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Research Article

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In-silico Study of Novel Dimeric flavonoid (OC251FR2) from the Seeds of *Garcinia kola* Heckel (Clusiaceae) Against Alpha Estrogen Receptor (ER- α) of Breast cancer

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Abstract:

In silico approach was used to investigate the inhibitory activities of a novel dimeric flavanone OC251FR2 (3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)-3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) isolated from the chloroform fraction of *Garcinia kola*, against alpha Estrogen receptor (ER- α); a major contributor to the growth of breast cancer. The docking was conducted using Maestro module 13.5 to obtain the ER- α PDB (5W9C) from NCBI. The OC251FR2 was docked using ligprep module with 4-hydroxytamoxifen being the reference drug. The qikpro was used to investigate the drug-likeness while ligand docking and induced fit docking were used to investigate the interaction and binding affinity of the ligands with the active sites of the PDB. The result shows that the isolated OC251FR2 interact more with amino acids in the active sites via H-bond, pi-pi interaction than the reference drug 4-Hydroxytamoxifen. The drug-likeness determined by qikpro shows that OC251FR2 violated three of the Lipinski rules of 5, and also have percent oral absorption. The quantum mechanics values show that OC251FR2 have similar properties comparable to the reference drug 4-hydroxytamoxifen. Hence, can serve as potential lead against alpha Estrogen receptor (ER- α).

Keywords: Alpha Estrogen receptor, OC251FR2, Docking, *Garcinia kola* seed, 4-hydroxytamoxifen, Breast cancer, flavonoid.

Graphical Abstract



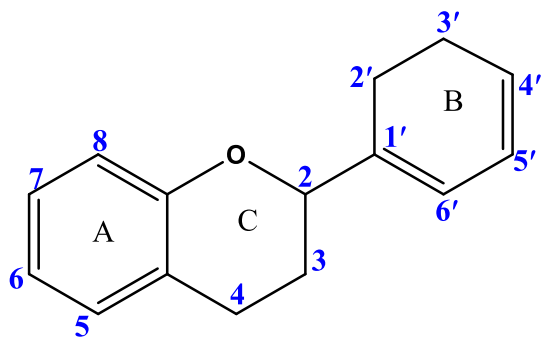
Introduction

Breast cancer (BC) is the most prevalent cancer and the second cause of death in women globally; with approximately 2.2 million new cases diagnosed in 2020 as reported by the World Cancer Research Fund International (<https://www.wcrf.org>). The BC incidence is higher in industrialized countries, but the mortality rate is more in less developed countries due to lack of standard healthcare facilities, limited access to oncology professionals, and means to afford the costly treatment required for breast cancer (DeSantis *et al.*, 2019; Tao *et al.*, 2015). In developed countries, access to medical care, screening equipment and advanced treatment, makes early detection of the disease possible and also increases the chances of recovery. The BC early detection remains a main issue in the fight against this cancer. Despite the fact that younger women exhibit more aggressive features and a worse prognosis, BC can be considered a prevalently post-menopausal disease (Giovannelli *et al.*, 2018). About 50–80% of female breast cancers have been established to be estrogen-dependent where estrogen binding to receptor has been reported to stimulate tumor cell proliferation (Cruceriu *et al.*, 2020; Leong and Zhuang, 2011; Smolarz *et al.*, 2022; Vogel, 2008). Thus, estrogen hormone dependence accounts for a major cause in the incidence of women breast cancer (Dania *et al.*, 2024). Two subtypes estrogen receptors, ER- α and ER- β , exist and exhibit distinct cellular and tissue distribution patterns in many cells and tissues in human thereby control key physiological functions (Farzaneh and Zarghi, 2016). ER- α is the major ER subtype in the mammary epithelium and plays a critical role in mammary gland biology as well as in breast cancer progression (Biswas *et al.*, 2022; Martinkovich *et al.*, 2014). Hence, the activation of ER- α by estrogens is considered to be accountable for enhanced

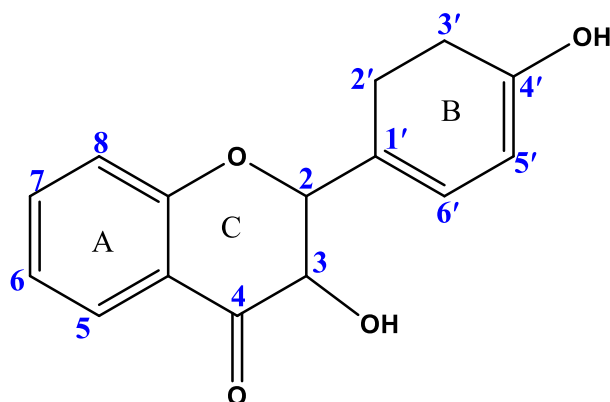
proliferation, making estrogen receptor ER- α , a target in endocrine treatment (Ervina *et al.*, 2021). ER- α status has been used to recognise breast cancer patients who are likely to respond to tamoxifen, the endocrine agent most commonly used to block the effects of estrogen at all stages of breast cancer, particularly in post-menopausal patients (Arnesen *et al.*, 2021). The anti-proliferative activity of tamoxifen, depend primarily on its ability to compete with estrogen for the ER- α , ligand binding site in breast tumor tissue (Chang, 2012).

Flavonoids, either natural or synthetic, are known to exhibit various biological activities such as antiviral, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities (Al-Khayri *et al.*, 2022; Kiyama, 2023; Kumar and Goel, 2019; Yahfoufi *et al.*, 2018). The chemical structure of flavonoids (Fig.1) is made up of 15 carbon atoms (C6-C3-C6) making two benzene rings (rings A and B) attached by a heterocyclic pyran ring (ring C) (Maleki *et al.*, 2019; Marín *et al.*, 2021); hence, they are grouped into different subclasses which comprise of flavonols, flavanonols, flavones, flavanones, chalcones among others (Braune *et al.*, 2016) depending on the connection of rings B and C, the conformation and oxidative status of ring C, as well as the total hydroxylation pattern (Magadan-Corpas *et al.*, 2024). Flavonoids, which are structurally similar to estrogens, are able to bind to the estrogen receptor and possess estrogenic or antiestrogenic activities (Chen *et al.*, 2022; Kiyama, 2023). It has been shown that antiestrogens repress the proliferation of breast tumor cells by competing with estrogens for binding to its nuclear receptors (Hanker *et al.*, 2020; Hurtado *et al.*, 2011). Studies showed that kolaviron, a biflavonoid from *Garcinia kola* seed, prohibited oxidative stress and lipid peroxidation in the commencement stage of colon cancer (Eboh *et al.*, 2015), while it showed strong inhibitory activity on aromatase of breast cancer (Elekofehinti *et al.*, 2016). Likewise, study has proven that the essential oil from *Garcinia kola* seed has in vitro antioxidant and anticancer properties (Folayan *et al.*, 2023; Olatunde *et al.*, 2021). In a separate study it was found that the seeds' oil components reduced the toxicity of the hepatorenal system in mice (Folayan *et al.*, 2022).

On this basis, in this study, the anti-proliferative potential against breast cancer of a newly isolated dimeric flavanonol OC251FR2 (Fig. 5) from *Garcinia kola* seed was predicted using in-silico approach targeting inhibition of ER- α .



