IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736. Volume 18, Issue 3 Ser. 1 (March 2025), PP 19-24
www.iosrjournals.org

Synthesis and Characterization of Novel Thiourea Derivatives of Cholesterol

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Abstract
In this study, six novel cholesterol-based thiourea derivatives were successfully synthesized via the reaction of cholesterol chloroformate with various thioureas. The synthesis aimed to explore the potential functionalization of cholesterol with thiourea moieties which are known for their diverse biological activities. The structures of the synthesized derivatives were confirmed using ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) and mass spectrometry. Additionally, FTIR (Fourier transform infrared spectroscopy) further validated the presence of key functional groups including, the thiourea moiety and the cholesterol backbone. Although no biological activity assays were conducted the successful synthesis and characterization of these thiourea derivatives provide a solid foundation for future studies exploring their biological and material applications.

Keywords: *Thiourea, cholesterol chloroformate, cholesterol derivatives, thiocarbamate, DMAP*

Date of Submission: 04-03-2025 Date of Acceptance: 14-03-2025

I. Introduction

Cholesterol[1] is a key biological molecule that plays a crucial role in maintaining cell membrane structure, stability and fluidity. It ensures that membranes do not become too rigid or too permeable, thus regulating membrane integrity under varying physiological conditions. Beyond its structural role, cholesterol serves as a precursor for steroid hormones, including those that regulate metabolism, immune response and reproductive functions. Additionally, cholesterol is a starting material for bile acid synthesis, which is essential for the digestion and absorption of dietary fats and fat-soluble vitamins (A, D, E and K) in the small intestines. Cholesterol also serves as a precursor for Vitamin D synthesis, which plays a critical role in calcium and phosphate balance in the body, supporting bone health and immune regulation. Consequently, cholesterol derivatives[2] have emerged as valuable compounds in biomedical research, chemical sensing, and materials science, due to their well defined structural features, tunable properties and biocompatibility[3-12].

Functionalisation of cholesterol by introducing thiourea[13-15] motifs can lead to novel bioactive derivatives with enhanced pharmacological potential. Thioureas exhibit diverse biological activities, including antibacterial, antifungal, antiviral and anticancer effects. These activities often stem from their ability to disrupt membrane function, inhibit key enzymes, or interfere with vital cellular processes. Given that cholesterol is an integral to cell membrane dynamics, thiourea-functionalised cholesterol derivatives could serve as a valuable platforms for developing novel therapeutic agents that combine the membrane affinity of cholesterol with versatile pharmacological actions of thiourea.

Cholesterol chloroformate readily reacts with thioureas to yield thiocarbamate derivatives through a nucleophilic substitution mechanism. In this reaction, the nucleophilic sulphur or nitrogen of the thiourea attacks the electrophilic carbonyl carbon of cholesterol chloroformate, displacing the chloride ion and forming a new CN bond. These derivatives are of significant interest due to their potential pharmacological activities including anti-inflammatory, anti-cancer, and cholesterol lowering effects. Additionally, structural modifications can improve the solubility, stability and bioavailability of cholesterol based drugs, making them more effective for therapeutic applications.

Modified cholesterol molecules are also valuable tools for studying cholesterol metabolism and function, particularly their effects on membrane fluidity, cellular signalling and lipid transport mechanisms. By attaching thioureas to cholesterol, researchers may develop probes or markers for investigating cholesterol-related pathways, which are critical in cardiovascular and neuro degenerative disorders. Furthermore, thioureas can be derivatized into heterocyclic compounds such as thiazoles and imidazoles, which are frequently found in biologically active molecules, expanding their potential applications in medicinal chemistry.

This study focuses on the synthesis and Characterization of novel thiourea derivatives of cholesterol. The synthesised cholesterol thiocarbamate derivatives[17-21] are structurally analysed using ^{1}H NMR, ^{13}C NMR, FTIR, and mass spectrometry to confirm their formation. These compounds could serve as a foundation for future studies on their biological and pharmaceutical potential.

