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Original article

Synthesis, antimicrobial evaluation and QSAR study of some 3-hydroxypyridine-4-one and 3-hydroxypyran-4-one derivatives

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ABSTRACT

A series of Mannich bases of 2-alkyl-3-hydroxy-pyridine-4-ones, namely 2-alkyl-3-hydroxy-5-*N*-piperidylmethyl or *N,N*-dialkylaminomethyl pyridine-4-ones **9**, **10** and **15**–**18**, two derivatives of *N*-aryl-2-methyl-3-hydroxy-pyridine-4-ones **19**, **20** and two *N*-alkyl derivatives of maltol, **21** and **22** were prepared. They were screened for their antibacterial and antifungal activities against a variety of microorganisms using micro plate Alamar Blue® assay (MABA) method. Multiple linear regressions (MLR) analysis was performed for the synthesized compounds as well as a series of pyridinone and pyranone derivatives **23**–**43** which have been synthesized and evaluated for antimicrobial activity by other researchers previously. Studied compounds showed a better quantitative structure–activity relationship (QSAR) model for the antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* in comparison with other tested microorganisms.

1. Introduction

It is almost 120 years that physicians have revealed that the coincidence of blood and bacteria in a wound may cause a life-threatening infection. It has also been shown that blood or hemoglobin enhances the lethality of intraperitoneal or subcutaneous inocula of bacteria such as *Escherichia coli*. The effective component of hemoglobin is iron, and various soluble iron compounds exert an equivalent effect [1]. Increased susceptibility to infectious disease is observed in iron-overloaded states such as ß-thalassemia major and in hemolytic states such as sickle cell disease. Administration of iron compounds to the host can increase the virulence of *E. coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and other pathogens [2]. In fact, iron is an essential element required for the growth and virulence of virtually all microbial pathogens [3,4]. Aerobic microorganisms need this element for a variety of cellular functions including reduction of oxygen for synthesis of ATP, replication of DNA, energy production and protection of the cell against oxygen reactive species [5,6]. The availability of iron is critically important in host-parasite interactions [7,8]. Vertebrate hosts withhold iron from microbial invaders as a major defence mechanism against infection [4,7,9]. This task is achieved by sequestration of iron with iron-binding proteins, the most abundant, haemoproteins which contain almost 80% of the total iron of the vertebrates [10]. Some natural antibiotics, called siderophores, have a sequestrating ability. They are low-molecular-weight chelating agents that form stable complexes with iron [11,12]. There are many reports of the antimicrobial activity of chelating agents with different chemical structures [13–19]. Kojic acid (5-hydroxy-2-hydroxymethyl-pyran-4-one) and 3-hydroxypyranones, derivatives of it are examples of these compounds [13]. The bidentate chelating ligand 3-hydroxypyranone, which has a catechol-like function, forms stable complexes with several metal ions such as Fe^{3+} [20,21]. In vitro antibacterial and antifungal activities of 3-hydroxypyridinones, bioisoster derivatives of 3-hydroxypyranones with metal chelating ability have been described. They have an inhibitory effect on the growth of *E. coli, Listeria inocua* and *Staphylococcus aureus* [19,22,23]. Cephalosporins possessing 2-(5-hydroxy-4-pyridon-2-yl) ethenyl moieties have shown strong activity against *Pseudomonas aeruginosa*. Another cephalosporin derivative with a 1,5-dihydroxy-4-pyridone-2-carboxamide moiety, MT0703, has excellent in vitro and in vivo antibacterial activity against *P. aeruginosa* as well as *E. coli*. The pyridinone ring in the structure of these cephalosporin derivatives plays a significant role in anti-pseudomonas activity [24,25]. More recently antibacterial and antifungal activities of carboxamide derivatives of 3-hydroxypyranones, 5-hydroxypyranones and 5-hydroxypyridinones have been reported [26,27].

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Quantitative structure–activity relationship (QSAR) research field has been widely developed because of its powerful ability to predict drug activity [28]. It gives information that is useful for molecular design and medicinal chemistry [29–33]. QSAR models are mathematical equations relating chemical structures to their biological activities as a linear regression model of the form $y = Xb + e$. This equation may be used to describe a set of predictor variables (X) with a predicted variable (y) by means of a regression vector (b). In the first step of a typical QSAR study one needs to find a set of molecular descriptors representing the higher impact on the biological activity of interest. Multiple linear regression (MLR), genetic algorithm (GA), partial least square (PLS) and principle component analysis (PCA) are some of the variable selection methods to build up such a set [34–37].

In the present paper, we describe the synthesis, structural properties and antimicrobial activities of 6 novel Mannich bases of 2-alkyl-3-hydroxy-pyridine-4-ones as well as the antimicrobial activity of 4 previously synthesized compounds that have this ring in their structures. Their antimicrobial activities were examined by micro plate Alamar Blue® assay method against various bacteria and fungi. In the chemometrics part of this study we explore QSAR model for a series of 3-hydroxypyridine-4-one and 3-hydroxypyran-4-one derivatives which have been synthesized and evaluated for antimicrobial activity by Aytemir et al. as well as the compounds prepared in this study [26,27].

Our QSAR analysis establishes mathematical relationship between biological activities and computable parameters such as topological, physicochemical, stereochemical or electronic indices.

