Steroids are a class of important polycyclic compounds which exhibit diverse biological activities. Except for the naturally occurring substances, most of steroidal pharmaceuticals are semi-synthetic compounds. It is proved that a number of biologically important properties of modified steroids are dependent upon structural features of the steroid ring system or side chain so this chemical modification of the steroid skeleton provides a way to alter the functional groups and numerous structure-activity relationships have been established by such synthetic alterations.

Steroids have always attracted considerable attention in bio-organic research because of being a fundamental class of biologically active molecules. Their profound physiological and clinical importance is now well validated. They can regulate a variety of biological processes and thus have the potential to act as drugs for the treatment of a number of diseases including cardiovascular, autoimmune, brain tumour, breast cancer, prostate cancer and osteoarthritis. The diversity in the biological action might be due to the presence of different functional groups located around the tetracyclic core which serve as substrates for different targets. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. Previous work from our laboratory described the synthesis of number of unknown steroidal derivatives in the cholestane series. These include azasteroids, oxasteroids, thia diazoles, oxadia zolines, pyrro lidine, pyrazolines etc.

In continuation of the above work, an attempt has been made to synthesize new heterosteroids with probable biological activities like antimicrobial, anticancer, antioxidant and simultaneously study their DNA binding behavior. The newly synthesized heterosteroids have been characterized by IR, $^1$H NMR, $^{13}$C NMR, MS and analytical data. In some cases, abnormal products have been obtained and this has offered scope for some mechanistic and stereochemical studies also. The whole work of the thesis is divided into five chapters namely,

Chapter 1 : Synthesis of steroidal pyrimidines
Chapter 2 : Synthesis of steroidal pyrans
Chapter 3 : Synthesis of steroidal pyrazoles and pyrazolones
Chapter 4 : Synthesis of steroidal benzothiazines and thiazoles
Chapter 5 : Biological activity (antimicrobial, anticancer and antioxidant) and DNA binding studies of newly synthesized heterosteroids
Synthesis of steroidal pyrimidines

Pyrimidine moiety containing molecules have been reported to exhibit a broad spectrum of biological activities such as anticancer, antiviral, antibacterial, antioxidant, anxiolytic, etc. As far as literature is concerned, little attention has been paid to the synthesis of steroidal based pyrimidine derivatives. This prompted us to carry out the synthesis of some steroidal pyrimidines and with this aim two series of reactions were carried out. First series involves the reactions of 5α-cholestan-6-one thiosemicarbazone (I), 3β-acetoxy-5α-cholestan-6-one thiosemicarbazone (II) and 3β-chloro-5α-cholestan-6-one thiosemicarbazone (III) with diethyl malonate to yield [4′, 6′-dioxo-2′-thioxo-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (IV), 3β-acetoxy [4′, 6′-dioxo-2′-thioxo-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (V) and 3β-chloro[4′, 6′-dioxo-2′-thioxo-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (VI), respectively while as the second series includes reactions of 5α-cholestan-6-one thiosemicarbazone and its analogues (I-III) with ethyl cyanoacetate to provide [6′-amino-2′-thioxo-4′-oxo dihydro-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (VII), 3β-acetoxy [6′-amino-2′-thioxo-4′-oxo dihydro-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (VIII) and 3β-chloro [6′-amino-2′-thioxo-4′-oxo dihydro-1H-pyrimidin-1-yl] 6-imino-5α-cholestan (IX), respectively.

