Chapter 4

Synthesis of steroidal benzothiazines and thiazoles
Theoretical
Benzothiazine is a heterocyclic compound consisting of a benzene ring attached to the 6-membered heterocycle thiazine. The molecular formula is C₈H₇NS. The name is applied to both the 2H isomer (1) and 4H isomer (2) which differ by the location of the double bonds. Benzothiazines were first reported in the 1960s. Subsequently, their preparation and intensive biological studies have been reported. In recent years benzothiazines have been of enormous interest to synthetic chemists. The enantioselective synthesis of such benzothiazines has been developed and formulated and transformations of these compounds were designed to target chiral, non-racemic building blocks as well as natural products.

![Diagram of structures 1 and 2](image)

Loev and Kormendy¹ in 1965 reported the first procedure for the synthesis of sulfostyryl (2, 1-benzothiazine 2, 2-dioxide) (4) during which 2-nitrophenethyl bromide (3) was converted in two steps to sulfonyl chloride. Alkaline hydrolysis gave the sodium sulfonate which was catalytically reduced to the amine. The amine upon trituration with PCl₅ yielded desired product sulfostyryl (4).

![Diagram of reaction steps from 3 to 4](image)

Prota and co-workers² reported the hydrolysis of (1-о-aminophenylthio-2, 2-diethoxy) ethane (5) resulted in the formation of (2-о-aminophenylthio)acetaldehyde (6) which after condensation formed ring in the form of 2H-1, 4-benzothiazine (7).
Corona et al.\textsuperscript{3} reported that N-substituted aminothiophenols (8 a-c) reacted with haloester and gave 3-oxo-3, 4-dihydro-2H-benzothiazine-2-carboxylic acid ethyl ester derivatives (9 a-c) which upon reaction with different amines provided 2H-1, 4-benzothiazine-3, 4-dihydro-3-oxo-2-carboxamides (10 a-h) in good yields.

Farag and co-workers\textsuperscript{4} reported that the aryl hydrazonoyl dichlorides (11 a-c) on reaction with 2-aminothiophenol in refluxing ethanol in the presence of triethylamine afforded 2, 3-bis(arylhydrazono)-2, 3-dihydro-4H-1, 4-benzothiazines (12 a-c) in good yields which on treatment with lead tetracetate in acetic acid at room temperature provided respective oxidation products (13 a-c).

\textbf{83}
Napolitano and co-workers\(^5\) reported that the oxidation of cysteinylcatehols (14 a-d) gave cysteinyldopaquinones (15 a-d) which after ring closure of the cysteine side chain under reflux conditions yielded 2H-benzothiazine derivatives (16 a-d).

Guarda et al.\(^6\) reported that the treatment of 2-chloro-5-nitroaniline (17) with sodium sulfide and sulfur gave 2-amino-4-nitrobenzenethiol sodium salt (18), which was cyclized to 2H-1, 4-benzothiazin-3-one (19), with chloroacetic acid. N-alkylation with octyl bromide and KOH in methanol afforded 4-octyl-6-nitro-2H-1, 4-benzothiazin-3-one (20). Reduction of the nitro group of 20 by SnCl\(_2\) in acidic medium gave 6-amino-4-octyl-2H-1, 4-benzothiazin-3-one (21). The N-acetylation of 21 resulted in acetylated product, acetylamino-4-octyl-2H-1, 4-benzothiazin-3-one (22).

Esmaili and co-workers\(^7\) reported that a magnetically stirred solution of 2-aminothiophenol in 40% ethyl acetate/hexane (5 mL) was allowed to react with the solution of dimethyl acetylenedicarboxylate (23 a, b) in 40% ethyl acetate/hexane (2 mL) that resulted in the formation of 3, 4-dihydro-3-oxo-2H-benzo-1, 4-thiazone derivatives (24 a, b).
Joanna Matysiak⁸ reported that reaction between substituted anilines (25 a-d) and sulfinyl bis (2, 4-dihydroxythiobenzoyl (STB) resulted in the formation of 2, 4-dihydroxy-N-(substituted phenyl) thiobenzamide derivatives (26 a-d) which in turn underwent intramolecular cyclization and gave 2-(2, 4-dihydroxy substituted phenyl)-4H-3, 1-benzo thiaazines (27 a-d).

