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Synthesis, Characterization and Studying the Anti-Fungal Activity of Resveratrol-Oxadiazole Derivatives

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Abstract

This study concerns with the antimicrobial properties of resveratrol-oxadiazole derivatives, with special emphasis on its antifungal activity linked oxadiazole 5-member aromatic heterocyclic compound which exhibits antifungal activity. The introduction of oxadiazole ring into target compounds effectively enhancing their bioactivity as antifungal. Resveratrol derivatives containing 1,3,4 oxadiazole ring had been synthesized and characterized successfully and their antifungal activity was estimated and showed that they have antifungal activity against C. albicans. The study results demonstrated that Compound 2 and 3 showed good activity against C. albicans when compared with resveratrol the positive control and DMSO the negative control, that the antifungal activity here is due to the synthesized compound and not to the solvent used, these compounds gave different inhibition zones at different concentration(0.05 mcg/ml, 0.025 mcg/ml, 0.01 mcg/ml, 0.005 mcg/ml) and this seemed to be beneficial as antifungal since they gave inhibition zone at even very low concentration 0.005 mcg/ml higher than parent compound. while compound 1 had the least inhibition zones among them. These compounds can be further investigated with other types of fungi and compared with different types of standard as antifungal agents.

Keywords

Synthesis, Antifungal, resveratrol, oxadiazole derivatives.

Around 1.6 million people each year pass away from major fungal illnesses such invasive candidiasis, according to WHO estimates, of the 13 million people who suffer from fungi-related disorders..

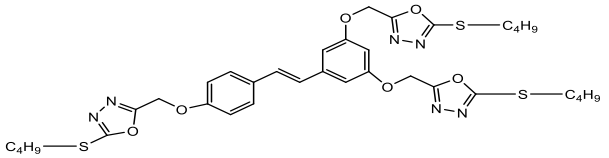
Fungal infection caused by Candida albicans is the most frequent cause of superficial and systemic candidiasis and is widely recognized as the most pathogenic yeast species. C. albicans causes 50%−60% of cases of systemic candidiasis[1]. Resveratrol has been extensively studied for a variety of different health-beneficial effects like anti-inflammation, anti-carcinogenesis, anti-obesity, anti- diabetes type 2, anti-aging, cardiovascular protection and neuro-protection etc. . Resveratrol has undergone significant research on a wide range of illnesses, but it has also been tested for its potential to fight against bacteria, fungus, and viruses. This review focuses on resveratrol's antimicrobial effects, particularly its antibacterial activities. properties[2, 3] [(1,2)]. Oxadiazole is a 5-member aromatic heterocyclic compound with molecular formula $C_2H_2ON_2$[4] [(3)]. It is consisting of two carbon atoms, two nitrogen atoms and one oxygen atom. Oxadiazoles can be contrasted with furan isosterically, but the substitution of two methine groups (−CH=) by two sp^2 nitrogen (−N=) decreases their aromaticity[5]. 1, 3, 4-oxadiazole exhibits a broad variety of behaviors, including antifungal activity[6, 7]. Reports indicated that the introduction of a oxadiazole ring into target

compounds could change their polarity, binding affinity with receptor, and flexibility, effectively enhancing their bioactivity[1, 8].

Experiments

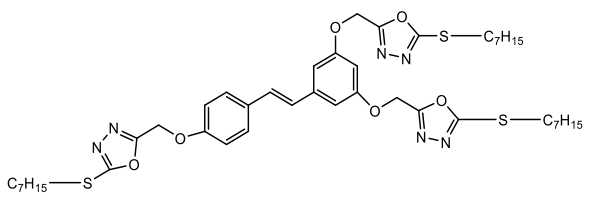
preparation of resveratrol 1,3,4-oxadiazol derivatives

General procedure for the synthesis of 5,5'-(((5-(4-((5-(butylthio)-1,3,4-oxadiazol-2-yl)methoxy)styryl)-1,3-phenylene) bis (oxy)) bis (methylene))bis (2-(butylthio)-1,3,4 oxadiazole)compound (1)



