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Original article

Synthesis, *in-vitro* antitubercular, antifungal activities and *in silico* molecular docking study of chalcone derivatives from 1-(2'-hydroxyphenyl)-3-(substituted- phenyl)-2-propenone

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ABSTRACT

Background: Tuberculosis (TB) is one of the major global health problems and faced to the increased resistance of *Mycobacterium tuberculosis* strains against existing antitubercular agents, it is important to look for new antitubercular drugs. **Aims:** The goal of this work was to synthesize four chalcones derivatives from 1-(2'-hydroxyphenyl)-3-(substituted-phenyl)-2-propenone, then evaluate their antitubercular and antifungal activities by standard computational and biological methods. **Methods:** These chalcones were synthesized by the Claisen-Schmidt condensation and their structures have been determined by Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C) and Fourier Transform Infrared (FTIR) spectroscopy. The in vitro antimycobacterial and antifungal assays were carried out by the microdilution method against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294), $H_{37}Ra$ (ATCC 25177), and *Candida albicans* (MTCC 1637), respectively. The molecular docking of these chalcones was performed by AutoDock vina program using *Mycobacterial tuberculosis* Thymidylate Kinase (PDB ID: 1G3U) and dihydrofolate reductase (PDB ID 1AI9) as target ligand. **Results:** All synthesized chalcones showed good and moderated antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) with a range of MICs ranging from 4 to 64 µg/mL. Regarding antifungal activity, all synthesized chalcones were active against *Candida albicans* strains (MTCC 1637) with a range of MICs ranging from 16 to 128 µg/mL. Based on absorption, distribution, metabolism, and excretion (ADME) properties, all chalcones synthetized satisfied the Lipinski rule. **Conclusion:** The results suggest that the synthesized chalcones, especially the (E)-3-(4- (dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one could be used, after in vivo and clinical tests, like antitubercular and antifungal supplement or even replace current drug therapies.

Introduction

For decades, tuberculosis (TB) caused by the *Mycobacterium tuberculosis* has been one of the major global health problems [1]. According to recent reports from the World Health Organization (WHO), TB is responsible for the death of approximately 1.4 million patients in which 208,000

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cases (or 14.86%) were associated with HIV [1-3]. This is explained by the increased growth of strains of *Mycobacterium tuberculosis* which are multi-resistant (in the form of Multidrug-resistant tuberculosis (MDR-TB), extensive drug-resistant tuberculosis (XDR-TB), and, in rare instances, fully drug-resistant tuberculosis to existing drugs such as rifampicin, ethambutol, etc. [1,4,5] In the Democratic Republic of Congo, the total incidence number of Tuberculosis amounts to 305,000 cases with a mortality number of 49,200 in 2021 [2]. Faced to this alarming situation but also with the resistance of certain fungi to existing antifungal drugs, much scientific research is directed towards the discovery of new antitubercular compounds with other mechanisms of action against *Mycobacterium tuberculosis* and new antifungal agents [1-6].

Natural medical compounds have always been considered as an inspiration for the development of new antimycobacterial, antibacterial, antifungal, antiviral, antoxidants, anti-inflammatory drugs [7-13]. Among these natural medical compounds with many biological activities, there is also the class of chalcones, which are known as 1,3-diphenylprop-2-en-1- one, are the aromatic ketones and the enones that form a variety of biological agents and they considered the main precursors for flavonoids and isoflavonoids biosynthesis in plants [14-21]. They are widely distributed in nature (in plants, bacteries, fungi, etc.) and are generally synthesized in the laboratory from aromatic aldehydes and aliphatic aldehydes or ketones via the condensation reaction Claisen-Schmidt in the presence of base or acid catalysts [17–22]. Chalcones have several biological activities such as antibacterial, antioxidant, anti-inflammatory, antiviral, antifungal, anti-ulceral, antimalarial, antileishmanial, anticancer, antitubercular, antihyperglycemic, anti-HIV, carboxygenase inhibitor, insecticidal, ect. And according to the literature these activities are due to the presence of the reactive function α,β-unsaturated keto present in the molecule [23-29]. According to the literature, chalcones have very good antioxidant activity which allows them to inhibit the *Mycobacterium tuberculosis* protein tyrosine phosphatases (PtpA and PtpB), 2-transenoyl-acyl carrier protein reductase (InhA), Fatty acid synthase type-II (FAS-II) gold Decaprenyl-phosphoribose 20- epimerase (DprE1) enzymes and therefore may underlie the antitubercular activity [1,5,6]. Thus, the presence of hydroxyl group (-OH) on the aryl rings of chalcones is essential for their antioxidant and anti-tubercular activities. The goal of this work is to synthesize 4 chalcone derivatives from 1-(2'-hydroxyphenyl)-3-(substituted-phenyl)-2-propenone, then evaluate their antitubercular and antifungal activities by standard computational and biological methods.

