Synthesis and antimicrobial screening of some 1, 3, 4-oxadiazoles and their molecular properties prediction through 'rule of five'

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Abstract: In the current study, a series of 5-(4-substituted phenyl) - 1, 3, 4-oxadiazole-2-thiols (4a-h) were prepared from 4-substituted benzoic acid hydrazides (3a-h). The chemical structures of synthesized compounds were elucidated by IR, ^{13}CNMR, Mass spectral techniques and nitrogen (%) analyses. All these synthesized compounds were investigated for their antibacterial activities against bacterial strains i.e. *Staphylococcus aureus*, *Bacillus substilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The strains, *Aspergillus niger* and *Candida albicans* were also examined for antifungal screening. The zone of inhibition was measured and percentage inhibition was calculated by comparing with standard drug. The minimum inhibitory concentrations (MICs) of potent synthesized compounds were determined. Then, all the synthesized compounds were computed to assess the drug-like properties through Lipinski's rule of five. The results of *in-vitro* assay showed that the compounds 4(a), 4(b) and 4(d) possess significant antibacterial activity whereas 4(a), 4(g) and 4(h) possess significant antifungal activity. The predicted drug likeness score of all these compounds were also meritorious among 1, 3, 4-oxadiazoles. The results recommended that these compounds might be used in future to generate derivatives for emergent antimicrobial agents with improved pharmacokinetic profile.

Keywords: Oxadiazole, antimicrobials, minimum inhibitory concentration, rule of five.

INTRODUCTION

The world is presently experiencing the challenges of augmented microbial resistance development against usually available antimicrobial agents. Owing to this challenge of such resistance, there is always a need of search for classes of novel antibacterial agents.

Azoles are a class of five membered heterocyclic compounds containing two heteroatoms out of which at least one must be a nitrogen atom (Acheson, 2008). Amongst them, 1, 3, 4-oxadiazoles proved to be a better pharmacophore showing a range of medicinal properties (Farshori *et al.*, 2010). The activities of 1, 3, 4-oxadiazoles reported by various researchers include antioxidant (Musad *et al.*, 2011), antimicrobial (Naveena *et al.,* 2010*;* Ramaprasad *et al.,* 2010.; Suresh Kumar *et al.,* 2010; Jha *et al.,* 2010), anticonvulsant (Almasirad *et al.,* 2004; Rajak *et al.*, 2010), Immunosuppressive (Yan *et al.,* 2012), anti-tubercular (Mamolo *et al.,* 2005; Shashikant *et al.,* 2009), anticancer (Kumar *et al.,* 2009; Padmavathi *et al.,* 2009) and anti-inflammatory activities (Jayashankar *et al.*, 2009; Singh *et al.,* 2013; Amir and Kumar, 2004).

Lipinski in his work (*Lipinski, 1997*) formulated the 'rule of five' for evaluating drug-like properties of molecules; an important tool for medicinal chemists used for quick assessment of compounds during discovery and optimization of drug molecules. Lipophilicity is one of the properties which affect nearly all the three phases of drug optimization i.e. pharmaceutical, pharmacokinetic and pharmacodynamic action (Rutkowska *et al.,* 2013; Bakht *et al.,* 2010).

Keeping in view, 1, 3, 4-oxadiazoles reported in this work, (4 a-h) were designed and prepared to study the antimicrobial action. Therefore, the synthesized compounds might be used in future to search the drug-like properties for developing their better pharmacokinetic profile.

MATERIALS AND METHODS

General

The chemicals were procured from Merck, Rankem Chemicals, Spectrochem (P) Ltd. and *Hi Media* Suppliers from Mumbai, India. Melting points of synthesized compounds were determined in open capillaries and are uncorrected. Purity of all synthesized compounds were analyzed by Thin Layer Chromatography (TLC) on Silica Gel G plates by means of iodine vapors as detecting agent and chloroform and methanol as solvent system (table 3). IR spectra (in KBr) were scanned on a Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{13}C NMR spectra in DMSO were recorded on a Jeol-JMS (400 MHz) spectrometer using TMS as an internal reference standard (chemical shift expressed in *ppm* on δ scale). Mass spectra were recorded on a Shimadzu LC-MS 2010 Spectrometer. Elemental analysis (%N) was carried out using Perkin Elmer 2400 analyzer. Intermediate compounds were established by determining the melting point and functional group test. The synthesized compounds were recrystallized from alcohol and water mixtures.