Synthesis of steroidal pyrans \(^3,^4\)

Pyran derivatives represent an important class of organic compounds with wide number of applications. They are not only used in cosmetics, pigments and biological agrochemicals but also constitute a structural unit of many natural products. These compounds have been reported to possess various pharmacological activities such as antiallergic, antitumor and antibacterial. The diversity in the biological action might be due to the presence of different functional moieties located around the pyran core which serve as substrates for different targets. Keeping this in consideration, the synthesis of some steroidal 4H-pyran has been carried out and with this aim two series of reactions were done. First series involves the reactions of cholest-5-en-7-one (X), 3β-acetoxycholest-5-en-7-one (XI) and 3β-chlorocholest-5-en-7-one (XII) with ethyl cyanoacetate to yield 2′-amino-3′-carboethoxycholest-6-eno [5, 7- d e] 4H-pyran (XIII), 3β-acetoxy-2′-amino-3′-carboethoxycholest-6-eno [5, 7 - d e] 4H-pyran (XIV) and 3β-chloro-2′-amino-3′-carboethoxycholest-6-eno [5, 7 - d e] 4H-pyran (XV) while as the second series includes reactions of cholest-5-en-7-one and its analogues (X-XII) with malononitrile to provide 2′-amino-3′-cyanocholest-6-eno [5, 7 - d e] 4H-pyran (XVI), 3β-acetoxy-2′-amino-3′-cyanocholest-6-eno [5, 7 - d e] 4H-pyran (XVII) and 3β-chloro-2′-amino-3′-cyanocholest-6-eno [5, 7 - d e] 4H-pyran (XVIII), respectively.


Chapter 3

Synthesis of steroidal pyrazoles and pyrazolones $^5,^6$

Pyrazoles have played a vital role in the development of theory in heterocyclic chemistry and are also used extensively as useful synthons in organic synthesis. Pyrazole derivatives also show number of biological activities like antimicrobial, anticancer, antioxidant, analgesic, anti-inflammatory, antipyretic, etc. This prompted us to carry out the synthesis of some of the steroidal pyrazoles and with this aim two series of reactions were done. First series involves the reactions of 5α-cholestan-6-one thiosemicarbazone (I), 3β-acetoxy-5α-cholestan-6-one thiosemicarbazone (II) and 3β-chloro-5α-cholestan-6-one thiosemicarbazone (III) with phosphorus oxychloride and acetamide to yield 5α-cholestan [6, 7-c] 5′-methyl-1′-carboxothioic acid amido pyrazole (XIX), 3β-acetoxy-5α-cholestan [6, 7-c] 5′-methyl-1′-carboxothioic acid amido pyrazole (XX) and 3β-chloro-5α-cholestan [6, 7-c] 5′-methyl-1′-carboxothioic acid amido pyrazole (XXI), respectively while as second series includes the reactions of 5α-cholestan-6-one (XXII), 3β-acetoxy-5α-cholestan-6-one (XXIII) and 3β-chloro-5α-cholestan-6-one (XXIV) with cyanoacetohydrazide to yield cholest-6 [5′-amino-1′, 2′-dihydropyrazol-3-one-1′-yl] 5-ene (XXV), 3β-acetoxysterol-6 [5′-amino-1′, 2′-dihydropyrazol-3-one-1′-yl] 5-ene (XXVI) and 3β-chlorocholesterol-6 [5′-amino-1′, 2′-dihydropyrazol-3-one-1′-yl] 5-ene (XXVII), respectively.

$^6$ Shamsuzzaman, Ayaz Mahmood Dar, I. A. Bhat, Y. Khan, DNA binding studies and in vitro cytotoxicity of newly synthesized steroidal pyrazoles. (Communicated)
Synthesis of steroidal benzothiazines and thiazoles

Benzothiazines represent an important class of compounds in medicinal chemistry because the presence of nitrogen-sulphur axis is one of the features responsible for their biological activity; hence they show bioactivities like anticancer, vasorelaxant, antidiabetic, antihypertensive, antimicrobial, etc. On the other hand, thiazole derivatives have also attracted continuing interest over the years because of their varied biological activities. They have been reported as antiallergic, antihypertensive, anti-inflammatory, antischizophrenic, antibacterial, anti-HIV, hypnotics and selective COX-2 inhibitors, fibrinogen receptor antagonists with antithrombotic activity and new inhibitors of bacterial DNA gyrase B. With this enthusiasm, an attempt for the synthesis of these compounds was made and hence two series of reactions were carried out. First series involves the one pot synthesis of 5α-cholestan-6-one and its analogues (XXII-XXIV) with iodine/2-aminothiophenol while as the second series involves the one pot synthesis of 2'-hydrazinocholestan-6-eno thiazole (XXXI), 3β-acetoxy-2'-hydrazinocholestan-6-eno thiazole (XXXII) and 3β-chloro-2'-hydrazinocholestan-6-eno thiazole (XXXIII) by reacting 5α-cholestan-6-one and its analogues with iodine/thiosemicarbazide.