Materials and Methods

Chemical materials

All the starting materials, reagents and solvents were commercially obtained (Merck). Thin- layer chromatography was carried out on silica gel plates (Merck Kieselgel 60 F254) and visualized by UV light (254 nm). The melting points are determined using a Büchi M-565 melting point apparatus (Büchi Labortechnick AG). NMR spectra were obtained using a Jeol ECA 400 (400 MHz) and Lambda 400 NMR spectrometers. All chemical shifts are reported in ppm. FT-IR spectra were taken in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer and the values were represented as wavenumber in cm^{-1}.

General procedure for the synthesis of chalcone derivatives from 1-(2'- hydroxyphenyl)-3-(substituted-phenyl)-2-propenone

These 4 substituted chalcones from 1-(2'-hydroxyphenyl)-3-(substituted-phenyl)-2-propenone have been synthesized by Claisen-Schmidt reaction using Sodium hydroxide (NaOH) as catalyst in anhydrous ethanol according to the literature [17-19].

To a solution of 2-hydroxyacetophenones or substituted 2-hydroxyacetophenones (1 eq) in ethanol (2.5mL/mmol), sodium hydroxide (3eq) was added. After10 min, substituted benzaldehydes (1.2eq) was added and the mixture was stirred for 30 min at room temperature, then left to stand for 24 h. After cooling the reaction mixtures with ice, the mixture was neutralized carefully using 1N hydrochloric acid. The crude mixture was extracted with ethyl acetate, washed with water and brine afforded chalcones, which were purified by column chromatography using hexane: ethyl acetate as eluent to give four pure chalcones (E)-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, (E)-3-(4- chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one, (E)-3-(4-bromophenyl)-1-(2-hydroxy-3, 4-dimethoxyphenyl) prop-2-en-1-one and (E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en1-one. The purity of

these 4 synthesized chalcones was evaluated by using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) methods and these compounds have been characterized by nuclear magnetic resonance (NMR) and FT-IR.

Determination of Mycobacterial and antifungal activities

The cultures of *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) and $H_{37}Ra$ (ATCC 25177) were obtained from Kabinda center hospital (Democratic Rebublic of Congo) and inoculated in Middlebrook 7H9 enriched (Difco, Becton, NJ, USA) media supplemented with 10% ADC-Tween-80 (Bovine serum albumin, dextrose, 0.2% glycerol and 0.05% Tween-80) and OD600 of cultures was measured, followed by dilution to achieve ~10^6 CFU/ mL whereas the cultures of *Candida albicans (C. albicans)* (MTCC 1637) were inoculated on potato dextrose agar media (20 mL). The antimycobacterial and antifungal assays were carried out by the microdilution method according to literature [1,4,6].

Microdilution method

The MICs (concentration which completely inhibit bacterial or fungal) of the (E)-1-(2- hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, (E)-3-(4-chlorophenyl)-1-(2- hydroxyphenyl) prop-2-en-1-one, (E)-3-(4-bromophenyl)-1-(2-hydroxy-3, 4-dimethoxyphenyl) prop-2-en-1-one and (E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one against *Mycobacterium tuberculosis* $H_{37}Rv$(ATCC 27294), $H_{37}Ra$ (ATCC 25177) and *Candida albicans* (MTCC 1637) were determined using the modified microdilution technique as described by the literature [1,4,6].

Under aseptic conditions, 96 wells microplates were used. All the wells of microplate were filled with 100 µL of supplemented Middlebrook 7H9 broth (Or potato dextrose broth for *C. albicans*). Test solutions (128µg/mL) of the synthesized chalcones were prepared in sterile dimethyl sulphoxide (DMSO) and 50 µL of this test solution were serially diluted to 0.25 µg/mL in the microplate's wells. Finally, 100 µL (10^6 cfu/mL) of the inoculums were added to each well of the microplates. One well was used as negative control of the growth of the microorganisms in the medium; whereas 50 µL of the antibiotic (Ethambutol for antitubercular test and fluconazole antifungal test) was used as positive control. The covered microplates were incubated at 37°C for 5 days. To indicate growth, 25 µL of resazurin dissolved in water was added to the microplate's wells and incubated at 37°C for 24 hours. Minimum Inhibitory Concentration (MIC) was detected on basis of visual color change of culture medium from blue to pink [1,30]. The MIC was defined as the lowest concentration of sample that prevents a color change to pink. All experiments were performed in triplicates.

The Minimum Bactericidal Concentrations (MBCs) and the Minimum Fungicidal Concentration (MFC) were determined by subcultivation according the literature [6, 30,31]. Ten microliter (10 µL) of well's contents where there was no growth were placed in petri dish which restrained 100 µL of Middlebrook 7H9 broth (Or potato dextrose broth for *C. albicans*) and incubated for 18-24 hours at 37°C for mycobacterial strain and 72 hours for *C. albicans*. The lowest concentration with no visible growth was defined as MBC for Mycobacterial strain and MFC for *C. albicans*, indicating = 99.9% killing of the original inoculum. All tests were performed in triplicate.