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Experimental

General Procedure for synthesis of methyl 4-substituted benzoate, 2(a-h)

The methyl benzoates, 2(a-h) were prepared according to reported method (Furniss *et al.,* 2005). (table 1)

General Procedure for synthesis of 4-substituted benzoic acid hydrazide, 3(a-h)

The acid hydrazides, 3(a-h) were synthesized according to reported method (Smith *et al.,* 1946; Furniss *et al.* 1989) with desirable modifications. The ester [2 (a-h), 0.1 mol] were dissolved in 100mL of methanol and transferred to the round bottomed flask fitted with a reflux condenser. Hydrazine hydrate (99%, 25mL) was slowly added to the reaction mixture and was refluxed for about 5-6 hours. The excess of solvent and hydrazine hydrate were distilled off. Now, product collected, was washed with distilled water, dried and recrystallized from ethanol & water (6:4) (table 2).

General Procedure for synthesis of 5-(4-substituted phenyl)-1, 3, 4-oxadiazole-2-thiol, 4(a-h)

The acid hydrazide [3 (a-h), 0.1 mol], carbon disulphide (0.12 mol), potassium hydroxide (0.1mol) and 250mL methanol were taken in a round bottomed flask and refluxed in the fume cupboard (Ainsworth *et al.,* 1956; Britsun *et al.,* 2005; Khan *et al.,* 2004). Heating was continued till evolution of hydrogen sulphide ceased. The excess of organic solvents were distilled off and then, distilled water was added. Dilute hydrochloric acid was added slowly to precipitate the crude product which was separated. The crude product was recrystallized using rectified spirit (table 3).

5-(4-Fluoro-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4a)

IR (KBr ν, cm^{-1}): 3086 (C-H stretching, aromatic), 2542 (S-H str), 1642 (C=N str, ring) 1576-1492 (C=C str, aromatic), 1179-1090 (C-O-C str, oxadiazole), 1056 (C-F str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 169.4 (C-2), 164.7 (C-5), 163.2 (C-4'), 130.2 (C-2',C-6'), 122.2 (C-1'), 115.6 (C-3',C-5'); LCMS: m/z 196 (M^+); Nitrogen found (calc.) for $C_8H_5FN_2OS$ (%):14.17 (14.28).

5-(4-Chloro-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4b)

IR (KBr ν, cm^{-1}): 3094 (C-H stretching, aromatic), 2548 (S-H str), 1654 (C=N str, ring), 1586-1482 (C=C str, aromatic), 1174-1088 (C-O-C str, oxadiazole), 772 (C-Cl str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 169.8 (C-2), 164.7 (C-5), 134.8 (C-4'), 129.6 (C-3',C-5'), 128.4 (C-2',C-6'), 124.4 (C-1'); LCMS: m/z 212 (M^+); Nitrogen found (calc.) for $C_8H_5ClN_2OS$ (%):13.07 (13.17).

5-(4-Bromo-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4c)

IR (KBr ν, cm^{-1}): 3096 (C-H stretching, aromatic), 2551 (S-H str), 1656 (C=N str, ring), 1584-1494 (C=C str, aromatic,), 1172-1086 (C-O-C str, oxadiazole), 654(C-Br str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 170.4 (C-2), 164.4 (C-5), 132.6 (C-3',C-5'), 129.8 (C-2',C-6'), 125.4 (C-1'), 123.2 (C-4'); LCMS: m/z 257 (M^+); Nitrogen found (calc.) for $C_8H_5BrN_2OS$ (%):10.81 (10.90).

5-(4-Iodo-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4d)

IR (KBr ν, cm^{-1}): 3099 (C-H stretching, aromatic), 2554 (S-H str), 1658, (C=N str, ring),1586-1496 (C=C str, aromatic), 1173-1088 (C-O-C str, oxadiazole), 540 (C-I str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 170.8 (C-2), 164.6 (C-5), 138.4 (C-3',C-5'), 129.6 (C-2',C-6'), 125.2 (C-1'), 94.3 (C-4'); LCMS: m/z 304 (M^+); Nitrogen found (calc.) for $C_8H_5IN_2OS$ (%):9.14 (9.21).

