Chapter 3

Part-A

Synthesis of A-ring substituted heterosteroids by epoxide ring opening reaction and their antimicrobial studies

3A.1 Introduction

The synthetic utility of epoxides resides mainly in their versatile reactivity. Nucleophilic addition to epoxides plays an important role in the stereoselective preparation of 1,2-disubstituted products.[1] Vicinal imidazolyl alcohols and their derivatives which can be obtained by epoxide ring opening reactions are very important class of compounds due to their wide range of biological activities.[2] For example, metronidazole, ornidazole, and secnidazole (Figure 3A.1) are found to have important antiprotozoal activity. Ornidazole has been investigated for use in Crohn's disease after bowel resection and secnidazole is a drug used for the treatment of dientamoebiasis.

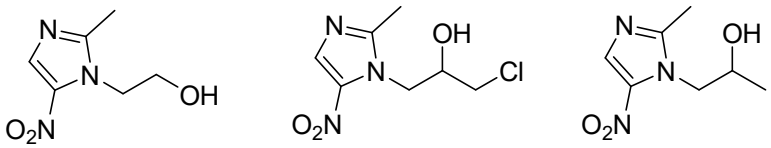
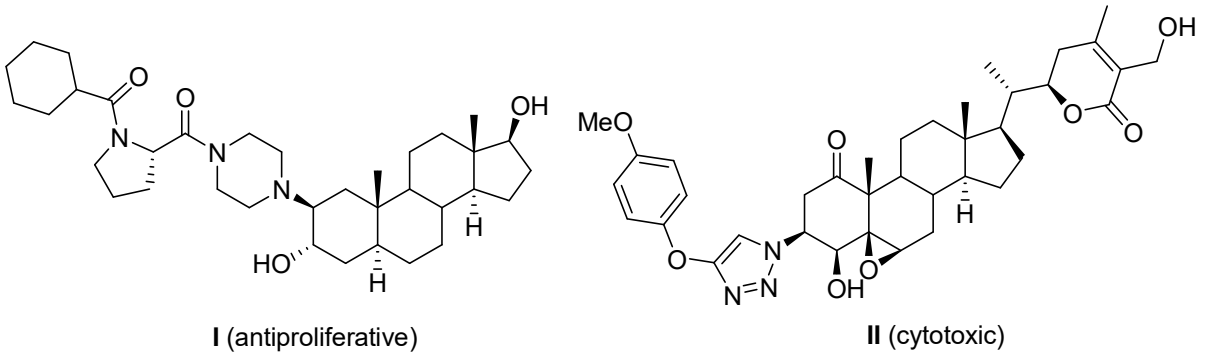


Figure 3A.1 Biologically important non-steroidal vicinal imidazolyl alcohols

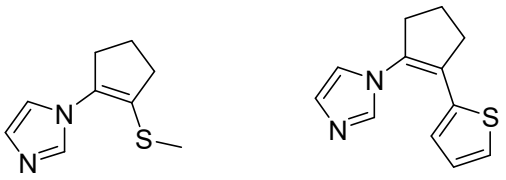
Heterocycle substituted steroidal compounds having *β*-hydroxyl group also have multifaceted pharmacological properties. For example 2*β*-piperazinyl-5*α*-androstane-3*α*,17*β*-diols (**I**, Figure 3A.2) inhibited HL-60 cell proliferation and exhibited a low toxicity on normal peripheral blood lymphocytes.[3] Again, 2,3-dihydrowithaferin A-3*β*-triazoles (**II**, figure 3A.2) have shown significant cytotoxic activity comparable to drug Mitomycin against human cancer cell lines.[4]

N-(1-cycloalkenyl)heterocycles are also important classes of compounds which have potential biological activity, for instance nonsteroidal compounds 1-[2-(methylsulfanyl)-1-cyclopentenyl]imidazole (**III**, Figure 3A.3) and 1-[2-(2-thienyl)-1-cyclopentenyl]imidazole (**IV**, figure 3A.3) are reported as neuronal injury inhibitors.[5]



I (antiproliferative) **II** (cytotoxic)

Figure 3A.2 Examples of steroidal vicinal imidazolyl alcohols

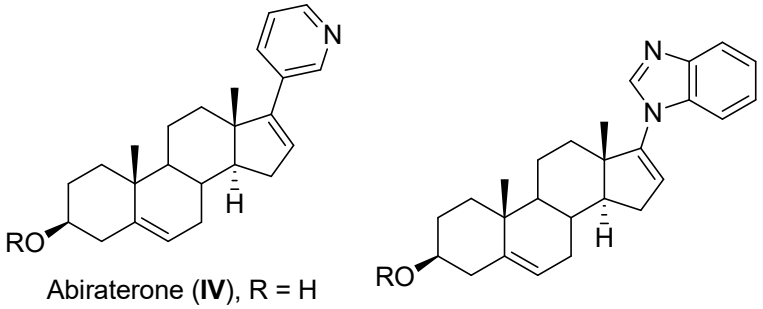


1-[2-(methylsulfanyl)-1-cyclopentenyl]imidazole (**III**, neuronal injury inhibitors)

1-[2-(2-thienyl)-1-cyclopentenyl]imidazole (**IV**, neuronal injury inhibitors)

Figure 3A.3 Biologically important non-steroidal *N*-(1-cycloalkenyl)heterocycles

Apart from non-steroidal *N*-(1-cycloalkenyl)heterocycles, the steroidal cycloalkenyl heterocycles are also found to have potent antitumor activities.[6,7] For example, abiraterone (**IV**), abiraterone acetate (**V**) and VN/124-1 (**VI**) are potent CYP17 inhibitors and show antitumor activity in the LAPC4 human prostate cancer cells (Figure 3A.4).



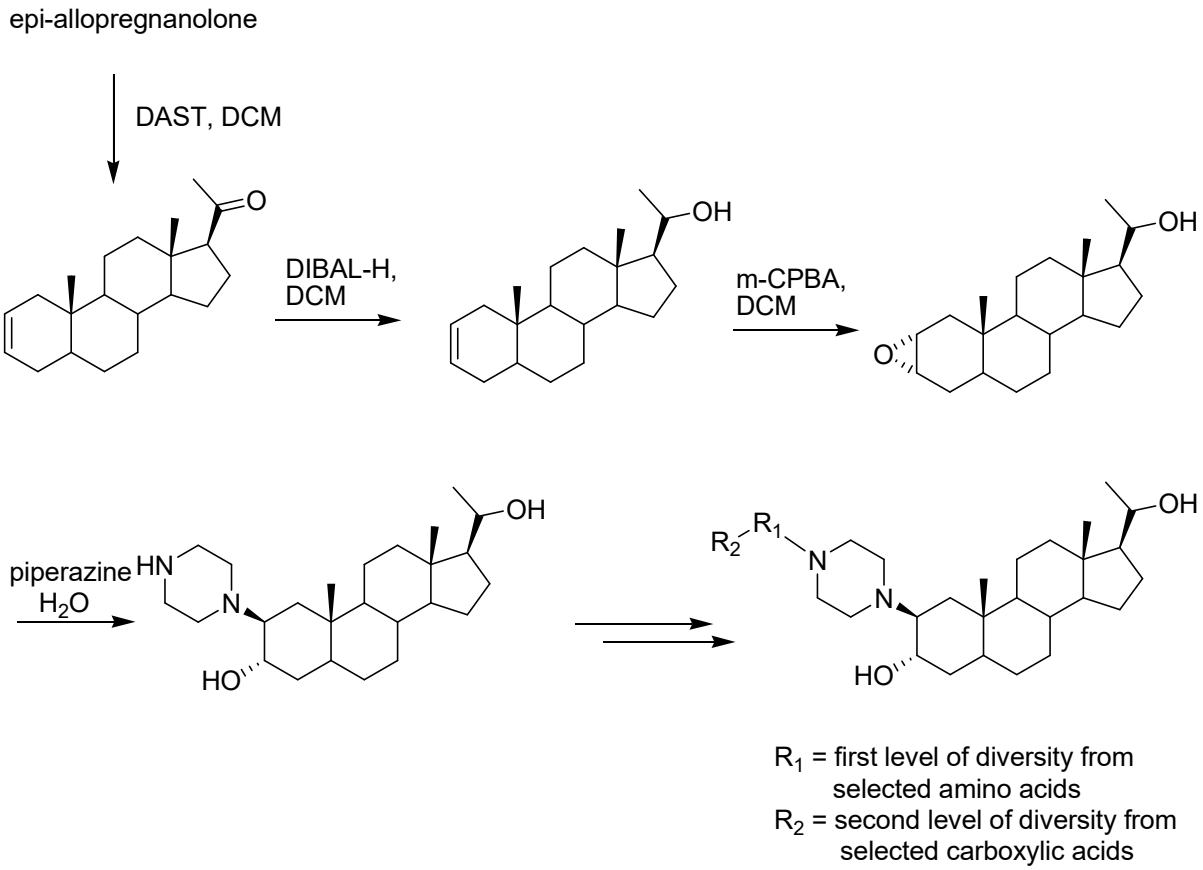
Abiraterone (**IV**), R = H
Abiraterone acetate (**V**), R = $COCH_3$
(CYP17 inhibitor)

VN/124-1 (TOK-001, **VI**)
(CYP17 inhibitory and AR ablative activities)

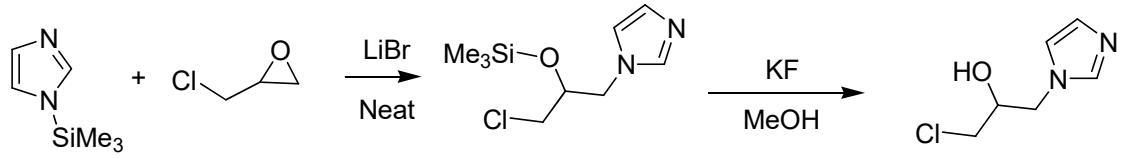
Figure 3A.4 Biologically important steroidal *N*-(1-cycloalkenyl)heterocycles

The chemistry of vicinal imidazolyl alcohols and *N*-(1-cycloalkenyl)heterocycles as well as epoxide ring opening reactions have motivated extensive investigations and a number of comprehensive synthesis has been extensively reviewed.

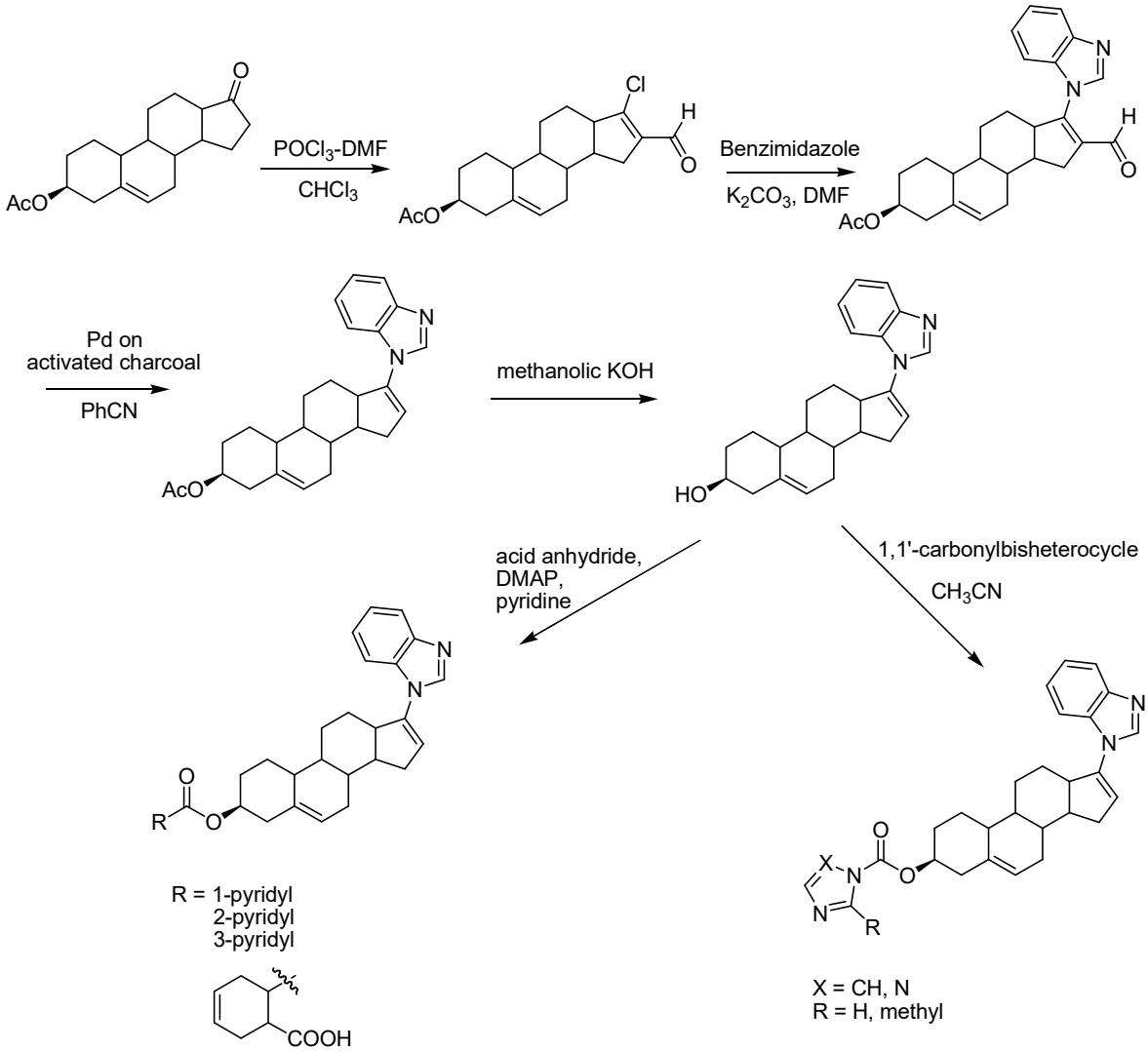
The regio- and stereo-selective aminolysis of 2,3-α-epoxide of epi-allopregnanolone followed by parallel coupling of amino acid and carboxylic acid results libraries of 2β-piperazino-(20R)-5α-pregnane-3α,20-diol-*N*-derivatives.[8] These compounds showed significant inhibition against HL-60 leukemia cell growth.



Jalil and co-workers[9] synthesized imidazolyl alcohols via one pot ring opening of epoxides with *N*-silylated imidazole catalyzed by LiBr under solvent-free conditions.

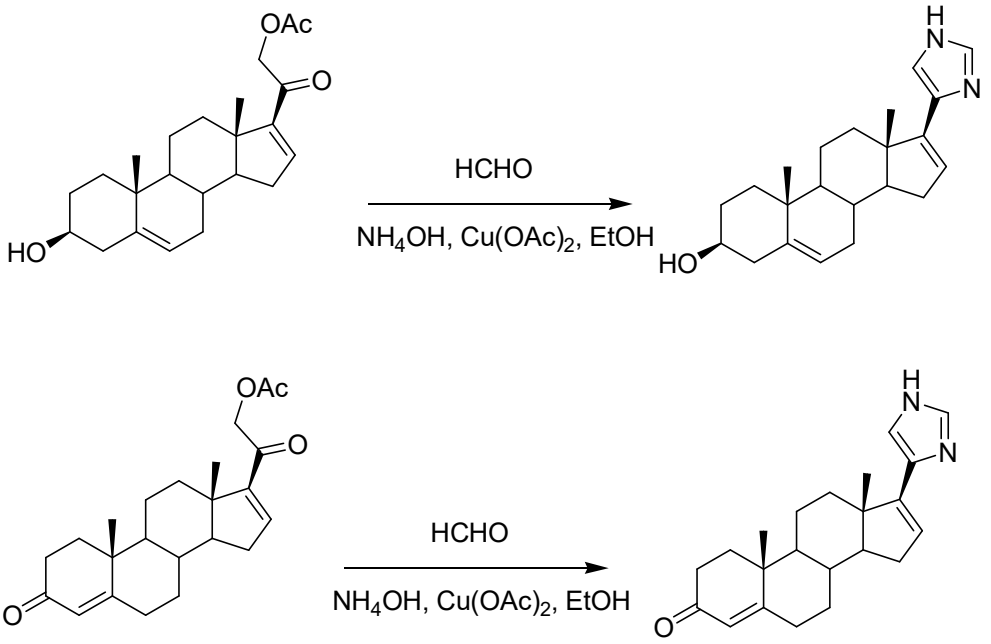


A few modifications of Prostate cancer drug candidate Galeterone and subsequent preparation of its C-3, C-16 and C-17 analogues were carried out by Purushottamachar and co-workers.[10] These modified compounds showed interesting antiproliferative and androgen receptor degrading activities against CWR22rv1 human prostate cancer cells.

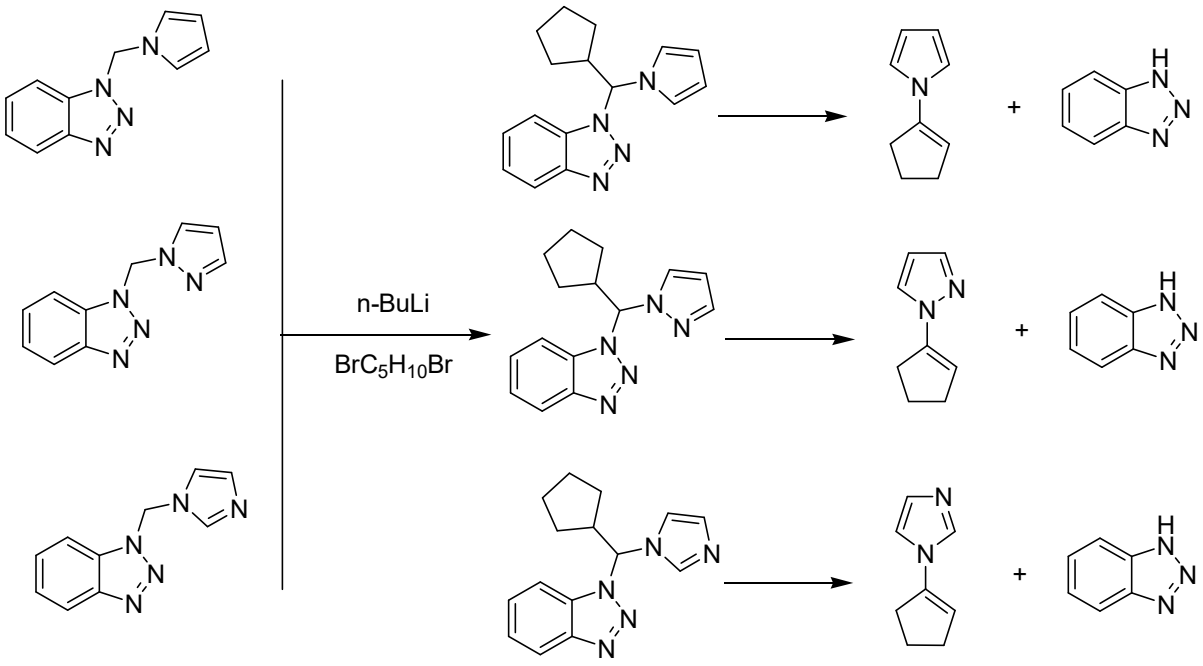


Ling and co-workers[11] synthesized 17-imidazolyl, pyrazolyl, and isoxazolyl androstene derivatives by condensing the corresponding 17-ketol acetate side

chain with formaldehyde and ammonium hydroxide. The 17α-(4′-imidazolyl) derivatives were found to be potent inhibitors against human testicular P45017R cell lines.



A series of novel *N*-(1-cycloalkenyl)-pyrroles, -pyrazoles, and -imidazoles were synthesized by Katritzky *et al.*[12] from 1-[1(heterocycyl)cycloalkyl]-benzotriazoles or 1-[1(heterocycyl)cyclohexyl]-5-phenyltetrazole1-[1(heterocycyl)cyclohexyl]-5-phenyltetrazole eliminating benzotriazole or 5-phenyltetrazole respectively.