2. Results and discussion

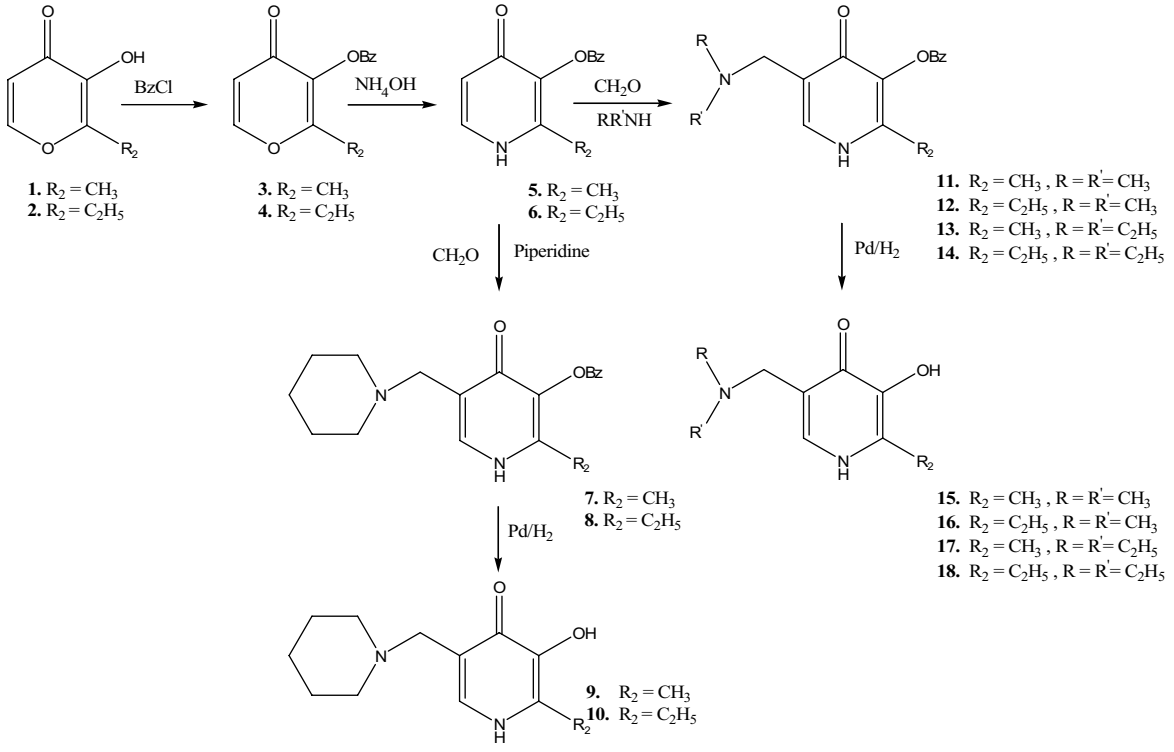
2.1. Chemistry

Mannich bases of 2-alkyl-3-hydroxy-pyridine-4-ones, namely 2-alkyl-3-hydroxy-5-*N*-piperidylmethyl or *N*,*N*-dialkylaminomethyl pyridine-4-ones **9**, **10** and **15**–**18**, were prepared by the methodology shown in Scheme 1. The commercially available maltol **1** or ethyl maltol **2** were benzylated in 90% aqueous ethanol to give **3** and **4**. Refluxing **3** or **4** with aqueous ammonia in ethanol gave 2-alkyl-3-benzyloxy pyridine-4-ones **5** and **6**. The Mannich bases **7**, **8** and **11**–**14** were obtained by the reaction of **5** and **6** with 40% formaldehyde solution and the appropriate secondary amine in ethanol. The benzyl protection was removed by catalytic hydrogenation of **7**, **8** and **11**–**14** to give the desired final Mannich bases **9**, **10** and **15**–**18**. Synthesis of *N*-aryl-2-methyl-3-hydroxy-pyridine-4-ones **19** and **20** was achieved by refluxing maltol **1** with an excess of the suitable primary aryl amines in acidic solution for 50–60 h (Scheme 2). The *N*-alkyl derivatives of maltol, **21** and **22** (Scheme 3) were prepared following the methodology as described by Harris from commercially available maltol **1** [38]. Formation of the desired compounds was confirmed on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. Some of the characterization data of the synthesized compounds are summarized in Table 1.

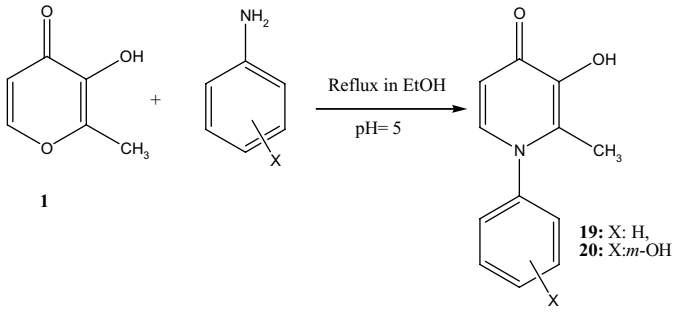
2.2. Antimicrobial activity

Minimum inhibitory concentrations (MICs) were determined by Micro plate Alamar Blue® Assay (MABA) method [39,40]. Tested bacteria were three Gram-positive bacteria: *S. aureus* PTCC 1337, *Bacillus subtilis* PTCC 1023 and *L. monocytogenes* PTCC 1165, three Gram-negative bacteria: *E. coli* PTCC 1338, *P. aeruginosa* PTCC 1074 and *Salmonella enteritidis* PTCC 1091 obtained from Persian type culture collection. The Muller–Hinton broth was used for bacteria [41]. All compounds were screened for their antifungal activity against one yeast-like fungus: *Candida albicans* PTCC 5027 and two molds: *Aspergillus niger* 5021 and *Aspergillus flavus* PTCC 5003 obtained from Persian type culture collection. RPMI 1640 medium buffered with MOPS at pH 7.0 was used for fungi [41]. The antibacterial and antifungal activity data are given in Table 2.

