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Inhibition of Cyclooxygenase-2 and Thymidylate Synthase by Dietary Sphingomyelins: Insights from DFT and Molecular Docking Studies

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Inhibition of cyclooxygenase-2 (COX-2) and thymidylate synthase (TS) enzymes plays a prominent chemopreventive and chemotherapeutic role in colorectal cancer studies. The basic computational investigation on the inhibition of these enzymes by sphingomyelin (SM) derivatives was carried out in silico using density functional theory (DFT) and molecular docking studies. Interactions between SM with unsaturated fatty acids, COX-2 and TS were compared with those of 5-fluorouracil (5-FU) and celecoxib, the standard anti-colorectal cancer drugs. The results showed that SM with alpha-linoleic acid derivative possesses the highest HOMO (-4.70 eV) and lowest LUMO (0.09 eV) energies, which may enhance their interactions with the target receptors. All SM molecules, irrespective of their fatty acid nature, have lower binding affinities (ΔG = -5.5 to - 6.8 kcal mol^{-1}) against COX-2 than celecoxib (-10.1 kcal mol^{-1}), indicating that the standard COX-2 inhibitor is much stronger than the natural SM. However, some of the natural SM are stronger inhibitors of thymidylate synthase than the standard drug, 5-FU, with SM having alpha-linoleic acid derivative (ΔG = - 6.2 kcal mol^{-1}) higher than 5-FU (ΔG = -5.28 kcal mol^{-1}), but lower than that of the active drug metabolite, 5-FdUMP (ΔG = - 7.4 kcal mol^{-1}). These ligand-protein interactions were all feasible and spontaneous.

Keywords: Cyclooxygenase-2, Thymidylate synthase, Sphingomyelin derivatives, DFT

INTRODUCTION

Cancer remains a big threat to human, as over a million people in the world are affected with this disease each year. The acquisition of effective scientific knowledge about complex biochemistry of cancer cells with development of innovative technologies to prevent, detect and treat the disease has increased considerably over the past few decades [1,2]. Colorectal cancer, among many other types of cancers, ranks the third most common causes of cancer-related death in the US [3]. Once metastases become clinically evident, prognosis is extremely poor and survival is often measured in months. Despite the high prevalence of colorectal cancer in the developed countries, its lowest incidence rate in West Africa has been reported [3,4]. This prevalence of colorectal cancer in advanced countries has been linked to genetic factors as well as environmental influences such as life-style patterns and diet.

Recently, more attention has been directed towards the use of natural dietary products for cancer prevention due to their various health benefits, noticeable lack of toxicity and side effects, and the limitations of chemotherapeutic agents [5]. Since diet has an important role in the etiology of colon cancer, dietary chemoprevention has received attentions for colon cancer prevention. Based on the findings on dietary sphingolipids, they can suppress the colon carcinogenesis [6,7]. We proposed that dietary sphingolipids, such as sphingomyelins, present in breast milk, which is exclusively being given to infants, and probably in other nuts and legume seeds such as melon and soybeans commonly consumed in Africa, may also be responsible for the low

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colorectal cancer incidence in the region.

Sphingolipids, a class of lipids with a backbone of sphingoid bases, are a set of aliphatic amino alcohols including sphingosine and sphinganine [8], which are identified by the presence of ceramide (a hydrophobic anchor) and a sphingoid base usually sphingosine-linked via an amino group to a fatty acid [9]. Different classes of sphingolipids, containing different head groups (sphingomyelins, glycosphingolipids, and gangliosides), showed similar effects [7]. Similarly, sphingolipid roles in the incidence of neurodegenerative diseases, such as Niemann-Pick and Gaucher diseases, have been previously studied [10,11], with sphingomyelin prominently inhibiting the activities of the proteins or enzymes.