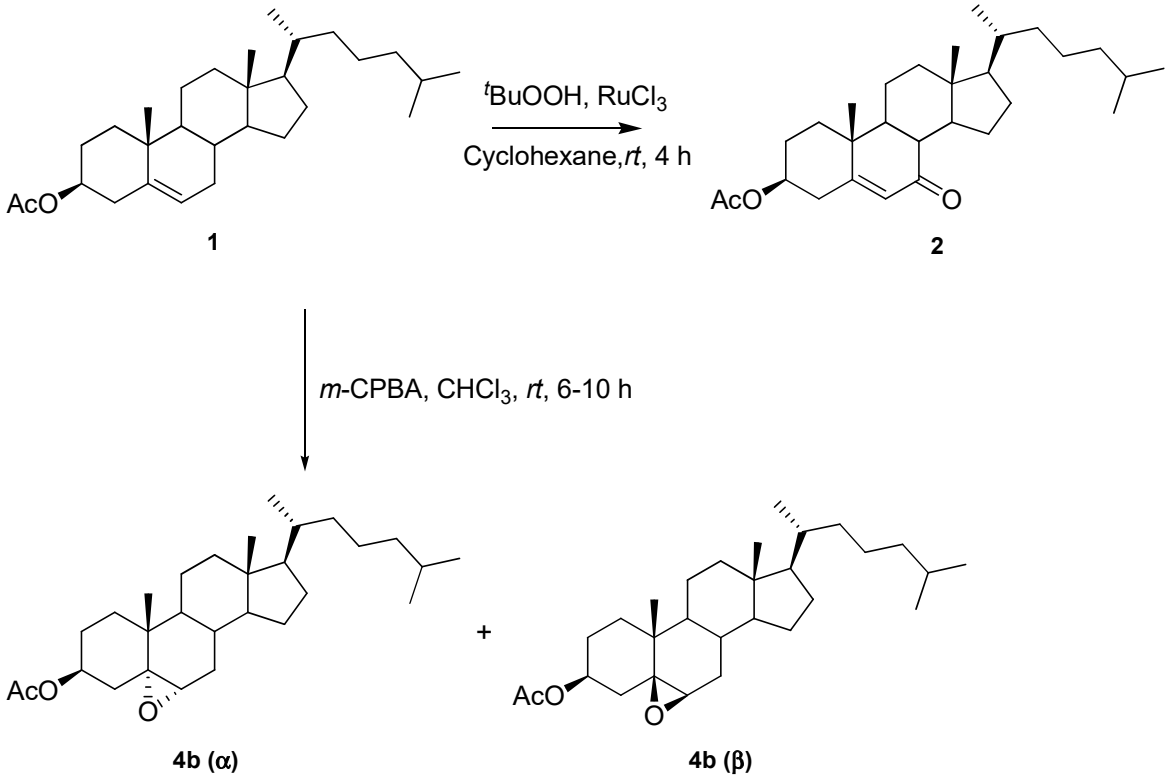


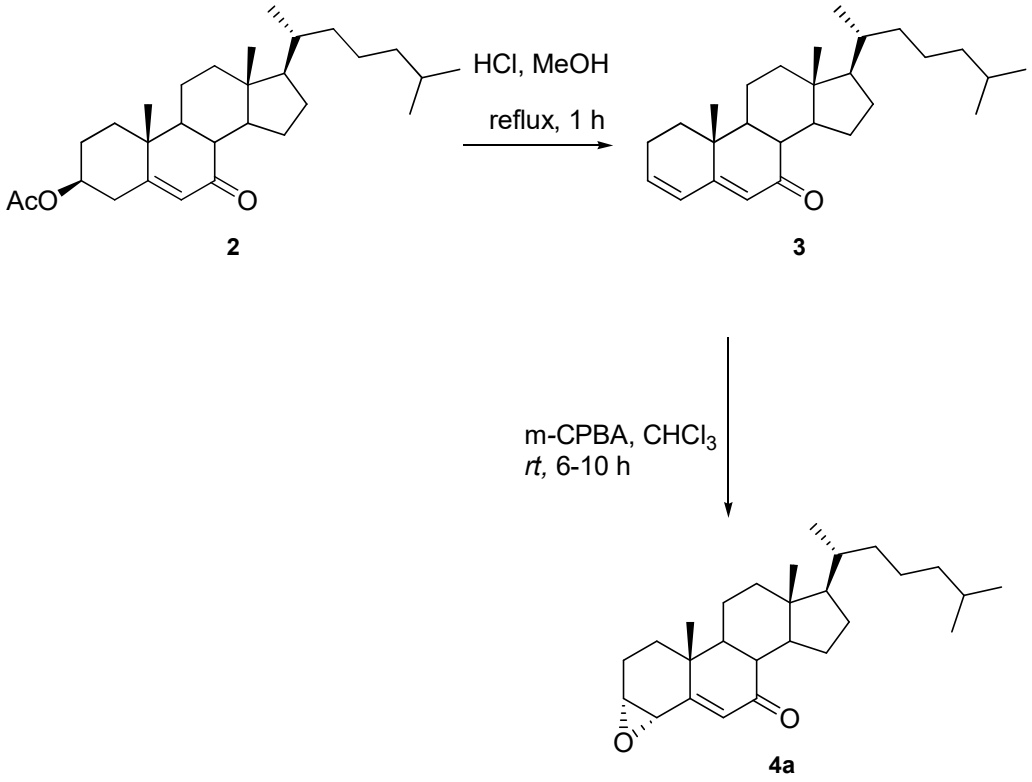
Owing to the importance of vicinal imidazolyl alcohols as well as *N*-(1-cycloalkenyl)heterocycles, it has been targeted for a novel strategy to synthesize hydroxy-(1*H*-imidazol-yl)/hydroxy-(1*H*-benzimidazol-yl)steroids and 2-(1*H*-imidazol-1-yl)/2-(1*H*-benzimidazol-1-yl)cyclohexanols. Under environmentally benign microwave irradiation, steroidal A/B- ring epoxide were explored for ring opening with imidazole and benzimidazole heterocycle. The methodology was extended with other nitrogen containing heterocycles also. Further, these steroidal/nonsteroidal *β*-hydroxy heterocyclic compounds were converted to the corresponding *N*-(1-cycloalkenyl)heterocycles *via* an acid catalyzed dehydration reaction in good yields. The *in-vitro* antimicrobial activities of these compounds were also investigated against five pathogenic strains, they are, *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (MTCC 426), *Pseudomonas syringae* (MTCC 673), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 29213) species.

3A.2 Results and discussion

3A.2.1 Chemistry

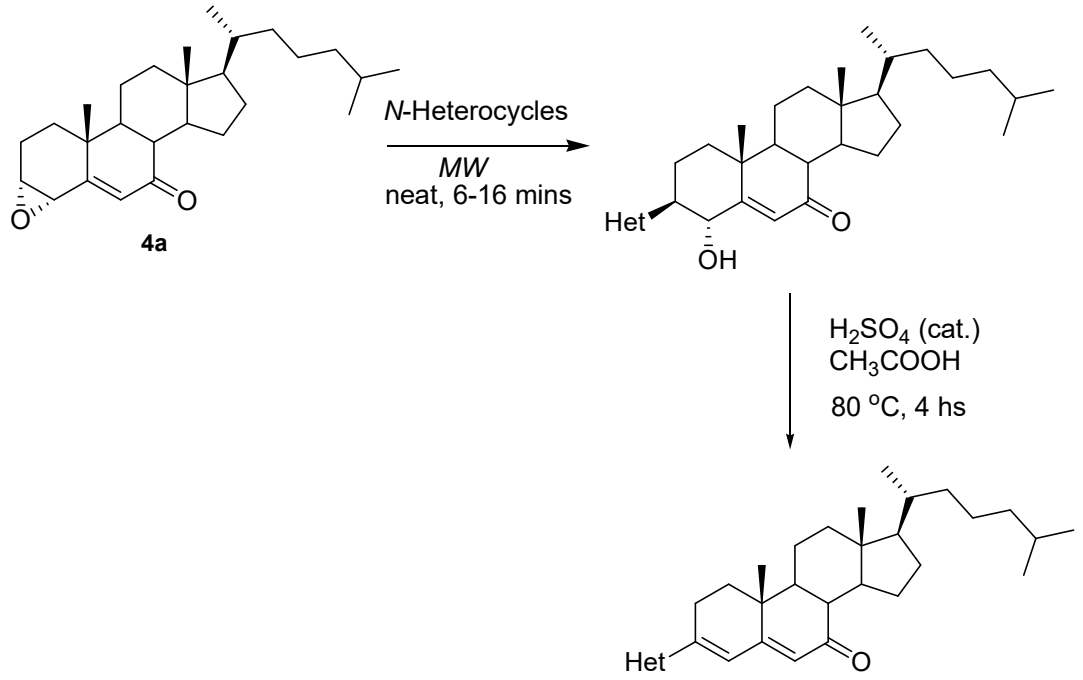
The synthesis of epoxide **4a** was carried out from commercially available cholesterol acetate (**1**) in 3-steps (Scheme 3A.1). Allylic oxidation of compound **1** with *tert*-butylhydroperoxide in presence of ruthenium chloride afforded ketone **2**. Treatment of compound **2** with hydrochloric acid in methanol followed by epoxidation of the conjugated ketone (**3**) thus obtained with *m*CPBA resulted epoxide **4a**. This epoxidation afforded only *α*-epoxide **4a** due to the presence of angular methyl group at C-10 position of the steroid moiety.[13] On the other hand cholesterol acetate (**1**) on epoxidation with *m*CPBA provided a mixture of epoxides **4b** (*α*:*β* = 4:1). The *α* and *β* ratio of compound **4b** was determined by the integration of the C-6 proton signals in the ^{1}H NMR spectra of the crude epoxide **4b** (δ = 2.84-2.91 for the *α*-epoxide and δ = 3.04-3.12 for the *β*-epoxide).[14]





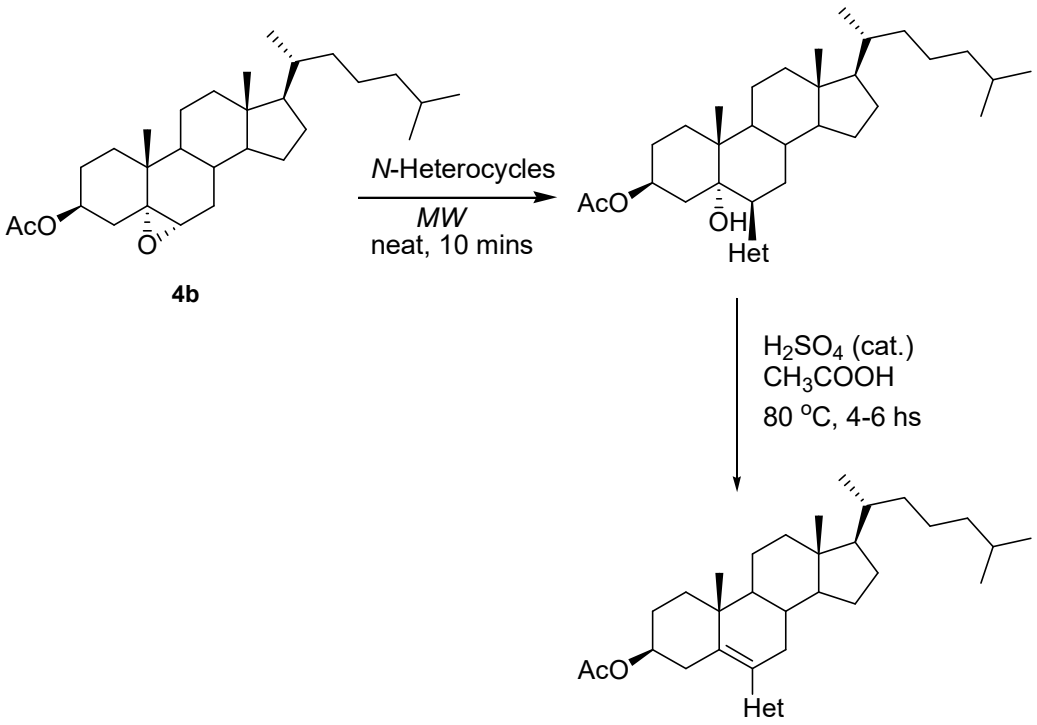
Scheme 3A.1

Finally, the microwave irradiation of an equimolar mixture of epoxide **4a** and imidazole in a closed vessel in a Synthos 3000 microwave reactor at 600 Watt (140 ^{o}C and 12 bar) for 6 minutes afforded compound 3-(1*H*-imidazol-yl)-4-hydroxy-5-en-cholest-7-one (**6a**) in 69% yield (Scheme 3A.2). Similarly, microwave reaction of steroidal epoxide **4a** with different imidazole derivatives **5b** and **5c** afforded hydroxy-(imidazol-yl)-steroids **6b** and **6c** (Entries 2-3, Table 3A.1). The reaction of steroidal epoxide **4a** was also performed with benzimidazole and other different *N*-containing heterocycles such as morpholine, thiomorpholine, tetrahydroisoquinoline and piperidine, which afforded 4α-hydroxy-3β-heterosteroids **6d-h** in good yields (Entries 4-8, Table 3A.1).



Scheme 3A.2

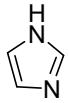
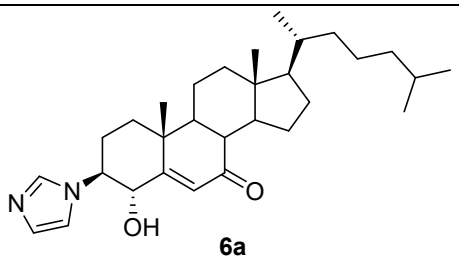
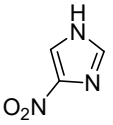
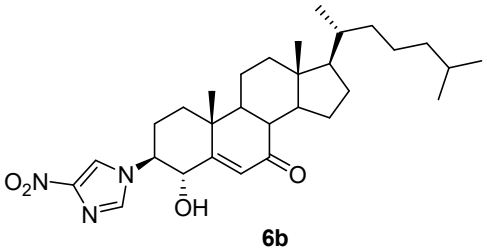
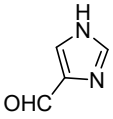
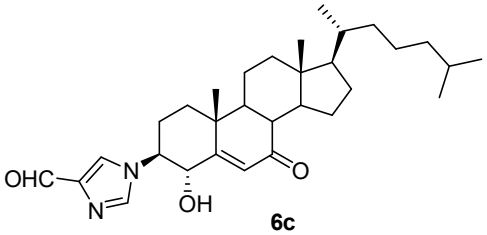
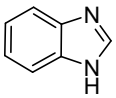
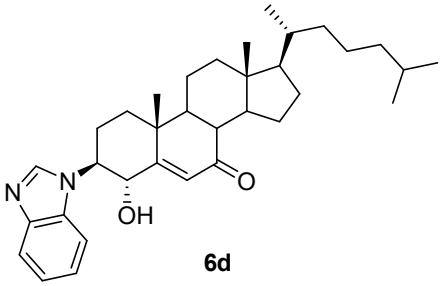
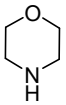
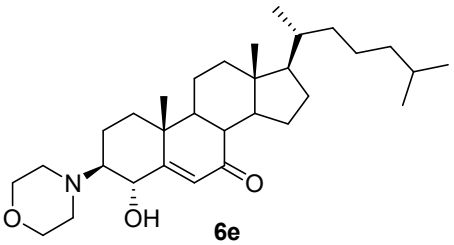
When equimolar mixture of epoxide **4b** was treated with imidazole by following same condition under microwave a diastereomeric mixture of imidazolyl alcohols was obtained, which on recrystalliztion in ethanol provided pure imidazolyl alcohol **6i** (Entry 9, Table 3A.1). The steroidal epoxides **4b** was also effectively opened by benzimidazole under the same reaction condition to afford compounds **6j** in good yield (Entry 10, Table 3A.1).

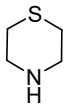
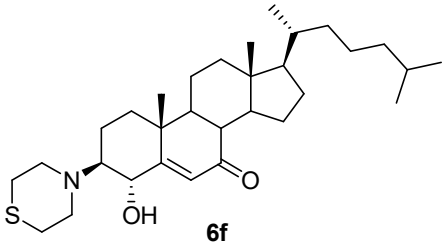
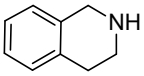
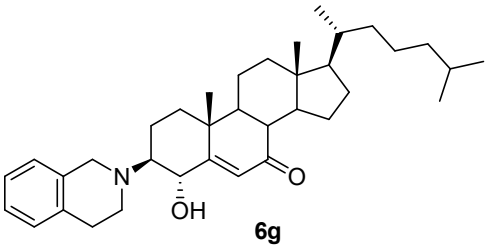
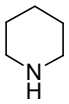
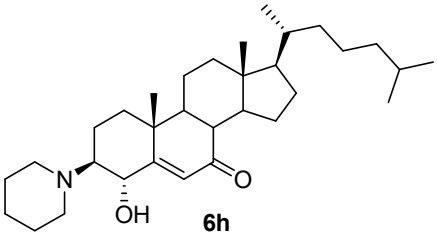
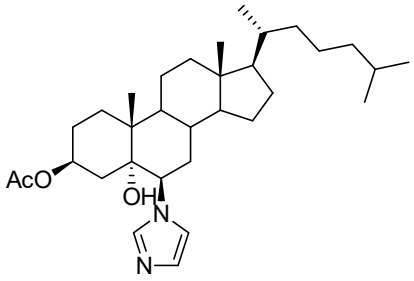
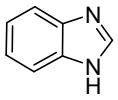
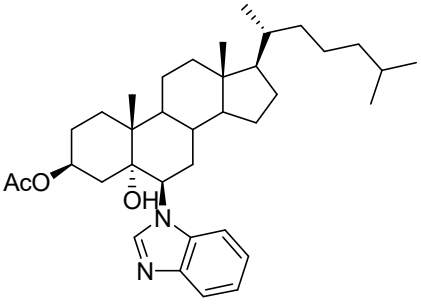
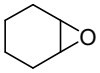
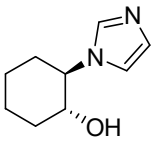


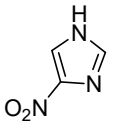
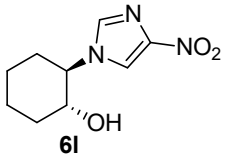
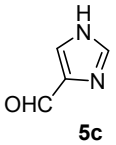
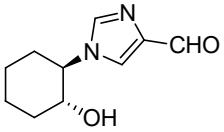
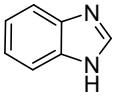
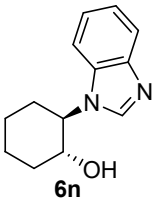
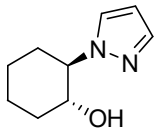
Scheme 3A.3

It was observered that the reaction was also effective for opening of nonsteroidal epoxide **4c** with different imidazole derivatives, which yielded compounds **6k-m (**Entries 11-13, Table 3A.1). The reaction of **4c** was also performed with *N*-containing heterocycles benzimidazole and pyrazole to afford compounds **6n** and **6o** in good yields (Entries 14-15, Table 3A.1).

