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Design, Synthesis and Biological Evaluation of 1,3,4-Oxadiazole derivatives

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An alarming increase in bacterial strains resistant to existing antimicrobial agents, demands a renewed effort to seek agents effective against pathogenic bacterias resistant to current antimicrobials. In this paper we report 3D QSAR analysis for the 21 molecules of 1,3,4-oxadiazoles by using k-Nearest Neighbor Molecular Field Analysis (kNN-MFA) combined with various selection procedures. Using kNN-MFA approach 30 3D-QSAR models were generated; one of these models was selected on the basis of q^2 and pred_r^2 values. The selected model has training set of 33 molecules and test set of 8 molecules with validation (q^2) and cross validation (pred_r^2) values of 0.6969 and 0.6148, respectively. The predicted activities by the developed models were in good accordance with the observed activities. In the present work, 1,3,4-oxadiazole derivatives (**5a-e**) were synthesized by the ring closure reactions of various acylhydrazides (**4a-e**) with carbon disulphide or with aromatic acids in $POCl_3$. The structures of all the compounds were elucidated by the spectral and elemental analysis. All the synthesized compounds were evaluated for their antimicrobial activity against *E. coli*, *S. aureus* and *S. epidermidis*.

Key Words: 1,3,4-Oxadiazole, Antimicrobial activity, 3D-QSAR, kNN-MFA.

INTRODUCTION

Several reports are available in literature on a variety of biological activities of substituted-1,3,4-oxadiazoles. These include antiinflammatory[1-4], hypoglycemic[5,6], antianxiety and antidepressant[7] and antimitotic[8] activities. In addition to these, a number of researchers have reported antimicrobial activities[9-13]. Keeping these above facts in view it is considered of interest to carry out 3D QSAR studies and synthesize a few 2,5-disubstituted-1,3,4-oxadiazole for their antimicrobial evaluation.

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The computer-aided prediction of biological activity in relation to the chemical structure of a compound is now commonly used technique in drug discovery[14-17]. Modern drug discovery also relies on the interface of chemical and biological diversity through high throughput screening[18]. Generation of functional molecular diversity for probing the biological activity space requires robust molecular scaffolds that are low in molecular weight and are easily modified to create a variety of chemically diverse, biologically active potential drugs. We do report our efforts to relate the dependence of the antimicrobial activity of new compounds on the nature of substitution in the 2,5-disubstituted-1,3,4-oxadiazoles. The present 3D QSAR study was carried out by using k-Nearest Neighbor Molecular Field Analysis (k-NNMFA) method for rationalizing the synthetic scheme. The better the description of a molecule in terms of structural parameters representing its activity, the better the results of pattern recognition and separation of molecules by activity. The predicted activities by the developed models should be in good accordance with the observed activities.

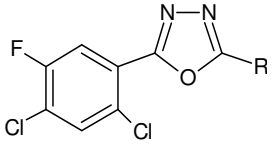
EXPERIMENTAL

Melting points of the synthesized compounds were taken by one end open capillary tubes melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 8400S spectrophotometer (KBr) and ^{1}H NMR spectra in DMSO-d_6 and chloroform on Brucker DRX-300 MHz and 400 MHz using TMS as internal standard. Chemical shifts were reported in parts per million (ppm) The homogeneity of the synthesized compounds were monitored by ascending thin layer chromatography (TLC) on silica gel G plates and visualized by using iodine vapour. Developing solvents were chloroform:methanol (4:1).