5-(4-Methyl-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4e)

IR (KBr ν, cm^{-1}): 3106 (C-H stretching, aromatic), 2558 (S-H str), 2914 (C-H str, aliphatic), 1670 (C=N str, ring), 1566-1462 (C=C str, aromatic), 1376 (C-H bend, aliphatic), 1175-1086 (C-O-C str, oxadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 171.6 (C-2), 164.5 (C-5), 131.8 (C-4'), 127.8 (C-2',C-6'), 125.8 (C-3',C-5'),123.4 (C-1'), 21.3 (Ar-$\underline{C}H_3$); LCMS: m/z 192 (M^+); Nitrogen found (calc.) for $C_9H_8N_2OS$ (%):14.48 (14.57).

5-(4-Hydroxy-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4f)

IR (KBr ν, cm^{-1}): 3256 (O-H, stretching), 3096 (C-H stretching, aromatic), 2539 (S-H str), 1664 (C=N str, ring), 1564-1490 (C=C str, aromatic), 1171-1088 (C-O-C str, oxadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 168.6 (C-2), 163.9 (C-5), 157.8 (C-4'), 118.8 (C-1'), 116.6 (C-3',C-5'),115.8 (C-2',C-6'); LCMS: m/z 194 (M^+); Nitrogen found (calc.) for $C_8H_6N_2O_2S$ (%):14.34 (14.42).

5-(4-Methoxy-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4g)

IR (KBr ν, cm^{-1}): 3104 (C-H stretching, aromatic), 2908 (C-H str, aliphatic), 2546 (S-H str), 1668 (C=N str, ring), 1558-1464 (C=C str, aromatic), 1368 (C-H bend, aliphatic), 1173-1090 (C-O-C str, oxadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 171.2 (C-2), 164.8 (C-5), 160.5 (C-4'), 118.7 (C-1'),116.5 (C-2',C-6'), 114.2 (C-3',C-5'), 56.4 (Ar-O-$\underline{C}H_3$); LCMS: m/z 208 (M^+); Nitrogen found (calc.) for $C_9H_8N_2O_2S$ (%):13.36 (13.45).

5-(4-Ethoxy-phenyl)-[1, 3, 4]-oxadiazole-2-thiol (4h)

IR (KBr ν, cm^{-1}): 3110 (C-H stretching , aromatic), 2924 (C-H str, aliphatic), 2552 (S-H str), 1672 (C=N str, ring), 1564-1471 (C=C str, aromatic), 1462 (C-H bend, aliphatic), 1178-1092 (C-O-C str, oxadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 170.2 (C-2), 165.2 (C-5), 159.8 (C-4'), 118.2 (C-1'), 115.4 (C-2',C-6'), 114.3 (C-3',C-5'), 64.5 (Ar-O-$\underline{C}H_2$-CH_3), 15.3 (Ar-O-CH_2-$\underline{C}H_3$); LCMS: m/z 222 (M^+); Nitrogen found (calc.) for $C_{10}H_{10}N_2O_2S$ (%):12.52 (12.60).

Table 1: Physical data of methyl 4-substituted benzoate, 2(a-h)

Compound Code	R	Yield (%)	M.P. (^oC)
2(a)	-F	80.4	ND
2(b)	-Cl	82.6	42-44
2(c)	-Br	85.6	80-82
2(d)	- I	85.2	112-14
2 (e)	$-CH_3$	89.2	33-35
2 (f)	-OH	88.4	39-41
2 (g)	$-OCH_3$	87.8	47-49
2 (h)	$-OC_2H_5$	87.2	51-53

Table 2: Physical data of 4-substituted benzoic acid hydrazide, 3(a-h)

Compound Code	R	Yield (%)	M.P. (^oC)
3(a)	-F	91.3	158-60
3(b)	-Cl	93.4	161-63
3(c)	-Br	94.2	162-64
3(d)	- I	93.8	174-76
3 (e)	$-CH_3$	94.3	116-18
3 (f)	-OH	94.1	125-27
3 (g)	$-OCH_3$	94.6	131-33
3 (h)	$-OC_2H_5$	94.0	141-43

Table 3: Physical data of 5-(4-substituted phenyl)-1, 3, 4-oxadiazole-2-thiol, 4(a-h)