Table 3A.1. Opening of steroidal/nonsteroidal epoxide rings by *N*-containing heterocycles

Entry	epoxide	Heterocycle	Vicinal heterocyclic alcohol	Time (min)	Yield (%)[a]
1	**4a**	 **5a**	 **6a**	6	69
2	**4a**	 **5b**	 **6b**	6	62
3	**4a**	 **5c**	 **6c**	6	66
4	**4a**	 **5d**	 **6d**	6	65
5	**4a**	 **5g**	 **6e**	10	74

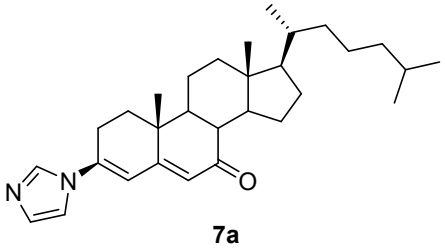
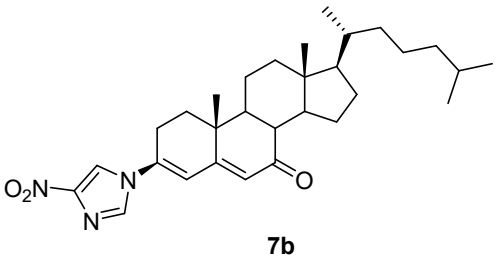
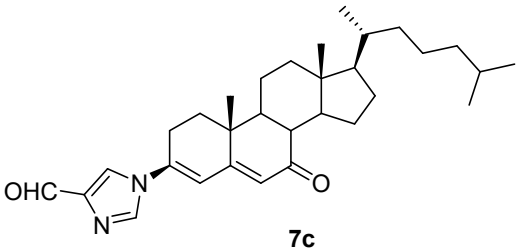
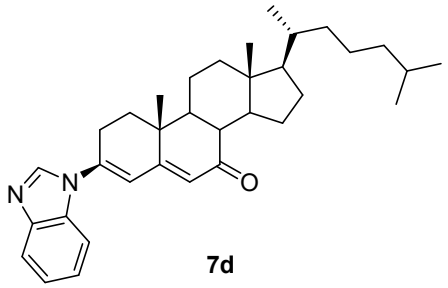
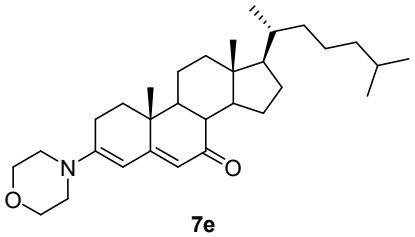
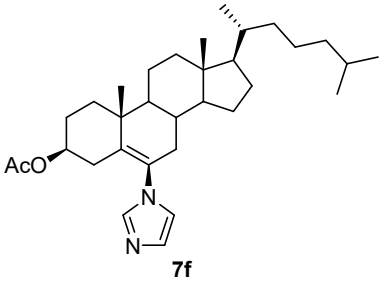
6	**4a**	 **5h**	 **6f**	10	69
7	**4a**	 **5e**	 **6g**	10	77
8	**4a**	 **5f**	 **6h**	10	68
9	**4b**	 **5a**	 **6i**	10	75
10	**4b**	 **5d**	 **6j**	16	71
11	 **4c**	 **5a**	 **6k**	10	79

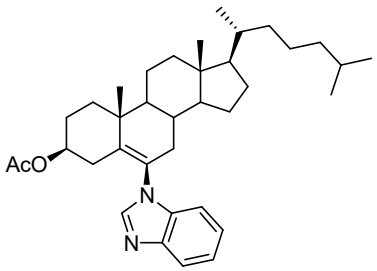
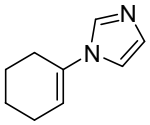
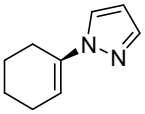
12	**4c**	 **5b**	 **6l**	10	75
13	**4c**	 **5c**	 **6m**	10	76
14	**4c**	 **5d**	 **6n**	10	78
15	**4c**	 **5i**	 **6o**	10	80

[a]Isolated yield

The dehydration reaction of compound **6** was studied in the next step. Increasing the time of the epoxide opening reaction from 6 minutes to 25 minutes for the synthesis of compounds **7a-d** afforded the dehydrated product **7a-d** in moderate yields (**7a** = 66%, **7b** = 55%, **7c** = 56% and **7d** = 61%). However, it was found that stirring a solution of compound **6a** with catalytic amount of sulfuric acid in acetic acid at 80 ^{o}C for 4 hours also provided compound **7a** in good yield (88%, Entry 1, Table 3A.2). This reaction condition was efficiently used for the dehydration reactions of steroidal hydroxyl compounds **6b-e**, **6i-j** as well as non-steroidal hydroxyl compounds **6k-o** to obtain steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycles **7b-i** in good yields (Entries 2-9, Table 3A.2).

Table 3A.2 Synthesis of steroidal and nonsteroidal *N*-(1-cycloalkenyl)heterocycles

Entry	Vicinal heterocyclic alcohol	Steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycles	Time (h)	Yield (%)[a]
1	**6a**	 **7a**	4	88
2	**6b**	 **7b**	4	83
3	**6c**	 **7c**	4	75
4	**6d**	 **7d**	4	88
5	**6e**	 **7e**	6	68
6	**6i**	 **7f**	4	81

7	**6j**	 **7g**	4	68
8	**6k**	 **7h**	4	79
9	**6o**	 **7i**	6	72

[a]Isolated product

3A.2.2 Biology

The antimicrobial activity of compounds **6a-o**, **7a-i** was evaluated and compared with standard drug kanamycin acid sulphate (Table 3A.3). As shown in Table 3 only few of the synthesized steroidal *N*-heterocyclic derivatives (**6b**, **6e-h**, **7b** & **7e**) showed moderate *in vitro* antimicrobial activity against the tested microorganisms. The steroidal derivatives **6e**, **6f** and **6h** were effective in inhibiting all the tested bacterial strains. The tetrahydroisoquinoline substituted steroidal derivative **6g** showed inhibition against three Gram-negative bacterial strains *E. coli, P. syringe and P. vulgaris*. Among the steroidal imidazoles only nitro group substituted steroidal imidazole **6b** showed inhibition against *S. aureus* and *B. subtilis*.

Table 3A.3 Antibacterial screening data

Compound		Zone of inhibition in mm[a,b]			
		Bacterial strains			
	E. coli	*P. syringe*	*P. vulgaris*	*S. aureus*	*B. subtilis*
6b	-	-	-	18	12
6e	12	16	16	10	14
6f	10	14	13	11	13
6g	12	12	15	-	-
6h	16	12	14	10	14
7b	-	-	-	14	-
7e	10	12	10	-	-
Kanamycin	32	36	32	33	29

[a]Zone of inhibitions less than 10 mm are not shown in the table

[b]Kanamycin (50 µg/well) was used as positive reference, synthesized compounds (50 µg/well)

Removal of hydroxyl group of this steroidal derivative led to decrease in the antimicrobial activity of the compounds as seen for compounds **7b** and **7e**. Compound **7b** showed inhibition only against bacterial strain *S. aureus* whereas compound **7e** showed inhibition only against three Gram-negative bacterial strains *E. coli, P. syringe and P.*

vulgaris. Compounds **6a, 6c-d**, **6i-o**, **7a**, **7c-d** and **7f-i** were not effective against any of the strains tested.

3A.3 Conclusion

In conclusion, a new solventless and catalyst-free green method has been developed for the steroidal/nonsteroidal epoxide ring opening reaction by nitrogen containing heterocycles under microwave irradiation. Moreover, some of these epoxide opening products were converted to their corresponding *N*-(1-cycloalkenyl)heterocycles by acid catalyzed dehydration reaction. All the epoxide opening and dehydrated compounds were screened *in vitro* for antibacterial activities against a panel of various bacterial strains. It was observed from the data that some of the epoxide opening and dehydrated compounds showed moderate inhibition activity against tested bacterial strains, indicating that these compounds are promising antibacterial compounds for further research.

3A.4 Experimental

3A.4.1 General experimental Procedure

Melting points were measured with a Buchi B-540 melting point apparatus and are uncorrected. IR spectra were recorded on Elmer FT-IR-2000 spectrometer using KBr pellets or on a thin film using chloroform. NMR spectra were recorded on Advance DPX 300 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Trace DSQ GCMS instrument. All the commercially available reagents were used as received. All experiments were monitored by thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates (Merck). Column chromatography was performed on silica gel (60-120 mesh, Merck Chemicals). All MW reactions were carried out in a Synthos 3000 (Anton Paar) microwave reactor.

3A.4.2 Biology

The antibacterial assay was performed following standard protocols and using test pathogens *E. coli*, *P. syringae*, *B. subtilis*, *P. vulgaris* and *S. aureus*. The antibacterial activity of all the compounds was tested using agar diffusion assay method[15] observing presence of inhibition zone (in mm) around the well. All tested bacteria were grown in Mueller Hinton broth (MHB) at 37 ºC for 24 hours. Whole experiments were carried out in a laminar flow to strictly maintain aseptic conditions. 100 µl of fresh inoculum from each culture was added on the plates and spread using sterilized spreader. Wells were punctured on freshly spread bacterial culture on MHA using sterile cork borer. The solution of test compound was prepared in DMSO solvent (Stock concentration 25 mg/mL) and the bored wells were filled with test compound (50 µg). Four wells were bored on each plate, each filled with same compound and two plates for each test compound were taken and the experiment was repeated twice. All the plates were incubated at 37 ºC for 24 h. Growth inhibition of test organisms was measured with standard scale, the mean values of inhibition zones were taken and the data were compared with standard drug kanamycin acid sulphate.

Chemical synthesis

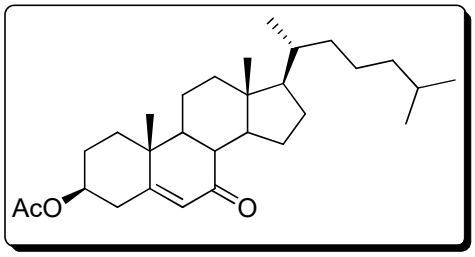
(a) Preparation and characterization of 3*α*,4*β*-epoxy-5-en-cholest-7-one

*Preparation and characterization of 3β-Acetoxy-7-oxo-cholest-5-en (**2**):*

To a solution of Cholesteroyl acetate (**1**, 10.0 g, 23.34 mmol) and $RuCl_3.H_2O$ (60 mg) in cyclohexane (40 mL), 70% *tert*-butyl hydroperoxide (20 mL) was added at room temperature within 1 hour and the reaction mixture was allowed to stir for another 5 hours. The reaction mixture was then treated with saturated $NaHCO_3$ solution, washed with water, extracted with EtOAc and dried over Na_2SO_4. The crude product obtained after removal of

EtOAc was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford pure keto compound 3*β*-Acetoxy-7-oxo-cholest-5-en **2**.

3*β*-Acetoxy-7-oxo-cholest-5-en (2)

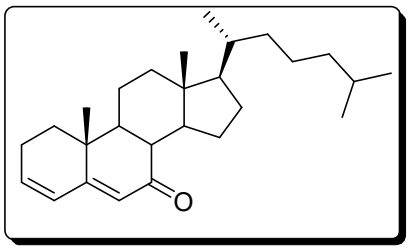


White solid, Yield 8.26 g (80%); R_f = 0.5 (EtOAc/Hexane = 1:9); m.p. 158-160 ^oC. IR (KBr, cm^{-1}) 2949, 1732, 1670, 1466, 1376, 1248, 758; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H), 0.65-2.55 (m, 29H), 0.86 (d, *J* = 6.3 Hz, 6H), 1.21 (s, 3H), 1.61 (s, 3H), 4.77-4.66 (m, 1H), 5.70 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.3, 18.9, 21.2, 21.3, 22.6, 22.8, 23.8, 27.3, 28.0, 26.3, 28.6, 35.7, 36.0, 36.2, 37.7, 38.3, 38.6, 39.5, 43.1, 45.4, 49.8, 49.9, 54.7, 72.2, 126.7, 163.9, 170.3, 202.0; MS (EI, *m/z*) = 382.3 [M^+-CH_3COOH]. Anal. Calcd. for $C_{29}H_{46}O_3$: C 78.68; H 10.47; Found: C 78.70; H 10.69.

*Preparation and characterization of 3β-Acetoxy-3,5-dien-cholest-7-one (**3**):*

Compound **2** (8.0 g, 18.09 mmol) was refluxed with hydrochloric acid (20 mL) in methanol for 1 hour. The residue obtained after removal of the solvent was washed with water, neutralised with $NaHCO_3$, extracted with DCM and dried over Na_2SO_4. The crude product obtained after removal of the DCM layer was purified by silica gel column chromatography using ethyl acetate/hexane (0.5:9.5) as the eluent to afford steroidal 7-keto 3,5-diene **3**.

3*β*-Acetoxy-3,5-dien-cholest-7-one (3)

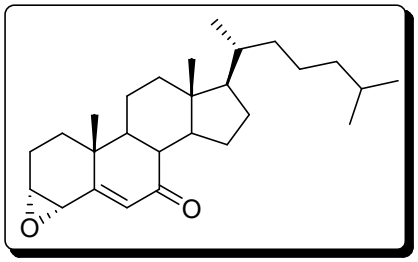


White solid, Yield 6.36 g (92%); R_f = 0.7 (EtOAc/Hexane = 1:9); m.p. 117-119 ^oC. IR (KBr, cm^{-1}): 2945, 1712, 1580, 1250; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.71 (s, 3H), 0.85-2.36 (m, 24H), 0.88 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.4 Hz, 3H), 1.11 (s, 3H), 5.60 (s, 1H), 6.06-6.22 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.6, 18.9, 21.2, 22.6, 22.9, 23.4, 23.9, 26.4, 26.5, 28.0, 28.6, 32.8, 35.8, 36.3, 39.0, 39.5, 43.4, 46.0, 49.6, 50.7, 54.9, 124.2, 127.7, 136.6, 160.7, 202.3; MS (EI, *m/z*) = 382; Anal. Calcd. for $C_{27}H_{42}O$: C 84.75; H 11.06; Found: C 84.77; H 11.28.

Preparation of and characterization 3α,4α-Epoxy-5-en-cholest-7-one (4a)

To a stirring solution of compound **3** (6.2 g, 16.22 mmol) in chloroform (15 mL), *m*-CPBA (4.2 g, 24.33 mmol) was added at 0 ^oC. The reaction mixture was then stirred for 4 hours at room temperature and after completion of the reaction, as indicated by TLC, 4% Na_2SO_3 was added into it and the whole reaction mixture was kept stirring for another 4 hours. The reaction mixture was then extracted with $CHCl_3$ wased with water, brine and dried over Na_2SO_4. The crude product obtained after removal of the solvent was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford pure steroidal epoxide **4a**.

3*α*,4*α*-Epoxy-5-en-cholest-7-one (4a)

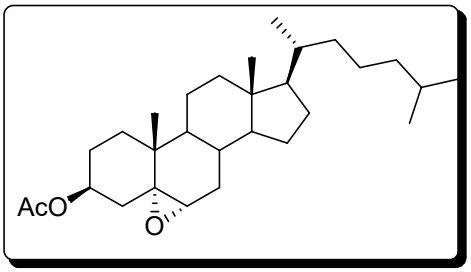


Yield 4.84 g (75%); R_f = 0.5 (EtOAc/Hexane = 1:9); m.p. 128-129 ^oC. IR (KBr, cm^{-1}): 2952, 1670, 1462, 1381; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.69 (s, 3H), 0.85-2.36 (m, 24H), 0.88 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.08 (s, 3H), 3.39-3.49 (m, 2H), 6.04 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.7, 18.8, 20.7, 21.2, 22.6, 22.8, 23.8, 26.2, 26.4, 28.0, 28.6, 35.6, 35.8, 36.2, 38.8, 39.5, 43.4, 46.5, 49.7, 50.5, 52.0, 52.4, 54.8, 131.4, 160.2, 201.3. MS (EI, *m/z*) = 398; Anal. Calcd. for $C_{27}H_{42}O_2$: C 81.35; H 10.62; Found: C 81.33; H 10.59.

(b) Preparation and characterization of 3*β*-Acetoxy-cholest-5,6-epoxide (4b, α:β = 4:1)

To a stirring solution of Cholesterol acetate **1** (3.0 g, 7.0 mmol) in chloroform (15 mL), *m*-CPBA (1.81 g, 10.5 mmol) was added at 0 ^oC. The reaction mixture was then stirred for 6 hours at room temperature and after completion of the reaction, as indicated by TLC, 4% Na_2SO_3 was added into it and the whole reaction mixture was kept stirring for another 4 hours. The reaction mixture was then extracted with $CHCl_3$ wased with water, brine and dried over Na_2SO_4. The crude product obtained after removal of the solvent was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford mixture of *α* and *β* steroidal epoxide **4b** (*α*:*β* = 4:1).

3*β*-Acetoxy-cholest-5*α*,6*α*-epoxide (4b)



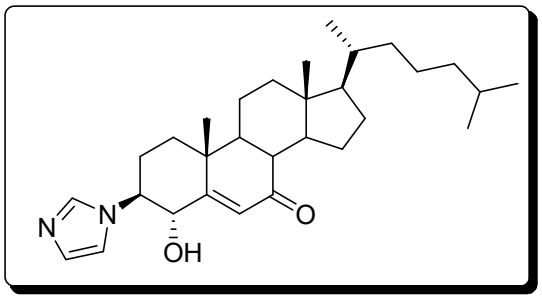
Yield 2.27 g (73%); m.p. 94-97 ^{o}C. IR (KBr, cm^{-1}): 2952, 1725; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.61 (s, 2.4H), 0.64 (s, 0.6H), 0.82-2.32 (m, 40H), 2.02 (s, 2.4H), 2.03 (s, 0.6H), 2.84-2.91 (m, 0.8H), 3.04-3.12 (m, 0.2H), 4.72-4.79 (m, 0.2H), 4.88-5.02 (m, 0.8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9, 15.9, 18.6, 21.3, 22.6, 22.8, 23.8, 27.2, 28.0, 28.1, 29.9, 35.0, 36.1, 39.4, 39.5, 42.3, 42.4, 55.8, 56.2, 56.8, 59.2, 62.5, 63.6, 65.2, 71.4, 170.2, 170.6; MS (EI, *m/z*) = 398 $[M]^{+}$.

Preparation of steroidal/nonsteroidal vicinal heterocyclic alcohols (6)

Epoxide **4** (0.5 mmol) and *N*-containing heterocycle **5** (0.5 mmol) were mixed intimately and the mixture was irradiated in a closed vessel in a Synthos 3000 microwave reactor at 600 Watt (140 ^{o}C and 12 bar) for 6-16 minutes. The residue obtained was purified by silica gel column chromatography using EtOAc/hexane as the eluent to afford the vicinal heterocyclic alcohols **6**.

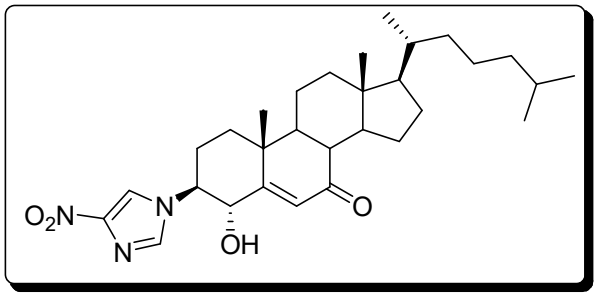
Characterization of steroidal/nonsteroidal vicinal heterocyclic alcohols (6)

3*β*-(1*H*-Imidazolo)-4*α*-hydroxy-5-en-cholest-7-one (6a)



Thick yellow gum, Yield 69%; IR ($CHCl_3$, cm^{-1}): 3421, 2952, 1672, 1451, 756; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.67 (s, 3H), 0.83-2.66 (m, 25H), 0.85 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.41 (s, 3H), 2.65-2.67 (m, 1H), 4.34 (bs, 1H), 5.83 (s, 1H), 6.90 (s, 1H), 7.11 (s, 1H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.7, 17.3, 18.8, 20.5, 22.6, 22.8, 23.8, 26.4, 28.2, 28.5, 29.6, 32.9, 35.7, 35.9, 38.6, 39.2, 39.5, 43.2, 45.8, 50.6, 52.3, 54.7, 66.7, 66.9, 74.8, 132.5, 133.8, 134.5, 162.7, 202.1; MS (EI, m/z) = 466 $[M]^+$. Anal. calcd. for $C_{30}H_{46}N_2O_3$: C, 77.21; H, 9.93; N, 6.00; Found: C, 77.45; H, 9.71; N, 6.37.

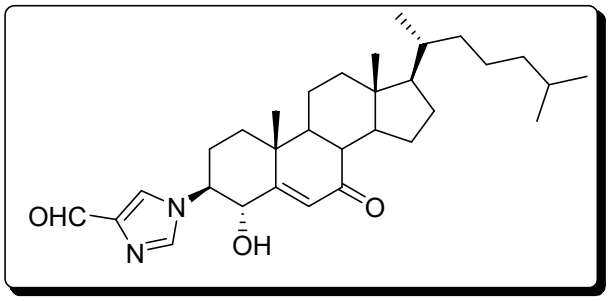
3*β*-(4-Nitro-1*H*-imidazolo)-4*α*-hydroxy-5-en-cholest-7-one (6b)



Thick yellow gum, Yield 62%; IR ($CHCl_3$, cm^{-1}): 3424, 2950, 1672, 754; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.69 (s, 3H), 0.86-2.64 (m, 25H), 0.84 (d, *J* = 6.4 Hz, 6H), 0.90 (d, *J* = 6.6 Hz, 3H), 1.42 (s, 3H), 2.65-2.68 (m, 1H), 4.36 (s, 1H), 5.84 (s, 1H), 8.20 (s, 1H), 8.23 (s, 1H);

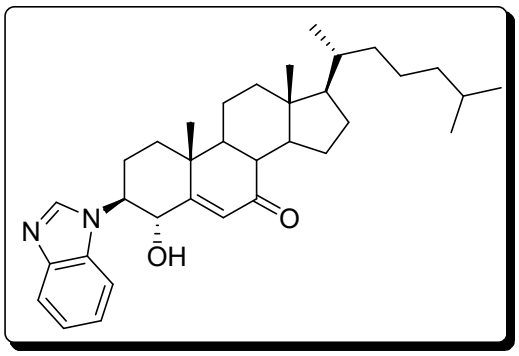
MS (EI, m/z) = 511 $[M]^+$. Anal. calcd. for $C_{30}H_{45}N_3O_4$: C, 70.42; H, 8.86; N, 8.21; Found: C, 70.52; H, 8.86; N, 8.35.