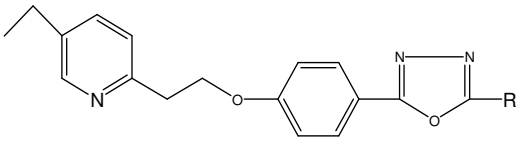
in vitro Minimum inhibitory concentration in mcg per mL of various 2,5-disubstituted-1,3,4-oxadiazoles against *E. coli* MTCC-443 strain by microdilution method were taken from the literature[19,20]. To these values, we have applied one of the modest kNN-MFA with various variable selection methods. Similar to many 3D QSAR methods[21,22] kNN-MFA requires suitable alignment of set of molecule. This is followed by generation of common rectangular grid around the molecules. The steric and electrostatic energies are computed at the lattice point of grid using methyl probe of charge +1, these interaction energy values at the grid point are considered for relationship generation using kNN method and utilized as descriptors to decide nearness between molecules[12].

All the 21 molecules taken in the study (Table-1) were drawn in Vlife QSAR Plus. They were optimized by using Merck Molecular Force Field (MMFF) and they were batch optimized also. After this all the 21 molecules were aligned (Fig. 1) using template based alignment method by choosing a minimum common structure as template (**I**) and the most effective one as the reference molecule (**II**). From the 21 molecules taken in the study, a training set of 17 molecules and test set of 4 molecules were generated using the various selection procedures. After the selection of the test and training sets, kNN methodology was applied to the descriptors generated over the grid as shown in the Show Point (Fig. 2).

TABLE-1
VARIOUS COMPOUNDS OF 3D QSAR STUDIES



I



II

Compound	R	Compound	R
1	C_6H_5-	11	C_6H_5-
2	4-OMe-C_6H_4-	12	4-Cl-C_6H_4-
3	4-Cl-C_6H_4-	13	2,3-Cl_2-C_6H_3-
4	2,4-Cl_2-C_6H_3-	14	2,4-Cl_2-C_6H_3-
5	2,4-Cl_2-5F-C_6H_2-	15	4-OMe-C_6H_4-
6	4-Cl-$C_6H_4OCH_2$-	16	4-NO_2-C_6H_4-
7	2,4-Cl_2-$C_6H_3OCH_2$-	17	2-NO_2-C_6H_4-
8	4-F-3-(OC_6H_5)-C_6H_3-	18	4-Me-C_6H_4-
9	5-(3-Cl-4-F-C_6H_3)-2-furanyl-	19	2-Me-C_6H_4-
10	5-(2-Cl-4-F-C_6H_3)-2-furanyl-	20	Pyridin-3-yl-
		21	Pyridin-4-yl-

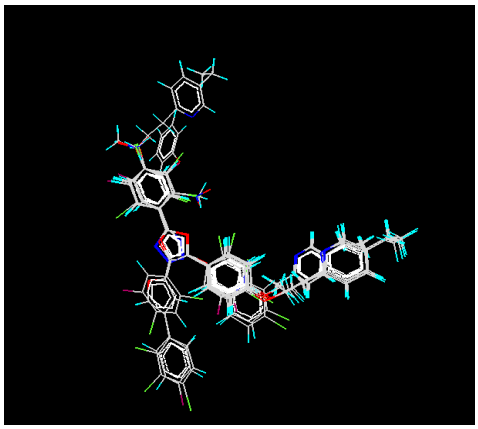


Fig. 1. Alignment of 21 molecules

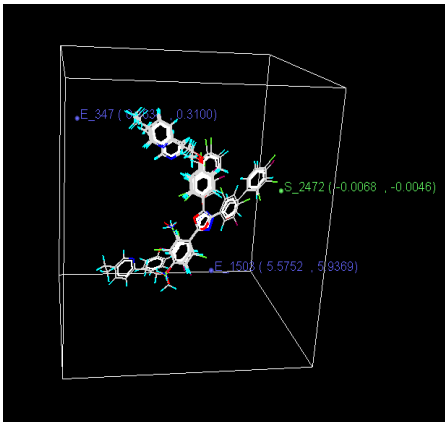


Fig. 2. Show points for the molecules

The QSAR models were evaluated using following statistical measures: *n*-number of descriptors; k-number of nearest neighbors; q^2-cross validated r^2 (by leave-one-out method); pred_r^2 - predicted r^2 for the external test.

