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Probing biological activity through structural modelling of ligand-receptor interactions of 2,4-disubstituted thiazole retinoids

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ABSTRACT

Retinoids, such as all-*trans*-retinoic acid (ATRA), regulate cellular differentiation and signalling pathways in chordates by binding to nuclear retinoic acid receptors (RARα/β/γ). Polar interactions between receptor and ligand are important for binding and facilitating the non-polar interactions and conformational changes necessary for RAR-mediated transcriptional regulation. The constraints on activity and RAR-type specificity with respect to the structural link between the polar and non-polar functions of synthetic retinoids are poorly understood. To address this, predictions from *in silico* ligand-RAR docking calculations and molecular dynamics simulations for a small library of stable, synthetic retinoids (designated GZ series) containing a central thiazole linker structure and different hydrophobic region substituents, were tested using a ligand binding assay and a range of cellular biological assays. The docking analysis showed that these thiazole-containing retinoids were well suited to the binding pocket of RARα, particularly *via* a favorable hydrogen bonding interaction between the thiazole and Ser232 of RARα. A bulky hydrophobic region (*i.e.,* present in compounds GZ23 and GZ25) was important for interaction with the RAR binding pockets. Ligand binding assays generally reflected the findings from *in silico* docking, and showed that GZ25 was a particularly strongly binding ligand for RARα/β. GZ25 also exhibited higher activity as an inducer of neuronal differentiation than ATRA and other GZ derivatives. These data demonstrate that GZ25 is a stable synthetic retinoid with improved activity which efficiently regulates neuronal differentiation and help to define the key structural requirements for retinoid activity enabling the design and development of the next generation of more active, selective synthetic retinoids as potential therapeutic regulators of neurogenesis.

1. Introduction

Retinoids are signaling molecules functionally related to all-*trans* retinoic acid (ATRA), a metabolite of Vitamin A (Fig. 1).[1,2] These small lipophilic molecules mediate cellular proliferation, differentiation and homeostasis in chordates[3] by acting as ligands for members of a family of nuclear receptors referred to as retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Given the range of biological processes regulated by retinoids, there is huge potential for synthetic retinoids as therapeutics. However, this potential has yet to be realized, mainly because of a lack of detailed understanding of RAR signaling mechanisms in biological processes and the design criteria for targeting synthetic retinoids to specific responses. Furthermore, although retinoic acid is used to treat a variety of skin conditions, acute promyelocytic leukemia, neuroblastoma and other cancers and metabolic diseases,[4–7] it is highly susceptible to photodegradation and readily isomerizes to a mixture of 9-*cis*-retinoic acid, 13-*cis*-retinoic acid and other isomers, as well as undergoing decomposition. The development of stable ATRA analogues is, therefore, of substantial importance for

Abbreviations: ATRA, all-*trans* retinoic acid; AF, activation function; ESI, electronic supplementary information; GZ, compound series code; H12, helix 12; LBD, ligand binding domain; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoid X receptor; TTN, 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene hydrophobic region.

