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Green synthesis, biochemical and quantum chemical studies of steroidal oximes

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Abstract−Microwave-assisted condensation of steroidal ketones with hydroxylamine under solvent-free condition in presence of basic alumina as solid support yielded the title compounds. The compounds were subjected for their *in vitro* antimicrobial against a set of pathogenic bacteria and fungi. The 3β-chloro-5α-cholestan-6-one oxime (**6**) showed highest antimicrobial activity. Steroidal oximes were also screened for their *in vitro* anthelmintic activity against earthworms. Chloro compound was found to be a more potent anthelmintic agent when compared with albendazole. The *in silico* docking technique has been applied to ascertain the potential binding sites of the compounds with the pathogenic receptor. The binding modes of a representative compound **6** were accessed on the basis of molecular docking studies. Moreover the structure–antimicrobial activity relationships were studied using some physicochemical and quantum-chemical parameters with GAMESS interface as well as WebMO Job Manager by DFT at B3LYP/6-31G & STO-3G level of theory.

Keywords: Solid State Synthesis, Steroidal Ketone, Antimicrobial, *in silico* Analysis, Quantum Chemical Analysis

INTRODUCTION

Oximes constitute a class of pharmacologically active compounds with a wide spectrum of activities [1-3]. Introduction of oximide to steroidal skeleton imparts robust chemical and biological activities, and these tailored pharmacophores are present in many therapeutic agents or drugs. Moreover, the position of oxime group(s) on the steroidal skeleton exerts remarkable difference in their cytotoxicities [4-9]. Therefore, several oximes and their derivatives are considered to be pharmacologically active and are successfully employed as anti-microbial [10], anthelmintic [11] anti-inflammatory [8], hypotensive [6], hypocholesterolemic [12], gestagenic and diuretic agents [13]. Aromatase inhibitors [14,15] have also been reported among them. Oximes are frequently used in synthetic organic chemistry as intermediate for the transformation of nitrile, amide, nitrile oxides, amines, nitro compounds and also serves as protecting group for carbonyl compounds [16-24]. The most widely used method for the synthesis of oxime is condensation of ketone with hydroxylamine-hydrochloride in the presence of sodium acetate or pyridine under refluxing condition in an alcoholic media [25-28]. Nevertheless, the drawback is low yield, high reaction time, toxicity of reagent and difficulties in product handling and isolation.

Any technological invention has many benefits but at the same time it also suffers from certain limitations, same as in the case with microwave. During the last decade, microwave-assisted synthesis has increasingly been used in organic synthesis because of higher yield, shorter reaction time and milder reaction conditions. Most importantly, microwave reactions can result in products which are almost impossible to obtain using conventional synthesis. Thus, a clean, mild and efficient method to synthesize oximes is developed using basic alumina as solid support under solvent free conditions. These conditions together constitute green synthesis. It not only improves yield but also saves the reaction time [29-31]. To the best of our knowledge there are neither reports about antimicrobial/anthelmintic activity nor any information of steroidal oximes synthesis under microwave irradiation conditions. Hence, the green synthetic procedure was employed to validate the synthesis of reported steroidal oximes. The identity of the synthesized compounds has been ascertained by their physical, analytical and spectral data. The *in vitro* biological activity and the subsequent molecular docking studies of the steroidal oximes have also been carried out. Finally, quantum-chemical and physicochemical calculations were carried out by DFT at B3LYP/6-31G & STO-3G model and Molinspiration software to study the relationship between the electronic properties and physicochemical parameters with antimicrobial activity of steroidal oximes analogues, respectively. In physicochemical parameters, such as partition coefficient (log P), molecular weight, topological polar surface area (TPSA), number of rotatable bonds, counts of hydrogen bond acceptors and donors of compounds, are frequently applied to predict bioavailability or membrane permeability. Lipinski's rule 5 is widely used to determine molecular properties that are important for a drug's pharmacokinetic properties *in vivo* [32]. It is used as a drug filter for drug that helps pharmaceutical scientists select the best candidates for development with a high probability of success. In quantum-chemical studies, density functional theory (DFT), molecular mechanics, semi-empirical and *ab-initio* methods are often used to calculate molecular properties. These methods give information regarding vibration modes, molecular geometry, heat of formation, force constants, electron densities, dipole moments, population analysis, chemical reaction pathways

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and thermodynamic properties etc.

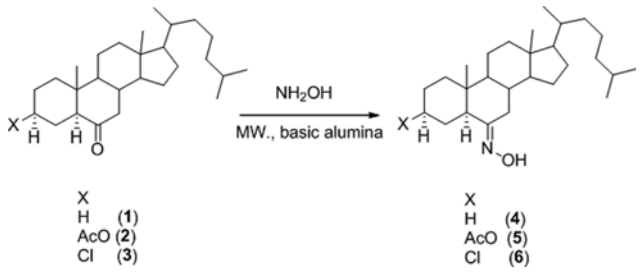
EXPERIMENTAL

1. Materials

Unless otherwise indicated, all common reagents and solvents were procured from standard sources and used without further purification. The starting materials 5α-cholestan-6-one (**1**), its 3β-acetoxy (**2**) and 3β-chloro (**3**) were synthesized by literature methods [33-36]. Melting points were determined on a Kofler apparatus and were uncorrected. The IR spectra were recorded on KBr pellets with Shimadzu IR-408 Perkin-Elmer 1800 (FTIR), and the values were given in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Avance-II 400 MHz instrument with TMS as internal standard and J values are in Hertz. Chemical shifts were reported in ppm (δ) relative to the solvent peak. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Elemental analyzes (C, H, N) were conducted using Carlo Erba analyzer model 1108. Thin layer chromatography (TLC) glass plates (20×5) were coated with silica gel (E-Merck G254, 0.5 mm thickness) and exposed to iodine vapours to check the purity as well as the progress of reaction.

