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Identification of a lead pharmacophore for the development of potent nuclear receptor modulators as anticancer and X syndrome disease therapeutic agents

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Abstract—A series of tetrahydroisoquinoline-*N*-phenylamide derivatives were designed, synthesized, and tested for their relative binding affinity and antagonistic activity against androgen receptor (AR). Compound **1b** (relative binding affinity, RBA = 6.4) and **1h** (RBA = 12.6) showed higher binding affinity than flutamide (RBA = 1), a potent AR antagonist. These two compounds also exerted optimal antagonistic activity against AR in reporter assays. The derivatives were also tested for their activities against another nuclear receptor, farnesoid x receptor (FXR), with most compounds acting as weak antagonists, however, compound **1h** behaved as a FXR agonist with activity slightly less than that of chenodeoxycholic acid (CDCA), a natural FXR agonist.

Nuclear receptors act as transcription factors to modulate transcription actions of target genes involved in maintenance of cellular phenotypes, metabolism, and cell proliferation, through homodimerizing or heterodimerizing with other nuclear receptors. Currently, there are more than 30 members in the nuclear receptor superfamily.[1] Most of these receptors exert their functions by ligand-activation and they usually contains six functional domains (A–F), including the first transcription activation domain (AF-1, A/B), DNA binding domain (C), hinge domain (D), and the second transcription activation domain (AF-2, E/F), also referred to as the ligand binding domain. Because of their importance in many diseases, including breast cancer, prostate cancer, and diabetes, they are viewed as potential targets for the development of specific therapeutic agents.

Androgens are steroid hormones that are responsible for the cellular proliferation and differentiation of male sexual organs and secondary sexual characteristics, and their action is mainly exerted through a nuclear receptor, androgen receptor (AR).[2] Androgen exerts its effects via a genomic mechanism in which androgen passively enters the target cells and binds to AR in the cytoplasm.[3] The androgen/AR complex translocates into the nucleus. In the nucleus, the AR complex dimerizes and binds to the promoter region of the androgen-regulated gene to initiate the transcription action and enhance the production of androgen-regulated proteins such as PSA, Bcl-2, and maspin.[4–6] Prostate cancer is the most common type of non-skin cancer and the second leading cause of cancer death in American men. In the early stage of prostate cancer, its growth highly relies on androgen, and the use of androgen deprivation therapy can significantly slow down the tumor growth. Numerous compounds that act as AR agonists like R1881, or antagonists like flutamide, nilutamide, cyperoterone acetate(CPA), and bicalutamide, have been developed.[7] Although flutamide has been used as a first line adjuvant monotherapy against prostate cancer for more than two decades, most patients who took flutamide eventually encounter a resistant stage.[8] Thus, there is an urgent need to develop new antiandrogens.

Farnesoid x receptor (FXR) is also a member of the nuclear receptor superfamily whose endogenous ligands have been identified as numerous bile acids, including chenodeoxycholic acid (CDCA), CA, and DCA.[9] The

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action of FXR is through heterodimerizing with another novel nuclear receptor, retinoid x receptor (RXR), to regulate the transcription of many essential genes involved in bile acid metabolism, including small heterodimer partner 1 (SHP), cholesterol 7α hydroxylase (CYP7A1), and bile salt export pump (BSEP).[10–12] Because these genes, which are regulated by FXR, are involved in bile acid related diseases, FXR modulators are viewed as potential treatments for bile acid and cholesterol homeostasis diseases including cardiovascular and lipid metabolism.

Most nonsteroidal antiandrogens, such as hydroxyflutamide, contain two structural moieties, a nitro (or a cyano) group at the phenyl ring (A ring) of the core scaffold that mimics the 3-carbonyl group of steroidal androgen, like R1881, and a hydrogen-bond donor moiety, such as a hydroxyl group, that mimics the 17β-hydroxyl group of R1881 (Fig. 1a).

In this study, we report a new core scaffold, 1,2,3,4-tetrahydroisoquinoline-*N*-phenylamide, as potential AR/FXR modulating ligand. The hydroxymethyl substituent at the 3 position mimics the important moiety of flutamide as shown in Fig. 1b. The phenylamide substituent might locate within helices 5 and 11 of AR to make extra protein interactions. Based on the core scaffold, we have generated derivatives by substituting various hydrophobic or hydrophilic substituents on the additional phenylamide group. Additionally, our core scaffold also superimposes closely with the steroid core scaffold of FXR native ligands, such as CDCA. We tested these compounds for both AR and FXR modulation. Our goal is to determine whether any of the new compounds is capable of serving as a lead pharmacophore for the development of AR and FXR modulators.

