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Research Article

Cholesterol, a Useful Framework for the Bottom-up Design of Molecular Motors

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Abstract

Cholesterol can be photochemically activated, by opening the B ring of the steroid skeleton at the C10 position, to produce an active species. This active species can further evolve through several photochemical pathways or thermally produce vitamin D. In particular the active species has a double bond at C6=C7, in a cis configuration, that can be photochemically isomerized to a trans form in a unidirectional way and a fast non-radiative deactivation pathway. As such, this precursor of vitamin D has the potential to work as a bio molecular motor. However, the competing reactions limit the potential of this application. Using the basic steroid skeleton of cholesterol, DFT/TDDFT calculations were used to bottom-up design a new steroid framework that can eliminate the drawbacks of pre-vitamin D. In particular, a demethylated version at C10 was tested in silico to confirm the absence of competing pathways and the main features of photochemical molecular motors: unidirectional rotation and non-radiative deactivation channels. This biocompatible molecular motor can be functionalized with pharmacologically active principles enhancing its ability to cross bilipid membranes. The 10-demethylated analog was found to lack thermal interconversion to vitamin D which prevents the retention of the C6=C7 double bond. DFT and TDDFT calculations were used to study the photo-physics of cis/trans isomerization which, in the S1 Potential Energy Surface, can be accomplished with a low activation barrier and a non-radiative deactivation pathway through a Conical Intersection. Stereochemistry drives a preferential rotation direction effectively making this molecule a molecular motor and not a switch. Small modifications to the chromophore area of this steroid enable the tunability of the activating wavelength to the purple area of the visible spectra.

Keywords: Cholesterol; DFT/TDDFT; Photochemical molecular motors; Steroids

Introduction

The use of molecular motors in biomedicine and the characteristics they should exhibit have been reviewed by Tour et al. [1], in particular those that are based on small organic molecules and fuelled by light. One possible applications of this technology are in drug delivery to improve passive lipoidal diffusion such as the crossing of biological membranes into cells [2]. Cholesterol has the ability to cross bilipid membranes and the kinetics of its diffusion have been studied to evaluate translocation rate constants which range from 3 to $7\mu s^{-1}$ [3]. However, these translocation rates are in the cellular fluid environment, are heavily dependent on Brownian motion, which could have a preferential direction of translocation if coupled to a nanomachine rotor [4].

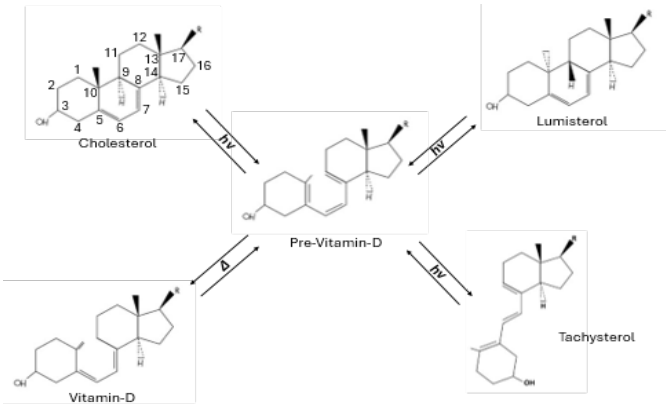
In this work we want to focus on the ability of cholesterol to be light activated to produce a precursor of vitamin D by opening ring B at C9, C10 positions [5,6]. This precursor, pre-vitamin-D, contains a C=C double bond in a cis configuration that has all the required characteristics to be a potential molecular motor driven by light energy [7]: i) unidirectional rotation around the C6=C7 double bond promoted by its stereochemistry arrangement; ii) a

non-radiative excited state deactivation pathway, through a Conical Intersection (CI) to convert the light energy into useful work [8]; iii) a locking mechanism by regeneration of cholesterol by light activation; a linkage at the 17 position that can be functionalized to attach other molecular fragments such as pharmacological active drugs [9]. Since these characteristics are common to other steroids, we will use the basic skeleton of cholesterol to bottom-up design a molecular motor with improved efficiency when compared to cholesterol. In particular pre-vitamin D, which results from the light activation of cholesterol, can have a competing equilibrium fuelled thermally that converts this molecule into vitamin D. This parasitic reaction involves the methyl group at C10 which transfers an H atom to C9 eliminating our required shaft double bond at C6=C7. As a result of this degradation pathway of our active molecular motor we tested an unmethylated equivalent system to prevent this reaction from occurring and we could modulate a working rotor cycle without competitive reactions converting light energy into useful work. One of the drawbacks of bio molecular motors activated by light energy is the need to avoid the harmful UVB and UVC radiation and preferentially, a useful range in the visible area of spectra [10]. The shaft in our motor is already conjugated with two other double bonds and the basic framework of the A, B, C and D rings allows for the extension of conjugation to two more bonds. We redesigned our model and succeeded at designing a working motor cycle with purple radiation in the boundary of the visible spectra. Although successful our approach to use demethylated analogs removes the chirality [11] of our molecule decreasing the driving force to unidirectional motion. However, we tested rotation in both directions of the dihedral angle defined by our shaft and due to steric crowding in the stereochemistry the activation energy is 7kJ/mol lower in the preferential negative direction of rotation.

