Antifungal screening and molecular docking simulation of silica supported synthesized sitosteryl hydrogen phthalate using microwave irradiation

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Abstract

In this study, steroidal sitosteryl hydrogen phthalate (stigmast-5-en-*3β*-yl hydrogen phthalate) was synthesized by the reaction of 3β-sitosterol and phthalic anhydride using silica gel as a solid support under microwave irradiation (MWI). The comparative study of microwave assisted synthesis and conventional synthesis of the steroidal compound in a hazardous solvent revealed that the former method provided shortened reaction times at increased yields. The compounds obtained by the two procedures were characterized by infrared spectroscopy, proton, carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) and high-resolution mass spectrometry. The synthesized compound was screened for *in vitro* antifungal activity against *Aspergillus niger* and *Candida albicans* by the Kirby-Bauer Well Diffusion method. The synthesized compound was subjected to the molecular docking simulation with a receptor (CYP51). The findings of the antifungal and docking studies revealed that the synthesized sitosteryl hydrogen phthalate could be considered as a suitable inhibitor of Lanosterol 14α-demethylase (CYP51). In addition, the molecular docking approach was applied to design hypothetical derivatives of sitosteryl hydrogen phthalate inhibitors against the antifungal target and to compare findings with the binding score of the molecular synthesized 3β-sitosteryl hydrogen phthalate.

Keywords: β-sitosterol; sitosteryl hydrogen phthalate; antifungal assay; molecular docking.

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1. INTRODUCTION

Microwave irradiation (MWI) is a rapidly growing field in synthetic organic chemistry, including solvent-free organic synthesis categorized under green chemistry, which is becoming increasingly popular in chemistry [1-4]. In most cases, the synthesis assisted by microwave irradiation was more efficient in terms of reaction time and product yields. Compared to the conventional approach (CP), it was also more environmentally friendly. In the last few decades, organic chemistry has attracted a number of researchers with the keen interest in the synthesis of heterocyclic compounds, which due to the presence of N and S in the moiety and its derivatives, exhibit a broad spectrum of biological activity [5-9]. Steroids are a class of variety of compounds that occur in nature and have the ability to penetrate cells, bind to the nucleus and membrane receptors. They exhibit a great variation in the structure and play a crucial role in life [10-11]. Survey of the literature reveals that synthesized modified steroids and their derivatives with various heterocyclic rings have seen an increase in the recent past [12-17]. A variety of steroids synthesized by the use of different approaches found the impressive possible applications such as drugs for the treatment of cardiovascuar and autoimmune diseases, brain tumors, breast cancer, prostate cancer and osteoarthritis [18-20] where they exhibited antitumor [21-22], antimicrobial

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[23-24], and anti-parasitic activities [25]. Gastroprotective activity of various β-sitosterol esters has been documented. In chronic and acetic acid-induced gastric ulcers, β-sitosterol and its glycosides have been shown to exhibit anti-ulcerogenic activity and it is believed that triterpenoids or sterols associated with derivatives of salicylic or benzoic acid demonstrate anti-ulcerogenic activity [26-29]. Additionally, certain phenolics are used as active antioxidants and cytotoxic agents; thus, derivatives of salicylic acid fall into similar classes that exhibit activities of this sort [30]. Some new biomedically active steroidal derivatives have recently been reported to incorporate the structural features of both sterols and salicylic acid derivatives [31]. Steroids are also well known for promoting growth and sexual development and for regulating metabolism [32-34]. In addition to pharmacologically significant usages of steroid compounds described above, nowadays there are many cases of anabolic steroid abuse. Anabolic steroid misuse can be associated with a wide variety of adverse side effects ranging from physically unattractive acne and breast development in men to life-threatening ones, such as heart attacks and liver cancer. Moreover, steroid misuse disturbs the normal secretion of hormones in various parts of the body causing reversible as well as permanent changes. Changes which can be reversed involve decreased sperm production and testicle shrinking (testicular atrophy), while the irreversible changes include baldness and breast development in men (gynecomastia) [35]. In continuation, steroids are also part of the plasma membrane, hence modification of these compounds result in modification of the cell membrane permeability in animals [36]. A variety of papers have been published in the literature on the transformation of steroid hydroxyl functionality by various anhydrides [29,37-44]. Therefore, our interest was preparation of steroidal sitosteryl hydrogen phthalate, by the reaction of an easily accessible steroidal; we focused on β-sitosterol with phthalic anhydride in the presence of pyridine under reflux conditions and to compare the synthesis by the application of MWI with the aim to obtain fair to good yields (Fig. 1). The structure of steroidal hydrogen phthalate (**2**) is identified on the basis of spectral (IR, NMR and HRMS) studies. The synthesized compound was tested for antifungal activity *in vitro*, followed by a molecular docking analysis to determine interactions between the receptor and the compound in order to discern the nature of the compound activity against fungi.