A) Basic structure of Flavonoid



B) Flavanonol structure: a subclass of flavanoid

Figure 1: Structures of flavonoid and flavanone subclass

MATERIALS AND METHODS

General Procedures

Purification and isolation was achieved using Preparative HPLC Agilent 1290 Infinity II coupled with G7165A 1260 multiwavelength detector. The HPLC system was controlled by Agilent OpenLab CDS software. Concentration of isolates was carried out using Genevac EZ-2 plus Personal Evaporator. Infra-red spectra determination was conducted using Perkin Elmer Spectrum-3 FTIR spectrometer. NMR spectra (1D- and 2D) were run on a Bruker 500 MHz and data obtained processed and analysed using Mnova 14.1.2 software. High resolution mass spectra determination was recorded on Agilent Quadrupole Time of Flight LCMS 6545 Q-TOF with Agilent 1260 Infinity II LC system. The LC/MS was operated in the positive-ion mode as MS/MS with m/z scan range 50-1000 and the data analysed with ACD Spectrus Processor

Chemicals and reagents: All solvents used were HPLC grade and all chemicals were obtained in the highest analytical quality available. HPLC grade Methanol (MeOH), and formic acid, were purchased from Merck (Darmstadt, Germany). Ultrapure Type 1 water was obtained from a Milli-Q purification system from Millipore (Bedford, MA, USA).

Plant Material: Fresh seeds of *Garcinia kola* were purchased from a local Bode market in Ibadan, Oyo State, Nigeria and authenticated by Mr D.P.O. Esimekhuai at the Department of Botany, University of Ibadan. A voucher specimen (UIH-23302) is available at the herbarium of the University of Ibadan, Ibadan, Nigeria.

Extraction and Isolation

G. kola (2 kg) was pulverised and extracted with 4 litres of absolute ethanol by soaking for three days (72 hours) using cold extraction process (Yeye *et al.*, 2023). The extraction was carried out three times to ensure complete removal of the constituents. This was filtered and the filtrate was concentrated using rotary evaporator at reduced pressure to give the crude extract (141.70 g). To the crude extract was added 4ml of water and 200 mL of absolute ethanol to give aqueous ethanolic crude extract. The aqueous ethanolic extract was successively partition with hexane, chloroform, Ethyl acetate. The hexane fraction (7.4 g), chloroform fraction (5.4 g), Ethyl acetate fraction (1.2 g) and Ethanol fraction (1.0 g) were obtained. The chloroform fraction was subjected to Column Chromatography CC on silica gel (Silica Gel 60 Angstrom Pore Size, 40-63 Micron, for Column Chromatography, Fisher Chemical™ 60 g) eluting with dichloromethane containing increasing concentration of methanol (0—20%, v/v) which gave three fractions OC240 (15.3mg), OC251(54.6 mg) and OC291(39.5mg) fractions at DCM/MeOH (9.6 : 0.4) solvent ratio.

Further separation of OC251 was achieved on Preparative HPLC Agilent 1290 Infinity II equipped with a binary pump fitted with Agilent 10 prep C₁₈ column (100 x 50 mm I.D.), a degasser and a 1260 multiwavelength detector (model G7165A). The HPLC system was controlled by Agilent OpenLab CDS software. Elution was achieved isocratically with the mobile phase, degassed ultra-pure Milli-Q Water 65%/ Methanol35% (65:35) at a flow-rate of 55.5 ml/min at room temperature and injection volume of 100µL for a run time of 50mins. Detection was effected by UV G7165A lamp from 210nm to 290nm. This resulted in separation of three isolates (OC 251FR1, OC251FR2 and OC251FR34) with automatic fraction collector. The most prominent of the peak was OC251FR2 while the remaining fractions have a smaller yield. The isolates were concentrated

under reduced pressure using rotary evaporator to reduce the solvent. Further concentration of the isolates was done using Genevac EZ-2 Personal Solvent Evaporator.

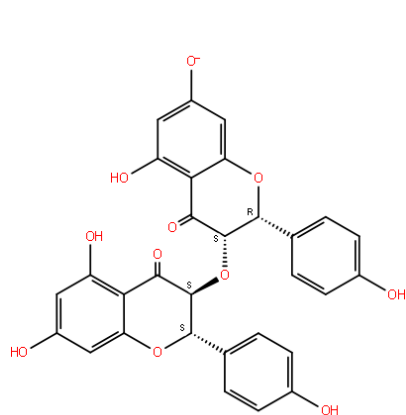
The isolate OC251FR2 was characterised and its structure was determined on the basis of FTIR, 1-D and 2-D NMR spectroscopic and mass spectroscopic data

Molecular Docking Protocol

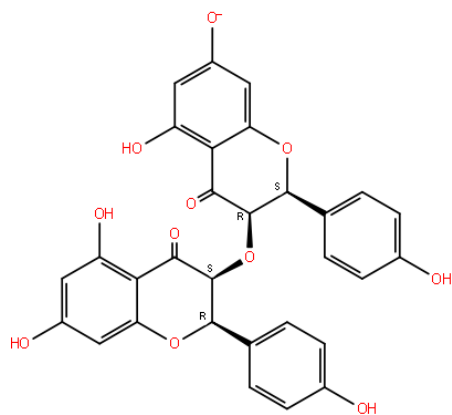
The in-silico approach to investigate the inhibitory potential of newly isolated OC251FR2 flavononol a subclass of flavonoid from the seed of *Garcinia kola* against Alpha Estrogen Receptor (ER- α) was carried out using 5W9CPDB.

Preparation of Ligand for Docking

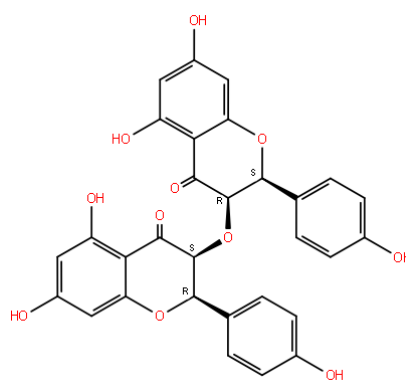
3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (OC 251 FR2) was drawn using Maestro 2-D Sketcher of the Schrodinger suite and moved to the 3-D workspace and then prepared using ligprep wizard to obtain forty-one (41) different conformers. Six of the conformers with the lowest energy were selected for docking and were labelled 1, 2, 3, 4, 5, and 6 (Fig. 2). The reference drug 4-Hydroxytamoxifen was obtained from the splitting of the ER- α (PDB ID: 5W9C). The reference drug was also prepared using the ligprep module on the Maestro 2023-1 of the Schrodinger suit which gives two conformers labelled 7 and 8 (Fig.3). The six selected conformers from OC251FR2 and the two conformers from 4-Hydroxytamoxifen, were docked against ER- α . R was selected as the reference compound for ER- α (5W9C) docking because it is found naturally binding to the site of the protein. The Ligprep wizard of the Schrodinger suite was then used to prepare all the ligands by assigning bond length, bond angle, generating possible conformers and ionization state at pH 7 using Epik and finally optimized using OPLS4 force field (Friesner *et al.*, 2004; Friesner *et al.*, 2006; Halgren *et al.*, 2004)



