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Research Article

BIOLOGICAL EVALUATION, QSAR AND MOLECULAR MODELING STUDIES OF 2,4-DICHLOROBENZOIC ACID DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT

Objective: The aim of this study was to evaluate 2,4-dichlorobenzoic acid derivatives as antimicrobial agents through *in vitro*, QSAR and molecular docking studies.

Methods: The compounds were subjected to *in vitro* antimicrobial screening by test tube dilution method and the structural characteristics governing the antimicrobial potential were studied using QSAR methodology. These compounds were also screened for docking simulation to find out binding confirmation of reported compounds with PDB 1aj0 and 5fsa using AutoDock tools and discovery studio.

Results: The antimicrobial evaluation data indicated that compounds 13 and 18 were found to be the most effective against all the bacterial strains and *Aspergillus niger* while compounds 1 and 14 exhibited more antifungal potential against *Candida albicans*. QSAR studies confirmed the role of molar refractivity and Balaban index (J) as controlling parameters for antimicrobial potential. Molecular modeling study revealed that compounds interact with the active site of PDB by hydrophobic, hydrogen bonding, and Van der Wall interactions.

Conclusion: These test compounds were identified as potent candidates for the control of microbial strains tested, and structural relationship with activity may provide valuable information for further design and synthesis of compounds with antimicrobial potential.

Keywords: Antimicrobial, QSAR, Molecular docking.

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INTRODUCTION

The multidrug resistance associated with life-threatening microbial infections is a re-emerging and alarming microbial threat and amenable for millions of deaths annually due to the inadequacy of efficacious antimicrobial drugs [1]. To improve the current antimicrobial therapies, several studies have been conducted to improve the available treatments, but due to the continuance of the antibiotic resistance, pathogens have become a critical and complicated health issue [2].

Sulfonamides, being antibacterial, having the prominent mechanism of action including attachment of sulfonamides with the dihydropteroate synthetase (DHPS) enzyme and alteration of bacterial pathways of folic acid synthesis in few eukaryotic cells, but in human beings, this mechanism is not followed [3,4]. The sulfonamides have been recognized due to various reported biological activities such as anticancer [5-9], anti-Alzheimer [10,11], anti-tubercular [12,13], antimicrobial [14-17], anti-inflammatory [18], carbonic anhydrase inhibitors [19-22], antidiabetic [11], anticonvulsant [23], and antimalarial [24-26].

Due to these facts, there is a prime need to shorten the duration of therapy and ascertain newer antimicrobial agents to avert the emergence of resistance. In continuation of our efforts dedicated toward development of antimicrobials, we have screened compounds (1-18) reported by Thakral and Singh [27] for antimicrobial potential along with QSAR and computational studies.

METHODS

Antimicrobial evaluation

The reported compounds were evaluated for their *in vitro* antimicrobial potential against bacterial and fungal strains through serial dilution technique to get their minimum inhibitory concentration [28]. The weighed amount of nutrient broth and Sabouraud dextrose broth was dissolved in distilled water to prepare nutrient medium for bacteria and fungus, respectively, and 1 ml of nutrient medium was transferred to each test tube. The test sample compound (0.01 g) was dissolved in 10 ml of dry dimethyl sulfoxide to give a stock solution of 100 µg/ml. The 1 ml solution of test compounds was transferred to test tubes having sterilized nutrient medium and diluted serially to get a set of five dilutions of test compounds having concentrations 50, 25, 12.5, 6.25, and 3.125 µg/ml. Minimal inhibitory concentration for each sample was investigated against Gram-positive bacterial strains; *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443), and *Staphylococcus epidermidis* (MTCC 435), two Gram-negative bacterial strains; *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 424), and two fungal strains; *Aspergillus niger* (MTCC 8189) and *Candida albicans* (MTCC 227). The freshly cultured strains of each organism were transferred in to test tubes and incubated at 37±1°C for 24 h for bacterial strains, 48 h for *C. albicans* and 7 days at 25±1°C for *A. niger*. Ciprofloxacin was considered as a standard for antibacterial and fluconazole for antifungal activity.

QSAR

The two-dimensional structures were drawn, and energy minimization was done by molecular mechanics force field (MM) process of hyperchem 6.03 (1993), and the pre-optimization of the energy minimized structures was done. The calculation of physicochemical parameters for each compound was done using TSAR 3.3 software for Windows (2000) and the SPSS software package (1999) was used for regression analysis [29,30].

