

IN-SILICO STUDIES OF NOVEL AZA-ANDROGEN DERIVATIVES; SYNTHESIS, PROCESS OPTIMIZATION AND CHARACTERIZATION OF 4-AZAANDROST-5-ENE-17-HYDROXY-3-ONE (PRECURSOR) FOR ITS COMMERCIAL APPLICATION.

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ABSTRACT

The 4-azasteroid scaffold remains a privileged framework for the design of steroid 5 α -reductase inhibitors and related bioactive agents, owing to the pharmacological versatility conferred by A-ring nitrogen substitution. In this study, a series of novel aza-androgen-amino-acid conjugates was designed by functionalizing the C-17 hydroxyl of the 4-azaandrost-5-ene-3-one core with alanine, glycine, leucine, phenylalanine, and lysine to explore variations in polarity, steric bulk, and hydrogen-bonding capacity. The designed conjugates were evaluated in silico for drug-likeness and ADME properties using Swiss ADME, and prioritized compounds were subjected to molecular docking against five biologically relevant targets (5 α -reductase type-2, androgen receptor, 17 α -hydroxylase, estrogen receptor, and DHFR) using CB-Dock2. Parallely, the key precursor 4-azaandrost-5-ene-17-hydroxy-3-one was synthesized from androst-4-ene-3,17-dione via a three-step sequence — ozonolytic A-ring cleavage, ammonia-mediated aza-cyclization, and sodium borohydride reduction — and the process was optimized and validated at 50 g scale with yields of 88–94% at each step. All intermediates and the target precursor were characterized by melting point, IR, ¹H/¹³C NMR, and HRMS. The combined computational and synthetic findings establish 4-azaandrost-5-ene-17-hydroxy-3-one as a robust, scalable, and structurally versatile intermediate for generating novel aza-androgen derivatives with predicted target-binding potential.

KEYWORDS: Aza-androgen; 4-azaandrost-5-ene-17-hydroxy-3-one; molecular docking; ADME/drug-likeness; process optimization

1 INTRODUCTION

A rigid tetracyclic cyclopentanoperhydrophenanthrene nucleus characterizes steroids, a significant class of physiologically active chemicals. They are useful scaffolds in medicinal chemistry because of their distinct three-dimensional structure, stereochemical variety, and capacity to interact with nuclear receptors and enzymes. Agents for hormonal disorders, cancer, inflammation, contraception, and other therapeutic uses are frequently developed using steroidal chemicals and their derivatives[1,2]. Additionally, semisynthetic methods that use important intermediates like androst-4-ene-3,17-dione and similar oxygenated steroids are frequently used to create steroidal active medicinal ingredients[3,4]. Azasteroids are created when a nitrogen atom is substituted for a carbon atom in the steroid nucleus. Specifically, 4-azasteroids have a nitrogen atom at the A-ring's C-4 location, which modifies the parent steroid framework's electrical characteristics, polarity, hydrogen-bonding capacity, and biological recognition[5]. Many biologically significant chemicals have been developed as a result of these structural alterations, particularly inhibitors of steroid 5 α -reductase, an enzyme that converts testosterone into the more powerful androgen dihydrotestosterone. Studies on the structure-activity relationship have demonstrated that the 4-aza-3-oxo steroidal arrangement can significantly affect enzyme-inhibitory and antiandrogenic action when combined with the proper substitution at C-17[6,7]. Thus, the 4-azaandrost-5-ene framework provides a desirable platform for the synthesis and design of steroidal derivatives with biological activity. While the 17-hydroxy group provides a functional site for additional derivatization, such as oxidation, esterification, etherification, and conversion into amide or other side-chain-containing derivatives, the conjugated 3-oxo group and a 5(6)-double bond provide a chemically useful A-ring system. The compound's lipophilicity, receptor affinity, metabolic stability, and biological activity may all be affected by these structural changes. There have been several documented synthesis methods for 4-azaandrostenes. Oxidation of testosterone to 4-androstene-3,17-dione, oxidative cleavage of the A-ring to form a seco-acid, and subsequent azacyclization utilizing an ammonium source are popular strategies. 4-azaandrost-5-ene-3,17-dione and similar derivatives have been prepared using this method[8].

