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Synthesis and biological evaluation of substituted hexahydroquinoline derivatives

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Abstract: It is known that microorganisms develop resistance to the drugs used in the treatment of diseases caused by bacteria and fungi. Therefore, the discovery of new drug molecules have great importance for the treatment of these diseases. In this study, compounds having dialkyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) general structure which are expected to exhibit antibacterial and antifungal activity were synthesized. ^{1}H-NMR and IR analyzes were performed to prove their structures. All compounds were tested against Gram (+) *Staphylococcus aureus* and Gram (-) *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* to evaluate their *in vitro* antibacterial activity; *Candida albicans* and *Cryptococcus neoformans var. grubii* to evaluate *in vitro* antifungal activity. According to the biological activity data, the most active derivative in the series was compound **2** as an antibacterial agent and compound **7** as an antifungal agent.

Keywords: Hexahydroquinoline; Hantzsch synthesis; antibacterial; antifungal; inhibition.

1. Introduction

Infection-related diseases, cardiovascular diseases and cancer are among the most important life-threatening diseases.[1] It is also known that microorganisms develop resistance to drugs used in the treatment of these diseases caused by bacteria and fungi. One of the reasons for the development of resistance is the misuse of antibiotics in the treatment of bacterial and fungal infections. In addition, carbapenems and glycopeptides also cause bacterial resistance. Therefore, the treatment of infections caused by bacteria and fungi becomes difficult. As a result the discovery of new drug molecules have great importance for the treatment of these diseases.[2,3]

The 1,4-dihydropyridine (1,4-DHP) structure, first synthesized in 1882, took part in the treatment as a calcium channel blocker. It is a molecule that has been the subject of research for many years due to its various pharmacological activities. 1,4-DHPs exhibit antihypertensive, anticonvulsant, antitubercular, anti-inflammatory, antioxidant, antiatherosclerotic, anti-Alzheimer, antiulcer and antimicrobial activity Depending on the structure-activity relationships, modifications were made in the structure of 1,4-DHPs and many 1,4-DHP derivatives were discovered including hexahydroquinolines. It was found that the hexahydroquinolines have a wide spectrum of pharmacological effects by interacting with different molecular targets.[4,5] One of the various modifications based on structure-activity on the hexahydroquinoline structure is the substitution of the phenyl ring at 4th position. This variation has been found to contribute to the activity. It has also been shown that the pharmacological activity is remained in hexahydroquinoline derivatives where the ester structure at 5th position of the

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1,4-DHP core is introduced into the ring system.[6] In addition, modification of the ester group has been shown to play an important role for activity. Biological activity varied between hexahydroquinoline derivatives bearing different ester groups.[7,8]

Based on this information the compounds containing two hexadroquinoline rings of the same molecule thus carrying two equivalents of the same pharmacophore were synthesized in this study. This combination is intended to increase biological activities. *In vitro* antibacterial and antifungal activities of the compounds were evaluated and the results were given.