Compounds **1a**–**2b** were synthesized by mixing commercial benzoic acid or phenyl acetic acid derivatives, (*S*)-(−)-1,2,3,4-tetrahydro-3-isoquinoline methanol, 1-hydroxybenzotriazole hydrate (HOBT), and 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide) hydrochloride (DEC), and allowed to react overnight under nitrogen gas, as shown in Scheme 1. Table 1 summarizes the relative binding affinity of compounds **1a**–**2b**. The fluorescence-based competitive binding assay purchased from Panvera (Madison WI) utilizes a synthetic androgen with high fluorescence polarization property when it binds to AR and recombinant AR ligand binding domain fused with GST. After the individual binding affinity is determined, it is further calculated as relative binding affinity with flutamide as standard comparison and CPA as a reference. All the compounds exhibited higher binding affinity than flutamide with both **1h** and **2b** showing 12-fold higher binding affinity. However, all the compounds showed weaker binding affinity than CPA.

The ligand binding pocket of AR consists of three essential hydrogen bonding areas for interaction between a AR modulator and AR LBD. This includes Arg 752 of helix 5 and Gln 711 of helix 3; Asn 705 of helix 3 and Thr 877 of helix 11.[13] Apart from these residues, the bound modulator is surrounded by hydrophobic residues. Most antiandrogens like flutamide and bicalutamide bind to AR LBD with two hydrogen-bond interactions between the nitro or cyano group at the 4 position of the A ring with Arg 752 and Gln 711, and between the hydroxyl group mimicking 17β-hydroxyl group of R1881 and Asn 705. Interestingly, even though our compounds do not contain a hydrogen-bond acceptor on the A ring, they still bind to AR with moderate binding affinity. This is likely due to potential hydrogen-bond interaction between the 3-hydroxymethyl group and the side chain of Asn 705 or the amide backbone of Leu 704. This is consistent with the fact that our core scaffold and hydroxyflutamide superimpose well in the 3-hydroxymethyl-1,2,3,4-tetrahydroisoquinoline structural portion where the latter has been observed to interact with the protein. On the other hand, these compounds could adopt a different orientation such that the D ring may reside within helix 3 and helix 5, allowing the substituted hydrophilic groups on the D ring to interact with Arg 752 and Gln 711, similar to the A ring of flutamide. The structure–activity relationship of flutamide and bicalutamide derivatives demonstrates that compounds with cyano or nitro substitution on the A ring have optimal binding affinity, which is at least 10-fold higher than those with fluoro or hydroxyl substituent.[14] If our compounds adopt the latter orientation mentioned above, they should not be expected to exert better binding affinity than flutamide because of lack of a cyano or nitro substituent. In addition, the binding orientation would also abolish the potential hydrogen-bond interaction with Asn 705, which is known to be critical for increased binding affinity. However, since our compounds show higher binding affinity than flutamide, they are unlikely to assume the latter orientation. Crystallographic studies of the compounds/AR LBD complexes should throw light on the binding mode of this new scaffold.

To evaluate the agonist and antagonist activities of these compounds, a transient transfection reporter assay in HepG2 cells was employed. Generally, HepG2 cells were transfected with three plasmids by using Superfect transfection kit (Qiagen, CA) including hAR expression plasmid pcDNA-hAR, a luciferase reporter plasmid containing androgen receptor response element pGL3-ARE-E4, and a normalization control, β-galactosidase reporter plasmid, pCMVβ (Clontech, CA). For comparison, DMSO for androgenic activity and flutamide for antiandrogenic activity were used as standard reference. The antiandrogenic activity of compounds **1a**–**2b** is shown in Fig. 2. Essentially, the compounds did not exhibit obvious androgenic effects when compared with DMSO and R1881 (data not shown). However, compound, **1b** (relative luciferase activity, RLA = 0.61 of R1881 effects) and **1h** (RLA = 0.62 of R1881 effects) exerted optimal antiandrogenic activity against hAR, with activity slightly less than flutamide's (RLA = 0.55 of R1881 effects). For the rest of the compounds, **1c** and **1g** acted as weak antiandrogens. Based upon these results, it seems that enhancement of antiandrogenic activity could be achieved by substituting hydrophilic and hydrophobic groups on the D ring to increase the