7. Shamsuzzaman, Ayaz Mahmod Dar, H. Khanam, M. A. Gato, Anticancer and antimicrobial evaluation of newly synthesized steroidal 5, 6 fused benzothiazines. Arabian Journal of Chemistry http://dx.doi.org/10.1016/j.arabjc.2013.06.027, in press
8. Shamsuzzaman, Ayaz Mahmod Dar, H. Khanam, M. A. Gato, Synthesis, Characterization and In Vitro Anticancer Activity of Newly Synthesized Steroidal 6, 7-Fused Thiazoles. Journal of Chemistry (Accepted) (2013)
Biological activities (antimicrobial, anticancer and antioxidant) and DNA binding studies of newly synthesized heterosteroids

Antimicrobial activity

In antimicrobial screening, the newly synthesized steroidal compounds showed moderate to potential behavior against different bacterial as well as fungal strains. During antimicrobial testing of steroidal pyrimidines (IV-VI), compound IV was found to be almost equally active as compared to the standard drug, Griseofulvin against Candida albicans. In case of screening of steroidal pyrans (XIII-XV) and steroidal pyrazolones (XXV-XXVII) against different microbial strains, the activity of the compound XIII and XXVI was found to be almost same as the standard drugs, Ciprofloxacin and Chloramphenicol, respectively against the Streptococcus pyogenes. When steroidal benzothiazines (XXVIII-XXX) were allowed to undergo the antimicrobial screening, all the three compounds showed moderate to good antibacterial activity but the compound XXVIII showed influential zone of inhibition i.e. 19.4 mm against the E-coli strain which is almost equal to the inhibition zone of Chloramphenicol i.e. 20.0 mm against the same strain.

Anticancer activity

During in vitro anticancer screening of steroidal pyrimidines (IV-VI), compound IV and V showed effective IC_{50} against A545, A549, HeLa and HepG2 cell lines hence showed potential cytotoxicity. In comet assay, compound V revealed maximum DNA damage against MCF7 cells. The nucleolytic experiment showed that reactive oxygen species ROS (‘OH) are responsible for cell death.² In case of cytotoxicity assay of steroidal pyrimidines (VII-IX), compound VIII and IX showed IC_{50} against A549, HeLa, HepG2 and A545 cell lines close to that of Cisplatin, hence also show endowed cytotoxicity. Compound VIII allowed maximum DNA degradation in comet assay.²

During the cytotoxic screening of steroidal pyrans (XIII-XV), IC_{50} for XIII against HeLa and MCF7 was found to be 13.73 μmol L^{-1} and 11.18 μmol L^{-1} in comparison with Doxorubicin (IC_{50} = 11.53 μmol L^{-1} and 12.41 μmol L^{-1}), respectively. Hence these compounds also have cytotoxic potency and thus presented maximum DNA damage against MCF7 cells in the comet assay. The nucleolytic experiments showed that ROS (‘OH) are responsible for cell death.³ During the cytotoxic screening of steroidal pyrans (XVI-XVIII),
compound XVII was found to be potentially cytotoxic with its IC<sub>50</sub> against MCF7 found to be 13.21 μmol L<sup>-1</sup> (close to the IC<sub>50</sub> of Doxorubicin 12.41 μmol L<sup>-1</sup>) and hence presented maximum DNA degradation of MCF7 cells in comet assay. Microscopic examination of cancer cells and compound XVII-treated cancer cells also showed the inhibition of growth by compound XVII.<sup>4</sup>