2. Preparation of Steroidal Oximes: General Procedure

A mixture of steroidal ketone (**1**, **2** or **3**) (1 mmol), hydroxylamine hydrochloride (1.2 mmol) and basic alumina (2.0 g) was ground thoroughly in a mortar. The reaction mixture in 25 ml reaction flask was heated in a microwave oven (power-time method) in time interval 30 s to 4.0 min, respectively, and monitored by tlc. After completion of the reaction, the beaker was cooled and the organic matter was diluted with water and filtered. The precipitate was dissolved in ether and washed with water and dried over anhydrous sodium sulfate; evaporation of solvent and crystallization from dry methanol afforded good yields of compound **4**, **5** or **6**, respectively (Scheme 1). The compounds obtained gave satisfactory C, H and N analysis and were characterized by comparison with authentic sample (m.p., mixed m.p., IR, ^{1}H &^{13}C NMR and MS) [37-40].



Scheme 1. Synthesis of steroidal oximes (4-6).

Comparison of conventional and microwave synthesis for **4**-**6**: (1) Conventional procedure; Compound **4**, yield: 60%, time: 4 hrs, compound **5**, yield: 85%, time: 4 hrs, compound **6**, yield: 65%, time: 6 hrs, (2) Microwave procedure; Compound **4**, yield: 78%, time: 3.5 min, compound **5**, yield: 90%, time: 2.5 min, compound **6**, yield: 85%, time: 3.0 min, respectively.

3. Antimicrobial Activity

3-1. Antibacterial Studies

The *in vitro* antimicrobial activities of steroidal oximes **4**-**6** were done using the bacterial cultures of *Staphylococcus pyogenes*, *Staphylococcus aureus* (MRSA +Ve), *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli* bacterial strains by disc diffusion method [41]. Ciprofloxacin was used as positive control, while the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls (Table 1).

3-2. Antifungal Studies

In vitro antifungal screening of the compounds against different strains of fungi *Penicillium marneffei*, *Aspergillus fumigates*, *Trichophyto nmentagrophytes*, *Candida albicans* and *Candida krusei* in

Table 1. Antibacterial screening data of synthesized steroidal oximes (4-6)

Compounds	Diameter of zones of inhibition (mm)				
	Gram positive bacteria		Gram negative bacteria		
	S. pyogenes	*S. aureus*	*S. typhi*	*P. aeruginosa*	*E. coli*
4	18.9	17.3	16.3	15.9	20.9
5	19.4	18.7	18.1	16.3	21.7
6	20.5	19.3	19.1	17.3	23.9
Standard	23.0	22.0	29.0	31.0	27.5

Negative control (DMSO) measured by the zone test (unit, mm)

Table 2. Antifungal activity of steroidal oximes (4-6); Diameter of zone of inhibition (mm)

Compounds	*P. marneffei*	*A. fumigatus*	*T. mentagrophytes*	*C. albicans*	*C. krusei*
4	11.7	16.8	14.6	21.4	14.1
5	14.1	19.4	16.3	22.4	13.5
6	15.1	22.6	17.6	25.5	16.6
Standard	21.5	26.5	24.0	30.5	18.5

Negative control (DMSO) measured by the zone test (unit, mm)

DMSO was also by using agar disk diffusion method [42]. The fungal activity of each compound was compared with Amphotericin B, a standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.

4. Anthelmintic Activity

Anthelmintic activities of the steroidal oximes (**4**-**6**) were tested at two different concentrations against the earthworms applying standard protocol [43]. Indian earthworms, phreretima posthuma, have been used widely for the *in vitro* anthelmintic screenings because of their anatomical and physiological resemblance with the most destructive of human parasitic helminths and for their easy availability. Earthworms were collected from moist soil and washed with normal saline to remove all the adhering debris. Worms of 3-5 cm in length and 0.1-0.2 cm in diameter were used for biological assay. The standard drug and test compounds were dissolved in the minimum amount of DMSO and adjusted the volume up to 10 ml with normal saline solution to get the concentrations of 0.20% w/v and 0.50% w/v. A group of three earthworms of nearly equal size were taken for each concentration and released into separate Petri dishes containing normal saline, standard drug solution, and test compound's solutions of the mentioned concentrations at room temperature. Normal saline was used as a control. Observations were made on the basis of time taken for paralysis and death of individual earthworms. The mean paralysis time and lethal time of the earthworms for different test compounds and standard drug are tabulated in Table 3. All the results were expressed as Mean± S.E.M. of three worms in each group.

5. *in silico* Studies

The retrieved protein 3H2X (pdb) was improved by using import and preparation option of MVD software, and missing bond order, hybridization state, angle and flexibility for achieving reliable potential binding site in receptor. The energy minimized ligands (or synthesized compounds) were drawn with ChemDraw Ultra (2D and 3D) and the prediction of ligand structural properties was carried by Discovery studio [44], MVD [45] and LigandScout [46]. These programs were used to perform molecular docking and energy profile of ligand-receptor interaction, independently. The GEMDOCK, which is a useful tool for molecular recognition, is also used to systematically evaluate and thus improve scoring functions. GEMDOCK provides interactive interfaces to prepare both the binding site of the target protein and the screening ligand library. Each ligand in the mentioned library was docked into the binding site. The scoring function obtained by Gemdock [47], which is based on a piece-wise linear potential and the scoring function, is now extended with a new term to take H-bond directionality into account. GEMDOCK generates and calculates the different interactions involved between receptor and guest, i.e., electrostatic, hydrogen and van der Waals interactions. Based on these interactions leading energy profiles of the docked molecules, GEMDOCK, finally, ranks and visualizes the docking compounds by combining the pharmacological interactions and energy based scoring function of GEMDOCK.

