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Original Article

Substituted Tetrahydrocarbazole Based on Indomethacin and Diclofenac with Heterocyclic Compound, Synthesis, Spectral and Antimicrobial Studies

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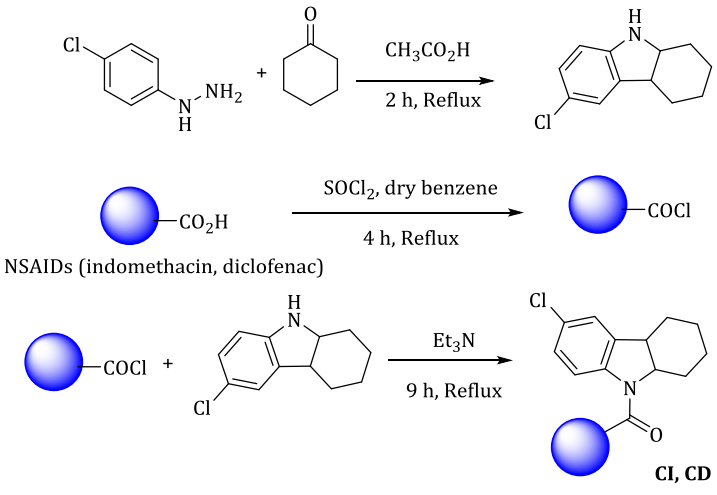
ABSTRACT

New 6-chloro-1,2,3,4-tetrahydrocarbazole compounds were prepared from the reaction of cyclohexanone with 4-chlorophenylhydrazine. The synthesis of heterocyclic compounds was carried out through the reaction of cyclohexanone with 4-chlorophenylhydrazine, then the produced compound was treated with two NSAIDs (indomethacin, diclofenac) by amide bond formation to form N-substituted THCZ by NSAIDs. The characterization of prepared compounds was identified using the ^{1}H, ^{13}C NMR and FT-IR spectroscopies. The antimicrobial activity of the synthesized compounds was tested *in vitro* to discover a good activity as antifungal and moderate as antibacterial, which was confirmed by the docking study of the compounds.

GRAPHICAL ABSTRACT



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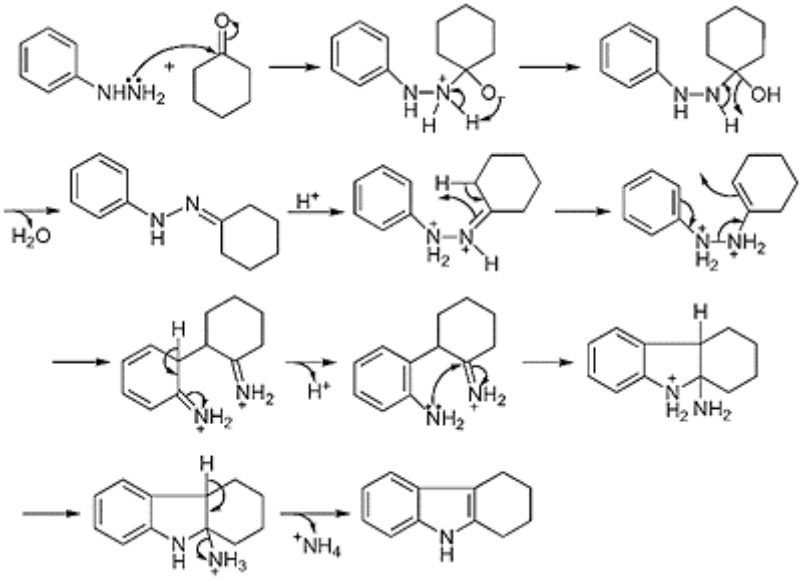
Introduction

This research was motivated by the rise in antimicrobial resistance worldwide and the inadequate generation of novel antimicrobial drugs [1]. It focused on the synthesis and antimicrobial activity of compounds produced by combining two biologically active compounds (NSAIDS and THCZ).

THCZ and Carbazole ring scaffolds are present in some alkaloids [2], they have a variety of biological effects such as antiviral [3], antitumor [4], antibacterial [5, 6] and broad-spectrum antifungal activity [7,8].

