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Research Article

SYNTHESIS, CHARACTERIZATION, COMPUTATIONAL STUDIES AND EVALUATION OF NOVEL SUBSTITUTED 1,3,4-OXADIAZOLES

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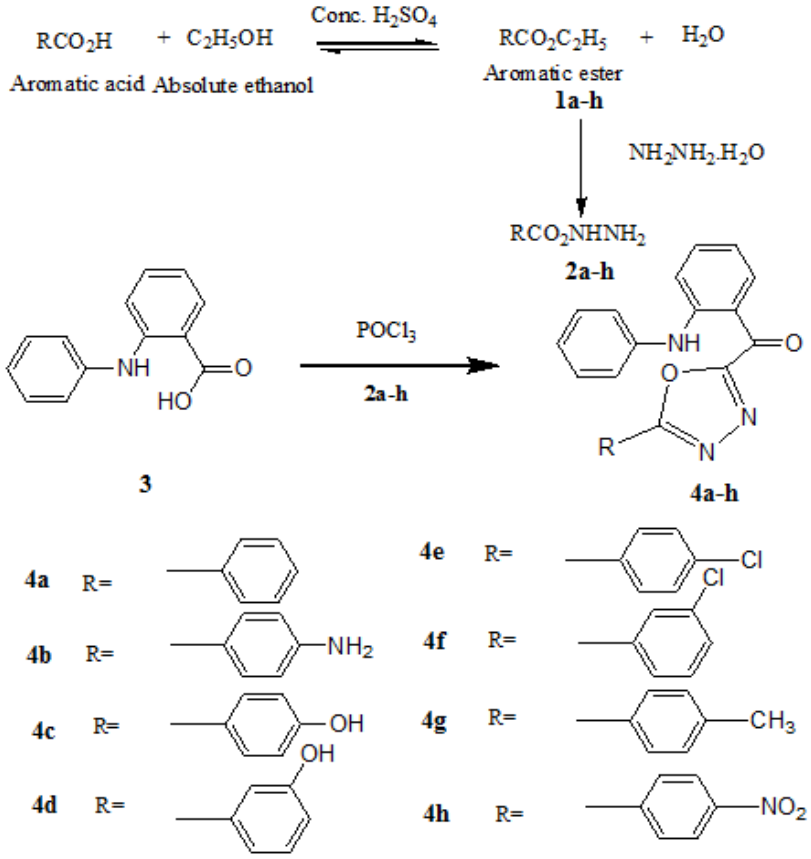
ABSTRACT

A new series of substituted 1,3,4-oxadiazole derivatives have been synthesized and authenticated by TLC, UV-Visible, FTIR, NMR and mass spectroscopic techniques. The physicochemical similarity of the novel derivatives with standard drugs was assessed by calculating from a set of 10 physicochemical properties using software programs. The information so obtained can be related to prediction of biological activity. All the target compounds were evaluated for their antimicrobial activity by using different *in-vitro* models. The test compounds demonstrated good similarity values with respect to the standard drugs. All test compounds effectively inhibited the investigated microbes and appear to be promising antimicrobial agents.

Keywords: Oxadiazole, Similarity studies, Antimicrobial activity, Minimum Inhibitory Concentration

INTRODUCTION

The present status includes that there is increasing interest of researchers in production of medicinally active lead molecule in terms of safety and efficacy for the treatment of various ailments. The antimicrobial nature of such safe compounds has received much more in the present scenario. The current treatment of infectious diseases associated with bacteria and fungi is quite troublesome due to resistance towards antimicrobial agents and their side effects. A number of various problems encountered with the use of antimicrobials such as local tissue irritation, toxicity, resistance, hypersensitivity etc. So there is an importunate need in the area of microbiological research for the development of antimicrobial agents in terms of safety and efficacy[1,2,3]. In the present scenario, a huge number of heterocycles have been explored for development of such potent as well as safe antimicrobials. From the literature survey 1,3,4-oxadiazole was found to be having enormous pharmacological activities such as anti-inflammatory, antimicrobial,[4,5] antifungal, antiviral, antidepressant, analgesic, anti-mycobacterial and anticancer etc[1-7]. So it was planned to synthesize and develop novel series of 1,3,4-oxadiazole derivatives and evaluate them for antimicrobial activity.