Table 3. Anthelmintic activity of steroidal oximes (4-6)

Compounds	Mean paralysis time (min) at conc. in % w/v		Mean death time (min) at conc. in % w/v	
	0.20	0.50	0.20	0.50
4	2.6±0.4	2.1±0.4	4.5±0.1	4.0±0.2
5	1.9±0.2	1.4±0.5	4.0±0.3	3.6±0.5
6	2.3±0.3	2.0±0.3	4.2±0.2	3.8±0.3
Albendazole	1.6±0.1	1.1±0.3	3.5±0.1	2.90±0.2
Control	-	-	-	-

6. Computational Methods

The frontier molecular orbitals (FMOs) and physicochemical properties of the compounds play a vital role in generation and escalation of bioactivity [48,49]. Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are important parameters; both are called frontier orbitals and determine the interaction with other species in biological as well as in chemical systems using their outermost orbitals through making a plate form of accepting and donor system. Therefore, the energy of the HOMO and LUMO is directly related to the ionization potential and the electron affinity, respectively. The physicochemical parameters of the synthesized compounds were determined by applying the following programs: CS ChemBioDraw Ultra, MarvinSketch and Molinspiration software. There are fourteen possible molecular descriptors consisting of logP, molecular weight, TPSA, nature of hydrogen bond, number of violation to Lipinski's rule, number of rotatable bonds, volume, drug likeness includes G protein coupled receptor (GPCR) ligand, ion channel ligand, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor, for any chemical compounds. CLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. TPSA is calculated based on the methodology published by Ertl et al. [50] as a sum of fragment contributions, oxygen and nitrogen centered polar fragments are considered. The maps of molecular lipophilicity potential (MLP) and TPSA were viewed in Molinspiration available online and ChemAxon software.

RESULTS AND DISCUSSIONS

1. Chemistry

A condensation reaction between steroidal ketones (**1**-**3**) and hydroxylamine hydrochloride was achieved by using microwave irradiation under solvent-free reaction conditions employing basic alumina as the solid support yielded the 5α-cholestan-6-one oxime (m.p., 205 °C, lit. m.p., 206 °C; [37]) (**4**), 3β-acetoxy-5α-cholestan-6-one oxime (m.p., 200 °C, lit. m.p., 206 °C; [38]) (**5**) and 3β-chloro-5α-cholestan-6-one oxime (m.p., 175 °C, lit. m.p., 175-176 °C; [39]) (**6**). In terms of reaction time, microwave irradiation needs only 30 s to 3 min, while conventional synthesis requires several hours and results in poor yields. The formation of compounds (**4**-**6**) is evidenced by the disappearance of carbonyl band of steroidal ketones at 1,731-1,735 cm^{-1} and appearance of intense bands due to C=N, N-O and OH of oxime (=N-OH) in the region 1,643-1,669, 1,371-1,415 and 3,160-3,169 cm^{-1}, respectively, confirming the change in functionality. The oximes (=N-OH) proton resonates as broad singlet in the region 12.09-12.15 ppm. The ^{13}C NMR assignments of

the compounds (**4**-**6**) also support the formation of desired products. ^{13}C NMR spectra displayed a characteristic signal in the region of 55.9-60.0 ppm due to the carbon atoms of C=N group. The spectral and elemental data of the compounds (**4**-**6**) were also found in close proximity to the literature data [37-40,51,52].

2. Antimicrobial Study

Steroidal oximes **4**-**6** were screened for their *in vitro* antimicrobial activity against *S. Pyogenes*, *S. aureus* (MRSA +Ve), *S. typhi*, *P. aeruginosa*, *E. coli*, *P. marneffei*, *A. fumigates*, *T. mentagrophyte*, *C. albicans* and *C. krusei*. The *in vitro* study demonstrated that the compound **5** was most active in antibacterial as well as antifungal activity. The zone of inhibition of compound **6** was 23.9 mm against *Escherichia coli*, while the zone of inhibition of **6** was 25.5 mm against *Candida albicans* (Table 1, 2). The high potency of **6** may be attributed to the presence of a certain number of H-bond acceptors with proper orientation and electron withdrawing nature of the present group at 3-position. To verify the above results, the *in silico* screening of compounds (**4**-**6**) was also performed.

3. Anthelmintic Activity

The efficiency of the tested compounds as anthelmintics agent against earthworms at two different concentrations, 0.20 and 0.50, are shown in Table 3 along with the standard drug used for comparison of the results obtained in an experiment. The result of this activity indicates that compound **6** has higher activity than that of compounds **4** and **5**. Compound **6** shows early paralysis and lethal time 1.9±0.2 & 1.4±0.5 and 4.0±0.3 & 3.6±0.5, respectively, at different concentrations of the tested steroidal oxime. The higher activity of **6** among analogous is due to the presence of chloro group at the 3-position of steroidal moiety. The better activity of the compound might be explained on the basis of the electron withdrawing nature of the present group in compound **6**, which plays an important role in biological activity. The functionalized system may be responsible for the enhancement of hydrophobic character and liposolubility of the molecules.

4. Structure Activity Relationship (SAR)

The structure activity relationship of the compounds can be ascertained on the basis of its 3D coordinative structures. This is further strengthened by analyzing their biological assay, notably by screening the synthesized compounds for their plausible antimicrobial activity. The study of SAR gives information of the functional groups present in the skeleton and their behavior in a targeted biological mechanism in the organism. Though, it is difficult to draw conclusions about molecular events from the structure activity relations. We have attempted to simplify the structure activity