The investigation of antimicrobial screening data revealed that some of the tested compounds showed moderate to good bacterial growth inhibition. Compounds **9**, **20** and **21** inhibited *S. aureus*



Scheme 1. Synthetic methodology adopted for the preparation of Mannich bases (**9** and **10**) and (**15**–**18**).



Scheme 2. Synthesis of *N*-aryl-3-hydroxypyridine-4-ones (**19** and **20**) *via* the single step synthetic pathway.

growth at 16 μg/ml concentration. Compound **9** inhibited *S. enteritidis* at the same concentration. It was also a moderate inhibitor of *B. subtilis*.

2.3. QSAR analysis

The biological data used in this study are antimicrobial activity, (in terms of −log MIC), of a set of ten 3-hydroxypyridine-4-one derivatives **9**, **10**, **15**–**22** which we prepared and twenty-one derivatives of 3-hydroxypyridine-4-one and 3-hydroxypyran-4-one **23**–**43** which have been reported before as antimicrobial agents [26,27].

The structural features of these compounds are listed in Table 3. The calculated descriptors for each molecule are summarized in Table 4. The antimicrobial activities are summarized in Tables 5–7 and then were used for subsequent QSAR analysis as dependent variables.

Separate stepwise selection-based MLR analyses were performed using different types of descriptors, and then, an MLR equation was obtained utilizing the pool of all calculated descriptors. The results are summarized in Tables 8–14. Correlation coefficient (r^2) matrices for the descriptors used in different MLR equations are shown in Tables 15–21. Collinear descriptors degrade the performance of MLR equations and such models have lowered prediction ability. Tables 15–21 show that no significant correlation exists between pairs of descriptors.

In Tables 8–14 the MLR analysis with different types of descriptors of tested compounds against *S. aureus*, *C. albicans* and *P. aeruginosa* are listed.

Table 8 provides the resulted equations for all of the compounds against *S. aureus*. In this series the chemical parameter did not have a significant impact on the antimicrobial activity. The equation E_1 shows that among quantum descriptors, MPC and DMy have a positive effect on the antimicrobial activity; this contribution suggests that electronic interaction plays an important role in inhibitory activity of these compounds. The positive coefficient of MPC reveals the presence of columbic interactions between the ligands and receptors. According to equation E_1 a negative region in receptor produces columbic interaction; ligands with most MPC could interact with receptor more efficiently. This could propose another mechanism; a receptor mediated one, if R^2 and Q^2 had significant values for the antimicrobial activity of these compounds.

The second equation of Table 8 was found by using constitutional descriptors (E_2). This equation explained the positive effect of number of double bonds (nDB) and number of rotatable bonds (RBN) on the antimicrobial activity of studied compounds.

The equation E_3 of Table 8 obtained from the pool of topological descriptors, explained the negative effect of average connectivity index chi-1 (X1A) and molecular multiple path count of order 7 (piPC07) on antimicrobial activity of the compounds.

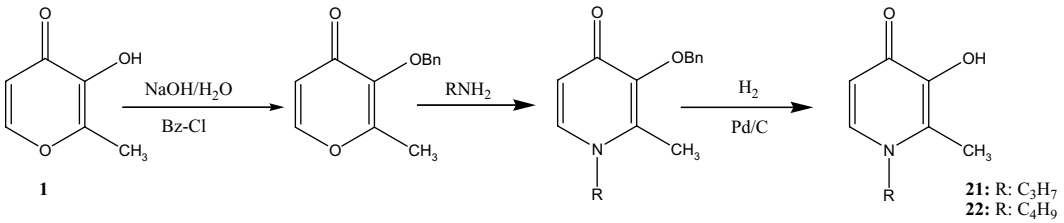
The equation E_4 of Table 8 was found by using geometrical descriptors. This equation explained the positive effect of molecular electrotopological variation (DELS) and the negative effect of 3D petijean shape index (PJI3) on the antimicrobial activity of compounds.

The effect of functional groups on the antimicrobial activity of the studied compounds has been described by equation E_5 of Table 8. This equation explained the positive effect of the number of secondary amides (aliphatic) (nCONHR) on the antimicrobial activity of the studied compounds. The positive sign of the coefficient of nCONHR proposed that an increase in the number of secondary amides (aliphatic) resulted in enhanced activity.

The last equation (E_6) was obtained from the all calculated descriptors. Stepwise selection and elimination of variables produced a three-parametric QSAR equation. In this model DMy and nDB have a positive effect and PJI3 has a negative effect on inhibitory activity.

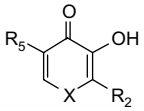
In Table 9 the resulted equations for all compounds against *C. albicans* are listed. The first equation of Table 9 was found by using quantum descriptors (E_1). It explained the positive effect of SUMPC and softness on antimicrobial activity of the compounds. The positive coefficient of SUMPC reveals the presence of columbic interactions between the ligands and receptors and demonstrates that ligands with most SUMPC could interact with receptor more efficiently. This could propose again the receptor mediated mechanism for the antimicrobial activity of these compounds if R^2 and Q^2 had significant values. The second equation of Table 9 was found by using chemical descriptors (E_2). It shows the positive effect of mass and the negative effect of surface area (SA) on the antimicrobial activity of the compounds. The negative coefficient of surface area indicates that increasing this parameter hinders the ligand to pass through the cell membrane and thus decreases the activity.