Molecular modeling

The advanced docking program, AutoDock Vina was used to evaluate the binding properties of test compounds into microbial targets [31]. The crystal structures of a ternary complex of *E. coli* dihydropteroate

synthase (1aj0) [32] and sterol 14-alpha-demethylase (CYP51) from a pathogenic yeast *Candida albicans* in complex with the antifungal drug posaconazole (5fsa) [33,34] were downloaded from the protein data bank (www.rcsb.org). All bound water molecules and cocrystallized ligands were removed from protein, and polar hydrogen atoms were added. 2D structure of ligands was penciled in MarvinSketch and saved in pdb format, then further saved into pdbqt format using AutoDock Vina. Energy minimization was performed using the MMFF94 force field. Then docking was performed according to specified conditions of grid box used by AutoDock tools. The Vina search space chosen was center_x = 39.66, center_y = 7.14, center_z = 2.63; center_x = 193.6, center_y = 1.0, center_z = 39.0 and size_x=40, size_y=40, size_z = 40 for 1aj0, 5fsa, respectively. The exhaustiveness was set to be 8.

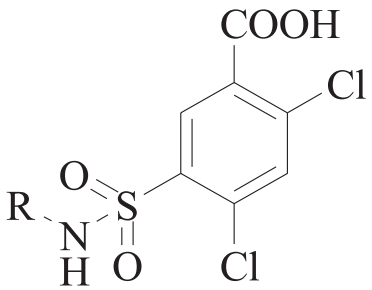
RESULTS AND DISCUSSION

In vitro antimicrobial studies

All the compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus*, *B. subtilis*, and *S. epidermidis* (Gram-positive bacteria), and *E. coli* and *P. aeruginosa* (Gram-negative bacteria), and antifungal potential against *C. albicans* and *A. niger*. The power management integrated circuit (pMIC) values obtained are recorded in Table 1.

Based on results, it could be inferred that compounds 13 and 18 have the highest and broad spectrum of antimicrobial potential while compound 2 exerted the highest activity against *S. epidermidis* with a pMIC value of 1.785 μM. Compounds 1, 3, and 11 exhibited good antimicrobial activity against all strains. Compounds 1 and 14 exhibited the most potent antifungal activity against *C. albicans* with a pMIC value of 2.102 μM. All other test compounds showed moderate antimicrobial potential against all bacterial and fungal strains. Compounds 13 and 18 also displayed more inhibition effect against *A. niger* species. Compounds 1, 3, 11, and 14 exhibited good antifungal potential against *A. niger*.

It is observed that in this series compound 2 with p-Cl substituent showed selective inhibition against *S. epidermidis* whereas the activity was diminished when substituted with the o-NO_2 group. Results revealed that chloro substituent on phenyl ring exerted higher activity against all microbial strains as compared to fluoro substituent. Apparently, the compound bearing methoxy group displayed more antimicrobial potential as compared to methyl substituted compound. Replacement of aromatic anilines with aliphatic amines as observed in compounds 15, 16, and 17, afforded a decrease in antimicrobial potential against all strains. A deep insight into the structure of test compounds revealed that their antimicrobial activity seems to be modulated by the effect of the substituent. Above all, it could be inferred that a significant broad spectrum of antimicrobial activity was allied with compounds incorporating electron withdrawing substitution [17].



General structure of 2,4-dichlorobenzoic acid derivatives.

QSAR study

In this present study, dataset of 18 compounds (1-18) was used to establish a quantitative relationship between antimicrobial activity and structural descriptors of substituted sulfonamide derivatives. The structural descriptors coding for lipophilic, steric, electronic, and molecular connectivity were used in this study, and the corresponding value for each molecular descriptor is presented in Table 2. A correlation matrix was constructed to find out the inter-relationship of different calculated molecular descriptors and antimicrobial activity as well as inter-relationship of different molecular descriptors with each other (Table 3).

It was observed from correlation matrix that highest correlation existed between Randic parameter (R) and first-order molecular connectivity index ($^1\chi$, r=1.000) and between Weiner index (W) and zero-order molecular connectivity index ($^0\chi$, r=0.995). The least interrelationship was observed for the energy of highest occupied molecular orbital (HOMO) and energy of lowest unoccupied molecular orbital (r=0.050) and energy of HOMO and third-order alpha shape indices ($k\alpha_3$, r=−0.063). In general high inter-relationship was observed for different calculated molecular descriptors with each other. The correlation of molecular descriptors with respective antibacterial and antifungal activity is presented in Table 4.