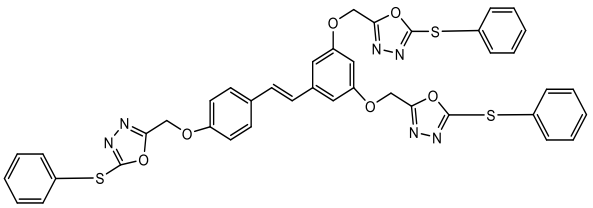
30 mL of DCM (dichloro methane) added to compound A [5,5'-((5-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)styryl)-1,3-phenylene)bis(methylene))bis(1,3,4-oxadiazole-2-thiol)] (1.00 g, 1.74 mmoL.) in 100 mL in size round bottom flask and stirred for 10 minutes until all compound (A) dissolved in dichloromethane and solution become homogenous. Potassium hydroxide (0.20 mL, 5.22 mmol,) was added drop wise via the dropper to the stirring solution and after 15 minutes butyl bromide ($C_3H_7CH_2Br$) (0.62 mL, 5.22 mmol) was added. The mixture heated at 30°C with stirring for two hours[9, 10]. Precipitate with brown color was formed, filtered with pump section, precipitate dried and washed with acetone to have the final product.

Synthesis of 5,5'-(((5-(4-((5-(heptylthio)-1,3,4-oxadiazol-2-yl)methoxy) phenethyl) -1,3-phenylene) bis (oxy))bis(methylene))bis(2-(heptylthio)-1,3,4-oxadiazole) compound (2)



(1.00g, 1.74mmoL.) of compound A [5,5'-((5-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)styryl)-1,3-phenylene) bis (methylene))bis(1,3,4-oxadiazole-2-thiol)] has been dissolved in 30 mL of dichloromethane in 100 mL in size round bottom flask, stirred for 10 minutes. Potassium hydroxide (0.2mL, 5.22 mmol,) was added drop wise to the stirring solution and after 15 minutes. The heptyl bromide ($C_6H_{13}CH_2Br$) (0.82 mL, 5.22 mmol) added and let the mixture heated at 30°C and stirring for two hours. [9,10] Brown precipitate was formed and filtered using pump section, washed with acetone and dried to get the desired product.

General procedure for the synthesis of 5,5'-(((5-(4-((5-(phenylthio)-1,3,4-oxadiazol-2-yl)methoxy)phenethyl)-1,3-phenylene)bis(oxy))bis(methylene))bis (2-(phenylthio)-1,3,4-oxadiazole)compound (3)



(1.00 g, 1.74 mmoL.) of compound A [5,5'-((5-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)styryl)-1,3-phenylene) bis (methylene))bis(1,3,4-oxadiazole-2-thiol)] was let to be dissolved in 30 mL of DCM (dichloro methane) in 100 mL in size round bottom flask and stirred for 10 minutes until the compound dissolve completely in solvent. Few drops from Potassium hydroxide (0.2mL, 5.22 mmol,) was added to the stirring solution drop wisely by using the dropper. Benzyl bromide (C_5H_5CBr) (0.52 mL, 5.22 mmol) added after 15 minutes and let the mixture heated at 30°C and stirred [9,10]. Brown precipitate was formed. The mixture was filtered with pump section after two hours, precipitate washed with acetone and dried to get the desired product.

Antifungal activity

Culture Media and Inoculum11, 12

The C. albicans strains were obtained from the Al- Nahrain University Center, and they were subsequently placed to tryptic soy broth (TSB). The applied TSB contained 2.5 g/L glucose, 5 g/L NaCl, 2.5 g/L K2HPO4, 17 g/L tryptone (pancreatic digest of casein), 3 g/L peptone (soybean digest), and 3 g/L peptone. The culture media was kept at 37 °C for 24 hours in an incubator to regenerate the C. albicans. According to the culture media data sheet, 1000 mL of distilled water was combined with 38 g of

powdered Мьller-Hinton agar (MHA) for fungal cultivation media and heated with a stirrer until a homogenous solution was created. The solution was then sterilized for 15 minutes at 121 °C in an autoclave. 25 mL or so of the prepared culture. SDA medium was prepared according to the manufacturer instructions, adjusted to pH 5, sterilized by autoclave at 121 °C, 20 minutes, then the medium was allowed to cool 45 єC. The medium was supplemented with chloramphenicol to inhibit the growth of Bacteria.