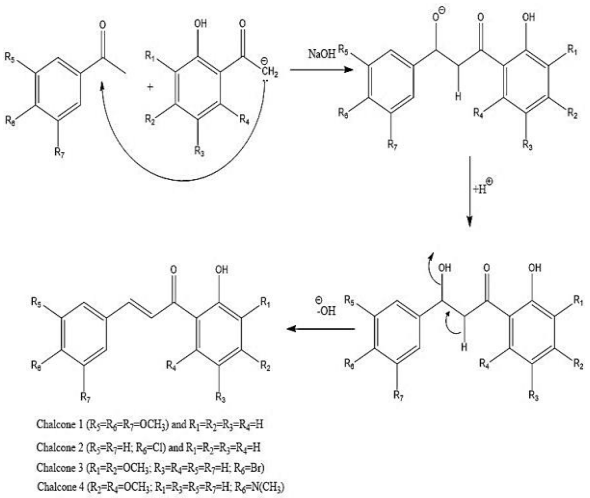
Molecular docking studies

The molecular docking of these 4 Chalcone derivatives from 1-(2'-Hydroxyphenyl)-3-(substituted-phenyl)-2-propenone on the target proteins of microorganisms was carried out by using Autodock vina program with standard protocol according the literature in order to understand the possible interactions between these compounds and the pathogenic microbial organisms [32]. The autodock tools were utilized for preparing the protein for docking. The polar hydrogen's, partial charges and Gastegier charges were added using these tools. The *Mycobacterial tuberculosis* target was Thymidylate Kinase (PDB ID: 1G3U) crystal structure of *Mycobacterium tuberculosis thymidylate kinase* complexed with thymidine monophosphate (tmp) whereas dihydrofolate reductase (PDB ID 1AI9) for *C. albicans*. These both target ligands were obtained from the protein data bank (http://www.rcsb.org).

In silico drug-likeness predictions

In *silico* drug-likeness helps to know whether a particular pharmacological agent has properties consistent with being an orally active drug. The properties of the of these 4 chalcone derivatives from 1-(2'-hydroxyphenyl)-3-(substituted-phenyl)-2-propenone were evaluated for their *in silico* parameters using Swiss ADME web [http://www.swissadme.ch/ (accessed on 20th December 2022).

Figure 1. General mechanism of the Claisen Schmidt reaction using sodium hydroxide (NaOH) as catalyst.



Chalcones synthesis

The characteristics, yield, physicochemical properties, FT-IR and NMR spectral data of 4 synthesized chalcones are represented in **table (1).**

The melting temperature and yield of these 4 synthesized chalcones were (156 ºC;87%), (150ºC; 81%), (152ºC; 70%) and (138ºC; 74%) for (E)-1-(2-hydroxyphenyl)-3-(3,4,5- trimethoxyphenyl)prop-2-en-1-one, (E)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1- one, (E)-3-(4-bromophenyl)-1-(2-hydroxy-3, 4-dimethoxyphenyl) prop-2-en-1-one and (E)-3- (4-(dimethylamino)phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one, respectively.

Mycobacterial (antitubercular) and antifungal activities

Antitubercular and antifungal activities of four synthesized chalcones from 1-(2'- hydroxyphenyl)-3-(substituted-phenyl)-2-propenone against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) and *C. albicans* (MTCC 1637) were determined by using the modified microdilution method. Ethambutol and fluconazole were used as reference compounds for Antitubercular and antifungal activity, respectively. Minimum Inhibitory Concentration, MFC and the ration MBC/MIC or MFC/MIC values are reported in **table (2).**

The concentrations of the test solutions were between 128µg/mL and 0.25µg/mL after serial dilution. The different chalcone test solutions exhibited varying degrees of antitubercular and antifungal activity.

The test solution of (E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (chalcone 4) exhibited the highest antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294), $H_{37}Ra$ (ATCC 25177) with the MICs of 4 µg/mL such as the standard (Ethambutol) followed by (E)-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (chalcone) with MIC of 8 µg/mL and 4 µg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294), and $H_{37}Ra$ (ATCC 25177), respectively. As for antifungal activity, both chalcones showed also the highest antifungal activity with the MIC of 16 µg/mL.

Molecular docking studies

The *in-silico* antitubercular activity results of synthetized chalcones (Ligands) against Thymidylate Kinase (PDB ID: 1G3U) crystal structure of *Mycobacterium tuberculosis thymidylate kinase* and the *in-silico* antifungal activity results of selected ligands against dihydrofolate reductase (PDB ID 1AI9) were reported in terms of binding energy and ligand interactions in order to predict the binding energy of ligands within the binding site of target proteins. These results are reported in **tables (3, 4)** and **figures (2 {a, b}; 3{a, b}).**

(E)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (chalcone 2) had high binding affinity (6.5 Kcal/mol) for Thymidylate kinase protein of *Mycobacterium tuberculosis* followed by (E)-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (chalcone 1) 6.5 Kcal/mol, whereas Ethambutol had binding affinity 4.1 Kcal/mol. The 3D and 2D interactions docking are shown **(Figures 2a, 2b).**

For dihydrofolate reductase (PDB ID 1AI9) of *C. albicans*, (E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (chalcone 4) had high binding affinity (6.8 Kcal/mol)followed by (E)-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (chalcone 1) 6.7 Kcal/mol, whereas fluconazole had binding affinity 7.3 Kcal/mol. The 3D and 2D interactions docking are shown in **figures (3a, 3b).**