The different molecular descriptors were subjected to linear free energy regression analysis with antibacterial activity against *S. aureus* and a mathematical model (Eq. 1) illustrating the importance of molar refractivity (MR) was obtained.

$$pMIC_{sa} = 0.052\ MR + 1.039 \quad (1)$$

n=18, r=0.970, r^2=0.940, q^2=0.929, s=0.009, F=256.482

The QSAR model represented by Equation 1 is monoparametric and based on the number of compounds included in the dataset and the rule of thumb directed us to go for development of multiparametric models which can quantify the activity of test molecules against *S. aureus*. The dataset was subjected to multiple linear regression analysis and Equation 2 was obtained which depicted the role of the energy of HOMO along with MR in modulating the observed antibacterial against *S. aureus*.

$$pMIC_{saMLR} = 0.0055\ MR - 0.0068\ HOMO + 0.954 \quad (2)$$

n=18, r=0.974, r^2=0.949, q^2=0.926, s=0.0087, F=139.817

An effort was made to further enhance the r value of QSAR model represented by Equation 2 and a detailed investigation of this model depicted that compound 6 was acting as an outlier whose response values were outside the expected limits.Hence, Compound 6 was excluded from the study (being an outlier), and a new QSAR model was obtained, represented by Equation 3, with improved r, r^2, q^2, F values, and low S values.

$$pMIC_{saMLR} = 0.0054\ MR - 0.0044\ HOMO + 0.983 \quad (3)$$

n=17, r= 0.981, r^2=0.963, q^2=0.937, s=0.0077, F=181.325

Equation 3 was used to predict the antibacterial activity of test compounds against *S. aureus* and a comparison of observed, predicted and residual antibacterial activity is presented in Table 5.

The results presented in Table 5 revealed that low residual values for antibacterial activity were obtained using QSAR model represented by Equation 3 which suggest that the developed model was a valid one. Same QSAR models were obtained for antibacterial activity of test compounds against *B. subtilis*, *P. aeruginosa*, *E. coli*, and antifungal activity against *A. niger*.

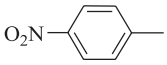
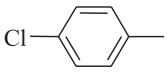
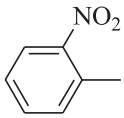
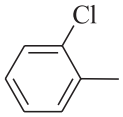
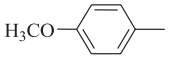
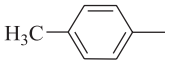
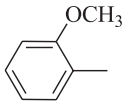
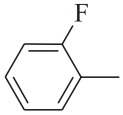
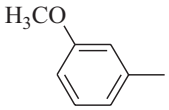
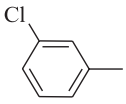
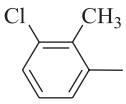
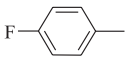
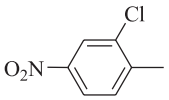
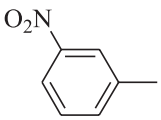
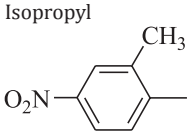
To develop QSAR model for antifungal activity of test compounds against *C. albicans*, the entire dataset was subjected to regression analysis and Equation 4 was obtained in which Balaban index (J) was found to be effectively controlling the antifungal activity.

$$pMIC_{ca} = -0.414\ J + 2.72\ 9 \quad (4)$$

n=18, r=0.643, r^2=0.414, q^2=0.325, s=0.131, F=11.300

The r value in Equation 4 was 0.643 which was less than the required limit (r≥0.7) and the outcomes of Equation 4 were studied in detail to

Table 1: *In vitro* antimicrobial activity of 2,4-dichlorosubstituted derivatives in terms of pMIC values (µmol/ml)