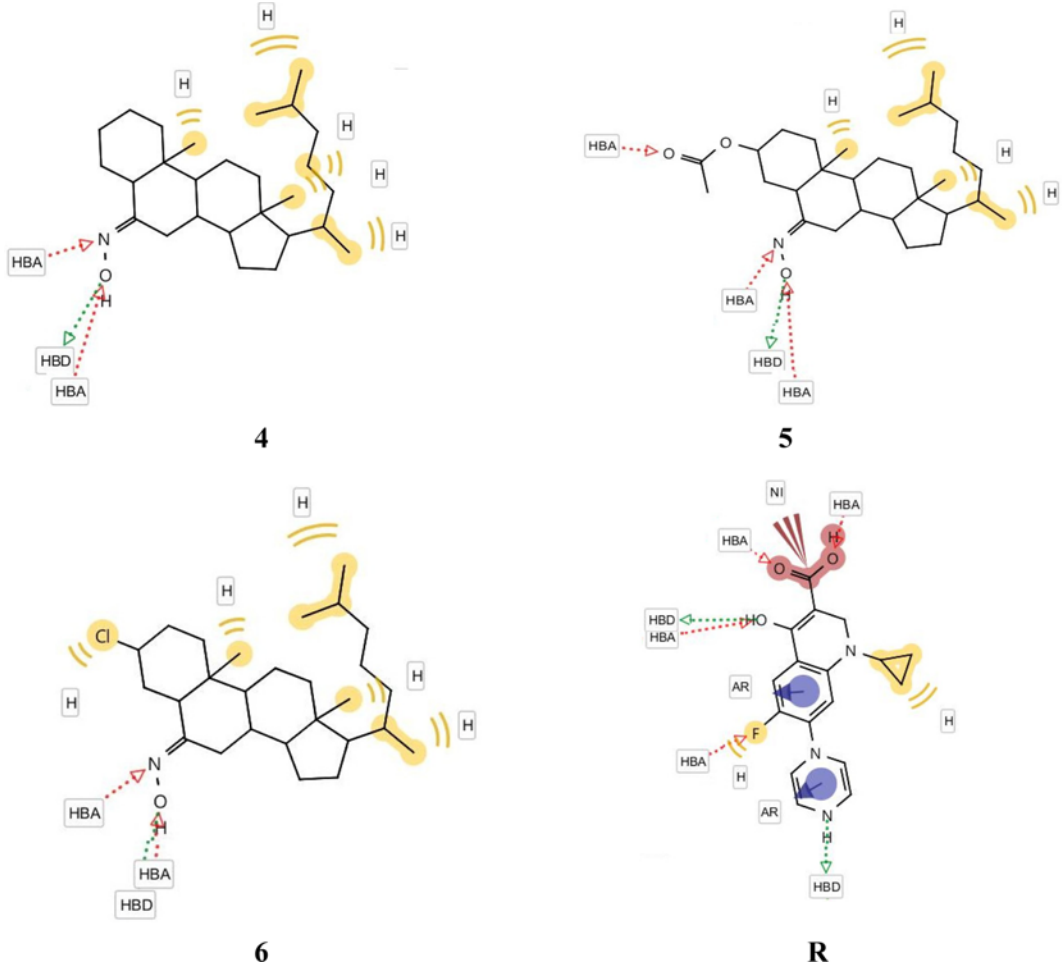


Fig. 1. Active sites of steroidal oximes (4-6) and reference drug (R) having four hydrogen acceptor sites including one hindrance site (Fluorine atom) while compound 6 with easily accessible sites.

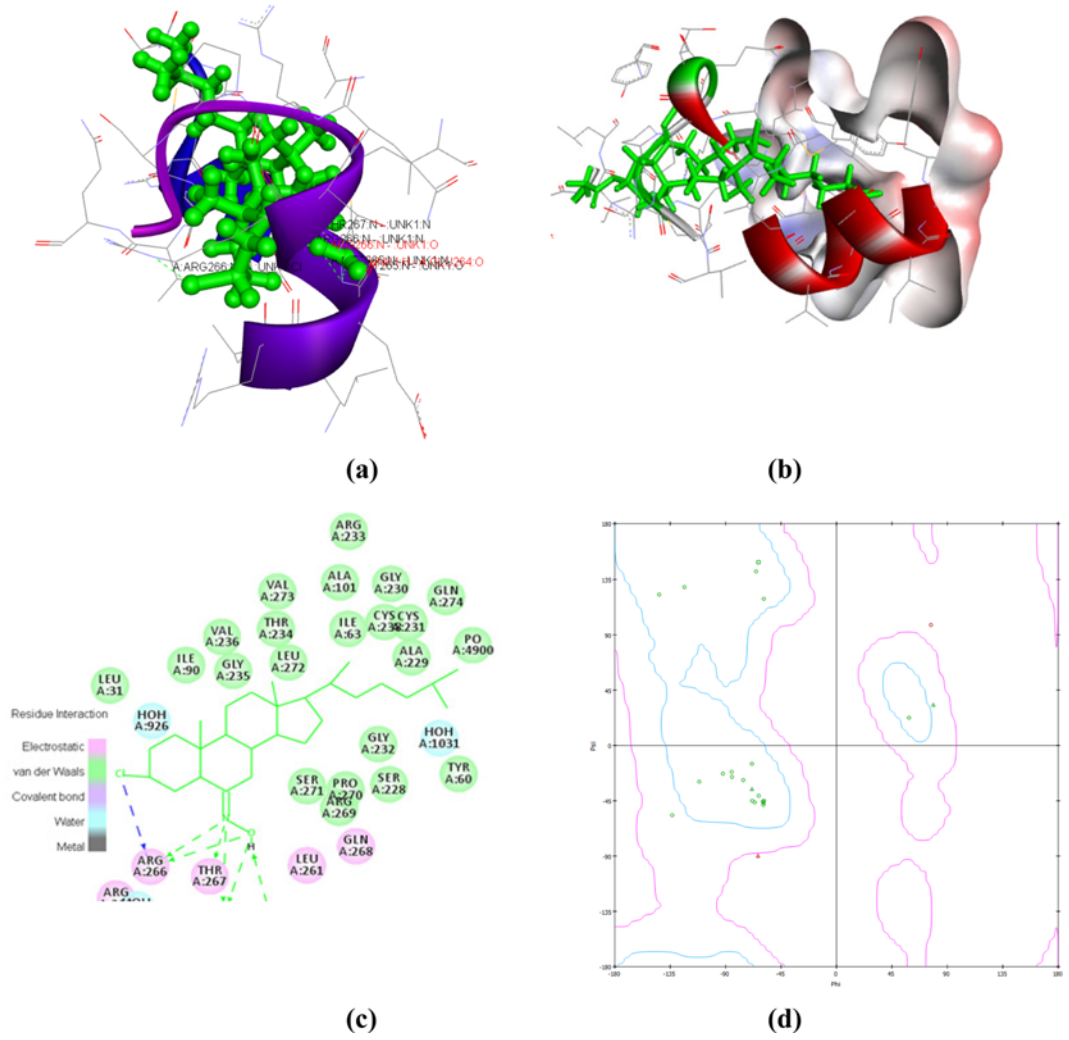


Fig. 2. (a) The binding mode of compound 6 inside the receptor active sites (3H2X pbd) showing hydrogen bonds between MET265:N - :UNK1:N, MET265:N - :UNK1:O, ARG266:N - :UNK1:N, ARG266:N - :UNK1:O, ARG266:NE - :UNK1:Cl, THR267:N - :UNK1:N, UNK1:H - A:GLU264:O residues and LIGAND (UNK) (b) receptor-ligand interaction surfaces including lipophilicity, H-bonding and solvent accessibility properties. (c) pharmacophore models of protein-ligand interactions: Red arrow represents the hydrogen bond acceptor (HBA); Green arrow represents the hydrogen bond donor (HBD); Brown color indicates hydrophobic (H) and (d) Ramachandran plot for the active residues after involving receptor and ligand interactions.