3*β*-(4-Formyl-1*H*-imidazolo)-4*α*-hydroxy-5-en-cholest-7-one (6c)



Thick brown gum, Yield 66%; IR ($CHCl_3$, cm^{-1}): 3421, 2954, 1676, 752; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.70 (s, 3H), 0.85-2.64 (m, 25H), 0.85 (d, *J* = 6.4 Hz, 6H), 0.92 (d, *J* = 6.6 Hz, 3H), 1.40 (s, 3H), 2.66-2.68 (m, 1H), 4.37 (s, 1H), 5.86 (s, 1H), 7.54 (s, 1H), 7.61 (s, 1H), 9.93 (s, 1H); MS (EI, m/z) = 494 $[M]^+$. Anal. calcd. for $C_{31}H_{46}N_2O_3$: C, 75.26; H, 9.37; N, 5.66; Found: C, 75.60; H, 9.71; N, 5.43.

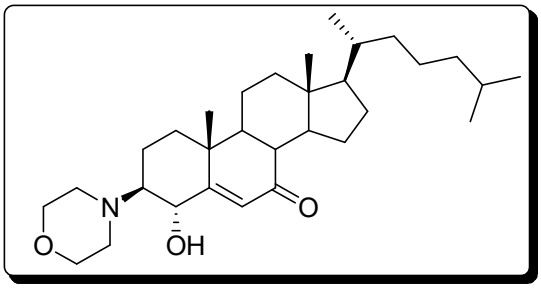
3*β*-(1*H*-Benzo[*d*]imidazolo)-4*α*-hydroxy-5-en-cholest-7-one (6d)



Thick yellow gum, Yield 65%; IR ($CHCl_3$, cm^{-1}): 3429, 2955, 1678; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.70 (s, 3H), 0.84-2.64 (m, 25H), 0.84 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.6 Hz, 3H), 1.40 (s, 3H), 2.66-2.68 (m, 1H), 4.35 (s, 1H), 5.87 (s, 1H), 7.13-7.91 (m, 5H); MS (EI, m/z) =

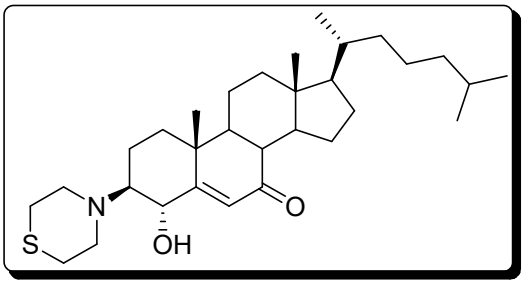
516 $[M]^+$. Anal. calcd. for $C_{34}H_{48}N_2O_2$: C, 79.02; H, 9.36; N, 5.42; Found: C, 78.82; H, 9.30; N, 5.67.

3*β*-Morpholino-4*α*-hydroxy-5-en-cholest-7-one (6e)



Yellow solid, Yield 74%; m.p. 121-123 ^oC; IR ($CHCl_3$, cm^{-1}): 3422, 2951, 1669, 1453, 756; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.84-2.66 (m, 25H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.42 (s, 3H), 2.38-2.42 (m, 4H), 2.66 (d, *J* = 2.0 Hz, 1H), 3.68 (t, *J* = 6.0 Hz, 4H), 4.33 (s, 1H), 5.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9, 17.4, 18.9, 20.6, 22.6, 22.8, 23.8, 26.3, 28.0, 28.5, 29.7, 32.9, 35.7, 36.1, 38.6, 39.2, 39.5, 43.1, 45.8, 50.6, 52.3, 54.8, 66.7, 66.9, 74.7, 132.7, 162.6, 202.2; MS (EI, *m/z*) = 485 $[M]^+$. Anal. calcd. for $C_{31}H_{51}NO_3$: C, 76.65; H, 10.58; N, 2.88 Found: C, 76.48; H, 10.66; N, 2.92.

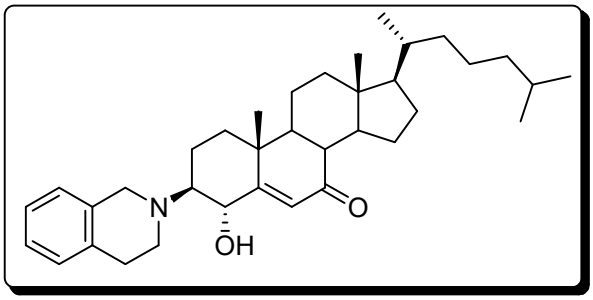
3*β*-Thiomorpholino-4*α*-hydroxy-5-en-cholest-7-one (6f)



Yellow thick oil; Yield 69%; IR ($CHCl_3$, cm^{-1}): 3423, 2954, 1665, 1457, 754; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.68 (s, 3H), 0.85-2.64 (m, 33H), 0.85 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 2.65 (d, *J* = 2.0 Hz, 1H), 4.36 (s, 1H), 5.84 (s, 1H); ^{13}C NMR

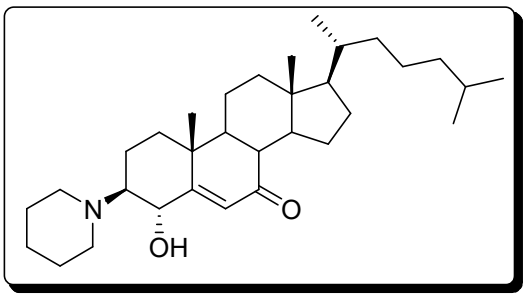
($CDCl_3$, 75 MHz) δ 11.7, 17.4, 18.9, 20.4, 22.6, 22.8, 23.8, 26.3, 28.5, 28.6, 29.5, 32.9, 35.7, 36.1, 38.7, 38.9, 39.7, 43.6, 45.8, 50.6, 52.3, 55.2, 58.3, 66.7, 66.9, 74.7, 132.9, 162.8, 202.4; MS (EI, *m/z*) = 501 $[M]^+$. Anal. calcd. for $C_{31}H_{51}NO_2S$: C, 74.20; H, 10.24; N, 2.79. Found: C, 74.13; H, 10.35; N, 2.98.

3*β*-Tetrahydroisoquinolino-4*α*-hydroxy-5-en-cholest-7-one (6g)



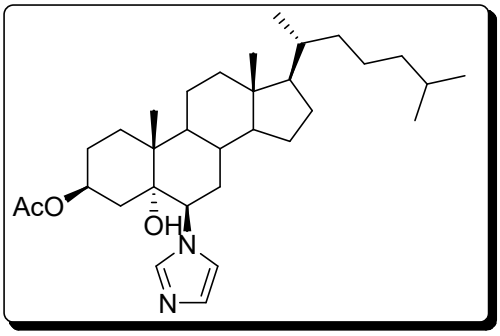
White solid, Yield 77%; m.p. 194-196 ^oC; IR ($CHCl_3$, cm^{-1}): 3395, 2924, 1670, 1461, 1374, 1156, 771; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.83-2.60 (m, 29H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.91 (d, *J* = 6.5 Hz, 3H), 1.08 (s, 3H), 2.84 (d, *J* = 2.1 Hz, 1H), 3.41-3.48 (m, 2H), 4.51 (s, 1H), 5.87 (s, 1H), 7.01-7.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 14.1, 17.7, 18.8, 21.2, 22.6, 22.7, 22.8, 26.2, 26.4, 28.0, 28.6, 29.7, 35.6, 35.7, 36.1, 38.8, 39.4, 43.4, 46.5, 49.7, 50.5, 54.8, 126.2, 127.8, 129.7, 129.9, 131.4, 160.2, 201.4; MS (EI, *m/z*) = 531 $[M]^+$. Anal. calcd. for $C_{36}H_{53}NO_2$: C, 81.30; H, 10.05; N, 2.63; Found: C, 81.38; H, 9.97; N, 2.89.

3*β*-Piperidino-4*α*-hydroxy-5-en-cholest-7-one (6h)



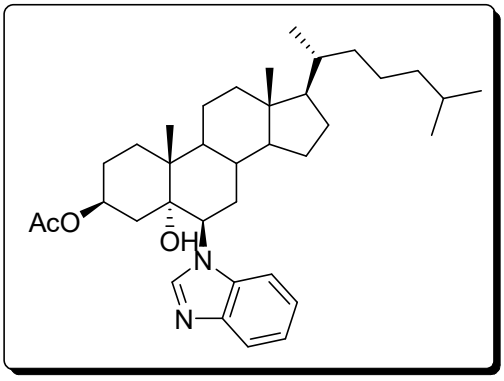
Brown solid; Yield 68%; m.p. 117-119 ^{o}C; IR ($CHCl_3$, cm^{-1}): 3418, 2929, 1670, 1466, 1382, 757; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.66 (s, 3H), 0.83-2.60 (m, 35H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.01 (s, 3H), 2.63 (s, 1H), 4.34 (s, 1H), 5.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.7, 18.8, 20.7, 21.2, 22.6, 22.8, 23.8, 26.2, 26.4, 28.0, 28.6, 29.7, 35.6, 35.7, 36.1, 38.8, 39.5, 43.4, 46.5, 49.7, 50.5, 52.0, 52.4, 54.8, 131.4, 160.3, 201.4; MS (EI, *m/z*) = 483 $[M]^+$, 465 $[M-18]^+$. Anal. calcd. for $C_{32}H_{53}NO_2$: C, 79.45; H, 11.04; N, 2.90; Found: C, 79.38; H, 11.26; N, 2.71.

3*β*-Acetoxy-5*α*-hydroxy--cholest-6*β*-(1*H*-imidazole) (6i)



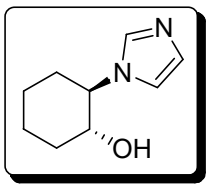
White solid, Yield 75%; m.p. 225-228 ^{o}C; IR ($CHCl_3$, cm^{-1}): 3205, 2945, 1729, 1381, 1245, 771; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.73 (s, 3H), 0.75 (s, 3H), 0.87-2.50 (m, 27H), 0.87 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.2 Hz, 3H), 2.04 (s, 3H), 3.11 (s, 1H), 4.00 (d, *J* = 5.3 Hz, 1H), 5.18-5.24 (m, 1H), 7.01 (s, 1H), 7.08 (s, 1H), 7.72 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.3, 15.7, 18.6, 21.1, 21.5, 22.6, 22.8, 23.9, 28.0, 28.3, 30.7, 32.6, 33.0, 35.8, 36.1, 38.4, 39.5, 42.8, 45.0, 53.4, 56.0, 56.2, 62.2, 71.0, 76.0, 76.6, 120.1, 128.0, 137.8, 171.1; MS (EI, m/z) = 512.4 $[M]^+$. Anal. calcd. for $C_{32}H_{52}N_2O_3$: C, 74.95; H, 10.22. N, 5.46. Found: C, 74.81; H, 10.28; N, 5.84.

3*β*-Acetoxy-5*α*-hydroxy-cholest-6*β*-(1*H*-benzo[*d*]imidazole) (6j)



White solid, Yield 71%; m.p. 157-159 ^{o}C; IR ($CHCl_3$, cm^{-1}): 3402, 2937, 1727, 1381, 1248, 771; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80 (s, 3H), 0.87-2.52 (m, 27H), 0.87 (d, *J* = 6.5 Hz, 6H), 0.94 (d, *J* = 6.2 Hz, 3H), 1.01 (s, 3H), 1.95 (s, 3H), 3.41 (bs, 1H), 4.36 (d, *J* = 6.7 Hz, 1H), 5.11-5.18 (m, 1H), 7.25-7.30 (m, 2H), 7.45 (m, 1H), 7.80 (m, 1H), 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.5, 17.1, 18.4, 18.6, 21.2, 21.4, 22.6, 22.8, 24.0, 28.0, 28.2, 33.8, 35.8, 36.2, 38.7, 39.5, 42.8, 44.8, 56.2, 56.3, 58.4, 60.4, 71.0, 111.2, 120.1, 122.2, 122.9, 136.0, 142.6, 142.9, 171.3; MS (EI, m/z) = 562.4 $[M]^{+}$. Anal. calcd. for $C_{36}H_{54}N_2O_3$: C, 76.82; H, 9.67; N, 4.98. Found: C, 76.89; H, 9.74; N, 4.73.

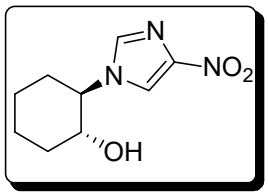
trans-2-(1*H*-Imidazol-1-yl)cyclohexanol (6k)



White solid, Yield 79%; m.p. 134-136 ^{o}C; IR ($CHCl_3$, cm^{-1}): 3447, 2923, 1437, 1260, 1119; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.22-2.14 (m, 8H), 3.52-3.74 (m, 2H), 4.94 (bs, 1H), 6.83 (s, 1H), 6.92 (s, 1H), 7.36 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.3, 25.0, 32.3, 34.4, 63.8, 72.7, 117.3, 127.9, 136.2; MS (EI, m/z) = 166 $[M]^{+}$. Anal. calcd. for $C_9H_{14}N_2O$: C, 65.03; H,

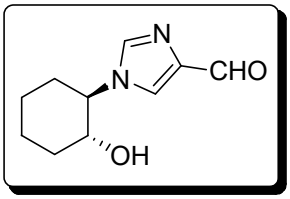
8.49; N, 16.85; Found: C, 65.06; H, 8.52; N, 16.88.

trans-2-(4-Nitro-1*H*-imidazol-1-yl)cyclohexanol (6l)



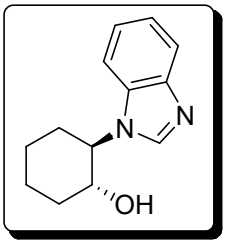
Yellow oil, Yield 75%; IR ($CHCl_3$, cm^{-1}): 3443, 2925, 1262, 1120; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.84-2.20 (m, 8H), 3.29-3.82 (m, 2H), 7.47 (s, 1H), 7.79 (s, 1H); MS (EI, m/z) = 211 $[M]^+$. Anal. calcd. for $C_9H_{13}N_3O_3$: C, 51.18; H, 6.20; N, 19.89; Found: C, 51.40; H, 6.28; N, 19.63.

trans-1-(2-Hydroxycyclohexyl)-1*H*-imidazole-4-carbaldehyde (6m)



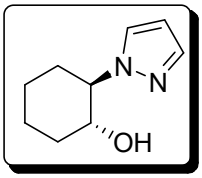
Colorless liquid, Yield 76%; IR ($CHCl_3$, cm^{-1}): 3451, 2923, 1723, 1434, 1262; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88-2.19 (m, 8H), 3.67-3.82 (m, 2H), 7.72 (s, 1H), 7.87 (s, 1H), 9.66 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.2, 24.9, 31.8, 34.1, 64.5, 72.9, 125.0, 139.2, 141.2, 184.9; MS (EI, m/z) = 194 $[M]^+$. Anal. calcd. for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42; Found: C, 61.96; H, 7.18; N, 14.40.

trans-2-(1*H*-Benzimidazol-1-yl)cyclohexanol (6n)



Brown solid, Yield 78%; m.p. 163-165 ^{o}C; IR ($CHCl_3$, cm^{-1}): 3090, 2926, 1615, 1494, 1458, 1253, 1075, 742; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.42-1.60 (m, 3H); 1.74-1.93 (m, 3H), 2.03-2.09 (m, 1H), 2.23-2.29 (m, 1H), 3.92–4.08 (m, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.50 (m, 2H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.5, 25.3, 31.9, 34.4, 62.5, 72.4, 110.6, 119.5, 122.2, 122.7, 133.8, 140.8, 142.8; MS (EI, m/z) = 216 $[M]^{+}$. Anal. calcd. for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95; Found: C, 72.21; H, 7.48; N, 12.99.

trans-2-(1H-Pyrazol-1-yl)cyclohexanol (6o)



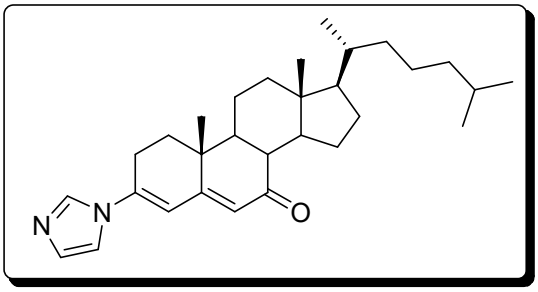
Thick colorless liquid, Yield 80%; IR ($CHCl_3$, cm^{-1}): 3447, 2931, 1245, 1123; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.29-1.35 (m, 3H), 1.70-1.77 (m, 3H), 2.00-2.07 (m, 2H), 3.69-3.85 (m, 2H), 4.07 (bs, 1H), 6.17 (d, *J* = 2 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.1, 24.8, 31.2, 33.6, 66.9, 72.9, 105.0, 128.5, 139.1; MS (EI, m/z) = 166 $[M]^{+}$. Anal. calcd. for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.85; Found: C, 65.25; H, 8.58; N, 16.99.

Preparation of steroidal/nonsteroidal N-(1-cycloalkenyl)heterocycles (7)

A stirring solution of hydroxy compound **6** (200 mg) and catalytic amount of sulfuric acid in acetic acid (0.5 mL) was heated at 80 ^{o}C for 4-6 hours. Acetic acid was removed in vacuo and ethylacetate (30 mL) and saturated solution of $NaHCO_3$ were added into the residue. The ethylacetate layer was washed with brine and the residue obtained after removal of the ethylacetate layer was purified by silica gel column chromatography using EtOAc/hexane as the eluent to afford the steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycle derivative **7**.

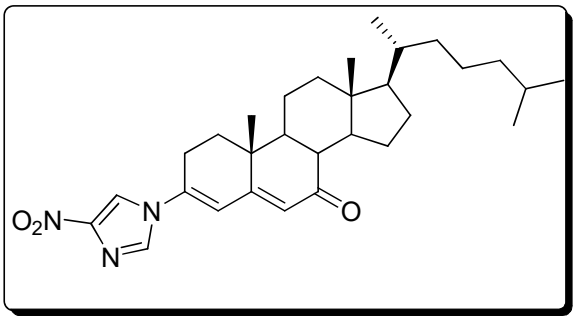
Characterization of steroidal/nonsteroidal N-(1-cycloalkenyl)heterocycles (7)

3-(1*H*-Imidazolo)-3,5-dien-cholest-7-one (7a)



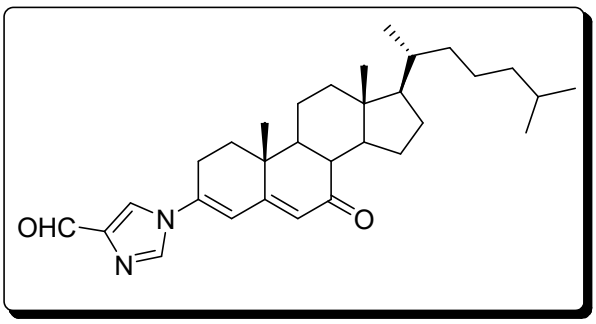
Yellow solid, Yield 88%; m.p. 182-186 ^{o}C; IR ($CHCl_3$, cm^{-1}): 2926, 1662, 1466, 772; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.80-2.50 (m, 24H), 0.85 (d, *J* = 6.7 Hz, 6H), 0.93 (d, *J* = 6.4 Hz, 3H), 1.22 (s, 3H), 5.09 (s, 1H), 6.25 (t, *J* = 4.2 Hz, 1H), 6.88 (s, 1H), 7.10 (s, 1H), 7.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 12.6, 14.1, 16.6, 18.9, 21.4, 22.6, 22.7, 22.8, 23.8, 26.2, 28.0, 28.5, 29.4, 29.7, 31.9, 32.4, 35.7, 36.1, 37.3, 39.5, 43.2, 45.7, 49.1, 50.2, 54.8, 114.1, 120.3, 122.4, 128.6, 133.4, 154.2, 156.5, 201.7; MS (EI, *m/z*) = 448 $[M]^{+}$. Anal. calcd. for $C_{30}H_{44}N_2O$: C, 80.31; H, 9.88; N, 6.24; Found: C, 80.44; H, 9.87; N, 6.39.

3-(4'-Nitro-1*H*-imidazolo)-3,5-dien-cholest-7-one (7b)



Yellow solid, Yield 83%; m.p. 90-92 ^{o}C; IR ($CHCl_3$, cm^{-1}): 2930, 1672, 1466; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.80-2.59 (m, 24H), 0.86 (d, *J* = 6.7 Hz, 6H), 0.93 (d, *J* = 6.4 Hz, 3H), 1.23 (s, 3H), 5.31 (s, 1H), 6.54 (t, *J* = 4.1 Hz, 1H), 8.22 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9, 13.0, 18.7, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.5, 29.0, 29.7, 35.7, 35.8, 36.2, 38.3, 39.5, 42.4, 51.1, 53.7, 56.2, 56.3, 114.3, 120.8, 122.6, 129.2, 134.4, 156.1, 156.0, 201.2; MS (EI, *m/z*) = 493 $[M]^{+}$. Anal. calcd. for $C_{30}H_{43}N_3O_3$: C, 72.99; H, 8.78; N, 8.51; Found: C, 72.79; H, 8.61; N, 8.64.