General procedure for the preparation of acid hydrazides (3a-d): The appropriate aromatic acids (0.01 mol) dissolved in absolute ethanol (10 mL). Hydrazine hydrate (0.02 mol) and few drops of conc sulphuric acid were added. The reaction mixture was refluxed for 6 h. The resulting solid obtained were filtered, dried and crystallized from methanol. The completion of reaction was monitored by thin layer chromatography and infrared spectrophotometer.

General procedure for the preparation of 5-(substituted)-2-thio-1,3,4-oxadiazoles (4a-e): To a solution of the hydrazides (0.01 mol) in ethanol (15 mL) at 0 ºC, carbon disulphide (2 mL) and potassium hydroxide (0.6 g) were added and the reaction mixture was refluxed until the evolution of H_2S gas ceased (*ca.* 12 h). Excess solvents were evaporated under reduced pressure and the residue was dissolved in water and then acidified with dilute hydrochloric acid (10 %) to pH ~5. The precipitate was filtered off, dried and crystallized from ethanol. The completion of reaction was monitored by thin layer chromatography and infrared spectrophotometer. Compound **4a**, **4c**, **4d**, **4e** were prepared with this method.

Compound 4a: 2-(5-Sulfanyl-1,3,4-oxadiazol-2-yl)phenol: Recrystallized from ethanol to yield 79 %, m.p. 205 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1612 (C=N, ring), 760 (C-O-C, ring), 1108 (C=S), 1590 (C=C Ar), 3250 (C-OH Ar), 3350 (NH). ^{1}H NMR (DMSO-d_6) δ: 6.92-6.95 (1H, m), 7.01-7.03 (1H, d), 7.38-7.42 (1H, m), 7.60-7.63 (1H, d), 10.43 (1H, s, -OH), 14.56 (1H, broad s, -SH). MS 194. Anal. CHN: calcd C 49.72, H 3.11, N 14.42, found C 49.76, H 3.16, N 14.49.

Compound 4c: 5-Phenyl-1,3,4-oxadiazole-2-thiol: Recrystallized from ethanol to yield 58 %, m.p. 185 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1610 (C=N, ring), 788 (C-O-C, ring), 1060 (C=S), 1502 (C=C Ar). ^{1}H NMR (DMSO-d_6) δ: 6.37-8.21 (5H, complex m, 5 Ar H), 14.138 (1H, s, SH), 7.38-7.42 (1H, m), 7.60-7.63 (1H, d), 10.43 (1H, s, -OH), 14.56 (1H, broad s, -SH). MS 178. Anal. CHN: calcd C 53.92, H 3.39, N 8.98, found C 54.01, H 3.45, N 15.78.

Compound 4d: 5-(4-Methylphenyl)-1,3,4-oxadiazole-2-thiol: Recrystallized from ethanol to yield 62 %, m.p. 193 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1618 (C=N, ring), 750 (C-O-C, ring), 1072 (C=S), 1510 (C=C Ar), 2947 (C-H), 3350 (NH). ^{1}H NMR (DMSO-d_6) δ: 3.39 (3H, m, Ar-CH_3), 7.39-7.41 (2H, s, fine splitting, Ar H), 14.69 (1H, s, -SH). MS 192. Anal. CHN: calcd C 56.23, H 4.19, N 14.57, found C 56.28, H 4.23, N 14.62.

Compound 4e: 5-(Pyridin-3-yl)-1,3,4-oxadiazole-2-thiol: Recrystallized from ethanol to yield 71 %, m.p. 169 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1635 (C=N, ring), 759 (C-O-C, ring), 1035 (C=S), 1592 (C=C Ar). ^{1}H NMR (DMSO-d_6) δ: 2.46 (1H, m, NH), 7.76-7.78 (2H, s, fine splitting), 8.76-8.77 (2H, s, fine splitting). MS 179. Anal. CHN: calcd C 46.92, H 2.81, N 23.45, found C 47.01, H 2.86, N 23.50.

