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Computational insights into oxysterols: MD and DFT applications in pharmaceutical research

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Abstract: Oxysterols, the oxidized derivatives of cholesterol, are biologically active molecules involved in a wide range of physiological and pathological processes. Small structural changes in the cholesterol backbone (such as additional hydroxyl or keto groups on the sterol rings or side chain) dramatically alter their biological effects, ranging from beneficial roles in cholesterol homeostasis and antiviral activity to harmful contributions to diseases like atherosclerosis, neurodegeneration, and cancer. Molecular dynamics (MD) simulations and density functional theory (DFT) calculations have emerged as powerful tools to investigate these molecules at an atomic level. By complementing experimental studies, computational approaches reveal how oxysterols interact with membrane lipids, providing mechanistic insight into their function. This review highlights key findings from MD and DFT studies on various oxysterols — including 7-hydroxycholesterol epimers, 7-ketocholesterol, 22-hydroxycholesterol epimers, 24(*S*)-, 25- and 27-hydroxycholesterol — in the context of pharmaceutical and medical research. We discuss how these methods uncovered differences in orientation, hydration, and intermolecular interactions of oxysterols in model membranes, lipid rafts, and complexes, explaining phenomena such as membrane domain destabilization or stabilization, rapid transbilayer "flip-flop" translocation, and stereochemistry-dependent biological activity. Understanding these molecular details is crucial for pharmacists and medical researchers, as it connects oxysterol structure–function relationships to their roles as potential biomarkers, therapeutic targets, or bioactive compounds involved in disease pathophysiology. Computational chemistry thus provides a valuable complement to experimental pharmacology, enabling the prediction of oxysterol behavior in biological systems.

Keywords: oxysterols, molecular dynamics simulations, density functional theory, membrane biophysics.

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Introduction

Cholesterol is not only an essential component of cell membranes that modulates membrane fluidity and signaling, but also a biochemical precursor for vitamin D, bile acids, and steroid hormones [1]. Its oxidized derivatives, known as oxysterols, can form enzymatically or via reactive oxygen species and carry additional polar functional groups (e.g. hydroxyl, carbonyl, or epoxy) either on the sterol ring system or the isooctyl side chain (Fig. 1) [2]. These seemingly minor structural modifications profoundly influence biological activity. Oxysterols are present at low levels in healthy organisms (orders of magnitude lower than cholesterol) and contribute to maintaining cholesterol homeostasis, for example by binding liver X receptors (LXRs) to regulate cholesterol metabolism [3]. Under pathological conditions, however, oxysterol concentrations can change significantly, and such changes have been linked to numerous diseases (diabetes, atherosclerosis, neurodegenerative diseases, osteoporosis, inflammatory conditions, and certain cancers) [2, 4, 5]. Intriguingly, oxysterols exert pleiotropic — and at times opposing — effects on cells, acting as either beneficial or detrimental agents depending on physiological or environmental conditions. For instance, some oxysterols assist in removing excess cholesterol and have neuroprotective or antiviral effects [6, 7], while others promote cytotoxicity or pathological changes [5]. Unlike cholesterol, many oxysterols can cross the blood–brain barrier and thereby directly influence the central nervous system [8, 9].

Experimental pharmacological and biochemical methods (e.g. microscopy, mass spectrometry, binding assays) have characterized many oxysterol effects in cells [10–13]. However, it is often challenging to directly observe how oxysterols behave at the molecular scale within membranes or complexes due to the complexity of biological systems. Here, computational chemistry provides a valuable complement. Molecular dynamics (MD) simulations and density functional theory (DFT) calculations allow researchers to probe the atomistic behavior of oxysterols and their interactions with lipids or proteins in controlled model systems. MD simulations track the motions of molecules over time based on force fields, which define the potential energy of a system through bonded and nonbonded interaction parameters. This approach provides insights into molecular orientation, conformation, and interactions within environments such as lipid bilayers [14]. DFT, a quantum mechanical approach, enables the calculation of optimized molecular geometries, electronic structures, and interaction energies of biomolecules and their complexes [15]. By integrating these approaches with experimental data, one can correlate macroscopic observations with microscopic mechanisms. For example, Langmuir monolayer experiments characterize macroscopic properties through surface pressure–area isotherms of lipid films, while MD simulations elucidate the specific intermolecular interactions responsible for the observed isotherm profiles [16]. This synergy of computation and experiment has proven especially fruitful in oxysterol research, where simulations have elucidated phenomena such as the orientation of oxysterol molecules at membrane interfaces, their propensity to form or disrupt membrane domains, and their ability to translocate (flip-flop) across bilayers [17].