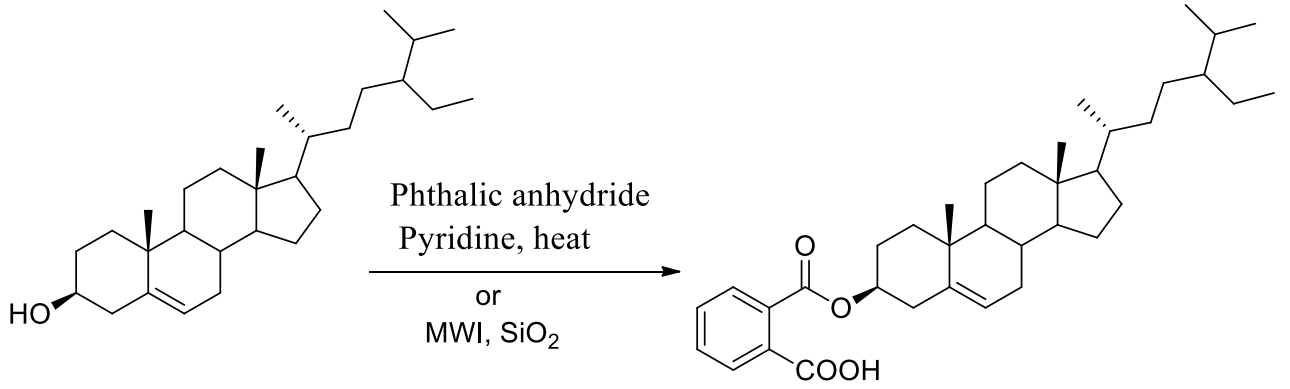


*Figure 1. Synthesis of the steroidal compound **2** from 3β-sitosterol*

2. MATERIALS AND METHODS

2. 1. Materials

3β-sitosterol (isolated from Ficus krishnae following the bibliographic procedure [45]), phthalic anhydride (SD Fine Chemicals, LR), pyridine (anhydrous, 99.8 %, Sigma Aldrich), dichloromethane (SD Fine Chemicals, AR), acetone (SD Fine Chemicals, LR) and silica gel (60-120 MESH, SD Fine Chemicals, LR), India and used as received. The reaction was also carried out using silica gel (2 g, 60-120 MESH) in conventional reaction in order to compare the results obtained from Microwave irradiation (MWI) at various reaction times (3-10 min) and reaction temperatures (100-130 °C).

2. 2. Experimental part

2. 2. 1.MWI synthesis

Adsorption of a slurry mixture of 3β-sitosterol (1 mmol), phthalic anhydride (1 mmol) and pyridine (0.75 cm^3) in a minimum amount of dichloromethane (DCM) over 2 g of silica gel (60-120 MESH, SD Fine Chemicals) was permitted. Then the contents were thoroughly mixed, and the extra solvent was evaporated openly in a fume hood. The impregnated silica gel was kept in a microwave and irradiated at 325 W (irradiated at 50 % power) during an appropriate time interval 3-10 min. The reaction mixture was cooled to room temperature after the completion of the reaction, as monitored by thin layer chromatography (TLC), and the product was extracted using methylene chloride. The organic layer was washed with water several times and finally dried over anhydrous Na_2SO_4, so that solvent evaporation and recrystallization resulted in the expected compound (**2**) as a yellow solid at a good yield of 80 %. Synthesis was performed three times to examine similar results.

2. 1. 2. Conventional procedure

3β-Sitosterol (1 mmol) was placed in a round bottom flask, followed by addition of pyridine (0.75 cm^3) and phthalic anhydride (1 mmol), and the mixture were refluxed on a hot plate for 3 h. The progress of the reaction was monitored by TLC. The resulting solution was poured into crushed ice water, stirred, filtered, dried, and finally crystallized with the use of acetone to provide a yellow solid, at a yield of 60 %, with a melting point of 140-142 °C (Reported: m.p. 145-146 °C) [29] (see Table 1 for reaction time and temperature). Another set of reaction was carried out at a temperature of 110 °C with stirring using silica gel in the reaction mixture.

2. 3. Characterization methods

The melting point was determined and is uncorrected on a Kofler apparatus. the infrared (IR) spectrum of sitosteryl hydrogen phthalate (**2**) was obtained by using KBr and the Pye Unicam SP3-100 spectrophotometer (Pye Unicam Ltd., UK). Proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance (^1H and ^{13}C NMR, respectively) were recorded by a Jeol Resonance spectrometer (Jeol Resonance Inc., Japan) at a frequency of 400 MHz for ^1H and 100 MHz for ^{13}C using $CDCl_3$ as a solvent and tetramethylsilane as the internal reference. Mass spectrum (MS) was measured by a JMS-D300 AIE MS-9 spectrometer (JEOL Ltd., Japan) using direct insertion technique at a source temperature of 250 °C. A commercially microwave oven model (NN-K571MN,Panasonic Inc, Canada). having a microwave frequency of 2450 MHz was used for the reaction. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the purity as well as the progress of the reaction.

2. 4. Antifungal activity screening

The sitosteryl hydrogen phthalate was screened for *in vitro* antifungal activity by using the Kirby-Bauer Well Diffusion method described in literature [46-48]. This protocol, also known as the disc-diffusion procedure, is the most frequently used antibiotic susceptibility test to determine which antibiotic should be exploited when treating an infection, and is based on the measured inhibition of bacterial growth under standard conditions [48]. The Kirby-Bauer well diffusion method was applied against two fungal strains: *Aspergillus niger* (MTCC-281, India) and *Candida albicans* (MTCC-183 India).