NSAIDs are considered as non-antibiotic drugs reported to display antibacterial activity [9], Some NSAIDs such as indomethacin and diclofenac have been shown to have synergistic effect with particular antimicrobial drugs [10]. Some NSAIDS like Diclofenac have an inhibitory impact on some bacteria like *S. aureus* [11].

The compounds 6-chloro-1,2,3,4-tetrahydrocarbazole (symbolized as 6C) and NSAID acid chloride reacted under reflux for 9 hours in the existence of triethylamine to form 6-chloro-1,2,3,4-tetrahydrocarbazole substituted at the heteroatom (N) by NSAIDs [12] as shown in Scheme 2. 6C was prepared by Borsche-Drechsel Reaction (Scheme 1) involving the addition of 4-chlorophenylhydrazine to cyclohexanone in acidic media (glacial acetic acid) with reflux for 2 hours [13]. NSAIDs (indomethacin and diclofenac) reacted with Thionyl chloride under reflux for 4 hours to produce NSAIDs acid chloride [14].



Scheme 1: Proposed mechanism of Borsche-Drechsel Reaction

Materials and Methods

Cyclohexanone and Benzene of ROMIL limited (Cambridge, UK), Indomethacin was obtained from SAFA Pharmaceutical Industries (Iraq), Diclofenac from Sama Al Fayhaa pharmaceutical industries (Iraq), 4-chlorophenylhydrazine from Hangzhou Hyper Chemicals limited (China), Thionyl chloride and methyl alcohol of CDH limited (India) and Diethyl Ether of Alpha Chemicals Private Limited (India). Other materials included the culture media (Sabouraud Dextrose Agar and Mueller-Hinton agar) of CONDA Pronadisa (Madrid, Spain), Ciprofloxacin disc of TMMEDIA (India) and DMSO of Alpha Chemicals Private Limited (India).

The Equipments used in this work included Hot plate stirrer of IKA (Germany), IR spectrometer of Shimizu (Japan), melting point system of Stuart (United Kingdom), Oven of Astell Hearso (England), Rotary evaporator of Bushi (Switzerland), ^{13}C NMR spectrophotometer of Varian inova (USA), ^{1}H-NMR spectrophotometer of Varian inova (USA).

Preparation of the compound 1-(6-chloro-1,2,3,4-tetrahydro-9H-carbazol-9-yl)-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) ethan-1-one symbolized as (CI)

The compound (CI) was prepared by adding (0.01 mole, 3.7 g) of indomethacin acid chloride in 20 mL dry benzene and 1 mL triethylamine with stirring to (2 g, 0.01 mole) 6-chloro-1,2,3,4-tetrahydrocarbazole in 30 mL dry benzene, as shown in Scheme 2. The mixture was refluxed for 9 hours and after the distillation of the solvent the precipitate was washed with 5% sodium bicarbonate and water [12].

Acid chloride of Indomethacin was prepared (as in Scheme 2) by addition of (0.01 mole, 0.7 mL) thionyl chloride slowly to (0.025 mole, 8.9 g) indomethacin solubilized in 25 mL dry benzene, the mixture was refluxed for 4 hour, left to dry, washed with 5 mL diethyl ether and dried to produce indomethacin acid chloride [14].

The compound 6C was prepared by addition of (7.1 g, 0.05 mole) 4-chloroPhenylhydrazine during one hour to a solution of (0.05 mole, 5.2 mL) cyclohexanone in 17 mL acetic acid under reflux with stirring. The mixture was refluxed for one more hour, and filtered. The solid crude was washed by water and 75% methyl alcohol and dried to obtain 6-chloro-1,2,3,4-tetrahydrocarbazole [15].