In silico techniques are now a crucial part of modern azasteroid drug creation and optimization, working in tandem with synthetic technology. Bulkier substituents at the C-17 position increase inhibitory potency, according to three-dimensional

quantitative structure–activity relationship (3D-QSAR) studies using CoMFA and CoMSIA models on a series of 4-azasteroidal 5 α -reductase inhibitors. This conclusion is supported by complementary docking analyses. More recent research has used molecular dynamics simulations to dock potential inhibitors and assess their binding interactions in relation to finasteride and dutasteride by utilizing homology- and AlphaFold-predicted structures of human SRD5A1. Similar to this, new candidate inhibitors of 5 α -reductase type 2 have been found using integrated workflows that combine scaffold hopping, fragment-based de novo design, molecular docking, and molecular dynamics[9]. These workflows have also identified the important active-site residues controlling ligand binding and complex stability, as well as predicted binding energies that are on par with or better than those of current medications [10]. These computational pipelines, which include docking, molecular dynamics, ADMET prediction, and QSAR modelling, allow for the logical prioritization of new aza-androgen derivatives before resource-intensive synthesis, reducing the amount of chemical space that needs to be investigated experimentally[9,10].

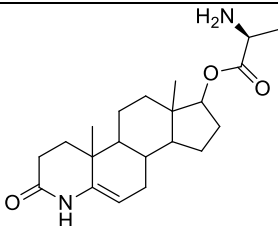
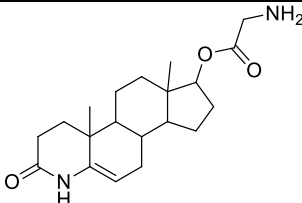
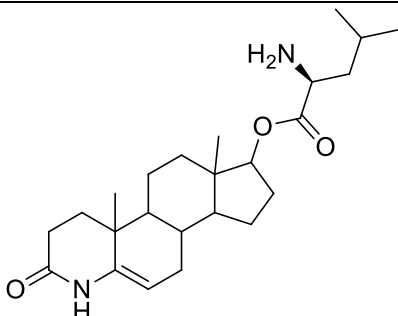
As it combines the pharmacologically significant 4-aza-3-oxo A-ring with a free hydroxyl group at C-17, the synthesis of 4-azaandrost-5-ene-17-hydroxy-3-one is particularly interesting for the synthesis of novel steroidal analogues with amide, ester, ether, or other pharmacophoric substituents, this molecule may be used as a flexible intermediate. Even though the 4-azaandrostene nucleus has been linked to antiproliferative and enzyme inhibitory actions, a derivative's biological characteristics cannot be inferred from its structural resemblance to known azasteroids. Therefore, this compound's production and thorough characterization are crucial first steps prior to biological evaluation. The current study uses computational screening of novel aza-androgen derivatives with a validated, optimized, and fully characterized synthetic route to their common precursor.

2 Experimental section

2.1 Design and Selection of Steroid–Amino-Acid Conjugates

Amino-acid conjugates were rationally designed to introduce systematic variations in polarity, hydrophobicity, steric bulk and hydrogen-bonding capacity at the C-17 position of the aza-androgen scaffold. Alanine, Glycine, leucine, phenylalanine and lysine were selected to provide a representative range of physicochemical characteristics. The resulting steroid–amino-acid conjugates (Table 1) were designed using Chem Draw and subjected to in-silico ADME and drug-likeness evaluation, and compounds displaying favorable predicted properties were subsequently prioritized for molecular docking[11].

