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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, biological evaluation and in silico screening of oxadiazinanones

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Abstract A series of novel oxadiazinan-5-one namely 2-methyl-2-phenyl-1,3,4-oxadiazinan-5-one (**6**), 2-(3-hydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**7**), 2-(4-hydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**8**), 2-(2,4-dihydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**9**) and 2-(2,5-dihydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**10**) have been synthesized by the reaction of acetophenone and its derivatives with cyanoacetic acid hydrazide. The structural assignments of the products were done on the basis of IR, ^{1}H NMR, ^{13}C NMR, MS, and analytical data. The in vitro antioxidant and antimicrobial activity of all the synthesized compounds (**6–10**) was tested by the DPPH and disk diffusion method, respectively. The synthesized compounds were screened against Gram-positive and Gram-negative bacterial and fungal strains. Compound (**7**) showed the highest inhibition comparable with the standard antibiotic drugs Ciprofloxacin and Amphotericin B. The antibacterial activity of the synthesized compounds was further investigated with the help of in silico docking study using Discovery studio 3.1, Molego Virtual Docker and LigandScout to predict the active sites.

Keywords Oxadiazinanone · Cyanoacetic acid hydrazide · Antimicrobial activity · Antioxidant activity · Docking study

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Introduction

The development of protocols for the preparation of novel heterocyclic derivatives have been a subject of great interest because of increasing bacterial resistance adopted by microorganism to traditional antimicrobial drugs (Rajganarendar *et al*., 2010). A handful reports are available on the synthesis of substituted oxadiazinanones with varied yields (Ranganarendar *et al*., 2005; Hassan *et al*., 2002, 2004; Rodrigues *et al*., 2005). One of the remarkable features of oxadiazinanones is the presence of twisted boat conformation (Zukerman *et al*., 2009) and conformational mobility of nitrogen in their ring structure (Rosling *et al*., 1997; Sibi and Liu, 2001; Sibi *et al*., 2001; Hitchcock *et al*., 2001). This flexibility makes these compounds an ideal candidate for the design of biologically potent molecules which can act as lead compound in the development of antioxidant and antimicrobial drugs.

It is well known that oxadiazinanes play a promising role for scavenging the antimicrobial activity. Thus, merging oxadiazinane skeleton in a variety of heterocyclic or non-heterocyclic system found application in drug development for treatment of bacterial, fungal, convulsant, inflammatory, malarial, tuberculotic infection. Recently, oxadiazinanthione derivatives are employed for treatment of crops protection from insect pests and some Lepidopteron pest species (Tomlin, 2006). They were also found to act as agonists of nicotinic acetylcholine receptor affecting synapse in the insect's Central Nervous System (Tomizawa and Casida, 2005; Tan *et al*., 2007). In silico molecular docking technique play a key role in the drug design and discovery to predict the orientation of the docked molecule(s) at the active site, so, the in silico studies of the synthesized compounds were carried out to the predict

the active site of pathogenic ClpS (301F pdb) protein and the results obtained are reported.

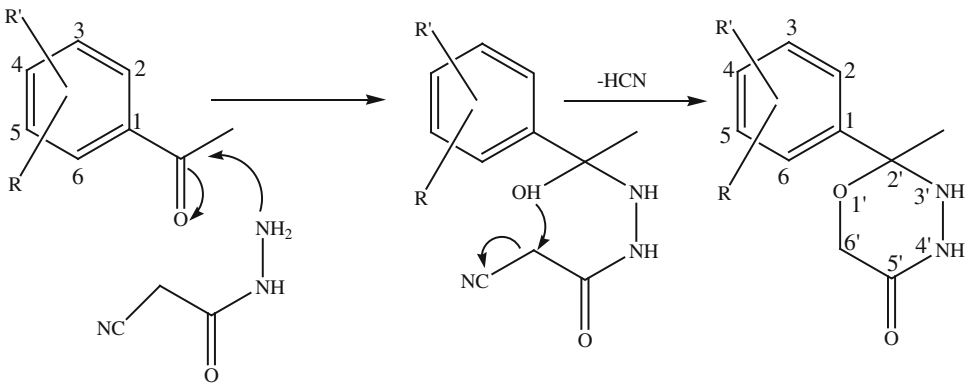
Results

Chemistry

With the aim of obtaining new candidates that may be of value in designing new, potent, selective and less toxic antioxidant and antimicrobial agent, we herein report the synthesis of oxadiazinan-5-ones (**6–10**) starting from acetophenones with versatile and convenient intermediate cyanoacetic acid hydrazide. The structural assignments of the products were done on the basis of IR, ^{1}H NMR, ^{13}C NMR, MS, and analytical data. The synthesized compounds were also explored for their plausible biological activities notably their antioxidant, antibacterial, and antifungal activities with respect to standard drugs. Cyanoacetic acid hydrazide reagent can act as an ambident nucleophile i.e., both an N- and C-nucleophile. Reaction of this reagent with various reactants leads to a number of polyfunctional heterocyclic compounds of pharmacological importance because of the availability of five active sites (Ried and Schleimer, 1958). Oxadiazinanones Scheme 1 were prepared from the reaction of respective acetophenones with cyanoacetic acid hydrazide in acetic acid as a solvent. The reaction does not demand rigorously dried solvents, reagents or inert atmosphere. The structures of the synthesized compounds were elucidated on the basis their spectral and micro analytical data. IR spectrum of synthesized compounds (**6–10**) gave useful information for resolving of structures of oxadiazinan-5-ones. All the compounds (**6–10**) showed two sets of absorption bands in the range of 3,248–3,250 and 3,382–3,385 cm^{-1} due to two N–H group stretchings and 1,680–1,695 cm^{-1} ascribed to C=O stretching frequencies, respectively. In addition, the absorption band at 3,500–3,549 cm^{-1} along with aromatic overtones was ascribed to the aromatic phenols. The shifting of band for carbonyl group in the IR spectra of all the synthesized compounds and emerging of weak band at 1,465–1,471 cm^{-1} (N–N) ascertains the formation of oxadiazinan-5-one. Moreover, the absence of absorption band at 2,200–2,250 cm^{-1} ascribed to C≡N group revealed the presence of cyclic compounds because of C≡N soft leaving group during S_N2 cyclization process in which a pair of electrons on the alkoxide ion is attracted to the carbon bonded to the cyanide group, which then leaves to generate the product. Furthermore, a strong band at 1,110–1,140 cm^{-1} explicated the formation of ether linkage during product formation. Although the formation of (**6–10**) may be interpreted through the nucleophilic attack either of hydroxyl group or hydroxyl ion of acetophenone formed during the attack of rear nitrogen of cyanoacetic acid hydrazide on cyano group (Scheme 1).