The effect of constitutional descriptors on the antimicrobial activity of the studied compounds has been described by equation E_3 of Table 9. It explained the positive effect of mean atomic Sanderson electronegativity (scaled on Carbon atom) (Me) and number of multiple bonds (nBM) on the antimicrobial activity. The MLR equation of Table 9 was obtained from the pool of topological descriptors (E_4). It includes the positive effect of Eigenvalue sum from Z weighted distance matrix (Barysz matrix) (SEigz) and of distance/detour ring index of order 10 (D/Dr10) and the negative effect of Balaban centric index (BAC) on the antimicrobial activity. The equation obtained from the effect of geometrical parameters on the antimicrobial activity of the studied compounds (E_5), shows



Scheme 3. Synthesis of *N*-alkyl-2-methyl-3-hydroxypyridine-4-ones **21** and **22** utilizing the methodology of Harris.

Table 1
Characterization data of the synthesized compounds.



No.	X	R_2	R_5	Mol. formula	M.p (°C)	Yield (%)	Analysis (%)		
							Found (Calculated)		
							C	H	N
9	NH	CH_3	CH_2–R[a]	$C_{12}H_{18}N_2O_2$	269–270	22	64.67 (64.84)	8.12 (8.16)	12.62 (12.60)
10	NH	C_2H_5	CH_2–R[a]	$C_{13}H_{20}N_2O_2$	270–272	25	65.96 (66.07)	8.32 (8.53)	11.83 (11.85)
15	NH	CH_3	CH_2–N$(CH_3)_2$	$C_9H_{14}N_2O_2$	252–253	25	59.11 (59.32)	7.70 (7.74)	15.43 (15.37)
16	NH	C_2H_5	CH_2–N$(CH_3)_2$	$C_{10}H_{16}N_2O_2$	254–255	30	60.06 (61.20)	8.25 (8.22)	14.35 (14.27)
17	NH	CH_3	CH_2–N$(C_2H_5)_2$	$C_{11}H_{18}N_2O_2$	252–253	23	62.81 (62.83)	8.59 (8.63)	13.43 (13.32)
18	NH	C_2H_5	CH_2–N$(C_2H_5)_2$	$C_{12}H_{20}N_2O_2$	256–257	23	64.11 (64.26)	8.86 (8.99)	12.46 (12.49)
19	N–Ph	CH_3	H	$C_{12}H_{11}NO_2$	221–222	35	71.55 (71.63)	5.35 (5.51)	7.08 (6.96)
20	*N*-*m*-OH–Ph	CH_3	H	$C_{12}H_{11}NO_3$	268–269	40	66.12 (66.35)	4.97 (5.10)	6.54 (6.45)

R[a] is  .

a negative effect of 3D Balaban centric index (J3D) and asphericity (ASP) on the antimicrobial activity.

The MLR equation of Table 9 obtained from the pool of functional group descriptors (E_6) explained the positive effect of the number of secondary amides (aliphatic) (nCONHR) and the number of substituted aromatic C (SP^2) (nCaR) and the negative effect of the number of tertiary amines (aliphatic) (nNR_2) on the antimicrobial activity. It shows that a decrease in the number of nNR_2 and an increase in the number of nCONHR and nCaR result in enhanced activity. The last equation E_7 was derived from the pool of all calculated descriptors. It shows the negative effect of J3D, ASP and superpendentic index (SPI) and the positive effect of the number of total secondary C (SP^3) (nCs) on the antimicrobial activity. This equation, which has a high statistical quality could explain and predict 0.81% and 0.73% of variance in pMIC data, respectively.

Table 10 describes that the QSAR models for the antimicrobial activity against *P. aeruginosa* resulted for all compounds by using different sets of molecular descriptors. The first equation (E_1) was found by using quantum descriptors and includes the negative effect of hardness on antimicrobial activity of compounds. The effect of constitutional descriptors on the antimicrobial activity of the studied compounds has been described by equation E_2 of Table 10. It shows the positive effect of average molecular weight (AMW), rotatable bond fraction (RBF) and number of 10-membered rings (nR10). The positive sign of the coefficient of the nR10 proposes that increasing the number of nR10 of the molecule results in an enhanced activity. The MLR equation of Table 10 obtained from the pool of topological descriptors (E_3) explained the positive effect of Eigenvalue sum from Z weighted distance matrix and the negative effect of information content index (neighborhood symmetry of 3-order) (IC3). The effect of geometrical descriptors on the antimicrobial activity of the studied compounds has been described by equation E_4 of Table 10. It explains the positive effect of maximal electrotopological negative variation (MAXDN) on the activity of these compounds. Equation E_5 shows that among the functional group descriptors, nNR_2 has a negative effect on the antimicrobial activity of these compounds. It shows that decreasing the number of nNR_2 results in enhanced activity. The equation E_6 was obtained from the pool of all calculated descriptors and it was similar to equation E_3.

