ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Antibacterial and Anticancer Activities of Oxadiazole Derivatives from N-Decanoic Acid.

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ABSTRACT

In the present study a series of 1,3,4-oxadiazole derivatives were synthesised by esterification of n-decanoic acid with absolute ethanol in presence of sulphuric acid then the ester was treated with hydrazine hydrate in presence of absolute ethanol to form acid hydrazide followed by the reaction of acid hydrazide with various aromatic acids in the presence of phosphorus oxychloride. The synthesised 1,3,4-oxadiazole **(3a-h)** were characterized by IR and ^{1}H NMR. The synthesized compounds have been evaluated for antibacterial activity against pathogenic bacteria *Bacillus subtilis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) using amoxicillin as a standard drug. Anticancer activity was screened against human breast cancer cell line MCF-7 and hepatoma cell line HEPG-2. The compounds were found to have significant antibacterial and anticancer activity.

Keywords: 1,3,4-oxadiazole, antibacterial activity, MCF-7, HEPG-2.

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ISSN: 0975-8585

INTRODUCTION

The treatment of infectious diseases still remains as major challenging problem. The clinical use of many compounds develops to fight against infectious diseases has been limited by their relatively high risk of toxicity and microbial resistance. A major research emphasis to counter this problem is the development of antimicrobials structurally unrelated to the existing compounds [1].

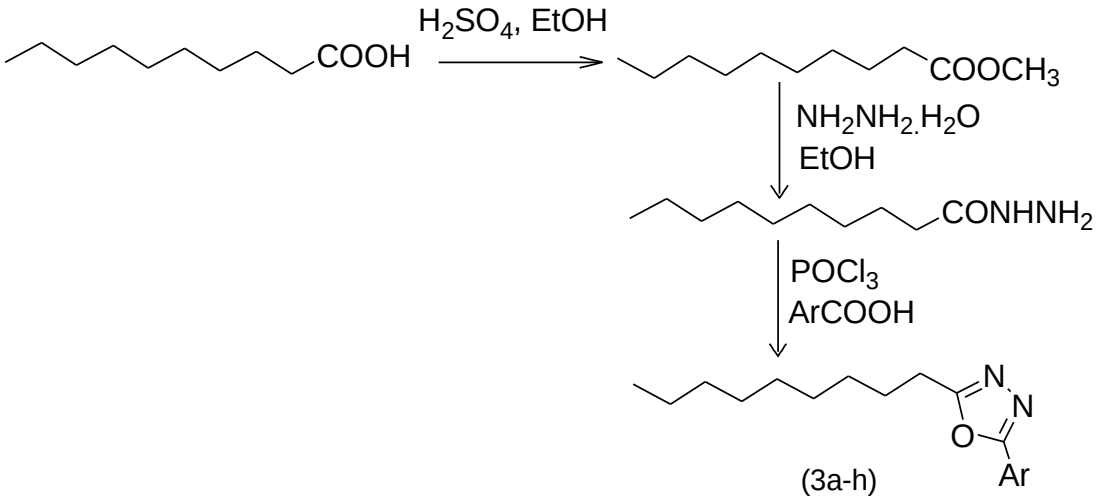
Cancer, a diverse group of diseases characterized by the proliferation and spread of abnormal cells, is a major worldwide problem. Its incidence in the developed countries is increasing and mortality occupies the second rank in the order of death causes [2]. In women, breast cancer is the most common cancer which affects 1 in 12 women and in the United States approximately 44,000 women dies annually from breast cancer [3]. Human liver carcinoma is the fifth most common type of cancer having more than 600,000 mortalities every year. Surgery and chemotherapy is the main treatment but the existing chemotherapeutic drugs have many side effects and restorative effects [4].

Oxadiazole is a heterocycle nucleus and considered to be derived from furan by replacement of two methane (-CH=) group by two pyridine type nitrogen (-N=). There are four possible isomers of oxadiazole depending on the position of nitrogen atom in the ring. It has appealing attention for last two decades, investigations in the field of oxadiazoles have been intensified due to their diverse therapeutic uses [5]. 1,3,4-Oxadiazole possess a variety of biological activities such as anti-inflammatory [6-8], hypoglycemic [9], antifungal, antibacterial [10-13] antimycobacterial, antitumor [14-15] insecticidal [16] activities. Tiodazosin, Nosapidil, Furamizole are oxadiazole derivatives already present in market [5].