The ^{1}H NMR Spectra of the compounds (**6–10**) exhibited a sharp down field singlet at δ 3.20-3.68 attributed to 4′-NH proton while the other singlets at 9.14–9.95 ascribed to 3′-NH proton, respectively. The methylene protons in between ether linkage and ketonic group were displayed as two distorted doublets in the range of δ 3.60–3.87 and 3.87–4.45 ppm due to two protons at CH_2 (H_a and H_b). The phenolic OH in case of (**7–10**) was found to resonate as independent singlets at δ 10.82, 10.58 and 10.24, 10.58 and 10.25, 10.68 ppm respectively. Aromatic protons appeared as a multiplet in each case at δ 7.32–7.71 ppm for five

Scheme 1 Tentative mechanism for the formation of titled compounds **6–10**



Cyanoacetic acid hydrazide

R	R'	
H	H	**1**
H	3-OH	**2**
H	4-OH	**3**
2-OH	4-OH	**4**
2-OH	5-OH	**5**

R	R'	
H	H	**6**
H	3-OH	**7**
H	4-OH	**8**
2-OH	4-OH	**9**
2-OH	5-OH	**10**

Table 1 Antibacterial activity of oxadiazinan-5-one compounds **6–10**

Compounds	Diameter of zone of inhibition (mm)				
	Gram-positive bacteria		Gram-negative bacteria		
	S. Pyogenes	*MRSA**	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
6	19.1 ± 0.2	18.2 ± 0.4	24.2 ± 0.4	14.1 ± 0.4	17.4 ± 0.2
7	19.4 ± 0.2	19.1 ± 0.4	26.2 ± 0.4	15.1 ± 0.2	25.4 ± 0.2
8	19.3 ± 0.2	18.3 ± 0.4	24.4 ± 0.4	14.6 ± 0.2	18.4 ± 0.4
9	18.1 ± 0.1	17.1 ± 0.2	24.4 ± 0.2	14.1 ± 0.2	17.2 ± 0.5
10	18.6 ± 0.2	17.6 ± 0.5	24.1 ± 0.2	14.8 ± 0.4	17.2 ± 0.1
Standard	22.0 ± 0.4	21.0 ± 0.4	31.5 ± 0.2	19.5 ± 0.2	27.5 ± 0.4
DMSO	–	–	–	–	–

Positive control (standard): Ciprofloxacin and negative control (DMSO) measured by the halo zone test (unit, mm)

* Methicillin resistant *Staphylococcus aureus* (MRSA +ve)

Table 2 MIC and MBC results of oxadiazinan-5-one compounds **6–10** positive control ciprofloxacin

Compounds	Gram-positive bacteria				Gram-negative bacteria					
	S. Pyogenes		*MRSA**		*P. aeruginosa*		*K. pneumoniae*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6	25	50	25	50	25	100	25	100	25	50
7	12.5	25	12.5	25	12.5	25	12.5	25	12.5	25
8	25	50	25	50	25	100	25	50	50	100
9	50	100	50	100	50	>100	50	100	50	100
10	50	100	50	100	50	100	50	100	50	100
Standard	6.2	12.5	6.2	12.5	12.5	25	6.2	25	6.2	25

MIC (μg/ml) minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of bacteria completely, *MBC* (μg/ml) minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely

protons (**6**), δ 6.76–7.24 ppm for four protons (**7**), δ 6.72–7.59 ppm for four protons (**8**), δ 6.72–7.59 ppm for three protons (**9**) and δ 6.73–6.80 and 7.25 ppm for three (**10**). ^{13}C NMR spectra provided a firm support for the formation of compounds and their signals were in good agreement with proposed structures of synthesized compounds. All the compounds exhibited signals at δ 165.5–173.3 due to (C=O). Signals at δ 72.8–73.8 due to CH_2 of the oxadiazinan-5-one ring were also observed. Further evidences of structures (**6–10**) were given by (+)-ESI mass spectroscopy. The mass spectra of (**6**), (**7**), (**8**), (**9**), and (**10**) showed the molecular ion peak at m/z 192 $(M)^+$, m/z 209 $(M + H)^+$, m/z 231 $(M + Na)^+$, m/z 258 $(M + H + Na)^+$, and m/z 236 $(M + 2H)^+$, respectively.

