Synthesis of Novel Cyclized C3-Azoles of Galeterone for Prostate Cancer Therapy

Shivani Mattikalli[1], Puranik Purushottamachar[2,3], Elizabeth Thomas[2], Vincent C. Njar[2,3]

[1]CMNS, University of Maryland, College Park, MD, USA

[2]Department of Pharmacology, [3]Center of Biomolecular Therapeutics (CBT), University of Maryland School of Medicine, Baltimore, MD, USA

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

UNIVERSITY OF MARYLAND | NIST INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

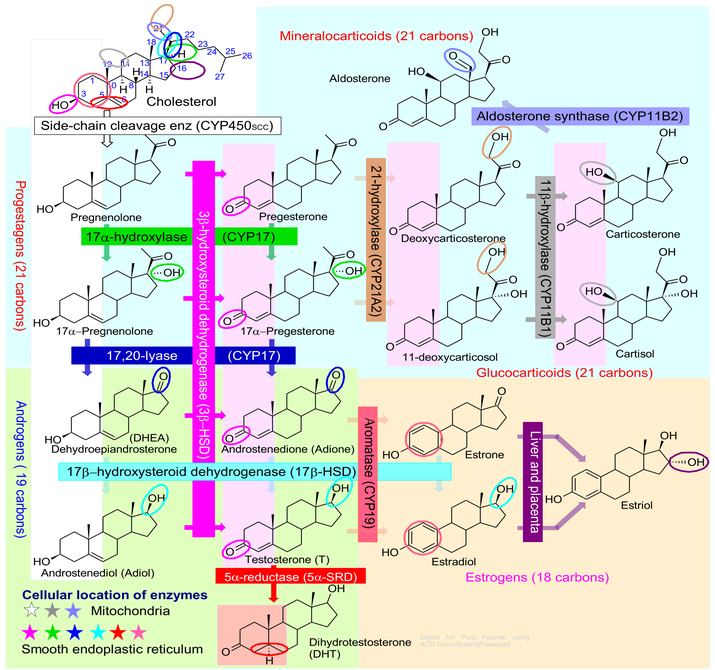
UNIVERSITY OF MARYLAND

Introduction

Prostate cancer (PC): PC is a significant health problem for men in the USA and worldwide. Nearly 30,000 American men die each year of advanced PC. The Androgen receptor (AR), a member of nuclear receptors family, is a key modulator in development and progression of PC.[1]

Steroidogenesis Pathway: In the steroidogenesis pathway, cholesterol undergoes two major modifications at the C17 and C3 positions for the biosynthesis of adrenal (male sex hormones) and gonadal (mineralocorticoids) hormones (**Fig 1)**.[2] The C17–hydroxylation/17,20-lyastion and C3-hydroxy oxidation are catalyzed by CYP17A1 and 3β–HSD enzymes, respectively. The formation of C3-oxo metabolite by 3β–HSD is essential for the further metabolism at C5 double bond by 5α-SRD enzyme to produce dihydrotestosterone (DHT), a most potent androgen[2]. These androgenic metabolites are important for the development and maintenance of male sex characters. The abnormal production of androgens and their aberrant interaction with androgen receptor is known for the development and progression of PC disease.[3]

Figure 1: Steroidogenesis pathway.[2]

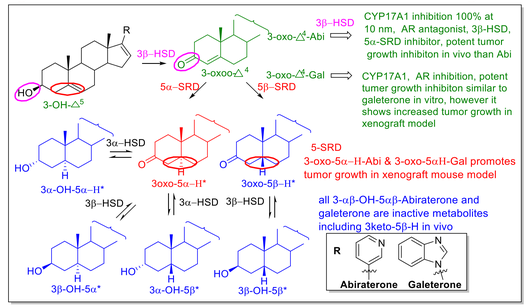


Lyase Inhibitors and their Pharmacokinetic Profiles: Clinical anti-PC agents abiraterone and galaterone (**Fig 2**) are 3-OH-Δ[5]-C17-heterocycles substituted steroids. Both agents exert their potent anti-PC activity by reducing androgen biosynthesis through CYP17A1 enzyme inhibition.[4a-c] The CYP17A1 enzyme catalyzes the steroid 17α-hydroxylation and 17,20-lyase action to form 17-oxo steroids and subsequently modified to 17β–OH by 17β-HSD enzyme (**Fig. 1**)[2]. Inhibiting 17-oxo formation is rate limiting for the 17β-OH formation.