Thymidylate synthase (TS) plays a critical role in the nucleotide metabolism of 2′-deoxyuridine-5′-monophosphate which could then be mistakenly incorporated into DNA, resulting in double and single-strand DNA break formation. Hence, it is an important target for 5-fluorouracil (5-FU), the standard chemotherapeutic drug for treatment of colorectal cancer [12]. 5-FU has remained the basis of therapeutic regimens used in the treatment of many human malignancies including colorectal cancer for many decades since it was introduced in 1958 [13-17]. Action of 5-FU is carefully mediated through the inhibition of thymidylate synthases (TS) [18]. Celecoxib is another active drug used for the treatment of colorectal cancer. Studies have demonstrated that colonic epithelial cells over expressing the cyclooxygenase-2 (COX-2) gene resist undergoing apoptosis and show altered adhesion and angiogenic properties [19]. These findings suggest that COX-2 may be involved in the progression of colorectal cancer. Furthermore, COX-2 is elevated in 40% of colon adenomas and 90% of colon carcinomas but not in normal colonic epithelium [20,21]. Using human colon carcinoma cell lines, investigators showed that COX-2 induces local immunosuppression by increasing prostaglandin E_2, a potent inhibitor of T lymphocyte proliferation, enabling colon cancer cells to escape host immune defense [22]. Nonsteroidal anti-inflammatory drugs (NSAIDs), a cyclooxygenase-2 (COX-2) selective inhibitor, such as celecoxib, have been reported as the drugs having potent the anticancer activities in laboratory models [23]. Inhibition of COX-2 by celecoxib resulted in loss of intra-tumor PGE_2 levels and reduced tumor growth in a dose-dependent manner. Celecoxib treated tumor showed a reduced proliferation and increased apoptosis of both tumor and stromal cells compared with vehicle controls. The major anti-tumor mechanism of celecoxib action is the inhibition of COX-2-derived prostaglandins, particularly PGE_2, suggesting that celecoxib acts as a novel therapeutic agent for the colorectal cancer.

Drug discovery processes are very complex and requires an interdisciplinary effort to design effective and commercially feasible drug. Computational chemistry method offers a unique ability for chemist to generate optimal geometry, structure and electronic properties of molecules and will help to make a decision as to which of the chemical transformation will occur in a reaction. Also, it serves as a fast and safe way for drug discovery whilst saving a lot financially as it reduces the number of laboratory experiments to be carried out [24,25].

Computational chemistry techniques such as density functional theory (DFT) and molecular docking have proved to be very useful tools in molecular recognition of biomolecules in the drug discovery process. These tools have been used in finding potential drugs/compounds for infectious diseases such Ebola and Zika [26-28]. It has also been used in drug discovery process for breast cancer [29]. Hence, it is a reliable tool to understand the application of SM with unsaturated fatty acid side chains in the management of colorectal cancer. The aim of this research is to carry out DFT and molecular docking studies on sphingomyelins with unsaturated fatty acid side chains as probable natural anti-colorectal cancer agents.

Computational Method and Molecular Docking Studies

A quantum chemical method via density functional theory (DFT) was employed to investigate the influence and interaction of sphingomyelins, naturally occurring compounds, with a promising chemopreventive properties against colorectal cancer. Conformation search was performed on modeled sphingomyelin molecules, and the lowest-energy conformer from each conformation search was taken for DFT calculations as implemented in Spartan 14 computational package on a core i5 computer with

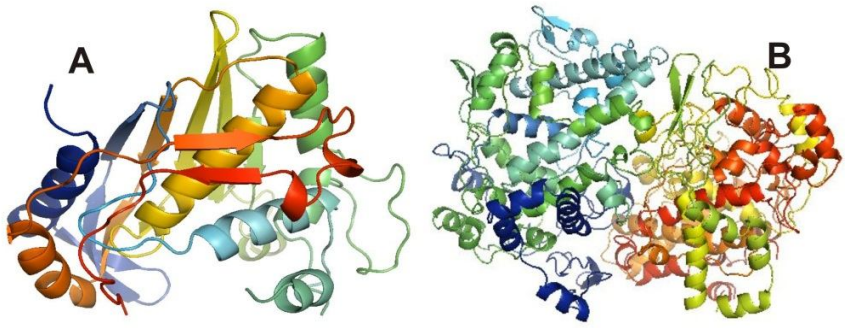

Fig. 1. The structure of (a) thymidylate synthase (PDB ID: 1HW4) and (b) cyclooxygenase-2 (PDB ID: 1CVU) as obtained from protein data bank.

2.60 GHz, 290 G hard disc and 4.00 GB ram specifications. The lowest-energy sphingomyelin conformers were optimized using DFT method with Becke's three-parameter hybrid functional, which employs the Lee, Yang, and Parr correlation functional, B3LYP [30], with 6-31G* basis set. Molecular descriptors, including highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), band gap (ΔE), dipole moment (DM), chemical hardness (η), chemical potential (μ), global nucleophilicity (ω), heteroatom (H), molecular weight (MW), lipophilicity (logP), area, volume, ovality, polar surface area (PSA), polarizability, hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) were obtained from the optimized sphingomyelins. The theoretical expressions for various descriptors and their relationship are given below:

$$\textit{Chemical Hardness}, \quad \eta = \frac{E_{LUMO} - E_{HOMO}}{2} \tag{1}$$

$$\textit{Band Gap}, \quad \Delta E = E_{LUMO} - E_{HOMO} \tag{2}$$

$$\textit{Chemical Potential}, \quad \mu = \frac{E_{HOMO} + E_{LUMO}}{2} \tag{3}$$