Results

Photo activation of cholesterol

The cholesterol molecule can be activated by irradiation to induce ring opening at the C9-C10 bond producing pre-vitamin D, in cis form, with a double bond at C6-C7, Scheme 1. This molecule can undergo several processes, either thermal or photochemical. In particular, ring closure can occur regenerating either cholesterol or its enantiomeric form lumisterol. Thermalization leads to vitamin D. A competitive process when irradiating the cis pre-vitamin D is the isomerization to the form trans. The x-ray structure of lumisterol is readily available from CCSD (Cambridge Structural Database) [12] and was used to model our complex by replacing the R group with a methyl. The calculated absorption band by TDDFT for both cholesterol and our model compound are 277nm which compares well with the experimental value of 282nm [13,14]. After excitation, relaxation in the S1 Potential Energy Surface (PES) leads to ring opening followed by deactivation to S0 at a conical intersection (CI) corresponding to a C9-C10 bond distance of 206pm, Figure 1.



Scheme 1: Photochemical pathways of cholesterol.

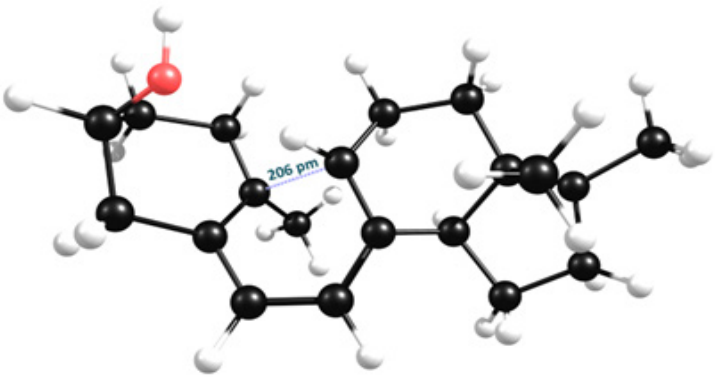


Figure 1: Structure of the relaxed form of Cholesterol in the S1 PES at the CI leading to S0 pre-vitamin D.

The C10 methyl group is facing the C9 carbon and thermally transfers a proton producing vitamin D, Figure 2. This process reorganizes the double bond system preventing the possibility of obtaining the transform tachysterol.

Figure 2: Thermal proton transfer from pre to vitamin D.

Redesigning pre-vitamin D to perform as a molecular motor

Since our aim is to design a molecular motor through rotation around the pre-vitamin D double bond, we need to avoid production of vitamin D. To achieve this goal, we replaced the methyl group at C10 by a hydrogen atom enabling cis / trans isomerization without competitive reactions, Figure 3.

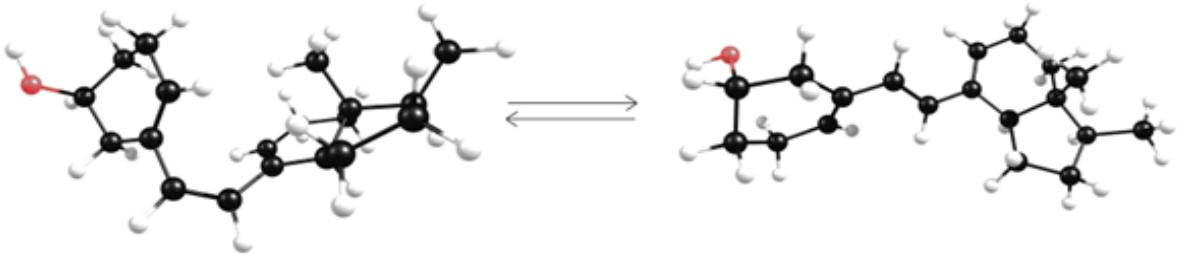
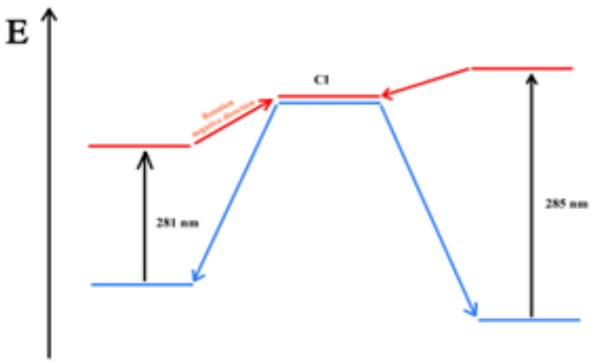