In this review, we summarize recent advances from MD and DFT studies of oxysterols, emphasizing how these approaches elucidate structure–activity relationships relevant to pharmaceutical and medical sciences. Particular attention is given to the behavior of oxysterols within model membranes, including lipid bilayers and raft-like domains, and to their interactions with other lipid species. By demonstrating the capacity of MD and DFT to reveal molecular mechanisms underlying oxysterol function, this review aims to promote their broader application to pharmacologically relevant systems, contributing to rational drug design and toxicological assessment.

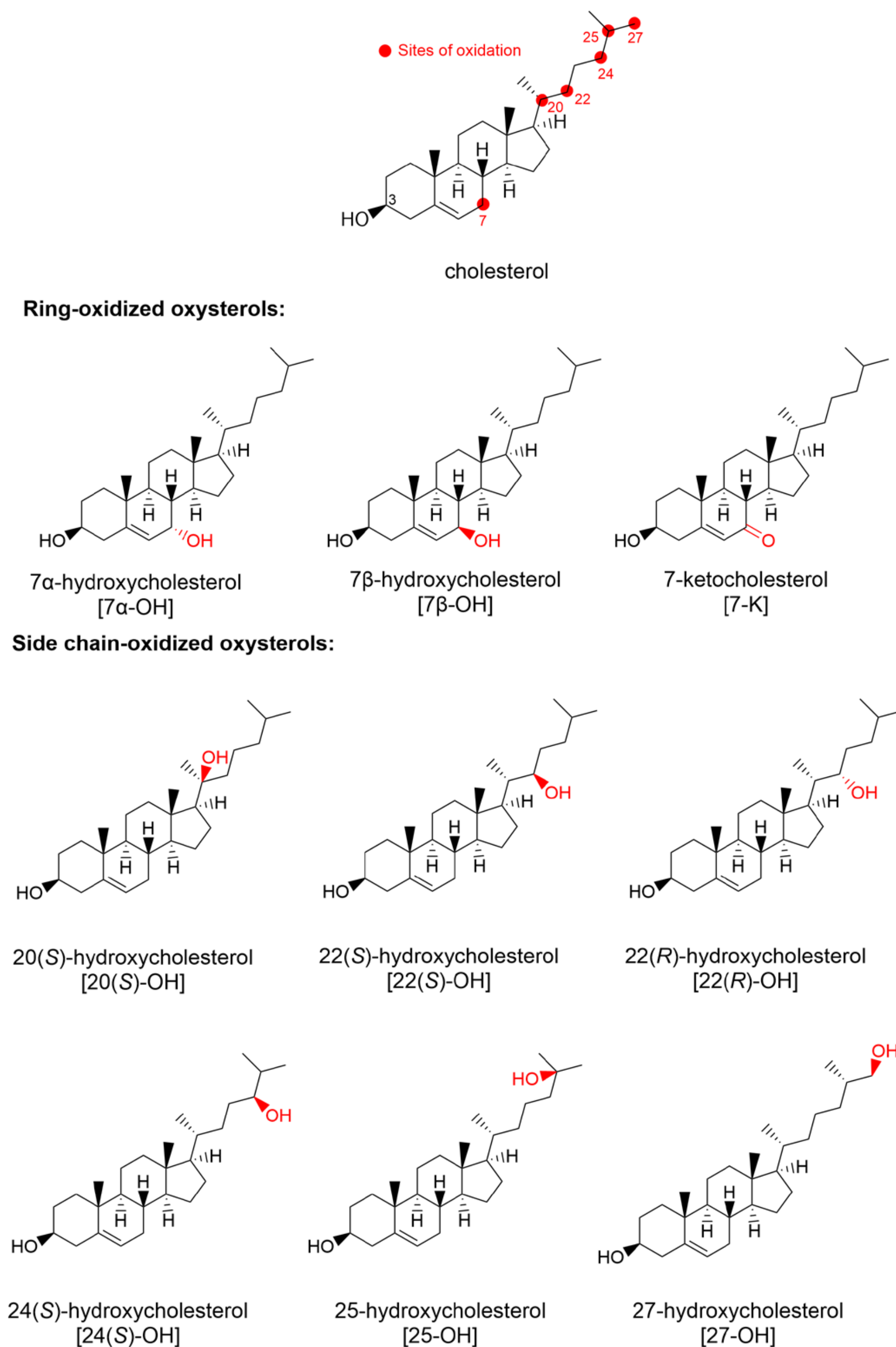

Fig. 1. Structural formulas of cholesterol and the oxysterols discussed in this review. Sites of oxidation are indicated and numbered on the cholesterol structure.