Scheme 1: Synthesis of substituted 1,3,4-oxadiazole derivatives

MATERIALS AND METHODS

The chemicals and solvents used were of analytical grade and procured from various authentic sources. Melting points of newly synthesized target compounds were determined on digital melting point apparatus (Flora; Perfit India) and were found uncorrected. Purity of compounds was checked by TLC on silica gel G. The structures of the synthesized compounds were supported by spectral data. UV spectra were recorded on Shimadzu double beam UV spectrophotometer 1800. The IR spectra were recorded on Shimadzu FTIR-8400S spectrophotometer. ^{1}H NMR spectras were recorded on Bruker Avance II-400MHz using TMS as internal standard; values were expressed in δ ppm. For mass spectra, solutions were made in HPLC grade methanol. Structural similarity studies were computed by Chem3D, version 10 molecular modeling software.

Experimental

Esters of aromatic acids (**1a-h**) and aryl acid hydrazides (**2a-h**) were prepared according to the literature method[8]. Aryl hydrazide **2a** (1 mol) was dissolved in phosphorous oxychloride (5 ml) and to it compound **3** (equimolar; 1 mol) was added. The reaction mixture after refluxing for 6-7 hours was cooled to room temperature and poured onto crushed ice. On neutralization of the contents with sodium bicarbonate solution (20%), a solid mass separated out. This was filtered and washed with water. It was crystallized from methanol to give **4a**. Similarly compounds (**4a-h**) were prepared (**Scheme 1**). Physical of synthesized compounds was given in Table 1.

Table 1: Physical constants of the synthesized compounds

S. No.	Molecular formula	%age yield	Molecular weight	Solubility	Melting point (°C)	λ_{max}	$R_f$value
4a	$C_{21}H_{15}N_3O_2$	89.90	341.36	DMSO, MeOH, $CHCl_3$	240- 243	373	0.54
4b	$C_{21}H_{16}N_4O_2$	83.00	356.38	DMSO, MeOH, $CHCl_3$	242-246	423	0.42
4c	$C_{21}H_{15}N_3O_3$	89.80	357.36	DMSO, MeOH, $CHCl_3$	255-258	379	0.47
4d	$C_{21}H_{15}N_3O_3$	80.80	357.36	DMSO, MeOH, $CHCl_3$	253-254	379	0.68
4e	$C_{21}H_{14}ClN_3O_2$	90.80	375.81	DMSO, MeOH, $CHCl_3$	270-272	385	0.40
4f	$C_{21}H_{14}ClN_3O_2$	90.00	375.81	DMSO, MeOH, $CHCl_3$	250-253	385	0.35
4g	$C_{22}H_{17}N_3O_2$	82.00	355.13	DMSO, MeOH, $CHCl_3$	280-283	355	0.42
4h	$C_{21}H_{14}N_4O_4$	81.00	386.36	DMSO, MeOH, $CHCl_3$	295-296	355	0.56

Assessment of structural similarity of synthesized compounds with standard drugs

Assessment of structural similarity studies was performed by means of physico-chemical and steric similarity between the standard drugs available and target compounds. Assessment of structural similarity of **synthesized** compounds (**4a-h)** was calculated with respect to the standard drugs[9]. Therefore we calculated a number of parameters for the **synthesized** compounds using molecular modeling software Chem 3D Ultra 10 after energy minimization and compared them to the values obtained for standard compounds[10].

The standard drugs used for assessment of similarity with synthesized compounds are cefixime and tosufloxacin tosylate. Various set of parameters were used for calculations given in Table 2.