***In-silico* drug-likeness predictions**

In silico drug-likeness helps to know whether a particular pharmacological agent has properties consistent with being an orally active drug. To be effective, a compound must have optimal hydrophilic and hydrophobic properties to carry in the blood before penetrating the cell membrane. A simple method for evaluating drug properties is to verify compliance with the Lipinski rule (rule of 5), which specifies the number of hydrophilic; molecular groups weight and hydrophobicity [33]. Lipinski rule proposed that drug target must have; the molecular weight (MW) ≤500, hydrogen bond acceptor (HBA)≤10, hydrogen bond donor (HBD)≤5, lipophilicity (logP)≤5. The absorption, distribution, metabolism and excretion (ADME) properties of synthetized chalcones are represented in **table (5).**

With regard to **Table 4**, all four chalcones synthetized satisfied the Lipinski rule, Ghose, Veber, Egan and muegge rule of five and also showed very good solubility because the logP is between 2 and 6 (2< log P<6). Thus, these derivative chalcones could be used as orally active drug.

Table 1. Characteristics of synthesized chalcones.

Compounds (IUPAC name)	Color	Yield(%)	Melting Point (ºC)	NMR Spectra data
(E)-1-(2-hydroxyphenyl)-3- (3,4,5-trimethoxyphenyl)prop-2-en-1-one	Yellow solid	87	156	^{1}H NMR (400 MHz, $CDCl_3$): δ 12.86 (s, OH), 7.92(dd, J= 1.52HZ, J= 8.21HZ, 1H), 7.84 (d, J= 15.21HZ, 1H), 7.53 (d, J= 15.21HZ, 1H), 7.48 (d, J= 8.21HZ, 1H), 7.03 (d, J= 8.82HZ, 1H), 6.94 (t, J= 8.21HZ, 1H), 6.88 (s, 2H), 3.93 (s, 6H, 2OCH_3), 3.91 (s, 3H, OCH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 193.59, 163.67, 153.61, 145.72, 140.91, 136.45, 130.13, 129.69, 120.09, 119.33, 118.89, 118.72, 108.02, 61.12, 56.35. IR (KBr, cm^1): 3433 (OH),
(E)-3-(4-				1636 (C=O), 1570 (CH=CH), 1127 (OCH_3) ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.75 (s, OH), 7.89(d, J=7.69, 1H), 7.84 (d, J= 15.11HZ, 1H), 7.55-7.63 (m, 3H), 7.49 (t, J= 7.41HZ, 1H), 7.39 (d, J= 8.26HZ,
chlorophenyl)-1-(2-hydroxyphenyl)	Golden yellow	81	150	2H), 7.02 (d, J= 8.26HZ, 1H), 6.94 (t, J=7.69 HZ, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 193.54, 163.71,
prop-2-en-1-one	Solid			144.01, 136.96, 136.65, 131.16, 129.88, 129.44, 120.65, 120.01, 118.99, 118.78. FR-IR (KBr, cm^{-1}):
(E)-3-(4-bromo				3435 (OH), 1647 (C=O), 1582 (CH=CH), 810 (C-Cl) ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.12 (s, OH), 7.82 (d,J= 15.49HZ, 1H), 7.67 (d, J=8.85, 1H), 7.49-7.59 (m,
phenyl)-1-(2-hydroxy-				5H), 6.54 (d, J= 8.85HZ, 1H), 3.96 (s, 3H, OCH_3),
3, 4-	Yellow			3.91 (s, 3H, OCH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ
dimethoxyphenyl)	Solid	70	152	192.23, 158.83, 158.43, 143.35, 136.85, 133.85,
prop-2-en-1-one				132.36, 129.99, 126.15, 125.15, 120.86, 115.61,
				103.03, 60.79, 56.27;
(E)-3-(4-				^{1}H NMR (400 MHz, $CDCl_3$): δ 14.03 (s, OH), 7.78(d, J=14.02, 1H), 7.70 (d, J= 9.18HZ, 2H), 7.50 (d,
(dimethylamino)phen				J=14.41, 1H), 6.67 (d, J= 9.18HZ, 2H), 6.04 (d, J=
yl)-1-(2-hydroxy-4,6-dimethoxyphenyl)	Dark orange	74	138	2.44HZ, 1H), 5.91 (d, J=2.44 HZ, 1H), 3.82 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.06 (s, 6H, 2xCH_3); ^{13}C
prop-2-en-1-one	Solid			NMR (400 MHz, $CDCl_3$): δ 192.64, 168.27, 166.18, 162.30, 161.17, 142.77, 129.86, 127.92, 125.18,
				114.37, 106.30, 93.38, 91.45, 55.78, 40.61, 40.28. IR
				(KBr); 3448 (-OH, Str), 1604 (C=O, Str), 1377 .22

Table 2. MIC, MBC (or MFC) and MBC/MIC or MFC/MIC of synthesized chalcones against the pathogenic bacteria by Microdilution assay.