Ring-Oxidized Oxysterols

7β-hydroxycholesterol and 7-ketocholesterol

The C7 position on the sterol B-ring is a common site of non-enzymatic oxidation. Two prevalent oxysterols formed via reactive oxygen species are 7β-hydroxycholesterol (7β-OH) and 7-ketocholesterol (7-K). These molecules are notable for their potent cytotoxicity *in vitro* and have been found enriched (along with cholesterol) in atherosclerotic plaques [18, 19]. MD simulations and monolayer studies have shown that adding a polar group at C7 dramatically alters the sterol's orientation and interactions in membranes. Unlike cholesterol, which inserts with a single C3-OH group anchoring at the membrane interface and its rigid rings perpendicular to the plane, 7β-OH and 7-K each possess two polar groups: the native hydroxyl group at C3 and an additional hydroxyl or carbonyl group at C7. Simulation and Langmuir film experiments indicate that 7β-OH anchors via both its C3-OH and C7-OH groups to the interface, resulting in the molecule adopting a tilted orientation within the monolayer [20]. In other words, the sterol cannot orient fully perpendicular to the interfacial surface (like cholesterol) because the additional polar moiety anchors it into a more horizontal, extended conformation to keep both hydrophilic groups in contact with water. This reflects a loosely packed and more fluid film — the 7β-OH molecules, being tilted and more hydrated, cannot pack as tightly with neighboring lipids, thus destabilizing the ordered lipid structure.

7-ketocholesterol behaves in an analogous way. Simulations suggest 7-K also lies tilted with both its C3-OH and C7-O groups at the interface, leading to film expansion and fluidization similar to 7β-OH [21]. The tilt angle for 7-K may be somewhat smaller due to the keto group's lower hydration (due to the lower average number of hydrogen bonds compared with the hydroxyl group) [22]. Nonetheless, both 7β-OH and 7-K disrupt membrane order. Langmuir monolayer experiments complemented by MD showed that 7β-OH and 7-K weaken intermolecular interactions in lipid rafts, the cholesterol–sphingolipid enriched domains, thereby destabilizing these domains [23]. In a model raft mixture of cholesterol and sphingomyelin (SM), replacing cholesterol with 7β-OH or 7-K leads to lower condensation and premature collapse of the monolayer, whereas replacing cholesterol with certain chain-oxidized sterols had the opposite effect (discussed below). This finding is in agreement with biological observations: 7β-OH and 7-K are pro-apoptotic and cytotoxic oxysterols, and their ability to disrupt lipid raft integrity — important for cell signaling and membrane protein function — may underlie some of their harmful effects [24, 25].

7α-hydroxycholesterol

The 7α-hydroxycholesterol (7α-OH) isomer, produced enzymatically (and also found in food [26]), contrasts with 7β-OH. Stereochemically, 7α-OH has the hydroxyl group oriented on the opposite site of the sterol ring. This seemingly small difference yields distinct behavior. Monolayer compression isotherms for 7α-hydroxycholesterol show a notable plateau region, a feature absent for 7β-OH (20). MD simulations explain this plateau as a phase transition in orientation: under lateral compression, 7α-OH molecules reorient from a state where both C3-OH and C7-OH groups are initially interfacial to a state where one of the polar groups (likely the 7-OH) flips out of the water, effectively adopting a different tilt. The observed differences can be rationalized based on geometric features obtained from DFT-optimized structures of 7α-OH and 7β-OH. In 7α-OH,