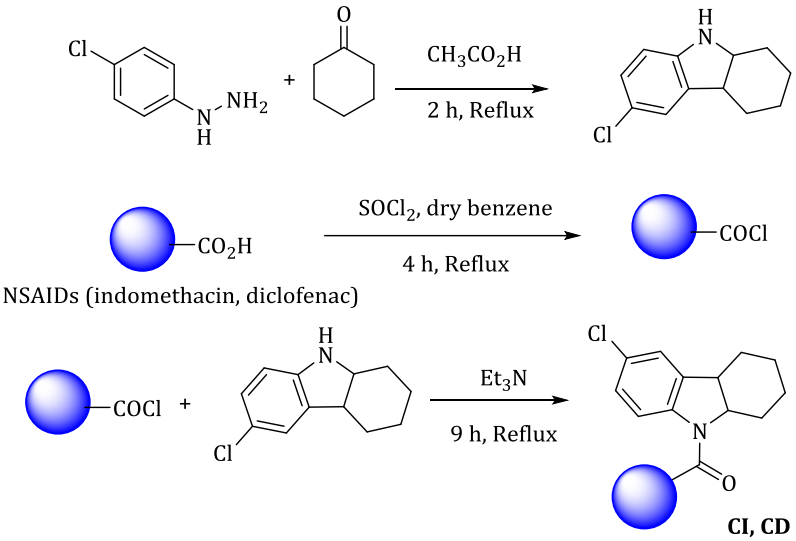
Preparation of the compound 1-(6-chloro-1,2,3,4-tetrahydro-9H-carbazol-9-yl)-2-(2-((2,6-dichlorophenyl) amino) phenyl) ethan-1-one Symbolized as (CD)

The compound (CD) was prepared by adding (0.01 mole, 3.1 g) of diclofenac acid chloride in 20 mL dry benzene and 1 mL triethylamine with stirring to (2 g, 0.01 mole) 6-chloro-1,2,3,4-tetrahydrocarbazole in 30 mL dry benzene. The mixture was refluxed for 9 hours. After the distillation of the solvent the precipitate was washed with 5% sodium bicarbonate and water [12].

Acid chloride of diclofenac was prepared by addition of (0.01 mole, 0.7 mL) thionyl chloride slowly to (0.025 mole, 7.4 g) diclofenac solubilized in 25 mL dry benzene. The mixture was refluxed for 4 hours, left to dry, washed with 5 mL diethyl ether and dried to produce diclofenac acid chloride [14].

Results and Discussion

The prepared compounds were summarized in the following Scheme 2.



Scheme 2: The general rout of compound synthesis

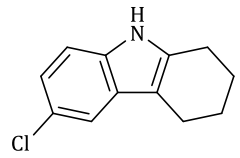
Identification of the compounds

The synthesized compounds were identified by ^{1}H, ^{13}C NMR and IR spectroscopies.

The ^{1}H NMR spectroscopy interpretation of compound 6-chloro-1,2,3,4-tetrahydrocarbazole

The ^{1}H NMR spectrum of the compound 6C Figure 3S displays Chemical shifts. The interpretation of these spectra was summarized in Table 1.

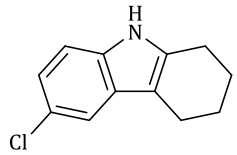
Table 1: [1]H NMR spectroscopy interpretation of the compound 6C

Chemical structure	Chemical shift	NO. of H	splitting	Interpretation
	7.35	1	singlet	Proton of benzene ring
	6.99	1	doublet	Proton of benzene ring
	7.26	1	doublet	Proton of benzene ring
	10.86	1	singlet	Proton of NH group
	2.72	2	triplet	Proton of CH2 group (Allylic)
	1.78	2	quintet	Proton of CH2 group (aliphatic)
	1.82	2	quintet	Proton of CH2 group (aliphatic)
	2.69	2	triplet	Proton of CH2 group (Allylic)

The [13]C NMR spectroscopy interpretation of the compound 6C

The [13]C NMR spectrum of the compound 6C Figure 4S displays Chemical shifts. The interpretation of these spectra was summarized in Table 2.

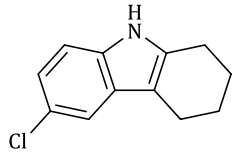
Table 2: 13C NMR spectroscopy interpretation of the compound 6C

Chemical structure	Chemical shift	Interpretation
	116.85	Aromatic Carbon
	123.13	Aromatic Carbon
	120.19	Aromatic Carbon
	112.33	Aromatic Carbon
	134.54	Aromatic Carbon
	136.96	Aromatic Carbon
	23.30	Allylic Carbon
	23.18	Aliphatic Carbon
	23.23	Aliphatic Carbon
	20.90	Allylic Carbon
	108.58	Aromatic Carbon
	128.92	Aromatic Carbon

The IR spectroscopy interpretation of the compound 6C

The IR spectrum of the compound 6C Figure 5S displays bands; the interpretation of these spectra was summarized in Table 3.