Synthesis of 2-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl acetate (4B): (Acetylation of 4a): The synthesized 2-(5-mercapto-1,3,4-oxadiazol-2-yl)phenol (0.01 mol) was treated with acetic anhydride (5 mL) and kept at room temperature for overnight. The mixture was poured into cold water, filtered, dried and crystallized from ethanol. The completion of reaction was monitored by thin layer chromatography and infrared spectrophotometer.

Compound 4b: 2-(5-Sulfanyl-1,3,4-oxadiazol-2-yl)phenyl acetate: Recrystallized from ethanol to yield 64 %, m.p. 132-134 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1751 (C=O), 1610 (C=N, ring) 760 (C-O-C, ring), 1080 (C=S), 1490 (C=C Ar), ^{1}H NMR (DMSO-d_6) δ: 2.43 (3H, m, $COCH_3$), 2.74 (1H, s, NH), 7.25 (1H, d, Ar H), 7.435

(1H, m, Ar H), 7.653 (1H, m, Ar H), 8.029 (1H, d, Ar H). MS 236. Anal. CHN: calcd C 50.84, H 3.41, N 11.86, found C 50.92, H 3.47, N 11.97.

General procedure for the preparation of 3,5-disubstituted-1,3,4-oxadiazoles (5a-e): The aromatic acid hydrazide (0.01 mol) and an appropriate aromatic acid (0.01 mol) was refluxed in $POCl_3$ (5 mL) for 8 h, cooled and poured on to crushed ice, made basic with sodium bicarbonate solution. The precipitate was filtered off, dried and crystallized from ethanol. The completion of reaction was monitored by thin layer chromatography and infrared spectrophotometer. Compound **5a-e** was prepared with this method.

Compound 5a: 2-[5-(Naphthalen-2-ylmethyl)-1,3,4-oxadiazol-2-yl]phenol: Recrystallized from ethanol to yield 80 %, m.p. 97 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1600 (C=N, ring) 759 (C-O-C, ring), 1055 (C=S), 1592 (C=C Ar), ^{1}H NMR (DMSO-d_6) δ: 2.50 (2H, s, CH_2), 5.13 (1H, s, OH), 6.94-8.19 (12H, m, 11Ar H, 1NH). MS 302. Anal. CHN: calcd. C 75.48, H 4.67, N 9.27, found C 75.55, H 4.70, N 9.38.

Compound 5b: 2-[5-(4-Methylphenyl)-1,3,4-oxadiazol-2-yl]phenol: Recrystallized from methanol to yield 75 %, m.p. 156 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1614 (C=N, ring) 779 (C-O-C, ring), 1055 (C=S), 1480 (C=C Ar), 3363 (C-OH). ^{1}H NMR (DMSO-d_6) δ: 2.435 (3H, s, Ar CH_3), 7.00-8.03 (8H, complex m, 8 Ar H), 10.20 (1H, s, Ar OH). MS 252. Anal. CHN: calcd C 71.42, H 4.79, N 11.10, found C 71.58, H 4.88, N 11.17.

Compound 5c: 2-[5-(2-Methylphenyl)-1,3,4-oxadiazol-2-yl]phenol: Recrystallized from ethanol to yield 59 %, m.p. 164 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1604 (C=N, ring) 781 (C-O-C, ring), 1553 (C=C Ar), 3330 (C-OH), 2921 (C-H). ^{1}H NMR (DMSO-d_6) δ: 2.79 (3H, s, Ar CH_3), 7.26-8.03 (8H, complex m, 8 Ar H), 10.59 (1H, s, Ar OH). MS 252. Anal. CHN: calcd. C 71.42, H 4.79, N 11.10, found C 71.58, H 4.88, N 11.17.