Literature survey revealed that minor modification in the structure of 1,3,4-oxadiazole can lead to quantitative as well as qualitative changes in the biological activity [3]. It prompted us to synthesize the various oxadiazole derivatives from n-decanoic acid with the goal of having lesser toxicity and improved activity. All the synthesized compounds have been supported by their spectral data and screened for their antimicrobial activity and anticancer activity.

MATERIAL AND METHOD

Melting points were determined by using open capillary method. The purity of the compounds was confirmed by TLC using solvent Chloroform: Methanol (1:9), hexane: ethyl acetate (6:4), (4:6) and petroleum ether and ethyl acetate (1:1). The spots were visualized in iodine chamber and ultraviolet lamp. IR spectra of synthesised compounds were recorded on Perkin Elimer IR 4000-400 (V_{max} in cm^{-1}) Spectrophotometer by using KBr pellets method. NMR spectra of synthesised compounds were recorded on Bruker Model Advance II 400 (400 MHz, ^1H NMR) and chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.



Scheme 1

ISSN: 0975-8585

Table 1: Characterization data of oxadiazole derivatives (3a-h)

Compound	Ar	Molecular formula	% yield	M.p. (°C)	R_f
3a	C_6H_5	$C_{16}H_{22}N_2O$	84	24	0.7
3b	4-ClC_6H_4	$C_{16}H_{21}ClN_2O$	86.6	42	0.76
3c	4-$CH_3OC_6H_4$	$C_{17}H_{24}N_2O_2$	98	39	0.67
3d	4-FC_6H_4	$C_{16}H_{21}FN_2O$	96	28	0.57
3e	3,4-$CH_3OC_6H_3$	$C_{18}H_{26}N_2O_3$	89	29	0.67
3f	2-Cl-5-$NO_2C_6H_3$	$C_{16}H_{20}ClN_3O_3$	82	40	0.60
3g	2,4-$ClC_6H_3CH_2$	$C_{17}H_{22}Cl_2N_2O$	80	45	0.57
3h	2-Cl $C_6H_4CH_2$	$C_{17}H_{23}ClN_2O$	85	50	0.61

EXPERIMENTAL

Procedure for synthesis of ester (1)

The esterification was carried out by using (0.1 Mol) of n-decanoic acid in 32 ml of absolute ethanol and 1.1 ml of H_2SO_4 and refluxed for 5-6 h. Mixture is poured into separating funnel containing diethyl ether and ether layer extracted, dried and used for further reaction.

Procedure for synthesis of acid hydrazide (2)

A mixture of ester **1** (0.1 mol) and hydrazine hydrate (0.11 mol) in 50 ml absolute ethanol was heated under reflux for 5-6 h. The reaction mixture was left overnight. Solid obtained was collected and recrystallized from absolute ethanol.

Procedure for synthesis of (3a-h)

An equimolar mixture of compound **2** and appropriate aromatic acids **(a-h)** in 10 ml phosphorus oxychloride ($POCl_3$) was refluxed for 5 h. The reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. The potassium hydroxide solution was added till the pH of the mixture raised to 8. The solid was collected by vacuum distillation, dried and recrystallized from absolute ethanol.

Spectral Analysis

2-nonyl-5-phenyl-1,3,4-oxadiazole (3a):

IR (KBr), ν cm^{-1}: 2938 (C-H str, Ar), 1596 (C=N str), 1152 (C-O-C str).
^{1}HNMR ($CDCl_3$) δ (ppm): 8.8 (m, 3H, Ar-H), 8.3 (m, 3H, Ar-H), 7.2-7.4 (s, 6H, CH_2), 4.5-4.7 (s, 2H, CH_2).

2-(4-chlorophenyl)-5-nonyl-1,3,4-oxadiazole (3b):

IR (KBr), ν cm^{-1}: 2926 (C-H str, Ar), 1601 (C=N str), 1104 (C-O-C str), 720 (C-Cl str). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.2-7.5 (m, 3H, Ar-H), 8.05-8.4 (m, 4H, Ar-H), 4.55-4.75(s, 2H, CH_2).