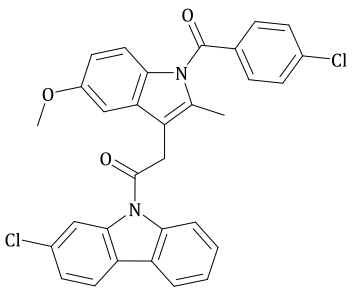
Table 3: IR spectroscopy interpretation of the compound 6C

Chemical structure	Band	Interpretation
	3404.88	NH band of secondary amine
	2938.37	Aromatic -H
	2905.99	Asymmetric H of cyclohexane
	2842.81	Symmetric H of cyclohexane
	1578.54	Aromatic C=C

The [1]H NMR spectroscopy interpretation of compound CI

The [1]H NMR spectrum of the compound CI Figure 6S displays Chemical shifts; the interpretation of these spectra was summarized in Table 4.

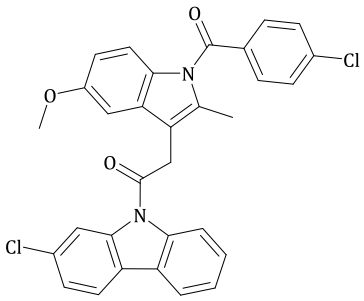
Table 4: ^{1}H NMR spectroscopy interpretation of the compound CI

Chemical structure	Chemical shift	NO. of H	splitting	Interpretation
	7.25	1	s	Proton of benzene ring
	7.05	1	d	Proton of benzene ring
	7.34	1	d	Proton of benzene ring
	2.60	2	t	Proton of CH_2 group (Allylic)
	1.84	2	q	Proton of CH_2 group (Aliphatic)
	1.85	2	q	Proton of CH_2 group (Aliphatic)
	2.69	2	t	Proton of CH_2 group (Allylic)
	3.67	2	s	Proton of CH_2-CO
	6.93	1	s	Proton of benzene ring
	3.77	3	s	Proton of CH_3-O
	6.71	1	d	Proton of benzene ring
	7.37	1	d	Proton of benzene ring
	7.69	2	t	Proton of benzene ring
	7.66	2	t	Proton of benzene ring
	2.23	3	s	Proton of CH_3 group(Allylic)

The ^{13}C NMR spectroscopy interpretation of the compound CI

The ^{13}C NMR spectrum of the compound CI Figure 7S displays Chemical shifts; the interpretation of these spectra was summarized in Table 5.

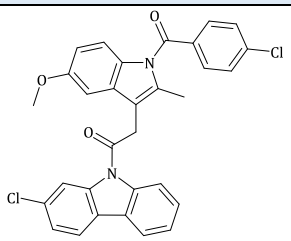
Table 5: ^{13}C NMR spectroscopy interpretation of the compound CI

Chemical structure	Chemical shift	Interpretation
	116.85	Aromatic Carbon
	123.11	Aromatic Carbon
	120.19	Aromatic Carbon
	113.95	Aromatic Carbon
	131.61	Aromatic Carbon
	136.97	Aromatic Carbon
	23.29	Allylic carbon
	23.16	Cyclic carbon
	23.21	Cyclic carbon
	20.89	Allylic carbon
	111.76	Aromatic Carbon
	128.78	Aromatic Carbon
	172.56	Carbonyl Carbon
	30.04	Next to carbonyl
	108.58	Aromatic Carbon
	134.53	Aromatic Carbon
	102.18	Aromatic Carbon
	156.01	Aromatic next to oxygen
	112.34	Aromatic Carbon
	115.04	Aromatic Carbon
	128.91	Aromatic Carbon
	134.63	Aromatic Carbon
	167.54	Carbonyl Carbon
	130.68	Aromatic Carbon
	131.22	Aromatic Carbon
	129.53	Aromatic Carbon
	138.07	Aromatic Carbon
	55.87	Aliphatic next to Oxygen
	13.67	Aliphatic Carbon

The IR spectroscopy interpretation of the compound CI

The IR spectrum of the compound CI Figure 8S displays bands; the interpretation of these spectra was summarized in Table 6.