Antimicrobial study

The evaluation of preliminary antimicrobial testing of compounds (**6–10**) is presented in Tables 1, 2, 3, and 4. The in vitro antimicrobial activity demonstrated that the compound (**7**) was most active among the five compounds in terms of antibacterial activity as well as antifungal activity. The MIC of compound (**7**) is 12.5 μg/mL with zone of inhibition 25.4 ± 0.2 against *Escherichia coli* (ATCC-8739) (bacterial strain) while as the MIC of compound **7** is 12.5 μg/mL with zone of inhibition 22.4 ± 0.4 against *Aspergillus fumigates* (fungal strain). The zones of inhibition (mm) of every compound against Gram-positive and Gram-negative bacteria are given in Table 1. The zones of inhibition (mm) of every compound against fungal

Table 3 Antifungal activity of oxadiazinan-5-one compounds **6–10** positive control (Amphotericin B) and negative control (DMSO) measured in terms of zone (unit, mm)

Compounds	CA	AF	TM	PM
6	21.4 ± 0.4	17.2 ± 0.4	14.5 ± 0.2	14.1 ± 0.4
7	25.5 ± 0.2	22.4 ± 0.4	17.2 ± 0.2	14.5 ± 0.4
8	22.4 ± 0.4	19.4 ± 0.4	16.3 ± 0.2	15.1 ± 0.4
9	20.2 ± 0.2	16.2 ± 0.5	14.1 ± 0.4	11.1 ± 0.2
10	21.4 ± 0.3	16.8 ± 0.8	14.8 ± 0.2	11.7 ± 0.4
Standard	30.5 ± 0.2	26.5 ± 0.2	23.5 ± 0.3	21.5 ± 0.5
DMSO	–	–	–	–

Diameter of zone of inhibition (mm)

CA Candida albicans, *AF Aspergillus fumigatus*, *TM Trichophyton entagrophytes, PM Penicillium marneffei*

Table 4 MIC and MFC of oxadiazinan-5-one compounds **6–10**

	CA		AF		TM		PM	
Compounds	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
6	25	25	25	50	25	50	25	100
7	12.5	25	12.5	25	12.5	25	12.5	50
8	25	100	50	100	25	50	50	100
9	50	100	50	100	50	100	50	>100
10	50	100	50	100	50	100	50	100
Standard	6.25	25	12.5	25	6.25	25	12.5	25

CA Candida albicans, *AF Aspergillus fumigatus*, *TM Trichophyton mentagrophytes, PM Penicillium marneffei, MIC* (μg/ml) minimum inhibitory concentration, i.e., the lowest concentration of the compound to inhibit the growth of fungus completely, *MFC* (μg/ml) minimum fungicidal concentration, i.e., the lowest concentration of the compounds for killing the fungus completely

strains are given in Table 3. MIC results of both bacterial and fungal strains are shown in Tables 2 and 4. It may be concluded that compound (**7**) bearing OH group at *meta* position of the benzene ring is most potent followed by compounds (**9**), (**8**), (**6**), and (**10**) bearing OH groups at different position, respectively. The high potency of (**7**) may be attributed to the presence of H-bond acceptor with proper orientation.

The antibacterial data of compounds (**6–10**) was further investigated on structural basis, molecular modeling and docking study of tested compounds into *P1* crystal form of pathogenic protein ClpS (301F pdb) using MVD and Discovery studio software was performed to predict the affinity, orientation and surrounding surface (Fig. 1) of the synthesized compounds at the active site. The different bonds i.e., hydrogen bonds, van der Wall forces and hydrophobic behavior formed with amino acids were in good agreement with the predicted binding affinities obtained by molecular docking studies as verified by antibacterial studies where compound (**7**) (Figs. 2, 3) was found to be the most active compound against *E. coli* strain compared with the standard drug (Figs. 4, 5) shows almost similar behavior in term of docking studies as well as Ramachandran plot. The activity of the compound (**7**) can also be explained, on structural basis (Fig. 6), which involves removal of hydrogen atom by hydroxyl group on the aromatic ring at the *meta* position as compared to *ortho*/*para* position. The increase in activity may be regarded due to low surface area which facilitates the guest relation with

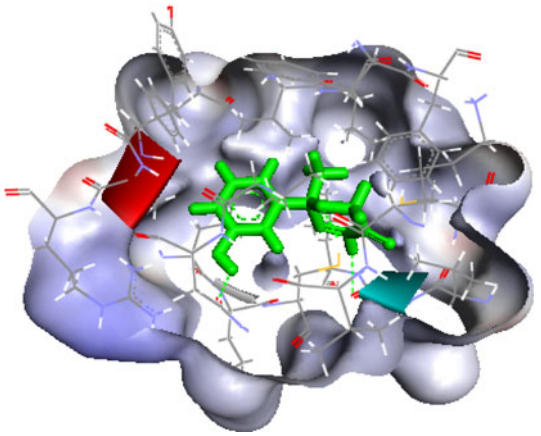


Fig. 1 Showing surface consist of different intra and interactions around the ligand (**7**)

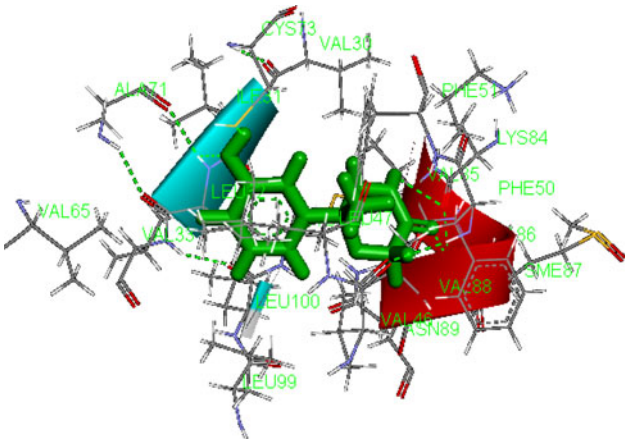


Fig. 2 Binding mode of compound (ligand) **7** in the binding site of P1 crystal form of *E. coli* ClpS (301Fpbd), it has 5 hydrogen bonds, it shows hydrogen bond one between A:CYS101:HG–LIGAND:N and LIGAND:H–A:CYS73:SG, and another hydrogen bonds between A:LEU32:H–LIGAND:O, A:VAL88:H–LIGAND:O and LIGAND:H–A:LYS84:O