Aspergillus niger and *Candida albicans* were inoculated at the concentration of 10^4 colony-forming units (CFU)/ml in Sabouraud's Dextrose broth and kept for 48 h at 28 °C. The inoculum was swabbed onto the Sabouraud's Dextrose Agar surface. 100 and 50 µl prepared from the stock (5 mg/ml) was loaded into the respective wells and the plates were incubated at 28 °C for 48 h and the diameters of the inhibition zones were measured. Each antimicrobial assay was performed in triplicate and the standard drug, Fluconazole (Pfizer, US) at the concentration of 1 µg cm^{-3} was used as a positive control for antifungal activity while DMSO was used as a negative control. Same concentrations of 3β-sitosterol were also applied for assessing antifungal activity.

2. 5. Molecular docking simulation

Ligand-protein interactions were studied by using the molecular docking software Autodock version 4.0 (The Scripps Research Institute, CA) [49]. For docking exploration, a 2D structure of (**2**) was drawn and converted into a 3D structure using ChemBiodraw Ultra and 3D ChemBiodraw (PerkinElmer Inc., USA), respectively. The sketched 3D structure of the steroidal compound **2** was optimized to minimize energy by using MM2 force and saved in pdb format for use in the Autodock 4.0 software [49]. The pdb file of (**2**) was then converted into the pdbqt format by AutoDock Tools (ADT 1.5.6) using default parameters. Gasteiger charge was added to the ligand by the ADT tools to generate the docking input files. Crystal structure of Lanosterol 14α-demethylase (PDB: 5V5Z) obtained from the pathogen *Candida albicans* is considered to be the responsible for fungal infection and was retrieved from the RCSB Protein Data Bank (PDB), acting as a receptor and the active sites of the targeting protein were identified from the literature [50]. Before the docking step, a protein (PDB: 5V5Z) was prepared in which the co-crystallized hetero atoms attached were removed to give a passage to the incoming steroidal compound during the docking. The grid dimensions and centre were set to 60×60×60 $Å^3$ and X = −47.7 Å, Y = −13.4 Å and Z = 22.9 Å, respectively, whereas the points were separated by 0.500 Å. The docked model with the minimum binding energy was considered the best and selected to visualize and explain the non-covalent bond between the compound and the receptor using the Discovery Studio visualizer (Dassault Systemes, France) [51]. In addition, the result of binding energy and amino acids involved in the interactions with the compound **2** was compared with a series of eleven structurally identical compounds. The structure of these compounds was drawn by using the ChemDraw software and the energy of the structure was minimized by using the MM2 force field. For the docking analysis, energy-minimized structures with *o*-, *p*-, and *m*-substitution of benzoic acids were used to predict binding energies and in-silico biological activities of various steroidal benzoic acid derivatives with the active binding domain of Lanosterol 14α-demethylase receptor target (PDB: 5V5Z). The molecular docking of the synthesized compound **2**, including eleven sketched compounds, in order to compare the binding energy and non-binding interactions between them in this study, was carried out by using AutoDock Vina under PyRx 0.8 interface [52] and applying the same grid and centre dimensions as for the compound **2.**

3. RESULTS AND DISCUSSION

3. 1. Chemistry

Easily accessible 3β-sitosterol reacted with phthalic anhydride in the presence of pyridine both under MWI and reflux conditions, which resulted in the desired product **2** having melting point (m.p.) 140-142 ^oC at good yields (Fig. 1). The structure of steroidal hydrogen phthalate (**2**) is identified based on spectral (IR, NMR and MS) and micro analytical studies. The FTIR spectrum of the compound **2** was used to identify functional groups present in the compound [29, 44]. It showed a broad peak at 3432 cm^{-1} assigned to the OH group of COOH and a strong band at 1721, and 1670 cm^{-1} corresponding to the carbonyl groups of -COOH and $–COO^-$, respectively. Other absorption peaks due to $-CH_2-$, -CH-, -C=C- and -C–O- groups were also observed in the spectrum at 2938, 2865, 1459 and 1290 cm^{-1}, respectively, supporting the assumption of the product formation by phthalylation of the hydroxyl group of the steroid. The structure **2** was also supported by the NMR spectral study (Fig. 2a) of the compound, which showed a doublet and multiplet at δ = 7.8, 7.7 and 7.5 ppm indicating the presence of aromatic protons belonging to the reagent phthalic anhydride linked to the steroid framework. ^1H NMR of the chemical shift of carboxylic proton was further shifted more downfield and appeared at δ = 8.2 ppm. The peaks resonating at δ = 3.5 (multiplet) and 5.5 ppm (doublet of doublets) are attributed to β-sitosterol-H3 and sitosterol-H6, whereas other peaks of the compound are found to resonate in accordance with sitosterol series. In the ^{13}C NMR spectrum (Fig. 2b), two peaks were seen at 170.8 and 167.7 ppm corresponding to carboxylic acid and ester carbonyl groups, respectively. Two carbon atoms appearing at δ = 139.6 and 123 ppm are assigned to C-5 and C-6 in the steroid skeleton, indicating the unsaturated center between the two carbons. Moreover, six carbon atoms appeared as peaks at δ = 121-141 ppm, indicating the presence of an aromatic ring. The chemical shift of C-3 that is part of the skeleton associated with the phthaloyl group appears at δ = 71.9 ppm. Chemical shifts of

carbon rings C and D, including side chains, are not significantly altered as compared to those of β-sitosterol. Also, the high-resolution MS (HRMS) correspond to the molecular formula determined as 562.40 (calculated) and 562.82 (found) for $C_{37}H_{54}O_4$. Based on the above discussion with the help of spectroscopic studies, the structure of the compound is identified as 3β-sitosteryl hydrogen phthalate.