Table 1 2D structure of compounds used for docking

Compound no.	Compound Name (IUPAC)	Structure
1	(2S)-4a,6a-dimethyl-2-oxo-2,3,4,4a,4b,5,6,6a,7,8,9,9a,9b,10-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl 2-aminopropanoate	
2	4a,6a-dimethyl-2-oxo-2,3,4,4a,4b,5,6,6a,7,8,9,9a,9b,10-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl 2-aminoacetate	
3	(2S)-4a,6a-dimethyl-2-oxo-2,3,4,4a,4b,5,6,6a,7,8,9,9a,9b,10-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl 2-amino-4-methylpentanoate	

4	(2S)-4a,6a-dimethyl-2-oxo-2,3,4,4a,4b,5,6,6a,7,8,9,9a,9b,10-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl 2-amino-3-phenylpropanoate	
5	(2S)-4a,6a-dimethyl-2-oxo-2,3,4,4a,4b,5,6,6a,7,8,9,9a,9b,10-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl 2,6-diaminohexanoate	

2.2 In-Silico ADME and Drug-Likeness Evaluation

The proposed aza-androgen amino-acid conjugates were subjected to in-silico absorption, distribution, metabolism, and excretion (ADME) evaluation using the SwissADME web-based platform[12]. The molecular structures of the designed compounds were submitted individually for prediction of their physicochemical, pharmacokinetic, and drug-likeness properties. The evaluated parameters included molecular weight, lipophilicity (LogP), topological polar surface area (TPSA), hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), rotatable bonds, aqueous solubility, gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate potential, and cytochrome P450 enzyme inhibition. Drug-likeness was assessed using Lipinski's rule of five and other medicinal-chemistry filters available within the platform. The predicted ADME profiles of the derivatives were compared to identify compounds exhibiting relatively favourable physicochemical and pharmacokinetic characteristics. Compounds satisfying the predefined selection criteria were prioritized for subsequent molecular docking studies. The computational ADME analysis was used as a preliminary screening step and was interpreted as predictive rather than experimental evidence of pharmacokinetic behaviour[13].

2.3 Molecular Docking Study

Molecular docking studies were performed to investigate the predicted binding modes and interactions of the selected aza-androgen amino-acid conjugates with biologically relevant protein targets. The three-dimensional structures of the selected target proteins were retrieved from the Protein Data Bank (PDB) using their corresponding PDB identification numbers[14]. The selected targets included 5 α -reductase type-2, androgen receptor, 17 α -hydroxylase, estrogen receptor, and human dihydrofolate reductase (DHFR), represented by PDB IDs 7BW1, 1T65, 3RUK, 1A52, and 1U72, respectively. The protein structures were inspected and prepared for docking by removing non-essential molecules and retaining the structural components required for ligand recognition. The three-dimensional structures of the selected ligands were prepared in a suitable format for docking. Protein–ligand docking was carried out using the CB-Dock2 web server, which performs blind docking through automated binding-cavity detection followed by docking of the ligand within the identified cavities[15]. The docking results were evaluated based on predicted binding affinity/docking score, binding cavity, ligand orientation, and the interactions established between the ligand and target-protein residues. The docked complexes were further analyzed to identify hydrogen-bonding, hydrophobic, π -related, van der Waals, and other relevant non-covalent interactions. The binding poses and interaction patterns were comparatively assessed to identify derivatives with favorable predicted binding characteristics.