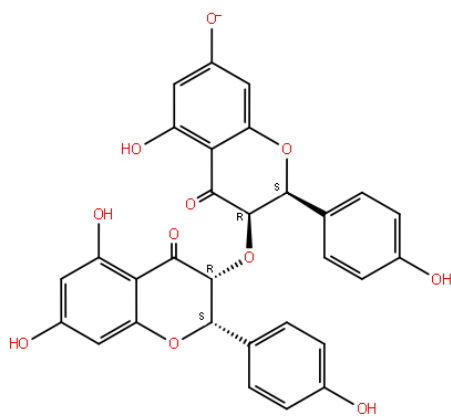
1



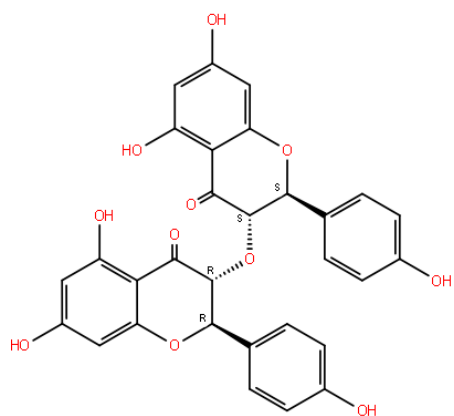
2



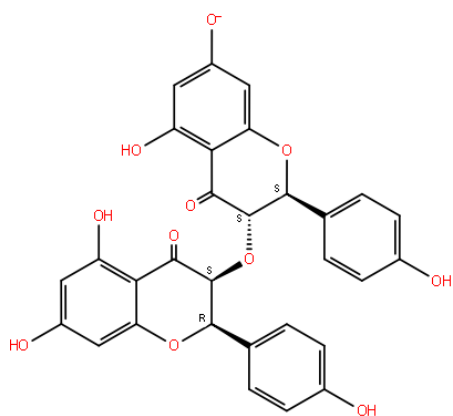
3



4



5



6

Figure 2: Selected Conformers of OC 251 FR2

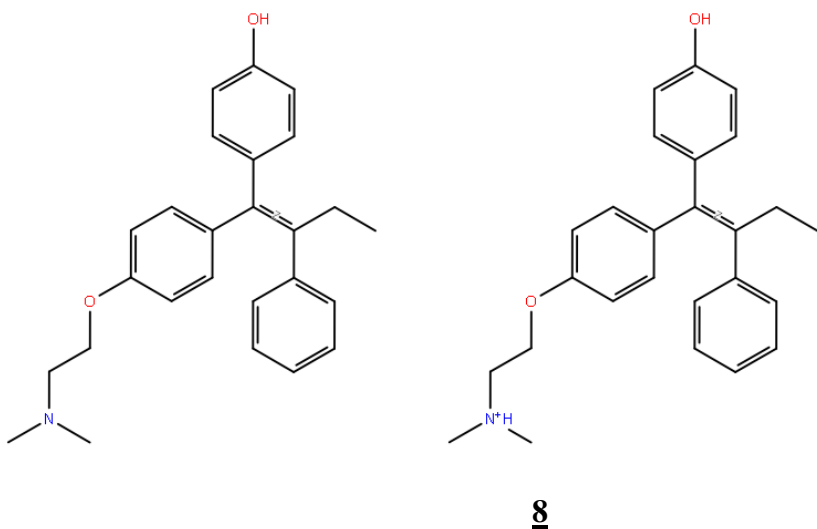


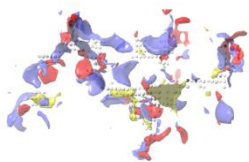
Figure 3: Conformers of the Reference Ligands

Protein Preparation

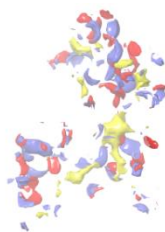
The protein structures human Estrogen Receptor-Alpha (ER α) (PDB ID: 5W9C), with resolution 1.59 Å was downloaded from a Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). The retrieved crystal coordinates were prepared in the protein preparation wizard of the Schrodinger suite. With default parameters of assigning bond order, optimizing, and minimizing using OPLS4 force field. The sitemaps were generated using the sitemap wizard. Five (5) sitemaps Table 1 (Fig. 4) were generated with different sizes, site scores, Dscores, volume, hydrophobicity and hydrophilicity using the sitemap tools of the Maestro Schrodinger suite. This gives the site volume, the site score, size, Dscore, philic and phobic values of the sitemap. This generates possible pockets for binding in the protein molecules. The active sites are lined with both hydrophilic and hydrophobic residues. Hydrophobic part is believed to be responsible for entrapping non-polar components of the drug while the hydrophilic part is believed to be responsible for entrapping the polar components of the drug. The Receptor Grid Generation was carried out on each sitemap to know the residual numbers that are involved in the binding process. This was actualized at 1 Å with a partial charge cut off 0.25 Å (Adegoke *et al.*, 2020a; Adegoke *et al.*, 2020b; Adeleke and Babatunde, 2020; Friesner *et al.*, 2004; Friesner *et al.*, 2006; Halgren *et al.*, 2004).

Table 1: Sitemap Analysis

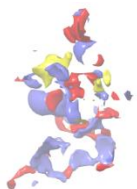
Sitemap	D score	Site Score	Volume	Size	Philic	Phobic
1	1.026	1.000	1089.025	378	0.996	0.471
2	1.071	1.014	873.621	299	0.789	0.697
3	0.980	1.005	553.259	196	1.175	0.504
4	1.025	1.028	239.414	106	1.093	1.110
5	1.036	1.024	180.418	96	1.040	0.794



Sitemap 1



Sitemap 2



Sitemap 3



Sitemap 5



Sitemap 6

Figure 4: 5W9C Sitemaps

Ligand Docking

The docking studies of OC 251 FR2, 4-Hydroxitamoxifen, with ER- α (5W9C) were carried out in Maestro (version 13.2) released 2023-1. The binding strength of the docked complexes were analyzed through docking score, glide e-model and ligand efficiency. These energies measure the degree of stability of the binding between the protein molecules and the ligand molecules. Also, an interaction with amino acid residue were taken note of via hydrogen bonding interaction, pi-pi stacking, metal coordination, salt bridge and solvent exposure. Docking calculation were executed in the Extra Precision (XP) mode of the Glide module in the Schrodinger suite molecular docking platform. The complex with the highest negative docking scores has better binding towards the respective proteins. Docking calculation of OC 251 FR2 conformers against the ER- α (with PDB 5W9C) isoforms will provide a selectivity profile that may be interesting for the development of a novel anti-breast-cancer agent. The 5W9C is an excellent target for the design of novel therapeutic strategies (Adegoke *et al.*, 2020a; Adegoke *et al.*, 2020b; Adeleke and Babatunde, 2020; Friesner *et al.*, 2004; Friesner *et al.*, 2006; Halgren *et al.*, 2004)

Induced Fit Docking

Computational biology tool called Induced Fit Docking, (IFD) provides a precise way of predicting the binding attraction of new inhibitors against the protein crystal (Folayan *et al.*, 2023; Iwaloye *et al.*, 2020).