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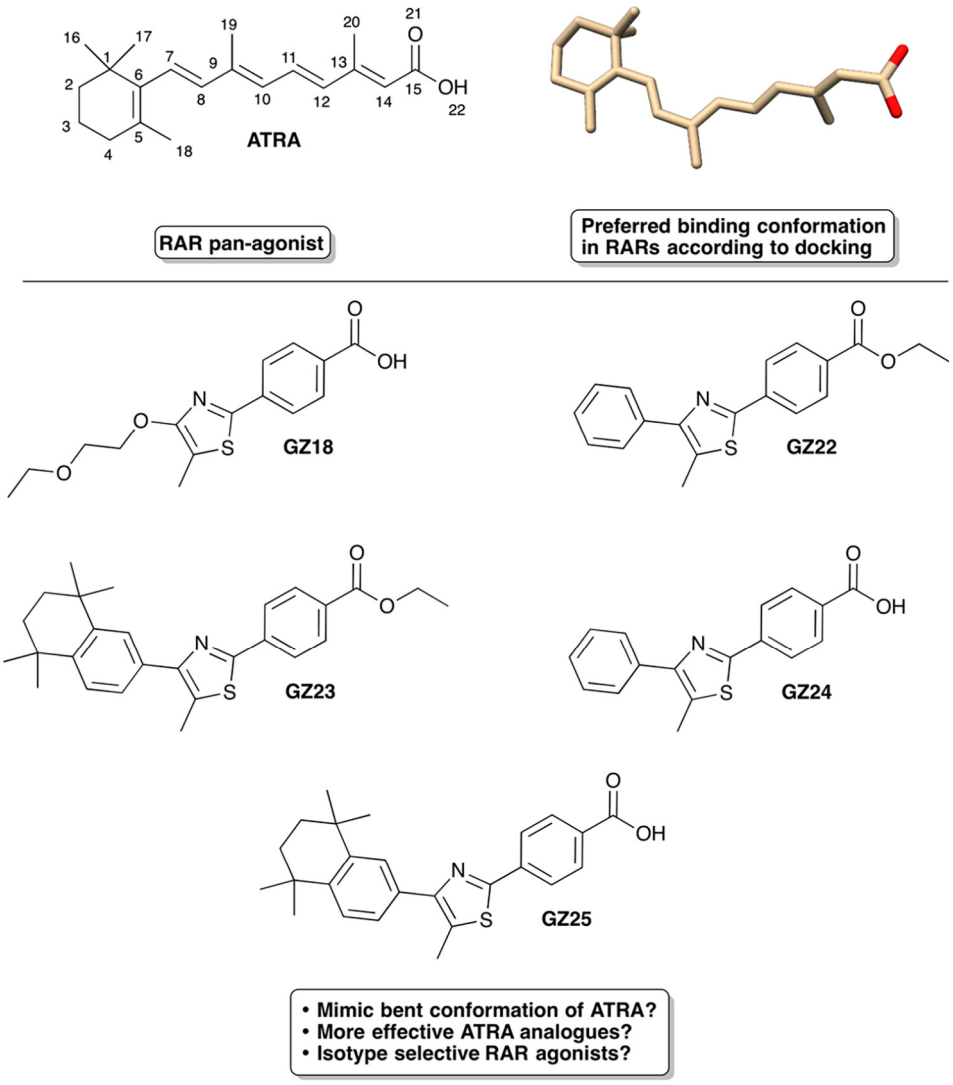

Fig. 1. Molecular structures of all-*trans*-retinoic acid (ATRA) and the synthetic thiazole retinoids GZ18, GZ22, GZ23, GZ24 and GZ25, and preferred binding conformation of ATRA according to previous molecular docking studies.[23]

improving the potential of RAR signaling pathways for drug development.[8,9]

Three closely related isotypes of each of the RARs (and RXRs) are known, and are designated RARα, RARβ, and RARγ, respectively.[1] They all share the same overall structure, with six domains, specified A to F.[1,10] The A and B domains comprise a ligand independent activation function (AF-1), responsible for recruiting coactivators necessary for gene regulation.[11] The DNA-binding C domain[12,13] is linked, *via* a hinge domain D, to the ligand-binding domain (LBD) E. The sequences of RAR isotypes are highly conserved, but subtle differences in the LBD give rise to distinct ligand binding pocket architectures, allowing for the design of isotype-specific retinoids.[14,15] In addition, alternative splicing of primary RAR transcripts generates, for each RAR isotype, subtypes with different A and B domains linked to the RARα-, RARβ- and RARγ-specific core C, D, E and F domains.

The activation mechanism of the RARs (and RXRs) by retinoids has been studied, in detail, by determination of a number of crystal structures of the LBDs. Upon retinoid binding to the LBD, a conformational change occurs allowing heterodimerisation to an RXR partner, and subsequent binding to DNA sequences known as retinoic acid response elements (RAREs).[4,10,16–18] A 'mouse-trap' process is thought to occur during retinoid binding to the RARs, in which the retinoid enters the pocket and associates with a cluster of polar residues at the end, facilitating a conformational change which brings the C-terminal helix 12 (H12) to enclose the ligand inside the pocket.[10] The interaction between H12 and the retinoid ligand is particularly important, since the *holo* positioning of H12 forms a binding surface (AF-2) that binds coactivators.[10,19,20] Effective stabilization of H12, therefore, leads to the promotion of gene transcription, and indeed, optimization of this interaction has become an important aim for the design of synthetic retinoids.[21]

The molecular structure of natural and synthetic retinoids can be described in terms of three distinct regions: a polar region (typically a carboxyl moiety) required for association with the polar cluster at the base of the pocket, a short linker that increases the length of the retinoid and can incorporate additional functionality for isotype-specific binding, and a hydrophobic region for interacting with H12 and the strongly hydrophobic opening to the binding pocket.[21,22] In recent molecular docking studies and receptor-binding assays, we showed that ATRA is able to bind to each of the RARs via a number of conformations with respect to the s-*cis* and s-*trans* orientations around formal single bond connections between double bonds. Indeed, a bent conformation with a single s-*cis* bond rotation about the C7–C8 alkene was the preferred conformation over the more linear, all-*trans* conformation frequently thought