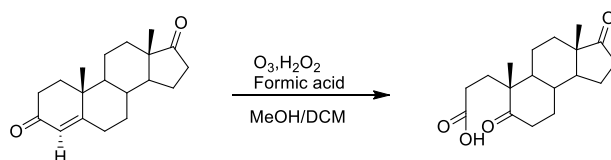
2.4 Materials for synthesis

H₂O₂ (30%), liq. ammonia(25%aq), formic acid (98%) were purchased from hyma synthesis, Methanol (99%), DCM (Dried 99.5%), Ethylene glycol (99%) Sodium borohydride were obtained from SRL. ¹HNMR (400 MHz), ¹³CNMR (400 MHz) spectra were recorded on VARIAN 400 MHz NMR. Using tetramethyl silane as an internal standard and the solvent resonance at (CDCl₃: ¹HNMR400MHz and ¹³CNMR400MHz): δ 7.2 ppm and δ 78 ppm, chemical shifts and coupling constants are given in parts per million (ppm) and Hertz (Hz), respectively. Peak multiplicities are assigned as follows: s (singlet), d (doublet), dd (double to doublet), dt (double to triplet), and m (multiplet), A BRUKER FT-IR spectrometer of 4000–500 cm⁻¹ range is used for IR spectroscopy. The functional groups contained in the synthesized compounds were identified using the distinctive absorption bands found in the spectra. A Waters Corporation mass spectrometer using Electrospray Ionization (ESI) technology was used to record the mass spectra. The molecular weight and structural characterization of the synthesized compounds were verified using the molecular ion peaks seen in the spectra. Kieselgel 60F254 plates covered with 0.25 mm silica gel were used for analytical thin-layer chromatography (TLC).

2.5 Synthesis of Precursor 1,2 and 3

2.5.1 5,17-dioxo-A-nor-3,5-secanrost-3-oic acid (1)

Androst-4-ene-3,17-dione (2.0 g, 6.98 mmol) was dissolved in methanol and/or dichloromethane (MeOH/DCM) and the resulting solution was cooled to approximately -40 to -30°C in a dry-ice cooling bath. Ozone (O_3), corresponding to approximately 3 molar equivalents (20.9 mmol), was then passed slowly through the reaction mixture over a period of about 2–3 h while maintaining the low temperature. Ozonolysis of the $\text{C}=\text{C}$ bond was allowed to proceed under continuous stirring. Low-temperature ozonolysis followed by oxidative work-up is a standard approach for oxidative cleavage of alkenes. After completion of ozonation, approximately 3 molar equivalents of hydrogen peroxide (H_2O_2) were added carefully to the reaction mixture. Then 4Meq of formic acid was added to the reaction mixture as indicated in the experimental procedure, and the mixture was stirred further at the same temperature. The reaction mixture was subsequently concentrated under reduced pressure to remove the volatile solvents (Scheme 1).

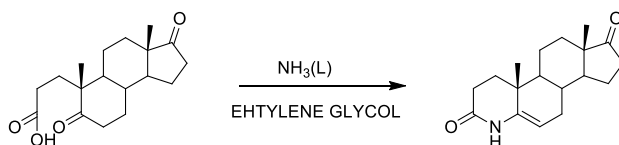


Scheme 1 Synthesis of 1 from androst-4-ene-3,17-dione

The concentrated reaction mixture was treated with approximately 1% aqueous sodium hydroxide solution, and the pH was adjusted to approximately 7–10. The mixture was transferred to a separating vessel and extracted with diethyl ether to remove neutral and non-polar organic impurities. The extraction was repeated twice, and the organic layers were separated. The remaining aqueous layer containing the ionized acidic product was then carefully acidified with dilute hydrochloric acid to approximately pH 1–3. Acidification regenerated the free carboxylic acid, which precipitated from the aqueous medium. The resulting solid was collected by filtration, washed appropriately with water, and dried to constant weight. The product was obtained as a pale-yellow solid. The isolated yield recorded in the experimental notes was approximately 1.89 g (88.4%).

2.5.2 4-azaandrost-5-ene-3,17-dione (2)

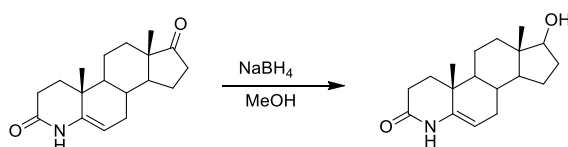
1 (2g, 6.9565 mmol) was dissolved in 10 ml Ethylene glycol under inert atmosphere. Cool the reaction mixture to 15 – 20°C and add aqueous ammonia (0.59g, 34.78 mmol) dropwise into the reaction mixture over a period of 15 to 30 minutes (Scheme 2). Then heat the reaction mixture to 120°C – 130°C and maintain for 5 hours. Check TLC and complies. The reaction mixture is cooled to room temperature and dilute with 20ml of water and acidify with dilute HCl to obtain our desired product. An off white amorphous solid was obtained with 1.816g.