Sample/ Standard	Microbial strains	Concentrations (µg/mL)								MIC (µg/mL)	MBC (µg/mL)	MFC (µg/mL)	MBC /MIC	MFC/ MIC
		12	64	32	16	8	4	2	1					
		8												
Chalcone	*Mt* $H_{37}Rv$	-	-	-	-	-	+	+	+	8	32	n.d	4	n.d
1	*Mt* $H_{37}Ra$	-	-	-	-	-	-	+	+	4	16	n.d	4	n.d
	C. albicans	-	-	-	-	+	+	+	+	16	n.d	64	n.d	4
Chalcone														
2	*Mt* $H_{37}Rv$	-	-	+	+	+	+	+	+	64	128	n.d	2	n.d
	Mt $H_{37}Ra$	-	-	-	+	+	+	+	+	32	64	n.d	2	n.d
	C. albicans	-	+	+	+	+	+	+	+	128	n.d	> 128	n.d	n.d
Chalcone														
3	*Mt* $H_{37}Rv$	-	-	-	-	+	+	+	+	16	64	n.d	4	n.d
	Mt $H_{37}Ra$	-	-	-	-	-	+	+	+	16	32	n.d	2	n.d
	C. albicans	-	-	-	+	+	+	+	+	32	n.d	64	n.d	4
Chalcone														
4	*Mt* $H_{37}Rv$	-	-	-	-	-	-	+	+	4	16	n.d	4	n.d
	Mt $H_{37}Ra$	-	-	-	-	-	-	+	+	4	16	n.d	4	n.d
	C. albicans	-	-	-	-	+	+	+	+	16	n.d	64	n.d	4
Ethambuto	*Mt* $H_{37}Rv$	-	-	-	-	-	-	+	+	4	16	n.d	4	n.d
l	*Mt* $H_{37}Ra$	-	-	-	-	-	-	-	+	4	8	n.d	2	n.d
Fluconazol	*C. albicans*	-	-	-	-	-	-	-	-	n.d	n.d	<1	n.d	n.d

(+) indicates microbial growth; (-) indicates no microbial growth; nd: not determined.

Table 3. Molecular docking simulation of synthetized chalcones (Ligands) against *Mycobacterium tuberculosis thymidylate kinase*.

Compounds	Binding affinity (Kcal/mol)	H-bond	Residual interactions					
			CH-bond	π-σ	π-alkyl	π- π	Metal-	π-cation
							Acceptor	π-anion
Chalcone	-6.3	Arg-A160,	Arg-A14	Ala-	Val-A17	-	-	-
1		Asp-A94,		A35				
		Thr-A33						
Chalcone	-6.5	Tyr-A39;	-	-	Arg-A160	-	Mg-A300	Glu-
2		Asp-A163						A166;
								His-A53
Chalcone	-5.9	Arg-A160	Phe-A36;	-	Ala-A35; Ala-A49;	Tyr-	-	-
3			Pro-A37;		His-A53	A39		
			Tmp-					
			A217					
Chalcone4	-5.7	Gly-A22	-	-	Lys-A19, Pro-A200; Ala-A204; Ala-A27,	-	-	-
					Ala-A208, Ala-A205,			
					Ala-A23			
Ethambutol	-4.1	So-A4220	TMP-A217	-	Val-A17, Arg-A14	-	-	-

Table 4. Molecular docking simulation of synthetized chalcones against dihydrofolate reductase (1AI9).

Compound	Binding affinity Kcal/mol)	H-bond	Residual interactions						
			CH-bond	π-σ	π-alkyl	VDW	π- π	Metal-Acceptor	**π-cation π-anion**
Chalcone 1	-6.7	-	Glu-B97, Lys-A192	Leu-B126	Arg-A191; Ile-B96, Leu- B100, Met- B1	Thr-A190, Glu-B120, Leu-B121, Asn- B124, Lys-B3, Ser-B125, Ser- A125, Asn-B101	-	-	-
Chalcone 2	-6.6	Tyr- A39; Asp-A163	-	-	Arg-A160	-		Mg-A300	**Glu-A166; His-A53**
Chalcone 3	-6.3	Ser-B95	Glu-B82	-	Arg-B79; Ala-A7; Arg-A108 Phe-A110, Phe-A167	-	His-A129	-	-
Chalcone 4	-6.8	Asn-A5	Val-A109 ; Arg-B79	-	Arg-A108;Ala-A7; Lys-A45, Phe-A167	-	-	-	**Glu-B82**
Fluconazo	-7.3	Val-	Arg-	-	His-	-	-	-	-

Table 5. ADME properties of synthetized chalcones.

Compound	Log P	MW (g/mol)	HBA	HBD
Chalcone 1	3.12	314.33	5	1
Chalcone 2	3.70	258.70	2	1
Chalcone 3	3.72	363.20	4	1
Chalcone 4	3.17	327.37	4	1

Figure 2. Three-dimensional and two-dimensional interactions of chalcone 2 (**2a**) and chalcone 4 (**2b**) with *Mycobacterium tuberculosis thymidylate kinase* protein amino acid residues.

