Point of VIEW

ACS chemical biology

Small-Molecule Triggers of Tadpole Metamorphosis

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ABSTRACT Small molecules that function as highly selective agonists and antagonists of cellular receptors comprise some of the most valuable therapeutic agents and molecular probes. A recent paper describes the design, synthesis, and evaluation of CO23, the first potent and specific agonist of thyroid hormone receptor-alpha (TRα), a member of the nuclear receptor (NR) superfamily of transcription factors. Together with previously reported TRβ-selective agonists such as GC-1, these compounds represent powerful new tools for studying gene expression, signaling, differentiation, and development controlled by this important NR.

The ability to achieve a high level of selectivity for a specific biological target is a key requirement for the generation of effective therapeutic agents and molecular probes. This endeavor can be quite challenging. Pockets that bind enzyme cofactors, hormones, and other key regulatory molecules are often highly conserved among protein family members that control diverse biological processes. However, our increasingly better understanding of the molecular recognition of protein–small-molecule interactions is beginning to allow the design of selective compounds that can target a single subtype among highly structurally similar members of protein families (*1*, *2*). In this issue of *ACS Chemical Biology*, Ocasio and Scanlan (*3*) report on the use of a rational design approach to identify the first specific agonist of thyroid hormone receptor (TR)-alpha (TRα). This agonist has profound effects on TRα-mediated metamorphosis of *Xenopus laevis*, and it represents an important new molecular probe for dissecting signaling pathways controlled by this nuclear receptor (NR).

Members of the NR superfamily of transcription factors share conserved ligand binding domains (LBDs). By binding small molecules, NRs play key roles in embryonic development, cellular differentiation, metabolism, and cell death. Many of these proteins represent major drug targets, and selective therapeutic agents targeting specific NR subtypes have the potential to revolutionize the treatment of diseases of the endocrine system (*4*). One such NR is the TR, which comprises two related subtypes, TRα and TRβ, encoded by two different genes (*5*). The LBDs of these subtypes are ~75% identical in amino acid sequence. The thyroid hormone 3,5,3′-triiodo-L-thyronine (Figure 1, T_3, **1**) binds the LBDs of both subtypes with high affinity (K_d = 0.06 nM) (*6*). Consequently, T_3 influences numerous physiological parameters, including growth, development, homeostasis, metabolism, heart rate, lipid levels, and mood (*7*). Pharmacological treatment with thyroid hormone has the potential to control body weight and lower cholesterol and triglycerides (*8*). However, this therapeutic approach has been plagued by side effects of hyperthyroidism, such as elevated heart rate and arrhythmia (*9*). Studies of patients resistant to thyroid hormone and knockout of the TR subtypes in mice have revealed that TRα mediates the effect of thyroid hormone on heart rate, whereas TRβ affects other responses to this hormone (*10*). Because of the potential of TRβ as a target of obesity, hyperlipidemia, depression, and osteoporosis, selective TRβ agonists are of significant interest as therapeutic agents (*11*). However, the design of compounds selective for only one of the two TR subtypes is difficult. The residues that define the hydrophobic ligand binding pockets (LBPs) of TRα and TRβ differ by only one amino acid; Ser277 in TRα is replaced by Asn331 in TRβ. Despite these similarities, TRβ-selective ligands such as GC-1 (**2**) (*12*) and compound **3** (Figure 1) (*13*) have been reported. A structural comparison of the LBDs of TRα bound to

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Published online October 20, 2006
10.1021/cb600398a CCC: $33.50

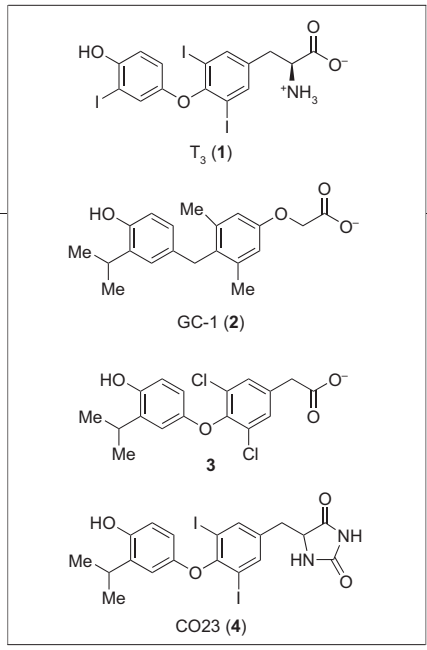

Figure 1. Structures of TR agonists. The thyroid hormone T_3 (1), which activates both TRα and TRβ, is shown on the top. The TRβ-selective thyromimetics GC-1 (2) and compound 3 are shown in the middle. The TRα-selective thyromimetic CO23 (4) is shown on the bottom.

compound **3** (*13*) and TRβ bound to T_3 (**1**) (*14*) is shown (Figure 2).