Compound code	R	Molecular Formula	Molecular Weight	Yield (%)	M.P. (^oC)	R_f Value	Chloroform: Methanol (ratio)
4(a)	-F	$C_8H_5FN_2OS$	196.20	71.2	174-176	0.74	6:4
4(b)	-Cl	$C_8H_5ClN_2OS$	212.66	78.5	180-182	0.82	6:4
4(c)	-Br	$C_8H_5BrN_2OS$	257.10	86.9	190-192	0.88	7:3
4(d)	- I	$C_8H_5IN_2OS$	304.10	88.6	212-214	0.80	7:3
4(e)	$-CH_3$	$C_9H_8N_2OS$	192.23	82.4	210-212	0.84	6:4
4(f)	-OH	$C_8H_6N_2O_2S$	194.20	80.6	184-186	0.86	1:1
4(g)	$-OCH_3$	$C_9H_8N_2O_2S$	208.23	84.8	202-204	0.82	1:1
4(h)	$-OC_2H_5$	$C_{10}H_{10}N_2O_2S$	222.26	87.8	206-208	0.86	6:4

Antimicrobial screening

The synthesized compounds 4(a-h) were evaluated for their antibacterial activity against *Staphylococcus aureus* (MTCC7443), *Bacillus subtilis* (MTCC121), *Escherichia coli* (MTCC118) and *Pseudomonas aeruginosa* (MTCC424) bacterial strains with Ciprofloxacin as a standard drug (Aneja, 2007; Chattha *et al.,* 2015).While, the antifungal activity was evaluated against *Aspergillus niger* and *Candida albicans* using disc diffusion method and Fluconazole was used as a standard drug (I.P., 1996, Verma, 1998).

Accurately weighed 10 mg of test compound; was dissolved in N, N-dimethyl formamide (DMF) and volume made up to 10mL in a volumetric flask (1.0 mg/mL). This test solution was suitably diluted with DMF to get 100 and 200µg/mL concentrations. Similarly, the 10 and 20µg/mL solutions of standard Ciprofloxacin and Fluconazole were also made from their stock solutions.The discs were prepared (about 6 mm diameter) from a Whatman No. 1 filter paper.

For antibacterial activity, nutrient broth having composition: beef extract (1.0g), yeast extract (2.0g), peptone (5.0g) and sodium chloride (5.0g) were transferred to a measuring flask and dissolved properly in distilled water and volume made up to 1000 mL. The nutrient agar medium was prepared by adding 2.0% w/v of agar to nutrient broth and adjusted the pH to 7.4. Sabouraud's dextrose medium with composition peptone (10.0g) and dextrose (40.0g) dissolved in 1000mL of distilled water and pH adjusted to 5.7 was used for antifungal studies. The agar medium was prepared by adding 1.5% w/v agar to above prepared broth.

The media and prepared discs were autoclaved and transferred to each petri plate under aseptic conditions. A standard inoculum (5×10^5 c.f.u./mL) was inoculated to both types of agar plates which were properly distributed. The sterile discs (approximately 6 mm in diameter) previously moistened with the test solution or the standard drug solution, were cautiously positioned on agar plates for desired activity. Bacterial and fungal agar plates were