2-(4-methoxyphenyl)-5-nonyl-1,3,4-oxadiazole (3c):

IR (KBr), ν cm^{-1}: 2935 (C-H str, Ar), 1604 (C=N str), 1103 (C-O-C str), 1259, 1027 (-OCH_3). ^{1}HNMR ($CDCl_3$) δ (ppm): 6.9-7.03 (m, 3H, Ar-H), 7.2-7.4 (m, 4H, Ar-H), 4.5-4.7(s, 2H, CH_2), 3.8-3.9 (s, 3H, CH_3).

2-(4-fluorophenyl)-5-nonyl-1,3,4-oxadiazole (3d):

IR (KBr), ν cm^{-1}: 2925 (C-H str, Ar), 1592 (C=N str), 1091 (C-O-C str), 1032 (C-F str). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.2-7.4 (m, 4H, Ar-H), 4.2-4.5 (s, 3H, CH_3), 3.4-3.7 (s, 3H, CH_3), 2.1-2.9 (s, 6H, CH_2), 1.2-1.8 (s, 8H, CH_2) 0.8-0.9 (s, 3H, CH_3).

2-(3,4-methoxyphenyl)-5-nonyl-1,3,4-oxadiazole (3e):

IR (KBr), ν cm^{-1}: 2929 (C-H str, Ar), 1610 (C=N str), 1179 (C-O-C str), 1249, 1030 (-OCH_3). ^{1}HNMR ($CDCl_3$) δ (ppm): 6.8-7.3 (m, 5H, Ar-H), 3.7-3.8 (s, 2H, CH2), 2.9 (s, 2H, CH_2), 2.3 (s, 2H, CH_2), 2.1 (s, 2H, CH_2) 1.3-1.6 (s, 6H, CH_2), 1.2 (s, 2H, CH_2), 0.8 (s, 3H, CH_3).

2-(2-chloro-5-nitrophenyl)-5-nonyl-1,3,4-oxadiazole (3f):

IR (KBr), ν cm^{-1}: 2925 (C-H str, Ar), 1590 (C=N str), 1150 (C-O-C str) 1534, 1345 (C-NO2 str), 739 (C-Cl str).
^{1}HNMR ($CDCl_3$) δ (ppm): 8.0-8.35 (m, 3H, Ar-H), 4.2-4.4 (s, 3H, CH_3), 3.5-3.7 (s, 3H, CH_3), 2.1-2.8 (s, 6H, CH_2), 1.2-1.8 (s, 8H, CH_2) 0.8-0.9 (s, 3H, CH_3).

2-(2,4-dichlorobenzyl)-5-nonyl-1,3,4-oxadiazole (3g):

IR (KBr), ν cm^{-1}: 2940 (C-H str, Ar), 1650(C=N str), 1460 (C-H bend, CH_2), 1385 (C-H bend, CH_3), 1133 (C-O-C str), 721 (C-Cl str).
^{1}HNMR ($CDCl_3$) δ (ppm): 7.21-7.40 (m, 3H, ArH), 4.4 (s, 2H, CH_2), 3.4 (s, 2H, CH_2), 2.9-3.0 (s, 2H, CH_2), 2.12 (s, 2H, CH_2), 1.81 (s, 2H, CH_2), 1.2-1.34 (t, 8H, CH_2), 0.8-0.9 (t, 3H, CH_3).

2-(2-chlorobenzyl)-5-nonyl-1,3,4-oxadiazole (3h):

IR (KBr), ν cm^{-1}: 2930 (C-H str, Ar), 1645(C=N str), 1455 (C-H bend, CH_2), 1383 (C-H bend, CH_3), 1130 (C-O-C str), 719 (C-Cl str).
^{1}HNMR ($CDCl_3$) δ (ppm): 7.10-7.40 (m, 4H, ArH), 4.5 (s, 2H, CH_2), 3.3 (s, 2H, CH_2), 2.8-3.0 (s, 2H, CH_2), 2.2 (s, 2H, CH_2), 1.71 (s, 2H, CH_2), 1.2-1.4 (t, 8H, CH_2), 0.8-0.9 (t, 3H, CH_3).