Drug-likeness

The ADME Tox calculations was done using the Qikpro module of the Maestro

Calculation of Physicochemical Properties

Qikprop module of the Schrodinger suite was used to calculate Adsorption, Distribution, Metabolism, Excretion and Toxicity (ADME-Tox) of some commonly used molecular descriptors such as the dipole moment, Logarithm of Octanol-Water Partition Coefficient (QPlogPo/W), percent Human Oral Absorption, Polar Surface Area (PSA), and Violation of the Lipinski's Rule of Five (Adegoke *et al.*, 2020a; Adegoke *et al.*, 2020b; Adeleke and Babatunde, 2020; Friesner *et al.*, 2004; Friesner *et al.*, 2006; Halgren *et al.*, 2004)

DFT Calculations

The density function theory (DFT) calculations were performed using the Jaguar Optimization module on the theory of B3LYP and functional basis of 6-31G on the Maestro 2023-1 of the Schrodinger suit. This gives the HOMO Energy (E_{HOMO}), LUMO Energy (E_{LUMO}). E_{HOMO} and E_{LUMO} were used to calculate the Ionization Potential (IP), Electron Affinity (EA), Energy Gap (ΔE), Chemical Hardness (η), Chemical Potential (μ), and Electrophilicity (ω). The HOMO and LUMO describe the ability of a compound to donate or accept electrons respectively. Energy Gap predicts the reactivity, biological activities and kinetic stability of the molecule. The higher the energy Gap the higher the stability. Chemical Hardness predicts the stability and reactivity. The lower the hardness the better the potential to exchange charges with amino acid. Chemical Potential on the hand predicts the reactivity and stability properties. The higher the chemical potential the lower the stability and the higher the reactivity. Highly negative chemical potential translates to easy acceptability of electrons and uneasy tendency to remove electron. Electrophilicity evaluates the biological potency of the molecule. The higher the electrophilicity the stronger the electrophilic property of the molecule

Results and Discussion

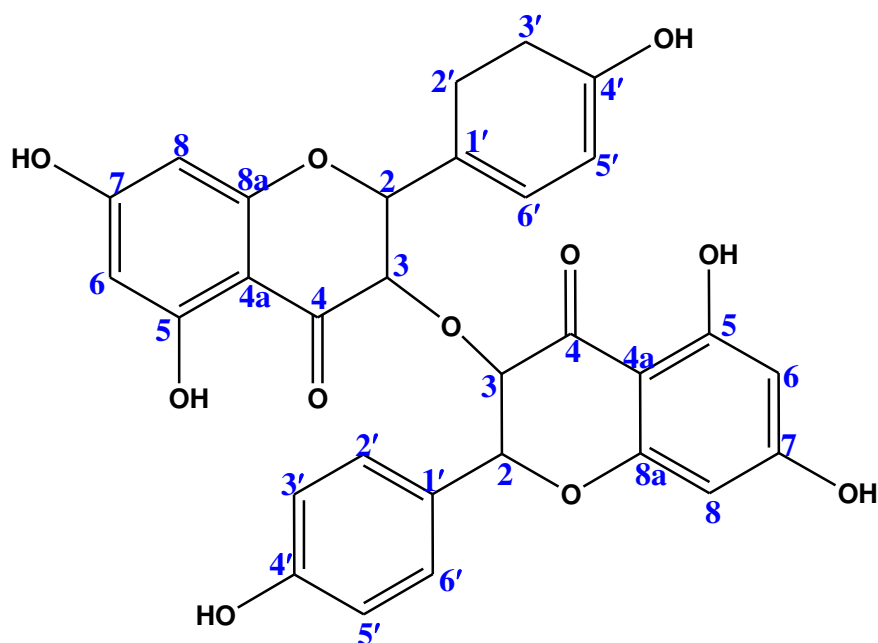


Figure 5: Structure of OC251FR2

3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)

Table 2: ^1H NMR and ^{13}C NMR of Compound OC251FR2 (DMSO- d_6)

Posit ion	^1H NMR	^{13}C NMR Experimental	^{13}C NMR Reported**	HMBC Correlations	COSY Correlations
2	5.50 (d, $J = 4.8$ Hz, 1H)	89.93	84.18		H-3
3	4.04 (d, $J = 4.8$ Hz, 1H)	72.74	73.18		H-2
4	-	197.32	197.95		
4a	-	101.72	101.24		
5	-, 12.14 (1H, s, OH-5)	164.05	164.04		
6	5.73 (d, $J = 4.5$ Hz, 1H)	96.11	96.72		
7	-, 9.41 (1H, s, OH-7)	167.10	167.18		

8	5.60 (d, $J = 4.5$ Hz, 1H)	95.54	96.07	
8a	-	164.10	163.55	
1'	-	129.30	129.60	
2'	6.68 (dd, $J = 6.5, 4.5$ Hz, 1H)	115.10	115.50	C-2, C-1', C-4', C-2'
3'	6.93 (dd, $J = 6.5, 4.5$ Hz, 1H)	128.57	128.70	
4'	-, 12.04 (1H, s, OH-4')	157.98	157.77	
5'	6.93 (dd, $J = 6.5, 4.5$ Hz, 1H)	128.57	128.70	
6'	6.68 (dd, $J = 6.5, 4.5$ Hz, 1H)	115.10	115.61	C-2'

Reported Hu *et al.*, 2017.**

Discussion

The compound OC251FR2 (Fig.5) was isolated as dark yellow grain-like solid with molecular peak ion of 559g/mol corresponding to the molecular formula $C_{30}H_{23}O_{11}$.

The ^1H -NMR spectrum (Supplementary Figure S1) of OC251FR2 (500 MHz; DMSO- d_6) displayed signals for OH functional groups (δ_H 12.14, OH-5; 12.04, OH-4'; 9.41, OH-7), this confirms the presence of three hydroxyl group at position 5, 7 and 4' on the aromatic ring. The existence of a chroman-4-one system was confirmed by δ_H 5.50 with $J = 4.8$ Hz, assigned to H-2 and 4.04 which appears as doublet, $J = 4.8$ Hz, was assigned H-3. This is typical of a flavanone class of flavonoids. Other aromatic proton peaks at δ_H 5.73 (H-6), appearing as a doublet with coupling constant $J = 4.5$ Hz, 1H, showed a meta coupling with 5.60 (H-8) which also appeared as a doublet with coupling constant 4.5 Hz. δ_H 6.68, which appears as double doublet, $J = 6.5, 4.5$ Hz, was assigned to H-2' and H-6' respectively and δ_H 6.93, which appears as double doublet, $J = 6.5, 4.5$ Hz, also assigned to H-3' and H-5' typical of 1,4-di-substitution on ring C (Table 2).

The ^{13}C -NMR spectrum (Supplementary Figure S2 and S3) of OC251FR2 (150 MHz; DMSO- d_6) showed a quaternary carbon δ_C 197.32 ppm which was assigned to the carbonyl carbon at position 4. The presence of 167.10, 164.10, 164.05 and 157.98 ppm are indication of (O-C) of a quaternary carbon, assigned to C-7, C-8a, C-5 and C-4' positions respectively. Other two quaternary carbons at δ_C 129.30 and 101.72 were assigned to C-1' and C-4a positions respectively. Four chemically and magnetically equivalent carbons belonging to two spin systems at δ_C 128.57 and 115.10 which were assigned to 3' and 5' and 2' and 6' respectively. Two non-aromatic carbon signals typical of a flavanone compounds at δ_C 89.93 and 72.74 ppm were assigned to C-1 and C-2 respectively (Table 2).

The HMBC spectrum (Supplementary Figure S4) of OC251FR2 showed a correlation of δ_H 6.68 (H-2') with 89.93 (C-2); 129.30 (C-1'); 157.98 (C-4'), 128.7 (C-2'); this suggests the presence of hydroxy group at position 4' and a flavanone system on ring B. Another correlation was found between δ_H 6.68 (H-6') with 128.7 (C-2') (Table 2).