Comp.	R	pMICsa	pMICbs	pMICse	pMICec	pMICpa	pMICca	pMICan
		pMIC (µmol)						
1		1.496	1.496	1.496	1.496	1.496	2.102	1.496
2		1.484	1.484	1.785	1.484	1.484	2.086	1.484
3		1.496	1.496	0.893	1.496	1.496	1.799	1.496
4		1.484	1.484	1.484	1.484	1.484	2.086	1.484
5		1.479	1.479	1.479	1.479	1.479	2.081	1.479
6		1.460	1.460	1.460	1.460	1.460	2.066	1.460
7		1.479	1.479	1.479	1.479	1.479	2.081	1.479
8		1.465	1.465	1.465	1.465	1.465	2.071	1.465
9		1.479	1.479	1.479	1.479	1.479	2.081	1.479
10		1.484	1.484	1.484	1.484	1.484	1.785	1.484
11		1.500	1.500	1.500	1.500	1.500	1.801	1.500
12		1.465	1.465	1.465	1.465	1.465	1.767	1.465
13		1.533	1.533	1.533	1.533	1.533	1.836	1.533
14		1.496	1.496	1.496	1.496	1.496	2.102	1.496
15	Butyl	1.417	1.417	1.417	1.417	1.417	1.719	1.417
16	Propyl	1.398	1.398	1.398	1.398	1.398	1.699	1.398
17	Isopropyl	1.398	1.398	1.398	1.398	1.398	1.699	1.398
18		1.511	1.511	1.511	1.511	1.511	1.812	1.511

pMIC: Power management integrated circuit

Table 2: Molecular descriptors used in regression analysis for 2,4-dichloro benzoic acid derivatives

Comp.	logP	MR	0χ	0χv	1χ	2χ	3χ	κ_1	κ_2	$\kappa\alpha_1$	$\kappa\alpha_2$	$\kappa\alpha_3$	R	J	W	LUMO	HOMO	μ
1	3.183	86.583	18.069	14.081	11.114	11.158	2.813	20.314	8.131	18.459	6.932	4.163	11.114	1.787	1385.000	−1.835	−10.130	4.979
2	3.747	84.063	16.491	14.013	10.203	10.259	2.602	18.340	7.266	17.208	6.536	3.994	10.203	1.802	1053.000	−1.569	−9.382	3.978
3	3.183	86.583	18.069	14.081	11.131	11.085	2.745	20.314	8.131	18.459	6.932	3.976	11.131	1.902	1295.000	−1.668	−10.106	4.639
4	3.747	84.063	16.491	14.013	10.220	10.155	2.512	18.340	7.266	17.208	6.536	3.801	10.220	1.849	1023.000	−1.508	−9.544	3.393
5	2.976	85.721	17.198	14.226	10.741	10.428	2.517	19.326	7.920	17.867	6.959	4.083	10.741	1.787	1218.000	−1.532	−8.901	3.552
6	3.696	84.299	16.491	13.818	10.203	10.259	2.602	18.340	7.266	16.923	6.356	3.865	10.203	1.802	1053.000	−1.467	−9.202	4.206
7	2.976	85.721	17.198	14.226	10.758	10.346	2.442	19.326	7.920	17.867	6.959	3.893	10.758	1.871	1158.000	−1.483	−9.007	4.651
8	3.368	79.475	16.491	13.196	10.220	10.155	2.512	18.340	7.266	16.854	6.312	3.647	10.220	1.849	1023.000	−1.523	−9.509	3.256
9	2.976	85.721	17.198	14.226	10.741	10.440	2.517	19.326	7.920	17.867	6.959	4.083	10.741	1.806	1188.000	−1.554	−9.141	3.833
10	3.747	84.063	16.491	14.013	10.203	10.271	2.602	18.340	7.266	17.208	6.536	3.994	10.203	1.813	1038.000	−1.561	−9.578	3.568
11	4.214	89.104	17.361	14.935	10.631	10.660	2.704	19.326	7.486	18.192	6.764	3.853	10.631	1.863	1154.000	−1.557	−9.497	2.926
12	3.368	79.475	16.491	13.196	10.203	10.259	2.602	18.340	7.266	16.854	6.312	3.834	10.203	1.802	1053.000	−1.568	−9.395	3.306
13	3.701	91.388	18.939	15.199	11.525	11.688	3.012	21.302	8.347	19.731	7.340	4.334	11.525	1.848	1512.000	−1.862	−10.165	4.410
14	3.183	86.583	18.069	14.081	11.114	11.170	2.813	20.314	8.131	18.459	6.932	4.163	11.114	1.810	1340.000	−1.749	−10.139	1.664
15	2.755	73.354	14.629	12.629	8.792	8.386	2.109	17.053	7.136	16.395	6.673	4.154	8.792	2.456	712.000	−1.374	−10.496	3.066
16	2.358	68.753	13.922	11.922	8.292	8.032	2.109	16.056	6.438	15.398	5.988	3.666	8.292	2.491	593.000	−1.369	−10.495	2.793
17	2.303	68.647	14.085	12.086	8.148	8.527	2.517	16.056	5.970	15.398	5.542	3.666	8.148	2.544	578.000	−1.359	−10.481	2.990
18	3.650	91.624	18.939	15.004	11.525	11.688	3.012	21.302	8.347	19.445	7.161	4.210	11.525	1.848	1512.000	−1.877	−9.930	6.882