Due to their structural similarities to endogenous steroids abiraterone and galeterone undergo metabolism at C3 position by 3–HSD enzymes (**Fig 2**). Consequently, these two agents have short half-life on oral administration ($t_{1/2}$~1h).[4a,c] .Moreover, both agents suffer from low oral bioavailability (abi-37% and gal-19%), and thus require very high dose to show clinical efficacy.[5]

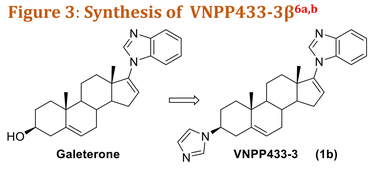
Synthesis

Fig 2: Abi and Gal's structure, metabolites and their biological activity[4b-c]



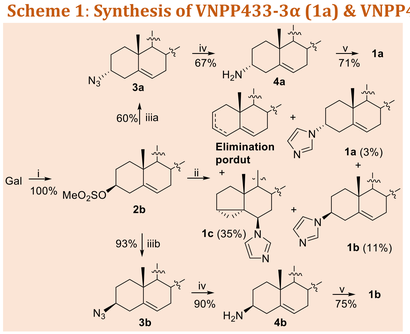
To prevent metabolism by 3-HSD, the metabolic soft-spot (C3–OH) of galeterone was appended with imidazole ring to obtain metabolically stable agent VNPP433-3β (**1b; Fig 2**) with no compromise in anti-prostate cancer properties.[6a-c] This modification has significantly improved biological half-life ($t_{1/2}$ 31 h) which is 24.75-fold superior to Gal's half-life ($t_{1/2}$~1h).[6c]

Figure 3: Synthesis of VNPP433-3β[6a,b]



Additionally, VNPP433-3β has improved oral absorption of 49.6% bioavailability which is 2.96 superior to Gal's oral absorption.[6c] Oral bioavailability of a drug depends on two interrelated factors: absorption and metabolism. Ideally a drug should be sufficiently absorbed from GI tract and should not undergo metabolism in GI tract or in liver (first pass) to have maximum systemic exposure.[7]

Scheme 1: Synthesis of VNPP433-3α (1a) & VNPP433-3β (1b) [6ab]

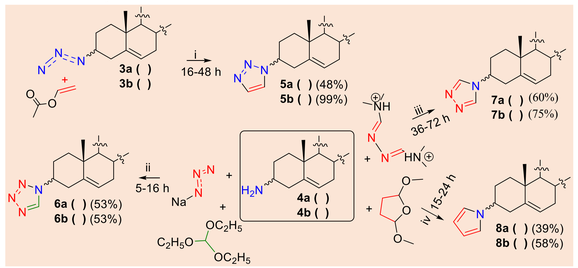


Reagents and conditions: (**i**) DCM, TEA, 4 °C, $MeSO_2Cl$, 30 min, rt, 16h; (**ii**) Toluene, Imidazole, Δ, 4h; (**iiia**) DMF, NaN_3, 80 °C, 3h; (**iiib**) DCM, $TMSN_3$, $BF_3.OEt_2$, rt, 5h, 2M aq. NaOH, rt, 6h; (**iv**) PPh_3, THF/MeOH/H_2O, 60 °C, 3h; (**v**) NH_3(aq. 50%), MeOH, glyoxal trimer dihydrate, HCHO (aq. 6%), 70 °C, 5h.

Considering the potential of C3 imidazole, at present we designed and synthesized various other C3 azole compounds such as triazole, tetrazole and pyrrole derivative (**Sch. 2**) of gal. We expected these novel azoles may further improve pharmacokinetic properties and anti-cancer activity.

In the literature it has been shown that azide and primary amine functions can be cyclized in one step reaction to obtain various azoles. Considering the advantages of cyclization methods and availability of stereospecific azide and amine intermediates we initiated our synthetic efforts to obtain target compound (**Sch. 2**) 1,2,3-triazol-1-yl[8] form azide (**3a-b**) and 1,2,3,4-tetrazole-1-yl[9], 1,2,4-triazole-4-yl,[10] pyrrole[11] and from primary amines (**4a-b**).

Scheme 2: Synthesis of novel azoles



Microwave irradiation of azide (**3a-b**) with vinyl acetate at 120° C for 16-48 h resulted in the formation of 1*H*-1, 2, 3-triazole-1-yl derivatives (**5a**, **5b**). The 1-substituted 1*H*-1, 2 ,3, 4-tetrazoles (**6a**, **6b**) were synthesized from the reaction of primary amines (**4a-b**) with triethyl orthoformate and sodium azide in presence of acetic acid at 80 ° C for 5 -16 h.