The ^1H - ^1H COSY spectrum (Supplementary Figure S5) of OC251FR2 showed a correlation of δ_H 5.50 (H-2) with 4.04 (C-3); this further confirms the compound as a compound with flavanone moiety (Table 2).

The compound was assigned based on 1D (^1H and ^{13}C NMR) and 2D (HSQC (Supplementary Figure S6), HMBC, COSY and NOESY (Supplementary Figure S8) experiments. OC251FR2 is a dimer with a flavanone unit.

The mass spectrometry experiment (Supplementary Figure S7a and b) of OC251FR2 indicated that the compound is a dimer with molecular formula of $C_{30}H_{23}O_{11}$ and it conforms to the molecular mass of 559 g/mol confirming the compound as 3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)-3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one). This is the first report of 3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)-3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) (OC251FR2); a dimer with flavanone moiety from *G. kola*. earlier, (Maňourová *et al.*, 2019), reported on the isolation and complete spectra characterisation of kolaviron, a biflavonoid as a major component in *G. kola* seeds. Stark *et al.*, 2013, isolated and characterised a series of 3,8''-linked bi-flavones from *Garcinia Buchananii*, compounds which are slightly different in functionality to the isolated compounds and are not dimeric in nature.

Docking Discussion

Docking Results

Sitemap 1

The docking score of the isolated OC251FR2 drug1 (-6.214) is having higher negative score than the reference drug (-5.289) (Table 3) which indicates that OC251FR2 is better located in the active site than the reference drug 7 and 8. Although 1 is having the same ligand efficiency with 7, but better glide emodel. The 2-D ligand amino acid interaction (Fig.6a&6b) and 3-D interaction (Fig.7a&7b) shows that the isolated OC251FR2 drug conformers have better hydrogen bond

interaction, although with longer bond length. The isolated OC251FR2 also have fairly good pi-pi interactions and salt bridge interactions. This means that the OC251FR2 compound can inhibit the active sitemap on the receptor.

Table 3: Ligand Docking Results with 5W9C

Sitemap	Compounds	D Score	Glide Emodel	Ligand Efficiency
1	OC251FR2	-6.214	-59.079	-0.182
	REFERENCE	-5.289	-53.782	-0.182
2	OC251FR2	-5.270	-69.723	-0.129
	REFERENCE	-5.270	-73.680	-0130
3	OC251FR2	-5.049	-73.316	-0.123
	REFERENCE	-4.975	-50.158	-0.168
4	OC251FR2	-3.377	-54.815	-0.082
	REFERENCE	-5.484	-46.006	-0.082
5	OC251FR2	-3.822	-54.034	-0.093
	REFERENCE	-2.601	-41.762	-0.090