LUMO: Lowest unoccupied molecular orbital, HOMO: Highest occupied molecular orbital

find out the reason for low r value and it was found that compounds 3, 10, 11, 12, and 18 were behaving as outliers as their response values were outside the experimental limits of dataset and QSAR model represented by Equation 5 was built up excluding these five compounds.

$$pMIC_{ca} = 0.525\ J + 3.015 \quad (5)$$

n=13, r=0.922, r^2=0.851, q^2=0.817, s=0.068, F=62.730

The QSAR model represented by Equation 5 has been built up using 13 compounds and it has got high r, r^2, q^2, F values, and low S values, which supported the fact that model represented by Equation 5 was valid and it can be used for prediction of antifungal activity of target compounds against *C. albicans,* and the predicted antimicrobial activity is presented in Table 5. Valid QSAR models were not obtained for antibacterial activity of test compounds against *S. epidermidis*.

Docking study

In silico molecular docking studies were executed to investigate the possible potential binding modes for these sulfonamide derivatives against microbial targets. In the present study, H-bonding, hydrophobic interactions, and free binding energy say, and dock score were considered for the analysis (Table 6).

In the case of antibacterial activity, all the docked compounds showed binding energy from -6.8 kcal/mol to -8.6 kcal/mol. In the binding mode of topmost active, compound 13 potently bound to 1aj0 through four hydrogen bonding and hydrophobic interactions (Fig. 1). Hydrogen bonding interactions are as: The oxygen atom of SO_2NH formed one hydrogen bond with Ser:222 (2.56Å), hydrogen atom of COOH created hydrogen bond with Thr:177 (2.50 Å) whereas oxygen atom of carbonyl of COOH engaged with Gly:191 (2.57 Å) amino acid residue and oxygen atom of NO_2 interacted with Arg:220 amino acid residue with 3.20 Å bond length. In case of hydrophobic interactions, Cl of 2,4-dichlorobenzoic acid was involved in alkyl interactions with Ala:151, Pro:152 whereas Cl of 2-Cl,4-NO_2 substituted phenyl ring interacted with Lys:221 amino acid residues. Pi-alkyl interactions were formed by 2,4-dichloro substituted phenyl ring and 2-Cl, 4-NO_2 substituted ring with Ala:151 and Pro:64, Lys:221 amino acid residues, respectively. Ser:222 amino acid was also found to engage with 2-Cl, 4-NO_2 substituted phenyl ring through pi-sigma interaction.

Second most active compound 18 showed six hydrogen bonding and hydrophobic interactions. Compound 18 maintained four hydrogen bonding interactions same as compound 13 but two additional bonds formed by hydrogen atom of COOH with Asn:144 with a bond length of 1.84 Å and protonated oxygen of NO_2 with Arg:220 with a bond length of 2.29 Å. In a comparison of compounds 8 (o-F) and 12 (p-F), halogen bond was established by ortho substituted fluorine may be contributing to the better activity of compound 8. Comparison of 2 (p-Cl) and 12 (p-F), 2 formed five hydrogen bond interactions whereas, in compound 12, three hydrogen bonds were observed. Compound 2 also formed two additional hydrophobic interactions such as alkyl interaction with Lys:221 (4.80 Å) and pi-alkyl interaction with Phe:190 (4.28 Å) by chlorine which resulted in increased in the activity of compound 2. In a comparison of compounds 5 (p-OCH_3), 7 (o-OCH_3), and 9 (m-OCH_3), pi-pi T-shaped interaction was only observed in compound 7 with Phe:190 amino acid residue (4.87 Å).