the orientation of the hydroxyl group at C7 reduces its ability to maintain simultaneous anchoring of both polar groups within the interfacial polar region as the area per molecule decreases [20]. This behavior was confirmed by Brewster angle microscopy images, which for 7α-OH revealed coexisting domains of differing brightness during the plateau — consistent with coexisting populations of molecules in distinct orientations. The ability to reorient likely makes 7α-OH films slightly more ordered (less fluid) than 7β-OH at high packing densities. Indeed, 7α-OH was found to interact strongly with sphingomyelin in mixed films — similarly to 25-OH — suggesting it can form specific complexes despite its ring oxidation. In contrast, 7β-OH's interactions with SM were weaker or nonspecific in comparable experiments [23]. This ties to observations in enzymatic assays: 7α-OH and 7β-OH, while comparably cytotoxic at high concentrations, differ in biochemical effects such as enzyme modulation. For example, 7α-OH is a much more effective activator of acyl-CoA:cholesterol acyltransferase (ACAT1) than 7β-OH (relative activities ~70% vs <10% in one study), presumably because the enzyme's steric requirements favor the 7α orientation [27]. The stereochemical placement of the hydroxyl alters how the oxysterol fits into protein binding sites (or membrane sites), echoing what MD studies showed about membrane orientation. Thus, the 7-hydroxy epimers illustrate how subtle stereochemical differences can lead to markedly different molecular interactions and, consequently, distinct biological effects. Computational analyses were essential for visualizing the altered orientation of the 7α-OH group, absent in 7β-OH, thereby linking these structural features to their divergent interaction patterns.

Side chain-oxidized oxysterols

Oxidation of the isooctyl side chain typically occurs via enzymatic pathways (cytochrome P450s), producing important signaling oxysterols such as 24-, 25-, and 27-hydroxycholesterol [28]. These side chain-oxidized sterols often have physiological regulatory roles and differ in behavior from ring-oxidized sterols. A key distinction is that chain oxidation creates a molecule with polar groups at opposite ends of the molecule. Such molecules are sometimes termed "bipolar" or "bolaform" sterols, analogous to bolaamphiphiles studied in colloid science [29, 30]. MD simulations and experimental studies have shown that chain oxysterols can orient in membranes in multiple ways — either with the additional polar group in the water phase or buried — and this flexibility has intriguing consequences for membrane organization and sterol translocation.

25-hydroxycholesterol

25-hydroxycholesterol (25-OH) is one of the most studied oxysterols computationally and experimentally, often used as a model chain-oxidized sterol. 25-OH (cholesterol with a hydroxyl group on C25, near the end of the side chain) is known for its role in innate immunity as a broad-spectrum antiviral and immunomodulator produced by macrophages. It is also one of the least toxic oxysterols to cells [24, 25]. A striking finding from MD and monolayer studies is that 25-OH does not adopt a single fixed orientation in membranes; instead, it can anchor in dual orientations. Specifically, 25-OH can insert either with its C3-OH group (ring end) facing the aqueous phase (much like cholesterol does) or with its C25-OH group (chain end) facing the aqueous phase [31, 32]. In a pure 25-OH monolayer at the water interface, approximately half the molecules were observed to orient one way and half the other, leading to a mixed orientational arrangement [32]. DFT calculations helped rationalize this by showing that 25-OH's two hydroxyl groups have very

similar hydrogen-bonding affinity to water (the calculated hydrogen bond energies for a water molecule with the C3-OH vs the C25-OH groups differed by only ~0.02 kcal/mol). Thus, neither end is distinctly favored for water anchoring. Further, DFT optimization of 25-OH dimers suggested that the most energetically favorable pairing was one where neighboring 25-OH molecules in a monolayer alternate orientations — one molecule up (anchored via the ring OH) next to one molecule down (anchored via the chain OH) [32]. This staggered arrangement maximizes complementary hydrogen bonding and packing. Subsequent MD simulations explicitly confirmed that, at equilibrium, 25-OH monolayers adopt an alternating mosaic of orientations rather than all molecules uniformly upright or inverted. In snapshots from simulations, the film looks like a patchwork where adjacent sterol molecules often orient oppositely, and this persisted over tens of nanoseconds [32].