Effects of MW irradiation on the product formation have been studied in terms of the yield at different reaction times and temperatures. Reaction time and yield were also compared to the conventional procedure (CP), including the use of silica gel in conventional reaction. The reaction was charged at different reaction times (3-10 min) and also at different reaction temperatures (100-130 °C). The maximum yield (80 %) was obtained at 125 °C when the reaction was performed for 5 min under the MWI (Table 1). At these conditions, purity and completion of the reaction were checked by using thin layer chromatography (TLC), which showed unresolved (Un) spots suggesting degradation of the product after formation. The product yield was also confirmed using silica gel under reflux with stirring in the conventional procedure (CP). Product yield was approximately the same but decreased in time in the presence of silica gel (2 g) as compared to the reaction without silica gel (Table 1). It has been well established that silica gel absorbs water produced in the esterification reaction. Consequently, the reaction rate increased.

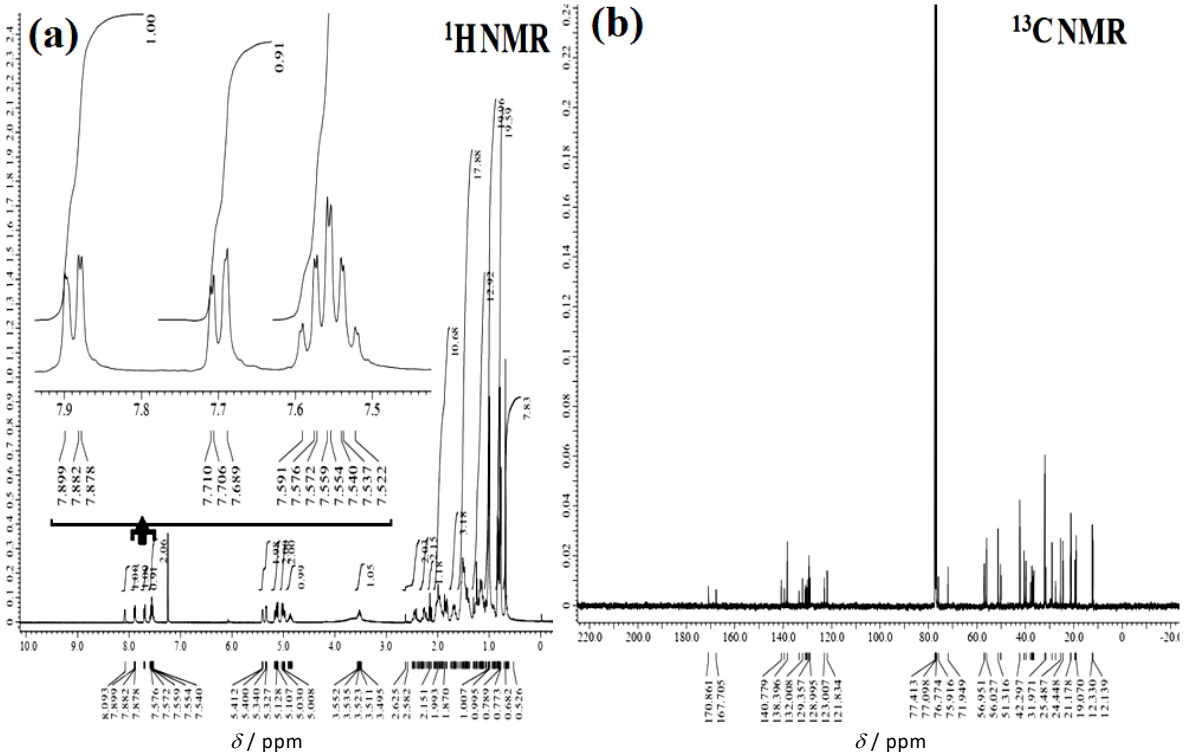


*Figure 2. NMR spectroscopy of the compound **2**: a) ^{1}H NMR spectrum; b) ^{13}C NMR spectrum*

Table 1. Effect of MWI synthesis conditions, reaction time and temperature, on the product yield including the effect of silica gel in the CP

Time, min	2	3	4	5	6	7	8
Yield, %	35	40	60	80	Un. multiple spots on TLC		
Temperature, °C	85	95	105	115	125	135	145
Yield, %	20	35	40	60	80	Degradation	
Conventional procedure (CP) without Silica gel	Yeld 60 % in 3 h under reflux						
Conventional procedure (CP) with Silica gel	Yeld 60-65 % in 2 h at 110 °C, with stirring						

3. 2. Antifungal activity screening

For evaluating the antifungal activity of the prepared compound against *Aspergillus niger* and *Candida albicans* fungi was carried out by the Kirby-Bauer Well Diffusion method. The inhibition zone was found around the wells filled with

the synthesized steroidal sitosteryl hydrogen phthalate by MWI at 125 °C (Table 2, Fig. 3). The result indicates that the modified sitosterol exhibited better antifungal activity against Aspergillus *niger* and *Candida albicans* strains compared to the starting compound, 3β-sitosterol and the standard drug as mentioned in literature [53-54]. In the conclusion of the experimental biological analysis, phthaloyl group linked to the sitosterol skeleton at the 3-position showed an increase in antifungal activity against the tested fungal strains.