In the case of antifungal activity, all the compounds displayed binding energy from −6.6 kcal/mol to −8.4 kcal/mol. The foremost active compound 1 formed two hydrogen bonding, hydrophobic and Van der Wall interactions with active binding site (Fig. 2). The protonated nitrogen of NO_2 created hydrogen bond with His:468 (2.12 Å) amino acid residue while NH of SO_2NH engaged with Pro:462 (2.24 Å) amino acid residue. NO_2 substituted phenyl ring established amide-pi-stacked interaction with Phe:463, pi-alkyl interaction with Ile:379 and pi-sulfur interaction with Cys:470 amino acid residues. The Cl of 2,4-dichloro substituted phenyl ring formed alkyl interaction with

Table 3: Correlation matrix for pMICsa with molecular descriptors of 2,4-dichloro benzoic acid derivatives

	logP	MR	0χ	1χ	2χ	$\kappa\alpha_3$	R	J	W	LUMO	HOMO	pMICsa
logP	1.000											
MR	0.718	1.000										
0χ	0.565	0.948	1.000									
1χ	0.587	0.959	0.989	1.000								
2χ	0.634	0.942	0.985	0.977	1.000							
$\kappa\alpha_3$	0.180	0.587	0.623	0.589	0.552	1.000						
R	0.587	0.959	0.989	1.000	0.977	0.589	1.000					
J	−0.722	−0.877	−0.831	−0.888	−0.877	−0.344	−0.888	1.000				
W	0.529	0.936	0.995	0.985	0.976	0.667	0.985	−0.828	1.000			
LUMO	−0.422	−0.748	−0.881	−0.829	−0.876	−0.682	−0.829	0.629	−0.897	1.000		
HOMO	0.435	0.493	0.329	0.440	0.367	−0.063	0.440	−0.702	0.325	0.050	1.000	
pMICsa	0.728	0.970	0.961	0.959	0.965	0.583	0.959	−0.863	0.948	−0.838	0.382	1.000

LUMO: Lowest unoccupied molecular orbital, HOMO: Highest occupied molecular orbital

Table 4: Correlation of molecular descriptors with antimicrobial activity of 2,4−dichloro benzoic acid derivatives

	pMICsa	pMICbs	pMICse	pMICec	pMICpa	pMICca	pMICan
logP	0.728	0.728	0.298	0.728	0.728	0.261	0.728
MR	0.970	0.970	0.098	0.970	0.970	0.534	0.970
0χ	0.961	0.961	−0.031	0.961	0.961	0.492	0.961
0χv	0.952	0.952	0.157	0.952	0.952	0.459	0.952
1χ	0.959	0.959	−0.001	0.959	0.959	0.561	0.959
1χv	0.951	0.951	0.166	0.951	0.951	0.464	0.951
2χ	0.965	0.965	0.015	0.965	0.965	0.505	0.965
2χv	0.899	0.899	0.241	0.899	0.899	0.339	0.899
3χ	0.823	0.823	0.021	0.823	0.823	0.269	0.823
3χv	0.561	0.561	0.262	0.561	0.561	0.075	0.561
κ_1	0.934	0.934	−0.073	0.934	0.934	0.452	0.934
κ_2	0.861	0.861	−0.085	0.861	0.861	0.504	0.861
κ_3	0.757	0.757	−0.023	0.757	0.757	0.427	0.757
$\kappa\alpha_1$	0.938	0.938	−0.026	0.938	0.938	0.386	0.938
$\kappa\alpha_2$	0.816	0.816	−0.006	0.816	0.816	0.404	0.816
$\kappa\alpha_3$	0.583	0.583	0.122	0.583	0.583	0.201	0.583
R	0.959	0.959	−0.001	0.959	0.959	0.561	0.959
J	−0.863	−0.863	−0.211	−0.863	−0.863	−0.671	−0.863
W	0.948	0.948	0.007	0.948	0.948	0.515	0.948
Te	−0.948	−0.948	0.036	−0.948	−0.948	−0.417	−0.948
ElE	−0.953	−0.953	0.097	−0.953	−0.953	−0.438	−0.953
NuE	0.951	0.951	−0.106	0.951	0.951	0.440	0.951
LUMO	−0.838	−0.838	−0.024	−0.838	−0.838	−0.314	−0.838
HOMO	0.382	0.382	0.316	0.382	0.382	0.596	0.382
μ	0.381	0.381	-0.148	0.381	0.381	0.231	0.381

Table 5: Comparison of observed, predicted, and residual activity of 2,4-dichloro benzoic acid derivatives