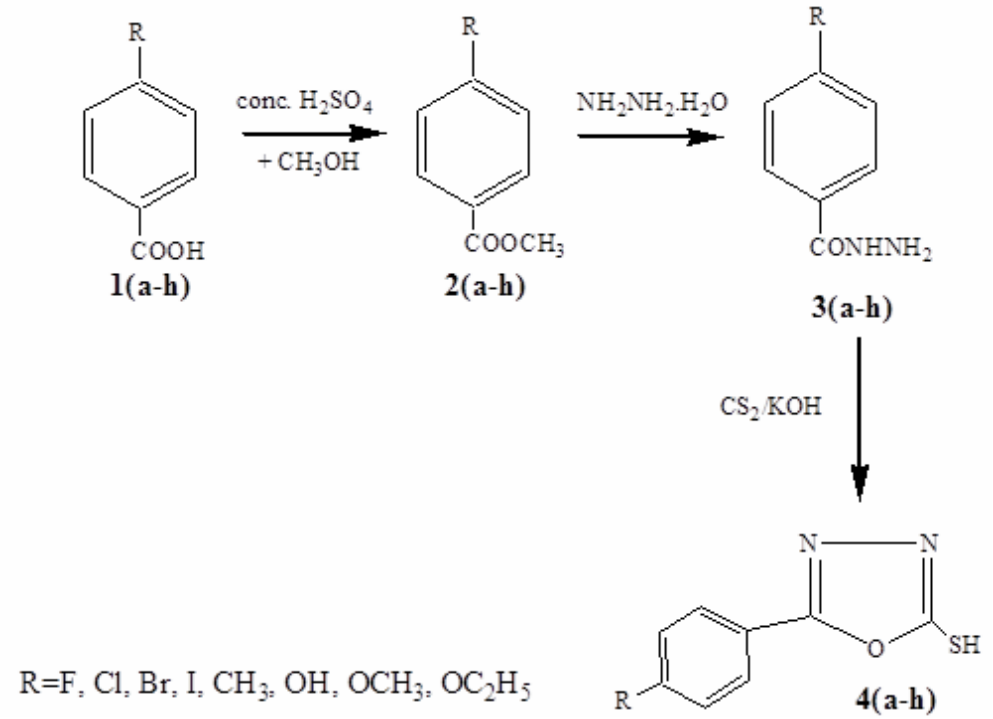

Fig: Synthetic path of 5-(4-substituted)-[1,3,4]-oxadiazole-2-thiol

incubated at 37±1°C for 24h and 25±1°C for 72h respectively.

The zone of inhibition was compared with that of standard drugs taken and percentage of inhibition was calculated (table 4). The zone of inhibition of DMF solvent control was found to be negligible and assumed to be zero mm.

Minimum inhibitory concentrations (MICs) were determined only for potent compounds (Chattha *et al.,* 2015). The various concentrations of test compounds in DMF like 90, 80, 70, 60, 50, 40, 30 and 20µg/mL were prepared and evaluated for inhibition. The concentration showing inhibition and that not showing inhibition were selected and further dilutions made and tested. This type of testing was continued till the actual concentration was evolved. The MIC values of compound which were found to the quite comparable with standard drug were determined by diluting the drug solution serially and observing their effectiveness in showing zone of inhibition (table 5).

Correlation of bioactivity of test compounds with drug like properties

In an attempt to correlate the biological activity with the physico-chemical properties of drugs, Lipinski (1997) formulated rule of five. He explained a chemical compound with certain biological activity which correlates molecular properties of orally active drug in humans (Lipinski *et al.,* 2000, 2001 and 2004). Lipinski's rule states that an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors
- Not more than 10 hydrogen bond acceptors
- A molecular mass less than 500 Daltons
- log *P* not greater than 5

In parallel to Lipinski, others proposed (Ghose *et al.,* 1999; Opera *et al.,* 2001) some extensions from rule of five which includes partition coefficient log *P* in -0.4 to +5.6 range; molecular weight (180-500 Daltons); molar refractivity (40 to 130 cm^3/mol) and number of atoms (20 to 70) and polar surface area which should not greater than 140 $A^{o\,2}$.

Veber *et al*. in 2002 reported his surprising finding that molecular flexibility also plays an important role in oral bioavailability. The more flexible the molecule, the less likely it is to be orally active (less than or equal to 10 rotatable bonds).

It was therefore, thought worthwhile to select as mentioned above, a few physico-chemical properties computed using Chem Draw ultra 6.0 and to correlate them with the biological activities.

RESULTS

The 1, 3, 4-oxadiazole leads were synthesized as per reaction scheme. The structures of compounds 4(a-h) of this series were elucidated by the spectral data (IR, ^{13}C-

Table 4: Data for Antimicrobial Activity-zone of inhibition and % growth inhibition of test compounds