This unique "bipolar" behavior has important implications. First, it means 25-OH forms monolayers of lower stability and packing order compared to cholesterol. Experimentally, 25-OH monolayers collapse at a lower surface pressure and exhibit features of a more compressible (less rigid) film. In MD simulations 25-OH was observed to have a different collapse mechanism: instead of the classic nucleation of 3D cholesterol crystals, 25-OH collapses by forming disordered multilayer structures (sometimes described as "branched" or "buckled" monolayers) [32]. The alternating orientations likely prevent the cooperative formation of a tightly interdigitated bilayer upon collapse, as cholesterol would do, and instead yield a tangled multilayer. Second, and perhaps most biologically significant, the dual orientation capability underpins a "flip-flop" mechanism for transbilayer movement. MD simulations demonstrated that chain-oxidized sterols like 25-OH can undergo rapid spontaneous translocation between membrane leaflets via a mechanism termed "bobbing" [17]. In this mechanism, the sterol remains roughly aligned in the membrane but intermittently exposes one polar end or the other to the aqueous phase, effectively rocking or "bobbing" within the membrane. Because 25-OH has polar groups on both ends, it can migrate across the hydrophobic core without ever flipping its entire molecule — it essentially threads its other polar end through to the opposite side. This is fundamentally different from cholesterol, which must rotate 180° to flip (a slow process). The simulations showed that 25-OH can cross a lipid bilayer almost 100 times faster than cholesterol [33]. In cellular context, this means 25-OH can rapidly equilibrate between the two leaflets of the plasma membrane or traverse internal membranes, contributing to its role as a quick-response signaling molecule in stress and immunity. The bobbing mechanism identified by MD provides a molecular explanation for experimental data showing chain-oxidized oxysterols have dramatically higher flip-flop rates than ring-oxidized ones. From a pharmaceutical perspective, this highlights how MD simulations uncovered a previously unappreciated mode of membrane transport, which could apply to any amphipath with dual polar groups — knowledge that could inform drug design for membrane permeability.

Importantly, 25-OH's ability to adopt mixed orientations also affects how it interacts with other lipids. Studies combining Langmuir monolayers, DFT, and MD examined 25-OH in mixtures with phospholipids characteristic of the two membrane leaflets. It was found that 25-OH interacts strongly with phosphatidylcholine (PC) lipids (outer leaflet) but weakly or repulsively with phosphatidylethanolamine (PE) lipids (inner leaflet) [33]. In a saturated PC (DPPC) monolayer, MD showed that 25-OH had no strong orientation preference — both orientations were populated — resulting in some molecules anchoring via the C3-OH and others via the C25-OH group when complexed with DPPC. By contrast, in an unsaturated PC (DOPC) monolayer, the looser molecular packing induced by kinked acyl chains, 25-OH preferentially adopts a single orientation anchored

via its C3-hydroxyl group. Evidently, the fluid environment of DOPC allowed 25-OH to tilt and insert in a cholesterol-like orientation more consistently, whereas DPPC's rigidity allowed both conformations. These differences suggest that membrane composition influences the orientational distribution of 25-OH, which in turn may modulate membrane properties and protein recognition. The same study noted that 25-OH's rapid transmembrane movement could be asymmetric — it may cross from an inner leaflet PE-rich environment to an outer leaflet PC-rich environment more readily than the reverse, since its presence is energetically preferred in PC-rich leaflets.

Another critical role of 25-OH is in modulating lipid rafts. As mentioned, 25-OH was found to stabilize ordered cholesterol–SM raft domains, in direct contrast to 7-oxysterols. In mixed monolayer simulations, 25-OH can form hydrogen bonds with SM (via its C3-OH to the SM amide, like cholesterol) and thereby substitute for cholesterol in the SM-rich phase [23]. MD simulations demonstrated that partial replacement of cholesterol with 25-hydroxycholesterol (25-OH) in a cholesterol/SM system preserved the integrity and order of the domain, consistent with experimental observations showing that moderate amounts of 25-OH do not disrupt lipid rafts and may even increase their rigidity by reducing water penetration [31]. The interaction energy calculations (DFT-based) for cholesterol/SM vs 25-OH/SM dimers were comparable, supporting the idea that 25-OH binds SM nearly as well as cholesterol does. However, owing to its bipolar character, an excess of 25-OH can give rise to additional, alternative structural arrangements. When 25-OH was mixed with SM at high proportions, Brewster angle microscopy and AFM imaging revealed multilayer structures described as "strings of beads" forming within the monolayer. These likely correspond to 25-OH-rich aggregates where some molecules invert orientation and stack, initiating bilayer budding. MD simulations confirmed that at high surface pressure, 25-OH and 27-OH can indeed start to form bilayer patches within a monolayer (see below) [34]. Biologically, this propensity might relate to how oxysterols promote or stabilize certain membrane curvatures or microdomain structures that facilitate processes like vesicle formation or viral fusion inhibition.