relations with compounds having substituent at the 3-position of steroidal skeleton. The SAR of the steroidal oximes was done by introducing the substituent at 3-position in steroidal skeleton of the products. The antimicrobial results reveal that compounds having additional functional groups in the cholestane moiety at position 3 (**5** and **6**) (Fig. 2) have potent inhibitory activity, while the non-substituted oxime **4** exhibited poor activity against most of the strains. This behavior may be attributed to the lack of interaction between compounds and receptor (amino acids). The poor activity of compound **4** may also be due to the unavailability of a lone pair to make certain number of hydrogen bonds (acceptor hydrogen bond AHB and donor hydrogen bond DHB) with receptor in compliance with the concept of drug-likeness Fig. 1.

5. *in silico* Screening

The molecular docking studies were performed to rationalize the obtained antimicrobial results. Hence, the antibacterial data of most active compound (**6**) was investigated on structural basis, molecular modelling and docking study against the pathogenic protein (3H2X pbd) using MVD and Discovery studio software to predict the affinity, orientation and surrounding surface (Fig. 2) of the synthesized compounds at the active site. The different bonds, *i.e.,* hydrogen bonds, van der Waals forces and hydrophobic behavior with amino acids, were in good agreement with the predicted binding affinities obtained by molecular docking studies as verified by antibacterial studies where compound **6** (Fig. 2) was the most active compared with standard drug (Fig. 1R). Based on the molecular docking studies, it was found that the MET265:N - :UNK1:N, MET265:N - :UNK1:O, ARG266:N - :UNK1:N, ARG266:N - :UNK1:O, ARG266:NE - :UNK1:Cl, THR267:N - :UNK1:N, and UNK1:H - A:GLU264:O residues (Fig. 2 and 3) interacted in proper orientation with comparatively high frequency with compound **6**. The empirical scoring function of GemDOCK (Fig. 4) is the estimated sum total of

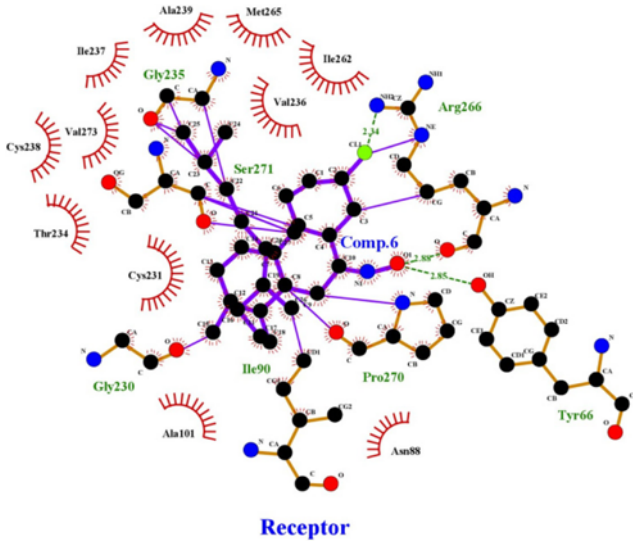
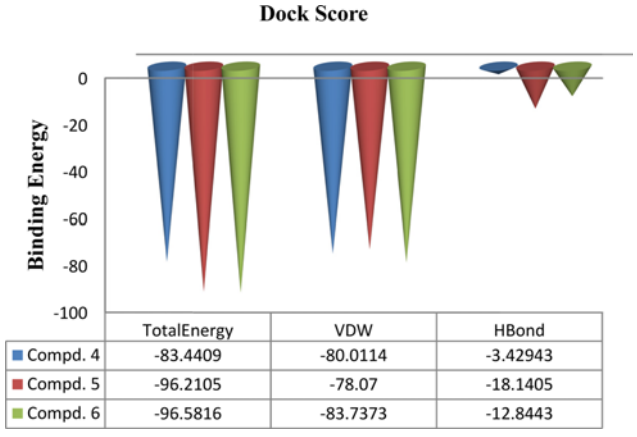


Fig. 3. Hydrophobic interactions (arcs) and hydrogen bonding between (dotted line) ligand (6) and receptor (3H2X pdb).



	TotalEnergy	VDW	HBond
Compd. 4	-83.4409	-80.0114	-3.42943
Compd. 5	-96.2105	-78.07	-18.1405
Compd. 6	-96.5816	-83.7373	-12.8443

Fig. 4. Estimated binding affinities of compounds (4-6) based on docked poses within the active site of target enzyme (PDB: 3H2X). (Where VDW=van der Waals' interaction energy, HBond=Hydrogen bonding interaction energy).

van der waals, H-bonding energies along with some important secondary forces. From the combined table and chart shown in Fig. 4, the compound **6** demonstrated better affinity to receptor and showed better docking score as it was buried well inside the cavity of the target protein. Moreover, the chloro-group present at the 3-postion of steroidal skeleton, which is electron withdrawing, creates electron deficiency around skeleton. This deficiency results in a strong interaction with biological molecules carrying a negative charge which brings the field of electromagnetic attraction into existence, causing either the death or check of microorganisms.

LigPlot software was used to understand the in-depth interaction between the docked ligands (synthesized compounds) and the active site residues. The number of hydrophobic and hydrogen bonds interactions and their pattern were matched with the help of diagram automatically generated by LigPlot [53]. Hence, LigPlot as an alternative ligand analysis tool is useful in knowing the hydrophobic interaction and hydrogen bond pattern after successful docking operations.