The distance d_i of a particular target compound *i* can presented as:

$$di^2 = \sum_{j=1}^{n} (1-X_{i,j}/X_{i,standard})^2 / n$$

Where, $X_{i,j}$ is value of molecular parameters *i* for compound *j*.

$X_{i,standard}$ is the value of the same molecular parameter *i* for standard drug.

n is the total number of the considered molecular parameters.

The similarity of the compounds can be calculated as:

$$\% \text{ age similarity} = (1-R) \times 100$$

Where R is quadratic mean also known as the root mean square and can be calculated as:

$$R = \sqrt{di^2}$$

Evaluation of antimicrobial activity

Antibacterial activity

The newly synthesized 1,3,4-oxadiazole derivatives (**4a-h**) were screened for their *in vitro* antibacterial activity against *E. coli*, *S. aureus*, *S. epidermidis* and *P. aeruginosa* by cup-plate method. Nutrient agar media was prepared by melting agar on water bath and then cooled it to 45 ºC with gentle shaking to bring about uniform cooling. Nutrient agar media was inoculated with fresh prepared culture media and mixed by gentle shaking before pouring on a sterilized petri dish. Poured the inoculated media into petridish and allowed to set for some time. Cups were made by punching the agar surface with a sterile cork bore (8 mm) and the punched part of the agar media was removed by scooping. Solutions containing 12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL of the test compound were added to each cup. Dimethyl formamide (DMF) was used as a solvent to prepare the stock solution of the test compounds. Amoxycillin and cefixime were taken as positive control and DMF was taken as blank (did not show any activity against test organism). The plates were incubated at 37 ºC for 24 h and the results were recorded. The zones of inhibition of the microbial growth produced by different concentration of test compounds (50µl/disc) were measured in millimetres (mm)[11,12]. The zone of inhibition data for antibacterial compounds was given in Table 4.

Antifungal activity

In vitro antifungal activity of test compounds **(4a-h)** was evaluated using *C. albicans* and *A. niger* strains, by cup plate technique, in Saboraud's dextrose broth culture media. The stock solution of test compounds were prepared in dimethyl formamide (DMF) and the serial dilution of test compounds were carried out for obtaining the concentration, ranging from 12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL. Fluconazole was taken as positive control and DMF was taken as blank (did not show any activity against test organism). The test compounds at various concentrations were added to the cup made by puncturing the agar dextrose media by sterilized cork bore. The plates were incubated at 37 °C for 48 h. The zones of inhibition of the microbial growth (50 µl/disc) produced by different concentration of test compounds were measured in millimetres (mm)[11,12]. The zone of inhibition data for antifungal compounds was given in Table 4.

Minimum Inhibitory Concentration (MIC)

Nutrient agar was prepared, sterilized and cooled to 45 °C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of culture and mixed well by gentle shaking before pouring into the sterilized petridishes. The poured materials were allowed to set and there after the cups were made by punching into the agar surface with sterile cork borer and scooping out the punched part of the agar. 0.1 ml of test compounds was added into the cups with the help of sterile

syringe. Two-fold diluted solutions of the target compounds and reference drugs were prepared (12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL) and allowed to diffuse for some time into the nutrient agar medium for 15 min. The plates were incubated at 30-35 °C for 48 hours. MIC values were determined at the end of the incubation period. The MIC values of test and standard drugs are given in Table 5. MIC_{50} were calculated mathematically depending upon the number of colonies inhibited with respect to novel 1,3,4-oxadiazole compounds orstandard drugs and compared to the colonies inhibited by control medium (without drugs)[13].