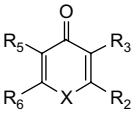
Table 11 lists the resulted equation for the antimicrobial activity of compounds **9**, **10**, **15**–**24** against *C. albicans*. The MLR equation of Table 11 was obtained from the quantum descriptors (E_1). It shows the negative effect of highest-occupied molecular orbital (HOMO) on the antimicrobial activity of compounds. This contribution suggests that electronic interaction plays important roles in the antimicrobial activity of these compounds. Molecules with low HOMO energy values are more able to accept electrons than

Table 2
Antibacterial and antifungal activities of synthesized compounds.

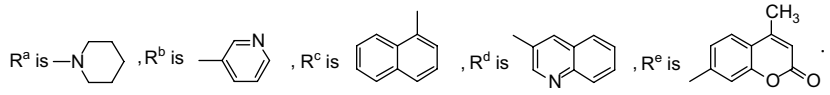
Compound	MIC μg/ml								
	E. coli	*S. enteritidis*	*P. aeruginosa*	*S. aureus*	*L. monocytogenes*	*B. subtilis*	*C. albicans*	*A. niger*	*A. flavus*
9	64	16	512	16	64	32	128	512	–[a]
10	512	512	256	–	–	512	128	512	256
15	512	512	256	–	512	–	–	512	–
16	512	256	256	512	512	–	512	512	512
17	512	128	256	512	–	–	512	512	512
18	512	512	256	-	512	512	512	512	512
19	128	128	512	128	512	256	–	–	–
20	512	64	512	16	–	–	512	512	256
21	128	128	512	16	512	256	256	512	256
22	512	256	512	256	–	512	256	256	256
23	–	–	–	–	–	–	512	128	16
24	–	–	256	–	512	–	128	512	512
Standard	0.25[b]	1[b]	0.5[b]	0.5[b]	1[b]	0.06[b]	2[c]	4[c]	8[c]

[a] Indicates bacteria are resistant to the compounds >512 μg/ml; MIC (μg/ml) = minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit bacterial growth.
[b] Ciprofloxacin.
[c] Ketoconazole.

Table 3
Chemical structure of the compounds used in QSAR analysis.



Compound	X	R_2	R_3	R_5	R_6
9	NH	CH_3	OH	CH_2–R^a	H
10	NH	C_2H_5	OH	CH_2–R^a	H
15	NH	CH_3	OH	CH_2–$N(CH_3)_2$	H
16	NH	C_2H_5	OH	CH_2–$N(CH_3)_2$	H
17	NH	CH_3	OH	CH_2–$N(C_2H_5)_2$	H
18	NH	C_2H_5	OH	CH_2–$N(C_2H_5)_2$	H
19	*N*-Ph	CH_3	OH	H	H
20	*N*-*m*-OH-Ph	CH_3	OH	H	H
21	*N*-C_3H_7	CH_3	OH	H	H
22	*N*-C_4H_9	CH_3	OH	H	H
23	O	CH_2Cl	H	OH	H
24	O	CH_3	H	OH	H
25	O	CH_2OH	OH	H	CH_3
26	O	CH_2OH	OCH_2Ph	H	CH_3
27	O	CHO	OCH_2Ph	H	CH_3
28	O	COOH	OCH_2Ph	H	CH_3
29	O	$CONHR^b$	OCH_2Ph	H	CH_3
30	O	$CONHR^c$	OCH_2Ph	H	CH_3
31	O	$CONHR^d$	OCH_2Ph	H	CH_3
32	O	$CONHR^b$	OH	H	CH_3
33	O	$CONHR^c$	OH	H	CH_3
34	O	$CONHR^d$	OH	H	CH_3
35	O	CH_2OH	H	OCH_2Ph	H
36	O	COOH	H	OCH_2Ph	H
37	O	CONHPh	H	OCH_2Ph	H
38	N–CH_3	CONHPh	H	OCH_2Ph	H
39	N–CH_3	CONHPh	H	OH	H
40	O	CONH–R^e	H	OCH_2Ph	H
41	N–CH_3	CONH–R^e	H	OCH_2Ph	H
42	N–CH_3	CONH–R^e	H	OH	H
43	O	CH_2OH	H	OH	H



molecules with high HOMO energy values. The effect of chemical descriptors on the antimicrobial activity of the studied compounds has been described by equation E_2 of Table 11. It explained the negative effect of surface area (SA) on the antimicrobial activity of the compounds. The negative coefficient of surface area indicates that an increase in the SA of the molecule hinders the ligand to pass through cell membrane and thus decreases the activity. Equation E_3, which was derived from a pool of constitutional constants, shows the positive effect of number of oxygen atoms (nO) on the antimicrobial activity. The positive sign of the coefficient of the nO

Table 4
Brief description of some descriptors used in this study.