Experimental Section

This section, describes the synthesis and characterization of six cholesterol derivatives **(1-6)** prepared by reacting cholesteryl chloroformate with six different thioureas. The structures were designed to explore how the effect of functional group modifications influence their reactivity, and are presented in **Scheme1**.

II. Materials And Methods

Chemicals and Reagents: All reagents and solvents were of analytical or HPLC grade and used without further purification. Cholesteryl chloroformate (98% purity) was purchased from Sigma-Aldrich and Dichloromethane (DCM, HPLC grade) was obtained from Fisher Scientific. 4-dimethyl aminopyridine (DMAP, 99% purity), was supplied by Sigma-Aldrich. Methanol (anhydrous, 99.8%) from VWR (Van Waters and Rogers) and Anhydrous Sodium Sulphate (Fisher Scientific) was used as a drying agent. Silica Gel (60-120 mesh, Acros Organics) was employed for column chromatography.

General Reaction Conditions: A polar aprotic solvent (DCM) was used to dissolve the reactants. Trimethyl amine served as a mild base to neutralise the HCl byproduct. Reactions were initiated at 0 ^{o}C (to control exothermic effects) and then allowed to warm to room temperature or ~ 40 to 60 ^{o}C to ensure efficient conversion. Thiourea was typically used in slight excess (1.1 equiv.) to drive the reaction to completion. Reaction progress was monitored by thin layer chromatography (TLC) on silica gel plates, and purification was achieved through column chromatography with methanol/chloroform as the solvent system.

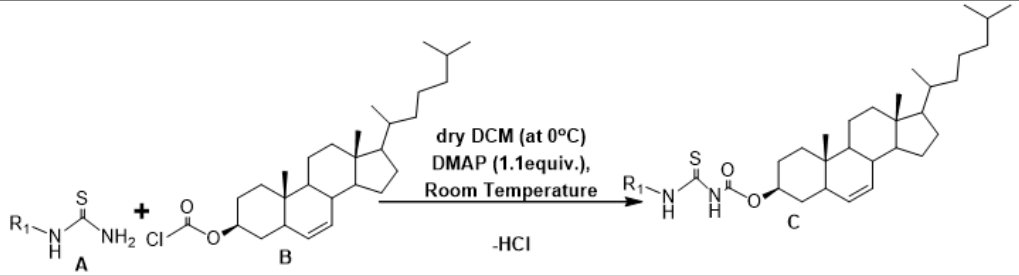
The reaction mechanism involves three steps – 1) nucleophilic attack- thiourea attacks the electrophilic carbonyl carbon of cholesterol chloroformate 2) substitution reaction - where the chloride ion is displaced forming a new carbon-hetero atom bond 3) The generated HCL is neutralized by the base.

General Procedure for the synthesis of cholesterol thiocarbamate derivatives: In a typical experiment, Dry DCM (10 mL) was placed in a round bottom flask under a nitrogen atmosphere. The selected thiourea (1.1equiv.) was added, followed by trimethyl amine (1.2equivalents). This mixture was cooled to 0 ^{0}C, and a solution of cholesteryl chloroformate (1.0 equiv.) in dry DCM was introduced dropwise over 30 minutes. The reaction mixture was stirred for an additional 30 minutes at 0 ^{0}C, after which a catalytic amount of DMAP was added. Stirring continued overnight (8-12) hours at room temperature or (or 40 to 60 ^{O}C) until TLC (silica gel plates, methanol/chloroform as the solvent system) indicated complete consumption of starting materials.

Upon completion, the reaction mixture was washed with deionized water (3 ×10 mL), and the organic layer was separated, dried over anhydrous sodium sulphate, and concentrated under reduced pressure to yield a crude product. This crude product was dissolved in a small volume of DCM, and methanol was added dropwise to induce precipitation. The precipitate was collected by filtration, dried under vacuum, and further purified by column chromatography (silica gel 60-120 mesh) using an ethyl acetate/hexane gradient. The resulting cholesterol thiocarbamate derivatives were isolated in yields ranging from 70% to 90%, depending on the specific thiourea used.