In case of the in vitro cytotoxic screening of steroidal pyrazoles (XIX-XXI), compounds XX and XXI showed potential cytotoxicity against SW480, HL-60 and MCF-7 cell lines by showing IC<sub>50</sub> close to that of Doxorubicin. Compound XXI showed IC<sub>50</sub> same as that of Doxorubicin against HL-60 cell line. Microscopic examination of cancer cells and compound XX and XXI-treated cancer cells showed the cytotoxic nature of these compounds. In comet assay, compound XX presented higher DNA damage against MCF7 cells.<sup>6</sup>

During the cytotoxic screening of steroidal benzothiazines (XXVIII-XXX), compound XXVIII showed effective IC<sub>50</sub> =13.73 μmol L<sup>-1</sup> against HeLa cell line. Compounds XXIX and XXX also showed minimum IC<sub>50</sub> of 15.83 μmol L<sup>-1</sup> (HepG2) and 16.89 μmol L<sup>-1</sup> (A549), respectively.<sup>7</sup> When the steroidal thiazoles (XXXI-XXXIII) were screened for the in vitro cytotoxicity, compound XXXII was found to be active against SW480, A549, HepG2, HeLa and HL-60 cells in comparison with the standard drug, Cisplatin.<sup>8</sup>

**Antioxidant activity**

2, 2-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay of new heterosteroids was studied during which the steroidal pyrimidines (IV-VI) exhibited potential antioxidant activity. DPPH assay of the steroidal pyrans (XIII-XV) was also carried out which showed that compounds were not significantly active. During the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging assay, steroidal pyrazolones (XVI-XVIII) showed significantly active antioxidant behavior. When the nitric oxide radical scavenging assay of steroidal thiazoles (XXXI-XXXIII) was carried out, all the compounds showed potential nitric oxide radical scavenging behavior.

**DNA binding studies**

The DNA-binding studies included gel electrophoresis, UV-vis absorption and fluorescence spectroscopy and molecular docking technique. The binding constants (K<sub>b</sub>) for the steroidal pyrimidines (IV-IX) were found to be 4.63 × 10<sup>-3</sup> M<sup>-1</sup>, 2.12 × 10<sup>-3</sup> M<sup>-1</sup>, 2.43 × 10<sup>-3</sup> M<sup>-1</sup>, 9.34 × 10<sup>3</sup> M<sup>-1</sup>, 6.56 × 10<sup>3</sup> M<sup>-1</sup> and 1.54 × 10<sup>4</sup> M<sup>-1</sup>, respectively. It was found that the absorption intensity in the compounds (IV-IX) increased with gradual addition of DNA and hence
showed hyperchromism, revealing the fact of exposure of more bases of DNA which is the indication of strong binding of compounds to CT DNA.\textsuperscript{1, 2}

The binding constants ($K_b$) for the steroidal pyrans XIV and XV were found to be $5.3 \times 10^3$ and $3.7 \times 10^2$ M\textsuperscript{-1} and for the steroidal pyrans (XVI-XVIII) $K_b$ was found to be $2.2 \times 10^3$ M\textsuperscript{-1}, $5.37 \times 10^3$ M\textsuperscript{-1} and $2.51 \times 10^3$ M\textsuperscript{-1}, respectively. The absorption spectra of these compounds also revealed strong hyperchromism implying their higher DNA binding propensity which results in partial uncoiling of DNA helix structure, exposing more bases of DNA which is the indication of strong binding of compounds to CT DNA.\textsuperscript{3, 4}

The gel electrophoresis also revealed the interaction of these compounds with DNA by showing the disappearance of the original band of DNA in gel plate. In molecular docking study, it was found that these type of compounds interacted with DNA through major as well as minor groove and their heterocyclic moiety (pyrimidine or pyran) shows electrostatic interaction between the nucleotide base pairs of DNA.\textsuperscript{1, 4}