Scheme 2 Synthesis of 2 from 1

2.5.3 4-azaandrost-5-ene-17-hydroxy-3-one (3)

2 (2g, 6.95 mmol) was dissolved in methanol (10ml) under an inert atmosphere in an ice bath setup. Then cool the reaction mixture to 0 – 5°C . Add Sodium Borohydride (2g, 52.8mmol) lot wise over a period of 1 hour at 0 – 5°C (Scheme 3). The reaction mixture was maintained for 5 hours. TLC was checked and complies. Subsequently the reaction mixture was distilled off under reduced pressure. 10 ml of water was added into the mass to maintain its pH 1 with (5–10 ml HCl). The precipitated product was then filtered off. An off-white solid product was obtained with 1.87g. of yield.



Scheme 3 Synthesis of 3 from 2

3 Result and Discussion

3.1 *In silico* ADME and drug-likeness profile

In silico ADME and drug-likeness profile of the proposed derivatives (compounds 1-5), predicted using the SwissADME web tool. All derivatives have molecular weights below 500 g/mol, TPSA values within the acceptable range (81.42-107.44 Å²), and satisfy Lipinski's rule of five with zero violations, indicating good oral drug-likeness (Table 2). All compounds are predicted to show high gastrointestinal absorption and no blood-brain barrier permeation, suggesting favorable oral bioavailability with minimal risk of CNS side effects.

Table 2 ADME and drug-likeness profile of the proposed derivatives

Compo und	Molecular weight (g/mol)	logP (A2)	TPSA	HBD	HBA	GI Absorption	BBB	Lipnski violation
1	360.49	2.71	81.42	2	4	High	No	0 violation
2	346.46	2.26	81.42	2	4	High	No	0 violation
3	402.57	3.46	81.42	2	4	High	No	0 violation
4	436.59	3.89	81.42	2	4	High	No	0 violation
5	417.58	2.58	107.44	3	5	High	No	0 violation

3.2 Molecular Docking Studies

The selected ligands were docked against five protein targets using CB Dock2. For each target, the top-ranked pose was selected for detailed analysis based on the docking score, location within the biologically relevant cavity, and chemical plausibility of the ligand–protein interactions. The docking scores of all ligands against the five targets (7BW1, 1T65, 3RUK, 1A52 and 1U72) are summarized in Table 3. The 3-D and 2-D interaction profiles of the phenylalanine conjugate within the active pocket of the androgen receptor (PDB ID: 7BW1) (Fig 1) are shown in Fig 1(A) and Fig 1(B), respectively.

Table 3 Docking scores of the ligands against five targets

Ligands no.	7BW1	1T65	3RUK	1A52	1U72
1	-10.0	-8.0	-9.1		-9.8
2	-10.4	-8.5	-9.0	-7.0	-9.1
3	-10.8	-7.7		-7.1	
4	-11.8	-9.5	-10.8		-9.7
5	-9.8	-7.0	-8.9	-6.9	-8.8