$$\frac{\textit{Gloobal Nucleophilicity}, \omega = \mu^2}{2\eta} \tag{4}$$

Molecular Docking Study

The target protein receptors, thymidylate synthase (TS, Fig. 1A) and cyclooxygenase-2 (COX-2, Figure 1B), used in this work were downloaded from the protein databank with PDB IDs: 1HW4 and 1CVU, respectively [31,32] and validated using Ramachandran plots using the MOLEMAN 2 program [33]. The outliers percentages were 2.6 and 3.3% for 1HW4 and 1CVU, respectively, which are within the accepted range (0-5%) for a protein of excellent quality. The protein was prepared by removing all water molecules and other complexes embedded in it before docking. The binding pocket of the initial inhibitor present in the original protein was used to determine the binding parameter as 46.643, -7.871 and 39.641 for 1HW4 and 28.364, 29.113 and 40.76 for 1CVU, regarding the X, Y, and Z axes, with the number of runs used for the molecular docking, 8.

Docking of sphingomyelin ligand molecules into the protein (target receptor) binding pockets was done using AutoDock and the binding energies (affinities) were obtained with AutoDock Vina software [34]. The binding energies and other parameters obtained from interactions between the ligands and target receptor enzymes of cyclooxygenase-2 (PDB ID: 1CVU), as shown in Fig. 2, and that of thymidylate synthase (PDB ID: 1HW4), as shown in Fig. 3, were compared with those of other popular colorectal cancer drugs such as 5-fluorouracil (5-FU) and celecoxib, the standard inhibitors of thymidylate synthase and cyclooxygenase-2, respectively.

RESULTS AND DISCUSSION

The molecular descriptors such as the energies of the highest occupied molecular orbital (E_{HOMO}), the lowest unoccupied molecular orbital (E_{LUMO}), also known as

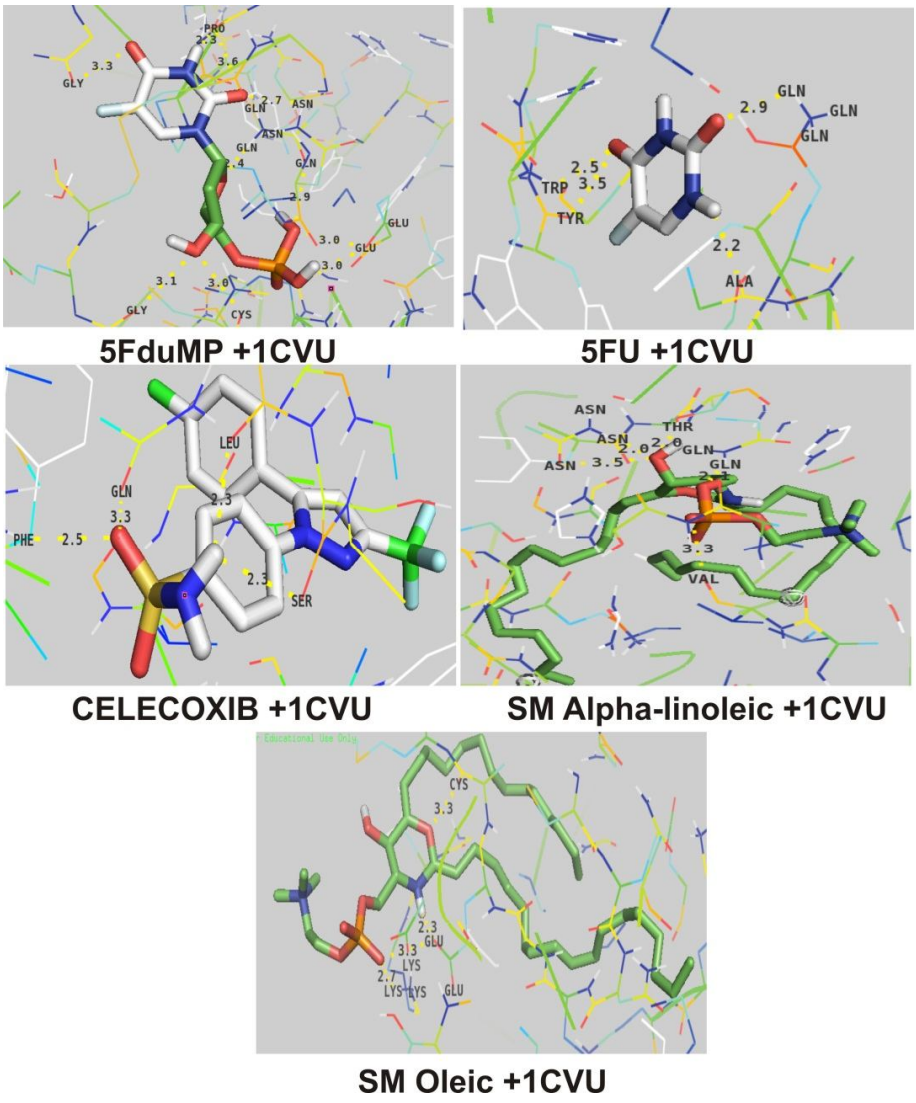

Fig. 2. Interactions between SM molecules, standard drugs and cyclooxygenase-2.

