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# Synthesis and evaluation of novel 17-indazole androstene derivatives designed as CYP17 inhibitors

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ABSTRACT

A series of novel 1*H*- and 2*H*-indazole derivatives of the commercially available dehydroepiandrosterone acetate have been synthesized and tested for inhibition of human cytochrome 17α-hydroxylase-$C_{17,20}$-lyase (CYP17), androgen receptor (AR) binding affinity, and cytotoxic potential against three prostate cancer (PC) cell lines.



## 1. Introduction

CYP17 is an endoplasmic reticulum membrane-bound multifunctional enzyme that exhibits 17α-hydroxylase and $C_{17,20}$-lyase activities on a single active site, both of which are crucial for human physiology [1–5]. The hydroxylase activity is involved in the conversion of pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone whereas the lyase activity is responsible for the side-chain cleavage of these hydroxy derivatives to afford dehydroepiandrosterone (DHEA) and androstenedione (AD), respectively. DHEA and AD are androgen precursors and can be further metabolized in steroidogenic tissues to more potent androgens such as testosterone and dihydrotestosterone (DHT).

The testis and the adrenal cortex are the two sites thought to produce most of the androgenic steroids in humans. The testis are responsible for about 90–95% of circulating androgens whereas the adrenals account for the remaining 5–10% [6].

In the adult prostate, androgens act directly on epithelial cells to maintain structural and functional viability. The secretory epithelial cells express the AR and require chronic androgenic stimulation for survival and functional integrity.

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Glandular involution occurs as a result of cell apoptosis when androgen levels drop below a threshold (as is the case in medical or surgical castration) [7].

Androgen deprivation as therapy for advanced PC was introduced by Huggins et al. in 1941 [8,9] and ever since it has been the mainstay for advanced PC treatment. At least 80% of the human prostate cancers show a favourable response to androgen deprivation as evidenced by the disappearance of symptoms or a decline of prostate specific antigen (PSA) levels [10,11]. However, relapses are seen invariably when tumors emerge as androgen-independent and apoptosis-resistant [7]. Mechanisms that may mediate this adaptation include AR amplification, AR mutation, alterations in the balance between transcriptional coactivators and corepressors, and activation of signal transduction pathways that by-pass the AR [12,13]. Gene amplification and amino acid substitutions in the AR are detected at a high frequency in recurrent tumors. These changes confer growth advantage to the tumor cells due to either hypersensitivity of AR to low, castrate-level androgens or a realignment of the receptor conformation, leading to altered ligand specificity that enables antiandrogens, adrenal androgens and non-androgen steroids to act agonistically to increase AR activity [7].

Enhanced intracellular conversion of adrenal androgens to testosterone and DHT has also been reported as an important mechanism for disease progression [14]. It may explain why the available AR antagonists do not have substantial activity against the androgen-independent tumor cells that emerge subsequent to androgen deprivation therapy, seeing that the AR antagonists will have a much lower affinity for the AR than the natural substrates. Inhibition of CYP17 is therefore a valuable approach for the treatment of androgen-dependent diseases such as PC as a means of inhibiting androgen biosynthesis both in the testis and adrenals. It should be noted that PC is a leading cause of mortality being the second most common cause of cancer-related death in both the USA and Australia (behind lung cancer), and the third most common cause of cancer-related death in the European Union (behind lung cancer and colorectal cancer) [15–17].

Several steroidal and non-steroidal compounds have been synthesized and evaluated as CYP17 inhibitors [18–23]. Out of these compounds, ketoconazole **1** (Fig. 1), an imidazole fungicide that has inhibitory activity towards CYP17 [24,25], has been used clinically in high dose (400 mg, every 8 h) for the treatment of advanced PC [26–28]. However, the fact that it concomitantly inhibits other steroidal P450 enzymes causing significant side effects [29] has limited its use. A recent investigation of the efficacy of low dose ketoconazole (200 mg, three times daily) found clinical benefit equal to high dose treatment, with a reduction in side effects [30]. Ketoconazole is still currently used alone or in combination with glucocorticoids as secondary hormonal therapy for hormone-refractory PC (HRPC) [31].

A common approach to the synthesis of potent steroidal inhibitors of CYP17 has been the design of substrate-like molecules bearing a heterocycle at the C17 position with privileged heteroatoms (N, S, O) which can interact as the sixth ligand with the heme iron of the enzyme. One of such compounds, abiraterone acetate **2** (Fig. 1), reported to be a very potent inhibitor of the enzyme [32], has successfully undergone Phase I clinical trials for PC treatment [33,34] and the first set of results of an open-label Phase II clinical trial have just been reported. Thus, 11 out of 18 patients have had PSA declines ≥50% at 3 months with 5 patients having a PSA decline ≥90% when 1000 mg of the drug were administered orally once daily to chemotherapy-naive castration-resistant PC (CRPC) patients, resistant to luteinizing hormone-releasing hormone (LHRH) analogues, antiandrogens, and frequently diethylstilbestrol (DES) and steroids [35].

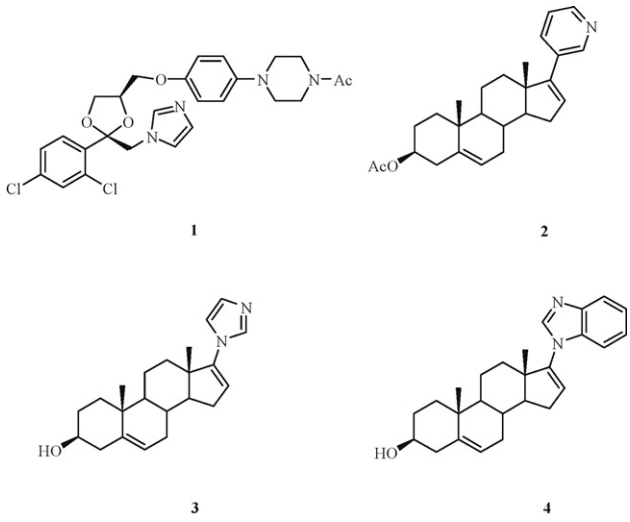


**Fig. 1 – CYP17 inhibitors.**

Another class of interesting steroidal inhibitors has been reported in which the azole group is attached to the C17 of the steroid nucleus through a nitrogen atom [36–38]. Both compound **3** (code named VN/85-1) (Fig. 1) and compound **4** (VN/124-1) potently inhibit CYP17. VN/124-1 has also been shown to have antiandrogenic properties against the androgen-dependent LAPC4 human prostate tumor xenograft, being actually more effective than castration in suppressing its growth [38].

Herein we report the synthesis and biological evaluation of novel 17-indazole androstene derivatives designed as CYP17 inhibitors. Other than their CYP17 inhibitory potential, their ability to bind to the AR, and cytotoxicity against three PC cell lines has also been evaluated.

## 2. Experimental

### 2.1. *Chemistry*

#### 2.1.1. *General*

Dehydroepiandrosterone acetate, indazole, bis(triphenylphosphine)rhodium(I)carbonyl chloride, 1,3-bis(diphenylphosphino)propane, aluminum isopropoxide, and *N*-methylpiperidone were obtained from Sigma–Aldrich Co. All solvents used were previously dried and purified according to standard procedures. For TLC analysis, Kieselgel $60HF_{254}$/Kieselgel 60G was used. Melting points were determined using a BUCHI Melting Point B-540 apparatus and are uncorrected. IR spectra were obtained using a JASCO