Compound 5d: 2-(5-Phenyl-1,3,4-oxadiazol-2-yl)phenol: Recrystallized from methanol to yield 60 %, m.p. 134-138 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1606 (C=N, ring) 781 (C-O-C, ring), 1548 (C=C Ar), 3060 (C-OH). ^{1}H NMR (DMSO-d_6) δ: 2.79 (3H, s, Ar CH_3), 7.26-8.03 (8H, Complex m, 8 Ar H), 10.59 (1H, s, Ar OH). MS. 238. Anal. CHN: calcd. C 70.58, H 4.23, N 11.76, found C 70.65, H 4.28, N 11.80.

Compound 5e: 3-(5-Phenyl-1,3,4-oxadiazol-2-yl)pyridine: Recrystallized from ethanol to yield 68 %, m.p. 182-188 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 1620 (C=N, ring) 772 (C-O-C, ring), 1552 (C=C Ar), 3090 (C-OH). ^{1}H NMR (DMSO-d_6) δ: 2.46 (1H, m, NH), 7.76-7.78 (2H, s, fine splitting), 8.76-8.77 (2H, s, fine splitting), 10.59 (1H, s, Ar OH). MS 239. Anal. CHN: calcd. C 65.27, H 3.74, N 17.56, found C 65.30, H 3.85, N 17.68.

Microbiology: The compounds were tested against bacterial strains *i.e. E. coli* (MTCC 443), *S. epidermidis* (ATCC12228) and *S. aureus* (ATCC25923). A standard inoculum (1-2 × 10^7 c.f.u./mL 0.5 McFarland standards) was introduced on to the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from

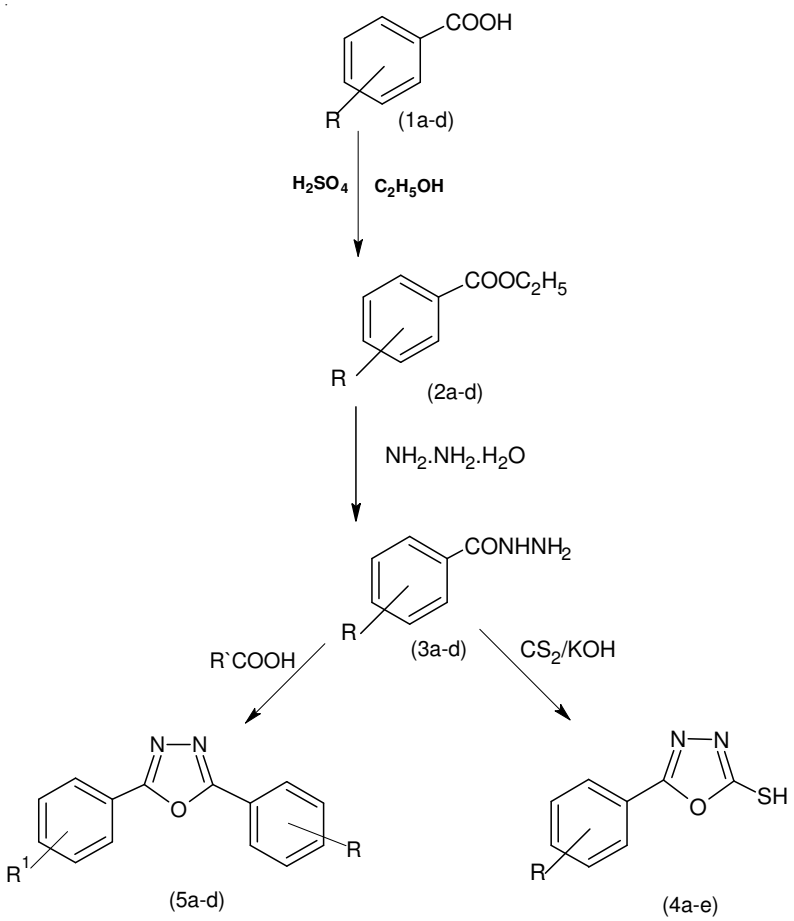
Whatman no. 1 filter paper and sterilized by dry heat at 140 ºC for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 ºC. Ciprofloxacin was used as a standard drug. Inhibition zones were measured and compared with the controls. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with *ca.* 5×10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Designing analysis: The importance and utility of the new 3D QSAR method *i.e.* kNN-MFA has been established by applying it to known sets of molecules as described above. It is reported that 30, 3D QSAR models were generated by kNN-MFA in conjunction with simulated annealing (SA), genetic algorithms and stepwise (SW) forward backward selection method. From these models, two of them were having good q^2 and pred_r^2 values, one of which was selected having good internal and external predictivity. For this model training and test sets were selected using random selection method and the descriptors were selected using simulated annealing method. The summary of the selected model can be given as: k = 2; q^2 = 0.6969; pred_r^2 = 0.6148; Descriptor range: E_34: 7 0.1634 to 0.3100; E_1503: 5.5752 to 5.9369; S_2472: -0.0068-0.0046.