Comps	Diameter of zone of inhibition (mm) and % growth inhibition[@]											
	S. aureus		*B. substilis*		*E. coli*		*P. aeruginosa*		*A. niger*		*C. albicans*	
T. Conc µg/mL	100	200	100	200	100	200	100	200	100	200	100	200
4 (a)	18 (90%)	21 (88%)	19 (86%)	22 (88%)	18 (78%)	21 (78%)	14 (70%)	18 (75%)	16 (73%)	20 (80%)	15 (71%)	19 (76%)
4 (b)	17 (85%)	20 (83%)	17 (77%)	20 (80%)	17 (74%)	21 (78%)	14 (70%)	17 (71%)	11 (50%)	13 (52%)	10 (48%)	14 (56%)
4 (c)	12 (60%)	16 (67%)	13 (59%)	16 (64%)	13 (57%)	17 (63%)	11 (55%)	16 (67%)	11 (50%)	14 (56%)	11 (52%)	14 (56%)
4 (d)	14 (70%)	18 (75%)	15 (68%)	19 (76%)	14 (61%)	19 (70%)	12 (60%)	17 (71%)	11 (50%)	14 (56%)	12 (57%)	14 (56%)
4 (e)	09 (45%)	11 (46%)	08 (36%)	11 (44%)	09 (39%)	12 (44%)	08 (40%)	11 (46%)	13 (59%)	17 (68%)	13 (62%)	16 (64%)
4 (f)	10 (50%)	13 (54%)	11 (50%)	14 (56%)	12 (52%)	15 (56%)	12 (60%)	14 (58%)	10 (45%)	13 (52%)	12 (57%)	17 (68%)
4 (g)	10 (50%)	14 (58%)	11 (50%)	14 (56%)	10 (43%)	13 (48%)	09 (45%)	12 (50%)	16 (73%)	19 (76%)	18 (86%)	21 (84%)
4 (h)	12 (60%)	15 (63%)	13 (59%)	15 (60%)	12 (52%)	16 (59%)	10 (50%)	13 (54%)	17 (77%)	21 (84%)	17 (81%)	20 (80%)
Cp[*]	20 (100%)	24 (100%)	22 (100%)	25 (100%)	23 (100%)	27 (100%)	20 (100%)	24 (100%)	--	--	--	--
Fu[*]	--	--	--	--	--	--	--	--	22 (100%)	25 (100%)	21 (100%)	25 (100%)
DMF	--	--	--	--	--	--	--	--	--	--	--	--

*Cp-Ciprofloxacin, Fu- Fluconazole, '--' indicates the zone of inhibition was not observed (assumed to be negligible).
* In this microbiological assay, the 10 µg/mL and 20 µg/mL concentrations of standard drug Ciprofloxacin and Fluconazole were taken. While, 100 µg/mL and 200 µg/mL of the test compounds were used.
[@] Percentage zone of inhibition of test compounds was calculated against various microbial strains with reference to standard and solvent control (DMF).

NMR and MS) and % nitrogen estimation confirming their established structures which is reported in experimental protocols.

In the synthesized compounds, Infra-red peaks appeared at 2640-2560 cm^{-1}, 1670-1640 cm^{-1} and 1090-1070 cm^{-1} for SH group, C=N linkage and C-O-C of oxadiazole respectively. The compound 4(a) showed IR peak at 1056 cm^{-1} due to C-F group. The presence of δ at about 171 ppm for C-2 and at 165 ppm approx. for C-5 in ^{13}CNMR spectrum can be justified by the formation of 1,3,4-oxadiazole nucleus. The nitrogen (%) and mass spectrum of title compounds reveals that the molecular ion peaks (M^+) were consistent with their assigned structures and molecular weights respectively.

Compounds 4(a) and 4(b) were found to be active showing average inhibitory action between 80- 90% as compared to Ciprofloxacin at concentrations of 10 and 20 µg/mL against *Staphylococcus aureus* and *Bacillus substilis*.

Compound 4(d) was moderately active showing average % inhibition between 71-79% whereas compounds 4(c) and 4(h) showed mild inhibitory effect ranging from 60-70%.

Rest of the compounds showed the inhibition from 40-59% considered to be inactive.

The compounds 4(a) and 4(b) showed promising activity ranging between 71-80% inhibition as compared to Ciprofloxacin against *Escherichia coli* and *Pseudomonas aeruginosa*. Moderate activity (61-70% of inhibition) was shown by compound 4(d). Compounds 4(c) and 4(h) showed mild inhibitory action (table 4).

Some of the compounds of the series were prominent in their action against *Aspergillus niger* and *Candida albicans* strains. The maximum inhibitory action (71-86% inhibition) was shown by the compounds 4 (a), 4(g) and 4(h). The remaining compounds of said series would have mild antifungal activities.