Madrid et al.⁹ reported the synthesis of diphenylamine derivatives (29 a-d) by the coupling of substituted aniline (28 a-d) with bromobenzene using Buchwald palladium coupling reaction. The compounds 29 (a-d) were then cyclized through reaction with sulfur and catalytic iodide under microwave irradiation and yielded 10H-phenothiazines (30 a-d).
Bakavoli and co-workers\textsuperscript{10} reported the reaction of 5-bromo-2, 4-dichloro-6-methyl pyrimidine (31) with 2-aminothiophenol which gave 2-(5-bromo-2-chloro-6-methyl pyrimidin-4-ylthio) benzenamine (32). The compound 32 reacted with secondary amines and yielded 2-(5-bromo-6-methyl-2-substituted-aminopyrimidin-4-ylthio) benzene amines (33 a-h) which on further reaction with sodamide in acetonitrile furnished pyrimido [4, 5 - b] [1, 4] benzothiazines (34 a-h) in good yield.

\[
\begin{align*}
\text{(31)} & \quad \text{(32)} & \quad \text{(33)} & \quad \text{(34)} \\
\text{R}^1 & \quad \text{R}^1 \\
(a) & \text{Pyrrolidine} & (a) & \text{Pyrrolidine} \\
(b) & \text{Piperidine} & (b) & \text{Piperidine} \\
(c) & \text{Methylpiperidine} & (c) & \text{Methylpiperidine} \\
(d) & \text{Methyl-6H-pyrimidine} & (d) & \text{Methyl-6H-pyrimidine} \\
(e) & \text{Ethyl-6H-pyrimidine} & (e) & \text{Ethyl-6H-pyrimidine} \\
(f) & \text{Morpholine} & (f) & \text{Morpholine} \\
(g) & \text{Piperidin-4-ol} & (g) & \text{Piperidin-4-ol} \\
(h) & \text{1-Phenyl-piperazine} & (h) & \text{1-Phenyl-piperazine} \\
\end{align*}
\]

Tandon and co-workers\textsuperscript{11} reported the reaction of 2-chloro-3-aryl sulfanyl-[1, 4] naphthoquinones (35 a-c) with aniline that provided 2-phenylamino-3-aryl sulfanyl-[1, 4] naphthoquinones (36 a-c) which in turn reacted with \( p \)-formaldehyde in the presence of tetrafluoroborate to yield quaternary intermediate that underwent intramolecular cyclization and gave dihydrobenzo [f] naphtha [2, 3 - b][1, 4] thiazepine-6, 11-dione (37 a-c).

\[
\begin{align*}
\text{(35)} & \quad \text{(36)} \\
\text{Quaternary salt} & \quad \text{(37)} \\
\text{Ar} & \quad \text{Ar} & \quad \text{R} \\
(a) & \text{C}_6\text{H}_5 & (a) & \text{H} \\
(b) & 3-\text{OMeC}_6\text{H}_4 & (b) & 3-\text{OMe} \\
(c) & \text{Naphthyl} & (c) & \text{C}_6\text{H}_{12} \\
\end{align*}
\]
Pratap and co-workers\textsuperscript{12} reported the synthesis of 1, 4-benzothiazine derivatives (38 a-j) by the condensation of 2-aminobenzenethiols and 1, 3-dicarbonyl compounds using biocatalyst, baker’s yeast under ultrasonic conditions.

\[
\begin{array}{c}
\text{R} \\
(a) \text{H} \\
(b) \text{CH}_3 \\
(c) \text{Cl}
\end{array}
\begin{array}{c}
\text{R}^1 \\
(a) \text{CH}_3 \\
(b) \text{CH}_3 \\
(c) \text{CH}_3
\end{array}
\begin{array}{c}
\text{R}^2 \\
\text{CH}_3 \\
\text{OC}_2\text{H}_5 \\
\text{Ph}
\end{array}
\begin{array}{c}
\text{R} \\
(a) \text{H} \\
(b) \text{H} \\
(e) \text{H}
\end{array}
\begin{array}{c}
\text{R}^1 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3
\end{array}
\begin{array}{c}
\text{R}^2 \\
\text{CH}_3 \\
\text{OC}_2\text{H}_5 \\
\text{Ph}
\end{array}
\]

Alam \textit{et al.}\textsuperscript{13} reported the synthesis of amino and phenyl amino derivatives of 5\textalpha-cholest-6-eno [6, 7 - d] thiazole (40 a-f) by reacting the lachrymatory 5-bromosteroidal ketones (39 a-c) with thiourea or phenylthiourea in methanol under reflux conditions.

\[
\begin{array}{c}
\text{X} \\
(a) \text{OAc} \\
(b) \text{C}_2\text{H}_5\text{COO} \\
(c) \text{H}
\end{array}
\begin{array}{c}
\text{X} \\
(a) \text{OAc} \\
(b) \text{C}_2\text{H}_5\text{COO} \\
(c) \text{H} \\
(d) \text{OAc} \\
(e) \text{C}_2\text{H}_5\text{COO} \\
(f) \text{H}
\end{array}
\begin{array}{c}
\text{R} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{Ph} \\
\text{Ph}
\end{array}
\]

Mushfiq \textit{et al.}\textsuperscript{14} modified the procedure reported by Alam \textit{et al.}\textsuperscript{13} and reported the one pot synthesis of 2'-amino-5\textalpha-cholest-6-eno [6, 7 - d] thiazole derivatives (42 a-c) by the reaction of steroidal ketones (41 a-c) with iodine and phenylthiourea under microwave conditions.
Kumar and co-workers\textsuperscript{15} reported the reaction of 2, 4-substituted aniline derivatives (43 a-d) with ammonium thiocyanate that yielded 2, 4-substituted phenylthiourea derivatives (44 a-d) which in turn reacted with bromine and gave substituted benzothiazol-2-ylamine bromate derivatives (45 a-d) which later upon reacted with liquid ammonia yielded benzothiazole derivatives (46 a-d).