RESULTS

Spectral data

*2-Phenylamino-phenyl-(5-phenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4a**)*

IR (nujol, cm^{-1}): 3400 (N-H), 3345, 3015 (C-H), 2950, 1690 (C=O), 1615 (C=N-N=C),1590 (C=C), 1455, 1180 (C-O-C), 760; ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.8(s, 1H, NH), 7.0-8.38 (complex multiplet, 13H, ArH); MS (m/z): 341.12 (M^+), 342.12 ($[M+1]^+$), 343.12 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(4-aminophenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4b**)*

IR (nujol, cm^{-1}): 3390 (N-H), 3250, 3010 (C-H), 2950, 1695 (C=O), 1610 (C=N-N=C),1590 (C=C), 1465, 1080 (C-O-C), 755; ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.05 (s, 2H, NH_2 of phenyl ring), 4.7 (s, 1H, NH), 7.0-8.38 (complex multiplet, 13H, ArH); MS (m/z): 356.13 (M^+), 357 ($[M+1]^+$), 358.12 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(4-hydroxyphenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4c**)*

IR (nujol, cm^{-1}): 3550 (O-H), 3005 (C-H), 1680 (C=O), 1610 (C=N-N=C), 1600 (C=C),1450, 1270, 1120(C-O-C), 810; ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.0 (s, 1H, NH), 5.05 (s, 1H, OH of phenyl ring), 7.0-8.38 (complex multiplet, 13H, ArH); MS (m/z): 357.11 (M^+), 358.11 ($[M+1]^+$), 359.12 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(2-hydroxyphenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4d**)*

IR (nujol, cm^{-1}): 3520 (O-H), 3400 (NH), 3000 (C-H), 1670 (C=O), 1600 (C=N-N=C),1598,1590 (C=C),1460, 1270, 1140 (C-O-C), 780; ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.4 (s, 1H, NH), 5.0 (s, 1H, OH of phenyl ring), 5.20 (s, 1H, NH), 7.0-8.38 (complex multiplet, 13H, ArH); MS (m/z): 357.11 (M^+), 358.11 ($[M+1]^+$), 359.12 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(4-chlorophenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4e**)*

IR (KBr, cm^{-1}): 3415(N-H), 2998 (C-H), 1675 (C=O), 1600 (C=N-N=C), 1590 (C=C), 1465, 1260, 1140 (C-O-C), 750, 610 (C-Cl); ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.8 (s, 1H, NH), 6.9-8.37 (complex multiplet, 13H, ArH); MS (m/z): 375.08 (M^+), 375.07 ($[M+1]^+$), 377.08 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(2-chlorophenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4f**)*

IR (KBr, cm^{-1}): 3415 (N-H), 2990 (C-H), 1695 (C=O), 1615 (C=N-N=C), 1560 (C=C), 1390, 1270, 1120 (C-O-C), 810, 610 (C-Cl); ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.2 (s, 1H, NH), 6.9-8.37 (complex multiplet, 13H, ArH); MS (m/z): 375.08 (M^+), 376.07 ($[M+1]^+$), 377.08 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(4-methylphenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4g**)*

IR (KBr, cm^{-1}): 3415 (N-H), 3000 (C-H), 1690 (C=O), 1610 (C=N-N=C), 1560 (C=C), 1270, 1090 (C-O-C), 825; ^{1}HNMR (DMSO-d_6), (δ, ppm): 2.5 (s, 3H, CH_3), 4.2 (s, 1H, NH), 6.9-8.37 (complex multiplet, 13H, ArH); MS (m/z): 355.13 (M^+), 356.14 ($[M+1]^+$), 357.14 ($[M+2]^+$).

*2-Phenylamino-phenyl-5-(4-nitrophenyl)-[1,3,4]oxadiazol-2-yl-methanone (**4h**)*

IR (KBr, cm^{-1}): 3410 (N-H), 3005 (C-H), 1690 (C=O), 1610 (C=N-N=C), 1560 (C=C), 1530 (assymmetric N-O), 1360 (symmetric N-O) 1270, 1110 (C-O-C), 815; ^{1}HNMR (DMSO-d_6), (δ, ppm): 4.0 (s, 1H, NH), 6.9-8.37 (complex multiplet, 13H, ArH); MS (m/z): 386.10 (M^+), 387.10 ($[M+1]^+$), 388.14 ($[M+2]^+$).