frontier molecular orbitals (FMO), and the global indices obtained from the optimized structures were used to investigate the chemical reactivity of sphingomyelin ligand towards the protein receptors. The principle of FMO is based on interaction and overlapping of two distinct reactants in which formation of two prominent molecular orbitals is the outcome of the interaction [35]; when a molecule possess high HOMO (nucleophile) energy, it indicates the tendency of such molecule to donate electrons to those molecules having high tendencies to accept electrons due to low LUMO (electrophile) energy. Hence, the increase in energy of HOMO and decrease in energy of LUMO are correlated to high inhibition efficiency of a ligand while a greater magnitude of LUMO with negative sign, representing the heat of formation, is related to the toxicity of the molecules [36-40]. Chemical descriptors of sphingomyelin with several unsaturated fatty acid derivatives as well as those of 5-FU and celecoxib are presented in Table 1. The HOMO energy values of SM with unsaturated fatty acid derivatives range from 0.27 eV to

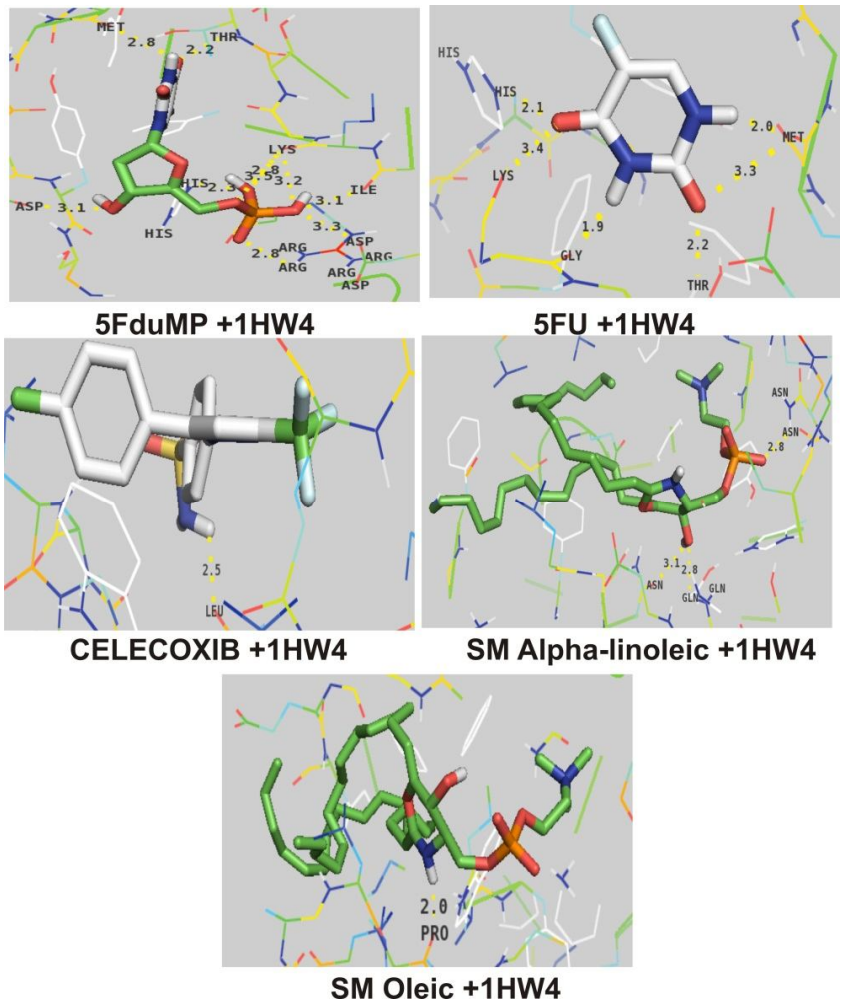

Fig. 3. Interactions between SM molecules, standard drugs and thymidylate synthase.

-4.70 Ev, indicating that SM series likely donate electrons to the receptor more readily than anti-colorectal cancer drugs (5-FU and Celecoxib) used as our standard in this study. The LUMO energy values range from -1.10 eV to 2.62 eV (Table 1), the LUMO energies of SM with alpha-linoleic acid side chain are higher than those of 5-FU and celecoxib, indicating that the anti-colorectal cancer drugs used as standards can readily accept electrons from the receptors.