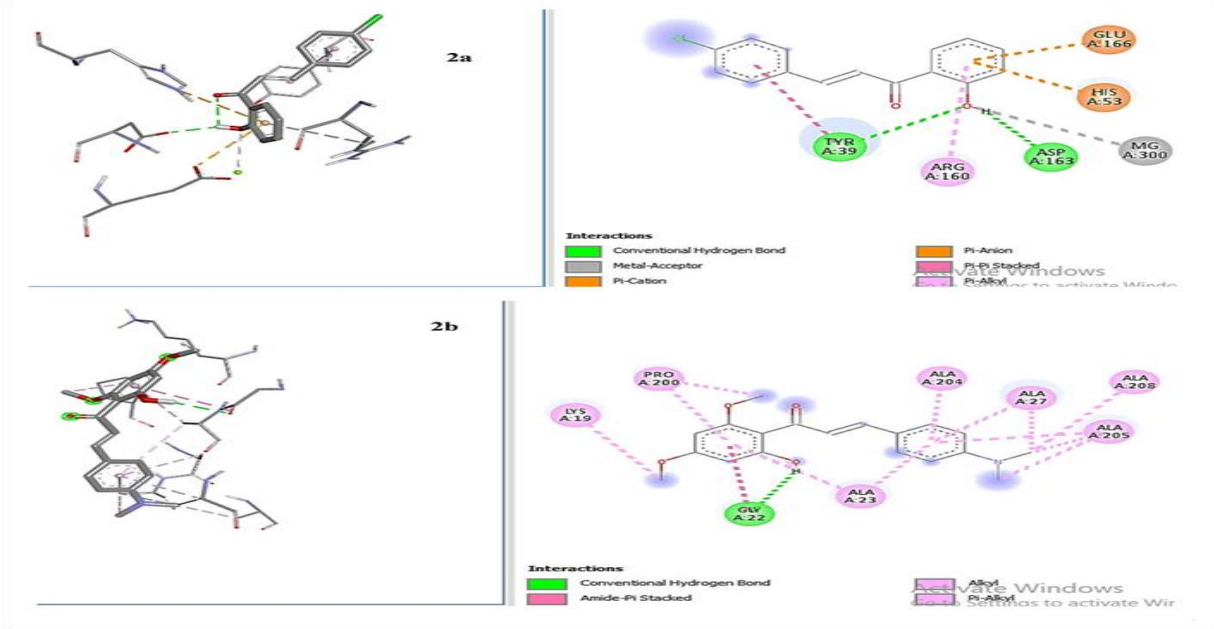
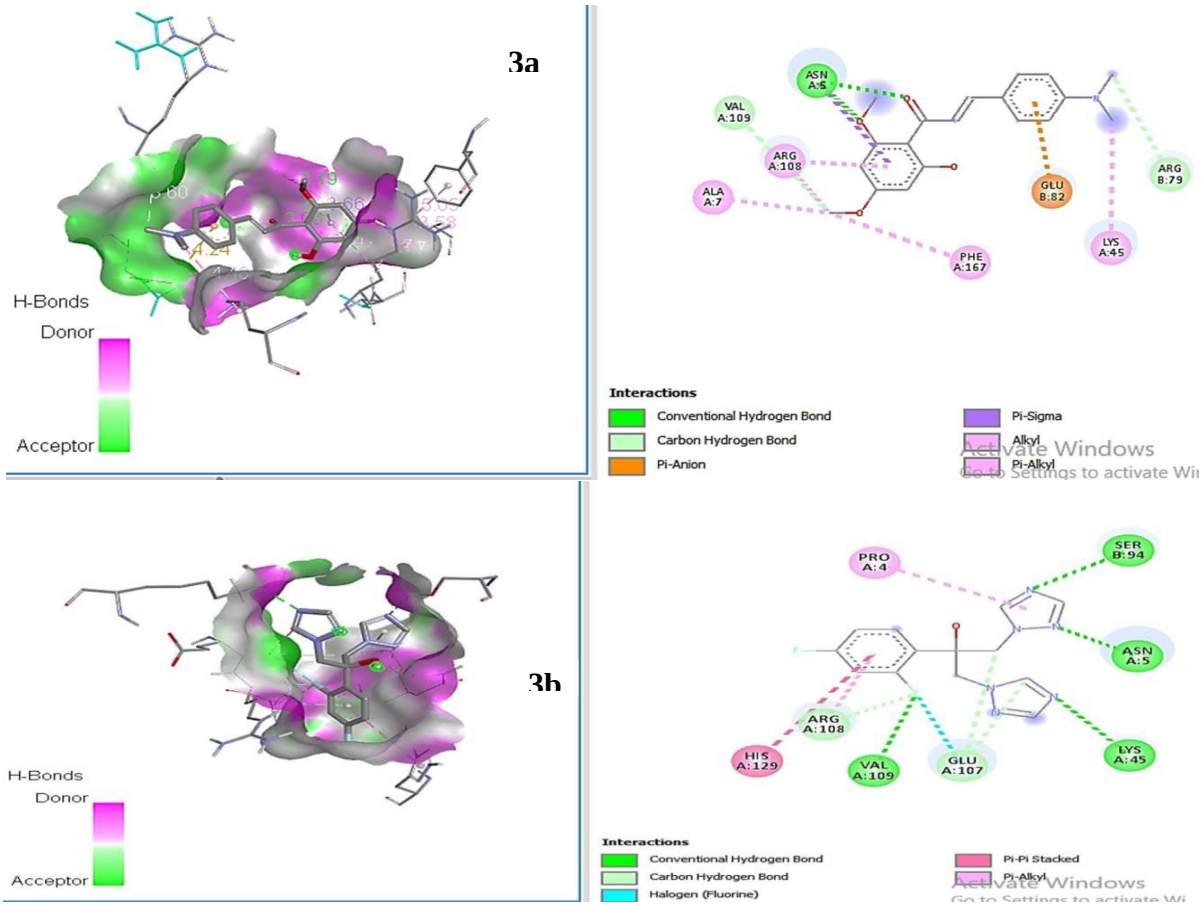