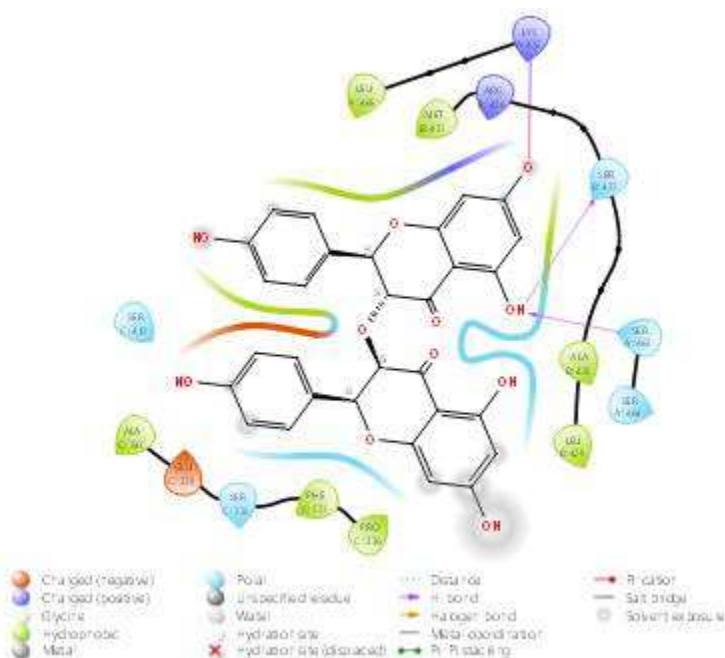


Figure 6a: 2-D ligand amino acid interaction of isolated OC251FR2 on sitemap1

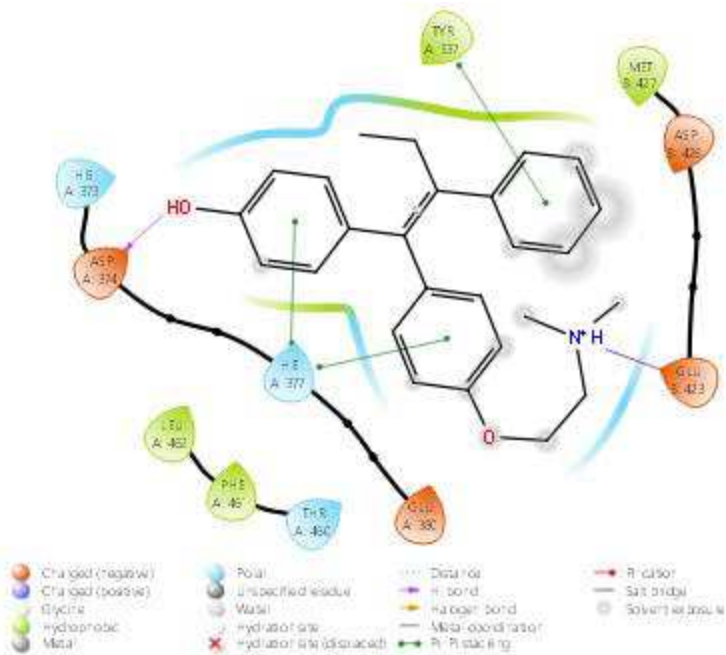


Figure 6b: 2-D ligand amino acid interaction of REFERENCE on sitemap1

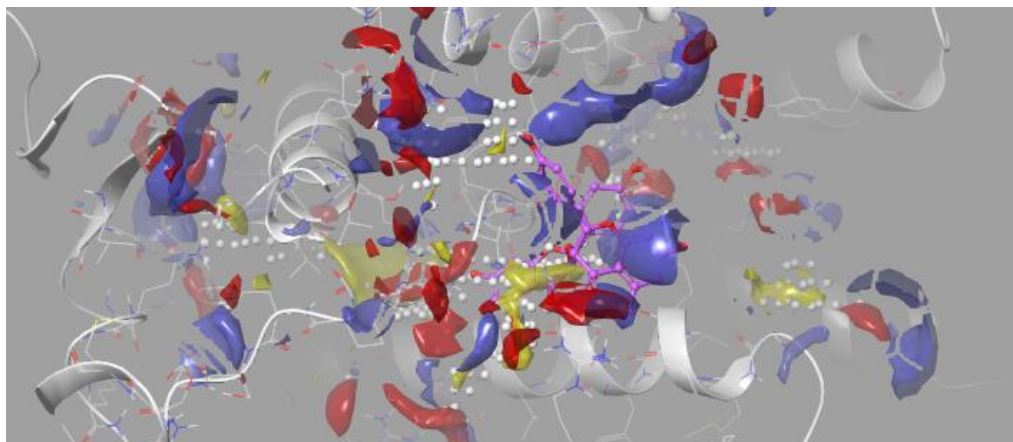


Figure 7a: 3-D ligand amino acid interaction of isolated OC251FR2 on sitemap1

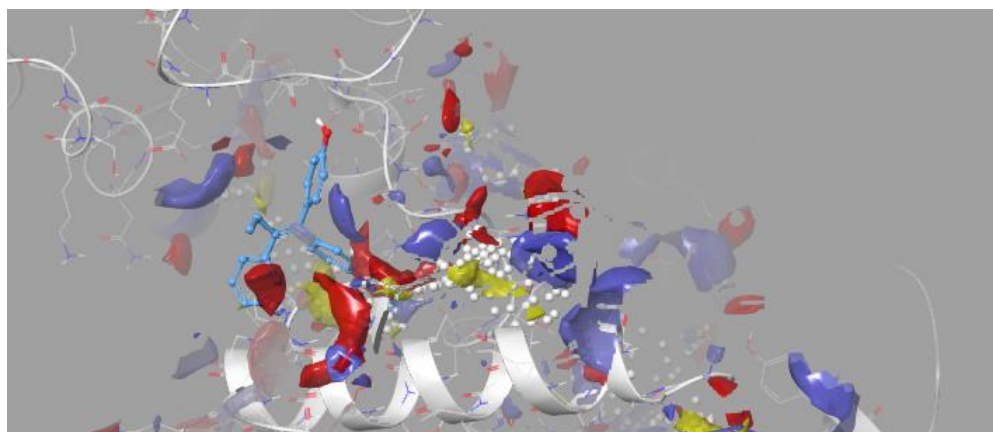


Figure 7b: 3-D ligand amino acid interaction of REFERENCE on sitemap1

Sitemap 2

The isolated OC251FR2 conformer 5 and reference conformer 7 have the same and the highest negative docking score (-5.270) (Table 3), although the ligand efficiency of the reference drug 7 is slightly higher (-0.130) than that of 5. This shows that they compete favorably for the active site and could perform similar level inhibition. The glide emodel of the two conformers 5 and 7 are also the best in sitemap 2. The 2-D (Fig. 8a&8b) and 3-D (Fig 9a&9b) interaction showed that the OC251FR2 drug conformers have more amino acid interactions with shorter bond length than the reference drug conformers. It means the isolated OC251FR2 can inhibit the sitemap two better than the reference drug.



Figure 8a: 2-D ligand amino acid interaction of isolated OC251FR2 on sitemap 2



Sitemap 3

The docking score of the isolated OC251FR2 compound 1 (-5.049) have higher negative score than the reference drug (-4.975) (Table 3) which indicates that the isolated OC251FR2 binds better into the active site than the reference drug 7 and 8. It also has higher ligand efficiency and glide emodel than reference drug. The 2-D (Fig. 10a & 10b) and 3-D (Fig. 11a & 11b) ligand amino acid interaction shows that OC251FR2 compound conformers have more hydrogen bond interaction than the reference drugs although the bond length are similar in lengths. The isolated OC251FR2 also have pi-pi interactions and salt bridge interactions with shorter bond length than the reference compound. This indicates that the isolated OC251FR2 compound has better inhibitory activities in the site than the reference drug.

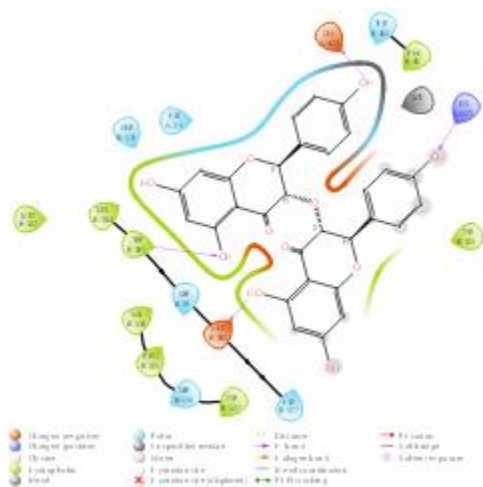


Figure 10a: 2-D ligand amino acid interaction of isolated OC251FR2 on sitemap 3

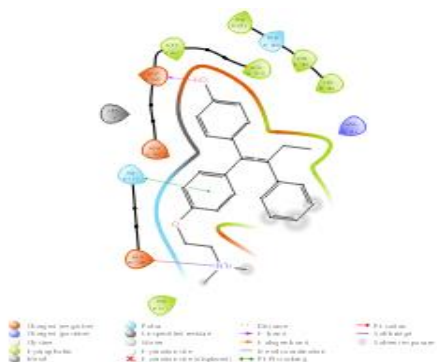


Figure 10b: 2-D ligand amino acid interaction of REFERENCE on sitemap 3

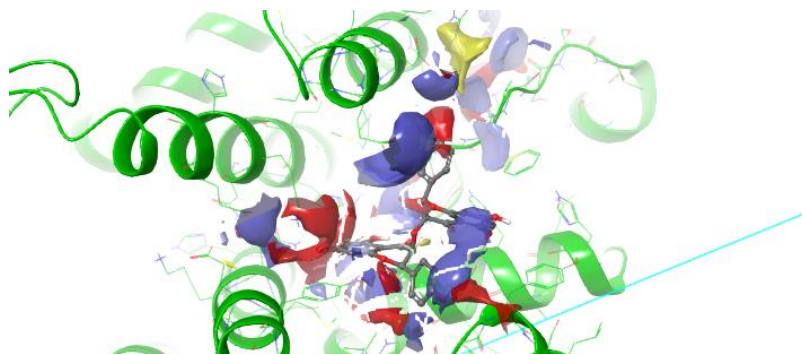


Figure 11a: 3-D ligand amino acid interaction of isolated OC251FR2 on sitemap 3

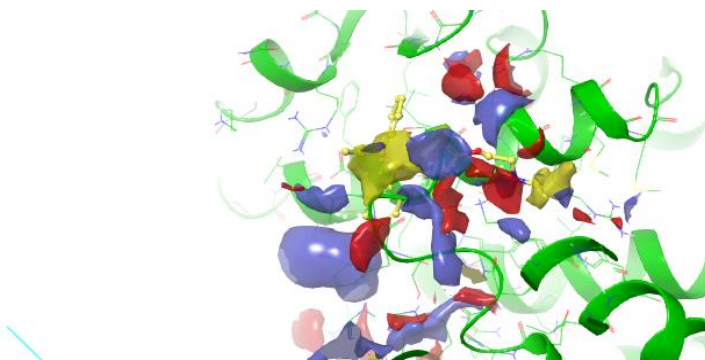


Figure 11b: 3-D ligand amino acid interaction of REFERENCE on sitemap 3

Sitemap 4

The docking score of the reference drug 7 is better than that of the isolated OC251FR2 drug conformers (Table 3). This because the active sitemap is having higher phobic value than the philic value i.e., the active site is lined with amino acids that interact more with less polar components of a compound, hence it interacts more with the reference drug which is less polar although the ligand efficiency and the glide emodel of the reference drug is better. The 2-D (Fig.12a &12b) and 3-D (13a &13b) ligand amino acid interaction shows that the isolated OC251FR2 drug conformers have better hydrogen bond interaction, although with longer bond length. The isolated OC251FR2 also have fairly good pi-pi interactions and salt bridge interactions with longer bond length. This indicate that despite lower docking score of OC251FR2, it can still compete favorably for the active site.