6. Computational Methods

For the antimicrobial study of steroidal oximes (**4-6**), quantum chemical and physicochemical calculations were carried out by DFT at B3LYP/6-31G and STO-3G using Gamess interface as well as WebMO Pro Job Manager for calculation and Avogadro for visualization and analysis. Quantum-chemical and physicochemical parameters of steroidal oximes are listed in Table 4. The calculated value of the energy of compound **6** (−5250369.76 KJ/mol) is lower than that of **4** (−3061044.43 KJ/mol) and **5** (−3649130.22 KJ/mol), indicating the more thermodynamic stability of **6**. The HOMO-LUMO energy difference of compound **6** is less than that of compound **4** and **5**. The energies of the FMOs are important properties in several chemical and pharmacological strategies. The difference in the energies of FMOs establishes properties of the system and gives information on the electron donating and accepting character of a compound bridging between HOMO-LUMO systems (Fig. 5). The energy of the highest occupied molecular orbital (E_{HOMO}) measures the electron-donating character of a compound, and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) measures its electron accepting character. From these definitions, the greater the E_{HOMO}, the greater will be the electron-donating capability, and the smaller the E_{LUMO}, the smaller will be the resistance to accept electrons. For the active compounds, the E_{HOMO} must bring negative values, whereas the less active/inactive compounds must have positive values, as shown in Table 4. Compounds showing less activity in a pharmacological process are more efficient electron donor compounds than that of the active ones. Though, the inactive compounds may interact through charge transfer mechanism with some compounds before reaching the biological receptor. Moreover, dipole moments as shown in Table 4 are highly correlated with the activity followed by molecular weight. It is observed that the higher the dipole moment and the higher the activity.

Lipophilic character of molecules plays an important role in determining molecular reactivity in a biological process and depends on two important factors: hydrophobicity and polarity. These factors of the molecules facilitate to cross the cellular membrane consisting of a number of heterogeneous phases or irreversibly damage the cellular membrane. Fig. 6 shows the molecular lipophilicity

Table 4. Comparison of Quantum-chemical and Physicochemical properties of steroidal oximes (4-6)

Comp.	Energy (kJ/mol)	Volume (Å^3)	LUMO (eV)	HOMO (eV)	Dipole (Db)	Log P	PSA
4	−3061044.43	435.774	0.56	−7.31	2.24	7.990	32.592
5	−3649130.22	480.329	0.38	−7.45	2.00	7.057	58.897
6	−4250369.76	449.335	0.27	−7.55	3.64	7.960	32.592

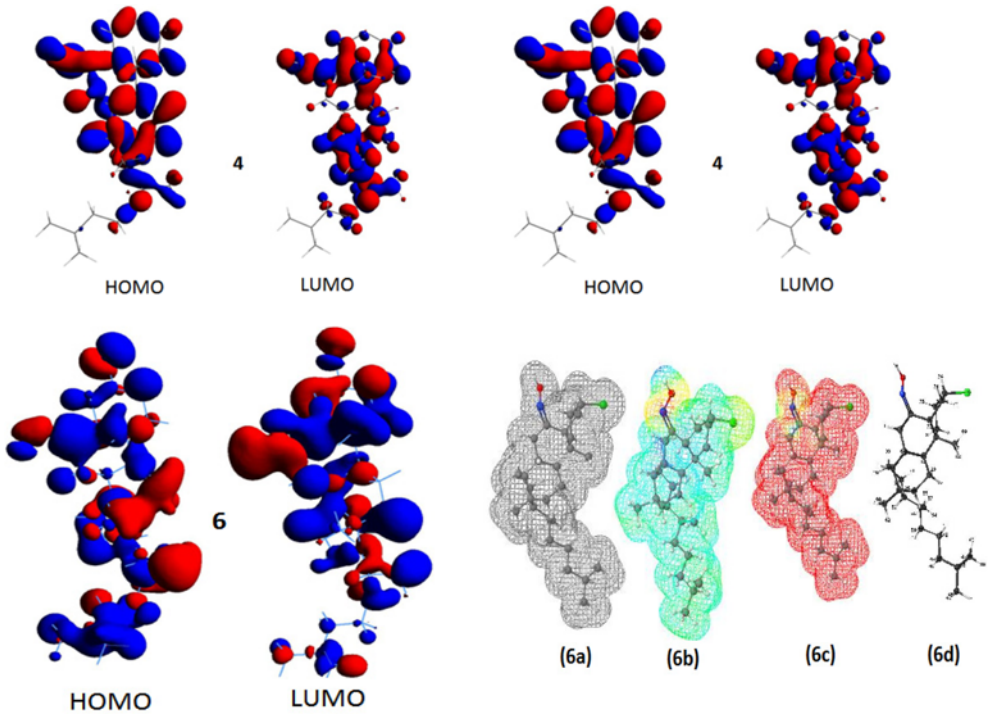


Fig. 5. HOMO and LUMO isosurface for 4-6 (Different surface colours show opposite signs of wave function along with Electron Density 6a, Electrostatic Potential 6b, Radical Frontier density surfaces 6c and Geometry optimized structure 6d.

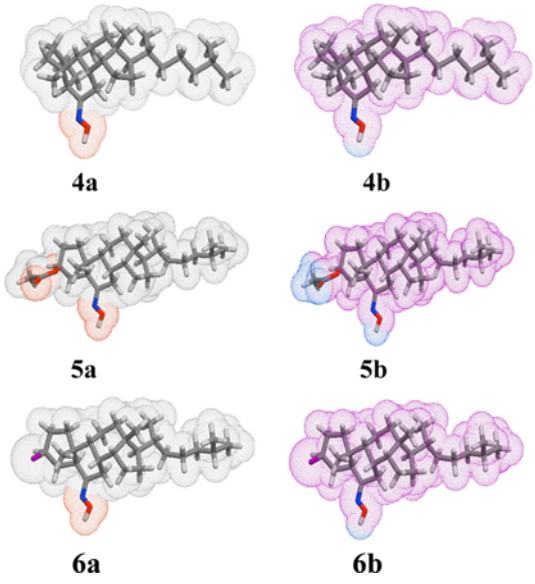


Fig. 6. 3D sketch of the compounds (4-6) with molecular lipophilicity maps, 4a-6a (Left Side) and polar surface pockets, 4b-6b (Right Side) showing the most lipophilic area (pink colour), intermediate lipophilic area (green colour), most hydrophobic area (blue colour), nonpolar area (gray white colour) and polar area (red colour).