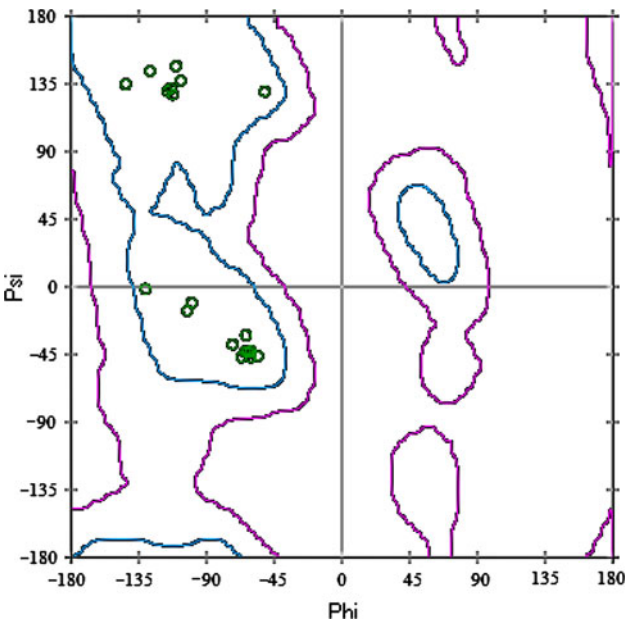


Fig. 3 Ramachandran *plot* of ClpS. The *figure* shows the pairs of (*psi*, *phi*) angles for all active binding amino acid residues with compound **7**

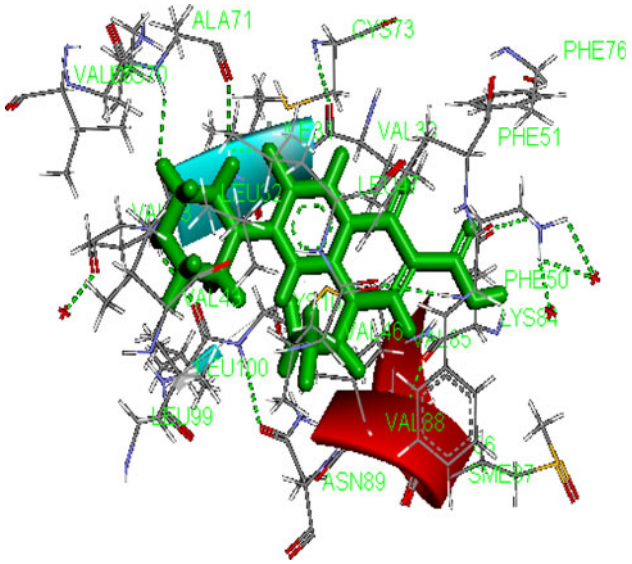


Fig. 4 Binding mode of standard drug Ciprofloxacin (ligand) in the binding site of P1 crystal form of *E. coli* ClpS (301Fpbd), it has 8 hydrogen bonds, it shows hydrogen bonds between A:LEU32:H–LIGAND:F, A:LYS84:HZ1–A:HOH169:O, A:LYS84:HZ3–A:HOH121:O, A:LYS84:HZ3–A:HOH169:O, A:LEU100:H–A:HOH1:O, A:HOH1:O–A:VAL33:O, A:HOH120:O–A:VAL33:O and A:HOH120:O–A:ASP35:OD2

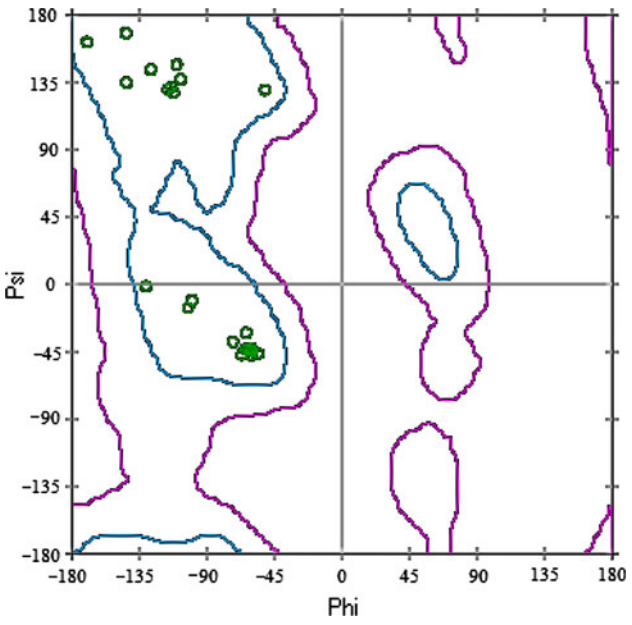


Fig. 5 Ramachandran *plot* of ClpS. The *figure* shows the pairs of (*psi*, *phi*) angles for all active binding amino acid residues with standard antibiotics Ciprofloxacin

receptor (host) in case of *ortho*/*para* positions. There are either large surface area or steric resistance (Fig. 6b, d) ceasing bonding with receptor. Moreover introduction of two hydroxyl groups on *ortho-para* positions on aromatic ring further decrease activity due to the lack of proper orientation which ceases available lone pair to make hydrogen bond with receptor (amino acids). Moreover compound (**10**) (Fig. 6b) makes intramolecular hydrogen bond between the ether linkage oxygen atom and phenolic group at the position of 6 which checked the formation of hydrogen bond with amino acids of pathogenic protein.