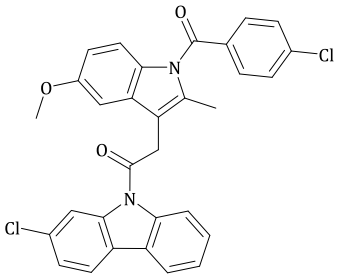
Table 6: IR spectroscopy interpretation of the compound CI

Chemical structure	Band	Interpretation
	3088.69	Aromatic C-H
	3034.66	Aromatic C-H
	2938.29	Asymmetric H
	2842.18	Symmetric H
	1579.12	Aromatic C=C
	1676.64	Carbonyl
	1696.90	Carbonyl

The [1]H NMR spectroscopy interpretation of the compound CD

The [1]H NMR spectrum of compound CD Figure 9S displays Chemical shifts; the interpretation of these spectra was summarized in Table 7.

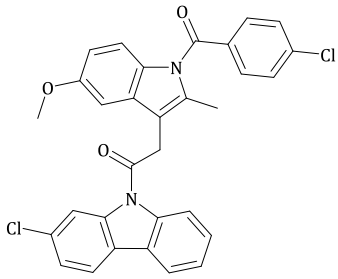
Table 7: [1]H NMR spectroscopy interpretation of the compound CD

Chemical structure	Chemical shift	NO. of H	splitting	Interpretation
	7.2	1	s	Proton of benzene ring
	7.21	1	d	Proton of benzene ring
	7.33	1	d	Proton of benzene ring
	2.7	2	t	Proton of CH_2 group (Allylic)
	1.78	2	q	Proton of CH_2 group (Aliphatic)
	1.83	2	q	Proton of CH_2 group (Aliphatic)
	2.67	2	t	Proton of CH_2 group (Allylic)
	3.88	2	s	Proton of CH_2-CO
	7.1	1	d	Proton of benzene ring
	6.4	1	t	Proton of benzene ring
	7.25	1	t	Proton of benzene ring
	7.23	1	d	Proton of benzene ring
	9.85	1	s	Proton of NH group
	7.37	2	q	Proton of benzene ring
	7.18	1	t	Proton of benzene ring

The [13]C NMR spectroscopy interpretation of the compound CD

The [13]C NMR spectrum of the compound CI Figure 10S displays Chemical shifts; the interpretation of these spectra was summarized in Table 8.

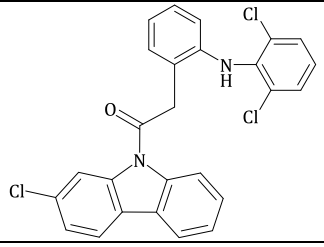
Table 8: [13]C NMR spectroscopy interpretation of the compound CD

Chemical structure	Chemical shift	Interpretation
	120.19	Aromatic carbon
	123.30	Aromatic carbon
	123.11	Aromatic carbon
	112.34	Aromatic carbon
	134.91	Aromatic carbon
	136.97	Aromatic carbon
	23.29	Allylic Carbon
	23.16	Aliphatic carbon
	23.22	Aliphatic carbon
	20.89	Allylic Carbon
	108.97	Aromatic carbon
	132.38	Aromatic carbon
	173.69	carbonyl group
	35.55	next to carbonyl
	125.45	Aromatic carbon
	28.281	Aromatic carbon
	116.86	Aromatic carbon
	30.341	Aromatic carbon
	129.85	Aromatic carbon
	128.91	Aromatic carbon
	143.26	Aromatic carbon
	134.53	Aromatic carbon

The IR spectroscopy interpretation of the compound CD

The IR spectrum of the compound CD Figure 11S displays bands; the interpretation of these spectra was summarized in Table 9.

Table 9: IR spectroscopy interpretation of the compound CD

Chemical structure	Band	Interpretation
	3404.20	NH of amine
	3137.53	Aromatic H
	3060.81	Aromatic H
	2937.60	Asymmetric aliphatic H
	2841.83	Symmetric aliphatic H
	1731.81	Carbonyl group
	1612.28	Aromatic C=C

The properties of the synthesized compounds are listed in Table (10).