Comp	pMICsaMLR (Eq. 3)			pMICca (Eq. 5)		
	obs	pre	res	obs	pre	res
1	1.496	1.495	0.001	2.102	2.077	0.025
2	1.484	1.478	0.006	2.086	2.069	0.017
3	1.496	1.495	0.001	-	-	-
4	1.484	1.479	0.005	2.086	2.045	0.041
5	1.479	1.485	−0.006	2.081	2.077	0.004
6	-	-	-	2.066	2.069	−0.003
7	1.479	1.486	−0.007	2.081	2.034	0.047
8	1.465	1.454	0.011	2.071	2.045	0.026
9	1.479	1.486	−0.007	2.081	2.068	0.013
10	1.484	1.479	0.005	-	-	-
11	1.500	1.506	−0.006	-	-	-
12	1.465	1.454	0.011	-	-	-
13	1.533	1.522	0.011	1.836	2.046	−0.210
14	1.496	1.495	0.001	2.102	2.065	0.037
15	1.417	1.425	−0.008	1.719	1.726	−0.007
16	1.398	1.401	−0.003	1.699	1.708	−0.009
17	1.398	1.400	−0.002	1.699	1.680	0.019
18	1.511	1.522	−0.011	-	-	-

Table 6: The Docking score of reported compounds with PDB (1aj0, 5fsa)

Comp	1aj0 (Kcal/mol)	5fsa (Kcal/mol)
1	−8.5	−8.4
2	−8.0	−8.1
3	−8.4	−7.7
4	−8.1	−7.9
5	7.5	−7.9
6	−8.2	−8.3
7	−8.3	−7.9
8	−8.6	−8.0
9	−7.9	−7.8
10	−7.9	−7.9
11	−8.3	−8.0
12	−8.4	−8.1
13	−8.5	−8.4
14	−8.2	−8.1
15	−6.8	−6.8
16	−6.8	−6.6
17	−6.9	−6.7
18	−8.6	−8.1
Ciprofloxacin	−8.2	-
Fluconazole	-	−6.9