The energy band gap ΔE ($\Delta E = E_{LUMO} - E_{HOMO}$) has been related to stability of the molecule; lower ΔE signifies the ease at which surface electron is removed from the molecule. Usually, when molecules possess a low ΔE, it is polarized, having high chemical reactivity, the kinetic stability would be low and chemical softness value would be high; thus kinetically labile. Those molecules having large band gaps are oftentimes unreactive and stable [41,42]. The energy band gaps of SM with unsaturated fatty acid side chains in this study range from 2.35-4.83 eV (Table 1). This suggests that SM series can readily interact with the target receptors during non-bonding chemical interactions; thus, higher inhibition efficiency than that of 5-FU and celecoxib. In addition, the band gaps obtained from SM with sapienic acid ($C16:1^B$) and alpha-linoleic acid side chains (SM C18:3) are 4.83 and 4.79 eV, respectively, as presented in Table 1; in a close range with those obtained for 5-FU and celecoxib (5.41 eV and 4.93 eV, respectively).

Table 1. Molecular Parameters Obtained from Sphingomyelin Containing Unsaturated Fatty Acid Chains and those Obtained from Colorectal Cancer Drugs Calculated *via* DFT at the B3LYP/6-31G* Level

Molecules	HOMO (eV)	LUMO (eV)	BG (eV)	DM (debye)	H (eV)	μ (eV)	Ω (eV)	MW (a.m.u)	Area (A^2)
SM C14:1	-4.14	0.11	4.25	18.36	2.125	-2.015	0.955	670.967	838.15
SM C16:1^A	0.27	2.62	2.35	30.45	1.175	-1.445	0.889	730.089	921.79
SM C16:1^B	-4.44	0.39	4.83	13.18	2.415	-2.025	0.849	699.011	874.15
SM C18:1^A	-4.15	0.33	4.48	15.52	2.240	-1.910	0.814	727.065	917.66
SM C18:1^B	-4.33	0.30	4.63	13.82	2.315	-2.015	0.877	727.065	917.84
SM C18:2	-4.37	0.30	4.67	13.45	2.335	-2.035	0.887	725.049	912.96
SM C18:3	-4.70	0.09	4.79	13.95	2.395	-2.305	1.109	723.033	911.97
SM C20:4	-3.70	-1.10	2.60	22.12	1.300	-2.400	2.215	749.071	944.05
SM C20:5	-4.42	0.18	4.60	13.39	2.300	-2.120	0.977	747.055	937.29
SM C22:1	-4.12	0.09	4.21	18.61	2.105	-2.015	0.964	783.173	1001.5
5-Fluorouracil	-6.79	-1.38	5.41	3.90	2.705	-4.085	3.085	130.078	128.71
Celecoxib	-6.52	-1.59	4.93	3.68	2.465	-4.055	3.335	381.378	363.91

The dipole moments obtained for SM with unsaturated fatty acid side chains range from 13.18-30.45 debyes (Table 1) are extremely high compared to those of 5-FU and celecoxib with dipole moments of 3.90 and 3.68. It has been argued that there is a specific correlation between dipole moment and the interactions between ligand drugs and receptor enzymes [43-45]. The hardness and softness of a molecule is also related to its polarizability which is a function of dipole moment; dipole moment describes the extent of separation of charges on the molecule. A hard molecule possesses a large band gap and a soft molecule has a relatively smaller band gap; a molecule with smaller value of chemical hardness would have the ability to act as an electron donor hence could act as an inhibitor for a biological species which could be responsible for neurodegenerative diseases [46,47]. The chemical hardness values of 5-FU and celecoxib were 2.705 and 2.465, respectively. The chemical hardness values obtained for SM with unsaturated fatty acid side chains are within the range of those obtained with the anti-colorectal cancer drugs used as standards in this study.

Molecular volume is obtained by a non-quantum mechanical method which is an integral of the areas inside a Van der Waals surfaces. It may also be determined by choosing an electron density isosurface and looking for the internal volume of that isosurface, thereby providing information on the percentage of electron density contained by a particular isosurface. Molecular volume is a pointer to predict whether a molecule would fit into the active site of an enzyme. It could also be used to predict the density as well as the cavity site for solvation [48]. The molecular volume of SM with unsaturated fatty acid side chains (Table 2) range from 078.39-914.25 A^3. SM with Erucic acid side chain has the highest molecular volume of 914.25 A^3; this was followed by SM with arachidonic acid side chain and Eicosapentaenoic acid side chain having molecular volume of 866.77 and 860.53 A^3, respectively.