Descriptor type	Molecular description
Constitutional	Molecular weight, no. of atoms, no. of non-H atoms, no. of bonds, no. of heteroatoms, no. of multiple bonds (nBM), no. of aromatic bonds, no. of functional groups (hydroxy, amine, aldehyde, carbonyl,nitro, nitroso, ..), no. of rings, no. of circuits, no of H-bond donors, no of H-bond acceptors, no. of Nitrogen atoms (nN), chemical composition, some of Kier–Hall electrotopological states (Ss), mean atomic polarizability (Mp), number of rotable bonds (RBN), mean atomic sanderson electronegativity (Me),
Topological indices	Molecular size index, molecular connectivity indices (X1A, X2v, X2Av, X3Av, X4Av), information content index (IC), Kier Shape indices, total walk count, path/walk-Randic shape indices, Zagreb indices, Schultz indices, Balaban J index (such as MSD) Wiener indices, topological charge indices, Sum of topological distances between F…F (T(F…F), Ratio of multiple path count to path count (PCR), Mean information content vertex degree magnitude (IVDM), Eigenvalue sum of Z weighted distance matrix (SEigz), reciprocal hyper-detour index (Rww), Eigenvalue coefficient sum from adjacency matrix (VEA1)
Geometrical	3D petijean shape index (PJI3), Gravitational index, Balaban index, Wiener index,
Quantum	Highest-occupied Molecular Orbital Energy (HOMO), Lowest Unoccupied Molecular Orbital Energy (LUMO), Most positive charge (MPC), Least negative charge (LNC), Sum of squares of charges (SSC), Sum of square of positive charges (SSPC), Sum of square of negative charges (SSNC), Sum of positive charges (SUMPC), Sum of negative charges (SUMNC), Sum of absolute of charges (SAC), Total dipole moment (DM_t), Molecular dipole moment at X-direction (DM_x), Molecular dipole moment at Y-direction (DM_Y), Molecular dipole moment at Z-direction (DM_Z), Electronegativity ($\chi = -0.5(\mathrm{HOMO} - \mathrm{LUMO})$, Electrophilicity ($\omega = \chi^2/2\eta$), Hardness ($\eta = 0.5(\mathrm{HOMO} + \mathrm{LUMO})$), Softness ($S = 1/\eta$)
Functional group	Number of total tertiary carbons (nCt), Number of H-bond acceptor atoms (nHAcc), number of total hydroxyl groups (nOH), number of unsubstituted aromatic C(nCaH), number of ethers (aromatic) (nRORPh)
Chemical descriptors	log *P*, Hydration Energy (HE), Polarizability (Pol), Molar refractivity (MR), Molecular volume (*V*), Molecular surface area (SA)

Table 5
Experimental and predicted activity of compounds against *Staphylococcus aureus*.

Compound	Experimental pMIC	Predicted pMIC	REP (%)
9	3.29	3.320549	0.917309
10	3.29	3.300702	0.324245
15	3.29	3.226554	−1.96638
16	3.29	3.397621	3.167543
17	4.19	3.749766	−11.7403
18	3.29	3.320459	0.917309
19	3.89	3.825538	−1.68504
20	3.29	3.26982	−0.61715
21	3.29	3.288552	−0.04402
22	3.89	3.928252	0.973778
23	3.59	3.620667	0.846993
24	3.59	3.725382	3.634035
25	3.59	3.506259	−2.38832
26	3.59	3.621242	0.862738
27	4.19	4.156255	−0.81191
28	3.59	3.561075	−0.81226
29	3.59	3.617666	0.764741
30	3.59	3.554753	−0.99153
31	3.89	3.895037	0.129311
32	4.19	4.099489	−2.20787
33	3.59	3.711696	3.278718
34	5.1	5.084036	−0.31399
35	3.59	3.553337	−1.03178
36	3.59	3.722266	3.553385
37	3.89	3.922216	0.821364
38	3.89	3.977879	2.209202
39	4.8	4.802176	0.045312
40	3.89	3.859087	−0.80105
41	3.59	3.490623	−2.84698
42	4.49	4.51052	0.454943
43	3.59	3.472806	−3.37462

Table 6
Experimental and predicted activity of compounds against *Candida albicans*.

Compound	Experimental pMIC	Predicted pMIC	REP(%)
10	3.29	3.41394	3.630409
16	3.29	3.46932	5.168736
17	3.89	3.892	0.051387
18	3.29	3.35912	2.057682
19	3.29	3.38349	2.763123
20	3.59	3.54768	−1.19289
21	3.29	3.32079	0.927189
22	3.59	3.32959	−7.82108
23	3.89	4.17263	6.773426
24	3.89	3.74806	−3.78703
25	3.89	3.90924	0.492167
26	3.89	3.70762	−4.91906
27	3.89	3.68921	−5.44263
28	3.89	3.84223	−1.24329
29	4.49	4.4661	−0.53514
30	4.49	4.50764	0.391336
31	3.89	3.70762	−4.91906
32	3.89	3.80144	−2.32964
33	3.89	3.9525	1.581278
35	3.89	3.74497	−3.87266
36	3.89	3.9956	2.642907
37	3.89	3.99694	2.675547
38	3.89	3.84885	−1.06915
39	3.89	3.75735	−3.53041
40	3.89	3.95029	1.526217
41	3.89	3.99638	2.661909
42	3.89	3.89782	0.200625
43	3.89	4.03217	3.525893

Table 7
Experimental and predicted activity of compounds against *Pseudomonas aeruginosa*.

Compound	Experimental pMIC	Predicted pMIC	REP(%)
9	3.59	3.443974	−4.24004
10	3.59	3.530871	−1.67462
15	3.59	3.472232	−3.3917
16	3.59	3.636632	1.282281
17	3.29	3.510631	6.284661
18	3.59	3.587978	−0.05636
19	3.29	3.723022	11.63093
20	3.29	3.747837	12.21603
21	3.89	3.653376	−6.47687
22	3.29	3.599309	8.593572
23	4.19	3.795735	−10.387
24	3.59	3.785079	5.153899
25	3.89	3.76962	−3.19344
26	3.59	3.715849	3.386829
27	3.89	3.742194	−3.94973
28	3.89	3.782049	−2.8543
29	3.89	3.888874	−0.02894
30	3.89	3.900399	0.266625
31	3.89	3.897727	0.198251
32	3.89	3.837207	−1.37581
33	3.89	3.873711	−0.42049
34	3.89	3.921648	0.807
35	3.89	3.744965	−3.8728
36	3.89	3.815239	−1.95954
37	3.59	3.682614	2.514894
38	3.59	3.578922	−0.30954
39	3.89	3.708554	−4.89264
40	3.59	3.68836	2.666772
41	3.89	3.617916	−7.52046
42	3.59	3.73787	3.955999
43	3.89	3.799604	−2.37908