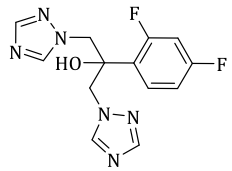
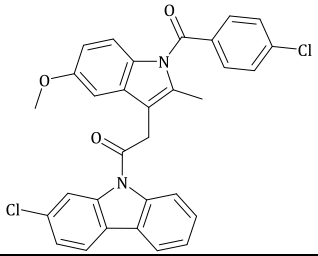
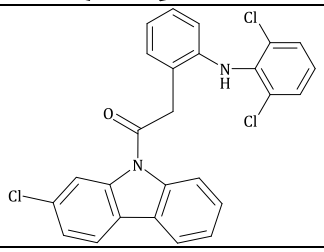
Table 10: properties of the compounds

Compounds	Chemical Formula	Molecular weight	Melting Point	Colour
6-chloro-1,2,3,4 tetrahydrocarbazole	$C_{12}H_{12}ClN$	205.68	144-147 °C	Wheat
CI	$C_{31}H_{26}C_{l2}N_2O_3$	545	108-111 °C	Brown
CD	$C_{26}H_{21}Cl_3N_2O$	483.8	99-103 °C	Dark Red

Docking study

Molecular docking experiments were conducted to investigate the binding modes of these compounds with the target enzyme Sterol 14-demethylase (CYP51), a cytochrome P450 enzyme necessary for sterol biosynthesis in eukaryotic cells and a significant target of therapeutic medications used to treat fungal infections [16]. The receptor used was CYP51 (PDB code 5TZ1) from the RCSB protein data bank. Genetic Optimization for Ligand Docking was used to dock the identified molecules (GOLD). GOLD searches binding ligand conformational space using a genetic algorithm and assigns a score of binding residues. Poses are ranked using GOLD scores [17].

Table 11: Docking score with Amino Acid of Fungal candida albicans, PDB: 5TZ1

ligand	Structure	fitness score	A.A (H bond interaction	A.A (Other interaction)
REF. Ligand (Fluconazole)		78.58	-	THR 311, HEM 601 (3), LYS 143, HIS 468, ILE 131 (3), TYR 132 (4)
C.I.		88.47	TYE 132, HEM 601	HEM 601 (3), PHE 233 (2), MET 508 (5), LEU 121 (3), TYR 118 (3), LEU 376 (3), TYR 132 (2), THR 311 (7), GLY 307 (2), ILE 131, PHE 288 (7)
C.D.		110.7	TYR 132	HEM 601 (5), THR 311 (4), GLY 307 (2), TYR 132 (7), ILE 131 (3), MET 508 (4), LEU 121 (2)

Docking scores of the standard compound (fluconazole) and the synthesized compounds (6-chloro-1,2,3,4-tetrahydrocarbazole, CI and CD) with Amino Acids of Fungal candida albicans and their interactions are listed in Table 11. The interactions of the CI and CD compounds with 5TZ1 protein are shown in Figures 1 and 2, respectively.