Zav’yalov and co-workers\textsuperscript{16} reported that 5, 5-dimethyl-cyclohexane-1, 3-dione (47) underwent reaction with bromine to provide 2-bromo-5, 5-dimethyl-cyclohexane-1, 3-dione (48) which in turn reacted with thiourea to yield 2-amino-5, 5-dimethyl-5, 6-dihydro-4H-benzo-1, 3-thiazol-7-one (49).
Discussion
Nitrogen containing steroids have the ability to regulate a variety of biological processes and thus are potential drug candidates for the treatment of a large number of diseases including breast cancer, prostate cancer, leukaemia, autoimmune diseases and osteoporosis.\textsuperscript{17-21} So is the case with the nitrogen containing derivative, benzothiazines in which the presence of a fold along the nitrogen-sulfur axis is one of the features responsible to impart their biological activity\textsuperscript{22}, hence they show broad spectrum of biological activities such as antagonists, anticancer, vasorelaxant, antidiabetic, antihypertensive and antimicrobial.\textsuperscript{23-28}

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, hypnotic, selective COX-2 inhibitors, fibrinogen receptor antagonists with antithrombotic activity and inhibitors of bacterial DNA gyrase B.\textsuperscript{29-38} The substituted thiazoles have a number of other characteristic pharmacological features such as relative stability and ease of starting materials built in biocidal unit, enhanced lipid solubility with hydrophilicity and easy metabolism of compounds.\textsuperscript{39}

The biological importance of these steroidal benzothiazines\textsuperscript{25-30} and steroidal thiazoles\textsuperscript{29-37} encouraged us to undertake the synthesis of new steroidal benzothiazines and aminothiazoles. The substrates selected for synthesizing these steroidal derivatives include 5\textalpha{}-cholestan-6-one\textsuperscript{40} (50), 3\beta{}-acetoxy-5\textalpha{}-cholestan-6-one\textsuperscript{41} (51) and 3\beta{}-chloro-5\textalpha{}-cholestan-6-one\textsuperscript{42} (52). The products obtained have been characterized on the basis of spectral (IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, MS) and elemental analyses.
Reaction of 5α-cholest-6-one (50) with iodine and 2-aminothiophenol.

The 5α-cholest-6-one (50) (1 mmol) in absolute ethanol (10 mL) was allowed to react with 2-aminothiophenol (1 mmol) and iodine (2 mmol). After completion of the reaction, the reaction mixture was taken in diethyl ether, diluted with Na₂S₂O₇ solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvents gave an oil which was crystallized from methanol to give compound 53, m.p 152 °C.

\[ \text{Iodine / 2-aminothiophenol} \]
\[ \text{EtOH, reflux, 19 h} \]

(50)  (53)

Characterization of the compound, m.p. 152 °C as 5α-cholestano [5, 6 - b] benzothiazine (53)

The compound 53 was correctly analyzed for the molecular formula C₃₃H₅₈NS. Its IR spectrum showed a band at 1628 cm⁻¹ assigned to C=O group while as the bands at 3062 and 1600 cm⁻¹ confirmed the presence of an aromatic moiety. The bands at 711 and 1385 cm⁻¹ were attributed to C-S and C-N groups, respectively. These values supported the presence of benzothiazine moiety⁴³ in the product molecule. The structure 53 was well supported by its \(^1\)H NMR spectrum which displayed a multiplet at \(\delta\) 6.43-6.24 integrating for four protons, indicating the presence of an aromatic ring. A two-proton doublet appeared at \(\delta\) 2.05 \((J = 8.0 \text{ Hz})\) for \(\text{C}_4-\text{H}_2\) and another doublet at \(\delta\) 2.04 \((J = 4.4 \text{ Hz})\) for \(\text{C}_7-\text{H}_2\). The prominent peaks for angular and side-chain methyl protons were observed at \(\delta\) 1.17, 0.97, 0.80 and 0.75. The \(^{13}\)C NMR spectrum of compound 53 displayed characteristic signals at \(\delta\) 163 showing the presence of C=O while as the signals at 149, 125, 124, 122.8, 122 and 120 confirmed the presence of an aromatic ring. Remaining carbon atoms were seen in accordance to the cholestane series. The structure of compound 53 was further supported by its mass spectrum in which the distinct molecular ion peak (M⁺ 491) was found.

On the basis of foregoing discussion and the mechanism proposed (Scheme 4.1), this compound can be best characterized as 5α-cholestano [5, 6 - b] benzothiazine 53.
Reaction of $3\beta$-acetoxy-$5\alpha$-cholestan-6-one (51) with iodine and 2-aminothiophenol.