Synthesis of other cholesterol thiocarbamate derivatives: The above procedure was applied uniformly to all six thioureas. Each thiourea (1.1 equiv.) was reacted with cholesterol chloroformate (1.0 equiv.) under identical conditions. The yields and melting points of the resulting derivatives **(1-6)** are summarised in **Scheme 1.**

SCHEME 1



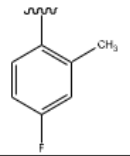
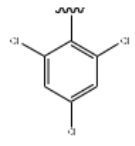
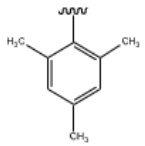
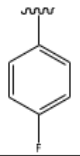
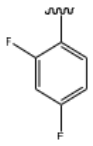
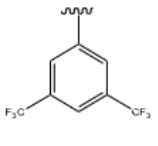
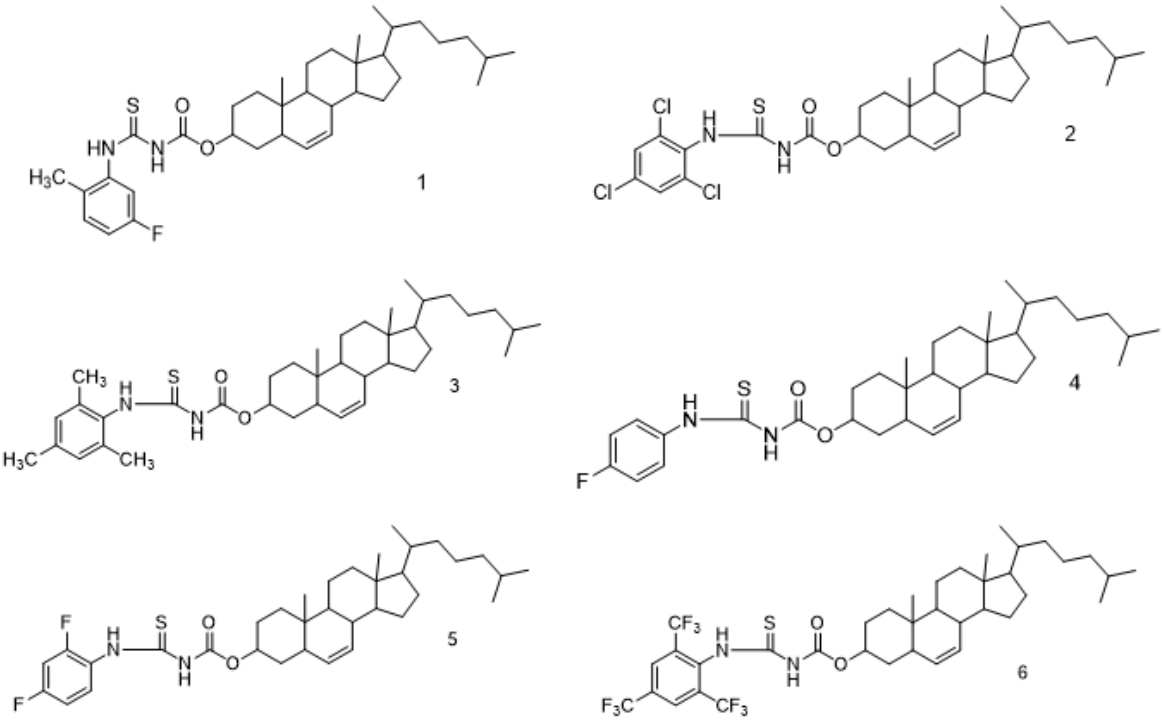
Compound A	1	2	3	4	5	6
R1						
M.P	93°C -95°C	160°C-162°C	150°C-153°C	95°C -98°C	115°C -117°C	160°C-180°C
% Yield	70-80%	75%	80%	70-80%	80%	75%
Color of the Compound formed	Light green solid	White solid	White solid	White solid	White solid	White solid

Figure.1: Chemdraw Structures of the six thiourea derivatives



Characterization of cholesterol thiocarbamate derivatives: All synthesized compounds were characterised by Nuclear Magnetic Resonance (NMR): ^{1}H NMR and ^{13}C NMR, Mass Spectrometry (MS) and Fourier Transform Infrared Spectroscopy (FTIR). These techniques confirmed the molecular structures and assessed the purity of the derivative. Representative spectra and data (Figure 1) support the successful formation of the desired cholesterol thiocarbamate products.