Similarity studies

Various sets of parameters were used for calculations are given in Table 2. All the results related to structural similarities of test compounds with standard drugs are given in Table 3.

Table 2: Calculations of various steric and physicochemical parameters (4a-h)

S. No.	SAS[a] (Å^2)	MSA[b] (Å^2)	SEV[c] (Å^3)	Ovality	MR[d]	MTI[e]	WI[f]	BI[g]	MW[h]	Log P
4a	596.60	317.11	261.588	1.6031	101.19	13529	1765	526866	341.371	4.54
4b	582.457	324.48	278.414	1.5735	105.89	15008	1983	635711	356.386	3.74
4c	576.296	321.19	276.126	1.5662	102,88	14789	1983	635711	357.371	4.15
4d	592.365	320.18	272.521	1.5715	102.88	14563	1943	623372	357.371	4.15
4e	617.510	330.59	276.335	1.6113	105.99	14570	1983	635711	375.816	5.09
4f	598.701	328.61	283.475	1.5746	105.99	14384	1943	623372	375.816	5.09
4g	629.020	337.21	279.754	1.6301	106.24	15227	1983	635711	355.398	5.02
4h	628.727	337.97	281.657	1.6246	-	18288	2475	908652	386.369	-
Cefixime	580.224	331.27	320.889	1.4615	103.69	15459	2353	1046550	453.45	-
Tosufloxacin Tosylate	570.267	318.96	282.947	1.5303	98.34	13519	2039	748946	404.350	2.41

[a]Connolly Solvent Accessible Surface Area; [b]Connolly Molecular Surface Area; [c]Connolly Solvent Excluded Volume; [d]Molar Refractivity (cm^3/mole)

[e]Molecular Topological Index; [f]Wiener Index; [g]Balaben Index; [h]Molecular Weight

Table 3: Assessment of structural similarities of synthesized compounds (4a-h) with standard drugs

S. No.	Compound	Similarity (%)	
		Cefixime	Tosufloxacin Tosylate
1.	4a	60.40	99.88
2.	4b	64.82	84.50
3.	4c	70.30	81.82
4.	4d	68.69	82.97
5.	4e	71.60	63.25
6.	4f	72.87	66.83
7.	4g	76.97	63.94
8.	4h	98.05	83.87

Pharmacological evaluation

Antimicrobial activity

The antimicrobial activity (antibacterial and antifungal) and MIC results are mentioned in Table 4 and 5 respectively. The best results were obtained at 200, 400 and 800 µg/mL.

Table 4: Antimicrobial activity of synthesized compounds (4a-h)

Compound	Concentration (µg/ml)	Zone of inhibition (mm)					
		E. coli	*S. aureus*	*P. aeruginosa*	*S. epidermidis*	*C. albicans*	*A. niger*
4a	200	26	16	-	13	18	24
	400	26	18	-	18	18	26
	800	28	22	-	20	22	27
4b	200	20	22	13	16	26	14
	400	22	24	16	18	28	16
	800	28	26	18	22	30	18
4c	200	22	16	-	-	12	-
	400	24	18	-	-	16	-
	800	28	19	-	-	18	-
4d	200	14	16	16	18	12	24
	400	18	20	20	20	14	24
	800	24	22	22	20	14	26
4e	200	14	24	16	-	-	24
	400	16	26	20	17	-	28
	800	18	26	22	20	-	30
4f	200	18	24	16	22	24	16
	400	24	25	22	24	28	18
	800	24	27	26	26	30	24
4g	200	14	16	16	16	14	18
	400	16	18	20	20	16	18
	800	18	22	22	22	18	20
4h	200	22	24	22	20	16	16
	400	24	26	24	22	20	18
	800	28	26	26	24	22	22
Amoxycillin	200	26	11	12	28	-	-
	400	30	14	14	32	-	-
	800	34	18	17	34	-	-
Cefixime	200	18	-	-	16	-	-
	400	22	12	12	20	-	-
	800	24	16	14	26	-	-
Fluconazole	200	-	-	-	-	24	-
	400	-	-	-	-	28	12
	800	-	-	-	-	30	16