Figure 3. Three-dimensional and two-dimensional interactions of chalcone 4 (**3a**) and fluconazole (**3b**) with dihydrofolate reductase (PDB ID 1AI9.



Discussion

Tuberculosis is one of the major global health problems and faced to the increased resistance of *Mycobacterium tuberculosis* strains which are multi-resistant (in the form of multidrug-resistant tuberculosis (MDR-TB), extensive drug-resistant tuberculosis (XDR-TB), and, in rare instances, fully drug-resistant tuberculosis (TDR-TB) against existing antitubercular agents, it is important to look for new antitubercular drugs.

In this study, we synthetized four chalcones derivatives (E)-1-(2-hydroxyphenyl)-3-(3,4,5- trimethoxyphenyl)prop-2-en-1-one (Called chalcone1); (E)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (Called chalcone2); (E)-3-(4-bromophenyl)-1-(2-hydroxy-3, 4-dimethoxyphenyl) prop-2-en-1-one (Called chalcone3); and (E)-3-(4- (dimethylamino)phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en1-one (Called chalcone1).

^{1}H-NMR spectrum of these four synthesized chalcones (1,2,3 and 4) each revealed the singlet at δ 12.86; 12.17; 13.12 and 14.03 ppm, respectively. This corresponds to the proton of the hydroxyl group close to the carbonyl group which is shielded and this difference could be due above all to the presence of two methoxy groups in the last two synthetized chalcones (3 and 4) but also to the presence of the 3 methoxy groups (OCH_3) in the other benzene cycle of the first synthetic chalcone, either the chloro group (Cl-) in the second chalcone, either the bromo group (Br-) in the third chalcone or a dimethylamino [-N(CH_3)$_2$] group in the other benzene of the fourth synthesized chalcone. The 3 protons of the two methoxy groups of the third synthetic chalcone have the chemical shift (δ) of 3.91 and 3.96 ppm, respectively whereas for those of the other two of the fourth chalcone, it is 3.82 and 3.79 ppm certainly because of their distance from the hydroxyl group but also from their position away from each other. The 6 characteristic protons of the fourth chalcone from two dimethylamino methyl groups have a chemical shift of 3.6 ppm. The infrared spectrum confirms the presence of the carbonyl group at 1636 and 1647 cm^{-1} and that of the hydroxyl group at 3433-3435 cm^{-1}.

The MIC is the lowest concentration of the extract at which no microbial survive. All synthesized chalcones showed good and moderated antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) with a range of MIC ranging from 4 to 64 µg/mL. However, the (E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (chalcone 4) and (E)-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl) prop- 2-en-1-one (chalcone 1) showed excellent activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) with Minimum Inhibitory Concentration (MIC) of 4; 4 and 8; 4 µg/mL, respectively. These Minimum Inhibitory concentration MIC values were the same to those of Ethambutol which is used as the standard antitubercular. This could be explained by the structure of these synthesized compounds that have electron donating groups (-OH, -OCH_3, -NCH_3) in their Aryl rings A and B. According the literature, the presence of electron donating (-OH, -OCH_3, -NCH_3, etc.) or electron withdrawing groups (-F, -Cl, -Br, etc.) in -ortho, -meta and para-aryl, enhance the penetrability of the compounds and support in the antitubercular activity of chalcones [1,34]. On the other hand, the chalcones 3 and 2 which have electron withdrawing groups (-Cl and - Br) in para position of the ring B, showed also the good antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) with the MIC of 16; 16 and 64; 32 µg/mL, respectively. Although having all the halogenated electron withdrawing groups (-Br and -Cl) in para-position of the ring B, chalcone 3 showed good anti-tubercular activity than that of chalcone 2 because it also has two methoxy electron donating groups in Meta and Para-position of ring A.