The $3\beta$-acetoxy-$5\alpha$-cholestan-6-one (51) (1 mmol) in absolute ethanol (10 mL) was allowed to react with 2-aminothiophenol (1 mmol) and iodine (2 mmol). After completion of the reaction, the reaction mixture was taken in diethyl ether, diluted with Na$_2$S$_2$O$_7$ solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvents gave an oil which was crystallized from methanol to give compound 54, m.p 163 °C.

Characterization of the compound, m.p. 163 °C as $3\beta$-acetoxy-$5\alpha$-cholestan-6-one [5, 6 - b] benzothiazine (54)

The elemental analysis of the compound corresponded to the molecular formula C$_{35}$H$_{51}$NO$_2$S. Its IR spectrum showed a band at 1650 cm$^{-1}$ which could be assigned to C=N group while as the bands at 3060 and 1603 cm$^{-1}$ confirmed the presence of an aromatic moiety. The IR spectrum of the compound 54 exhibited strong absorption bands at 1714 and 1206 cm$^{-1}$ indicating the presence of an acetoxy group while as the bands at 750 and 1388 cm$^{-1}$ were attributed to C-S and C-N groups, respectively. These values suggested the presence of benzothiazine moiety$^{43}$ in the product molecule. The structure 54 was well supported by its $^1$H NMR spectrum which displayed a multiplet at δ 6.33-6.28 integrating for four protons, indicating the presence of an aromatic ring. A broad multiplet ($W_{1/2} = 15$ Hz, axial) for one proton was observed at δ 4.7 which could be assigned to C$_{3\alpha}$-H. The three acetoxy group protons appeared at δ 2.03 as a sharp singlet. A doublet for two protons appeared at δ 1.8 ($J = 8.0$ Hz) for C$_4$-H$_2$ and another doublet integrating for two protons at δ 1.9 ($J = 5.2$ Hz) for C$_7$-H$_2$. Other prominent peaks for angular and side-chain methyl protons were observed at δ 1.18, 0.97, 0.83 and 0.70. The $^{13}$C NMR spectrum of compound 54 displayed characteristic signals at δ 174 and 163 showing the presence of C=O and C=N, respectively while as the signals at 148, 129, 128, 126, 124 and 122 confirmed the presence of an aromatic carbons. The signal at δ 73 was assigned to the C$_3$ of the steroidal molecule.
with acetoxy group attached to it. Remaining carbon atoms were seen in accordance to the cholestane series. The structure of compound 54 was further supported by its mass spectrum in which the distinct molecular ion peak (M+ 549) was observed.

On the basis of above studies and its analogy with earlier compound 53, this compound can be best characterized as 3β-acetoxy-5α-cholestan-6-one [5, 6 - b] benzothiazine 54.

**Reaction of 3β-chloro-5α-cholestan-6-one (52) with iodine and 2-aminothiophenol.**

The 3β-chloro-5α-cholestan-6-one (52) (1 mmol) in absolute ethanol (10 mL) was allowed to react with 2-aminothiophenol (1 mmol) and iodine (2 mmol). After completion of the reaction, the reaction mixture was taken in diethyl ether, diluted with Na2S2O7 solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvents gave an oil which was crystallized from methanol to give compound 55, m.p 146 °C.

![Chemical structure of 52 and 55](image)

**Characterization of the compound, m.p. 146 °C as 3β-chloro-5α-cholestan-6-one [5, 6 - b] benzothiazine (55)**

The compound 55 was correctly analyzed for the molecular formula C33H46ClNS (Beilstein positive). Its IR spectrum showed a band at 1626 cm⁻¹ which could be assigned to C=N group while as the bands at 3058 and 1598 cm⁻¹ confirmed the presence of an aromatic moiety and the bands at 710, 1380 and 740 cm⁻¹ were attributed to C-S, C-N and C-Cl groups, respectively. These values supported the presence of benzothiazine moiety in the product molecule. The structure 55 was well supported by its ¹H NMR spectrum which displayed a multiplet at δ 6.43-6.24 for four protons, indicating the presence of an aromatic ring. A broad multiplet (W₁₂ = 17 Hz, axial) for one proton was observed at δ 3.5 which could be assigned to C₃α-H. A doublet for two protons appeared at δ 2.07 (J = 8.0 Hz) for C₄-H₂ and another doublet at δ 1.87 (J = 4.8 Hz) for two C₇-methyl protons. The prominent peaks for angular and side-chain methyl protons were observed at δ 1.18, 0.97, 0.80 and 0.75.
The $^{13}$C NMR spectrum of compound 55 displayed a characteristic signal at $\delta$ 164 showing the presence of $C=\equiv N$ while as the signals at 146, 127, 126, 125, 123 and 122 confirmed the presence of an aromatic moiety. The signal at $\delta$ 59 was assigned to the C$_3$ of the steroidal molecule with chlorine attached to it. Remaining carbon atoms were seen in accordance to the cholestane series. The structure of compound 55 was further supported by its mass spectrum in which the distinct molecular ion peak ($M^+$ 525/527) was found.