Table 5: MIC_{50} (µg/ml) of synthesized compounds (4a-h)

Compound	R	MIC (µg/ml)					
		E. Coli	*P. aeruginosa*	*S. aureus*	*S. epidermidis*	*C. albicans*	*A. niger*
4a	H	25	25	-	200	200	50
4b	4-NH_2	25	25	400	100	100	25
4c	4-OH	25	25	-	100	-	200
4d	2-OH	200	200	200	50	100	200
4e	4-Cl	200	200	200	25	400	-
4f	2-Cl	100	100	100	50	200	50
4g	4-CH_3	25	25	50	50	100	50
4h	4-NO_2	100	100	200	100	200	200
Control	-	-	-	-	-	-	-
Amoxycillin	-	12.5	200	100	12.5	-	-
Cefixime	-	50	400	400	50	-	-
Fluconazole	-	-	-	-	-	12.5	400

DISCUSSION

In this work, total eight derivatives of 1,3,4-oxadiazole (**4a-h**) were prepared by reaction of N-phenyl anthranilic acid with hydrazide in the presence of phosphorus oxychloride (cyclodehydrating agent). The physicochemical properties of the synthesized derivatives were computed using software programme Chem3D Ultra after energy minimization. All compounds showed good percentage similarity with cefixime and tosufloxacin tosylate (60-98%).

The antimicrobial sensitivity testing of the synthesized compounds assayed using cup plate technique in the nutrient agar at 12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL concentrations was shown in Table 4. Amoxycillin, cefixime and fluconazole were taken as standard drugs in same concentration as that of test compounds. From antibacterial screening, it was concluded that at concentration of 800 µg/ml, compounds **4h**, **4f**, **4b**, and **4e** showed larger zone of inhibition as compared to standard drug amoxycillin against *E. coli*

(28, 24, 28, 18 mm), *P. aeruginosa* (26, 26, 18, 22 mm), and Gram positive bacteria *S. aureus* (28, 27, 26, 26 mm) and *S. epidermidis* (24, 26, 22, 22 mm). The compounds **4b**, **4f**, **4h** at concentration of 800 µg/ml exhibited good antifungal activity against *C. albicans* (30, 30, 22 mm) and compounds **4a**, **4d**, **4e** were highly active against *A. niger* (27, 26, 30 mm) as compared to standard drug fluconazole. Minimum inhibitory concentrations of the synthesized compounds are given in Table 5.

CONCLUSION

A series of oxadiazole derivatives were synthesized and evaluated for their antimicrobial as well as antioxidant activity. The compound **4h** was emerged to be very potent antimicrobial agent and also showed very good similarity with cefixime and tosufloxacin tosylate (98.05 and 83.87%). The highest antibacterial activity against gram positive species *and S. epidermidis* were shown by **4h**, **4f**, **4b**, **4e** and gram negative *species P. aeruginosa* and *E. coli* were shown by compounds **4h**, **4f** and **4b**. The compounds **4a**, **4d**, and **4e** also exhibited good antifungal activity against *C. albicans*. The compounds **4a**, **4d** and **4e** were active against *A. niger* as compared to standard drug fluconazole. All compounds showed good percentage similarity with cefixime and tosufloxacin tosylate. So, the significant activity of compound may be due to the presence of nitrophenyl, chlorophenyl, aminophenyl and hydroxyphenyl moiety in addition to oxadiazole moiety.

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