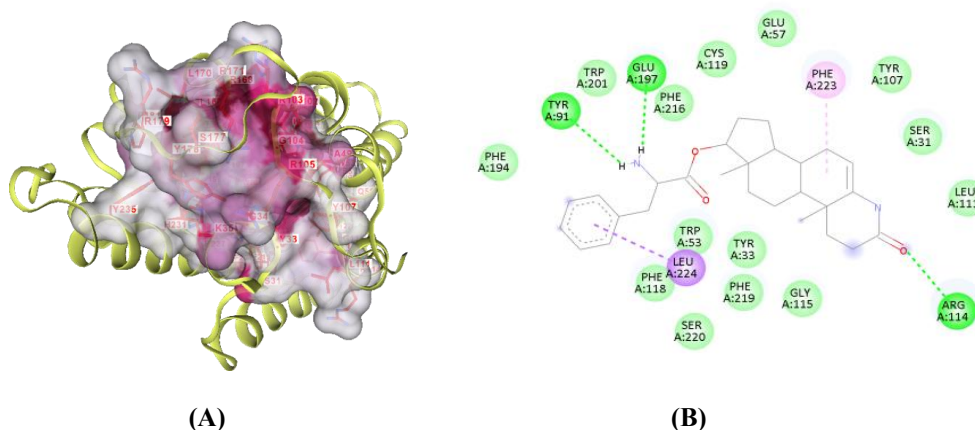


Fig 1 Molecular docking analysis of phenylalanine conjugate with androgen receptor (PDB ID: 7bw1): (A)3-D representation within the active pocket (B) 2-D representation showing ligand-amino acid residues interaction

3.3 Scale up reaction

The synthesis of the target aza-androgen steroid was successfully accomplished through a sequential oxidative cleavage, aza-ring formation, and selective reduction strategy, with the reaction conditions subsequently validated at 50 g scale as summarized in Table 3.1. In the first step, S-1 was subjected to ozonolysis using 25.0 g of ozone in a methanol/dichloromethane solvent system (250/250 mL) at -40 to -30 °C for approximately 2–3 h. The oxidative work-up was performed using 17.8 g of hydrogen peroxide and 32.1 g of formic acid, affording 46.9 g of the corresponding oxidized steroidal intermediate (S-2), demonstrating the successful conversion of the starting material under the optimized

ozonolysis conditions. The obtained S-2 was subsequently subjected to aza-ring formation using 13.9 g of liquid ammonia in 500 mL of ethylene glycol at 120–130 °C for 5 h. These conditions were established through systematic optimization of reagent stoichiometry, solvent, temperature, and reaction time and were found to provide efficient conversion to the desired aza-steroidal intermediate. On scaling up the optimized reaction to 50 g of S-2, the reaction proceeded smoothly and afforded 44.3 g of S-3, demonstrating good reproducibility and scalability of the process. In the final step, S-3 was subjected to selective reduction using 50.0 g of sodium borohydride in 500 mL of methanol at 0–5 °C for 5 h. Controlled low-temperature reduction afforded 47.2 g of the desired reduced aza-androgen steroid intermediate. The successful execution of all three transformations at 50 g scale demonstrates the robustness and reproducibility of the optimized synthetic sequence. In particular, the controlled temperature, appropriate reagent loading, solvent selection, and reaction time were found to be critical parameters for achieving efficient transformation at larger scale. Overall, the scale-up study confirmed the feasibility of the developed synthetic route and established a practical process for the preparation of the target aza-androgen steroid intermediate, providing a suitable basis for further process development and potential commercial-scale application. The isolated quantities and corresponding reaction parameters are summarized in Table 4.

Table 4 Scale up reaction

Precursor	Starting Material(g)	Reagent/eq	Solvent/Volume	Temp	Time	Obtained product(g)	% yield
1	50	O ₃ , 25.0 g; H ₂ O ₂ , 17.8 g; HCOOH, 32.1 g	MeOH/DCM, 250/250 mL	–40 to –30 °C	2-3h	46.9	93.8
2	50	Liq. NH ₃ , 13.9 g	Ethylene glycol, 500 mL	120–130 °C	5h	44.3	88.6
3	50	NaBH ₄ , 50.0 g	Methanol, 500 mL	0–5 °C	5h	47.2	94.4

3.4 Analytical Data interpretation

3.4.1. 5,17-dioxo-A-nor-3,5-secandrostan-3-oic acid (1) The obtained product was isolated as [4-aza-androst-5-ene-3,17-dione] slight yellow to orange semi solid; yield: 94.4%; ¹H NMR (400 MHz, CDCl₃) δ: 1.1(3-H, s, 19-CH₃), 0.9(3-H, s, 18-CH₃). ¹³C NMR (400 MHz, CDCl₃, δ ppm): 220.2, 214.1, 179.2, 50.6, 50.3, 47.9, 47.5, 37.6, 35.6, 34.3, 30.8, 29.8, 29.1, 27.01, 21.7, 20.6, 20.3, 13.6.

