ORIGINAL RESEARCH PAPER

Chemistry

BIOLOGICALLY ACTIVE OXADIAZOLE DERIVATIVES

KEY WORDS: Oxadiazole, Antibaceterial, Antifungal, Cytotoxicity

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ABSTRACT

The synthesis of novel thioether 1,3,4-oxadiazole derivatives having long alkyl chain is reported. All the synthesized compounds are characterized by NMR, FTIR and Mass spectral techniques. Synthesized compounds are screened for microbial and cytotoxic activities.

INTRODUCTION

Oxadiazole derivatives are very well known as biologically active compounds and many of these compounds have shown a wide spectrum of antimicrobial activity[1]. Some oxadiazoles with different substituents on the heterocyclic ring resulted fungicidal[2] and antibacterial agents[3]. The use of these "wonder drugs", combined with improvements in sanitation, housing, and nutrition, and the advent of widespread immunization programmes[4], and the development of numbers of antimicrobial agents for treatment of microbial infections[5] has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable, and frequently fatal. Worldwide emergence of multi-resistant microbial strains is a growing concern which requires a multi-pronged research strategy[6]. Some material applications of 1,3,4-oxadiazole derivatives lie in the fields of photo-sensitizers and liquid crystals[7-9].

Microwave is non-conventional energy source. It can be used to carry out a wide range of reactions in high yield and in short time than conventional heating. The reactions which are not possible under conventional conditions can sometimes be affected by the high energy of MWI[10].

In this paper, oxadiazole derivatives are synthesized by conventional method and microwave method to get effective method. They are also screened for microbial and cytotoxic activities. Concept behind to evaluate the microbial activity is because of expected mesomorphic property of synthesized compound. It is well known that cell wall that is phospholipids having of liquid crystalline properties. Hence, compound having similar type of properties may easily penetrate to cell and might be having potential microbial activities.

MATERIALS AND METHODS

Starting materials were procured from Aldrich. Analytical TLC was conducted on Merck aluminium 0.2mm of silica gel 60 F-254. Microwave synthesis was carried out by using Samsung GW71B domestic equipment.

METHODS

Hydrazide (II) was synthesized by condensing ethyl 4-hydroxyphenylbenzoate and hydrazine hydrate (80%).

CONVENTIONAL METHOD

5-(4-HYDROXY)PHENYL-3H-1,3,4-OXADIAZOLINE-2-THIONE (III)[11]

A solution of KOH (1.6g) in water (10mL) was added dropwise to a stirred suspension of hydrazide (II) (28mmol) in ethanol (80mL) at 25°C. After all of the hydrazide has dissolved, carbon disulfide (35mmol,) was added at the same temperature. The solution was evaporated in vacuum using a rotatory evaporator. The residue was poured into a mixture of 400g ice and 100mL concentrated hydrochloride acid. The precipitate formed was filtered off, and crystallized from ethanol/water (4/1) yielding thione (III). Yield 68% B.P. 247°C

5-(4-HYDROXY)PHENYL-2-*N*-HEXADECYLTHIO-1,3,4-OXADIAZOLE (IV)

Triethylamine (3.67mmol) and 1-bromo hexadecane (3.67mmol) were successively added dropwise to a stirred solution of (III) (3.67mmol) in absolute ethanol (10mL). After heating the mixture for 4h under reflux, the solvent was evaporated on a rota-evaporator. The residue was poured into 100mL of water, the resulting precipitate was collected and crystallized from ethanol/water (1/1) yielding compound IV Yield 60%.

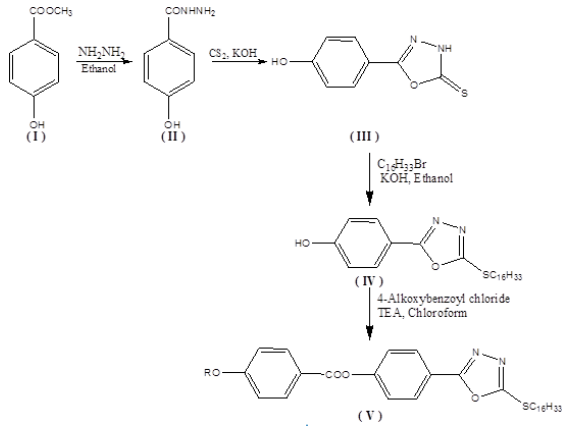
Ester Ia–f was synthesized by the condensation of Hydroxyoxadiazole (IV) with 4-*n*-alkoxybenzoyl chloride. The products were purified by column chromatography using the mixture of ethyl acetate and hexane as an eluant. Products were crystallized by mixture of ethanol/water (2/1). The following yields were obtained: Ia (42%), Ib (54%), Ic (45%), Id (39%), Ie (48%), If (50%).