Formation of steroidal benzothiazines (53-55) under the condition case may be shown according to the proposed mechanism (Scheme 4.1). The mechanism for the formation of these benzothiazines involves formation of 5α-iodocholest-6-one in situ as an intermediate, which on further reaction with 2-aminothiophenol undergoes $SN^\#$ reaction at C$_5$ and condensation at C$_6$ resulting in cyclization that leads to the formation of corresponding product. The remarkable feature of the reaction is the formation of 5α-iodoketone in situ as an intermediate which might be obtained separately by the reaction of ketones with iodine.

Scheme 4.1. Mechanism for the formation of steroidal benzothiazine derivatives (53-55)

Work published;
Reaction of 5α-cholestan-6-one (50) with iodine and thiosemicarbazide.

The 5α-cholestan-6-one (50) (1 mmol) in absolute ethanol (15 mL) was allowed to react with thiosemicarbazide (1 mmol) and iodine (2 mmol). After completion of the reaction, the reaction mixture was taken in diethyl ether, diluted with Na₂S₂O₇ solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave an oil which was crystallized from methanol to afford compound 56, m.p 129 °C.

(50)

Iodine/Thiosemicarbazide
Ethanol, reflux

(56)

Characterization of the compound, m.p. 129 °C as 2′-hydrazinocholestan-6-eno [4, 5 - d] thiazole (56)

The compound 56 was correctly analyzed for the molecular formula C₂₈H₄₇N₃S. Its IR spectrum showed bands at 3376 and 3328 cm⁻¹ which could be assigned to NH and NH₂ groups, respectively while as the bands at 1617, 1557, 1328 and 634 cm⁻¹ were attributed to C=O, C=N, C-N and C-S groups, respectively. These values suggested the presence of aminothiazole moiety⁴ in the product molecule. The structure 56 was well supported by its ¹H NMR spectrum which displayed a broad singlet integrating for two protons at δ 6.2 (exchangeable with D₂O) depicted the presence of NH₂ while as the singlet integrating for one proton at δ 3.8 (exchangeable with D₂O) showed the presence of NH. A doublet appeared at δ 2.74 (J = 16.9 and 5.5 Hz) depicting the presence of C₅α-H. The prominent peaks for angular and side-chain methyl protons were observed at δ 1.18, 0.97, 0.83 and 0.70. The ¹³C NMR spectrum of compound 56 displayed characteristic signal at δ 163 showing the presence of C=N while as the signals at δ 130 and 120 assigned to C₆ and C₇, respectively. Remaining carbon atoms were seen in accordance to the cholesane series. The structure of compound 56 was further supported by its mass spectrum in which the distinct molecular ion peak (M⁺ 457) was found.

On the basis of foregoing discussion and the mechanism proposed (Scheme 4.2 a, b), this compound can be best characterized as, 2′-hydrazinocholestan-6-eno [4, 5 - d] thiazole 56.
Reaction of 3β-acetoxy-5α-cholestan-6-one (51) with iodine and thiosemicarbazide.

The 3β-acetoxy-5α-cholestan-6-one (51) (1 mmol) in absolute ethanol (15 mL) was allowed to react with thiosemicarbazide (1 mmol) and iodine (2 mmol). After completion of reaction, the reaction mixture was taken in diethyl ether, diluted with Na₂S₂O₇ solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave an oil which was crystallized from methanol to give compound 57, m.p 136 °C.

![Chemical structures of 51 and 57](image)

Characterization of the compound, m.p. 136 °C as 3β-acetoxy-2'-hydrazinocholestan-6-eno [4, 5 - d] thiazole (57)

The elemental analysis of compound 57 corresponded to the molecular formula C₃₉H₄₉N₅O₂S. Its IR spectrum showed bands at 3395 and 3310 cm⁻¹ which could be assigned to NH and NH₂ groups, respectively. The IR spectrum of the compound exhibited strong absorption bands at 1730 and 1210 cm⁻¹ indicating the presence of acetate group while as the bands at 1625, 1555, 1320 and 645 cm⁻¹ were attributed to C=O, C=N, C-N and C-S groups, respectively. These IR values supported the presence of aminothiazole moiety in the product molecule. The structure 57 was well supported by its ¹H NMR spectrum which displayed broad singlet integrating for two protons at δ 6.8 (exchangeable with D₂O) indicating the presence of NH₂ while as a singlet integrating for one proton at δ 4.4 (exchangeable with D₂O) showed the presence of NH. A broad multiplet (W½ = 15 Hz, axial) for one proton was observed at δ 4.7 which could be assigned to C₅α-H. The three acetoxy group protons appeared at δ 2.03 as a sharp singlet. A double doublet for one proton appeared at δ 2.7 (J = 15 and 5 Hz) for C₅α-H. Other prominent peaks for angular and side-chain methyl protons were observed at δ 1.18, 0.97, 0.83 and 0.70. The ¹³C NMR spectrum of compound 57 displayed characteristic signals at δ 171.2, 163 showing the presence of C=O and C=N while as the signals at δ 132, 120 and 70.2 were assigned to C₆, C₇ and C₃, respectively. Remaining carbon atoms were seen in accordance to the cholestan series. The
structure of compound 57 was further supported by its mass spectrum in which the distinct molecular ion peak (M⁺ 515) was found.

