeling or functionalization of proteins of interest.[2] Biotin conjugates are also employed in the developing technology of genome-wide analysis. For example, ChIP-sequencing (ChIP-seq) combines chromatin immunoprecipitation (ChIP) with massively parallel sequencing to identify the binding sites of DNA-binding proteins in a genome-wide manner,[3,4] but chemical affinity capture, such as biotin-avidin interaction, can also be used as an alternative to the antigen-antibody interaction. Such chemical affinity-based sequencing (Chem-seq) is a useful technique to identify genomic sites where biologically active compounds and their target proteins interact.[5,6] The Chem-seq method was first employed in 2014 to investigate bromodomain (BRDs), CDK9 and DNA intercalator, based on the use of BRDs inhibitor JQ1, CDK inhibitor AT7519 and the intercalator psoralen, respectively,[7] and further work been reported since then.[8] Though the Chem-seq method is in principle a powerful tool for identifying DNA target sites, its practical application has been limited, probably due to the difficulty of developing suitable biotin conjugates as Chem-seq probes, because introduction of a biotinylated moiety is likely to cause loss of ligand activity. Therefore, development of novel biotin conjugates of transcription-modulating compounds that retain the biological activity is necessary. Here, we report the rational design, synthesis, and biological evaluation of biotin conjugates of the synthetic retinoid Am580.[9,10] Retinoids, which are ligands of retinoic acid receptors (RARs), regulate various key physiological processes, including cell fate decision, by regulating expression of their target genes.[11] To date, a number of retinoid analogues have been developed as drug candidates, and several natural and synthetic retinoids are in clinical use as therapeutic agents.[12,13] The development of retinoid-biotin conjugates as chemical probes would be useful for genome-wide analysis of their action mechanisms.

As a model to guide the development of the retinoid probes, we focused on the retinoid-induced differentiation of mouse embryotic carcinoma (EC) cell line P19. Activation by all-*trans* retinoic acid (ATRA: **1**) of the cognate gene regulatory network in P19 cells induces differentiation into neuronal precursor cells.[14,15] We first confirmed that a synthetic retinoid could induce the neural-differentiating signals in P19 in the same manner as the endogenous RARs ligand, ATRA. Indeed, we found that synthetic retinoid Am580 (**2**) induced the expression of neural differentiation markers (vide infra). Based on this finding, we selected Am580 as the retinoid moiety of the desired conjugates because of its ready availability and high chemical stability in comparison to ATRA.

In order to design the Am580-biotin conjugates, we focused on the

* Corresponding authors at: Institute for Quantitative Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan (Shinya Fujii).
E-mail addresses: fujiis@iam.u-tokyo.ac.jp (S. Fujii), marco@igbmc.fr (M.A. Mendoza Parra).

https://doi.org/10.1016/j.bmcl.2018.06.011
Received 27 April 2018; Received in revised form 4 June 2018; Accepted 5 June 2018
Available online 06 June 2018
0960-894X/

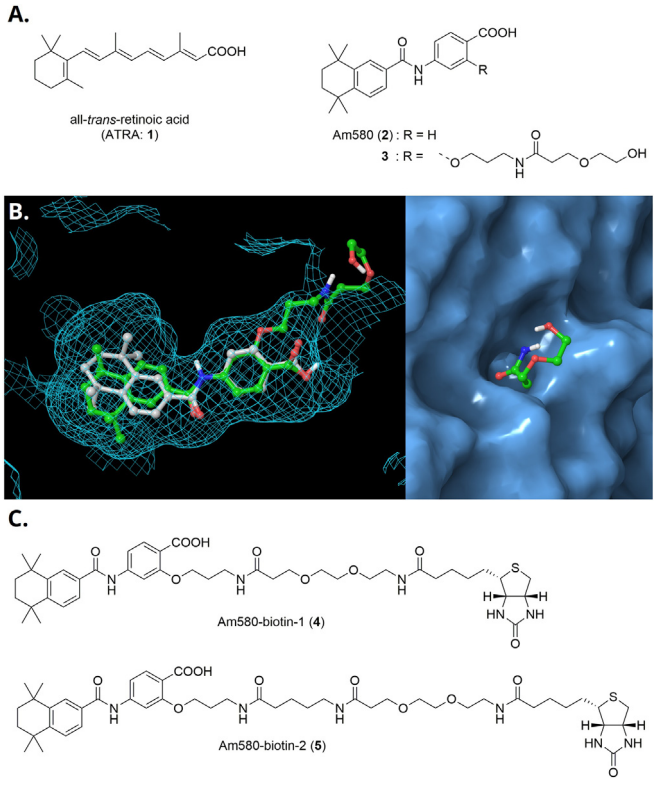

Fig. 1. A) Structures of ATRA (**1**), Am580 (**2**) and the model compound **3** used in the docking simulation. B) Docking model of **3** with hRARα LBD (PDB ID: 3KMR) obtained with AutoDock.17 The hRARα LBD complex with **2** (gray) is superimposed on the docking model of **3** (green). (Left) The protein surface of the ligand-binding pocket is indicated as a blue mesh. Note that the side chain moiety of **3** extends outside the protein. (Right) The protein surface is indicated as a blue solid. C) Structures of the designed Am580-biotin conjugates.

X-ray co-crystal structure of Am580 bound to RARα.[16] The co-crystal exhibits a tunnel structure with conserved water molecules, directed from the receptor surface to the ligand-binding pocket in close proximity to the carboxylic acid of **2**. Based on these considerations, we conducted docking simulation of model compound **3** bearing a long side-chain substructure. In the docked structure, the side chain moiety of **3** accesses the receptor surface, suggesting that a linker moiety positioned at the *ortho*-position of the benzoic acid would be acceptable. This is consistent with the structures of previously developed RAR-targeting retinoid conjugates such as RAR-degradation inducers[18] (SNIPERs: Specific and Nongenetic IAPs-dependent Protein ERasers).[19] Based on these considerations, we designed two Am580-biotin conjugates, namely Am580-biotin-1 (**4**) and Am580-biotin-2 (**5**), bearing biotinylated side chains at the *ortho*-position of the benzoic acid moiety (Fig. 1).