However, it was observed that SM with palmitoleic acid

Table 2. Molecular Parameters Obtained from Sphingomyelin Containing Unsaturated Fatty Acid Chains and those Obtained from Colorectal Cancer Drugs Calculated *via* DFT at the B3LYP/6-31G* Level

Molecules	Volume (A^3)	Ovality	PSA (A^2)	Polarizability	HBD	HBA
SM C14:1	766.61	2.07	74.458	102.56	2	8
SM C16:1^A	078.39	2.12	78.388	109.90	2	8
SM C16:1^B	803.02	2.09	73.222	105.38	2	8
SM C18:1^A	839.89	2.13	74.250	108.45	2	8
SM C18:1^B	840.04	2.13	73.233	108.43	2	8
SM C18:2	835.95	2.13	73.315	108.08	2	8
SM C18:3	832.52	2.13	77.026	107.78	2	8
SM C20:4	866.77	2.15	73.647	110.84	2	8
SM C20:5	860.53	2.14	73.288	110.10	2	8
SM C22:1	914.25	2.20	74.541	114.55	2	8
5-Fluorouracil	105.85	1.19	51.774	48.68	2	4
Celecoxib	336.82	1.55	72.529	67.53	1	6

side chain has the least molecular volume, 78.39 A^3.

Polar surface area (PSA) is commonly used in medicinal chemistry metric for theoptimization of a drugs ability to permeate cells. Molecules with a polar surface area greater than 140 $Å^2$ tend to be poor at permeating cell membranes [49]. For a molecule to penetrate the blood-brain barrier, a PSA less than 90 $Å^2$ is usually needed. The PSA values of all the derivatives of sphingomyelin with unsaturated fatty acid chains under study range from 61-78 $Å^2$ (Table 2). These values showed that they could pass through the highly selective permeable membrane which serves to prevent the circulating blood from reaching the brain. Chemical potential reveals the ability of the molecule to cause either a chemical or electrochemical reaction [50]. Generally, particles (electrons) would flow from molecules with higher chemical potential to those molecules with lower chemical potentials. The chemical potential of the molecules under study range from -1.445 to -2.400, however, the chemical potential of 5-FU and celecoxib, the standard anti-colorectal cancer drugs, were -4.085 and -4.055, respectively. The negative values of chemical potentials of the molecules under study indicate the willingness of such molecules to accept electrons.

Hydrogen bond is an important chemical tool in highlighting the physicochemical activities of a particular molecule. The chemical bonding resulting from H-bond may be intermolecular or intra-molecular depending on the environment the interaction is taking place and its strength depends on the electronegativity of other atoms involved. The classification of strong and weak hydrogen bond is based on the proton acceptor and proton donor group [51,52]. Hydrogen bond donor (HBD) indicates the ability to donate the hydrogen bond to the neighboring atoms, either intra-molecularly or inter-molecularly, and would

depend on the electronegativity of that element present in the molecular interaction; presence of highly electronegative elements, such as F, O, N would influence the H-bond donor capacity. Similarly, hydrogen bond acceptor (HBA) signifies the hydrogen bond acceptor; presence of electron lone pair is a requisite for such adjacent atom(s) to accept hydrogen bond. It is observed that the SM series has a higher number of HBA than HBD, this might be a signal that the derivatives under study possess atom(s) carrying lone pair of electrons within their molecules. The HBD number is equal to 2 and HBA is equal to 1 in all ligands under study as presented in Table 2.

Docking of molecules, a drug design approach by taking advantage of computer is a suitable tool to predict the interaction between the receptor enzyme and the donor ligand. An effective docking can be utilized to screen large derivatives of compounds, and rank the result in order to propose the inhibition trend of the target enzyme by the ligand. The aim is to identify ligands that would interestingly bind strongly to a certain receptor and to ensure that a particular ligand does not bind mistakenly to another site so as to guide against interference with other functioning molecules in the body. Drug metabolism study, transport and excretion are also of major consideration [53]. Tables 3 and 4 represent the parameters obtained from docking the existing anti-colorectal cancer drugs (5-FU and celecoxib) with cyclooxygenase-2 isozyme and thymidylate synthase enzymes; this serves as a basis for comparing the effectiveness of sphingomyelin with unsaturated fatty acid derivatives under study with the target enzymes. The binding affinity (ΔG), hydrogen bond distance, and number of binding sites per molecule were considered. Binding affinity is a parameter with a great importance in the field of pharmaceutical chemistry; ease and quick estimation of affinity would be of a great benefit to drug design and discovery processes. Similarly, bond distance becomes more important when there is a strong affinity between two molecules and it is the integral of all Van der Waals radii within their molecules [53].