Where condensation of amine (**4a-b**) with N,N'-diemthylformamide- azine in pyridine at 125 ° for 36-72 h provided 4*H*-1,2,4-triazole-4-yl (**7a**, **7b**). The modified Clauson-Kaas reaction of amine (**4a-b**) with 2,5-dimethoxytetrahydrofuran, acetic acid is applied to achieve the synthesis of pyrrole derivatives (**8a-b**). Reaction of alpha azide and alpha amine functional group requires more time and they provide lower yield of products in comparison to beta substrates. This agrees with our previous observation that equatorial function is easily accessible than axil.[6b]

Methods

General method A: Synthesis of 1H-1,2 3-triazol-1-yl substituted compounds (5a, 5b) by cyclization of azide 3a and 3b: Azide (**3a** or **3b**) (0.3g) and vinyl acetates (2 mL) were mixed and sealed in Biotage vial and irradiated under microwave at 120 °C for 16 - 48 h. Then solvent evaporated, crude product purified by flash column chromatography using 1% MeOH in ethyl acetate to obtain solid products.

General method B: Synthesis of 1*H*-1,2,3,4-teraazol-1-yl substituted compounds (6a, 6b) by cyclization of amine 4a and 4b: A mixture of amine (**4a** or **4b**) (0.2g, 0.516 mmol), Triethyl orthoformate (0.8 ml, 4.8 mmol), sod azide (0.31g, 4.8 mmol), acetic acid (~ 2 mL) was stirred at 80 °C for 5-12 h. Reaction mixture evaporated, treated with water, neutralized with sodium bicarbonate, suspension extracted with ethyl acetate, organic layer dried with sod. sulfate, evaporated and purified on a short flash silica column using 5% MeOH in ethyl acetate.

General method C: Synthesis of 4*H*-1,2,4-triazol-4-yl substituted compounds (7a, 7b) by cyclization of amine 4a and 4b: A solution of amine (**4a** or **4b**) (0.1g, 0.258 mmol), N,N-dimethylformamide azine (0.04 g, 0.310 mmol) and pyridine (1.5 mL) were stirred at 125 °C for 36-72 h. RM concentrated and crude product purified by short flash silica column using 5-10% MeOH in ethyl acetate.

General method D: Synthesis of Pyrrole substituted compounds (8a, bb) by cyclization of amine 4a and 4b: For 1 mole equivalent amine substrate: prepare a solution of 0.85 mole equivalent 2,5-dimethoxytetrahydrofuran and 10 equivalent (v/v) water by gently refluxing for two hours. Cool the solution to room temperature before adding 10 equivalent (v/v) DCM, 1 mole equivalent of sod. acetate, and 1 mole equivalent of acetic acid mix well before addition of amine substrate. Reaction mixture stirred vigorously under dark for 15 h then neutralized with sod. carbonate solution, extracted with DCM, dried and evaporated to obtain off-white crude product which purified by flash silica chromatography using 30% ethyl acetate in pet ether.

Conclusions

- Presence of 3-OH-△[5]-function in steroid based therapeutic agent leads to phase-1 metabolism by 3-HSD and 5-SRD enzymes.
- Modification of metabolic soft-spot (C3–OH) with heterocyclic ring is an innovative concept of obtaining metabolically stable agents with no compromise in anti-PC properties.
- C3-imidazole compound exhibited major improvement in oral bioavailability (%F) and elimination half-life ($T_{1/2}$).
- Considering the biological potential of C3 imidazole we designed and synthesized various azoles by cyclization method with excellent to good yield.
- We observed that β-azide and amine undergoes reaction quickly and provides higher yield than α-substrates.
- These newly synthesized tetrazoles, triazoles, and diazole are under evaluation for biological activity.

References

1. Ross RK. et al. *Can. Res.*, **1998**, *58*, 4477
2. Hanukoglu. *JSBMB*, **1992**, *43*, 779-04
3. Purushottamachar P. et al. *J. Med. Chem.*, **2013**, *56*, 4880-98
4. a) Handratta VD. et al. *J. Med. Chem.*, **2005**, *48*, 2972 b) Li Z. et al. *Nature.*, **2016**, *533*(7604), 547-5 c) Alyamani M. et al. *Cell. Chem. Biol.*, **2016**,*24*,825-2
5. Bruno RD. *Steroids*, **2011**, *76*, 1268
6. a) Purushottamachar P. et al. *ACS Med. Chem. Lett.*, **2016** *7* (7), 708–713 b) Purushottamachar P. et al. *Org. Process Res. Dev.* **2016**, *20* (9), 1654–1661c) Kwegyir AK. et al. *Cancers,* **2018**, *24*, 1627
7. Chaturvedi PR. *Curr. Opin. Chem. Biol.* 2001, *5*, 452.
8. Hansen S. et al. *Synlett* **2009**, *22*, 3275–3278
9. N Yilmaz S. et al. *Therm. Anal. Calorim.* **2015**, *119*, 2321–2328
10. Abdellah I. *Curr. Top. Electrochem.* **2011**, *16*, 81–91
11. Gourlay, BS. et al. *Tet.Lett.*, **2006**, *47*, 799-801