Compound 1: Cholest-5-en-3-yl N-(4-fluorophenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.45(s, 1H, NH-Ar), 8.04 (s, 1H, NH-CS,), 7.56- 6.99 (m, 3H, ArH, overlapping H-F coupling), 5.42 (s, 2H, olefinic protons), 4.88– 4.54 (m, 1H, OCO-CH), 2.50-0.68 (m,45H) aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.11 (C=S, thiourea group), 152.26(C=O, carbamate), 141.48 (C-F, ArH), 138.82 (CNH, ArH), 133.56 (CNH, ArH), 126.49(CNH, ArH), 126.40 (C -olefinic), 123.49 (C -olefinic), 115.84 (CNH, ArH), 115.61 (CNH, ArH), 80.01 (C-O, methine), 56.66, 56.10, 49.97, 42.30, 39.67, 39.50, 37.99, 36.53, 36.16, 35.77, 31.89, 31.80, 29.70, 28.21, 28.01, 24.27, 23.81, 22.82, 22.56, 21.03, 19.27, 18.71, 11.86. 2.13, 0.31 (cholesterol backbone and side chain). IR (ν, cm^{-1}) : 3505, 3476 , 3193 (N-H stretching of thiourea), 2933, 2867 C-H stretching vibration methyl or methylene groups), 1778 and 1717 (C=O stretching, thiocarbamate ester functionalities) 1605 and 1565 (C=N stretching, confirming the presence of the thiourea group), 1247, 1198 (C-N stretching, typical of thiourea derivatives) 802 to 574 (out of plane or bending vibrations associated with C=N /C=S stretch in thiourea). MS (ESI, m/z): 597 $[M+H]^+$ calculated for $C_{36}H_{53}FN_2O_2S$ 596.8904.

Compound 2: Cholest-5-en-3-yl N-(2,4,6-trichlorophenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) $\boldsymbol{\delta}$ 10.91 (s, 1H, NH), 8.22 (s, 1H, NH), 7.43 (s, 2H), 5.44-5.41 (d, J = 4.8 Hz, 2H, olefinic), 4.70 – 4.62 (m, 1H, OCO-CH), 2.46–0.87 (m, 42H aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.00, 162.09, 152.32, 138.96, 135.21, 134.69, 132.22, 128.60, 127.33, 123.32, 79.02, 56.62, 56.08, 49.90, 42.28, 39.66, 39.49, 37.87, 36.83, 36.50, 35.77, 31.88, 31.79, 28.20, 28.00, 27.62, 24.26, 23.80, 22.82, 22.55, 21.00, 19.24, 18.70, 11.83. IR (ν, cm^{-1}) : 3505, 3476 , 3193 (N-H stretching of thiourea), 2933, 2867 C-H stretching vibration methyl or methylene groups), 1782 and 1717 (C=O stretching, thiocarbamate ester functionalities) 1605 and 1565 (C=N stretching, confirming the presence of the thiourea group), 1247, 1198 (C-N stretching, typical of thiourea derivatives) 700 to 600 (aromatic C-Cl stretch, the aliphatic C-H stretch at 2933 confirms the steroid backbone and side chain of cholesterol). MS (ESI): m/z found at 669.10 $[M+1]^+$; calculated for $C_{35}H_{49}Cl_3N_2O_2S$ 668.19.