Docking studies

In the present investigation, an attempt was made to know the ligand (synthesized compounds)-receptor interaction of oxadiazinanones against pathogenic protein (ClpS; 301F pdb) by performing docking studies using discovery studio 3.1 (Accelrys Software Inc, 2011), Molego Virtual Docker (Thomsen and Christensen, 2006) and LigandScout (Wolber *et al*., 2006). These are one of the most accurate and commonly used tools to predict the binding orientation, energy calculation, and surrounding interactions between compound and protein. All the compounds were designed and structure was analyzed using Chem Draw Ultra 3D software and then these structures were energetically minimized using MOPAC, and coordinates of compounds were checked using PRODRG. The *P1* crystal structure of ClpS (301F pdb) was taken from PDB (www.rcsb.org/pdb). Docking studies were carried out to distinguish the nature and the extent of interaction of title product oxadiazinanones with *P1* crystal of pathogenic strain. The docking studies of a typical compound (**7**) are presented in Table 5. It shows the interaction between protein and compound (**7**) in the form of MolDock scores, hydrogen bond interactions. The obtained data indicates that the MolDock score is minimum indicating more than 75 % host–guest relation between compound-receptor. Therefore, the compound (**7**) shows maximum inhibitor action. Moreover, the obtained docking result of (**7**) mentioned in Table 1 along with bond distance showed three hydrogen bond interactions between compound and receptor. The maximum inhibitor action and drug-likeness of (**7**) can be predicted using Lipinski Rule of Five (Table 7) which explains the molecule in terms of drug and it must contain minimum number hydrogen donors group (<5) and lipophilicity (Log $P > 5$). Compound (**7**) fully satisfies this criteria of drug, showing almost similar behavior in term of docking studies, Ramachandran plot as well as structure performance (Figs. 2, 3) as compare with the reference drug (Figs. 4, 5).

Antioxidant study

The synthesized compounds (**6–10**) were subjected to free radical scavenging activity by DPPH methods (Nami *et al*., 2012; Shirwaington *et al*., 2004). This model of scavenging activity by DPPH radical is extensively applied to evaluate

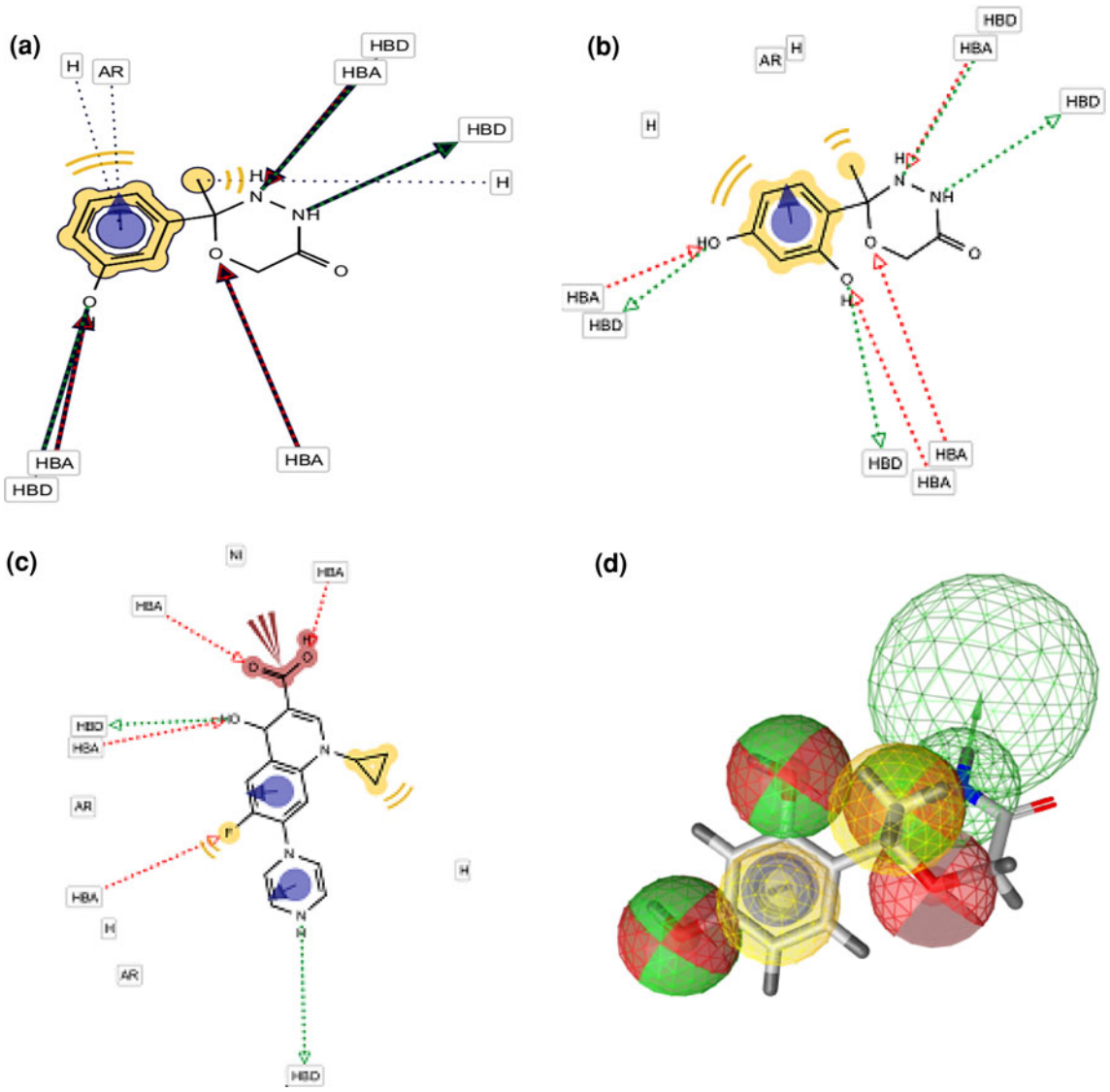


Fig. 6 Ligand interaction **a** showing hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), aromatic (AR), and hydrophobic behavior (H), **b** showing intramolecular hydrogen bond between OH of Ar and ether linkage including HBA, HBD, AR, and H, **c** ciprofloxacin showing HBA, HBD, AR, and H and **d** showing steric resistance between methyl and 2-OH of Aromatic

Table 5 Amino acid residues and atoms of ligand **7** involved in hydrogen bonding, bond length, MolDock, ReRank, and HBond calculation

Residues	Atom ID	Pdb atom name	Ligand atom	Distance (Å)	MolDock score	rerank score	HBond
Cys	101 (A)	S (donor)	O (acceptor)	3.22			
Cys	73 (A)	S (donor)	O (both)	3.52	−72.418	−57.34	−0.8004
Leu	32 (A)	N (donor)	O (acceptor)	2.01			
Ala	71 (A)	O (acceptor)	O (both)	3.59			

the antioxidant activity in shorter time that as compared to other methods. The odd electron in the DPPH free radical gives a strong absorption band at $\lambda = 517$ nm, which is purple in color. This property makes it suitable for spectrometric studies. The DPPH assay has often been used to estimate the antiradical activity of antioxidant. The free radical scavenging capabilities of the compounds were measured in term of hydrogen donating of free radical scavenging ability after adding methanolic solution of (DPPH) to the sample solution of different concentrations.