Figure 3: *Cis / trans* optimized structures for the model compound with H-atom at C10.

Although calculations show that the trans form is thermodynamically more stable by 14.9kJ/mol than the cis form, thermalization cannot occur due to an activation energy of 129/136kJ/mol (respectively for the negative/positive variation of the dihedral angle). However, in the S1 state rotation around the double bond leads to a CI only 13kJ/mol above the S1 cis structure.



Scheme 2: Working cycle for the rotating propeller.

Since we have asymmetric energetics for the rotation around the C=C double bond we can expect a preferential rotation in the negative direction creating a molecular propeller instead of a switch [15], Scheme 2.

The S1 state is characterized by a HOMO→LUMO π-π* transition located in the C10 to C8 conjugated system, Figure 4. In order to red shift the operating wavelength range of our propeller we tested the modification of our skeleton to extend the conjugated system to C1-C2. Further modification was to extend this later modification either to C11-C12 or to oxidize the alcohol C-OH group to a carbonyl C=O. The results for the trans isomer showed a red shift from 285nm to 336nm (C1=C2), 355nm (C1=C2 and C=O) or 382nm (C1=C2 and C11=C12). The carbonyl oxidized molecule has an extra n-π* transition at 403nm, in the visible purple/blue area, but with a much lower (almost forbidden) oscillator strength, Figure 5. The carbonyl compound is not eligible as a molecular motor since after excitation to S2 there is a quick relaxation to S1 not allowing free rotation around the C=C bond. The absence of a CI between S0 and the S1 (n-π*) states does not open an efficient non-radiative channel that prevents emission or the formation of the triplet T1 state.

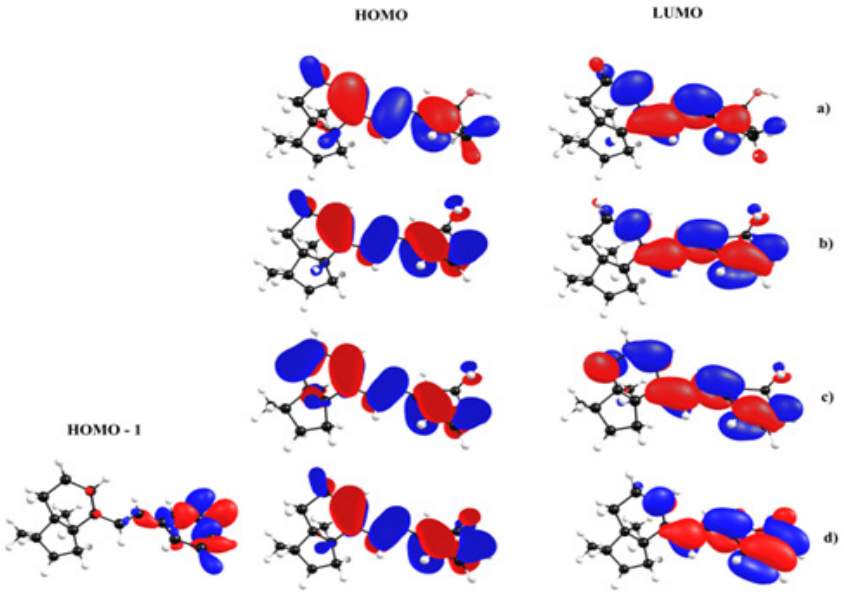


Figure 4: Frontier orbital for model compounds. a) base compound; b) C1=C2; c) C1=C2 and C11=C12; d) C1=C2 and C=O.