Compound 3: Cholest-5-en-3-yl N-(2,4,6-trimethylphenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.73 (s, 1H,NH), 8.10 (s, 1H, NH), 6.93 (d, J = 4.4 Hz, 2H), 5.41 (dd, J = 19.1, 5.2 Hz, 2H,olefenic), 4.85 – 4.62 (m, 1H, OCO-CH), 2.29 (s, 6H), 2.27 - 0.67(m, 45H). aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.46, 162.11, 152.39, 138.98, 135.22, 132.73, 132.61, 129.27, 129.07, 123.39, 79.04, 56.67, 56.12, 50.20, 42.31, 39.50, 38.04, 37.89, 36.53, 36.17, 35.78, 31.89, 31.81, 28.21, 28.01, 27.77, 24.27, 23.81, 22.82, 22.56, 21.04, 19.29, 18.71, 11.85. IR (KBr,ν, cm^{-1}) : 3508, 3155 (broad N-H stretching of thiourea), 2939, 2868 (aliphatic C-H stretching vibration methyl or methylene groups from cholesterol backbone or side chain), 1780 and 1712 (C=O stretching, thiocarbamate ester functionalities) 1609 and 1529 (C=N stretching, confirming thiourea moiety), 1464 (possibly rig stretching or CH bending for aromatic ring with alkyl substituents), 1241, 1187 (C-N stretching, typical of thiourea derivatives) -800 to 600 (732, 681, 657 peaks for out of plane or bending vibrations of aromatic substituents and C=S bending modes). HRMS (ESI-TOF): m/z found at 607.4303 $[M]^+$ calculated for $C_{38}H_{58}N_2O_2S$ 607.4297.

Compound 4: Cholest-5-en-3-yl N-(4-fluorophenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.45 – 11.31 (s, 1H,NH), 8.04 (s, 1H,NH), 7.58 – 7.54 (s, 1H), 7.44 – 7.38 (m, 1H), 7.16 – 7.12 (m, 1H), 7.11 – 7.06 (m, 1H), 5.42 (s, 2H,olefinic), 4.88 – 4.54 (m, 1H OCO-CH), 2.50-0.68 (m,42H) aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$) $\boldsymbol{\delta}$ 178.47, 152.26, 141.48, 138.82, 133.56, 126.49, 126.40, 123.49, 115.84, 115.61, 80.01, 56.66, 56.10, 49.97, 42.30, 39.67, 39.50, 37.99, 36.53, 36.16, 35.77, 31.89, 31.80, 29.70, 28.21, 28.01, 24.27, 23.81, 22.82, 22.56, 21.03, 19.27, 18.71, 11.86. IR (KBr, ν, cm^{-1}) : 3507,3675 (broad N-H stretching of thiourea), 2966, 2942, 2868, 2848 (aliphatic C-H stretching vibration from cholesterol backbone or side chain), 1780,1734 and 1711 (C=O stretching, thiocarbamate ester functionalities), 1615,1575,1535,1507 (C=N stretching, confirming thiourea moiety also aromatic ring contributions if fluoro phenyl is present), 1462,1438, 1367,1324, (possibly CH bending or ring stretching, 1263,1242,1187 (C-N and N-CS stretching, typical of thiourea frame) -800 to 600 (out of plane or bending vibrations of aromatic substituents and C=S bending modes). MS (ESI, m/z) found at 583.87 $[M+H]^+$ calculated for $C_{35}H_{51}FN_2O_2S$, 582.87.

Compound 5: Cholest-5-en-3-yl N-(2,4-difluorophenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) $\boldsymbol{\delta}$ 11.42 (s, 1H,NH), 8.24 – 8.12 (m, 1H,NH), 8.10 (s, 1H), 7.47 – 7.38 (m, 2H), 6.92 (m, 2H), 5.42 (d, J = 4.9 Hz, 2H,olefinic), 4.75 – 4.59 (m, 1H, OCO-CH), 2.40- 0.68 (m ,42H).aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$) $\boldsymbol{\delta}$ 182.72, 162.16, 152.33, 138.94, 128.83, 123.34, 121.71, 112.44, 105.52, 79.07, 56.62, 56.08, 49.90, 42.28, 39.65, 39.49, 37.87, 36.50, 36.15, 35.77, 31.87, 31.79, 31.63, 28.20, 28.00, 24.25, 23.87, 22.81, 22.55, 21.01, 19.23, 18.69, 11.83. IR (KBr, ν, cm^{-1}) : 3512, ~3174 (broad N-H stretching of thiourea), 2938, 2871 (aliphatic C-H stretching vibration from cholesterol backbone or side chain), 1714 (C=O stretching, thiocarbamate linkage), 1615,1573,1539,1502 (C=N stretching, confirming thiourea moiety), 1363, 1282, 1227, 1172 (C-N stretching, typical of thiourea frame also aromatic ring contributions if fluoro phenyl is present) -800 to 600 (out of plane bending vibrations of aromatic ring and possible C=S wagging). HRMS (ESI-TOF): m/z found at 601.3635 $[M]^+$ calculated for $C_{35}H_{50}F_2N_2O_2S$ 601.8538.