5-FU has notable impacts on colorectal cancer as a single agent or in combination with other drugs. It interferes with DNA synthesis by blocking thymidylate synthase. The binding energies obtained from docking 5-FU with thymidylate synthase and cyclooxygenase-2 was -5.2 and -5.7 kcal mol^{-1}. Comparison between these energies and those obtained from binding SM with unsaturated fatty acid side chains with cyclooxygenase-2 isozyme and thymidylate synthase showed a close range between their binding affinities. However, SM with alpha-linoleic side chain interestingly showed a higher binding capacity (-6.8 kcal mol^{-1}) than that of 5-FU and other SM derivatives. Furthermore, it has the strong hydrogen bond interactions with more amino acid residues than those of 5-FU when docked with cyclooxygenase-2 as shown in Table 3.

Similarly, binding affinities obtained from interaction of SM with Sapienic acid, Myristolic acid, Eicosapentaenoic acid, and Linoleic acid side chains were -6.1, -5.9, -6.0 and -5.9 kcal mol^{-1}, respectively. These were higher than those of 5-FU when interacted with cyclooxygenase and formed non-bonding interactions with more amino acid residues. The binding affinities of SM with unsaturated fatty acid derivatives compared with those obtained from 5-FU on interaction with thymidylate synthase showed that alpha-linoleic has higher binding affinity (-6.2 kcal mol^{-1}) than the established cancer drug (5-FU). Also, interactions of SM with side chains of linoleic acid, palmitoleic acid and sapienic acid showed higher affinities (-5.9 kcal mol^{-1} and -5.7 kcal mol^{-1}, respectively) and more hydrogen bond interactions with amino acid residues compared to those of 5-FU. It is important to note that other drugs has higher binding affinities than 5-FU. It has been reported that 5-FU by itself is inactive and it must be intra-cellularly converted into various nucleotide metabolites, one of the prominent metabolites is 5-fluoro-2'-deoxyuridine-5'-monophosphate (5dUMP) which is more active against cancer cell [54]. Molecular docking studies of 5dUMP with cyclooxygenase-2 and thymidylate synthase receptors showed higher binding affinities of -8.4 and -7.4 kcal mol^{-1} and interacted with more amino acid residues than 5-FU, as shown in Tables 3 and 4, respectively.

Celecoxib is a non-steroidal anti-inflammatory drug (NSAIDs) which selectively inhibits cyclooxygenase-2 (COX-2), this ligand has been reported to possess anticancer activity [23]. The binding affinity obtained from the interaction of celecoxib and cyclooxygenase-2 was -10.1 kcal mol^{-1}. This value was higher than the binding affinities obtained from interaction of other ligands (natural and artificial origins) with cyclooxygenase-2 as considered

Table 3. Affinity and Binding Site between Sphingomyelin Derivatives with Cyclooxygenase-2 (COX-2)

Sphingomyelin derivatives	Cyclooxygenase-2 (COX-2)		
	Binding affinity (kcal mol^{-1})	Hydrogen bonding	Distance (Å)
		O^P = HIS'386	2.8
		O^P = TYR'385	3.3
SM-C14:1	-5.9	O^I = TYR'385	3.2
		O^H = GLN'203	3.5
		O^I = TYR'385	3.6
		O^H = TYR'122	3.2
SM-C16:1^A	-5.5	O^H = ARG'44	2.2
		H^O = ARG'44	2.2
		O^I = ASP'125	3.6
		O^H = TYR'122	3.2
SM-C16:1^B	-6.1	O^H = ARG'44	2.2
		H^O = ARG'44	2.2
		O^P = LYS'137	2.7
SM-C18:1^A	-6.1	O^P = GLU'46	3.3
		O^P = SER'121	2.5
SM-C18:1^B	-5.8	O^I = THR'118	3.2
		O^H = THR'118	3.0
		O^P = LEU'472	3.3
		O^I = ASN'43	3.2
SM-C18:2	-5.9	O^H = ASN'43	2.9
		O^P = VAL'447	3.3
		O^I = GLN'452	2.1
SM-C18:3	-6.8	O^H = ASN'382	3.5
		O^H = ASN'382	2.0
		O^H = THR'212	2.0
			3.4
		O^P = ASN'43	2.5
SM-C20:4	-5.7	O^I = ARG'44	
		O^P = LEU'472	3.2
SM-C20:5	-6.0	O^P = LYS'468	3.4
		O^H = ASN'43	2.8
		O^P = ASP'125	3.2
SM-C22:1	-6.2	O^P = ARG'44	2.2
		ALA'202	2.6
5-FU	-5.7	THR'206	2.1