Table 2. Antifungal activity of the synthesized steroidal compound 2 under MWI, the starting compound β-sitosterol and a standard drug; DMSO served as a negative control

Fungi	Inhibition zone diameter, mm			
	β-sitosteryl hydrogen phthalate	β-sitosterol	Standard drug Fluconazole (1 μg cm^{-3})	DMSO as NC
Aspergillus niger	18±0.17	15±0.15	18±0.23	NZI
Candida albicans	24±0.21	NZI	25±01.9	NZI

NZI: No zone of inhibition, NC: Negative control. The results are reported as mean ± SD (n = 3).

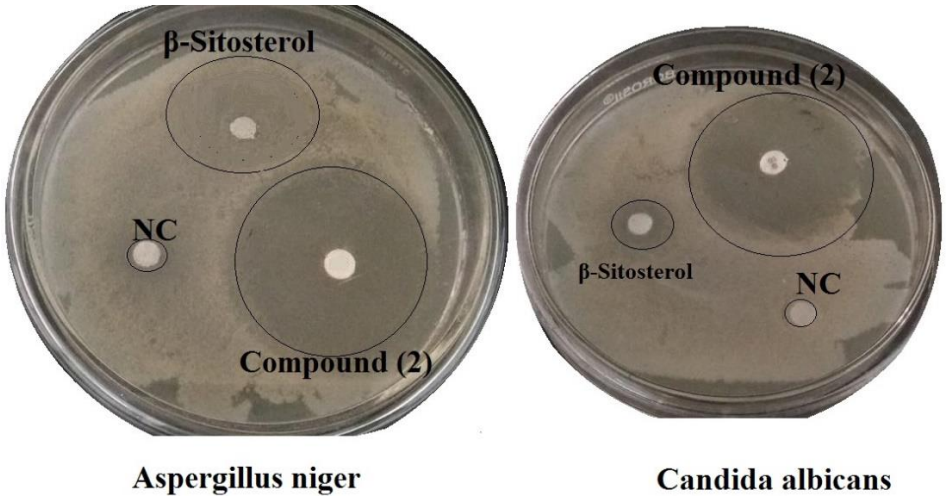


*Figure 3. Results of the antifungal activity screening of sitosteryl hydrogen phthalate (**2**) synthesized by microwave irradiation , 3β-sitosterol and negative control (NC)*

3. 3. Molecular docking analysis

Molecular docking was performed between β-sitosteryl hydrogen phthalate **2** (Fig. 4a) and the receptor CYP51 from *Candida spp*. (Fig. 4b) to investigate the binding pattern of the synthesized compound to receptor binding sites. It was shown that the compound is bound within the active site in the same way as the co-crystal ligand docked into the amino acid cavity of the target protein [48]. When the binding mode of β-sitosteryl hydrogen phthalate to the CYP51 of *Candida spp*. was developed, the best-high affinity model with the lowest binding energy (−11.0 kcal mol^{-1}) among the docked poses was assumed to have a favorable orientation within the CYP51 binding site. Such a model was selected and analyzed for visualization and interpretation of docking findings (Fig. 4). The docked pose of the synthesized compound was found to be inserted into the active pocket of the receptor and interacted with amino acids PHE380 and TYR118 by hydrogen bonds (Fig. 4c and d) as well as the groups of SER378, ILE379, PHE233, THR122, GLY307, GLY303, GLY308, LEU376, and GLY472 involved, so to make a pocket around the compound by van der Waals forces, which led to stabilization of the docked pose including π-sulfur interaction with MET508 (Fig. 4e) at the binding site. Other amino acids belonging to hydrophobic groups such as ILE304, ILE471, ALA476, CYS470, LEU204, PHE475, TYR132, LEU150, ILE131 and PHE126 are involved in alkyl and π-alkyl interactions. These interactions stabilize the compound in the proper orientation within the active pocket of the targeting protein and impede the growth of pathogenic fungi stains. A 2D docked model is represented in Figure 4e showing various amino acids and their interactions with the steroidal compound **2**.

Molecular docking is an important and widely used tool in the structure-based drug design (SBDD) because of its ability to predict the conformation of low molecular weight organic compounds within the specific target binding sites with a significant degree of precision. Structure-based modeling is accompanied by identification of a compound of a therapeutic interest in the

database. In general, biological activity data are connected to structural information once a ligand-receptor complex has been identified. In a molecular docking procedure, specific search algorithms perform quantitative predictions of binding energies, providing docked compound rankings based on the binding affinity of ligand receptor complexes [55-56]. In order to predict and compare antifungal activity of the steroidal compounds few of which were synthesized and published in the literature [57], the remaining compounds were hypothetically designed for SBDD. So molecular docking was performed in order to compare the binding profiles of the synthesized compound **2** and designed structures of eleven analog inhibitors having the substituted group at various position (*o*-, *p*- and *m*-) of the aromatic ring with the target receptor.