27-hydroxycholesterol

Oxidation at carbon 27 yields another physiologically important oxysterol, 27-hydroxycholesterol (27-OH), which is abundant in circulation and links cholesterol metabolism with bile acid pathways [35]. 27-OH is also known as the first identified selective estrogen receptor modulator of cholesterol origin, and it has been implicated in osteoporosis (promoting bone resorption) and in endocrine-related cancers [36]. From a biophysical standpoint, 27-OH is very similar to 25-OH in that the hydroxyl is at the extreme end of the side chain (C27 being the terminal carbon). Thus, one might expect 27-OH to behave like 25-OH — and indeed, simulations show 27-OH also exhibits dual orientations and can form tail-to-tail hydrogen bonds with other 27-OH molecules [34]. Both 25-OH and 27-OH readily underwent a monolayer-to-bilayer transition upon compression — essentially, they tend to form multilayer aggregates — facilitated by hydrogen bonding between adjacent oxysterol molecules. DFT calculations showed that two 27-OH molecules can form a dimer stabilized by hydrogen bonds involving their C27-OH and C3-OH groups (or alternatively via a bridging water molecule) forming a stable dimeric structure that may act as a nucleation center for bilayer assembly. Thus, when a monolayer of 27-OH is compressed, it is more likely to fold into a bilayer structure compared to 24-OH (which cannot hydrogen bond tail-to-tail, as discussed below). MD simulations confirmed that, whereas cholesterol consistently formed a bilayer under self-assembly conditions, 25-OH and 27-OH instead formed branched

networks of monolayer and bilayer segments, reflecting a geometrically constrained packing [32]. This could be visualized as the "strings of beads" observed in microscopy for oxysterols/SM systems: the beads are essentially oxysterol multilayer blobs connected by monolayer "strings." The propensity of 27-OH to form such structures may relate to its biological activities. It has been noted that 25-OH and 27-OH have unique antiviral and antimicrobial effects not seen in other oxysterols [6]. One hypothesis is that the propensity of 27-OH to induce transient membrane defects or form rigid microdomains — stemming from its bilayer-forming tendency — may hinder membrane fusion events or pathogen entry. In this way, 27-OH can modify membrane architecture in manners distinct from cholesterol, potentially increasing resistance to viral fusion. Although the precise *in vivo* mechanism remains complex, molecular simulations indicate that 27-OH readily self-associates through hydrogen bonding and can reorganize membrane structure. Conversely, this same capacity for self-association and membrane restructuring may underlie the deleterious effects of 27-OH in bone tissue: accumulation within osteoblast membranes could perturb signaling microdomains or alter membrane protein distribution — particularly given its weak interactions with sphingomyelin — thereby promoting bone resorption processes [37].

24(S)-hydroxycholesterol

The primary brain oxysterol, 24(*S*)-hydroxycholesterol (24(*S*)-OH), is produced by the enzyme CYP46A1 in neurons to eliminate cholesterol from the brain, as the metabolite crosses the blood–brain barrier into the circulation [38]. It has a hydroxyl group at C24, a position slightly closer to the ring than 25-OH or 27-OH. Interestingly, 24(*S*)-OH behaves more like cholesterol in many respects. MD simulations show that 24(*S*)-OH tends to maintain a single orientation (C3-OH group at interface, C24-OH group buried in the hydrocarbon region) and forms stable, condensed monolayers nearly as well as cholesterol [34]. Unlike 25-OH or 27-OH, 24(*S*)-OH does not show evidence of bilayer formation upon collapse — it appeared to simply collapse by the same mechanism as cholesterol rather than forming multi-layer folds. The likely explanation is that the C24-OH group is not positioned at the extreme terminus of the side chain but rather embedded within it. Consequently, if 24(*S*)-OH were to invert its orientation, the C3-OH group would be forced deeper into the hydrophobic core while the C24-OH group would be exposed to the aqueous phase — an arrangement that is both sterically constrained and energetically unfavorable. In essence, 24(*S*)-OH is an oxysterol with only one effective polar group — the other OH is close enough to the hydrophobic part that it likely remains embedded. DFT and MD analyses suggest 24(*S*)-OH cannot easily hydrogen-bond with a neighbor's 24-OH molecules the way 25-OH or 27-OH can with each other; the steric constraints of the side chain prevent two 24-hydroxyl groups from approaching closely without significant conformational distortion. As a consequence, 24(*S*)-OH cannot participate in the tail-to-tail hydrogen-bonding network and therefore does not display the bilayer-forming tendency characteristic of other side-chain oxidized oxysterols [34]. From a biological standpoint, this may underlie the comparatively low cytotoxicity of 24(*S*)-OH and its more benign interaction with membranes, even though it is one of the predominant oxysterols in the brain. 24(*S*)-OH behaves as a slightly more polar analogue of cholesterol, maintaining its characteristic condensing and ordering effects on phospholipid membranes. Experimental and computational studies have shown that 24(*S*)-OH preserves bilayer order and rigidity to an extent comparable to cholesterol, consistent with its interfacial orientation and retention within raft-like domains. Owing to its higher polarity, however, 24(*S*)-OH is more readily transported across cellular membranes, particularly in neurons,