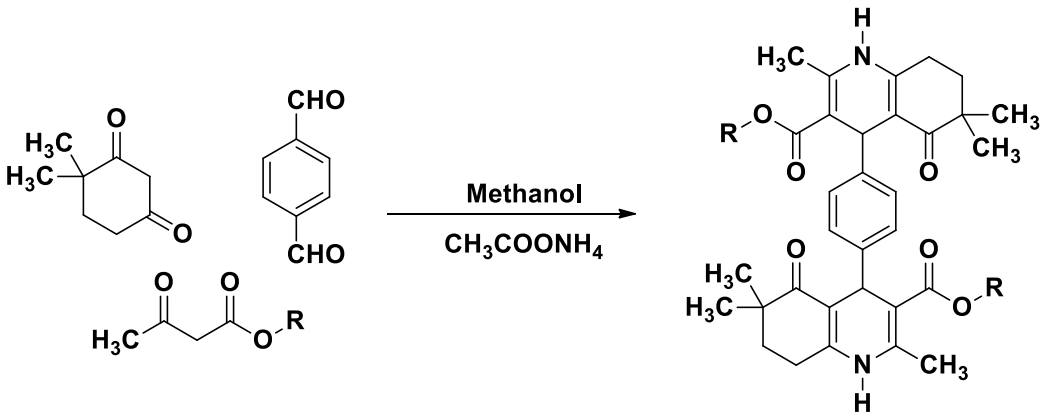
2. Experimental

2.1. Chemical Material and Apparatus

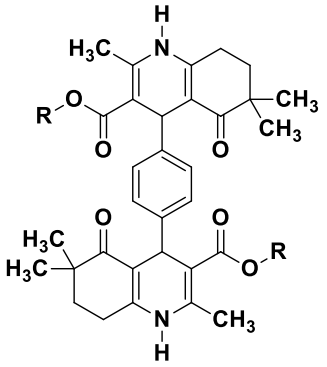
All chemicals used in this study were purchased from Aldrich (Germany). Melting points were determined with *Thomas Hoover Capillary Melting Point Apparatus* and were not corrected. Infrared spectra were recorded on a *Perkin Elmer Spectrum BX* (ν, cm-1). The ^{1}H-NMR spectra were recorded on a *Varian Mercury 400 MHz Ultra Shield Spectrophotometer* (DMSO-d6; tetramethylsilane as internal standard) spectrometer. TLC was carried out on Merck 0.2 mm silica gel 60 F254 analytical aluminum plates. All compounds reported in this paper were in racemic form. Other chemicals or solvents used in this study were HPLC or analytical grade.

2.2. Chemistry

Synthesis of compounds having a general structure of alkyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) was taken place in a flask by refluxing 4,4-dimethyl-1,3-cyclohexadione (2 mmol), terephthalaldehyde (1 mmol), appropriate alkyl acetoacetate (2 mmol) and ammonium acetate (10 mmol) in the presence of 10 mL of methanol for eight hours.[9] The reaction was followed by the TLC method. The reaction mixture was cooled to room temperature and poured into ice-water. The precipitated solids were filtered off. Crystallization was carried out in a methanol-water mixture for purification (Scheme 1).



Scheme 1. General synthesis of the compounds **1-7**.



Compound	R
1	Methyl
2	Ethyl
3	Isopropyl
4	Isobutyl
5	*t*-Butyl
6	2-(methacryloyloxy)ethyl
7	Benzyl

Figure 1. Structure of the compound **1-7**

Methyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahidroquinoline-3-carboxylate) ***(1):*** Yellowish solid, m.p. decomp 300°C, yield: 67,9 %. FTIR (ν, cm^{-1}) 3303 (N-H stretching), 3082 (C-H stretching, aromatic), 2928 (C-H stretching, aliphatic), 1683 (C=O stretching, ester), 1646 (C=O stretching, ketone), 1470 (C-H, bending), 1185 (C-O stretching) and 807 (C-H, bending, p-disubstituted benzene).^{1}H NMR (δ, DMSO-d_6): 0.88 (3H, s, 6-CH_3), 0.94 (3H, s, 6-CH_3), 1.68-1.69 (2H, m, quinoline H-7), 2.22 (3H, s, 2- CH_3), 2.45-2.48 (2H, m, quinoline H-8), 3.51 (3H, s, $COOCH_3$), 4.78 (1H, s, quinoline H-4), 6.82-6.88 (2H, m, aromatic), 9.06 (1H, s, NH).

Ethyl4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) ***(2):*** Yellowish solid, m.p: m.p. decomp 300°C, yield: 71,1 %. IR (ν, cm^{-1}) 3277 (N-H stretching), 3086 (C-H stretching, aromatic), 2965 (C-H stretching, aliphatic), 1691 (C=O stretching, ester), 1651 (C=O stretching, ketone), 1485 (C-H, bending), 1189 (C-O stretching) and 818 (C-H, bending, p-disubstituted benzene).^{1}H NMR (δ, DMSO-d_6): 0.86 (3H, s, 6-CH_3), 0.94 (3H, s, 6-CH_3), 1.67-1.71 (2H, m, quinoline H-7), 2.21 (3H, s, 2- CH_3), 2.44-2.48 (2H, m, quinoline H-8), 3.93 (1H, dq, $COO\underline{CH_{2A}}\underline{CH_3}$), 3.99 (1H, dq, $COO\underline{CH_{2B}}\underline{CH_3}$), 4.76 (1H, s, quinoline H-4), 6.89 (2H, s, aromatic), 9.00 (1H, s, NH).