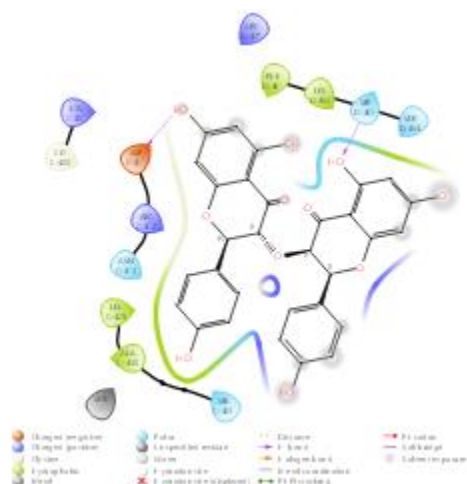


Figure 12a: 2-D ligand amino acid interaction of isolated OC251FR2 on sitemap 4

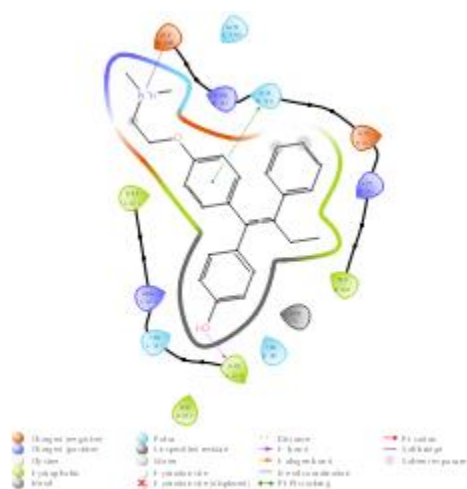


Figure 12b: 2-D ligand amino acid interaction of REFERENCE on sitemap 4

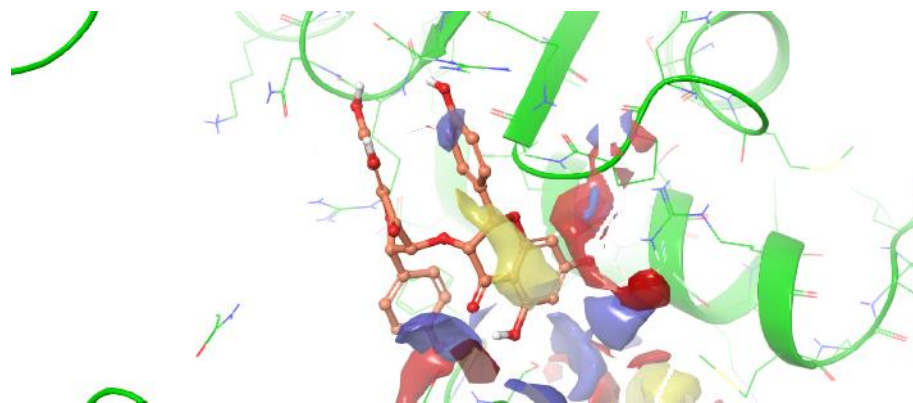


Figure 13a: 3-D ligand amino acid interaction of isolated OC251FR2 on sitemap 4

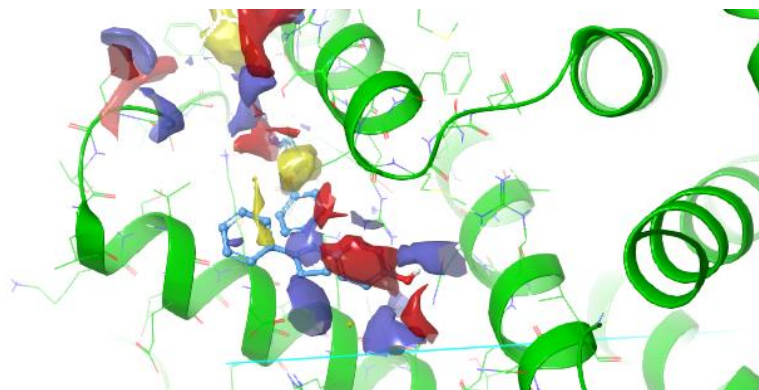


Figure 13b: 3-D ligand amino acid interaction of REFERENCE on sitemap 4

Sitemap 5

The docking score of the isolated OC251FR2 drug 1 (-3.822) have higher negative score than the reference drug (-2.601) (Table 3) which indicates that the OC251FR2 drug is better located in the active site than the reference drug 7 and 8. Conformer 1 have better ligand efficiency with 7 and also better glide emodel. The 2-D (Fig. 14a & 14b) and 3-D (Fig.15a & 15b) ligand amino acid interaction show that the isolated OC251FR2 drug conformers have better hydrogen bond interaction, although with longer bond length. The isolated OC251FR2 compound exhibits fairly good pi-pi interactions and salt bridge interactions. This means that the isolated OC251FR2 compound also inhibits the active sitemap on the receptor.

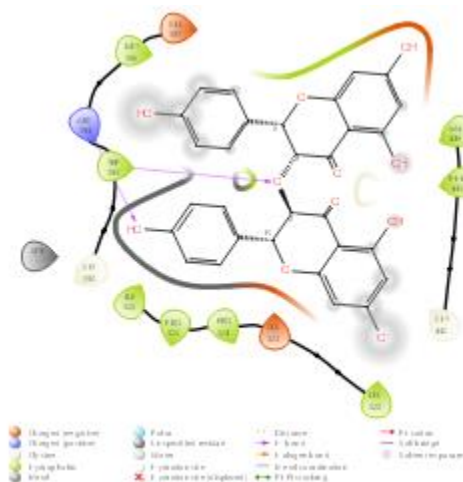


Figure 14a: 2-D ligand amino acid interaction of isolated OC251FR2 on sitemap 5



Table 4: 2-D Interaction with 5W9C Sitemaps

Sitemap	S/N	HBond	Pi-Pi stalking/Pi-Cation	Salt Bridge
1	1	VAL ₍₄₅₈₎ 2.08, SER ₍₄₆₃₎ 2.30 PHE ₍₃₃₇₎ 1.73	ARG ₍₄₃₆₎ 5.93, LYS ₍₄₇₂₎ 3.36	
	2	ASP ₍₃₇₄₎ 1.63	HIE ₍₃₇₇₎ 4.77, HIE ₍₃₇₇₎ 4.89 TYR ₍₅₃₇₎ 5.03	GLU ₍₄₂₃₎ 2.85
2	1	ASP ₍₄₂₆₎ 2.08	ARG ₍₄₃₆₎ 5.82	
	2	LYS ₍₄₇₂₎ 1.96, ARG ₍₄₃₆₎ 2.15	LYS ₍₄₇₂₎ 4.85	
3	1	GLU ₍₄₂₃₎ 1.95, ASP ₍₄₂₆₎ 1.67, LYS ₍₅₂₀₎ 1.82, GLU ₍₃₈₀₎ 1.93		
	2	ASP ₍₄₂₆₎ 1.90	HIE ₍₃₇₇₎ 5.38	GLU ₍₃₈₀₎ 2.87
4	1	GLN ₍₅₀₆₎ 2.32, ASP ₍₄₇₂₎ 2.21, SER ₍₄₆₃₎ 2.73, LYS ₍₄₇₂₎ 2.55		
	2	ALA ₍₄₃₀₎ 1.95	HIE ₍₄₇₆₎ 4.83	ASP ₍₄₈₀₎ 3.55
5	1	PRO ₍₃₂₄₎ 1.80, GLU ₍₄₄₇₎ 2.02, GLU ₍₃₉₇₎ 2.08, TRP ₍₃₉₃₎ 2.46		
	2	GLU ₍₃₉₇₎ 1.52		GLU ₍₃₅₃₎ 3.88