potential map (MLP) of the synthesized compounds (**4-6**), suggesting that the compound with acetoxy group at 3-position of steroidal skeleton in cholestane series is more lipophilic than that of its analogus (**4** and **6**). In the present study, the *log P* of the most active compounds is higher under the certain limits than those of less active compounds in a biological process. Among the three compounds (**4-6**), a trend was observed in which the antimicrobial activity decreases with increasing the log P. Log P is usually correlated with the biological activity. According to the Lipinski rule of five, the majority of "drug-like" molecules have log P≤5, number of hydrogen bond acceptors ≤10, molecular weight ≤500 and number of hydrogen bond donors ≤5. Molecules violating more than one of these rules may have problems with bioavailability. However, there are some exceptions to this rule, and a compound is likely to be orally active as long as it does not break more than one of his rules because some orally active drugs such as atorvastatin, cyclosporin do not obey the rule of five. Partition coefficient or *Log P* is an important parameter used in rational drug design to measure molecular hydrophobicity. Hydrophilic/lipophilic nature of drug molecule affects drug absorption, bioavailability, drug-receptor interactions, metabolism of molecules, as well as their toxicity. The log P values of derivatives were found to be in the range of 7.857-8.43 and are a clear violation of Lipinski's rule of five. None of the compounds (**4-6**) fulfilled Lipinski's rule as their Log P score was above **5** and **6** suggesting these compounds are highly lipophilic with very poor aqueous solubility. Moreover, molecular volume also plays an important role in SAR studies to model molecular properties and biological activity. It was also observed that the active compounds **5** and **6** have higher molecular volume as compared to **4**. Molecular polar surface area (PSA) is closely related to the hydrogen bonding potential of a molecule and is a very useful parameter for prediction of drug transport properties. From Fig. 6 and Table 4, all the compounds (**4-6**) were found in the range of 32.592-58.897 and below the limit, that is, 160 A^{o} in respect of PSA, which showed that molecules are fulfilling the optimal requirement for

drug absorption.

CONCLUSIONS

The present finding provides a convenient, eco-friendly, short reaction time and simple work-up method for the preparation of steroidal oximes in the presence of basic alumina using microwave irradiation under solvent-free conditions. The *in vitro* biological screening data was further investigated by *in silico* analysis that proves that steroidal oximes are better antimicrobial and anthelmintic agents against different strains and earthworms, respectively. Quantum-chemical and physicochemical calculations indicate that antibacterial activity correlates well with HOMO-LUMO energy difference of molecules and calculated log *P* under certain limits. The highest antibacterial activity of compound **5** and the results of computational studies would be helpful in synthesis of a large library of oximes analogues for extensive antimicrobial and anthelmintic studies, which would be used to develop a more appropriate drug candidate.