thereby facilitating cholesterol turnover rather than accumulation. MD simulations further corroborate that 24(*S*)-OH, when incorporated into model membranes, exerts an ordering influence nearly equivalent to that of cholesterol. Notably, 24(*S*)-OH is one of the neurodegenerative disease biomarkers, as its plasma concentration reflects brain cholesterol turnover. Understanding its behavior within membranes is therefore of pharmacological relevance [39].

20(S)-hydroxycholesterol

20(*S*)-OH oxysterol, featuring a hydroxyl group at C20, has gained attention for its role in bone biology [40]. 20(*S*)-OH is an intermediate in certain cholesterol metabolism pathways and has been shown to promote osteoblast differentiation and counteract osteoporosis in combination with 22(*S*)-OH [41]. Recent studies using MD and Langmuir monolayers provided insight into how 20(*S*)-OH interacts with membranes differently from 24(*S*)-OH or 27-OH [42]. It was found that 20(*S*)-OH has a special affinity for sphingomyelin (SM), the raft-forming sphingolipid. In mixed monolayers of SM and oxysterols, 20(*S*)-OH uniquely forms a well-defined 1:1 complex with SM, as evidenced by a distinct inflection in the surface pressure–area isotherm and a minimum in the interaction free energy at a 1:1 molar ratio. By contrast, 24S-OH and 27-OH showed only weak, non-specific interactions with SM, without distinct complex stoichiometry. MD simulations provided an explanation: self-interactions between 20(*S*)-OH molecules are weaker than those observed for 24(*S*)-OH or 27-OH, indicating that 20(*S*)-OH exhibits a lower tendency to self-associate. This reduced self-clustering permits SM molecules to intercalate and form hydrogen bonds with 20(*S*)-OH more readily, resulting in the formation of a stable 20(*S*)-OH/SM complex. In contrast, 24(*S*)-OH and 27-OH tend to preferentially interact with themselves (especially 27-OH, which can dimerize) rather than with SM, making their mixing less favorable. The simulations showed that in 20(S)-OH/SM systems, SM molecules could insert between 20(*S*)-OH molecules and form hydrogen bonds (likely between the SM amide and the C20-OH and/or C3-OH of 20(*S*)-OH). In 27-OH/SM system, however, the 27-OH molecules often remained clustered and SM was excluded from those clusters. These molecular differences may underpin the unique biology: 27-OH is known to induce osteoporosis, while 20(*S*)-OH is protective (37,40). The strong interaction of 20(*S*)-OH with SM suggests that it may modulate membrane microdomains involved in osteogenic signaling, thereby enhancing cell survival and differentiation pathways. Conversely, the weak interaction of 27-OH with SM may destabilize lipid raft microdomains or prevent its proper incorporation into signaling regions of the membrane, thereby perturbing osteogenic signaling and shifting the balance toward bone resorption. It is hypothesized that 20(*S*)-OH and 22(*S*)-OH act synergistically as a complex (termed the "SS" complex) that is particularly potent in promoting osteoblast differentiation [43]. In pharmacological research, these findings suggest that modulating oxysterol interactions with membrane microdomains could represent a potential therapeutic strategy. Structural analogues of 20(*S*)-OH may be developed as bone-anabolic agents, whereas inhibitors targeting the deleterious actions of 27-OH could help counteract its bone-catabolic effects.

22(R)- and 22(S)-hydroxycholesterol

The 22(*R*)- and 22(*S*)-hydroxycholesterol epimers (22(*R*)-OH and 22(*S*)-OH) are particularly noteworthy for their divergent interactions with liver X receptors (LXRα/β): 22(*R*)-OH acts as an LXR agonist, whereas 22(*S*)-OH has been shown to function as an LXR antagonist [44]. Furthermore,

22(*S*)-OH has been investigated as a therapeutic candidate: it has demonstrated capacity to reduce lipogenesis and lipid accumulation, indicating potential relevance for treating obesity and type 2 diabetes [45]. Although these differences most likely originate from how each epimer fits within the LXR ligand-binding site, one might expect their behavior in membranes to be similar. However, it was demonstrated that 22(*R*)-OH and 22(*S*)-OH also exhibit distinct behaviors in membrane models [46], which could in turn modulate their accessibility and distribution to nuclear receptors.