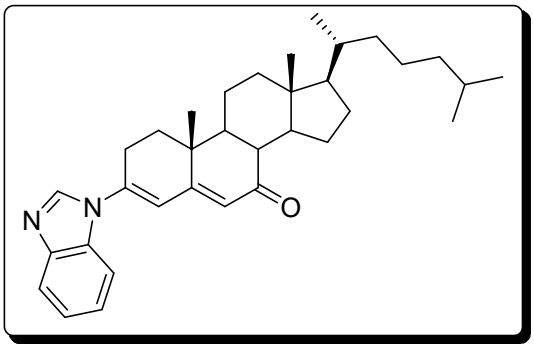
3-(4'-Formyl-1*H*-imidazolo)-3,5-dien-cholest-7-one (7c)



Yellow solid, Yield 75%; m.p. 108-110 ^{o}C; IR ($CHCl_3$, cm^{-1}): 2952, 1671, 1536, 1466; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.81-2.55 (m, 24H), 0.86 (d, *J* = 6.6 Hz, 6H), 0.93 (d, *J* = 6.4 Hz, 3H), 1.27 (s, 3H), 5.33 (s, 1H), 6.33 (t, *J* = 4.3 Hz, 1H), 7.53 (d, *J* = 0.8 Hz, 1H), 7.58 (d, *J* = 1.0 Hz, 1H), 9.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.7, 18.9,

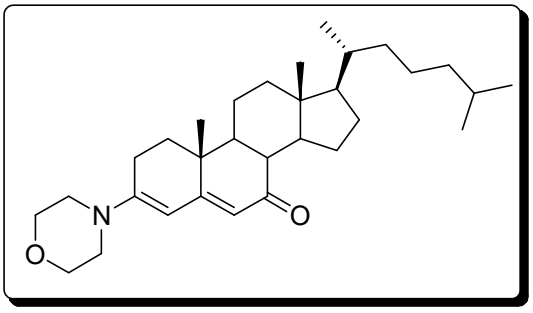
21.4, 22.6, 22.8, 23.8, 28.0, 28.5, 29.7, 31.0, 32.2, 35.7, 36.1, 37.3, 38.6, 39.5, 43.2, 45.8, 49.0, 50.1, 54.8, 122.4, 125.3, 132.8, 134.6, 139.0, 142.2, 155.7, 186.0, 201.3; MS (EI, *m/z*) = 476 $[M]^+$. Anal. calcd. for $C_{31}H_{44}N_2O_2$: C, 78.11; H, 9.30; N, 5.88 Found: C, 78.14; H, 9.33; N, 5.91.

3-(1*H*-Benzo[d]imidazolo)-3,5-dien-cholest-7-one (7d)



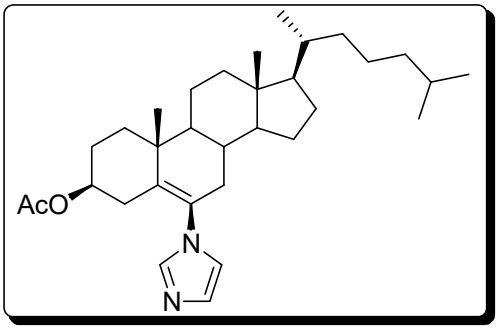
Brown solid, Yield 88%; m.p. 126-128 ^oC; IR ($CHCl_3$, cm^{-1}): 3418, 2929, 1670, 1466, 1382, 757; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.73 (s, 3H), 0.80-2.60 (m, 24H), 0.86 (d, *J* = 6.6 Hz, 6H), 0.93 (d, *J* = 6.5 Hz, 3H), 1.25 (s, 3H), 5.21 (s, 1H), 6.38 (s, 1H), 7.10-7.86 (m, 4H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.9, 18.9, 22.6, 22.8, 26.2, 28.0, 29.7, 35.8, 36.2, 38.7, 39.5, 42.8, 51.8, 56.0, 56.6, 114.1, 120.5, 121.9, 122.6, 122.8, 129.2, 134.4, 156.2, 156.7, 201.7; MS (EI, *m/z*) = 498 $[M]^+$. Anal. calcd. for $C_{34}H_{46}N_2O$: C, 81.88; H, 9.30; N, 5.62; Found: C, 81.98; H, 9.21; N, 5.75.

3-(Morpholino)-3,5-dien-cholest-7-one (7e)



Thick yellow liquid; Yield 68%; IR ($CHCl_3$, cm^{-1}): 2952, 1672, 1457, 1355, 1177, 923; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.80-2.45 (m, 24H), 0.86 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.4 Hz, 3H), 1.41(s, 3H), 2.46 (t, *J* = 4.0 Hz, 4H), 3.67 (t, *J* = 4.2 Hz, 4H), 5.27 (s, 1H), 5.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.4, 18.9, 22.6, 22.7, 22.8, 22.9, 26.3, 28.0, 29.7, 35.7, 36.1, 38.5, 38.9, 39.0, 39.5, 43.0, 45.9, 50.0, 52.3, 54.8, 66.7, 71.9, 123.9, 132.8, 159.7, 201.8; MS (EI, *m/z*) = 467 $[M]^+$. Anal. calcd. for $C_{31}H_{49}NO_2$: C, 79.60; H, 10.56; N, 2.99; Found: C, 79.81; H, 10.42; N, 3.27.

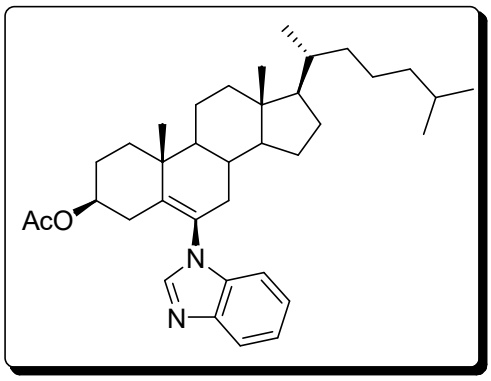
3*β*-Acetyl-6-(1*H*-imidazolo)-cholest-5-en (7f)



Yellow solid, Yield 81%; m.p. 99-102 ^oC; IR ($CHCl_3$, cm^{-1}): 2928, 1733, 1584, 1020; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.71 (s, 3H), 0.80-2.40 (m, 28H), 0.85 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.24 (s, 3H), 2.00 (s, 3H), 4.47-4.55 (m, 1H), 6.85 (s, 1H), 7.10 (s, 1H), 7.43 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.8, 18.7, 19.6, 21.2, 22.5, 22.8, 23.8, 24.1, 27.4,

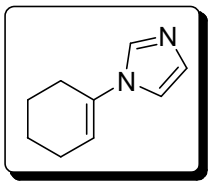
28.0, 28.1, 31.9, 35.7, 36.1, 36.9, 37.4, 39.5, 42.3, 49.5, 56.0, 56.2, 72.8, 119.2, 129.0, 129.3, 136.7, 136.9, 170.2; MS (EI, *m/z*) = 494.4 $[M]^+$. Anal. calcd. for $C_{32}H_{50}N_2O_2$: C, 77.68; H, 10.19; N, 5.66; Found: C, 77.51; H, 10.04; N, 5.84.

3*β*-Acetyl-6-(1*H*-benzo[d]imidazolo)-cholest-5-en (7g)



Yellow solid, Yield 83%; m.p. 105-108 ^oC; IR ($CHCl_3$, cm^{-1}): 2948, 1733, 1462, 1225; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.70 (s, 3H), 0.80-2.42 (m, 28H), 0.85 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.23 (s, 3H), 2.11 (s, 3H), 4.74-4.85 (m, 1H), 7.00-7.23 (m, 3H), 7.64-7.68 (m, 1H), 7.70 (s, 1H); MS (EI, *m/z*) = 544.4 $[M]^+$. Anal. calcd. for $C_{36}H_{52}N_2O_2$: C, 79.36; H, 9.62; N, 5.14; Found: C, 79.42; H, 9.67; N, 5.06.

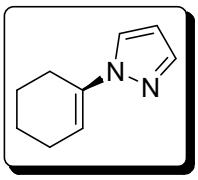
1-(Cyclohex-1-en-1-yl)-1*H*-imidazole (7h)



Brown oil, Yield 79%; IR ($CHCl_3$, cm^{-1}): 2927, 1661, 773; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.64-1.72 (m, 2H), 1.78-1.86 (m, 2H), 2.17-2.22 (m, 2H), 2.41-2.43 (m, 2H), 5.83 (s, 1H), 7.07 (s, 1H), 7.09 (s, 1H), 7.66 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.6, 22.3, 24.0, 27.3,

29.7, 116.4, 116.5, 129.2, 133.7, 134.5; MS (EI, m/z) = 148 $[M]^+$. Anal. calcd. for $C_9H_{12}N_2$: C, 72.94; H, 8.16; N, 18.90; Found: C, 72.98; H, 8.01; N, 18.72.

1-(Cyclohex-1-en-1-yl)-1*H*-pyrazole (7i)



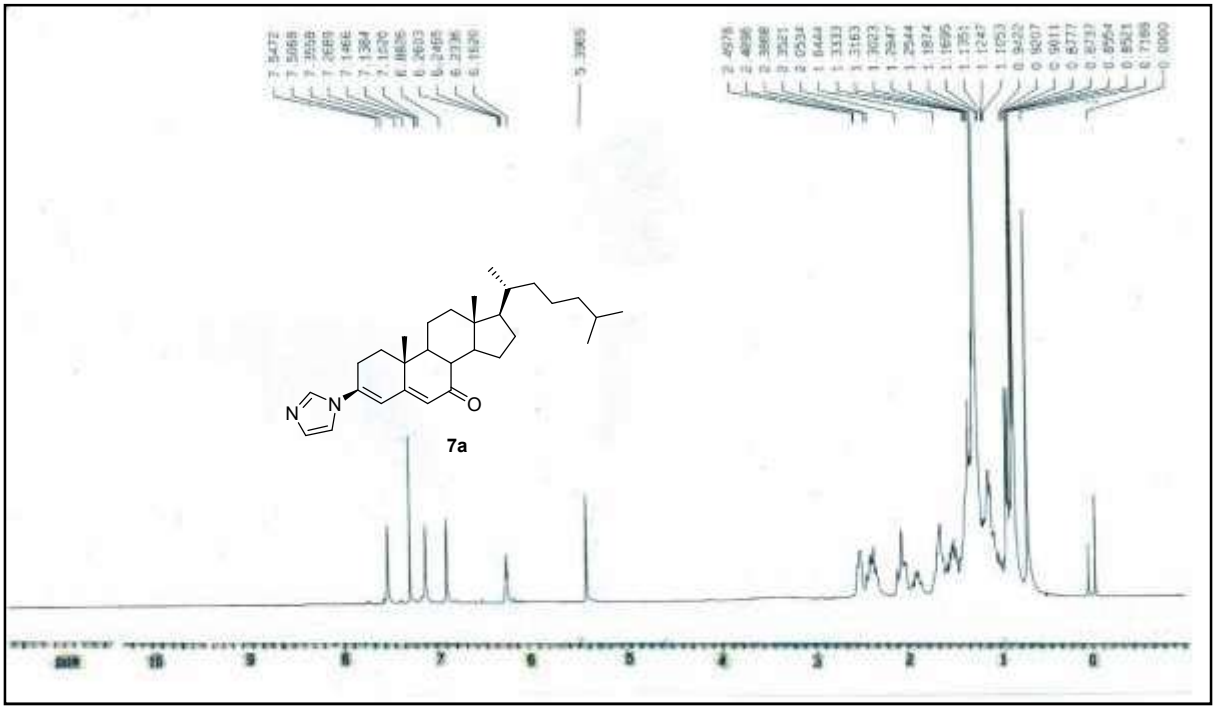
Brown liquid, Yield 72%; IR ($CHCl_3$, cm^{-1}): 2926, 1657, 768; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.61-1.71 (m, 2H), 1.80-1.89 (m, 2H), 2.18-2.27 (m, 2H), 2.53-2.60 (m, 2H), 6.11-6.13 (m, 1H), 6.31-6.32 (m, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.8, 22.4, 24.1, 25.9, 105.9, 113.8, 125.8, 136.5, 139.7; MS (EI, m/z) = 148 $[M]^+$. Anal. calcd. for $C_9H_{12}N_2$: C, 72.94; H, 8.16; N, 18.90; Found: C, 72.81; H, 8.11; N, 18.58.

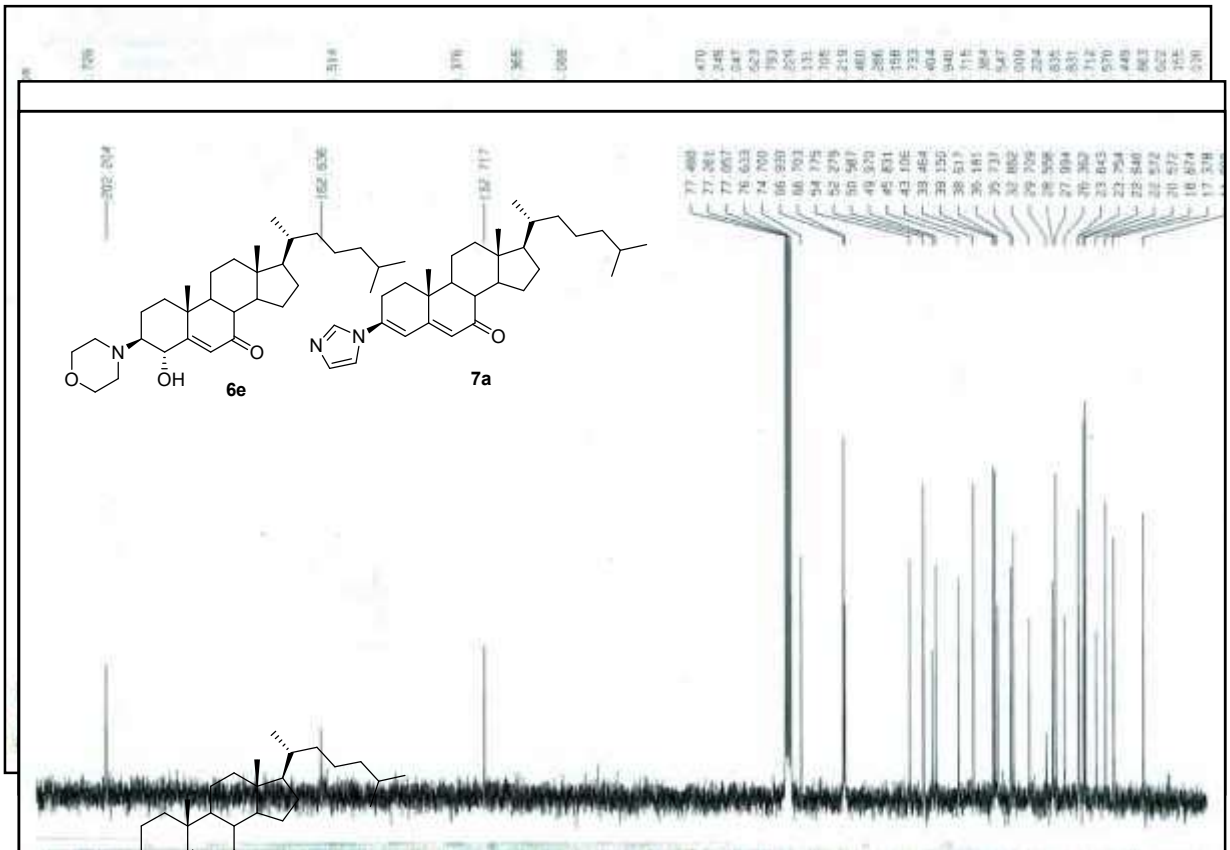
References:

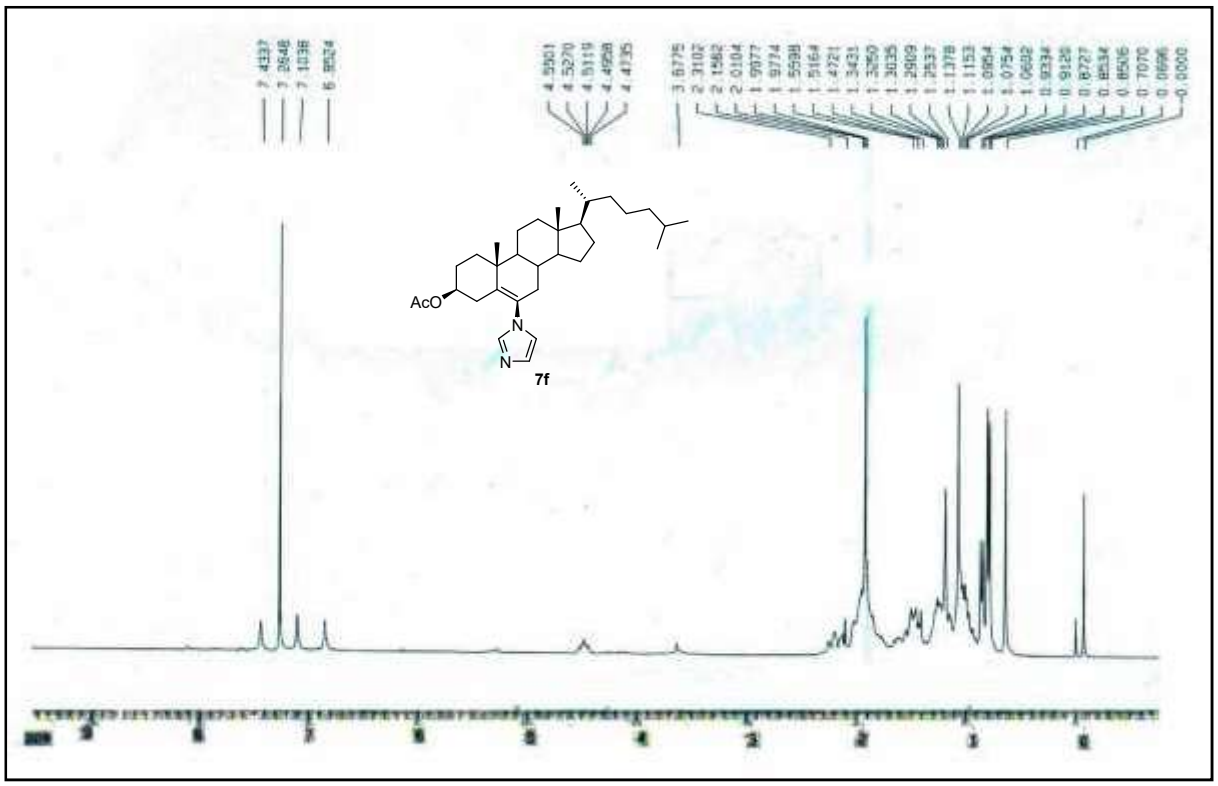
1. Bonollo, S.; Lanari, D.; Vaccaro, L. *Eur. J. Org. Chem.,* **2011**, 2587.
2. De, M.; Guiseppe, L. R.; Alessandra, D. P.; Rino, R.; Alberto, B.; Chiara, C.; Anna, S.; Giovanni, M.; Emmanuele, C.; Marino, A.; Romano, S. *J. Med. Chem.,* **2005**, *48*, 4378.
3. (a) Roy, J.; Roy D. P.; Poirier, D. *J. Comb. Chem*., **2007**, *9*, 347; (b) Jegham, H.; Maltais, R.; Dufour , P.; Roy, J.; Poirier, D. *Steroids*, **2012**, *77*, 1403.
4. Yousuf, S. K.; Majeed, R.; Ahmad, M.; Sangwana, P.; Purnimaa, B.; Saxsenab, A. K.; Suria, K. A.; Mukherjeea, D.; Tanejaa, S. C. *Steroids*, **2011**, *76*, 1213.
5. Hoelscher, P.; Rehwinkel, H.; Burton, G.; Moewes, M.; Hillmann, M. *Ger Offen DE 19627310*, **1998**; *Chem. Abstr.*, **1998**, 128, 114949h.
6. Vogiatzi, P.; Claudio, P. P. *Expert Rev. Anticancer Ther.*, **2010**, *10*, 1027.
7. Handratta, V. D.; Vasaitis, T. S.; Njar, V. C.; Gediya, L. K.; Kataria, R.; Chopra, P. *et al. J. Med. Chem.*, **2005**, *48*, 2972.
8. Jegham, H.; Maltais, R.; Dufour, P.; Roy, J.; Poirier, D. *Steroids*, **2012**, *77*, 1403.
9. Jalil, M. A.; Masumb, S. M. *Tetrahedron Lett.*, **2012**, *53*, 3049.
10. Purushottamachar, P.; Godbole, A. M.; Gediya, L. K.; Martin, M. S.; Vasaitis, T. S.; Kwegyir-Afful, A .K.; Ramalingam, S.; Alagoz, Z. A.; Njar, V. C. O. *J. Med. Chem.*, **2013**, *56*, 4880.
11. Ling, Y.; Li, J. S.; Liu, Y.; Kato, K.; Klus, G. T.; Brodie, A. *J. Med. Chem.*, **1997**, *40,* 3297.
12. Katritzky, A. R.; Maimait, R.; Xu, Y. J.; Gyoung, Y. S. *J. Org. Chem.*, **2002**, *67*, 8230.
13. Stary, I.; Kocovsky, P. *Chem. Commun.*, **1985**, *50*, 1227.
14. Zhao, Y. C.; Xiang, Y. Z.; Pu, L.; Yang, M.; Yu, X. Q. *Appl. Catal. A.*, **2006**, *301*, 176.