Compound 6: Cholest-5-en-3-yl N−(2,4,6−tris(trifluoromethyl)phenyl) thiocarbamoyl carbamate

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.85 (s,NH 1H), 8.23 (s, NH,1H), 8.11 (s, 1H), 7.74 (s, 1H), 5.41 (m, 2H, olefinic protons), 4.48 (m, 1H, OCO-CH), 2.39- 0.68 (m, 42H). aliphatic protons from the side chain and steroid ring). ^{13}C NMR (100 MHz, $CDCl_3$**)** $\boldsymbol{\delta}$ 178.21, 155.14, 152.39, 139.31, 139.08, 138.65, 132.32, 131.92, 124.15, 123.87, 123.64, 123.01, 121.53, 119.84, 77.87, 56.66, 56.10, 49.96, 42.45, 39.69, 39.49, 37.93, 36.51, 36.16, 35.77, 31.88, 31.81, 28.21, 28.00, 27.67, 24.26, 23.81, 22.81, 22.55, 21.02, 19.25, 18.69, 11.69. IR (KBr, ν, cm^{-1}) : 3707, 3675, 3512, 3276 (broad N-H stretching of thiourea), 2943, 2867(aliphatic C-H stretching vibration from cholesterol backbone or side chain), 1746,1720 (C=O stretching, thiocarbamate linkage), 1651,1628,1599 (C=N stretching, confirming thiourea moiety), 1531,1469, 1381,1362 (ring stretching, C-N modes, typical of thiourea frame also C-F or CF3 influences in the finger print region. 1275,1255-1189 (C-N stretching possibly C-F stretches from -CF3 groups and thiocarbamate link vibrations), -800 to 600 (out of plane bending vibrations of aromatic ring and possible C=S wagging, C=S deformation). HRMS (ESI-TOF): m/z found at 768.6371 $[M]^+$ calculated for $C_{38}H_{49}F_9N_2O_2S$ 768.3371.

III. Results And Discussion

Six novel thiourea derivatives of cholesterol were successfully synthesized by reacting cholesterol chloroformate with a series of substituted thioureas. The reactions for carried out in dry dichloromethane in the presence of trimethylamine and catalytic amount of DMAP under controlled temperature conditions. The use of a slight excess of the substituted thiourea helped drive the reaction to completion. The products were isolated by

precipitation and subsequently purified through column chromatography resulting in yields ranging between 70% to 80%. The melting points of the derivatives varied significantly depending on the substituents on the aromatic ring attached to the thiourea moiety. Specifically, the compound 6 bearing the strongly electron withdrawing and bulky 2,4,6−tris(trifluoromethyl)phenyl) group exhibited high melting point of 160-180 ^{o}C, closely comparable to the melting point of the compound with the 2,4,6-trichlorophenyl substituent containing compound 2, which showed a melting point of 160 - 162 ^{o}C. These two derivatives possess strong intermolecular interactions due to their electron withdrawing substituents, resulting in efficient packing arrangement of atoms and subsequently elevated melting points. In contrast, the derivative featuring electron-donating and bulky methyl groups 2,4,6-trimethylphenyl (compound 3), exhibited a slightly lower melting point of 150 - 153 ^{o}C. The melting point differences among these derivatives clearly reflect how subtle variations in electronic effects and steric bulk influence their crystal packing, intermolecular interactions, and thermal stability.