DISCUSSION

The MIC values were determined for the potent compounds in the statistical terms (Mean ± SD). The most potent compounds were found to be 26 to 42µg/mL for *Staphylococcus aureus*, 24 to 38µg/mL for *Bacillus subtilis*, 28 to 40µg/mL for *Escherichia coli* and 38 to 58 µg/mL for *Pseudomonas aeruginosa*. Compounds 4(a), 4(b) and 4 (d) were most active antibacterial compounds

Table 5: Data for Minimum Inhibitory Concentrations (MIC) of test and standards

Compounds	[#]Minimum Inhibitory Concentrations (MIC), µg/mL (Mean[$] ± SD)					
	S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
4 (a)	26±1.00	24±1.15	28±2.00	38±0.58	42±2.00	46±0.58
4 (b)	42±0.58	46±1.00	40±0.58	58±1.15	ND	ND
4 (d)	42±1.15	38±2.00	38±0.58	50±1.15	ND	ND
4 (g)	ND	ND	ND	ND	38±0.58	36±058
4 (h)	ND	ND	ND	ND	40±1.15	42±2.00

[#] ND indicates the MIC of those compounds were not determined
[$]Data was rounded off to whole numbers

Table 6: Computation of drug like properties for 1, 3, 4-Oxadiazole leads

Compound code	Lipinski's Rule of five				Variants of Drug likeness			Veber's Rule
	Molecular Weight (Daltons)	LogP	No. of HBD	No. of HBA	CLogP	Molar Refractivity (cm^3/mol)	Total no of atoms	Number of rotatable bonds
4(a)	196.20	2.54	1	3	1.76	50.45	18	1
4(b)	212.66	2.94	1	3	2.33	55.04	18	1
4(c)	257.10	3.21	1	3	2.48	57.86	18	1
4(d)	304.10	3.74	1	3	2.74	62.64	18	1
4(e)	192.23	2.87	1	3	2.10	55.28	21	1
4(f)	194.20	1.99	2	4	1.22	51.93	19	1
4(g)	208.23	2.26	1	4	1.67	56.70	22	2
4(h)	222.26	2.59	1	4	2.20	61.45	25	3

in the synthesized series. The range of MIC was 38 to 42 µg/mL for *Aspergillus niger* and 36 to 46µg/mL for *Candida albicans*. Compounds 4(a), 4(g) and 4 (h) were most active antifungal compounds within the series (table 5). From the above comparisons; it was found that compound 4(a) has highest antimicrobial activity among the series of synthesized compounds.

The 4-substituted phenyl group on C-5 of the oxadiazole nucleus affects antibacterial and antifungal activities of synthesized compounds. The synthesized compound 4(a) (R=4-fluorophenyl) on C-5 position of oxadiazole nucleus showed highest antibacterial and antifungal action. The compound 4(d) (R=4-iodophenyl) possess significant antibacterial and moderate antifungal activity. Therefore, the electron attracting groups (e.g. F, Cl, Br and I) present on benzene ring at C-5 position of oxadiazole nucleus is required for the potent antibacterial activity.

In contrast to this, the compounds 4(g) (R=4-methoxy phenyl) and 4(h) (R=4-ethoxy phenyl) showed moderate antibacterial but prominent antifungal activities. The activities of compounds 4(e) (R=4-methylphenyl) and 4(f) (R=4-hydroxyphenyl) were found to have feeble activity. The enhancement in the antifungal activities of the synthesized compounds may be due to the effect of electron releasing groups (i.e. CH_3, -OH, $-OCH_3$ and $-OC_2H_5$).

Table 6 contains calculated Lipinski parameters of the investigated compounds, 4 (a-h). From this table, it was found that all the title compounds 4 (a-h) were found to have the molecular weights (192-222 Daltons) and log *P* values (1.99–3.74) which are in the acceptable range following rule of five but compounds 4 (a-h) violates the said rule in terms of H-bond acceptors (3-4) and H-bond donors (1-2) which affect pharmacokinetic properties. Therefore, only two compounds 4(g) and 4(h) were close to rule of five which will be useful to develop the clinically important drug like candidates.

The fact that the physico-chemical properties of drugs determine their cellular permeability. Since, there is less change in lipophilicity affecting the drug's permeability. It establishes the background for the clinical development of the drug like molecule.

CONCLUSION

In the present study, it is reported that the synthesized 1, 3, 4-oxadiazoles are developed from acid hydrazides through simple synthetic approaches to search newer antimicrobial agents. For this, antimicrobial screening against various bacterial and fungal strains using disc diffusion method were studied. The antimicrobial compounds 4 (a-h) were subjected to assess drug-like properties. All the title compounds 4 (a-h) were within complete harmony of these parameters as established by 'Lipinski's rule'.

The results of this microbiological assay have been further investigated in order to explore the mode of action of these outstanding antimicrobial agents along with toxicity studies.

In conclusion, it is possible that auxiliary modifications in these bioactive compounds shall be of great effort to develop the selective antimicrobial agents.

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