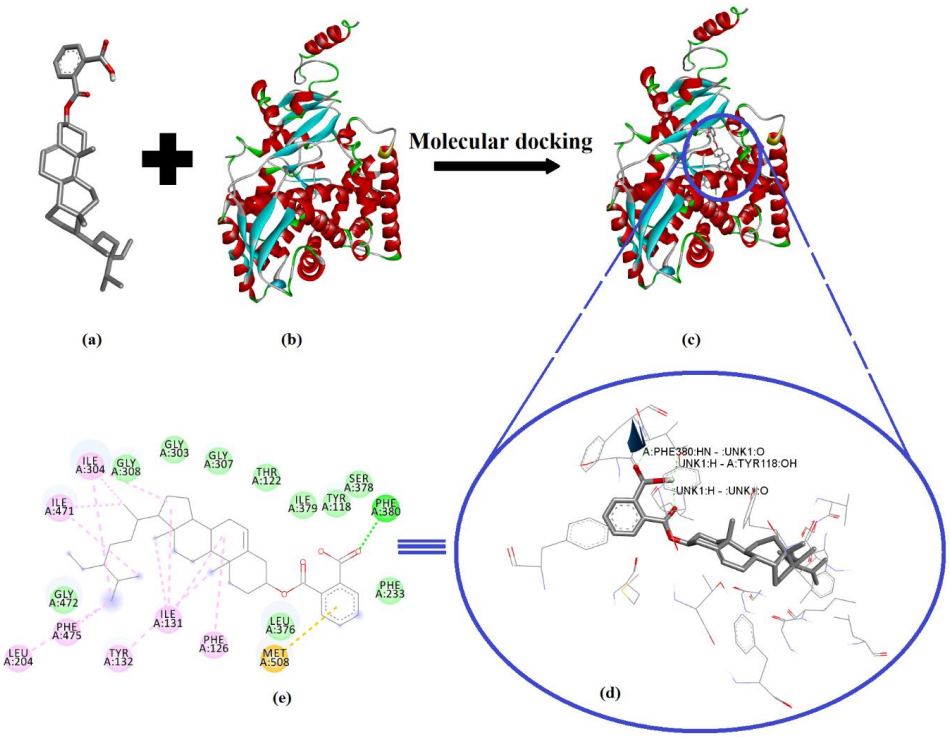


*Figure 4. Schematic representation of interactions of the compound **2** (sitosteryl hydrogen phthalate) with the targeting protein, CYP51 (PDB ID 5v5z); molecular docking interactions of (a) compound **2**, (b) receptor; (c) the best docked pose showing the active site where the compound **2** interacts; (d) the binding pose of the compound **2** to the receptor showing various interactions; (e) 2D interactions between the structure of CYP51 and the compound **2**.*

The molecular docking results are based on the docking score (-7.8 to -11.0 kcal mol^{-1}) and amino acids interacting with steroid compounds shown in Table 3 in order to classify the lead compound in an agreeable manner. As stated in Table 3, the binding affinity values of the steroidal derivatives in the study (SN 1 to 12) are between -7.8 and -11.0 kcal mol^{-1}. These values suggest that most steroidal compounds docked with different conformations into the cavity of the active receptor site (Fig. 5) displayed a rank of binding energies suggesting that the biological activity of all compounds can be categorized as high to moderate. The compound **2** (SN 1), as shown in Table 3, has the highest docking score against CYP51 (PDB ID 5v5z) as compared to the other designed structures (SN 2 to 12). This result is due to the involvement of the hydrogen bonding interaction between the carboxyl group of the compound **2** in the ortho position of the aromatic ring and the amino acid residue as shown in Figure 4. The participation of hydrogen bonds in designed steroidal compounds (SN 2-12) has not been identified or has not been properly coordinated so that the compounds are not very stable in the receptor cavity and lead to different levels of biological activity following the predicted order 1 > 12 > 2 = 11 > 3 > 9 > 6 > 7 > 10 > 4 > 8. Hydrophobic amino acid residues of the receptor (Table 4 and Fig. 4) wrapped the compound **2** at a proper distance *via* non-covalent interactions that lead to a good docking score followed by better fungal activity.

Quantitative Structure-Activity Relationship (QSAR) is typically studied and calculated by making slight changes to the lead structure to develop analogues followed by evaluation of the impact of these structural changes on biological

activity. QSAR was determined in a small series of twelve steroidal compounds with an aromatic ring bearing the -COOH, -OMe, -Br or $–NO_2$ group at different positions in the benzene ring, such as *ortho*-, *para*- or *meta*-position. There are several descriptors that were applied in the QSAR study, such as electronic characteristics, steric effects, solvent partitioning (Log P), and also molecular mass. Among these, the electronic effect of the substituted groups on the chemical skeleton has a unique role to assess stereochemistry and the binding constant stability, which are the most critical factors influencing drug activity [58]. The *ortho*-, *meta*- or *para*-substituted group on the aromatic ring is either electron withdrawing or electron donating group and its electronic effects within the molecule may influence its electron distribution, thus directly influencing how easily and permanently the ligand binds to its target receptor. In our study, -COOH, $-NO_2$, -Br and –OMe are at the *o*-, *p*- or *m*-position of the benzene ring and first two groups, carboxyl substituent and nitro substituent, are strongly electron withdrawing groups, the bromo substituent is a weak electron withdrawing group, while the methoxy substituent is electron releasing at the *para*-position but electron withdrawing at the *meta*-position according to the Hammett equation classification. It is clear from Table 3 that derivatives with the electron withdrawing character of the substituent exhibited higher docking scores than those with the electron donating groups. Also, when the methoxy substituent was shifted at the *para*-position, the docking score value decreased due to the changed electron withdrawing character to the electron donating indicating that electronic effects might not be exempt from molecular docking. Participation of the other descriptors such as steric effects, polarity, charge transfer phenomena, hydrogen bonding ability, etc. also plays an important role in determining whether the drug molecule is capable of getting close enough to its target site to bind, which, in turn, determines the docking score and biological character of the chemical compound.