On the basis of above studies and its analogy with earlier compound 56, this compound can be best characterized as 3β-acetoxy-2'-hydrazinoocholest-6-eno [4, 5 - d] thiazole (57).

**Reaction of 3β-chloro-5α-cholestan-6-one (52) with iodine and thiosemicarbazide.**

The 3β-chloro-5α-cholestan-6-one (52) (1 mmol) in absolute ethanol (15 mL) was allowed to react with thiosemicarbazide (1 mmol) and iodine (2 mmol). After completion of reaction, the reaction mixture was taken in diethyl ether, diluted with Na₂S₂O₇ solution, subsequently washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave an oil which was crystallized from methanol to give compound 58, m.p 143 °C.

![Chemical structure of 52 and 58](image)

**Characterization of the compound, m.p. 143 °C as 3β-chloro-2'-hydrazinoocholest-6-eno [4, 5 - d] thiazole (58)**

The compound 58 was correctly analyzed for the molecular formula C₂₈H₄₆ClN₅S (Beilstein positive). Its IR spectrum showed bands at 3370 and 3320 cm⁻¹ which could be assigned to NH and NH₂ groups, respectively. The IR spectrum of the compound 58 exhibited absorption bands at 1622, 1560, 1323, 745 and 635 cm⁻¹ which were attributed to C=C, C=N, C-N, C-Cl and C-S groups, respectively. These IR values depicted the presence of aminothiazole moiety in the product molecule. The structure 58 was well supported by its ¹H NMR spectrum which displayed broad singlet integrating for two protons at δ 6.63 (exchangeable with D₂O) indicating the presence of NH₂ while as a singlet integrating for one proton at δ 4.45 (exchangeable with D₂O) showed the presence of NH. A broad multiplet (W₁/₂ = 15 Hz, axial) for one proton was observed at δ 3.9 which could be assigned to C₅α-H. A double doublet for one proton appeared at δ 2.8 (J = 17.05 and 5.3 Hz) depicting the presence of C₅α-H. The prominent peaks for angular and side-chain methyl protons were observed at δ 1.18, 0.97, 0.83 and 0.70. The ¹³C NMR spectrum of compound 58 displayed
characteristic signal at \( \delta \) 162 showing the presence of \( C=\text{N} \) while as the signals at \( \delta \) 134, 120 and 57.7 were assigned to \( C_6, C_7 \) and \( C_3 \), respectively. Remaining carbon atoms were seen in accordance to the cholestane series. The structure of compound 58 was further supported by its mass spectrum in which the distinct molecular ion peak (\( M^- 489/491 \)) was found.

The mechanism for the formation of steroidal aminothiazole derivatives (56-58) can be explained by considering that during the reaction the \( \alpha \)-iodoketone 1a formed \textit{in situ} undergoes allylic displacement of iodine \( \textit{via} \) enolization and the subsequent attack of sulfur of thiosemicarbazide followed by cyclization leads to the formation of products 56-58 (Scheme 4.2a). An enol tautomeric form 1b might be the driving force to accelerate the reaction towards product formation.

\[ \text{Scheme 4.2a. Mechanism for the formation of steroidal aminothiazole derivatives (56-58) via the allylic displacement of iodine.} \]
The formation of products 56-58 may also be explained by an alternate route considering that during the reaction 1, 3-shift of iodine from C5 to C7 leads to the formation of intermediate 1c in situ followed by S_N^2 attack of sulfur of the reagent and subsequent cyclization provides the desired products 56-58 (Scheme 4.2b).

Scheme 4.2b. Mechanism for the formation of steroidal aminothiazole derivatives (56-58) via 1, 3-shift of iodine.

Work accepted;

Synthesis, characterization and in vitro anticancer activity of newly synthesized steroidal 6, 7-fused thiazoles, Shamsuzzaman, Ayaz Mahmood Dar, et al., Journal of Chemistry (accepted)
Experimental
All the melting points were determined in degrees Celsius on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Perkin Elmer RXI Spectrophotometer and values are given in cm\(^{-1}\). \(^1\)H and \(^13\)C NMR spectra were run in CDCl\(_3\) on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in ppm (\(\delta\)). Mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Petroleum ether refers to a fraction of boiling point 60-80 °C. Sodium sulfate (anhydrous) was used as a drying agent.