Isopropyl4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) ***(3):*** Yellowish solid, m.p. decomp 300°C, yield: 63,2 %. IR (ν, cm^{-1}) 3274 (N-H stretching), 3093 (C-H stretching, aromatic), 2982 (C-H stretching, aliphatic), 1690 (C=O stretching, ester), 1648 (C=O stretching, ketone), 1454 (C-H, bending), 1197 (C-O stretching) and 820 (C-H, bending, p-disubstituted benzene). ^{1}H NMR (δ, DMSO-d_6): 0.88 (3H; s; 6-CH_3), 0.97 (3H; s; 6-CH_3), 1.06 (3H, d, $CH(\underline{CH_3})_{2a}$), 1.16 (3H, d, $CH(\underline{CH_3})_{2b}$), 1.68-1.72 (2H, m, quinoline H-7), 2.26 (3H, s, 2-CH_3), 2.49-2.51 (2H, m, kinolin H-8), 4.77-4.83 (1H, m, $\underline{CH}(CH_3)_2$), 4.85 (1H, s, kinolin H-4), 7.19-7.58 (2H, m, aromatic), 9.06 (1H, s, NH).

Isobutyl4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-car boxylate) ***(4):*** Yellowish solid, m.p. decomp 300°C, yield: 59,8 %. IR (ν, cm^{-1}) 3299 (N-H stretching), 3075 (C-H stretching, aromatic), 2956 (C-H stretching, aliphatic), 1686 (C=O stretching, ester), 1604 (C=O stretching, ketone), 1481 (C-H, bending), 1213 (C-O stretching) and 852 (C-H, bending, p-disubstituted benzene). ^{1}H NMR (δ, DMSO-d_6): 0.78 (3H, d, J= 8 Hz, $CH_2CH(\underline{CH_3})_a$), 0.79 (3H, s, 6-$CH_3$), 0.81 (3H, d, J= 4.8 Hz $CH_2CH(\underline{CH_3})_b$), 0.99 (3H, s, 6-$CH_3$), 1.78-1.85 (H, m, $CH_2\underline{CH}(CH_3)_2$), 1.98 (H, d, J= 16 Hz, quinoline H-8a), 2.15 (H, d, J= 16 Hz, quinoline H-8b), 2.28 (H, d, J= 16 Hz, quinoline H-6a), 2.31

(3H, s, 2-CH_3), 2.4 (H, d, J= 16 Hz, quinoline H-6b), 3.71 (2H, m, $\underline{CH_2}CH(CH_3)_2$), 4.90 (1H, s, quinoline H-4), 7.21-7.58 (2H, m, aromatic), 9.10 (1H, s, NH).

t-Butyl l4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-car-boxylate) ***(5)****:* Yellowish solid, m.p. decomp 300°C, yield: 66,7 %. IR (ν, cm^{-1}) 3284 (N-H stretching), 3088 (C-H stretching, aromatic), 2969 (C-H stretching, aliphatic), 1693 (C=O stretching, ester), 1602 (C=O stretching, ketone), 1487 (C-H, bending), 1160 (C-O stretching) and 821 (C-H, bending, p-disubstituted benzene).^{1}H NMR (δ, DMSO-d_6): 0.80 (3H, s, 6-CH_3), 0.83 (3H, s, 6-CH_3), 1,24 (9H, s, COO($\underline{CH_3}$)$_3$), 1.64-1.66 (2H, m, quinoline H-7), 2.17 (3H, s, 2- CH_3), 2.40-2.43 (2H, m, quinoline H-8), 4.67 (1H, s, quinoline H-4), 6.89 (2H, s, aromatic), 8,84 (1H, s, NH).

*2-(methacryloyloxy) ethyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate)****(6)****:* Yellowish solid, m.p. decomp 300°C, yield: 63,4 %. IR (ν, cm^{-1}) 3300 (N-H stretching), 3072 (C-H stretching, aromatic), 2926 (C-H stretching, aliphatic), 1701 (C=O stretching, ester), 1597 (C=O stretching, ketone), 1483 (C-H, bending), 1167 (C-O stretching) and 806 (C-H, bending, p-disubstituted benzene).^{1}H NMR (δ, DMSO-d_6): 0.86 (3H, s, 6-CH_3), 0.94 (3H, s, 6-CH_3), 1.67-1.70 (2H, m, quinoline H-7), 2.25 (3H, s, 2- CH_3), 2.45-2.49 (2H, m, quinoline H-8), 4.83 (1H, s, quinoline H-4), 4.93-5.09 (2H, m, COO$\underline{CH_2}C_6H_5$), 6.84 (2H, s, aromatic), 7.11-7.16 (2H, m, $COOCH_2C_6H_5$), 7.23-7.27 (3H, m, $COOCH_2C_6H_5$), 9.10 (1H, s, NH).

Benzyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) ***(7)****:* Yellowish solid, m.p: 287-290°C, yield: 63,4 %. IR (ν, cm^{-1}) 3300 (N-H stretching), 3072 (C-H stretching, aromatic), 2926 (C-H stretching, aliphatic), 1701 (C=O stretching, ester), 1597 (C=O stretching, ketone), 1483 (C-H, bending), 1167 (C-O stretching) and 806 (C-H, bending, p-disubstituted benzene).^{1}H NMR (δ, DMSO-d_6): 0.86 (3H, s, 6-CH_3), 0.94 (3H, s, 6-CH_3), 1.67-1.70 (2H, m, quinoline H-7), 2.25 (3H, s, 2- CH_3), 2.45-2.49 (2H, m, quinoline H-8), 4.83 (1H, s, quinoline H-4), 4.93-5.09 (2H, m, COO$\underline{CH_2}C_6H_5$), 6.84 (2H, s, aromatic), 7.11-7.16 (2H, m, $COOCH_2C_6H_5$), 7.23-7.27 (3H, m, $COOCH_2C_6H_5$), 9.10 (1H, s, NH).

2.3. Antimicrobial Assay

The antimicrobial activity of the all compounds were tested against *Staphylococcus aureus ATCC 43300, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Acinetobacter baumannii ATCC 19606* and *Pseudomonas aeruginosa ATCC 27853.*

2.3.1. Preparation of Microbial Cultures and for Antimicrobial Activity Test of Alkyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahidroquinoline-3-carboxylate)

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 $\mu g/^{mL}$ or 20 µM (unless otherwise indicated in the data sheet), in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO. All the sample-preparation where done using liquid handling robots. Compounds that showed solubility issues during stock solution preparation are detailed in the data sheet.

2.3.2. Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600), then added to each well of the compound containing plates, giving a cell density of 5´105 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

2.3.3. Antimicrobial Data Collection

Analysis Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

2.4. Antifungal Assay

The antifungal activity of the all compounds were tested against *Candida albicans ATCC 90028 and Cryptococcus neoformans var. Grubii ATCC 208821.*

2.4.1. Preparation of Microbial Cultures and for Antifungal Activity Test of Alkyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahidroquinoline-3-carboxylate)

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 µg/mL or 20 µM (unless otherwise indicated in the data sheet), in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO. All the sample-preparation where done using liquid handling robots. Compounds that showed solubility issues during stock solution preparation are detailed in the data sheet.

2.4.2. Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1 x 106 to 5 x 106 CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 ´103 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 24 h without shaking.

2.4.3. Antifungal Data Collection

Growth inhibition of C. albicans was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of C. neoformans was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

3. Results and Discussion

3.1. Chemistry

In this study seven compounds having a general structure of alkyl 4,4'-(1,4-phenylene)bis(2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate) were synthesized by modified Hantzsch reaction. Ammonium acetate was used instead of ammonia as nitrogen source. The compounds carry alkyl chains bearing an increasing number of carbon atoms, benzyl and methacryloyloxy which include unsaturated moiety as ester functional group. Exact structures of the all compounds were elucidated by IR and ^{1}H-NMR analysis. The absorption bands at around 3300 cm^{-1} confirmed N-H in the ring system, around 1700 confirmed ester groups and around 1600 cm^{-1} confirmed ketone groups. As expected ^{1}H-NMR spectra of the compounds displayed singlet signals belonging to $–CH_3$ groups at 0.9 ppm, multiplet signals belonging to aromatic protons at 7.0 ppm and singlet signals belonging to –NH groups at 9 ppm. The peaks belonging to other protons were seen in expected values of the compounds' spectras.

3.2. *Antimicrobial and Antifungal Activity*

The *in vitro* antimicrobial activity of the all compounds were tested against *Staphylococcus aureus ATCC 43300, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Acinetobacter baumannii ATCC 19606* and *Pseudomonas aeruginosa ATCC 27853.* The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate.

The antifungal activities of the all compounds were tested against *Candida albicans ATCC 90028 and Cryptococcus neoformans var. Grubii ATCC 208821.* The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate (Table 1 and Table 2).