The synthesized compounds react with DPPH and convert it into 1,1-diphenyl-2-picrylhydrazine. The extent of decolourization is an indicative of potentiality of antioxidant behavior of a particular compound. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate. The compounds (**6–10**) were tested for antioxidant property by DPPH method. The compound (**7**) showed the highest IC_{50} value in antioxidant activity followed by the compounds (**9**), (**8**), (**6**), and (**10**) respectively and result were reported in Table 6.

Table 6 Quantitative screening of antioxidant activity by DPPH assay method ($n = 3$)

S.no	Compounds	Absorbance	Absorbance at 517 nm					IC_{50}
			2 μg/mL	4 μg/mL	6 μg/mL	8 μg/mL	10 μg/mL	
1	Control	($Abs_{control}$)	0.9428 ± 0.08	0.9428 ± 0.08	0.9428 ± 0.08	0.9428 ± 0.08	0.9428 ± 0.08	
2	**6**	Abs_{sample}	0.8498 ± 0.05	0.7189 ± 0.04	0.6143 ± 0.08	0.5016 ± 0.06	0.3815 ± 0.04	8.25
		(AA %)	9.86	23.75	34.84	46.80	59.53	
3	**7**	Abs_{sample}	0.7850 ± 0.07	0.6433 ± 0.06	0.4698 ± 0.08	0.3180 ± 0.05	0.1625 ± 0.03	5.99
		(AA %)	16.73	31.76	50.16	66.27	82.76	
4	**8**	Abs_{sample}	0.8305 ± 0.03	0.7059 ± 0.07	0.5920 ± 0.05	0.4805 ± 0.06	0.3545 ± 0.08	8.02
		(AA %)	11.91	25.13	37.21	49.03	62.40	
5	**9**	Abs_{sample}	0.7950 ± 0.05	0.6579 ± 0.07	0.4805 ± 0.03	0.3389 ± 0.08	0.1785 ± 0.05	6.28
		(AA %)	15.67	30.21	49.03	64.05	81.06	
6	**10**	Abs_{sample}	0.8805 ± 0.06	0.7668 ± 0.04	0.7050 ± 0.04	0.6568 ± 0.07	0.6380 ± 0.05	12.95
		(AA %)	6.61	18.67	25.22	30.34	32.33	
7	Standard	Abs_{sample}	0.7689 ± 0.02	0.6135 ± 0.07	0.4396 ± 0.04	0.2890 ± 0.06	0.1226 ± 0.04	4.45
		(AA %)	18.44	34.92	53.37	69.34	86.99	

Materials and methods

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Shimadzu IR-408 Perkin-Elmer 1800 (FTIR) and its values are given in cm^{-1}. The ^1H NMR and ^{13}C NMR spectra were run in DMSO-d_6 on a Bruker Avance-II 400 MHz instrument with TMS as internal standard: *J* values are in Hertz. Chemical shifts are reported in ppm (δ) relative to the solvent peak. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Elemental analyzes (C, H, N) were conducted using Carlo Erba analyzer model 1108. Thin layer chromatography (TLC) glass plates (20 × 5) were coated with silica gel (E-Merck G254, 0.5 mm thickness) and exposed to iodine vapors to check the purity as well as the progress of reaction. Acetophenone, *m*-hydroxy acetophenone, *p*-hydroxy acetophenone, and 2,4-dihydroxy acetophenone were purchased from Sigma–Aldrich Chemicals Pvt. Ltd. Other chemicals used were of analytical grade without further purification. Cyanoacetic acid hydrazide reagent was synthesized by usual method.

Experimental

General method for the preparation of oxadiazinan-5-one derivatives (**6–10**)

To a solution of acetophenone or its derivatives (**1–5**), (1 mmol) in acetic acid (10 mL) was added cyano acetic hydrazide in equimolar ratio in same solvent. The reaction mixture was stirred for 4 h. The progress as well as purity of reaction was monitored by TLC. On completion of reaction the precipitate was formed. The precipitate was suspended in water (30 mL), neutralized with saturated aqueous $NaHCO_3$ and filtered using suction. It was washed with water and ether was added. The ethereal layer was

washed with water and dried over anhydrous sodium sulfate. Crude product was obtained by removing the solvent and was crystallized from chloroform–methanol to furnish oxadiazinanone-5-one (**6–10**).

2-methyl-2-phenyl-1,3,4-oxadiazinan-5-one (**6**)

It was crystallized with $CHCl_3$–EtOH as white colored solid; Yield: 65 %, m.p. 162–65 °C; Anal. Calc. for $C_{10}H_{12}N_2O_2$: C, 62.49; H, 6.29; N, 14.57; O, 16.65. Found: C, 62.49; H, 6.30; N, 14.48; O, 16.73; IR ν_{Max}^{KBr} cm^{-1}: 3244 (N–H), 3382 (N–H), 1680 (conjugated C=O), 1465 (N–N), 1110 (–O–); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.80 (3H, s, CH_3), 3.28 (1H, s, NH, D_2O exchangeable), 3.74, 4.0 (H_a, H_b dis. d, CH_2), 7.32–7.71 (5H, m, Ar–H), 9.95 (1H, s, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.1 (CH_3), 72.8 (C6′), 94.1 (C2′), 126.3 (C2 & 6), 128.4 (C3 and 5), and 129 (C4), 149.6 (C1) and 165.5 (C=O); MS (ES+) m/z: 192 $(M)^+$.