15. Lemriss, S.; Marquet, B.; Gineste, H.; Lefeuvre, L.; Fassouane, A.; Boiron, P. *J. Mycol. Med.*, **2003**, *13*, 189.

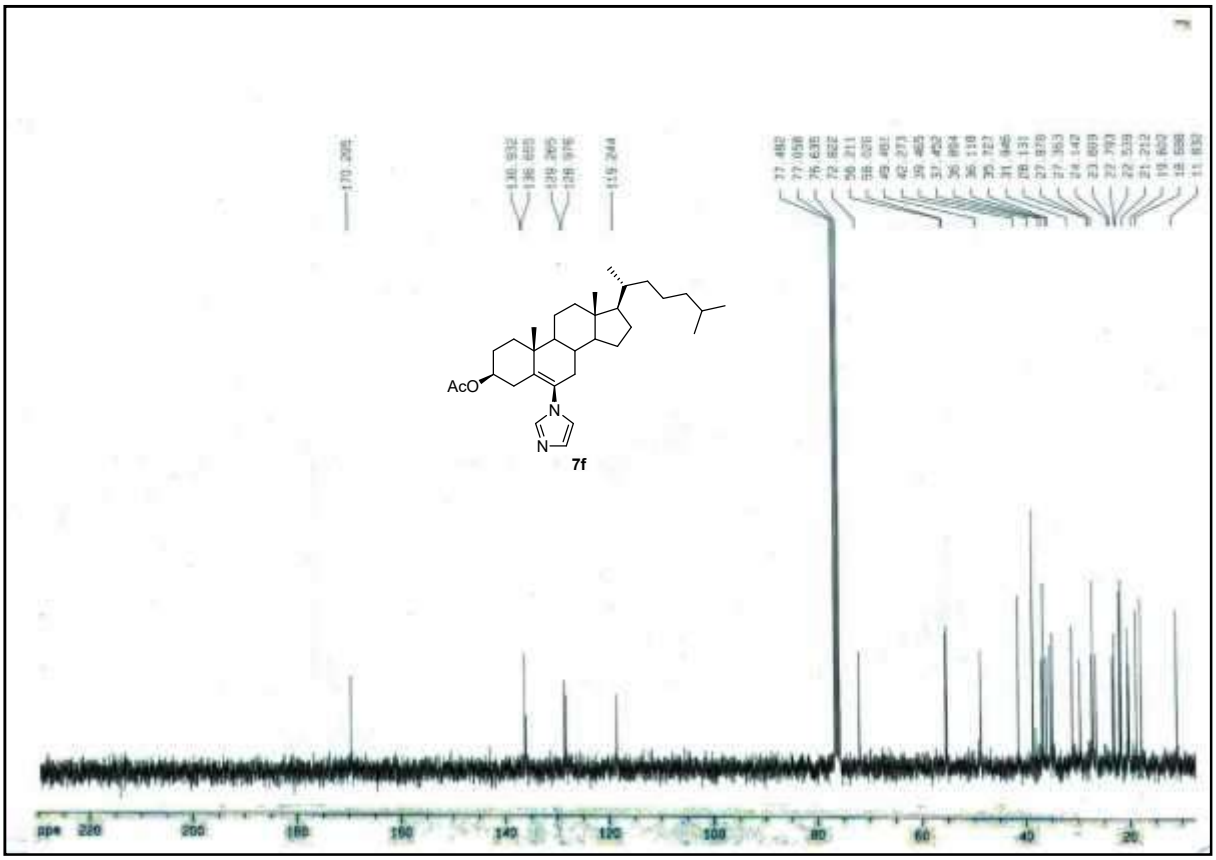
^{1}H NMR and ^{13}C NMR of some selected synthesized compounds



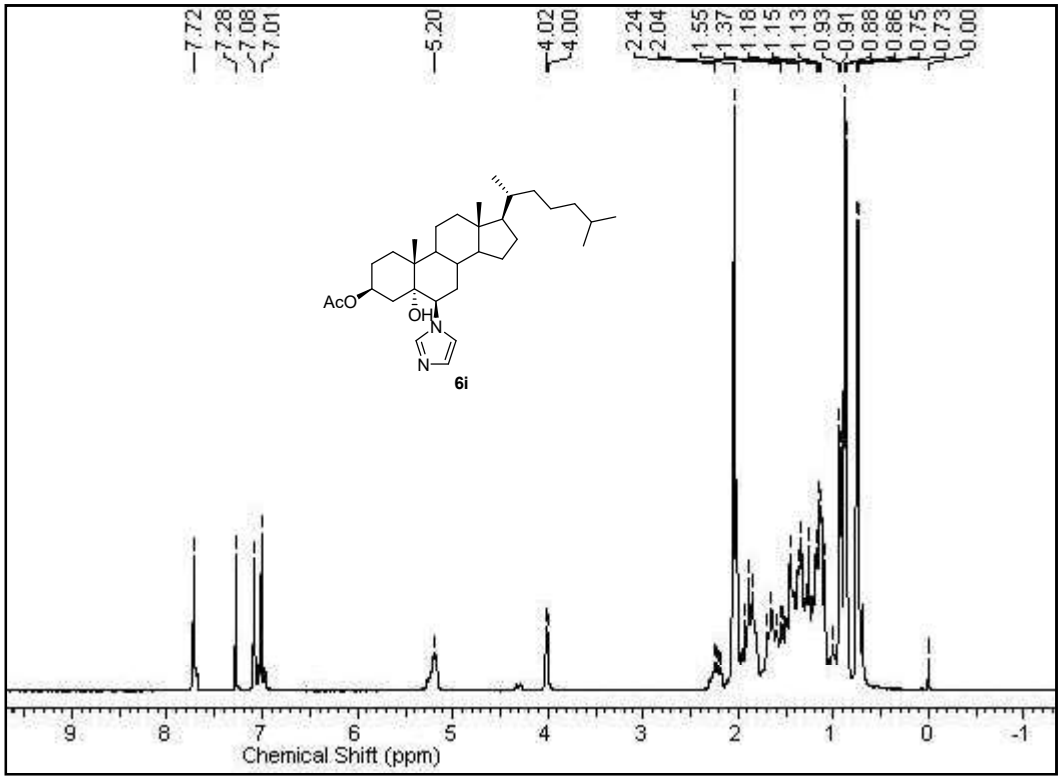


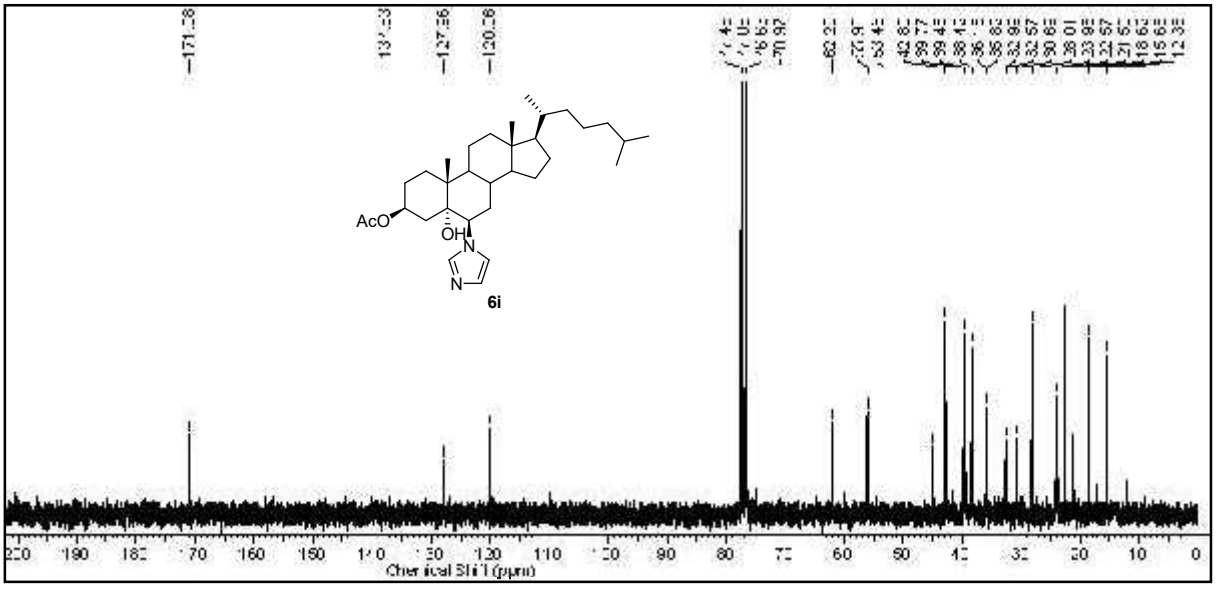


7f



7f


7.72
7.28
7.08
7.01
5.20
4.02
4.00
2.24
2.04
1.55
1.37
1.18
1.15
1.13
0.93
0.91
0.88
0.86
0.75
0.73
0.00
AcO
OH
N
N
6i
9
8
7
6
5
4
3
2
1
0
-1
Chemical Shift (ppm)


171.28
137.33
127.36
120.26
82.23
70.92
AcO
OH
N
N
6i
Chemical Shift (ppm)

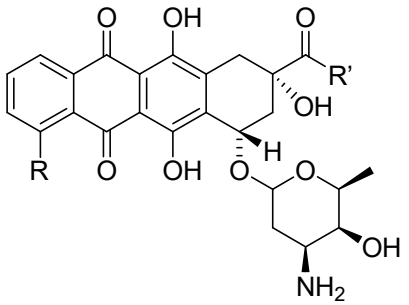
Chapter 3

Part-B

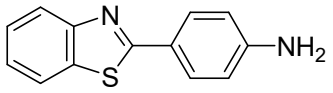
Biological studies of some Michael adducts of D-ring steroids

3B.1 Introduction

Cancer is the most prevalent killer disease in the history of mankind. Cancer cells in particular have irregular cell cycle progression profiles due to the mutagenic nature and the presence of growth factors.[1] Destruction of the checkpoints is a favourable property in formulating an anticancer drug because the cells are more susceptible and sensitive to more damage. Thus, it is not only important to inhibit tumor growth, it is also as equally vital to prevent the cells from metastasizing. Since Richard Nixon launched a global war to eradicate cancer, research to find the perfect cure has been widely expanding. A variety of chemotherapeutic drugs are being progressively formulated especially in treating metastatic cancer. Figure 3B.1 shows some anthracycline class of molecules which are used for the treatment of metastatic cancer. Epirubicin (II) is used to treat breast cancer, while daunorubicin (III) and idarubicin (IV) are most commonly used to treat different types of leukemia. One antitumor derivative of 4-(benzo[d]thiazol-2-yl)benzenamine is currently in phase 1 clinical trials in Great Britain.[2]



Doxorubicin (**I**), R = OCH_3 , R'=CH_2OH
Epirubicin (**II**), R = OCH_3 , R'=CH_2OH
Daunorubicin (**III**), R = OCH_3 , R'=CH_3
Idarubicin (**IV**); R=H, R'=CH_3

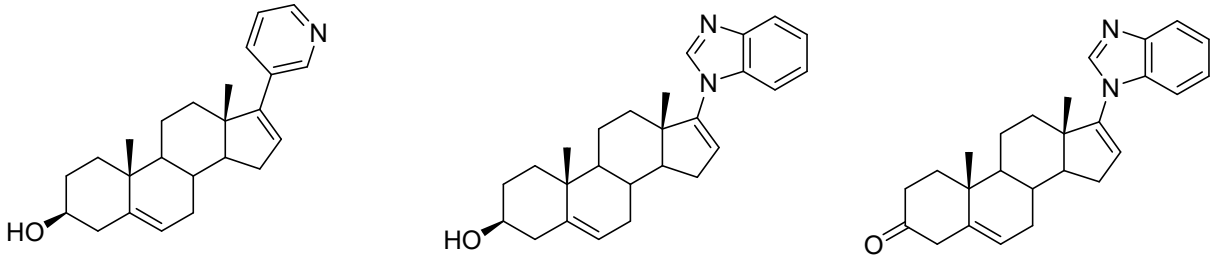


4-(benzo[*d*]thiazol-2-yl)benzenamine

Figure 3B.1 Examples of some drugs used for the treatment of cancer

In fact, one of the most famous drugs used to treat breast cancer is doxorubicin (**I**), an anthraquinone/anthracycline[3,4]. Nevertheless, despite doxorubicin's effectiveness in treating breast cancer, it comes with adverse side effects including cardiac toxicity.[3,4]

Heterocycle attached to steroidal core also exhibit interesting anticancer properties. The importance of danazole, 17-imidazolyl steroid as anti-tumor drug is already discussed in the introduction chapter of the thesis. Abiraterone is used to treat prostate cancer which is active against 17α-hydroxylase and C 17-20 lyase components of the target enzyme obtained from human testis. Recently, steroidal C-17 benzoazoles (**I** and **II** in figure 3B.2) are found to inhibit the growth of DHT-stimulated LNCaP and LAPC4 prostate cancer cells with IC_{50} values in the low micromolar range (i.e., < 10 μM).[5] In fact, **I** is the important antihormonal agent that is significantly more effective than castration in suppression of androgen-dependent prostate tumor growth.

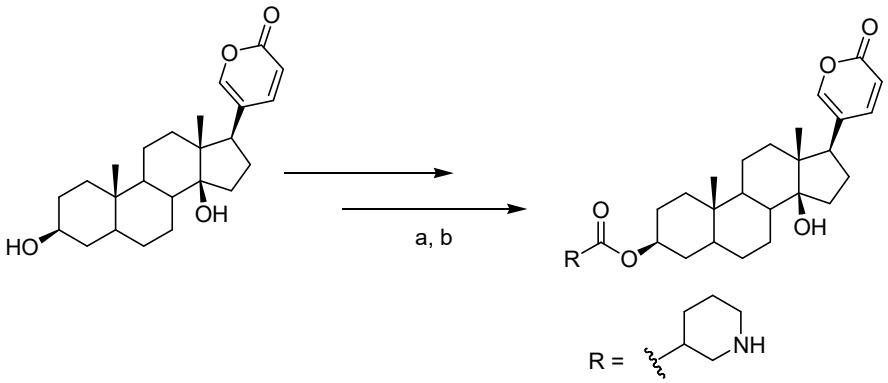


Abiraterone **I** **II**

Figure 3B.2 Compounds having steroidal core active against tumor growth

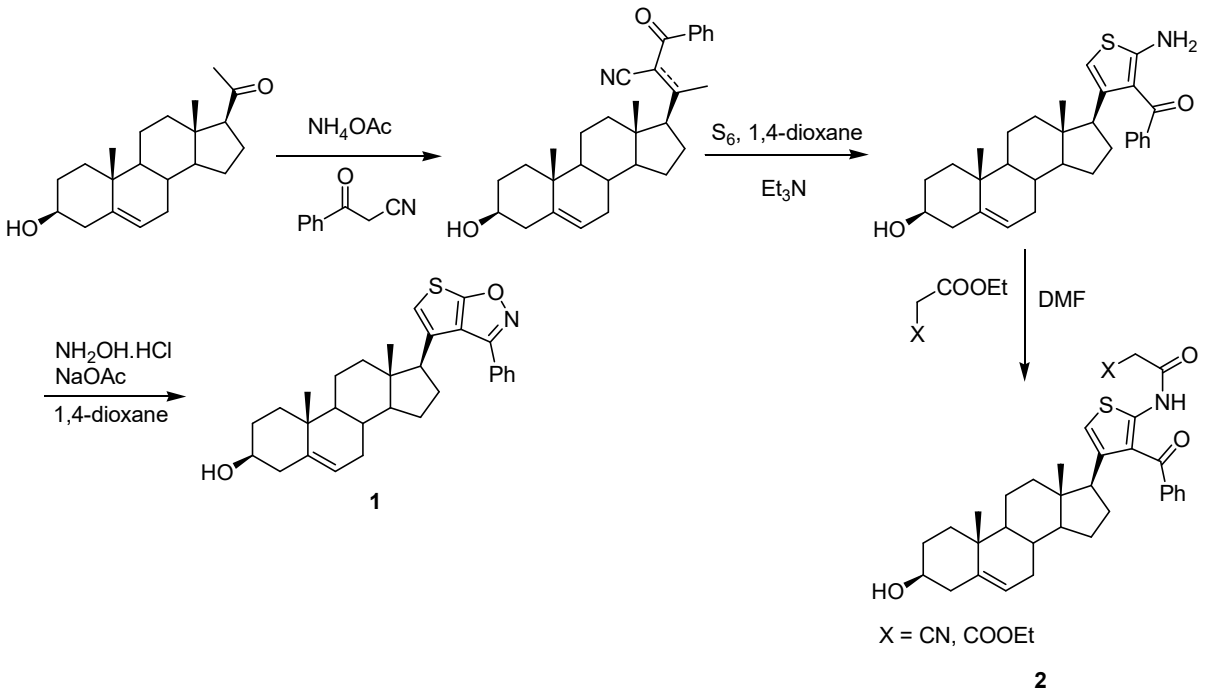
In recent years, there has been a great research interest focused on developing new anti-tumor molecules in steroidal moiety with an aim to discover unique compound that might be effective in metabolism of cancer cells, while leaving normal healthy cells largely unaffected.

Ma and co-workers synthesized[6] bufalin 3-nitrogen-containing-ester derivatives and evaluate their proliferation inhibition activities against human cervical epithelial adenocarcinoma (HeLa) and non-small-cell lung cancer (A549) cell lines. On two cell lines, the cytotoxic potency of bufalin-3-piperidinyl-4-carboxylate compound was found to be significant (IC_{50} values on HeLa and A549 cell lines were 0.76 nM and 0.34 nM, respectively) compared to the parent compound bufalin.



Reagents and conditions: (a) RCOOH, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, DCM, 12 h, rt and (b) TsOH, MeOH, 2 d, rt.

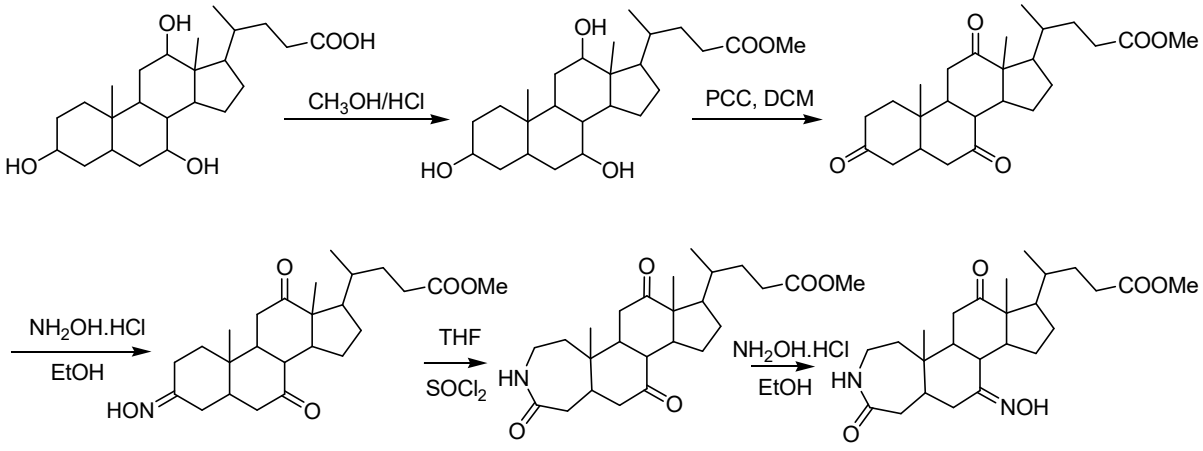
The cytotoxic activities of condensation product of pregnenolone (compound 1 and 2) were found to be interesting against six cancer cell lines compared to the drug CHS-828.[7] These compounds were synthesized by Mohareb et al by Knoevenagel reaction of pregnenolone with cyanomethylene reagents.