It has been established that a protein's binding site is made up of important residues within its active pocket. A good evaluation of these residues provides more information on the molecular interaction between proteins and ligands. Of the several amino acid residues contained in the single-chain polypeptide, ER- α , ASP 462, LYS 472, ALA 430, VAL 458, GLU 397, HIE 377, SER 463 are the most common. Table 4 gives an overview of the residues that interacted within the complex formed by ligand and protein. Our research findings revealed that most of the interactions were H-bond interaction with few pi-pi cation and salt bridge interactions with different amino residues as shown in Figures 6a & 6b, 8a & 8b, 10a & 10b, 12a & 12b and 14a & 14b respectively. Furthermore, the 3D binding pose of the isolated OC251FR2 compound and the

reference drug with ER- α as shown in Figures 7a & 7b, 9a & 9b, 11a & 11b, 13a & 13b and 15a & 15b revealed same respectively.

Table 5: Induced Docking Results with 5W9C

Active sites	Molecules	Dorking Scores	IFD Dorking Score
1	OC251FR2	-13.374	-2040.14
	REFERENCE	-12.273	-2041.99
2	OC251FR2	-10.703	-2035.01
	REFERENCE	-12.428	-2040.24
3	OC251FR2	-12.404	-2038.10
	REFERENCE	-12.392	-2042.10
4	OC251FR2	-07.458	-2028.69
	REFERENCE	-13.126	-2041.55

IFD (Table 5) offers a better way for estimating binding affinity since it enables the protein to rotate while it is bound to a ligand using the existing active sites. 5W9C have four existing binding sites, and it was noticed that the isolated OC251FR2 compound has higher docking score in each of the active site 1 and 3 but lower IFD scores in all the active site. This implies that the isolated OC251FR2 compound bound more intimately to the active sites in active sites 1 and 3 of ER- α hence indicating a stronger molecular interaction.

Drug-likeness

The ADME Tox calculations was done using the Qikpro module of the Maestro Schrodinger suite (Table 6) shows that the isolated OC251FR2 compound is more polar than the reference drug because they all have higher dipole moment. The partition coefficient of the OC251FR2 compound is very good as it falls within the standard limit and even better than the reference drug. The polar surface area (PSA) is higher than the standard limit of $\leq 140 \text{ \AA}^2$. The rule of five is violated.

Table 6: ADME Tox Table

S/N	Dipole	QPLogPo/W (a)	PSA (b)	Rule of Five	Percent Oral Absorption (c)
1	7.393	2.032	195.857	3	11.621
2	4.950	1.909	201.197	3	10.250
3	2.863	2.482	196.144	3	14.377
4	8.629	1.832	199.398	3	10.435
5	2.590	2.552	194.110	3	18.146
6	7..838	1.969	194.110	3	13.938
7	1.070	5.653	35.966	1	100
8	0.856	5.832	34.663	1	100

a= Logarithm of the partition coefficient of the compound between n-octanol and water (recommended value <5).

b=Polar surface area (recommended value $\leq 140 \text{\AA}^2$).

c=Percentage of human oral absorption (<25% is weak and >80% is strong).

Quantum Studies

The frontier Molecular Orbital energies and the global reactivity parameters of the isolated OC251FR2 and reference drug are presented in Table 7. The E_{HOMO} (-0.264) of the reference drug is lower than that of the isolated OC251FR2 compound (-0.211) suggesting that the isolated OC251FR2 compound is less nucleophilic and more stable in reaction when compare to the reference drug (Manoj et al., 2022). The Energy Gap (ΔE) of the reference drug was higher than that of isolated OC251FR2 compound implying that the isolated OC251FR2 drug could interact with the receptor via the formation of stronger bonds that could lead to ligand-receptor complex, improved binding affinity and effectiveness of the drug. The chemical Hardness (η) and global softness values of both the reference and the isolated OC251FR2 drugs suggests the that the isolated OC251FR2 drug is relatively soft hence have a low resistance to altering of their electronic distribution during a reaction. This implies that it could exchange charges with the amino acids better than the reference drug. The chemical potential (μ) as presented in Table 7, shows negative values for the reference drug and the ligands, indicating a good complex stability with the receptor. The electronegativity (χ) of a molecule measures its ability to attract electrons (Karton and

Spackman, 2021). From Table 7, the electronegativity of the ligand (0.085) indicates that its ability to attract electron is low therefore might exhibit weak biomolecular interaction. Electrophilicity index (ω) on the other hand predicts the electrophilic nature of a chemical specie and measures the tendency of a molecule to accept an electron such that high ω values denote good electrophilicity (Umar and Uzairu, 2022). Organic molecules based on electrophilicity are graded as follows; molecules having $\omega < 0.8$ eV are weak electrophiles, molecules having $\omega > 0.8$ eV but < 1.5 eV are moderate electrophiles while molecules with $\omega > 1.5$ eV are strong electrophiles (Umar and Uzairu, 2022). The electrophilicity value of the isolated OC251FR2 drug and the reference drug shows that the compounds are weak electrophiles. The DFT properties show that the isolated compound OC251FR2 have similar properties when compared with those of the reference drug.

Table 7: Frontier Molecular Orbital Energies and Global Reactivity Parameters

S/N	Parameters	Isolated OC251FR2	Reference Drug
1	EHOMO	-0.211	-0.264
2	ELUMO	0.041	0.111
3	Energy Gap	0.253	0.375
4	Ionization Potential	0.211	0.264
5	Electron Affinity	-0.041	-0.111
6	Chemical Hardness	0.126	0.187
7	Global Softness	3.947	2.660
8	Chemical Potential	-0.085	-0.076
9	Electronegativity	0.085	0.076
10	Electrophilicity Index	0.028	0.015

Conclusion

A novel dimeric flavanone from the chloroform fraction of *G. kola*, 3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one)-3,3'-oxybis(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) (OC 251 FR2), a naturally occurring flavanone was isolated for the first time from this plant. The 1-D and 2-D NMR and the mass spectrometry data confirms the structure of the compound. The molecular docking studies conducted against alpha Estrogen receptor (ER- α) showed that the isolated OC251FR2 interact more with amino acids in the active sites via H-bond, pi-pi interaction than the reference drug 4-Hydroxytamoxifen. The drug-likeness determined by qikpro shows that OC251FR2 violated three of the Lipinski rules of 5, and also have percent oral absorption. The quantum mechanics values show that OC251FR2 have comparable properties to the reference drug 4-hydroxytamoxifen.

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Authors' contribution

Conceptualization and methodology: Tunmise T. Eugene-Osoikhia.; Docking and validation: Mohammed Isiaka, Nnenna W. Odozi.; Review and editing: Tunmise T. Eugene-Osoikhia, Nnenna W. Odozi.; Writing of manuscript: Tunmise T. Eugene-Osoikhia, Emmanuel O. Yeye , Mohammed Isiaka. Supervision: Ibrahim A Oladosu

Declaration of conflict of interest

The authors declare no conflict of interest

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