2-(3-hydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**7**)

The compound **(7)** was crystallized with $CHCl_3$–MeOH as yellowish white colored solid; Yield: 70 %, m.p.172–75 °C; Anal. Calc. for $C_{10}H_{12}N_2O_3$; C, 57.67; H, 5.81; N, 13.46; O, 23.06; found: C, 57.69; H, 5.81; N, 13.45; O, 23.09; IR ν_{Max}^{KBr} cm^{-1}: IR ν cm-1: 3435 (OH), 3248 (N–H), 3383 (N–H), 1685 (C=O), 1468 (N–N), 1120 (–O–); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.81 (3H, s, CH_3), 3.67 (1H, s, NH, D_2O exchangeable), 3.87, 3.94 (H_a, H_b, dis. d, CH_2), 6.76–7.24 (4H, m, H_2, H_4, H_5, H_6, Ar–H), 9.14 (1H, s, NH, D_2O exchangeable), 10.82 (1H, s, 3-OH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.2 ($\underline{C}H_3$), 73.2 (C6′), 93.0 (C2′), 113.2 (C2), 113.4 (C4), 115.3 (C6), 117.7 (C5), 149.9 (C1), 159.2 (C3) and 172.7 (C=O); MS (ES+) m/z: 209 $(M + H)^+$.

2-(4-hydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**8**)

It was crystallized with $CHCl_3$–MeOH as white colored solid; Yield: 70 %, m.p. 193–95 °C; Anal. Calc. for $C_{10}H_{12}N_2O_3$; C, 57.67; H, 5.81; N, 13.46; O, 23.06; found: C, 57.70; H, 5.84; N, 13.43; O, 23.03; IR ν_{Max}^{KBr} cm^{-1}: 3440 (OH), 3249 (N–H), 3384 (N–H), 1690 (C=O), 1470 (N–N), 1134 (–O–); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.73 (3H, s, CH_3), 3.33 (1H, s, NH, D_2O exchangeable), 3.60, 3.87 (H_a, H_b, dis. d, CH_2), 6.72-6.78 (2H, m, H_3, H_5, Ar–H), 7.49–7.59 (2H, m, H_2, H_6, Ar–H), 9.29 (1H, s, NH, D_2O exchangeable), 10.58 (1H, s, 4-OH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.0 (CH_3), 73.4 (C6′), 93.5 (C2′), 113.4 (C3), 115.5 (C5), 128.4 (C2), 128.6 (C6), 139.1 (C1), 158.5 (C4) and 173.0 (C=O); MS (ES+) m/z: 231 $(M + Na)^+$.

2-(2,4-dihydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**9**)

It was crystallized with $CHCl_3$–EtOH as yellowish colored solid; Yield: 65 %, m.p. 185–87 °C; Anal. Calc. for $C_{10}H_{12}N_2O_4$; C, 53.55; H, 5.40; N, 12.50; O, 28.55; found: C, 53.51; H, 5.43; N, 12.51; O, 28.52; IR ν_{Max}^{KBr} cm^{-1}: 3466 (OH), 3250 (N–H), 3385 (N–H), 1695 (C=O), 1470 (N–N), 1140 (–O–); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.65 (3H, s, CH_3), 3.33 (1H, s, NH, D_2O exchangeable), 3.75, 4.11 (H_a, H_b, dis. d, CH_2), 6.72–6.78 (2H, m, H_2, H_6, Ar–H), 7.51–7.59 (1H, d, Ar–H), 9.28 (1H, s, NH, D_2O exchangeable), 10.24 (1H, s, 4-OH, D_2O exchangeable), 10.58 (1H, s, 2-OH, D_2O exchangeable) ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.2 (CH_3), 73.3 (C6′), 91.3 (C2′), 108.1 (C3), 113.4 (C5), 121.0 (C1), 129.3 (C6), 153.1 (C2), 157.5 (C4) and 173.1 (C=O); MS (ES+) m/z: 235$(M + H)^+$.

2-(2,5-dihydroxyphenyl)-2-methyl-1,3,4-oxadiazinan-5-one (**10**)

It was crystallized with $CHCl_3$–EtOH as yellowish colored solid; Yield: 65 %, m.p. 197–200 °C; Anal. Calc. for $C_{10}H_{12}N_2O_4$; C, 53.55; H, 5.40; N, 12.50; O, 28.55; found: C, 53.52; H, 5.43; N, 12.49; 0, 28.54; IR ν_{Max}^{KBr} cm^{-1}: 3465 (OH), 3248 (N–H), 3386 (N–H), 1693 (C=O), 1471 (N–N), 1140 (–O–); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.67 (3H, s, CH_3), 3.68 (1H, s, NH, D_2O exchangeable), 3.76, 4.13 (H_a, H_b, dis. d, CH_2), 6.73–6.80 (2H, m, H_3, H_4, Ar–H), 7.25 (1H, d, H_6 Ar–H), 9.96 (1H, s, NH, D_2O exchangeable), 10.25 (1H, s, 5-OH, D_2O exchangeable), 10.68 (1H, s, 2-OH, D_2O exchangeable) ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.3 (CH_3), 73.8 (C6′), 91.5 (C2′), 108.1 (C3), 113.8 (C4), 121.9 (C1), 129.9 (C6), 153.5 (C2), 157.9 (C5) and 173.3 (C=O); MS (ES+) m/z: 257 $(M + Na)^+$.