1

2

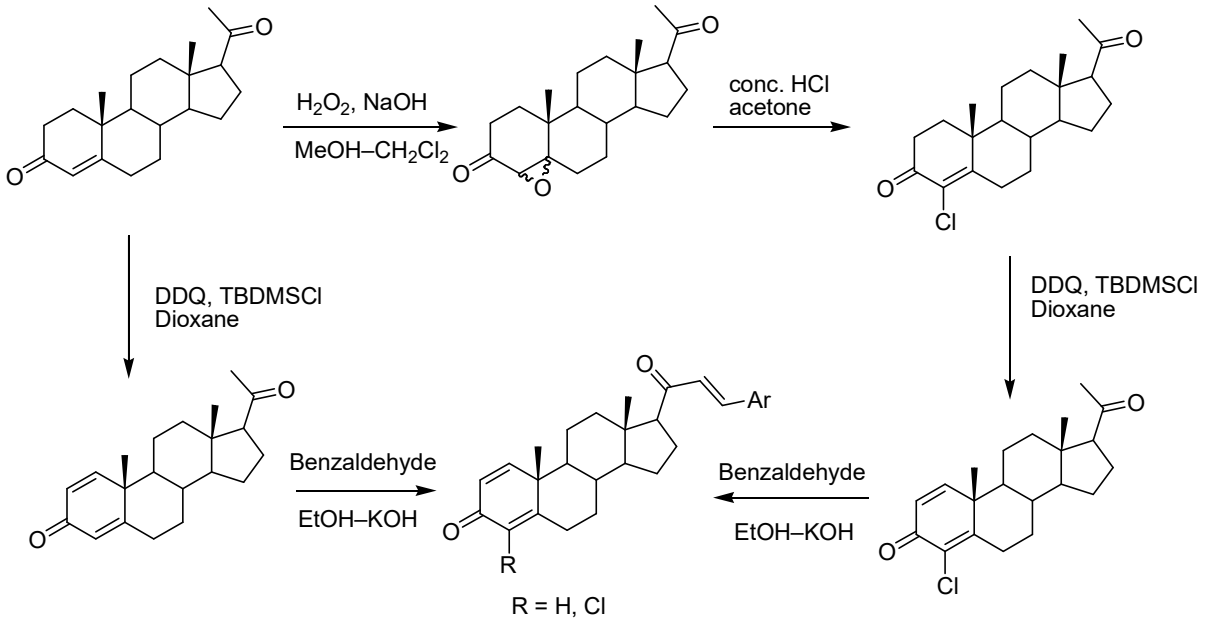
A series of 3-aza-A-homo-4-one bile acid derivatives were synthesized by Huang and co-workers[8] using cholic acid as starting materials. The bile acid derivatives with 3-aza-A-homo-4-one configuration bearing a 6-hydroximino group displayed a distinct cytotoxicity to Hela tumor cell line compared to the drug cis-platin.



In vitro cytotoxicity of 2-substituted 17β-hydroxy/17-methylene compounds were studied by Panchapakesan and co-workers[9] against four different cell lines from colon, lung, glioma and breast cancers.

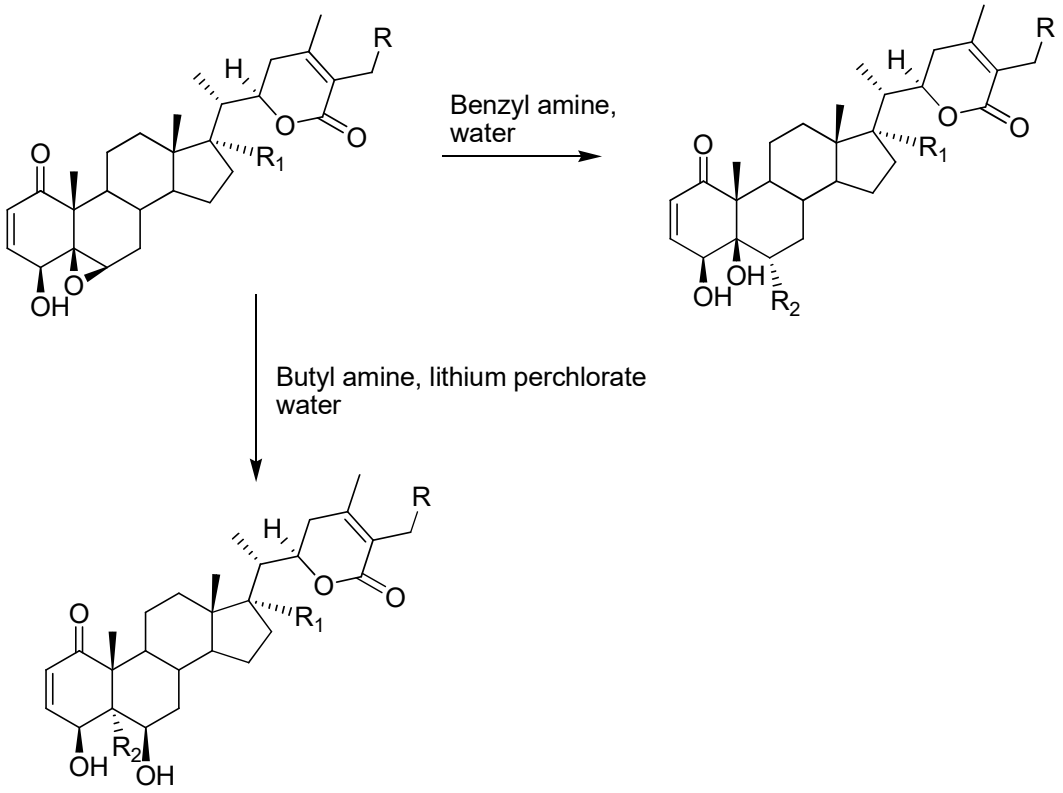
Tong *et al*[10] isolated and identified seven steroidal saponins from the rhizomes of DZW: diosgenin, trillin, diosgenin diglucoside, deltonin, zingiberensis saponin (ZS), protodeltonin and parvifloside. These compounds showed interesting cytotoxic activity against human and murine cancer cell lines very close to doxorubicin.

A series of novel derivatives of 21E-benzylidene-pregn-1,4-diene-3,20-dione and 21E-benzylidene-4-chloro-pregn-1,4-diene-3,20-dione was synthesized by Fan *et al*[11] from the commercially available progesterone. These compounds were evaluated for their cytotoxic activity against brine shrimp (Artemia salina) and murine Lewis lung carcinoma cells (LLC).

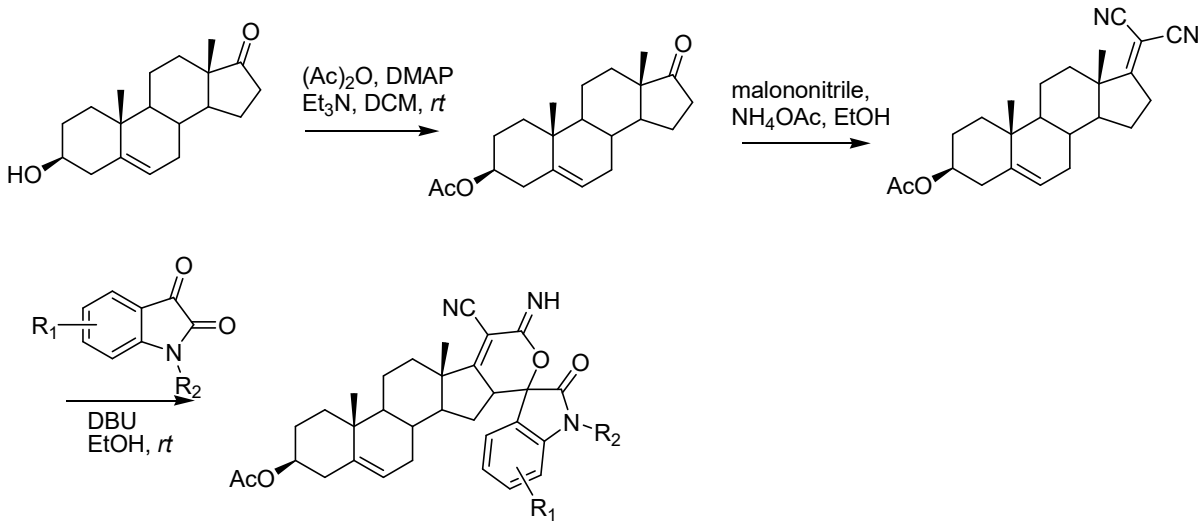


Joshi and co-workers[12] synthesized a series of derivatives with modifications at carbons 5, 6, and 7 in ring B of withanolides, a major steroidal constituent of *Withania*

somnifera to study the role of the epoxide group towards the cytotoxic property. Withanolides were converted into the respective thiiranes, amino alcohols and alcohols by selective reactions at the epoxide ring and were evaluated for in vitro anticancer activity against four cancer cell lines to study the structure activity relationships.



A series of novel steroidal pyran–oxindole hybrids were efficiently synthesized through the vinylogous aldol reaction of vinyl malononitrile with substituted isatins by Yu and co-workers.[13] The cytotoxic activities of these compounds were studied further and displayed moderate to good cytotoxicity against T24, SMMC-7721, MCF-7 and MGC-803 cells.



In continuation of our interests for the search of a new chemical entity, some studies of the cytotoxic activities of chemically synthesized 3*β*-acetoxy-16α-(1*H*-imidazol-1-yl)-17*β*-acetyl-androst-5-ene and 3*β*-acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene and their oxime derivatives on three cancer cell lines cervical HeLa, prostate DU 205 and MCF-7 were attempted.

3B.2 Results and discussions

The main objective of the present work is to evaluate the *in vitro* cytotoxic activities of compounds 3*β*-acetoxy-16α-(1*H*-imidazol-1-yl)-17*β*-acetyl-androst-5-ene and 3*β*-acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene and their oxime derivatives against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and MCF-7 cancer cell-line in comparison to the drug doxorubicin.

The oxime derivatives of 3*β*-acetoxy-16α-(1*H*-imidazol-1-yl)-17*β*-acetyl-androst-5-ene (**4**) and 3*β*-acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene (**5**) were synthesized by oximation reaction of compound **2** and **3** with hydroxylamine hydrocholoride in ethanol (Figure 3B.3). The preparation of compound **2** and **3** were accomplished by

Michael addition of imidazole and benzimidazole to 16-dehydropregnenolone acetate under microwave irradiation following a procedure developed at our laboratory.[14]

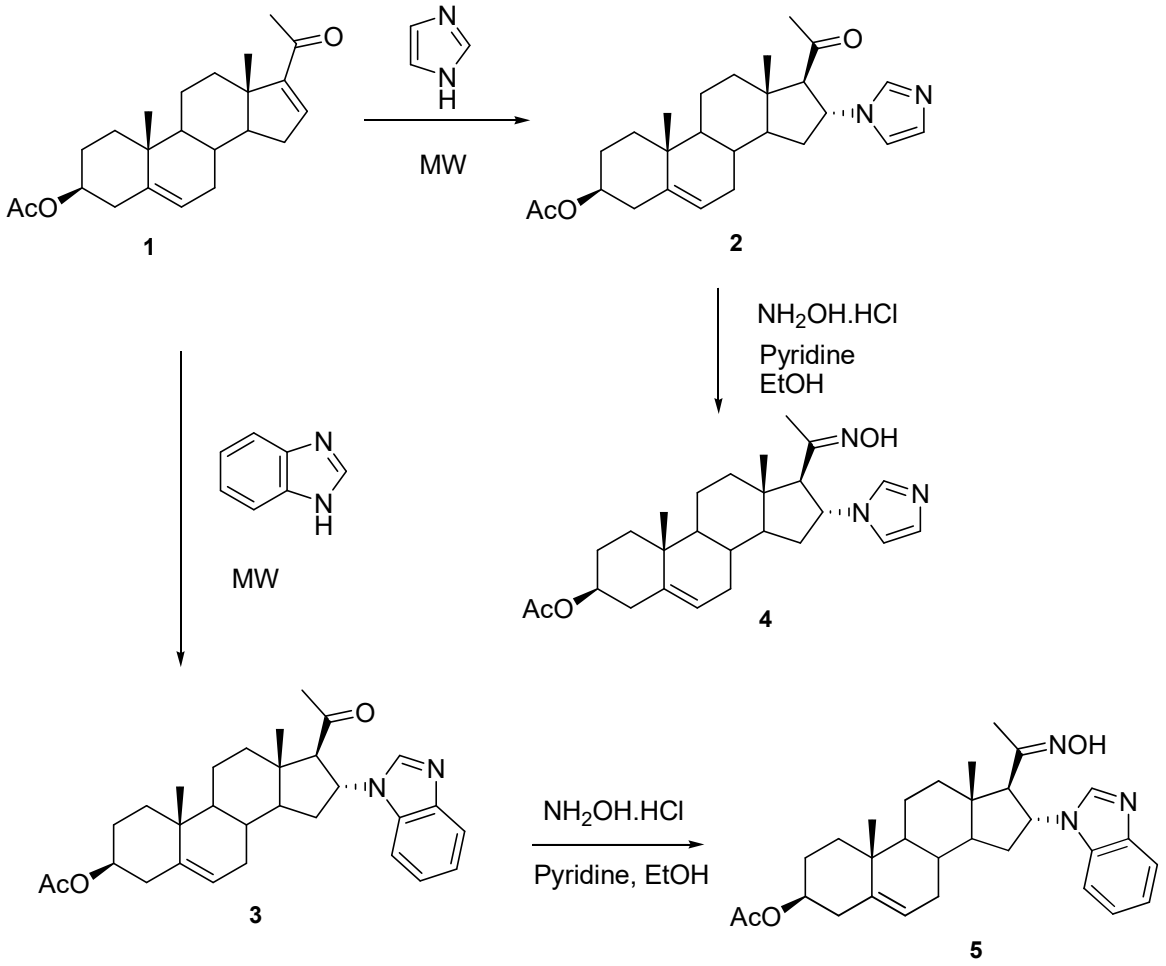


Figure 3B.3

The stereochemistry of the 16α-substituted imidazole and benzimidazole was further studied. The optical rotational data of these hetero steroids were compared with the reported 16-substituted steroidal heterocycles.[15] It was determined that the formation of 16α-substituted *N*-heterocycles having trans orientation with respect to the acetyl group at C-17 position of the steroid moiety. The α orientation of imidazole/benzimidazole at 16-position and the β orientation of acetyl group at 17-position was then confirmed by NOESY spectrum

of compound **2** and **3**. In the NOESY spectrum of both the compounds **2** and **3**, the presence of correlation of CH_3-18 protons with H-16 and CH_3-18 protons with CH_3-21 protons and the absence of correlation between CH_3-18 protons with H-17 also indicated that H-16 and H-17 were β and α oriented respectively in both the compounds (Figure 3B.4).

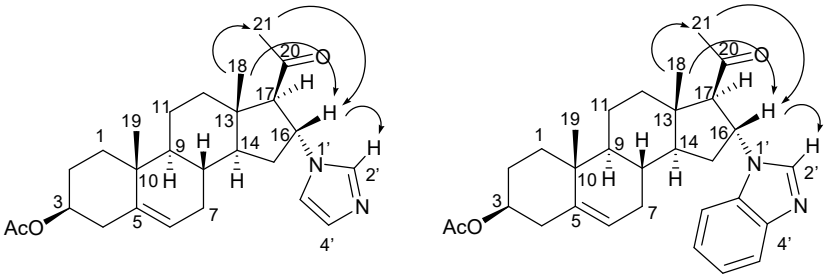
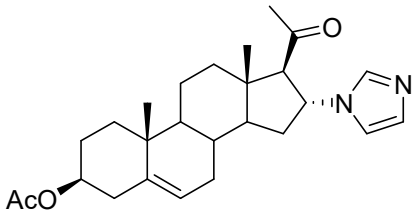
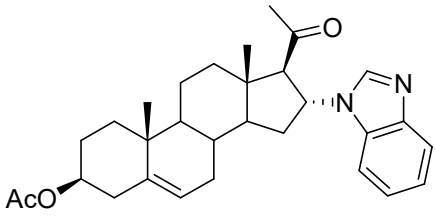
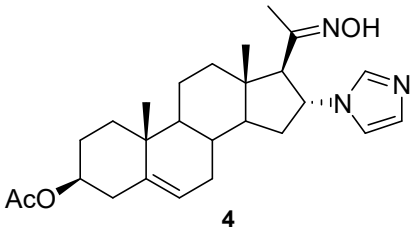
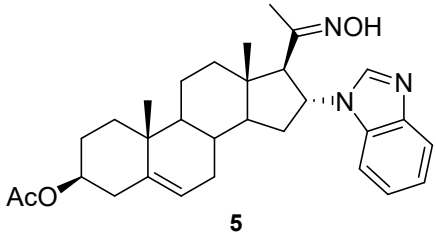


Figure 3B.4 Key NOESY correlations and relative stereochemistry of compound 2 and 3

The 16α-substituted imidazole and benzimidazole derivatives were further evaluated according to standard protocols for their in vitro cytotoxicity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line. The IC_{50} values of all the compounds were listed in Table 3B.1.

Table 3B.1 IC_{50} values of compounds determined by MTT assay

Compound	IC_{50} in μM HeLa cell line at 10 μM conc.	IC_{50} in μM DU 145 cell line at 10 μM conc.	IC_{50} in μM MCF-7 cell line at 10 μM conc.
 **2**	48.2584	37.8069	-
 **3**	8.3317	12.0192	8.2855
 **4**	29.8783	15.7615	-
 **5**	7.8234	10.2380	-
Doxorubicin	7.8126	9.1194	7.5094

It was observed that steroidal ketone **3** has shown significant cytotoxicity against HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line with IC_{50} = 8.3317 nM, 12.0192 nM and 8.2855 nM respectively. Again oxime derivative of the compound **3** showed promising cytotoxicity against HeLa cancer cell line with IC_{50} value

7.8234. The reference compound used for this study is doxorubicin which is an anthracycline class of antitumor drug.

3B.3 Conclusion

In summary, the cytotoxicities of two newly synthesized Michael adduct of 16-dehydropregnenolone acetate 3*β*-Acetoxy-16α-(1*H*-imidazol-1-yl)-17*β*-acetyl-androst-5-ene and 3*β*-Acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene and their oxime derivatives were studied. The cytotoxicities were evaluated against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line. The results showed that 3*β*-Acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene exhibited the optimal cytotoxic effect against the three cancer cell lines, with IC_{50}s in the nM range compared to the drug Doxorubicin.

3B.4 Experimental

3B.4.1 General experimental Procedure

Melting points were measured with a Buchi B-540 melting point apparatus and are uncorrected. IR spectra were recorded on Elmer FT-IR-2000 spectrometer on a thin film using chloroform. NMR spectra were recorded on Avance DPX 300 MHz FT-NMR spectrometer or Bruker Avance III 500 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Trace DSQ GCMS instrument or Bruker ESQUIRE 3000 LCMS instrument. All the commercially available reagents were used as received. All experiments were monitored by thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates (Merck). Column chromatography was performed on silica gel (100-200 mesh, Merck Chemicals).

3B.4.2 Biology

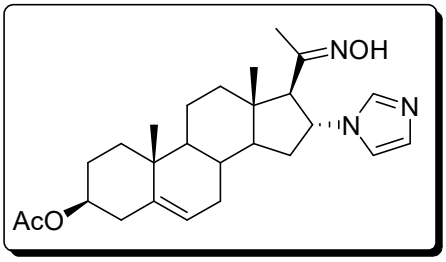
Cellular viability in the presence of test compounds was determined by MTT-micro cultured tetrazolium assay. The cells seeded to flat bottom 96 (1000cells/100µl) well plates and cultured in the medium containing 10% serum and allowed to attach and recover for 24 hours in a humid chamber containing 5% CO_2. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; sigma catalog No M2128) was dissolved in PBS at 5 mg/mL, filtered to sterilize and remove a small amount of insoluble residue present in MTT. Then different concentrations of the compound were added to the cells. After 48 hours, stock MTT solution (10 µl) was added to the culture plate. Cells were again kept in CO_2 incubator for 2 hours. After incubation, 100 µl of DMSO was added and mixed. The absorbance was read at 562 nm in a plate reader. The results were represented as percentage of cytotoxicity/viability. All the experiments were carried out in triplicates. From the percentage of cytotoxicity the IC_{50} value was calculated[16].