Descriptor range (Fig. 1) for the selected model of the Series elaborates that; (i) 1,3,4-oxadiazole ring is essential for the activity. (ii) Negative range of steric field indicates that less bulky substituent would be favourable for the activity. (iii) Positive range of electronic field indicates that less electronegative substituent would be favourable for the activity as already the basic moiety taken in the study is substituted with high electronegative groups like chlorine and fluorine so the other substituents employed should be less electronegative.

1,3,4-Oxadiazole derivatives were synthesized by the ring closure reactions of various aroylhydrazides (**3a-d**) with carbon disulphide (**4a-e**) or with aromatic acids in $POCl_3$ (**5a-e**) in good quality and yield (**Scheme-I**). The aroylhydrazides (**3a-d**) were synthesized by esterification reaction of various aromatic acids by using sulphuric acid as catalyst. The synthesized 1,3,4-oxadiazoles were characterized by IR, ^{1}HNMR, mass and elemental analysis. The IR spectrum of final compounds showed the absence of amide and carbonyl frequency in the region 1760-1650 cm^{-1} and the NH frequency in the region 3400-3200 cm^{-1} and showed a new band in 1620-1564 cm^{-1} region due to ν(C=N).



Scheme-I

Antimicrobial activity: The newly prepared compounds were screened for their antibacterial activity against *E. coli* (MTCC 443), *S. epidermidis* (ATCC12228) and *S. aureus* (ATCC25923) bacterial strains by disc diffusion method. In all the determinations tests were performed in triplicate and the results were taken as a mean of 3 determinations. The compounds **4b**, **4e** and **5e** have shown significant inhibition comparable to standard while compound **5a** and **5c** have shown moderate activity[23] (Table-2).

Conclusion

The 3D-QSAR study has shown that less electronegative substituent would be favourable for the activity. As already the basic moiety taken in the study is substituted with high electronegative groups like chlorine and fluorine so the other substituents employed should be less electronegative. Hence the future molecules should be designed with less electronegative group to result in potentially active molecules. We have synthesized 2,5-disubstituted-1,3,4-oxadiazole derivatives and evaluated

TABLE-2
ANTIBACTERIAL ACTIVITIES OF THE COMPOUNDS (**4a-4e**) AND (**5a-5e**)

Strain	Zone of inhibition in (mm)*		
	E. coli	*S. epidermidis*	*S. aureus*
Std. (ciprofloxacin)	26	30	29
4a	18	22	24
4b	25	26	24
4c	20	18	22
4d	19	16	20
4e	21	26	25
5a	21	25	23
5b	19	22	20
5c	23	24	23
5d	19	18	21
5e	24	25	24

for their antimicrobial activities. The compounds **4b**, **4e** and **5e** have shown significant inhibition comparable to standard while compound **5a** and **5c** have shown moderate activity.

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