Table 1. General Information of Microorganisms

Abbreviation	Code	Name	Description	Strain	Organsim	Type
Sa	GP_020	*Staphylococcus aureus*	MRSA	ATCC 43300	Bacteria	G+ve
Ec	GN_001	*Escherichia coli*	FDA control	ATCC 25922	Bacteria	G-ve
Kp	GN_003	*Klebsiella pneumoniae*	MDR	ATCC 700603	Bacteria	G-ve
Ab	GN_034	*Acinetobacter baumannii*	Type strain	ATCC 19606	Bacteria	G-ve
Pa	GN_042	*Pseudomonas aeruginosa*	Type strain	ATCC 27853	Bacteria	G-ve
Ca	FG_001	*Candida albicans*	CLSI reference	ATCC 90028	Fungi	Yeast
Cn	FG_002	*Cryptococcus neoformans var. grubii*	Type strain	H99; ATCC 208821	Fungi	Yeast

Table 2. Biological activity results of all compounds

Compound	Organism	Inhibition	Z-score	Concentration (μg/mL)
1	*Staphylococcus aureus*	14.60	-0.55	32.00
1	*Escherichia coli*	-3.07	0.09	32.00
1	*Klebsiella pneumoniae*	10.51	-0.24	32.00
1	*Acinetobacter baumannii*	5.18	0.44	32.00
1	*Pseudomonas aeruginosa*	3.82	1.04	32.00
1	*Candida albicans*	4.71	-1.21	32.00
1	*Cryptococcus neoformans*	-27.93	-1.65	32.00
2	*Staphylococcus aureus*	22.49	-0.90	32.00
2	*Escherichia coli*	-0.10	-0.39	32.00
2	*Klebsiella pneumoniae*	21.63	-1.00	32.00
2	*Acinetobacter baumannii*	28.63	-1.38	32.00
2	*Pseudomonas aeruginosa*	5.61	0.80	32.00
2	*Candida albicans*	-3.95	1.31	32.00
2	*Cryptococcus neoformans*	-65.54	-0.75	32.00
3	*Staphylococcus aureus*	N/D	N/D	32.00
3	*Escherichia coli*	N/D	N/D	32.00
3	*Klebsiella pneumoniae*	N/D	N/D	32.00
3	*Acinetobacter baumannii*	N/D	N/D	32.00
3	*Pseudomonas aeruginosa*	N/D	N/D	32.00
3	*Candida albicans*	N/D	N/D	32.00
3	*Cryptococcus neoformans*	N/D	N/D	32.00
4	*Staphylococcus aureus*	N/D	N/D	32.00
4	*Escherichia coli*	N/D	N/D	32.00
4	*Klebsiella pneumoniae*	N/D	N/D	32.00
4	*Acinetobacter baumannii*	N/D	N/D	32.00
4	*Pseudomonas aeruginosa*	N/D	N/D	32.00
4	*Candida albicans*	N/D	N/D	32.00
4	*Cryptococcus neoformans*	N/D	N/D	32.00
5	*Staphylococcus aureus*	13.41	-0.18	32.00
5	*Escherichia coli*	-9.31	1.77	32.00
5	*Klebsiella pneumoniae*	0.32	0.73	32.00
5	*Acinetobacter baumannii*	-5.14	1.30	32.00
5	*Pseudomonas aeruginosa*	20.80	-1.27	32.00
5	*Candida albicans*	0.64	0.18	32.00
5	*Cryptococcus neoformans*	-100.41	0.47	32.00
6	*Staphylococcus aureus*	18.72	-0.89	32.00
6	*Escherichia coli*	0.95	-0.63	32.00
6	*Klebsiella pneumoniae*	17.66	-0.68	32.00
6	*Acinetobacter baumannii*	21.74	-0.95	32.00
6	*Pseudomonas aeruginosa*	15.90	-0.60	32.00
6	*Candida albicans*	15.23	-4.16	32.00
6	*Cryptococcus neoformans*	-91.25	0.19	32.00
7	*Staphylococcus aureus*	11.06	0.00	32.00
7	*Escherichia coli*	-2.34	0.13	32.00
7	*Klebsiella pneumoniae*	3.12	0.50	32.00
7	*Acinetobacter baumannii*	0.04	0.39	32.00
7	*Pseudomonas aeruginosa*	10.79	0.09	32.00
7	*Candida albicans*	18.00	-4.99	32.00
7	*Cryptococcus neoformans*	-93.19	0.25	32.00

According to biological activity results all inhibition values are below 50 % and Z-Scores are below 2.5.

4. Conclusion

We synthesized seven substituted hexahydroquinoline derivatives with modified Hantzsch reaction. The *in vitro* antibacterial and antifungal activity were evaluated with Gram-positive, Gram-negative organisms and fungi. Although no significant antimicrobial activity was observed in the compounds, the compound 2 had the highest inhibition value against Gram-positive bacteria and Gram-negative bacterias. The compound with the highest inhibition value against fungi is compound 7. The antimicrobial activity of the compound 3 and 4 could not determined.

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