The structures of the synthesised derivatives were confirmed by ^{1}H and ^{13}C Nuclear magnetic resonance spectroscopy. In proton NMR data, key signals included down field resonances in the region (10.8-11.8 ppm) corresponding to the N-H protons of the thiourea functionality. Olefinic protons from the cholesterol skeleton consistently appeared around 5.4 ppm as singlets, whereas complex multiplets between 0.68 and 2.50 ppm were attributed to the numerous aliphatic protons present in the steroid framework and side chain. Additionally, aromatic proton resonances varied according to the nature of the aromatic substituents, notably displaying characteristic coupling patterns in fluorine containing derivatives. The ^{13}C NMR spectral data supported the structural assignments by exhibiting distinctive resonances. For instance, the thiocarbonyl carbon (C=S) of the thiourea moiety appeared consistently near 185 ppm, and the thiocarbamate carbonyl (C=O) resonated around 152 -155 ppm. Signals corresponding to the olefinic carbons and numerous aliphatic carbons in the cholesterol skeleton, appeared in their expected regions, further corroborating the structures.

Fourier-transform infrared spectroscopy (FTIR) data further supported the structural characterization by identifying key functional groups. Prominent bands around 3505- 3476 and ~3193 cm^{-1} indicated N-H stretching bands of the thiourea groups. The aliphatic C-H stretching vibrations of the cholesterol backbone appeared at 2933 and 2867 cm^{-1}. Additionally, the characteristic C=O stretching vibrations of thiocarbamate functionalities appeared prominently near 1778 and 1717 cm^{-1}, while the C=N stretching absorptions (conforming the thiourea group) were noted around 1605 and 1565 cm^{-1}. Furthermore, fingerprint region bands between 1247 and 1198 cm^{-1} were assigned to C-N stretching vibrations typical of thiourea derivatives. Lower frequency bands (802-574 cm^{-1}) corresponded to out of plane bending modes and were also influenced by aromatic substituents

The mass spectral data (ESI-MS) for all synthesised derivatives were in excellent agreement with calculated molecular weights, further confirming their identity and purity. For example the mass spectrum of 4-fluorophenyl derivative displayed a clear $[M+H]^{+}$ peak at m/z 597, which corresponds closely to the calculated mass (596.89). Similar correlations were observed for the other derivatives, providing strong evidence for the successful formation of the target components.

Collectively, these observations underscore the critical influence of substituents on melting points, stability, and spectroscopic behaviour. Electron withdrawing substituents e.g., 2,4,6-trichloro and 2,4,6−tris(trifluoromethyl) notably enhance molecular rigidity, intermolecular interactions, and atomic packing efficiency resulting in substantially elevated melting points. Conversely electron donating substituents (e.g., trimethyl) also contribute to robust intermolecular interactions, albeit slightly weaker than their electron withdrawing counterparts, leading to moderately high but relatively low melting points.

In conclusion the study illustrates that even subtle electronic and steric modifications in thiourea substituted cholesterol derivatives significantly influence the physical properties, such as melting points and solid atomic packing suggesting potentially important implications for their biological and material applications.

IV. Conclusion

In summary, a series of novel cholesterol based thiourea derivatives were synthesized via a straightforward reaction between cholesterol chloroformate and various substituted thioureas. The structural integrity and purity of these compounds was confirmed using ^{1}H and ^{13}C NMR spectroscopy, FTIR and mass spectrometry which collectively validating the successful formation of thiourea and thiocarbamate functionalities on the cholesterol framework. The observed variations in melting points particularly among derivatives baring 2,4,6−tris(trifluoromethyl)**,** 2,4,6-trichloro and 2,4,6-trimethyl subtsituents, highlight the significant impact of substitution effects on atomic packing, intermolecular interactions and overall molecular stability.

Although no biological assays were conducted in the study, the successful synthesis and thorough characterization of these derivatives provide a robustt foundation for future investigation into their potential applications in drug design, nanomaterials, and the study of cholesterol related biological pathways.

Acknowledgements:

We thank Osmania University for providing infrastructural facilities and IICT Hyderabad for spectral data assistance.

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