The synthesis of 3\(\beta\)-chlorocholest-5-ene, cholest-5-ene, 6-nitrocholest-5-ene and 5\(\alpha\)-cholestan-6-one is shown in chapter 1, page 23, 24. The synthesis of 3\(\beta\)-acetoxycholest-5-ene, 3\(\beta\)-acetoxy-6-nitrocholest-5-ene and 3\(\beta\)-acetoxy-5\(\alpha\)-cholestan-6-one is shown in chapter 1, page 24, 25. The synthesis of 3\(\beta\)-chloro-6-nitrocholest-5-ene and 3\(\beta\)-chloro-5\(\alpha\)-cholestan-6-one is shown in chapter 1, page 25, 26.

**Reaction of 5\(\alpha\)-cholestan-6-one derivatives (50-52) with iodine and 2-aminothiophenol:**

To a solution of 5\(\alpha\)-cholestan-6-one derivatives (50-52) (1 mmol) in absolute ethanol (10 mL) was added 2-aminothiophenol/ thiosemicarbazide (1 mmol) and iodine (2 mmol) in the same solvent (25 mL) and the reaction mixture was refluxed for 13-21 h. The progress of the reaction was monitored by TLC. After completion of reaction the excess solvent was reduced to three fourths of the original volume under reduced pressure. Then it was cooled to room temperature, diluted with Na\(_2\)S\(_2\)O\(_7\) solution and subsequently with water. The mixture was extracted with diethyl ether, washed with water and finally dried over anhydrous sodium sulfate. Evaporation of solvents and crystallization of the oily residue from methanol afforded corresponding products (53-58).

**5\(\alpha\)-Cholestanol [5, 6 - b] benzothiazine (53):**

Yield 83%; m.p. 152 °C; Analysis found: C 80.59, H 10.04, N 2.85%. C\(_{33}\)H\(_{48}\)NS requires: C 80.12, H 9.86, N 2.63%; IR (KBr): \(\nu\) max 3062, 1600 (aromatic), 1628 (C=N), 1385 (C-N), 711 (C-S); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 6.43-6.24 (m, 4H, aromatic), 2.05 (d, 2H, C\(_4\)-H\(_2\), \(J = 8.0\) Hz), 2.04 (d, 2H, C\(_7\)-H\(_2\), \(J = 4.4\) Hz), 1.17 (s, 2H, C\(_{10}\)-CH\(_2\)), 0.75 (s, 3H, C\(_{13}\)-CH\(_3\)), 0.97 and 0.80 (other methyl protons); \(^13\)C NMR (CDCl\(_3\)): \(\delta\) 163 (C=N), 149, 125, 124, 122.8, 122, 120 (aromatic carbons), 48 (C\(_3\)), 46 (C\(_{14}\)), 42.2 (C\(_4\)), 39 (C\(_{10}\)), 35 (C\(_3\)), 26 (C\(_{19}\)), 24 (C\(_{11}\)), 22 (C\(_{18}\)), 20 (C\(_{15}\)); MS: m/z 491 [M\(^+\)].
3β-Acetoxy-5α-cholestan-6b-benzothiazine (54):
Yield 80%; m.p. 163 °C; Analysis found: C 76.45, H 9.35, N 2.55%. C35H41NO2S requires: C 76.17, H 9.08, N 2.32%. IR (KBr): ν max 3060, 1603 (aromatic), 1714 (OCOCH3), 1650 (C=N), 1388 (C-N), 1206 (C-O), 750 (C-S); 1H NMR (CDCl3): δ 6.33-6.28 (m, 4H, aromatic), 4.7 (m, 1H, C3α-H, W½ = 15 Hz), 2.03 (s, 3H, OCOCH3), 1.9 (d, 2H, C7-H2, J = 5.2 Hz), 1.8 (d, 2H, C4-H2, J = 8.0 Hz), 1.18 (s, 3H, C10-CH3), 0.70 (s, 3H, C13-CH3), 0.97 and 0.83 (other methyl protons); 13C NMR (CDCl3): δ 174 (OCOCH3), 163 (C=N), 148, 129, 128, 126, 124, 122 (aromatic carbons), 73 (C3), 46 (C14), 44 (C13), 42 (C4), 39 (C10), 35 (C5), 26 (C19), 24 (C11), 22 (C18); MS: m/z 549 [M+].

3β-Chloro-5α-cholestan-6b-benzothiazine (55):
Yield 85%; m.p. 146 °C; Analysis found: C 75.32, H 9.19, N 2.66%. C33H28CINS requires: C 75.07, H 9.03, N 2.61%. IR (KBr): ν max 3058, 1598 (aromatic), 1626 (C=N), 1380 (C-N), 740 (C-Cl), 710 (C-S); 1H NMR (CDCl3): δ 6.43-6.24 (m, 4H, aromatic), 3.5 (m, 1H, C3α-H, W½ = 17 Hz), 2.07 (d, 2H, C7-H2, J = 8.0 Hz), 1.87 (d, 2H, C4-H2, J = 4.8 Hz), 1.18 (s, 2H, C10-CH3), 0.75 (s, 3H, C13-CH3), 0.97 and 0.80 (other methyl protons); 13C NMR (CDCl3): δ 164 (C=N), 146, 127, 126, 125, 123, 122 (aromatic carbons), 59 (C3), 47 (C14), 46 (C13), 42.6 (C4), 39 (C10), 35 (C5), 26 (C19), 24 (C11), 22 (C18), 20 (C15), 17 (C16); MS: m/z 525/527 [M+].