MICROWAVE METHOD

5-(4-HYDROXY)PHENYL-2-*n*-HEXADECYLTHIO-1,3,4-OXADIAZOLE (IV)

Triethylamine (0.36mmol) and 1-bromo dodecane (0.36mmol) were successively added dropwise to a stirred solution of (III) (0.36mmol) in absolute ethanol (1mL). Reaction mixture was kept under microwave for 40s at 760W. The solvent was evaporated on a rotatory evaporator. The residue was poured into 100mL of water, the resulting precipitate was collected and crystallized from ethanol/ water (1/1) yielding the compound IV. Yield 90%.

Esters Ia–f was synthesized by the mixing of amino-oxadiazole (IV) (0.01mol) with 4-*n*-alkoxybenzoyl chloride (0.01mol) in 1mL pyridine. The resulting mixture was kept under microwave for 40s. (1:1) cold HCl was added to the reaction mixture and filtered. The obtained products, after drying, were purified by column chromatography using the mixture of ethyl acetate and hexane as an eluent. Products were crystallized by the mixture of ethanol/water (2/1). The following yields were obtained: Ia (90%), Ib (88%), Ic (85%), Id (85%), Ie (87%), If (90%)



R= - C_nH_{2n+1}, Where n= 4, 5, 6, 7, 8, 10 **(I a-f)a**

Scheme 1: Synthetic route for series I compound

CHARACTERIZATION

The structures were confirmed by ^{1}H NMR; Bruker AC-250P spectra using $CDCl_3$, TMS, 250MHz and Fourier transform infrared (FTIR; Nicolet550) spectra using KBr disc; the purity of the final products was evaluated by thin layer chromatography (TLC).

Characterization of compound (IV)

FTIR cm^{-1}= 3300 (br, -OH), 3092 (Csp^2–H); 2915 (Csp^3–H); 1608 (C=C); 1521 (C=N). Elemental analysis: Calculated for $C_{24}H_{38}N_2O_2S$: C,68.89; H,9.09; N,6.70%. Found: C,69.89; H,9.04; N,6.60%.

Characterization of esters Ia–h compound (V)

	^{1}H NMR (ppm)	FTIR (cm^{-1})	Elemental Analysis
Ia	0.90 (t, 3H, CH_3); 0.95 (t, 3H, CH_3); 1.15–1.45 (m, 28H, 14xCH_2); 1.71 (m, 4H, OCH_2–CH_2 and SCH_2–CH_2); 3.20 (t, 2H, SCH_2); 3.95 (t, 2H, OCH_2); 6.90 (d, 2H, Ar-H); 7.68 (m, 4H, Ar-H); 7.85 (d, 2H, Ar-H)	2920(Csp^3–H); 1730(-COO-); 1650(C=O); 1605(C=C)	Calculated for $C_{35}H_{50}N_2O_4S$: C,70.70; H,8.41; N,4.71%. Found: C,70.67; H,8.35; N,4.69%
Ib	0.80 (t, 3H, CH_3); 0.95 (t, 3H, CH_3); 1.20–1.43 (m, 30H, 15xCH_2); 1.75 (m, 4H, OCH_2–CH_2 and SCH_2–CH_2); 3.19 (t, 2H, SCH_2); 3.95 (t, 2H, OCH_2); 6.91 (d, 2H, Ar-H); 7.80 (m, 4H, Ar-H); 7.85 (d, 2H, Ar-H)	2925(Csp^3–H); 1725(-COO-); 1645(C=O); 1605(C=C)	Calculated for $C_{36}H_{52}N_2O_4S$: C,71.05; H,8.55; N,4.60%. Found: C,71.01; H,8.52; N,4.59%
Ic	0.85 (t, 3H, CH_3); 0.92 (t, 3H, CH_3); 1.24–1.45 (m, 32H, 16xCH_2); 1.79 (m, 4H, OCH_2–CH_2 and SCH_2–CH_2); 3.26 (t, 2H, SCH_2); 3.99 (t, 2H, OCH_2); 6.85 (d, 2H, Ar-H); 7.80 (m, 4H, Ar-H); 7.95 (d, 2H, Ar-H).	2930(Csp^3–H); 1750(-COO-); 1670(C=O); 1600(C=C).	Elemental analysis: Calculated for $C_{37}H_{54}N_2O_4S$: C,71.38; H,8.68; N,4.50%. Found: C,71.28; H,8.59; N,4.49%
Id	0.83 (t, 6H, 2xCH_3); 1.21–1.80 (m, 38H, 19xCH_2); 3.20 (t, 2H, SCH_2); 4.01 (t, 2H, OCH_2); 7.00 (d, 2H, Ar-H); 7.81 (d, 2H, Ar-H); 7.86 (d, 2H, Ar-H); 8.00 (d, 2H, Ar-H).	2920(Csp^3–H); 1730(-COO-); 1655(C=O); 1610(C=C).	Calculated for $C_{38}H_{56}N_2O_4S$: C,71.70; H,8.81; N,4.40%. Found: C,71.68; H,8.78; N,4.35%
Ie	0.91 (t, 6H, 2xCH_3); 1.25–1.75 (m, 40H, 20xCH_2); 3.28 (t, 2H, SCH_2); 4.05 (t, 2H, OCH_2); 7.01 (d, 2H, Ar-H); 7.90 (d, 2H, Ar-H); 7.92 (d, 2H, Ar-H); 7.99 (d, 2H, Ar-H).	2910(Csp^3–H); 1735(-COO-); 1640(C=O); 1605(C=C)	Calculated for $C_{39}H_{59}N_3O_3S$: C,72.00; H,8.92; N,4.31%. Found: C,71.90; H,8.89; N,4.28%
If	0.90 (t, 6H, 2xCH_3); 1.25–1.80 (m, 44H, 22xCH_2); 3.26 (t, 2H, SCH_2); 4.04 (t, 2H, OCH_2); 7.00 (d, 2H, Ar-H); 7.82 (d, 2H, Ar-H); 7.88 (d, 2H, Ar-H); 8.10 (d, 2H, Ar-H)	2910(Csp^3–H); 1740(-COO-); 1630(C=O); 1605(C=C).	Calculated for $C_{41}H_{63}N_3O_3S$: C,72.57; H,9.14; N,4.13%. Found: C,75.49; H,9.09; N,4.08%