In Langmuir monolayer experiments, both 22(*R*)-OH and 22(*S*)-OH were miscible with SM and had comparable overall interaction strength with SM at the air–water interface (the surface pressure measurements indicated similar depletion pressures). However, the composition of the mixed monolayer at the strongest interaction point differed: one epimer might form a tighter complex requiring a different proportion of SM than the other. It turned out that the minimum in free energy for 22(*S*)-OH/SM systems occurred at a slightly different mixture ratio than for 22(*R*)-OH/SM, suggesting subtle differences in how each epimer interacts with SM. MD simulations revealed that 22(*R*)-OH formed more hydrogen bonds with neighboring sterol molecules than 22(*S*)-OH did [46]. This leads to greater hydration of the C22-OH group of 22(*R*)-OH — water molecules could more easily approach and hydrogen bond with the C22-OH of 22(*R*)-OH compared to the C22-OH group of 22(*S*)-OH. MD simulations revealed that 22(*R*)-OH exhibits greater conformational flexibility at the membrane interface, consistent with higher hydration and looser molecular packing. In contrast, 22(*S*)-OH forms a more constrained, hydrogen-bonded network with neighboring molecules, leading to a more condensed and ordered monolayer structure.

Stereochemistry matters

Epimeric oxysterols demonstrate that not only the position of an oxygen substituent but also its stereochemical orientation can alter membrane behavior. MD simulations in combination with monolayer experiments have shown differences in hydration, hydrogen bonding, and packing for epimers like 7α/7β and 22(*R*)/22(*S*) hydroxycholesterols. These molecular differences help explain why epimers often have distinct pharmacological profiles (agonist vs antagonist, toxic vs protective). For pharmacists and medicinal chemists, these findings emphasize the importance of stereochemical purity and consideration when dealing with sterol-like drugs or nutraceuticals: the wrong epimer might integrate into membranes differently or fail to reach the correct site of action efficiently. Computational approaches provide a means to predict these subtleties before *in vivo* tests, guiding the design of stereospecific interventions.

Conclusions

The examples discussed in this review illustrate how molecular modeling approaches — particularly molecular dynamics simulations and density functional theory calculations — have provided atomistic insight into the biophysical behavior of oxysterols, complementing and extending experimental observations. Through atomistic simulations, it was possible to visualize phenomena such as the dual-orientation "bobbing" of 25-OH at a membrane surface [32], the tilt and packing differences induced by a 7-OH vs a 7-K on the sterol ring [47], and the subtle hydrogen-bond networks distinguishing stereoisomeric molecules [46]. DFT computations have provided quantitative insight into intermolecular forces — for instance, confirming that two 25-OH molecules can hydrogen-bond in an alternating orientation, or that the hydration energies of different oxysterol

hydroxyls are nearly equal [32]. Collectively, these computational approaches provide mechanistic explanations for experimental observations. For example, MD simulations indicate that 7β-OH induces greater membrane fluidization than cholesterol because its interfacial anchoring prevents proper vertical alignment within the bilayer [47]. Likewise, 25-OH traverses membranes rapidly owing to its bipolar character, which enables translocation without molecular inversion [17]. Furthermore, oxysterols such as 27-OH exhibit antiviral and osteotoxic properties, likely due to their propensity to form hydrogen-bonded assemblies that perturb membrane organization, as demonstrated by simulation studies [34].

While it is well established that the addition of a functional group or a change in stereochemistry can markedly alter the biological activity of a compound, MD and density functional theory analyses elucidate the molecular origins of these differences. By identifying the specific intermolecular interactions and energetic determinants that govern distinct membrane behaviors, computational approaches provide a mechanistic framework for understanding the divergent physiological and pharmacological effects of bioactive molecules. In pharmacological and biomedical research, such knowledge bridges molecular structure with therapeutic outcome, guiding the rational design of novel compounds that harness beneficial oxysterol functions or mitigate their pathological effects. The example of oxysterols illustrates how the integration of computational and experimental methodologies transforms empirical observations into predictive understanding, reaffirming the role of molecular modeling as an indispensable tool in modern drug discovery and mechanistic biochemistry.

Conflict of interest

None declared.

Author's contribution

Conceptualization: J.K., M.Ś., W.J; Literature search and analysis: J.K.; Writing — draft preparation: J.K.

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