Minimum bactericidal concentration (MBC) of a test solution is the lowest dilution level needed to completely inhibit bacterial growth and it depends on the solvent and the bacteria. All synthesized chalcones showed moderate bactericidal activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (virulent strains ATCC 27294), $H_{37}Ra$ (the attenuated strains ATCC 25177) with MBC ranging from 16 to 128 µg/mL and their ratios of MBC/MIC are below 4. This is a clear indication of their large bactericidal activity according the literature [9].

Regarding antifungal activity, all synthesized chalcones were active against *Candida albicans* strains (MTCC 1637) with a range of MIC ranging from 16 to 128 µg/mL and their minimum fungicidal concentration (MFC) were 64 µg/mL for (E)-1-(2-hydroxyphenyl)-3- (3,4,5-trimethoxyphenyl) prop-2-en-1-one (chalcone 1), (E)-3-(4-bromo phenyl)-1-(2- hydroxy-3, 4-dimethoxyphenyl) prop-2-en-1-one (chalcone 3) and (E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (chalcone 4). Whereas for (E)- 3-(4-chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (chalcone 2), the MFC was over 128 µg/mL. Their ratios of MFC/MIC are below to 4 except for chalcone 2. This is a clear indication of their Fungicidal activity.

The in *silico* antitubercular results indicated that all synthesized chalcones (1–4) showed strong binding affinity (ranges from −5.7 to −6.3, given in Table 3) towards the amino acid residues in active pocket Thymidylate Kinase (PDB ID: 1G3U) protein through H-bond and residual interactions, compared to standard drug Ethambutol (-4.1 Kcal/mol). Chalcone 2 [(E)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one] showed better affinity 6.5 Kcal/mol and interacted with Tyr-A39; Asp-A163 amino acid residue through the H-bond and with Arg-A160, Glu-A166; His-A53 amino acid residues through residual interaction such as π-alkyl, metal Acceptor, π-cation and π-anion **(Figure 2a).** This could be explained by the presence of the electron withdrawing group (Cl-) in the aromatic ring.

Chalcone 4 [(E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en- 1-one], which exhibited good anti-tubercular activity, also showed good affinity by interacting with 8 target protein amino acids including Gly-A22 through H-bond interaction. And the seven other amino acids of the target protein (Lys-A19, Pro-A200; Ala-A204; Ala-A27, Ala- A208, Ala-A205, Ala-A2) by π-alkyl interaction **(Figure 2b).** Whereas the antitubercular drug reference (Ethambutol) was only interacting with 4 target protein amino acids, two (Val-A17, Arg-A14) of which by residual interaction (π-alkyl interaction).

The in *silico* antifungal results indicated that all synthesized chalcones (1–4) showed strong binding affinity (ranges from −6.3 to −6.8, given in **table (4)** towards the amino acid residues in active pocket dihydrofolate reductase (PDB ID 1AI9) protein through H-bond and residual interactions.

The Chalcone 4 showed H-bond interactions with Asn-A5 and residual interactions for Val-A109; Arg-B79; Arg-A108; Ala-A7; Lys-A45, Phe-A167; and Glu-B82 **(Figure 3a)** but the standard drug Fluconazol has H-bond interaction with Val-A109,Lys-A45, Asn-A5,Ser-B94 amino acid residues and has residual interaction (CH-bond and π-alkyl) with Arg-A108, Glu-A107, His-A129,Pro-A4 amino acid residues **(Figures 3b).** Chalcone 1 showed also a good affinity against dihydrofolate reductase (6.7 Kcal/mol) with only residual interactions (Glu-B97, Lys-A192, Leu-B126, Arg-A191; Ile-B96, Leu-B100, Met-B1, Thr- A190, Glu-B120, Leu-B121, Asn-B124, Lys-B3, Ser-B125, Ser-A125,Asn-B101) **(Table 4).**

Based on absorption, distribution, metabolism and excretion (ADME) properties, all chalcones synthetized satisfied the Lipinski rule, Ghose, Veber, Egan and muegge rule of five and also showed very good solubility because the logP is between 2 and 6 (2< log P<6). The results suggest that the synthesized chalcones, especially the (E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (Chalcone 4) could be used, after in vivo and clinical tests, like antitubercular and antifungal supplement or even replace current drug therapies.

Conclusion

Our findings indicate that these four chalcone derivatives synthetized from 1-(2'-Hydroxyphenyl)-3-(substituted-phenyl)-2-propenone have good antitubercular and antifungal activities against *Mycobacterium tuberculosis* (H_{37}Rv virulent strains ATCC 27294, H_{37}Ra the attenuated strains ATCC 25177) and *Candida albicans* strains (MTCC 1637). In addition, the in *silico* antitubercular and antifungal results indicated that all synthesized chalcones (1–4) showed strong binding affinity towards the amino acid residues in active pocket Thymidylate Kinase (PDB ID: 1G3U) and dihydrofolate reductase (PDB ID 1AI9) protein through H-bond and residual interactions. These data support the idea that the synthesized chalcones, especially the (E)-3-(4-(dimethylamino) phenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2- en-1-one (Chalcone 4) could be used, after in vivo and clinical tests, like antitubercular and antifungal supplement or even replace current drug therapies.

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Conflict of interest

There are no conflicts of interest.

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