Antifungal Activity Assessment

Agar-well diffusion is one of the widely used and recognized bioassessment techniques. [13]. The zone diameter of inhibition was therefore calculated using the agar-well diffusion method. For the culture of the C. albicans fungus, the SDA (65 g/L) was employed. To pre-sterilized Petri plates, 25 mL of molten medium that had been cooled to 45 °C was added. Then, to achieve a uniform dish surface growth, 24-hour-old cultures of Candida albicans were dispersed using a sterile cotton swab and loop, and the microbe was distributed across the whole surface of the agar dish. The contents of the Petri plate (or plates) were then chilled and dried. Using a sterilized glass Pasteur pipette, five 5-mm-deep wells were punched into the agar to hold the samples and dimethyl sulfoxide (DMSO) as the control sample. Using a syringe, the solution was injected into a 0.45 micrometer solve Poured through a filter into a glass vial. The diluted chemicals were dispensed at varied concentrations into the appropriate wells in an amount of about 75 l. After 30 minutes at room temperature, the Petri plates underwent a 24-hour incubation period at 37 °C. After incubation, a ruler was used to measure the diameter of the inhibitory zones, and the results were given in millimeters (mm). The average result was calculated after each test was carried out in triplicate.

Statistical analysis

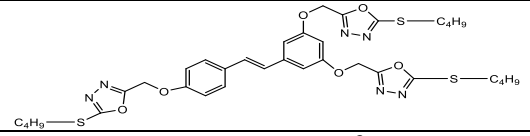
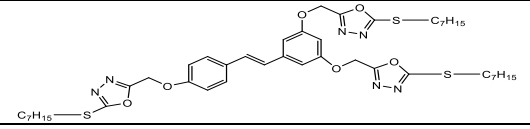
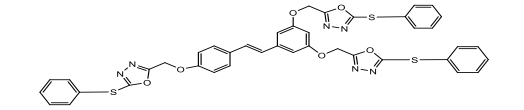
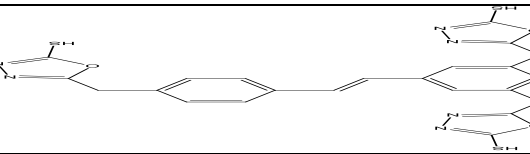
The statistical analysis was performed using excel 2010 to estimate the mean ± SD and t-test analysis to compare between the tested compounds in the study.

Results and discussion

preparation of compounds

Compound 1-3 prepared successfully according to the method provided by dhiaa et al, compound 1 showed the following characteristics: Brown crystal, yield 78%, M.P = 269-271°C. IR KBr (cm^{-1}): appearance of 1593 (C=N), 1055 (Oxadiazole ring stretching) and disappearance of 2480 S-H stretching. ^{1}H-NMR (DMSO): δ2.00 (Trip, 6H, CH_2), δ 1.05-1.883 (Mult, 12H, CH_2), δ 0.84 (Trip, 9H. CH_3). ^{13}C-NMR (DMSO): δ 24.30-26.81 (aliphatic carbons that replace the hydrogen atom from binding to sulfur atom), δ 151.08 (aromatic carbon alpha to oxygen of oxadiazole ring), δ152.75 (Aromatic carbon alpha to SR group) [9,10].

Table 1: Names and structures of the starting and prepared compounds

Compound	Name	Structure
1	5,5'-(((5-(4-((5-(butylthio)-1,3,4-oxadiazol-2-yl)methoxy)styryl)-1,3phenylene)bis(oxy))bis (methylene))bis(2-(butyllthio)-1,3,4 oxadiazole)	
2	5,5'-(((5-(4-((5-(heptylthio)-1,3,4-oxadiazol-2-yl)methoxy) styryl)-1,3-phenylene) bis(oxy)) bis(methylene))bis(2-(heptylthio)-1,3,4-oxadiazole)	
3	5,5'-(((5-(4-((5-(phenylthio)-1,3,4-oxadiazol-2-yl)methoxy)styryl)-1,3-phenylene)bis (oxy)) bis(methylene))bis(2-(phenylthio)-1,3,4-oxadiazole)	
A	5,5'-((5-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)styryl)-1,3-phenylene)bis (methylene))bis(1,3,4-oxadiazole-2-thiol)	