proposes that an increase in the number of (nO) of the molecule resulted in activity enhancement. The equation E_4 of Table 11 was found by using topological descriptors. This equation explained the positive effect of Balban centric index (BAC) on antimicrobial activity of compounds **9**, **10**, **15**–**24**. The effect of geometrical descriptors on the antimicrobial activity of these compounds has been described by equation E_5 of Table 11. It explained the negative effect of 3D petijean shape index (PJI3) and positive effect of sum of geometrical distances between N…N, i.e., G(N…N) on the antimicrobial activity. The effect of functional groups on the antimicrobial activity of these compounds has been described by equation E_6 of Table 11. This equation describes the structure–activity relationship better than those obtained from the quantum, chemical, constitutional and topological descriptors. The two-parametric QSAR equation has correlation coefficient and standard error equal to 0.81 and 0.13, respectively. It explained the negative effect of number of total primary C (SP^3) (nCp) and number of unsubstituted aromatic C (SP^2) (nCaH) on the antimicrobial activity of the compounds. The last equation E_7 was obtained from the all calculated descriptors. Stepwise selection and elimination of variables produced again a two-parametric functional group QSAR equation. This equation could explain and predict 0.81% and 0.61% of variance in pMIC data, respectively.

In Table 12 the resulted equation for the antimicrobial activity of compounds **9**, **10**, **15**–**24** against *P. aeruginosa* are listed. It should

Table 8
The results of MLR analysis with different types of descriptors of all compounds (*Staphylococcus aureus*).

No.	Descriptor source	MLR equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Quantum	$pIC_{50} = 2.505(\pm0.647) + 3.507(\pm1.691)$ MPC + $0.121(\pm0.048)$DMy	31	0.45	0.34	0.37	0.31	11.52
E_2	Constitutional	$pIC_{50} = 3.775(\pm0.254) + 0.148(\pm0.036)$nDB − $0.115(\pm0.049)$RBN	31	0.41	0.36	0.42	0.18	8.74
E_3	Topological	$pIC_{50} = 20.128(\pm4.595) - 35.306(\pm9.853)$X1A − $0.001(\pm0.001)$piPCO7	31	0.34	0.37	0.39	0.23	7.20
E_4	Geometrical	$pIC_{50} = 4.531(\pm0.570) + 0.031(\pm0.009)$DELS − $1.859(\pm0.688)$ PJI3	31	0.37	0.36	0.40	0.21	8.51
E_5	Functional group	$pIC_{50} = 3.605(\pm0.081) + 0.521(\pm0.151)$nCONHR	31	0.30	0.38	0.40	0.17	11.95
E_6	Molecular descriptor	$pIC_{50} = 4.544(\pm0.495) + 0.130(\pm0.040)$DMy + $0.082(\pm0.031)$nDB − $1.275(\pm0.584)$PJI3	31	0.55	0.50	0.36	0.35	11.24

Table 9
The results of MLR analysis with different types of descriptors of all compounds (*Candida albicans*).

No.	Descriptor source	MLR equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Quantum	$pIC_{50} = 5.727(\pm0.572) + 0.391(\pm0.093)$Softness $+ 0.117(\pm0.049)$SUMPC	28	0.50	0.22	0.24	0.35	12.05
E_2	Chemical	$pIC_{50} = 4.241(\pm0.252) + 0.006(\pm0.001)$Mass $- 0.005(\pm0.001)$SA	28	0.62	0.19	0.21	0.52	20.65
E_3	Constitutional	$pIC_{50} = -3.598(\pm2.072) + 0.0.039(\pm0.007)$nBM $+ 6.897(\pm2.019)$ME	28	0.62	0.19	0.21	0.51	20.96
E_4	Topological	$pIC_{50} = 3.724(\pm0.190) + 0.003(\pm0.001)$D/Dr10 $- 0.023(\pm0.005)$BAC $+ 0.360(\pm0.160)$SEigz	28	0.73	0.16	0.19	0.60	22.16
E_5	Geometrical	$pIC_{50} = 5.173(\pm0.202) - 0.370(\pm0.056)$J3D $- 0.983(\pm0.301)$ASP	28	0.51	0.18	0.19	0.60	25.88
E_6	Functional group	$pIC_{50} = 3.781(\pm0.065) - 0.312(\pm0.077)$nNR2 $+ 0.247(\pm0.078)$nCONHR $+ 0.095(\pm0.032)$nCaR	28	0.67	0.18	0.20	0.53	16.16
E_7	Molecular descriptor	$pIC_{50} = 6.192(\pm0.292) - 0.714(\pm0.097)$J3D $- 1.519(\pm0.269)$ASP $+ 0.249(\pm0.065)$nCs $- 0.001(\pm0.00)$SPI	28	0.82	0.14	0.15	0.73	25.30

Table 10
The results of MLR analysis with different types of descriptors of all compounds (*Pseudomonas aeruginosa*).