Selective TRα agonists are of significant interest as molecular probes of TR signaling pathways. On page 585 of this issue of *ACS Chemical Biology*, Ocasio and Scanlan (*3*) describe the first such TRα-selective thyromimetic, termed CO23 (Figure 1, **4**). CO23 (**4**) is a selective TRα agonist despite binding to both of the purified TR subtypes with equal affinity. This similarity in affinity is perhaps not surprising given that the LBPs are identical except for the single Ser277 to Asn331 substitution. In contrast, GC-1 (*12*) and compound **3** (*13*) are selective agonists of TRβ because they bind substantially more tightly to this protein subtype. Several available X-ray structures of TRs bound to ligands (*13*–*16*) show that the selective binding of GC-1 (**2**) and compound **3** to TRβ appears to result from participation of the carboxylate moiety in a hydrogen bonding network that includes the TRβ Asn331 residue.

When evaluated against TRs expressed in mammalian cells, CO23 (**4**) selectively activates gene expression controlled by TRα. Previous studies of related estrogen receptors (*17*, *18*) show that this selectivity may arise from subtle differences in the induced conformations of amino acid side chains that define the LBP. Minor effects on amino acid side chains can influence the dynamics of the conformationally mobile helix-12, the most carboxy-terminal helix of NR LBDs (*19*). Perturbation of helix-12 can affect the recruitment of coactivator proteins to the LBD, potentiate interactions with the transcriptional machinery, and regulate gene expression.

Because of its selectivity for TRα, CO23 (**4**) has unique effects on TR signaling *in vivo*. When tadpoles of the frog *X. laevis* are treated with CO23 (**4**), this compound triggers only part of the developmental program that regulates metamorphosis; hind limbs of tadpoles grow, but other developmental changes are not activated. These changes include resorption of the head and tail observed upon treatment with the pan-agonist T_3 (**1**) or the TRβ-selective agonist GC-1 (**2**) (*20*). Moreover, sequential treatment of tadpoles with CO23 (**4**) followed by GC-1 (**2**) revealed that these isoform-selective agonists can be used for temporal control of tadpole morphogenesis; the proper sequence of activation of the two TR subtypes is critical for correct execution of the developmental program. Furthermore, it was also confirmed by real-time PCR analysis of gene expression that selective activation of TRα *in vivo* by CO23 (**4**) is controlled by this TR subtype.

GC-1 (**2**) and CO23 (**4**) represent members of a growing family of small molecules that affect development or cellular differentiation by interacting with defined molecular targets. Among the compounds known to affect NRs in this way, BMS493, a pan-antagonist of the retinoic acid receptors alpha, beta, and gamma, affects hindbrain patterning in chick embryos (*21*). Rosiglitazone and other members of the thiazolidinedione family of antidiabetic drugs can control the differentiation of adipocytes by functioning as selective agonists of the peroxisome proliferator-activated receptor-gamma (PPARγ) (*22*). Conversely, the selective PPARγ antagonist T0070907 inhibits differentiation of adipocytes (*23*). Other compounds that affect development and differentiation through well-characterized mechanisms include purmorphamine (*24*, *25*) and cyclopamine (*26*). These compounds function as agonists and antagonists, respectively, of Smoothened, a key regulator of the Hedgehog signaling pathway. Other examples of the use of chemical genetics to probe developmental biology have also been reviewed (*27*).

Activation of gene expression mediated by the genomic effects of TRα and TRβ can now be dissociated *in vivo* through the use of specific small molecules. This chemical-genetics approach provides new tools to elucidate the complex biology regulated by NRs. However, in addition to their genomic effects, TRs and many other NRs activate distinct nongenomic signaling pathways (*28*). Given the previous achievements of using small molecules to separate genomic from nongenomic actions of estrogen receptors (*29*, *30*), identifying compounds that isolate these specific functions of TR subtypes to provide even finer resolution

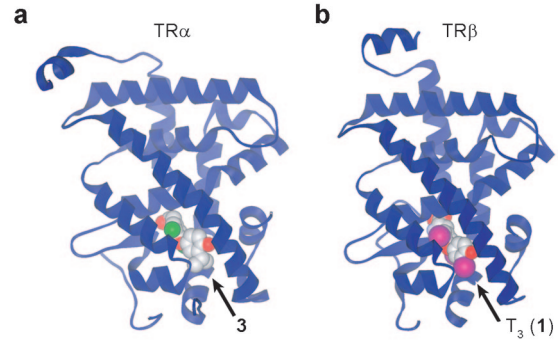

Figure 2. X-ray structures of TR LBDs. a) TRα bound to compound 3 (PDB code 1NAV). b) TRβ bound to T_3 (1, PDB code 1XZX).

should be possible. Further development of this strategy has the potential to lead to highly selective therapeutic agents for various life-threatening diseases and to powerful molecular probes of signaling pathways yet to be fully elucidated.

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