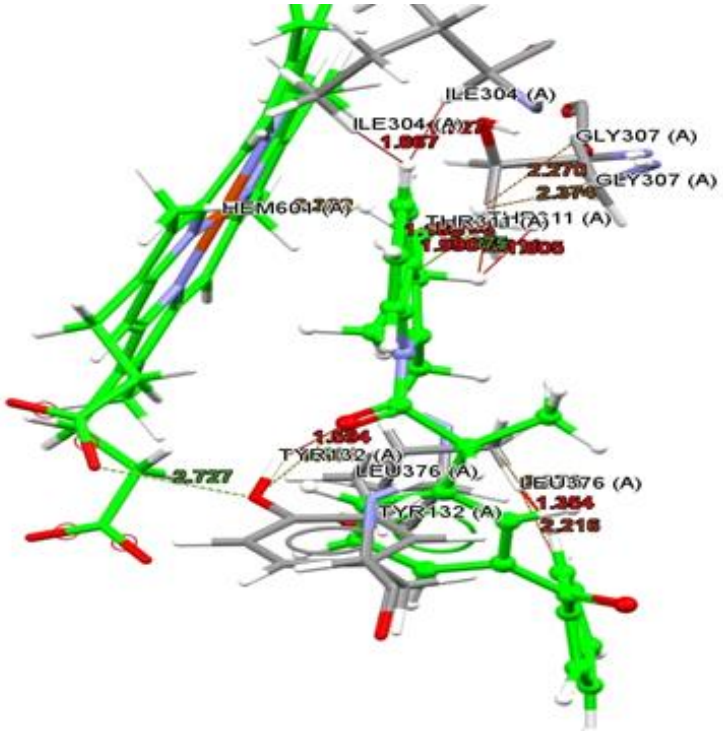


Figure 1: docking of CI with 5TZ1 protein

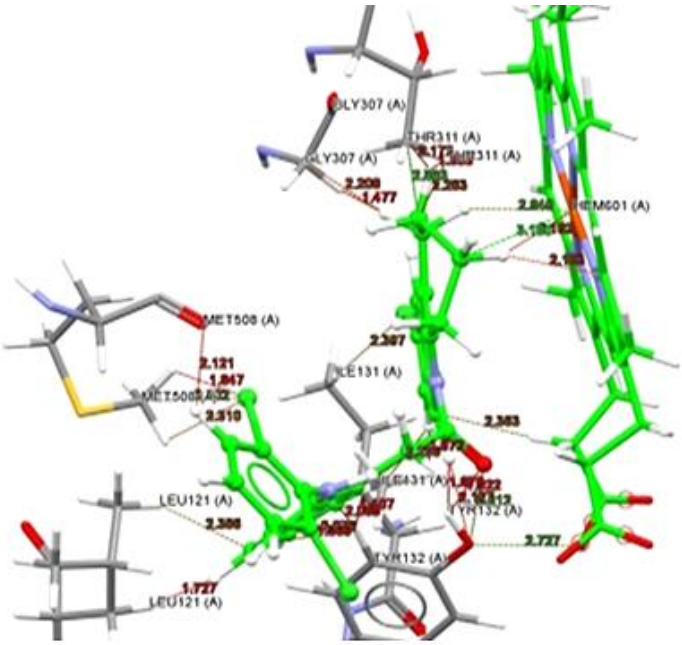


Figure 2: docking of CD with 5TZ1 protein

Antimicrobial activity

The antibacterial activity of the new compounds was tested *in vitro* depending on well diffusion assay against 3 types of gram negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli*), one type of gram positive bacteria (*S. aureus*) and one type of fungi (*candida albicans*) using agar plate (Sabouraud Dextrose Agar for the

fungi and Mueller-Hinton agar for the bacteria) and 4 serial dilutions of the new compounds with DMSO as solvent. Ciprofloxacin as antibacterial and fluconazole as antifungal were used as reference drugs for the comparison.

The compound 6C showed reasonable activity against all types of bacteria except S. aureus as listed in Table 12.

Table 12: Antimicrobial activity of the newly synthesized compounds based on well diffusion assay expressed as inhibition diameter zones in millimeters (mm)

	Cipro	Flu	6C					CI					CD				
			stock	1	2	3	4	stock	1	2	3	4	stock	1	2	3	4
S. aureus.	18		0	0	0	0	0	15	14	12	11	7	16	14	9	7	3
P. aeruginosa	20		17	16	14	12	11	0	0	0	0	0	10	0	0	0	0
E.coli	23		20	18	0	0	0	0	0	0	0	0	0	0	0	0	0
K. pneumoniae	11		16	14	12	10	7	14	12	9	8	5	14	12	10	9	3
C. albicans		14	12	11	8	7	4	14	13	11	10	6	20	18	14	13	8

Conclusions

The synthesized compounds (6C, CI and CD) were identified and confirmed by ^{1}H, ^{13}C NMR and IR spectroscopies.

In vitro tests against fungi showed that they have good biological activity against candida albicans as expected from the docking study which show that the compounds CI and CD have higher docking scores than the control compound (fluconazole), confirming the in vitro tests.

In vitro test against bacteria showed that the compounds CI and CD exhibits no activity against *P. aeruginosa* and *E. coli* but good to moderate activity against *S. aureus* and *K. pneumonia*.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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