3.4.2 4-azaandrost-5-ene-3,17-dione (2) The obtained product was isolated as [4-aza-androst-5-ene-17-hydroxy-3-one] off white amorphous solid; yield: 94%; Melting point: 288.4°C –292.9°C; IR (cm⁻¹): 3055, 2940, 1736.6, 1657.4. ¹H NMR (400 MHz, CDCl₃) δ: 8.4(1H, s, -NH), 4.9(1H, s, 6-H), 1.1(3H, s, 19-CH₃), 0.9(3H, s, 18-CH₃). ¹³C NMR (400 MHz, CDCl₃, δ ppm): 220.5, 169.8, 140.08, 102.8, 51.4, 48.03, 47.5, 35.7, 34.1, 31.4, 31.2, 31.1, 28.5, 28.3, 21.7, 20.2, 18.6, 13.5. HRMS(ESI) calculated for C₁₈H₂NO₂ [M+H]⁺ + m/z: 288.5; found [M+H]⁺: 288.3.

3.4.3 4-azaandrost-5-ene-17-hydroxy-3-one (3) The obtained product was isolated as [4-aza-androst-5-ene-17-hydroxy-3-one] off white amorphous solid; yield: 94%; Melting point: 291°C –294°C; IR (cm⁻¹): 3063, 2937, 1659.4. ¹H NMR (400 MHz, CDCl₃) δ: 7.4(1H, s, -NH), 4.8(1H, s), 3.7(1H, t, C-6-H), 1.12(3H, s, 19-CH₃), 0.79(3H, s, 18-CH₃). ¹³C NMR (400 MHz, CDCl₃, δ ppm): 169.3, 140.01, 103.04, 81.6, 51.1, 48.0, 42.8, 36.2, 34.3, 31.7, 31.5, 30.4, 29.2, 28.4, 23.2, 20.5, 18.7, 11.03. HRMS(ESI) calculated for C₁₈H₂NO₂ [M+H]⁺ + m/z: 290.5; found [M+H]⁺: 290.3.

4 CONCLUSION

The present study successfully integrates computational screening with synthetic process development for a pharmacologically important aza-androgen precursor. In silico ADME evaluation identified aza-androgen-amino-acid conjugates with favorable drug-likeness profiles, and molecular docking against 5α-reductase type-2, androgen receptor, 17α-hydroxylase, estrogen receptor, and DHFR revealed promising binding affinities, particularly for the phenylalanine conjugate, supporting their potential as multi-target bioactive candidates. On the synthetic front, a practical three-step route to 4-azaandrost-5-ene-17-hydroxy-3-one — comprising ozonolysis, aza-cyclization, and selective reduction — was developed from androst-4-ene-3,17-dione and successfully scaled to 50 g with consistently high yields (88–94%) and reproducible quality, as confirmed by spectroscopic and mass-spectrometric characterization. These results demonstrate that the developed process is robust, scalable, and suitable for further process development toward commercial-scale manufacture. Together, the computational and experimental findings position 4-azaandrost-5-ene-17-hydroxy-3-one as a versatile precursor for the design and synthesis of next-generation aza-androgen therapeutics warranting further biological validation.

Author contributions

Shaik Mahmmad Raja: Formal analysis, compiling spectral characterizations, writing—original manuscript. Ruby Singh: Conceptualization, procurement of funds, supervision, and writing—reviewing and editing. Rimpal Satpathy: Formal analysis, writing—original manuscript. Pradeep Kumar Swain: Conceptualization, procurement of funds and supervision.

Sai Deepak Pasuparth: Writing—reviewing and editing. All authors have approved the final version of the manuscript, Rama S. Lokhande: Reviewing and Editing.

Conflicts of interest

The authors declare no conflict of interest.

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