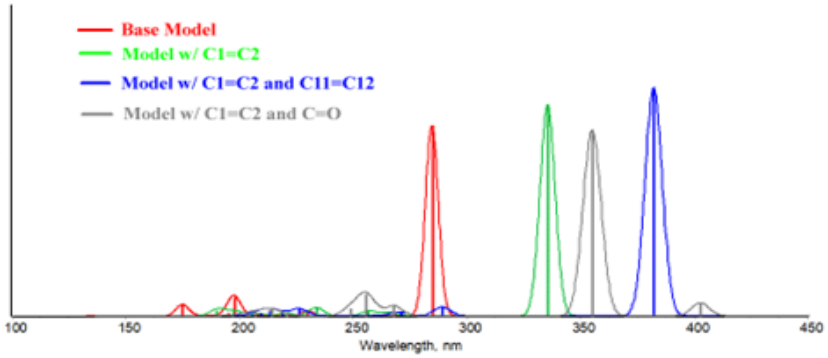


Figure 5: TDDFT simulated UV/VIS spectra for modified model compounds.

Discussion

Drug delivery faces the challenge of creating a pharmacologically effective concentration of the active agent inside the cells or organs. The active molecule has to cross blood organs barriers in particular bilipid membranes which, in many instances, require a high concentration of the active principle in the blood stream, also enhancing the side effects of the drug. The crossing of these barriers is usually driven by Brownian motion which is highly ineffective at promoting a forward movement of the drug inside the bilayer [4]. A molecular motor of few nanometers coupled with our drug molecule can provide the forward thrust needed to facilitate barrier crossing, increasing the availability of the active principle inside the cell and consequently decreasing the concentration in the blood stream with all the inherent advantages. Cholesterol is a bio molecule already present in the human body and can be photochemically activated to produce a species with all the characteristics of a photochemical molecular motor: The B ring opening divides the cholesterol into two subframes linked by a double bond (ene function). The A ring sub-frame can act as a rotor and the C, D rings as a stator. Rotor and stator are linked by the shaft at C6=C7. However, the stereochemical configuration of this species leaves the methyl group at C10 in the best spatial position to transfer a proton to C9 reorganizing the conjugated system of double bonds eliminating the ene function at C6-C7 (production of vitamin D). This process is not only favorable but can be driven thermally, even in absence of radiation (dark). Our aim in this work is to enhance the stability of the photochemical molecular motor species by redesigning the basic cholesterol skeleton retaining a modified steroid frame and testing the new species in silico as a promising candidate to be a photochemically, driven by light, molecular motor. This new species has to be able to harvest energy from light, use it to produce torque in a unidirectional way and convert it into useful work. Our designed active molecule will retain the double bond of pre-vitamin D with an intersection of

the S0 and S1 Potential Energy Surfaces to have a non-radiative CI channel to convert the light energy of the cis form in heat of the trans form. This CI should be located at, or near, the peak of the S0 isomerization reaction coordinate.

DFT/TDDFT calculations starting in a basic steroid skeleton model, based on cholesterol, were used to bottom-up design a molecular motor with its shaft located in the C6=C7. After opening ring B, rings C and D become the stator part of the motor while ring A can act as the rotor. The stator can be functionalized at 17 position of ring D but, in the model the linkage was kept as a single methyl group. This linkage point can be used to attach other molecular fragments such as pharmacological active principles. The known ability of steroids to cross bilipid membranes [3] associated to the driving force produced by the rotor will enhance the bio availability of the active drug in the inner cell plasmatic fluid. Calculations have shown that by replacing the methyl group at C10 by a hydrogen, blocks the competitive reactions of pre-vitamin D, retaining the C6=C7 double bond. The photo physics associated with the chromophore block of our molecule are compatible with a working cycle activated by UV irradiation: The excited cis molecule can reach, by rotation, a CI at which, non-radiatively, the rotation can proceed to the trans form. The transform also exhibits similar behavior leading back to the cis form. Since stereochemistry favors a preferential direction of rotation, continuous irradiation keeps the rotor part spinning around the C=C double bond.

The basic skeleton was further modified to extend the conjugated π-system to induce a red shift to the visible spectra. Without further functionalization of the chromophore, a wavelength of 382nm was achieved, in the purple area of the visible spectra.

Since there are many steroids with the cholesterol hydroxyl group replaced by a carbonyl, we tested a carbonyl derivative but it was proved not to be effective as a molecular motor, lacking either a rotating shaft or a non-radiative deactivation channel, limiting the family of potential compounds to the hydroxyl derivatives.