2′-Hydroxinocholest-6-eno [4, 5-d] thiazole (56):
Yield 73%; m.p. 129 °C; Analysis found: C 73.52, H 10.28, N 9.19%. C28H27N3S requires: C 73.47, H 10.19, N 9.13%. IR (KBr): ν max 3376, 3328 (NH, NH2), 1617 (C=C), 1557 (C=N), 1328 (C-N), 634 (C-S); 1H NMR (CDCl3): δ 6.2 (br s, 2H, NH2, exchangeable with D2O), 3.8 (s, 1H, NH, exchangeable with D2O), 2.74 (dd, 1H, C3α-H, J = 16.9 Hz, 5.5 Hz), 1.18 (s, 3H, C10-CH3), 0.70 (s, 3H, C13-CH3), 0.97 and 0.83 (other methyl protons); 13C NMR (CDCl3): δ 163 (C=N), 130 (C6), 120 (C7), 50 (C5), 46 (C14), 42.2 (C4), 39 (C10), 35 (C5), 26 (C19), 24 (C11), 22 (C18), 20 (C15), 17 (C16); MS: m/z 457 [M+].

3β-Acetoxy-2′-hydranocholest-6-eno [4, 5-d] thiazole (57):
Yield 82%; m.p. 136 °C; Analysis found: C 69.90, H 9.51, N 8.15 %. C30H29N3S2 requires: C 69.84, H 9.39, N 8.11%. IR (KBr): ν max 3395, 3310 (NH, NH2), 1730 (OCOCH3), 1625 (C=C), 1555 (C=N), 1320 (C-N), 1210 (C-O), 645 (C-S); 1H NMR (CDCl3): δ 6.8 (br s, 2H, NH2, exchangeable with D2O), 4.7 (m, 1H, C3α-H, W½ = 15 Hz), 4.4 (s, 1H, NH, exchangeable with D2O), 2.7 (dd, 1H, C3α-H, J = 15 Hz, 5 Hz), 2.03 (s, 3H, OAc), 1.18 (s,
3H, C_{10}-CH_{3}), 0.70 (s, 3H, C_{11}-CH_{3}), 0.97 and 0.83 (other methyl protons); $^{13}$C NMR (CDCl$_3$): $\delta$ 171.2 (OCOCH$_3$), 163 (C=N), 132 (C$_6$), 120 (C$_7$), 70.2 (C$_3$), 46 (C$_{14}$), 44 (C$_{13}$), 42 (C$_4$), 39 (C$_{10}$), 35 (C$_5$), 26 (C$_{19}$), 24 (C$_{11}$), 22 (C$_{18}$), 20 (C$_{15}$), 17 (C$_{16}$); MS: $m/z$ 515 [M$^+$].

**3ß-Chloro-2'-hydrazinocholest-6-eno [4, 5 - d] thiazole (58):**

Yield 76%; m.p. 143 °C; Analysis found: C 68.43, H 9.36, N 8.54%. C$_{28}$H$_{46}$ClN$_3$S requires: C 68.37, H 9.29, N 8.49%; IR (KBr): $\nu_{\text{max}}$ 3370, 3320 (NH, NH$_2$), 1622 (C=C), 1560 (C=N), 1323 (C-N), 745 (C-Cl), 635 (C=S); $^1$H NMR (CDCl$_3$): $\delta$ 6.63 (br s, 2H, NH$_2$, exchangeable with D$_2$O), 4.45 (s, 1H, NH, exchangeable with D$_2$O), 3.9 (m, 1H, C$_3$$\alpha$-H, $W_2$ = 17 Hz), 2.8 (dd, 1H, C$_5$$\alpha$-H, $J$=17.05 Hz, 5.3 Hz), 1.18 (s, 3H, C$_{10}$-CH$_3$), 0.70 (s, 3H, C$_{11}$-CH$_3$), 0.97 and 0.83 (other methyl protons); $^{13}$C NMR (CDCl$_3$): $\delta$ 162 (C=N), 134 (C$_6$), 120 (C$_7$), 57.7 (C$_3$), 46 (C$_{14}$), 45 (C$_{13}$), 42.6 (C$_4$), 39 (C$_{10}$), 35 (C$_5$), 26 (C$_{19}$), 24 (C$_{11}$), 22 (C$_{18}$), 20 (C$_{15}$), 17 (C$_{16}$); MS: $m/z$ 489/491 [M$^+$].
References
References

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