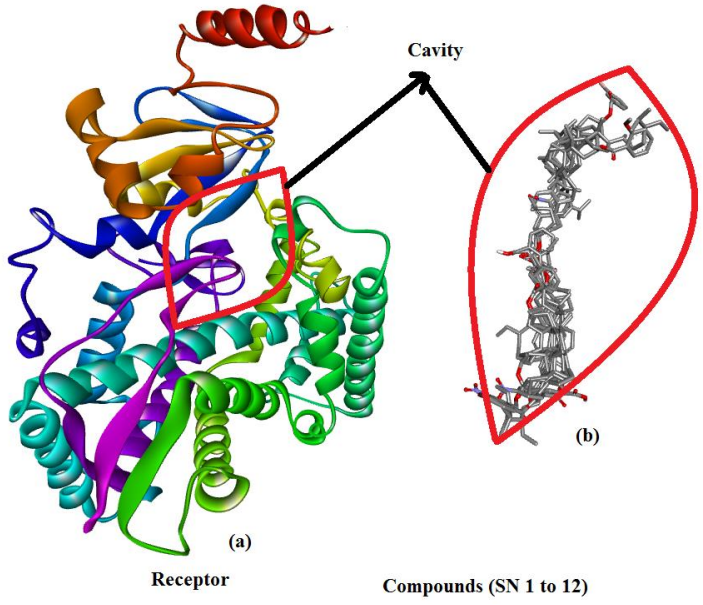
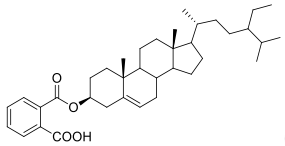
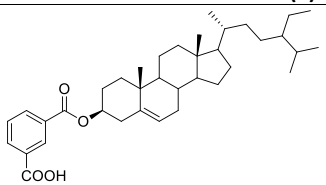
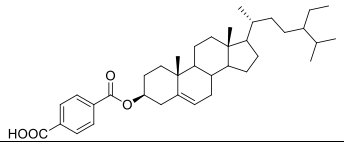
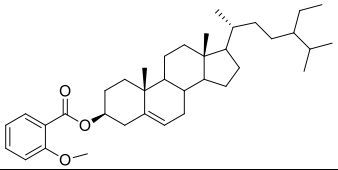
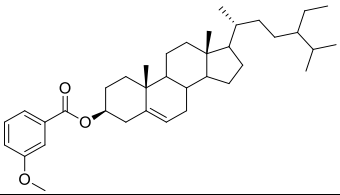
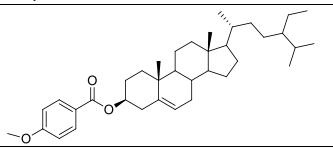
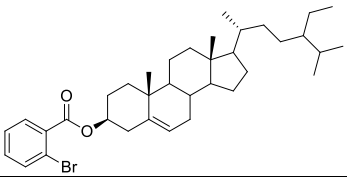
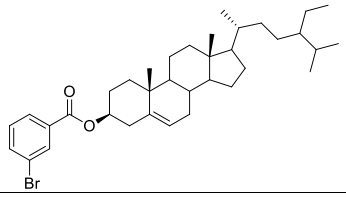
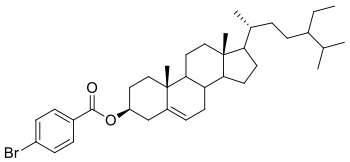
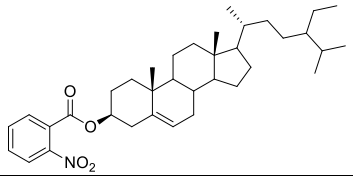
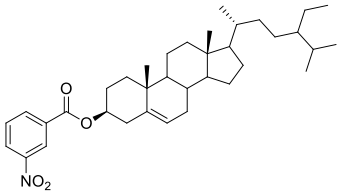
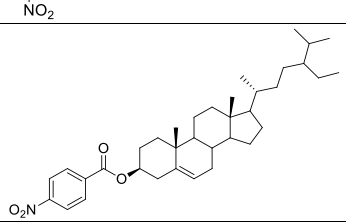


Figure 5. (a) Receptor with a cavity as a site for compounds binding and (b) 3D structures of the prepared compounds (SN 1 to 12 as shown in Table 3)

Table 3. Molecular docking results of the synthesized compound 2 and the designed structures of steroidal derivatives

SN	Compounds	$G_{binding}$ / kcal mol^{-1}	Compounds surrounded by interactive amino acid residues
1	 **(2)**	-11.0	PHE380, TYR118, SER378, ILE379, PHE233, THR122, GLY307, GLY303, GLY308, LEU376, GLY472, MET508, ILE304, ILE471, ALA476, CYS470, LEU204, PHE475, TYR132, LEU150, ILE131, PHE126
2		-10.6	GLN479, SER312, GLY308, GLY307, GLY303, ALA476, THR311, GLY472, ILE471, ILE304, PHE475, LEU204, TYR132, LEU121, THR122, LEU5786, PHE228, SER378, HIS377, PHE126, GLY307, PHE233, MET508, TYR118,