Antibacterial Activity

Antimicrobial activity of the synthesised compounds was performed against gram positive bacteria (*B. subtilis*) and gram negative bacteria (*E. coli*) by bore and well method using amoxicillin as standard drug. The standard and test solutions were prepared in dimethyl sulphoxide (DMSO) at the two concentration of 10µg/ml and 20µg/ml. The strains of microorganism were taken from Pharmacy Department, Banasthali Vidyapith, Banasthali, Rajasthan and were inoculated in sterile nutrient broth (about 100 ml) at 37°C ±1°C for 24 hours. 21.1gm of agar media was mixed in 500 ml of distilled water and autoclaved at 121°C for 15 min and then pour the agar medium into sterile peteridishs under aseptic conditions. Three wells were made in solidified agar media in each petri dish by a steel borer. Standard and test compound solutions were filled in wells. The seeded broth (0.2 ml) containing 10^6-10^7 colony forming units/ml of the test organism was inoculated on solidified agar plate and incubated at 37°C ±1°C for 24 hours. The bacterial potential of test compounds was determined as zone of inhibition (mm) and each compound was tested thrice.

Table 2: Antibacterial activity of oxadiazole derivatives

Compound	*B. subtilis*		*E. coli*	
	10µg/ml	20µg/ml	10µg/ml	20µg/ml
3a	18	21	22	25
3b	24	27	21	23
3c	19	23	20	23
3d	23	26	23	25
3e	22	25	17	20
3f	18	22	19	20
3g	19	20	17	22
3h	25	27	23	25
Amoxicillin	26	28	25	27

Anticancer activity

The *in-vitro* anticancer activity of synthesized oxadiazole derivatives was determined against human hepatoma (HEPG2) and breast (MCF7) cancer cell lines using SRB assay protocols [17]. All compounds were screened at 4 dose levels viz. 10, 20, 40 and 80 µg/ml and each experiment were repeated thrice. Adriamycin was used as positive control and results were recorded in term of growth inhibition (GI_{50}).

RESULTS AND DISCUSSION

The structures of the synthesized compounds (3a-3h) were ascertained on the basis of their consistent IR and NMR spectral characteristics. Results of antimicrobial evaluation of the synthesized compounds indicated that compound **3b, 3d 3e** and **3h** showed excellent antimicrobial activity against the gram positive *B. subtilis* bacteria at the concentration of 10µg/ml and 20µg/ml whereas **3a**, **3d** and **3h** showed excellent antimicrobial activity against the gram negative *E. coli* bacteria at the concentration of 10µg/ml and 20µg/ml.

Table 4: Anticancer activity of oxadiazole derivatives against Human breast (MCF7) and Hepatoma (HEPG2) cell line measured as GI_{50}

Compound	MCF7	HEPG2
3a	>80	>80
3b	44.11	40.15
3c	44.50	>80
3d	>80	>80
3e	48.4	>80
3f	>80	>80
3g	70.33	>80
3h	48.10	36.40
Adriamycin	<10	<10

The anticancer activity of the synthesized 2-nonyl-5-aryl-1,3,4-oxadiazole derivatives was determined against human breast (MCF7) and hepatoma (HEPG2) cell lines using SRB assay protocols [17] and the results are presented in Table **4**. The compounds **3b, 3c and 3e** were found to be most active with GI_{50} values **44.11, 44.50** and **48.40 µg/ml** respectively but have moderate activity in comparison to standard drug against human breast cancer cell line (MCF7). The compound **3g** and **3h** also found to be active against MCF7 cell line. The compound **3b** and **3h** showed moderate activity against hepatoma (HEPG2) cancer cell line with GI_{50} value **40.15** and **36.40 µg/ml**.

CONCLUSION

The antimicrobial and anticancer activity of synthesized compounds may be due to the presence of different pharmacophores, which might alter the lipophilic character of the of the molecules and facilitate the passage across the membrane of the micro-organism/cell.

Thus, compound **3b** and **3h** may be taken as a lead compound for the discovery of new drug molecules for the treatment of bacterial infection, breast and hepatoma cancer.

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