Chemical synthesis

(a) Preparation and characterization of 3*β*-Acetoxy-16*α*-(1*H*-imidazol-1-yl)-17*β*-acetyl oxime-androst-5-ene (4):

To a stirring solution of hydroxylamine hydrochloride (57 mg, 0.82 mmol) and molecular sieves (500 mg) in anhydrous ethanol (10 mL), pyridine (2 mL) was added. The reaction mixture was stirred at room temperature for 10 minutes and then 3*β*-Acetoxy-16α-(1*H*-imidazol-1-yl)-17*β*-acetyl-androst-5-ene **2** (350 mg, 0.82 mmol) was added into it. The reaction mixture was heated at 50 ^{o}C for 8 hours. Ethanol and pyridine were removed in vacuo and the crude product obtained was purified by column chromatography using EtOAc-hexane (1:3) as the eluant to afford compound **4** in 83% yield.

3*β*-Acetoxy-16*α*-(1H-imidazol-1-yl)-17*β*-acetyloxime-androst-5-ene (4)

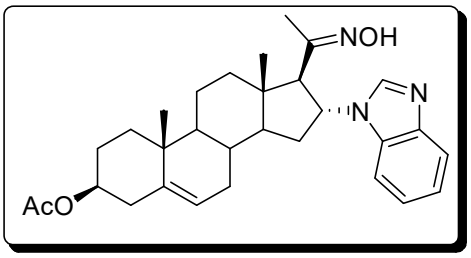


Yellow thick liquid, Yield 83% ; $[\alpha]_D$ = -10.0 (c, 0.5, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: ν 3338, 2941, 1728, 1246, 755; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.70- 2.92 (m, 18H), 0.75 (s, 3H), 1.03 (s, 3H), 1.87 (s, 3H), 2.10 (s, 3H), 2.81 (d, 1H, *J* = 7.8 Hz), 4.52-4.68 (m, 1H), 5.34-5.41 (m, 2H), 6.95 (d, 1H, *J* = 6.6 Hz), 7.06 (d, 1H, *J* = 6.6 Hz), 7.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.6, 15.6, 19.4, 21.4, 27.7, 29.7, 31.5, 36.6, 38.6, 44.7, 49.9, 55.4, 62.2, 73.7, 120.2, 122.8, 132.1, 139.9, 140.0, 153.4, 171.2; MS (EI) m/z = 439.3 $[M]^+$. Anal. calcd. for $C_{26}H_{37}N_3O_3$: C, 71.04; H, 8.48; N, 9.56; Found: C, 71.26; H, 8.60; N, 9.29.

(b) Preparation and characterization 3*β*-Acetoxy-16*α*-(1 -benzimidazol-1-yl)-17 *β*-acetyloxime-androst-5-ene (5):

To a stirring solution of hydroxylamine hydrochloride (43 mg, 0.63 mmol) and molecular sieves (500 mg) in anhydrous ethanol (8 mL), pyridine (2 mL) was added. The reaction mixture was stirred at room temperature for 10 minutes and then 3*β*-Acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyl-androst-5-ene **3** (300 mg, 0.63 mmol) was added into it. The reaction mixture was heated at 50 ^oC for 8 hours. Ethanol and pyridine were removed in vacuo and the crude product obtained was purified by column chromatography using EtOAc-hexane (1:3) as the eluant to afford compound **5** in 81% yield.

3*β*-Acetoxy-16*α*-(1*H*-benzimidazol-1-yl)-17*β*-acetyloxime-androst-5-ene (5)



Yellow solid, Yield 81%; m.p. 158 ^{o}C. R_f = 0.3 (EtOAc/Hexane = 1:4); $[\alpha]_D$ = -8.9 (c, 0.5, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: ν 2929, 2855, 1730, 1657, 1033, 762; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.84 (s, 3H), 2.36-0.90 (m, 17H), 1.07 (s, 3H), 1.82 (s, 3H), 2.05 (s, 3H), 2.84 (d, 1H, *J* = 9.7Hz), 4.70-4.60 (m, 1H), 5.41-5.37 (m, 1H), 5.74-5.64 (m, 1H), 7.38-7.25 (m, 2H), 7.52 (dd, 1H, *J* = 7.1 & 3.2 Hz), 7.80 (dd, 1H, *J* = 5.7 & 2.0 Hz), 8.32 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.5, 15.5, 19.4, 20.7, 21.5, 27.7, 29.7, 30.5, 31.8, 36.6, 36.9, 38.1, 38.6, 44.7, 49.9, 55.3, 55.4, 62.0, 73.8, 119.9, 121.8, 122.5, 123.0, 131.8, 140.0, 142.5, 144.3, 145.2, 153.1, 170.6; MS (EI) m/z = 489.3 $[M]^+$. Anal. calcd. for $C_{30}H_{39}N_3O_3$: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.60; H, 8.33; N, 8.56.

References:

1. Abu, N.; Akhtar , M. N.; Ho, W. Y.; Yeap, S. K.; Alitheen, N. B. *Molecules*, **2013**, *18*, 10367.
2. (a) Bradshaw, T. D.; Westwell, A. D. *Curr. Med. Chem.,* **2004**, *11*, 1009; (b) Brantley, E.; Antony, S.; Kohlhagen, G.; Meng, L.; Agama, K.; Stinson, S. F.; Sausville, E. A.; Pommier, Y. *Cancer Chemother. Pharmacol.*, **2006**, *58*, 62.
3. Beslija, S. *Breast Cancer Res. Treat.*, **2003**, *81*, 25.
4. Lown, J. W. *Pharmacol. Ther*., **1993**, *60*, 185.
5. Handratta, V. D.; Vasaitis, T. S.; Njar, V. C. O.; Gediya, L. K.; Kataria, R.; Chopra, P.; Newman, D.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. H. *J. Med. Chem.*, **2005**, *48*, 2972.
6. Ma, B.; Xiao, Z. Y.; Chen, Y. J.; Lei, M.; Meng, Y. H.; Guo, D. A.; Liu, X.; Hu, L. H. *Steroids*, **2013**, *78*, 508.
7. Mohareb, R. M.; El-Sayed, N. N. E.; Abdelaziz, M. A. *Steroids*, **2013**, *78*, 1209.
8. Huanga, Y.; Chen, S.; Cui, J.; Gan, C.; Liu, Z.; Wei, Y.; Song, H. *Steroids*, **2011**, *76,* 690.
9. Panchapakesan, G.; Dhayalan, V.; Moorthy, N. D.; Saranya, N.; Mohanakrishnan, A. K. *Steroids*, **2011**, *76*, 1491.
10. Tong, Q. Y.; He, Y.; Zhao, Q. B.; Qing, Y.; Huang, W.; Wua, X. H. *Steroids*, **2012**, *77*, 1219.
11. Fan, N. J.; Bai, Y. B.; Zhang, F. Y.; Luo, B.; Tang, J. J.; Zhang, Q. Z.; Gao, J. M. *Steroids*, **2013**, *78*, 874.

12. Joshi, P.; Misra, L.; Siddique, A. A.; Srivastava, M.; Kumar, S.; Darokar, M. P. *Steroids*, **2014**, *79*, 19.

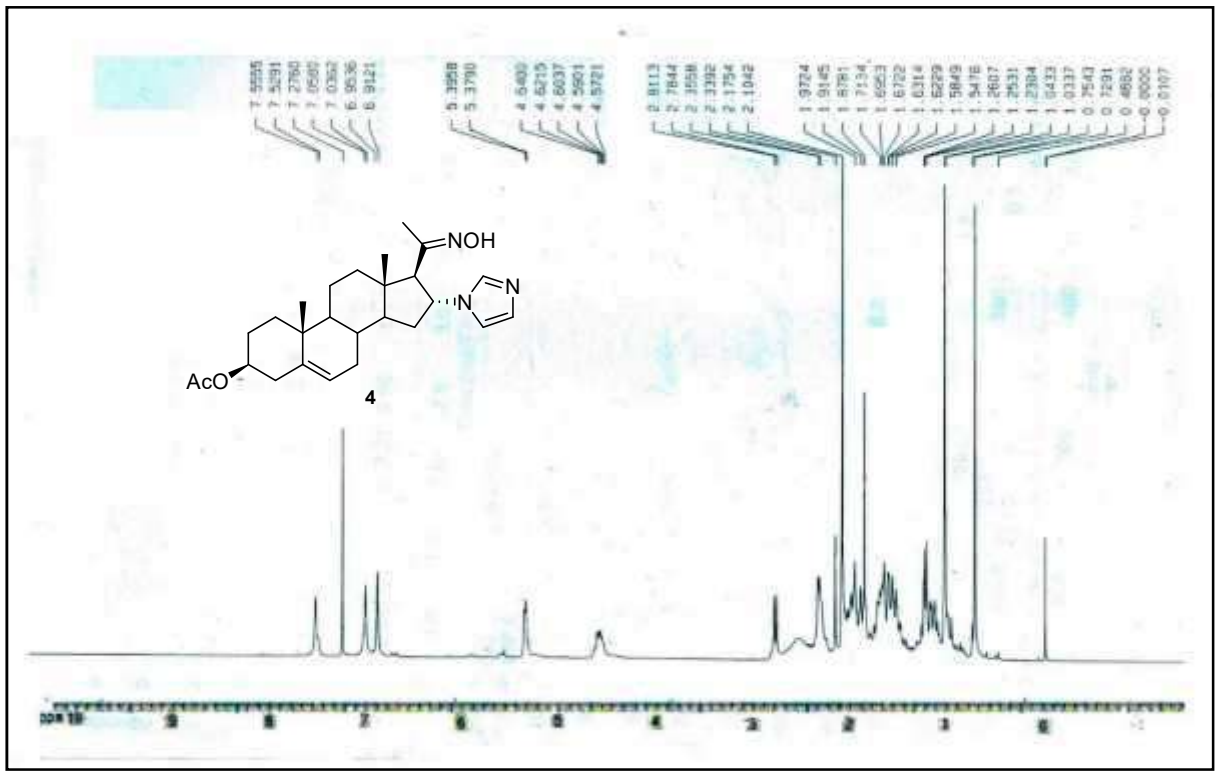
13. Yu, B.; Qi, P. P.; Shi, X. J.; Shan, L. H.; Yu, D. Q.; Liu, H. M. *Steroids*, **2014**, *88*, 44.

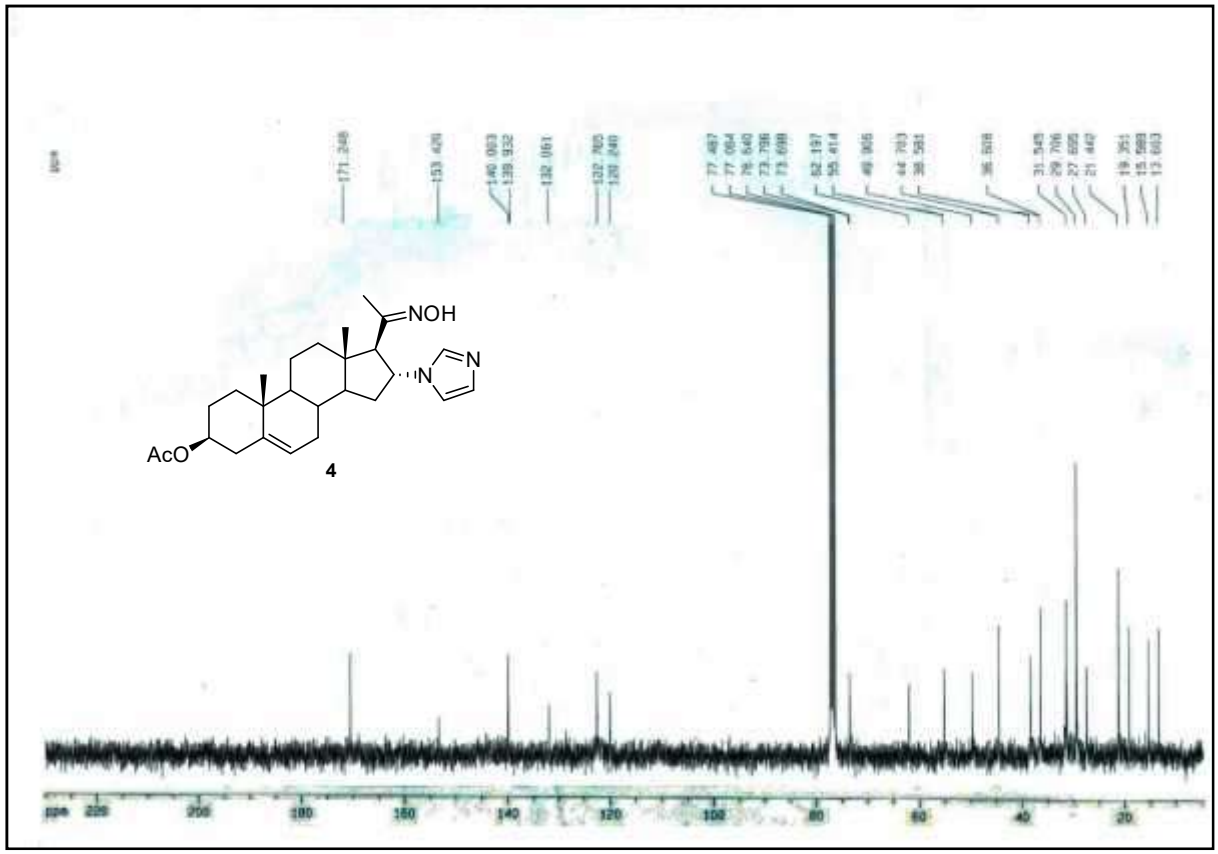
14. PhD thesis, S. Gogoi, DU, 2013

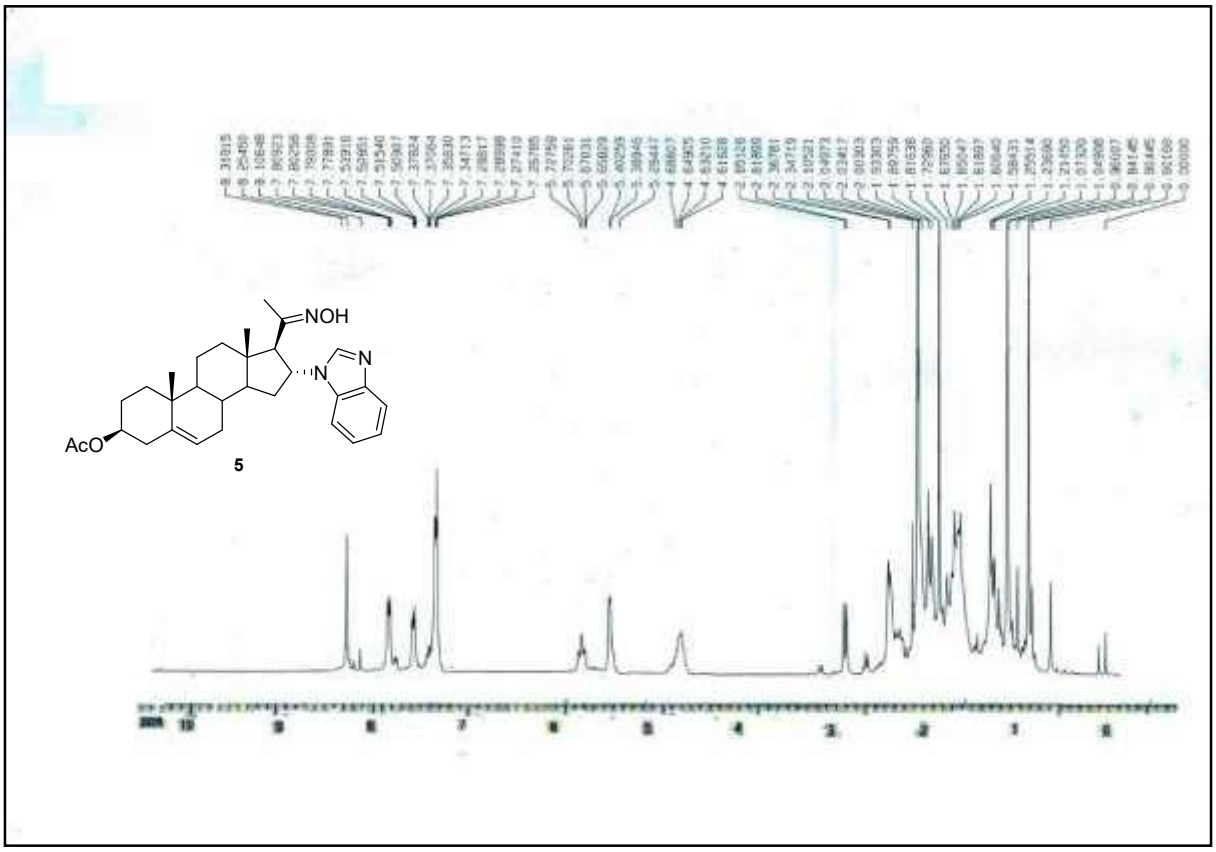
15. Gould, D.; Shapiro, E. L.; Finckenor, L. E.; Gruen, F.; Hershberg, E. B. *J. Am. Chem. Soc.*, **1956**, *78*, 3158.

16. Myadaraboina, S.; Alla, M.; Saddanapu, V.; Boena V. R.; Addlagatta, A. *Eur. J. Med. Chem.*, **2010**, *45*, 5208.

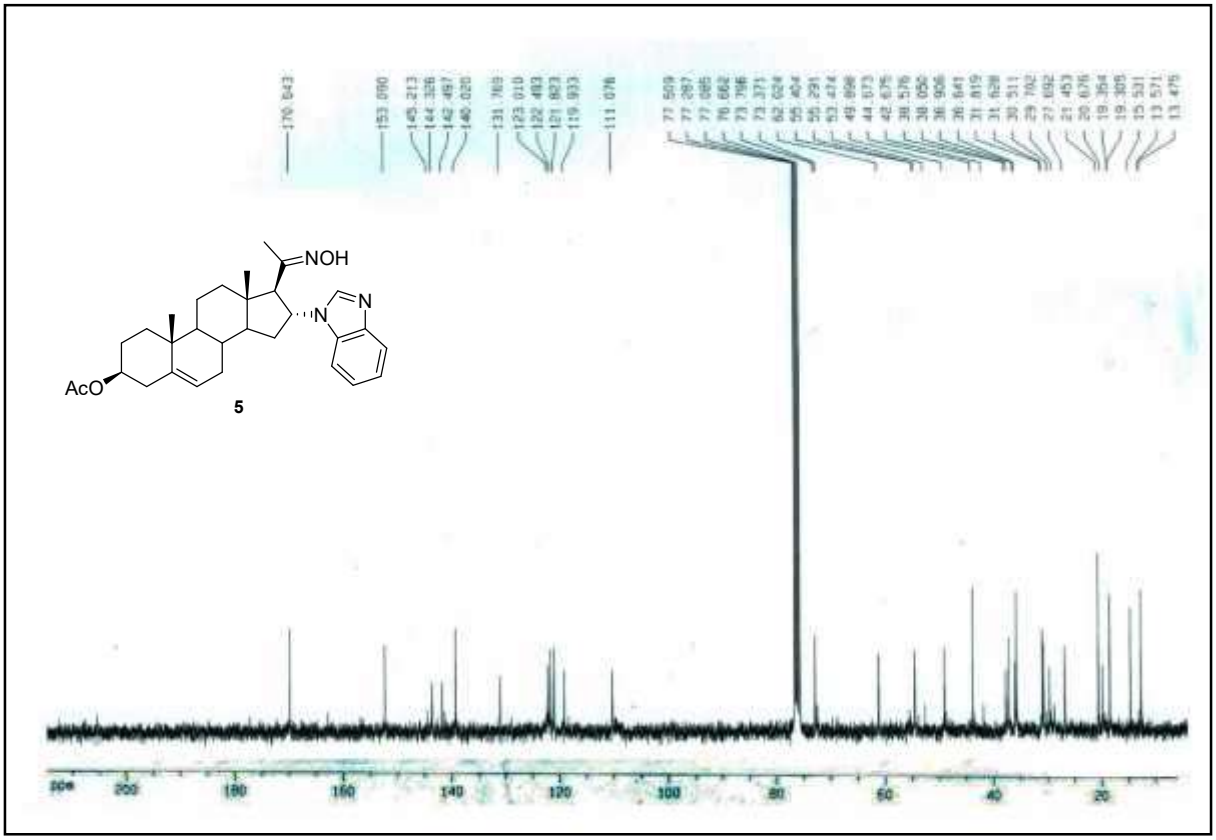
^{1}H NMR and ^{13}C NMR of synthesized compounds







5



5