SN	Compounds	$G_{binding}$ / kcal mol^{-1}	Compounds surrounded by interactive amino acid residues
3		-10.5	SER378, ILE379, PHE380, MET508, PHE233, TYR118, VAL509, LEU376, TYR132, ILE131, PHE126, PHE228, GLY307, GLY303, ILE471, CYS470, LEU150, LEU204, PHE475, ILE304, GLY472, GLY308
4		-8.3	PHE235, TYR243, HIS242, HIS377, PHE233, PHE380, PRO230, SER506, LEU238, LEU240, ILE231
5		-10.3	MET508, SER378, ILE131, VAL509, PHE228, TYR132, GLY303, GLY307, LEU204, ALA146, LEU376, TYR118, HIS377, CYS470, PHE475, GLY472, LEU150, ILE304, GLY308, ILE471.
6		-9.3	GLN479, GLY308, GLY472, ILE471, CYS470, TYR118, GLY303THR311, LEU121, HIS377, SER378, LEU376, PHE233, THR122, MET508, TYR132, PHE228, GLN309, PHE475, ILE304, PHE126, GLY307
7		-8.9	PHE58, ALA61, GLY65, PRO230, LEU87, ILE231, TYR64, PHE233, LEU88, SER506, MET508, HIS377, SER378, LEU376, PHE380, TYR118
8		-7.8	PHE380, TYR505, PHE233, PRO230, HIS377, SER506, ILE231
9		-9.8	HIS377, SER3788, MET308, SER506, PRP230, PHE233, ILE231, TYR118, PHE380, LEU376, THR122.
10		-8.4	HIS242, LEU240, LEU238, PHE233, ILE231, PHE233, PHE380, TYR505, HIS377, SER506, PRO230, THR229, TYR243
11		-10.6	LEU121, MET508, TYR118, ILE304, GLY308, LEU204, ALA476, PHE475, GLY303, GLY307, ILE471, TYR132, ILE131, PHE126, THR122, LEU376, PHE228, SER378, PHE233. GLY472, SER312, GLN479, THR311
12		-10.9	PRO230, HIS377, PHE233, MET508, SER378, PHE380, LEU376, TYR118, TYR132, PHE126, THR122.

4. CONCLUSIONS

A biologically active steroidal compound, sitosteryl hydrogen phthalate, was prepared by MWI as well as by the conventional method and characterized using physicochemical techniques. The MWI technique proved to be advantageous in terms of being environmentally friendly, requiring less time and reagents and resulting in higher yields than the traditional process. The synthesized steroidal compound was screened against two fungal strains for the in-vitro antifungal activity. The molecular docking study of the sitosteryl hydrogen phthalate including the eleven designed steroidal derivatives was performed to elucidate mechanisms of the interaction with the active site of the targeting protein. The binding mode and free energy analysis of the synthesized and hypothesized compounds indicate that the synthesized steroid 2 has the potential to act as an effective inhibitor. The highest binding score (-11.0 kcal mol^{-1}) was determined for this compound binding effectively to the CYP51 structure from the pathogen *Candida albicans* and acting as a receptor that binds to Lanosterol 14α-demethylase to inhibit the proper fungal growth. Therefore, the docked 3β-sitosteryl hydrogen phthalate can be considered as a potential antifungal agent for further examination.

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SAŽETAK

Ispitivanje antimikotičnog dejstva i simulacija molekulskog dokinga β-sitosteril hidrogenftalata sintetisanog pod dejstvom mikrotalasnog zračenja na silicijum-dioksidu

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(Naučni rad)

U ovom radu je steroid sitosteril-hidrogenftalat sintetisan u reakciji 3β-sitosterola i anhidrida ftalne kiseline pod dejstvom mikrotalasnog zračenja, korišćenjem silikagela kao čvrste podloge. U poređenju sa konvencionalnom sintezom ovog steroidnog jedinjenja, koja podrazumeva upotrebu toksičnog rastvarača, sinteza pomoću mikrotalasnog zračenja obezbedila je veće prinose za kraće vreme reakcije. Jedinjenja dobijena primenom dva pomenuta postupka okarakterisana su infracrvenom spektroskopijom, nuklearnom magnetnom rezonancom (^{1}H i ^{13}C NMR) i masenom spektrometrijom visoke rezolucije. Ispitano je *in vitro* antimikotično delovanje sintetisanog jedinjenja na gljivice *Aspergillus niger* i *Candida albicans* difuzionim testom Kirbi-Bauera. Pored toga, sintetisano jedinjenje je modelovano radi određivanja najpovoljnijeg mesta vezivanja sa receptorom (CYP51) metodom molekulskog dokinga. Rezultati ispitivanja antimikotičnog dejstva i doking studija pokazali su da se sintetisani sitosteril-hidrogenftalat može smatrati pogodnim inhibitorom receptora CYP51 (lanosterol 14α-demetilaze). Pored toga, pristup molekulskog dokinga primenjen je i za dizajniranje hipotetičkih inhibitora derivata sitosteril-hidrogenftalata i poređenje rezultata sa simulacijama sintetisanog 3β-sitosteril-hidrogenftalata kao antimikotika.

Ključne reči: β-sitosterol; sitosteril-hidrogenftalat; antimikotično dejstvo; molekulski doking.