Materials and Methods

Theoretical calculations

All theoretical calculations were of the density functional theory (DFT) type and carried out using GAMESS-US version R3 [16]. A range corrected LC BPBE (ω=0.20 au-1) functional and 6-31G** basis set, as implemented in GAMESS-US [16], was used in both ground- and excited-state calculations. TDDFT calculations, with similar functionals, were used to probe the excited-state potential energy surface (PES). The results obtained with the LC-BPBE (20) functional are unscaled raw data from calculations. For the resulting optimized geometries time dependent DFT calculations (using the same functional and basis set as in the previously calculations) were performed to predict the vertical electronic excitation energies. Molecular orbital contours were plotted using ChemCraft 1.7 program. Optimized geometries were confirmed to be at least a local minimum by checking for absence of imaginary frequencies in the Hessian.

Conclusion

We aim to design from the bottom-up a biocompatible photochemically driven molecular motor, that can be used as a carrier for small-molecule pharmaceutical drugs, based on a steroid skeleton as similar as possible to cholesterol. By selective modification of the cholesterol frame we achieved a C10 demethylated molecule that can fulfil all the requirements for an enhanced drug carrier to cross bilipid membranes.

A. It can be functionalized at C17 providing the linkage between the active drug fragment and the stator of the motor.

B. Can be activated/locked by light irradiation induced B-ring opening/closure.

C. Can unidirectionally rotate around the C6=C7 double bond, driven photochemically, eliminating the random rotation switch possibility.

D. Has a non-radiative photochemical pathway (CI) to convert light energy into useful work.

E. Can be further modified to red shift the working wavelength to purple avoiding the harmful UV spectra.

Aside from providing a potential candidate for a photochemical bio molecular motor, we tested a methodology to further improve the design of new or existing bio molecules with potential to become drug carriers for in vivo crossing of blood/cell barriers.

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References

1. García-López V, Liu D, Tour JM (2019) Light-activated organic molecular motors and their applications. Chemical Reviews 120(1): 79-124.
2. Smith D, Artursson P, Avdeef A, Di L, Ecker GF, et al. (2014) Passive lipoidal diffusion and carrier-mediated cell uptake are both important mechanisms of membrane permeation in drug disposition. Molecular pharmaceutics 11(6): 1727-1738.
3. Filipe HA, Javanainen M, Salvador A, Galvão AM, Vattulainen I, et al. (2018) Quantitative assessment of methods used to obtain rate constants from molecular dynamics simulations-translocation of cholesterol across lipid bilayers. Journal of Chemical Theory and Computation 14(7): 3840-3848.
4. Gao C, Vargas Jentzsch A, Moulin E, Giuseppone N (2022) Light-driven molecular whirligig. Journal of the American Chemical Society 144(22): 9845-9852.
5. Havinga E (1973) Vitamin D, Example and Challenge. Experientia 29(10): 1181-1193.
6. Jäpelt RB, Jakobsen J (2013) Vitamin D in plants: A review of occurrence, analysis and biosynthesis. Frontiers in Plant Science 4: 136.
7. Ryota Iino, Kazushi Kinbara, Zev Bryant (2020) Introduction: Molecular motors. Chemical Reviews 120(1): 1-4.
8. Keller D, Bustamante C (2000) The mechanochemistry of molecular motors. Biophysical Journal 78(2): 541-556.

9. Albuquerque HM, Santos CM, Silva AM (2018) Cholesterol-based compounds: Recent advances in synthesis and applications. Molecules 24(1): 116.

10. Wang H, Bisoyi HK, Zhang X, Hassan F, Li Q (2022) Visible light-driven molecular switches and motors: Recent developments and applications. Chemistry 28(18): e202103906.

11. Sheng J, Pooler DR, Feringa BL (2023) Enlightening dynamic functions in molecular systems by intrinsically chiral light-driven molecular motors. Chemical Society Reviews 52(17): 5875-5891.

12. Groom CR, Bruno IJ, Lightfoot MP, Ward SC (2016) The Cambridge structural database. Acta Cryst B 72: 171-179.

13. Orlova TN, Terenetskaya IP (2009) Possible use of provitamin D3 photoisomerization for spectral dosimetry of bioactive antirachitic UV radiation. Journal of Applied Spectroscopy 76: 240-244.

14. Cisneros C, Thompson T, Baluyot N, Smith AC, Tapavicza E (2017) The role of tachysterol in vitamin D photosynthesis–a non-adiabatic molecular dynamics study. Physical Chemistry Chemical Physics 19(8): 5763-5777.

15. Feringa BL, Koumura N, Van Delden RA, Ter Wiel MKJ (2002) Light-driven molecular switches and motors. Applied Physics A 75: 301-308.

16. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, et al. (1993) General atomic and molecular electronic structure system. Journal of computational chemistry 14(11): 1347-1363.