BIOLOGICAL ACTIVITIES[12]

The compounds were tested in vitro for their antifungal activity against *Aspergillus oryzae* and *Aspergillus niger* by cup-plate agar diffusion method. For anti bacterial activity, we had taken 20g of luria broth (Hi media M-575) and 25g of agar–agar in 1000mL distilled water and heated till it dissolved. Then, the mixture was sterilized by autoclaving at 15lbs pressure and 121°C for 15min. Here, agar–agar was used to solidify the solution. After that, six Petri dishes having flat bottom were taken and filled with about 18mL of the above solution. Overlay the plate with 4mL soft agar–agar containing 0.1mL test culture. Bored four well of 8mm diameter in each plate. We had then dissolved the compound in DMF having 1000ppm concentration and added 0.1mL of testing solution into each well. This solution was allowed to diffuse at 4°C. After 20min of diffusion, the plate was incubated at 37°C overnight. After incubation, we observed the zone of inhibition and measured the diameter of the zone. For anti fungal activity, we had taken 20g Sabouraud dextrose instead of lubria broth and followed the same procedure as above. All the synthesized compounds showed good antimicrobial activity (Table 1).

CYTOTOXICITY TEST

Brine shrimp lethality bioassy (BSLT):

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of medicinal plants. Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial sea water under constant aeration for 38h. After hatching, active nauplii free from egg shells were collected from brighter portion of the chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5mL of the brine solution. In each experiment, test substances whose activities are to be checked were added to the vial according to their concentrations and maintained at room temperature for 24h under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations (1-5000µg/mL) of the test substances in a set of three tubes per dose. Replicas should be maintained to get accurate results (Table 1).

TABLE 1:DATA FOR SYNTHESIZED SERIES I

Sr. No.	Anti Bacterial Blank 12mm		Anti Fungal Blank 10mm		CYTOTOXIC ACTIVITY*
	E. coli	*S. aureus*	*A. niger*	*A. oryzae*	ED_{50} µg/mL
	14.75	14.75	12.00	12.00	Podophyllotoxin: 3.88
I-a	13.00	12.50	10.25	10.50	22.32
I-b	13.00	12.25	10.25	10.25	43.25
I-c	13.25	12.25	10.50	10.25	35.20
I-d	12.75	12.75	10.50	10.25	45.39
I-e	12.50	12.00	10.25	10.50	48.40
I-f	12.50	12.50	10.50	10.00	45.60

Furacin (As a Standard): *E. coli*. : 14.75; *S. aureus:* 14.75; *A. niger*: 12.00; *A. oryzae* : 12.00

*Soluble in DMSO

CONCLUSION

All the derivatives of series I were synthesized by conventional and microwave methods. Synthesis compounds give more yield by the microwave method in less. So, the microwave synthesis method is better than the conventional method. All the synthesized compounds of series I was screened for the microbial activity. All synthesized compounds showed moderated to good microbial activities. Activity increases as the number of carbon increases in alkyl chain. All the compounds were found to possess cytotoxic activity. The newly synthesized oxadiazole derivatives having good to moderate anti-bacterial and anti-fungal activities, they may be used for the development of new drugs for the treatment of bacterial and fungal diseases.

ACKNOWLEDGE

Author is thankful to Parul University for providing the experimental work facility.

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