These conjugates were synthesized as shown in Scheme 1. Condensation of tetramethyltetrahydronaphthalene carboxylic acid (**6**) and methyl 4-aminosalicylate afforded amide **7**. An *N*-protected aminopropyl moiety was introduced at the phenolic group of **7** to afford **8**, and removal of the Boc group under acidic conditions gave the intermediate **9**. The biotinylated side chain was connected to the aminopropyl moiety of **9** to afford **10** using the commercially available biotinylation reagent **14**, and then hydrolysis of the ester group afforded the target compound **4**. The conjugate **5** was also synthesized via the intermediate **9**. Six-atom elongation of the side chain of **9** gave **11**, and removal of the Boc group under an acidic condition gave **12**. The biotinylated moiety was connected to the side chain of **12** under the same conditions as used for **10**, and hydrolysis of the ester group afforded the target compound **5** (Scheme 1).

The biological potency of the synthesized retinoid-biotin conjugates as retinoids was assessed in terms of the neural differentiating activity toward P19 cells. We investigated the expression levels of retinoid-responsive genes, including neuronal differentiation markers *Ascl1*[20,21] and *Brn2* (*Pou3f2*),[22,23] by quantitative RT-PCR (RT-qPCR). As shown in Fig. 2, treatment with ATRA (**1**) for 72 h significantly induced the expression of *Rarb*, *Cyp26a1*, *Ascl1* and *Brn2*, and Am580 (**2**) exhibited similar gene expression-inducing potency, as mentioned above. As for the biotin conjugates, we found that Am580-biotin-1 (**4**) exhibited significant gene expression-inducing activity. Conjugate **4** induced *Rarb* expression in a dose-dependent manner, and the response to 10 μM **4** was similar to that in the case of 1 μM **1** or **2**. Regarding the three other genes, conjugate **4** also significantly induced *Cyp26a1*, *Ascl1* and *Brn2* gene expression, and 1 μM or 10 μM **4** induced similar responses to those seen with 1 μM **1** or **2**. On the other hand, in contrast to conjugate **4**, the gene expression-inducing activity of conjugate **5** was quite weak. At 10 μM concentration, **5** induced expression of *Rarb* and *Cyp26a1* to some extent, but the mRNA levels were significantly lower than those induced by **1**, **2**, and **4**. No significant increase of *Ascl1* and *Brn2* expression was observed (Fig. 2).

In the course of the neural differentiation of P19 cells, the expression levels of retinoid-responsive genes exhibit markedly different time courses. For example, *Rarb* and *Cyp26a1* increase rapidly in response to retinoid, while other genes including *Ascl1* and *Brn2* show a slower increase of expression level.[24] Based on this background, we investigated the time courses of *Rarb*, *Cyp26a1*, *Ascl1* and *Brn2* expression in P19 cells induced by these compounds. Fig. 3 shows the expression levels of these genes after 6, 24 and 72 h treatment with each compound. ATRA (**1**) induced a rapid increase of *Rarb* and *Cyp26a1* mRNAs, followed by a decrease at the time point of 72 h. On the other hand, the expression of *Ascl1* was only weakly induced at 6 h, and then continued to increase up to 72 h. Similarly, induction of *Brn2* expression was not observed at 6 h, but was observed at 24 and 72 h. Am580 (**2**) induced essentially the same gene expression patterns as **1**. Conjugate **4** also exhibited similar gene expression patterns, namely, a rapid increase and then decay of *Rarb* and *Cyp26a1* mRNAs, and a relatively slow increase of *Ascl1* and *Brn2* mRNAs. On the other hand, conjugate **5** induced only modest increases of *Rarb* and *Cyp26a1* mRNAs, and did not induce expression of *Ascl1* and *Brn2* (Fig. 3).

The above results show that conjugate **4** retains neural differentiation-inducing potency toward P19 cells, and thus could serve as a probe to investigate retinoid function. Thus, connecting the biotinylated side chain at the proximal position of the carboxylic acid appears to be a reasonable design strategy for functional retinoid probes. On the other hand, conjugate **5** exhibited significantly weaker biological activity than **4**. It is interesting that the biotin conjugate with a longer linking substructure exhibited weaker potency than the conjugate bearing a shorter linking substructure. A possible explanation is that the protein surface of RAR, including the tunnel moiety, contains many hydrophilic amino acid residues, so that the relatively hydrophobic character of the linking substructure adjacent to the retinoid part of conjugate **5** in comparison with **4** may be disadvantageous for binding to the receptor. Also, the relatively rigid nature of the two serial amide moieties of **5** might inhibit proper folding of the receptor. Nuclear receptors share the common domain structure and high sequence similarity in the LBD. Therefore, this structure-activity relationship could represent a useful clue for designing chemical probes for other nuclear receptors.

In summary, we rationally designed and synthesized Am580-biotin conjugates, and found that conjugate **4** retains the biological activity of the retinoid, at least as regards potency for induction of P19 cell differentiation. Conjugate **4** exhibited the same gene induction pattern as retinoids **1** and **2**, and should be available as a probe for investigation of retinoid function. The linking substructure proved to be critical for retaining the biological activity. This design rationale and the structure-activity relationship could also be helpful for developing biotin-conjugates of other nuclear receptor ligands. We are planning to conduct