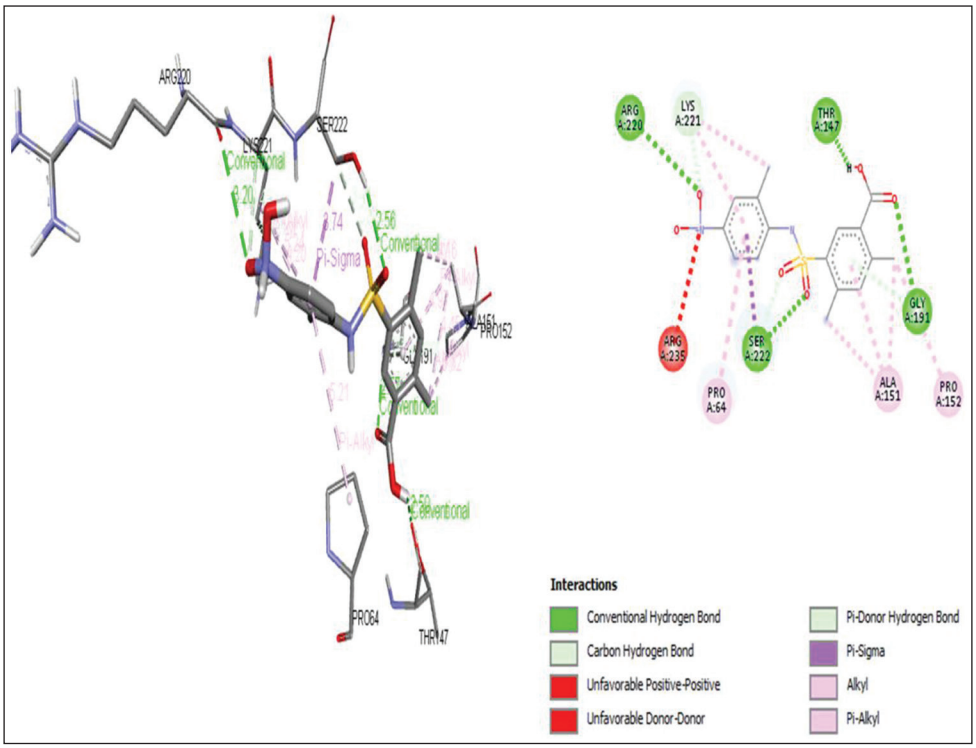


Fig. 1: Binding interactions of compound 13 with the active site of 1aj0

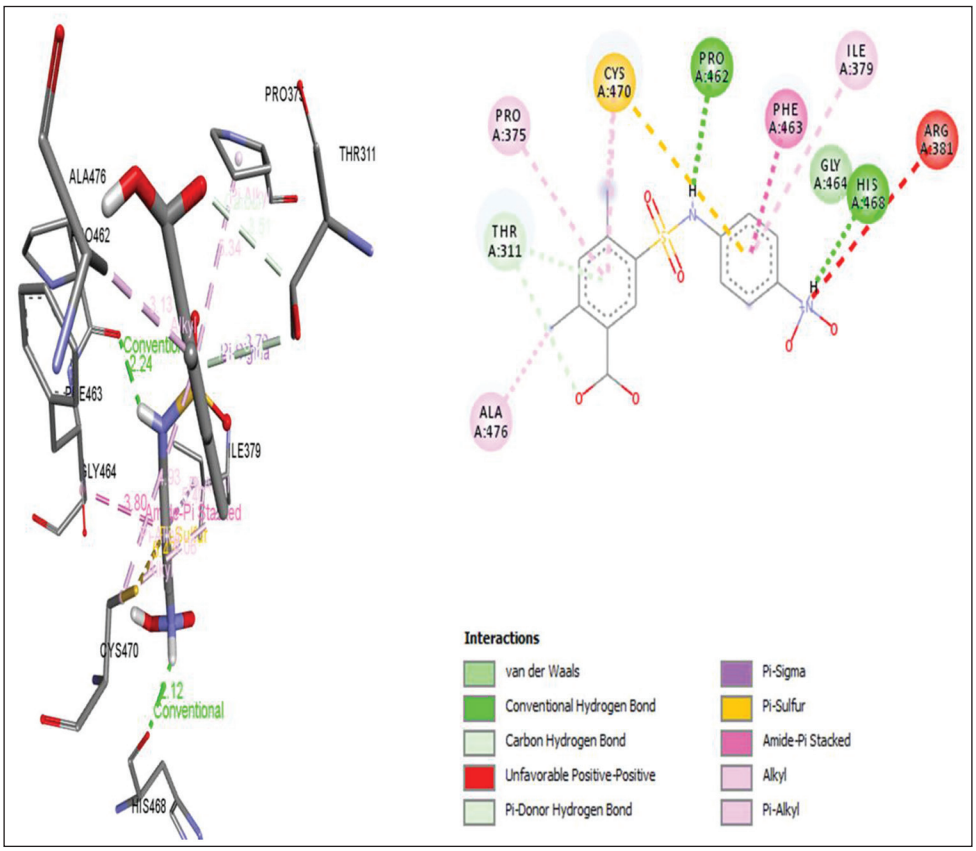


Fig. 2: Binding interactions of compound 1 with active site of 5fsa

Ala:476, Cys:470 while 2,4-dichloro substituted phenyl ring engaged with Cys:470, Thr:311 amino acid residues by forming pi-alkyl and pi-sigma interactions, respectively. Gly:464 amino acid residue formed Van der wall interaction with this compound.

In a comparison of a compound containing NO_2 substitution, compounds 1, 3, and 14, amide-pi-stacked interaction was only found in compound 1, substituted with p-NO_2 group. Compound 2 (p-Cl) formed a similar type of interactions as compared to compound 12 (p-F) with two more

interactions such as alkyl and pi-alkyl interaction with Ile:379 and Phe:105 amino acid residues, respectively, that resulted in an increase in antifungal potential. Compounds with aliphatic substitutions such as 15, 16, and 17, amide-pi-stacked, and pi-pi stacked interactions were not observed which might have contributed for the decrease in antifungal potential.

CONCLUSION

The set of 18 test compounds was subjected to antimicrobial evaluation and results depicted that compounds with NO_2 substitution were most active and para-substituted nitro group effectively increase the antimicrobial potential as compared to substitution at ortho and meta positions. The compounds with chloro substitution were found to be better in activity in comparison to other halogen substituents. The QSAR studies depicted the involvement of lipophilic parameter MR and Balaban index (J) as governors of antimicrobial potential in case of antibacterial and antifungal activity, respectively. Docking studies correlated the activity with structural features by depicting various interaction modes through which the test compounds were interacting with microbial targets such as hydrophobic, hydrogen bonding, and Van der Wall interactions.

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AUTHORS' CONTRIBUTIONS

The authors ST and VS have carried out the *in vitro* evaluation and computational studies.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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