Table 5. Continued

		ASN' 39	2.7
		CYS' 41	3.0
		GLY' 95	3.1
		GLY' 135	3.3
5-FDUMP	-8.4	PRO' 154	2.3; 3.6
		GLN' 461	2.4; 2.9
		GLU' 465	3.0
		GLN' 192	3.3
Celecoxib	-10.1	LEU' 352	2.3
		SER' 353	2.3
		PHE' 518	2.5

Table 5. Affinity and Binding Site between Sphingomyelin Derivatives with Thymidylate Synthase (TS)

Sphingomyelin derivatives	Thymidylate synthase (TS)		
	Binding affinity (kcal mol^{-1})	Hydrogen bonding	Distance (Å)
SM-C14:1	-5.8	O^M = ALA'144	3.4
		O^P = HIS'196	2.4
		O^I = ASP'218	2.4
SM-C16:1^A	-5.8	O^H = ASN'226	2.3
		O^P = SER'216	2.6
		O^P = SER'216	3.5
SM-C16:1^B	-5.7	O^I = HIS'256	2.3
		O^H = ASN'226	2.7
		H^N = PRO'193	2.0
SM-C18:1^A	-5.6		
		OP = PRO'193	3.2
SM-C18:1B	-5.4	OH = PRO'193	2.9
		OH = TRP'182	2.4
		OP = SER'216	3.0
		OP = HIS'196	2.3
SM-C18:2	-5.9	OI = ASP'218	2.3
		OI = ASN'226	3.3

Table 6. Continued

		O^P = ASN'183	2.8
		O^H = GLN'214	2.8
SM-C18:3	-6.2	O^H = ASN'226	3.1
		O^I = ASP'218	3.5
		O^H = TYR'135	2.6
SM-C20:4	-5.2	O^K = ASN'226	2.3
		O^K = ASN'226	3.2
SM-C20:5	-6.1	Nil	Nil
		O^P = SER'216	2.7
SM-C22:1	-5.2	O^P = ASP'218	2.3
		O^I = HIS'196	2.8
		O^K = HIS'141	2.2
5-FU	-5.2	O^K = LYS'93	3.5
		H^N = MET'149	2.3
		ILE' 92	3.1
		LYS' 93	2.8; 3.2; 3.5
		THR' 96	2.2
		ARG' 140	2.8
5-FDUMP	-7.4	HIS' 141	2.3
		MET' 149	2.8
		ASP' 152	3.1
		ASP' 289	3.3
Celecoxib			
	-7.9	LEU' 189	2.5

SM = Sphingomyelin, C14:1 = Myristolic, C16:1^A = Palmitoleic, C16:1^B = Sapienic, C18:1^A = Oleic, C18:1^B = Vaccenic, C18:2 = Linoleic, C18:3 = Alpha-Linoleic, C20:4 = Arachidonic, C20:5 = Eicosapentaenoic, C22:1 = Erucic, O^P = Oxygen on phosphocholine, O^I = Oxygen attached to phosphocholine, O^H = Oxygen attached to hydrogen, H^N = Hydrogen attached to nitrogen, O^C = Oxygen attached to carbon, O^K = Oxygen forming ketone group, O^M = Oxygen forming methoxyl group, N = Nitrogen, H^O = Hydrogen attacked to oxygen.

under this study. Likewise, the binding affinity (-7.9 kcal mol^{-1}) of celecoxib with thymidylate synthase was also higher than that of SM series. The negative sign indicates the spontaneity of the interaction between the ligand and the enzyme. It is similar to the thermodynamic parameter $\Delta G < 0$, which is a negative value. It implies that the interaction between the reacting species would occur as predicted.

CONCLUSIONS

Density functional theory and molecular docking studies have shown to be the preliminary sets of investigations and screening tools in drug design and discovery in pharmaceutical and related fields. Sphingomyelins containing unsaturated fatty acid derivatives, the important molecules derived from our diets, showed potential binding capacity with thymidylate synthase (PDB ID: 1HW4) as well as cyclooxygenase-2 (PDB ID: ICVU). They could be further investigated for their potential to defeat cancer (colorectal cancer) diseases, probably as alternatives to known cancer drugs; due to their lower risk or side effects (less toxic and derived from consumable agricultural sources), or to reinforce other established cancer drugs for effective cure of cancer diseases.

The results show that SM with alpha-linoleic acid side chain surpasses other derivatives in terms of binding affinity and interaction with amino acids in cyclooxygenase-2 enzyme. It was a similar case when it interacted with thymidylate synthase enzyme. The interaction (binding) between the ligand (sphingomyelin with unsaturated fatty acid side chains) and the receptor (cyclooxygenase-2; ICVU and thymidylate synthase; 1HW4) was spontaneous and feasible.

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