REFERENCES

1. J. Kassa, K. Kuca, J. Karasova and K. Musilek, *Mini-Rev. Med. Chem*., **8**, 1134 (2008).
2. V. O. Kuryanov, T. A. Chupakhina, A. A. Shapovalova, A. M. Katsev and V. Y. Chirva, *Russ. J. Bioorg. Chem*., **37**, 231 (2011).
3. F. Worek and H. Thiermann, Strategies for the development of effective broad spectrum oximes, In defence against the effects of chemical hazards, Toxicology, Diagnosis and Medical Countermeasures (pp. 30-1-30-6). Meeting Proceedings RTO-MP-HFM-149, Paper 30. Neuilly-sur-Seine, France: RTO.
4. J. Cuia, L. Fana, L. Huanga, H. Liub and A. Zhou, *Steroids*, **74**, 62 (2009).
5. J.-G. Cuia, L. Fana, Y. Huanga, Y. Xinb and A. Zhou, *Steroids*, **74**, 989 (2009).
6. N. M. Krstić, M. S. Bjelaković, Ž. Žižak, M. D. Pavlović, Z. D. Juranić and V. D. Pavlović, *Steroids*, **72**, 406 (2007).
7. Y. Huang, J. Cui, Q. Zheng, C. Zeng, Q. Chen and A. Zhou, *Steroids*, **77**, 829 (2012).
8. D. P. Jindal, R. Chattopadhaya, S. Guleria and R. Gupta, *J. Med. Chem*., **38**, 1025 (2003).
9. R. Bansal, S. Guleria, C. Ries and R. W. Hartmann, *Arch. Pharm*., **343**, 377 (2010).
10. M. I. Sikharulidze, N. Nadaraia, M. L. Kakhabrishvili, N. N. Barbakadze and K. G. Mulkidzhanyan, *Chem. Nat. Compd*., **46**, 493 (2010).
11. Y. Horii, Y. Otsuka, M. Tateishi, S. Makimura and K. Kusano, *J. Vet. Med. Sci*., **60**, 271 (1998).
12. E. W. Cantrall, R. Littell, S. M. Stolar, W. P. Cekleniak, H. J. Albers, S. Gordon and S. Bernstein, *Steroids*, **1**, 173 (1963).
13. N. V. Kovganko, S. N. Sokolov, Y. G. Chernov, Z. N. Kashkan and V. L. Survilo, *Chem. Nat. Compd*., **46**, 750 (2010).
14. S. Dubey, D. P. Jindal and P. Piplani, *Ind. J. Chem*., **44B**, 2126 (2005).
15. M. Pokhrel and E. Ma, *Molecules*, **16**, 9868 (2011).
16. N. Iranpoor, H. Firouzabadi and G. Aghapour, *Synth. Commun*., **32**, 2535 (2002).
17. L. G. Donaruma and W. Z. Helst, *Org. React*., **11**, 1 (1960).
18. (a) P. A. S. Smith and J. E. Robertson, *J. Am. Chem. Soc*., **84**, 1197 (1962); (b) E. J. Buehler, *Org. Chem*., **32**, 261 (1967); (c) E. F. Schoenewaldt, R. B. Kinnel and P. Davis, *J. Org. Chem*., **33**, 4270 (1968).
19. (a) Y. H. Chiang, *J. Org. Chem*., **36**, 2146 (1971); (b) K. C. Liu, B. R. Shelton and R. K. Howe, *J. Org. Chem*., **45**, 3916 (1980).
20. A. R. Hajipour and N. Mahboubghah, *J. Chem. Res. (S),* 122 (1998).
21. P. R. Dave and F. Forshar, *J. Org. Chem*., **61**, 8897 (1996).
22. J. K. Whitesell and M. A. Whitesell, *Synthesis*, 517 (1983).
23. T. Shinada and K. Yoshihara, *Tetrahedron Lett*., **37**, 6701 (1995).
24. (a) J. P. Ley and H. J. Bertram, *Bioorgan. Med. Chem*., **9**, 1879 (2001); (b) C. Shen and D. S. Sigman, *Arch. Biochem. Biophys*., **286**, 596 (1991).
25. I. L. Finar and G. H. Lord, *J. Chem. Soc*., 3314 (1957).
26. (a) J. T. Li, X. L. Li and T. S. Li, *Sonochem*., **13**, 200 (2006); (b) J. J. Guo, T. S. Jin, S. L. Zhang and T. S. Li, *Green Chem*., **3**, 193 (2001); (c) H. Sharghi and M.-H. Sarvari, *J. Chem. Res*. (S), 24 (2000).
27. (a) K. Niknam, B. Karimi and A. R. Kiasat, *Bull. Korean Chem. Soc*., **26**, 975 (2005); (b) H. Eshghi, M. H. Alizadeh and E. Davamdar, *J. Korean Chem. Soc*., **52**, 52 (2008); (c) I. Damljanovic, M. Vukicevic and R. D. Vukicevic, *Monatsh. für Chem*., **137**, 301 (2006).
28. (a) J. J. Xia and G. W. Wang, *Molecules*, **12**, 231 (2007); (b) H. Zang, M. Wang and B. W. Cheng, *J. Song, Sonochem*., **16**, 301 (2009).
29. M. Kidwai, B. Dave and K. R. Bhushan, *Chem. Papers*, **54**, 231 (2000).
30. M. Kidwai, K. R. Bhushan, P. Sapra, R. K. Saxena and R. Gupta, *Bioorg. Med. Chem*., **8**, 69 (2000).
31. M. Kidwai, *Pure Appl. Chem*., **73**, 147 (2001).
32. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Deliv. Rev*., **23**, 4 (1997).
33. D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc*., 2876 (1955).
34. C. W. Shoppee , R. H. Jenkins and G. H. R. Summers, *J. Chem. Soc*., 1657 (1958).
35. R. M. Dodson and B. Riegel, *J. Org. Chem*., **13**, 424 (1948).
36. A. Windaus and O. Dalmer, *Ber*., **52**, 162 (1919).
37. (a) D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc*., 2876 (1955); (b) H. Suginome and H. Takahashi, *Bull. Chem. Soc. Jpn*., **48**, 576 (1975).
38. (a) C. E. Anagnostopoulos and L. F. Fieser, *J. Am. Chem. Soc*., **76**, 532 (1954); (b) J. Barnett, B. R. Ryman and F. Smith, *J. Chem. Soc*., 528 (1946); (c) L. Knof, *Justus Liebigs Ann. Chem*., **642**, 194 (1961).
39. (a) Shafiullah, M. R. Ansari and S. Husain, *Bull. Chem. Soc. Jpn*., **60,** 3411 (1987); (b) E. G. Ford, P. Chakravorty and E. S. Wallis, *J. Am. Chem. Soc*., **60**, 413 (1938).
40. H. Duddeck, J. Frelek , C. Kriiger, G. Snatzke,W. J. Szezepek, P. Wagner and S. Werner, *Tetrahedron: Asymmetry*, **3**, 613 (1992).
41. (a) I. Galani, F. Kontopidou, M. Souli, P. D. Rekatsina, E. Koratzanis, J. Deliolanis and H. Giamarellou, *Int. J. Antimicrob. Agents*, **31**, 434 (2008); (b) A. W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turk, *Am. J. Clin. Pathol*., **45**, 493 (1966).
42. NCCLS - *Clinical laboratory standards institute method for antifungal disk diffusion susceptibility testing of yeasts*, Proposed Guideline - M. 44A, 23 (2004).
43. E. O. Ajaiyeoba, P. A. Onocha and O. T. Olarenwaju, *Pharm. Biol*., **39**, 217 (2001).

44. Accelrys Software Inc., *Discovery Studio Modeling Environment*, Release 3.1. (2011).
45. R. Thomsen and M. H. Christensen, *J. Med. Chem*., **11**, 3315 (2006).
46. G. Wolber, A. A. Dornhofer and T. Langer, *J. Comput. Aided Mol. Des*., **20,** 773 (2007).
47. J. M. Yang and C. C. Chen, *Proteins*, **55**, 288 (2004).
48. M. S. Alam, L. Liu, Y. E. Lee and D. U. Lee, *Chem. Pharm. Bull., (Tokyo)*, **59**, 568 (2011).
49. K. Sharma, S. B. Mishra and A. K. Mishra, *Helv. Chim. Acta*, **94**, 2256 (2011).
50. P. Ertl, B. Rohde and P. Selzer, *J. Med. Chem*., **43**, 3714 (2000).
51. S. Uzzoman, H. Khanam, A. Mashrai, Y. N. Mabkhot and A. Husain, *Acta Cryst*., **E68**, o3037 (2012).
52. K. A. Ketuly, A. H. A. Hadi, S. W. Ng and E. R. T. Tiekink, *Acta Cryst*., **E67**, o773 (2011).
53. A. C. Wallace, R. A. Laskowski and J. M. Thornton, *Prot. Eng*., **8**, 127 (1995).