Antimicrobial activity

The in vitro antimicrobial activities of acetophenone oxadiazinanones (**6–10**) were evaluated using the bacterial cultures of *Staphylococcus Pyogenes* (clinically isolated), Methicillin resistant *Staphylococcus aureus* (MRSA +Ve), *Pseudomonas aeruginosa* (ATCC-9029), *Klebsiella pneumonia* (clinically isolated), and *Escherichia coli* (ATCC-25922) and fungal cultures of *Candida albicans* (ATCC-90028), *Candida albicans, Aspergillus fumigates,* and *Aspergillus niger* by the disk diffusion method (Cruickshank *et al*., 1975; Collins, 1976; Khan, 1997; Varma, 1998) and subsequently the Minimum Inhibitory Concentration (MIC) of all the compounds were determined. Ciprofloxacin was used as standard drug in case of bacteria and Amphotericin B in case of fungi, whereas the disk poured in DMSO (dimethyl sulphoxide) was used as a

negative control. The MIC was assisted by the macro dilution test using standard inoculums of 10^5 c.f.u./mL. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 3.

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 approximately 5×10^5 c.f.u./mL 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). To obtain the minimum bacterial concentration (MBC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9 % of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are summarized in Table 4.

Antifungal studies

Antifungal activity was also done by disk diffusion method using *Candida albicans, Aspergillus, fumigatus, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The fungal activity of each compound was compared with Amphotericin B used as standard drug. Inhibition zones were measured and compared with the control. The fungal zones of inhibition values are given in Table 5. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4–6×10^4 c.f.u./mL. The cultures were incubated for 48 h at 35 °C and the growth was monitored. To obtain the minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. were counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9 % of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 4.

Antioxidant studies

The compounds (**6–10**) were tested for their antioxidant property using 1,1-diphenylpicrylhydrazyl (DPPH) method. In this procedure, drug stock solution (1 mg/mL) was diluted to final concentration of 2, 4, 6, 8, 10 and 12 in methanol. Methanolic DPPH solution (1 mL, 0.3 mmol) was added to 3.0 mL of drug solution of different concentrations. The tube was kept at an ambient temperature for 30 min and the absorbance was measured at 517 nm. The scavenging activity was calculated by following formula: % inhibition $= [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$. Where A_{Control} is the absorbance of the L-ascorbic acid (Standard) and A_{Sample} is the absorbance of different compounds. The methanolic DPPH solution (1 mL, 0.3 mM) was used as control. The inhibitory concentration (IC_{50}) value represents the concentration required to exhibit 50 % antioxidant activity (Fig. 7) The IC_{50} values were calculated by the linear regression of plots where the abscissa represents the concentration of the compounds (μg/mL). Explicitly, IC_{50} is the average percentage of antioxidant activity. Results in the form of percent inhibition are tabulated in the Table 6.

In silico studies

The retrieved protein ClpS (301F pdb) was improved using import and preparation option of MVD software, and

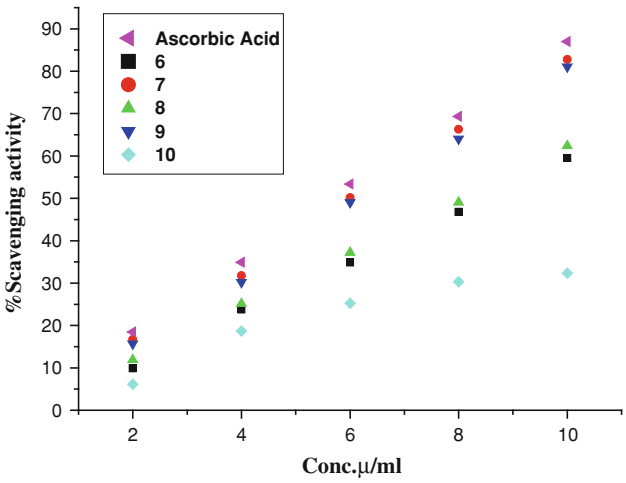


Fig. 7 Antioxidant activity of compounds **6–10**

Table 7 In silico ADMET properties of synthesized compounds

Compound	Mutagenic	Tumerogenic	Irritation	Reproductive effect	cLog *P*	Solubility	Drug-likeness	Drug score
6	No	No	No	No	0.19	−1.28	4.18	0.96
7	No	No	No	No	−0.10	−0.98	2.34	0.93
8	No	No	Medium	Medium	−0.10	−0.98	1.58	0.57
9	No	No	No	No	−0.40	−0.68	2.28	0.93
10	No	No	No	No	−0.40	−0.68	2.56	0.94

Table 8 Lipinski rule of five for compounds **6–10**

Molecular weight (compound)	Hydrogen bond (donor)	Hydrogen bond (acceptor)	Log *P*	Molar refractivity
192.00 (**6**)	2	3	0.514	51.025
208.00 (**7**)	3	4	0.219	52.689
208.00 (**8**)	3	4	0.219	52.688
224.00 (**9**)	4	5	−0.075	54.353
224.00 (**10**)	4	5	−0.075	54.353

missing bond order, hybridization state, angle, and flexibility for achieving reliable potential binding site in receptor. The energy minimized ligands (synthesized compounds) drawn with ChemDrwa Ultra (2D & 3D) were predicted ADMET Table 7, drug-likeness and ligand structural properties (Fig. 6, c for ciprofloxacin) achieved through organic chemistry portal Lipinski's Rule of Five Table 8 and LigandScout, respectively. Discovery studio, MVD, and Ligand scout were used to perform molecular docking and energy profile of ligand-receptor interaction, independently.

Conclusions

The present work reports the in vitro antimicrobial and antioxidant activities of synthesized oxadiazinan-5-one derivatives of acetophenones. It is inferred from biological and in silico docking studies that all compounds exhibit substantial antibacterial and antifungal activity against different strains of bacterium and fungus, respectively. Moreover, oxadiazinanones moiety also showed good antioxidant activity.

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