Compound 2 showed the following characteristics Brown crystal, yield 89%, M.P = 282-284°C. IR KBr (cm^{-1}): appearance of 1585 (C=N str), 1070 (Oxadiazole ring stretching) and disappearance of 2480 S-H stretching. ^{1}H-NMR (DMSO): δ2.47 (Trip, 6H, CH_2), δ 1.66-2.38 (Mult, 30H, CH_2), δ 1.25 (Trip, 9H. CH_3). ^{13}C-NMR (DMSO): δ 11.67-28.70 (aliphatic carbons

that replace the hydrogen atom from binding to sulfur atom), 152.41 (aromatic carbon alpha to oxygen of oxadiazole ring), δ159.04 (Aromatic carbon alpha to SR group) [9,10].

Compound 3 showed the following characteristics Brown crystal, yield 86%, M.P = 288-289°C. IR KBr (cm^{-1}): appearance of 1593 (C=N), 1055 (Oxadiazole ring stretching) and disappearance of 2480 S-H stretching. ^{1}H-NMR (DMSO): δ 6.07 (Singlet, 6H, CH2). ^{13}C-NMR (DMSO): δ87.89-83.71 (Aliphatic CH_2 alpha to Exo oxygen and oxadiazole ring), δ157.91 (Aromatic C=N alpha to oxygen of oxadiazole ring), δ 159.04 (Aromatic carbon alpha to SR group) [9,10].

Compound names, structure and physical characteristics showed in table 1 and 2

Table 2: The description, melting point, and yield % of the intermediates and finished product.

Compound no.	Molecular formula	Molecular weight (M.wt)	Description	Yield%	Melting point (m.p)
1	$C_{35}H_{42}N_6O_6S_3$	738.94	Brown crystal	78	269-271
2	$C_{44}H_{60}N_6O_6S_3$	865.18	Brown crystal	89	282-284
3	$C_{41}H_{30}N_6O_6S_6$	798.91	Brown crystal	86	288-289

Antifungal activity

Based on the diameter of the inhibitory zones as determined by a ruler, the results were reported in millimeters (mm). Compound 1 showed little activity against the used fungi, while compound 2 and 3 showed significant antifungal activity against the used fungi with (10mm) inhibition zone at low concentration (0.005 mcg/ml) used when compared to compound 1, this confirm that the synthesized resveratrol derivatives gave better activity than resveratrol with (8mm) at the same concentration, these results compared with DMSO as negative control to prove that the antifungal activity attributed to the prepared compound and not to the solvent used. As shown in table 3.

Table 3: anti-fungal activity of the prepared compound

compound	Concentration 1	Concentration 2	Concentration 3	Concentration 4
	0.05 mcg/ml± SD	0.025 mcg/ml± SD	0.01 mcg/ml± SD	0.005 mcg/ml± SD
1	10 mm±1*	5 mm ±1*	5 mm±1*	5 mm±1*
2	15 mm±1	15 mm±1	10 mm±1	10 mm±2
3	20 mm±1	15 mm±2	10 mm±1	10 mm±2
DMSO	0 mm	0 mm	0 mm	0 mm
Resveratrol	13 mm	10 mm	8 mm	8 mm
* P< 0.05				

Conclusion

There is a huge clinical unmet need in treating fungal infection. Currently antifungal agents need to improve its safety and efficacy. Adding oxadiazole moiety will improve the antifungal activity significantly leading to optimum managements for fungal infections. The study results demonstrated that Compound 2 and 3 showed good activity against C. albicans when compared with DMSO the negative control, that the antifungal activity here is due to the synthesized compound and not to the solvent used, these compounds gave different inhibition zones at different concentration(0.05 mcg/ml, 0.025 mcg/ml, 0.01 mcg/ml, 0.005 mcg/ml) and this seemed to be beneficial as antifungal since they gave inhibition zone at even very low concentration 0.005 mcg/ml higher than parent compound. while compound 1 had the least inhibition zones among them. These compounds can be further investigated with other types of fungi and compared with different types of standard as antifungal agents.

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