No.	Descriptor source	MLR equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Quantum	$pIC_{50} = 2.177(\pm0.430) - 8.999(\pm2.507)$Hardness	31	0.55	0.19	0.19	0.25	12.88
E_2	Constitutional	$pIC_{50} = 1.870(\pm0.314) + 0.172(\pm0.030)$AMW $+ 2.720(\pm0.753)$RBF $+ 0.175(\pm0.071)$nR10	31	0.62	0.14	0.19	0.25	14.76
E_3	Topological	$pIC_{50} = 4.138(\pm0.347) + 0.807(\pm0.143)$SEigz $- 0.288(\pm0.096)$IC3	31	0.53	0.16	0.17	0.44	16.11
E_4	Geometrical	$pIC_{50} = 2.859(\pm0.307) + 0.396(\pm0.141)$MAXDN	31	0.21	0.20	0.21	0.13	7.94
E_5	Functional group	$pIC_{50} = 3.839(\pm0.046) - 0.270(\pm0.068)$nnr2	31	0.35	0.18	0.19	0.26	15.69
E_6	Molecular descriptor	$pIC_{50} = 4.138(\pm0.347) + 0.807(\pm0.143)$SEigz $- 0.288(\pm0.096)$IC3	31	0.53	0.16	0.17	0.44	16.11

Table 11
The results of MLR analysis with different types of descriptors of compounds **9**, **10**, **15**–**24** (*Candida albicans*).

No.	Descriptor source	MLR Equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Quantum	$pIC_{50} = -0.16(\pm1.353) - 11.201(\pm4.207)$HOMO	10	0.46	0.21	0.22	0.32	6.89
E_2	Chemical	$pIC_{50} = 4.891(\pm0.523) - 0.004(\pm0.001)$SA	10	0.46	0.21	0.23	0.31	6.87
E_3	Constitutional	$pIC_{50} = 2.674(\pm0.360) + 0.373(\pm0.154)$nO	10	0.42	0.22	0.25	0.19	5.87
E_4	Topological	$pIC_{50} = 3.943(\pm0.162) - 0.022\ (\pm0.008)$BAC	10	0.49	0.20	0.24	0.27	7.76
E_5	Geometrical	$pIC_{50} = 6.213(\pm0.544) - 3.633(\pm0.748)$PJI3 $+ 0.095(\pm0.034)$G(N…N)	10	0.78	0.14	0.20	0.43	12.90
E_6	Functional group	$pIC_{50} = 4.014(\pm0.098) - 0.244(\pm0.046)$nCp $- 0.076(\pm0.025)$nCaH	10	0.81	0.13	0.18	0.61	14.98
E_7	Molecular descriptor	$pIC_{50} = 4.014(\pm0.098) - 0.244(\pm0.046)$nCp $- 0.076(\pm0.025)$nCaH	10	0.81	0.13	0.18	0.61	14.98

Table 12
The results of MLR analysis with different types of descriptors of compounds **9**, **10**, **15**–**24** (*Pseudomonas aeruginosa*).

No.	Descriptor source	MLR Equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E1	Quantum	$pIC_{50} = 0.167(\pm1.381) - 10.775(\pm4.373)$HOMO	12	0.37	0.22	0.18	0.28	6.07
E2	Topological	$pIC_{50} = 5.70(\pm0.452) - 0.855(\pm0.182)$IDDE $+ 0.190(\pm0.072)$PHI	12	0.72	0.15	0.18	0.54	11.50
E3	Molecular descriptor	$pIC_{50} = 5.70(\pm0.452) - 0.855(\pm0.182)$IDDE $+ 0.190(\pm0.072)$PHI	12	0.72	0.15	0.18	0.54	11.50

Table 13
The results of MLR analysis with different types of descriptors of compounds **25**–**43** (*Staphylococcus aureus*).

No.	Descriptor source	MLR equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Quantum	$pIC_{50} = 3.927(\pm0.081) + 0.731(\pm0.049)$DMy	19	0.42	0.35	0.39	0.24	12.63
E_2	Constitutional	$pIC_{50} = 3.477(\pm0.203) + 0.095(\pm0.040)$ nDB	19	0.25	0.40	0.19	0.15	5.68
E_3	Topological	$pIC_{50} = 3.412(\pm0.183) + 0.035\ (\pm0.011)$BAC	19	0.35	0.37	0.19	0.26	9.45
E_4	Functional group	$pIC_{50} = 4.509(\pm0.143) - 0.422\ (\pm0.152)$ nRORPh $- 0.626(\pm0.175)$nCs $- 0.159(\pm0.062)$nCaR	19	0.61	0.30	0.36	0.40	7.89
E_5	Molecular descriptor	$pIC_{50} = 8.241(\pm0.820) + 0.124(\pm0.023)$DMy0.103 (±0.120)nAB $- 21.398(\pm3.590)$RBF $- 0.177(\pm0.034)$L/BW $- 0.130$ (±0.035)VRA2 $+ 0.204(\pm0.089)$SPAN	19	0.93	0.15	0.19	0.82	25.48

Table 14
The results of MLR analysis with different types of descriptors of compounds **25**–**33**, **35**–**43** (*Candida albicans*).

No.	Descriptor source	MLR equations	*N*	R^2	S.E	RMS_{CV}	Q^2	*F*
E_1	Chemical	$pIC_{50} = 3.602(\pm0.167) + 0.001(\pm0.001)$Mass	18	0.23	0.17	0.20	0.14	7.60
E_2	Constitutional	$pIC_{50} = 3.769(\pm0.055) + 0.027(\pm0.006)$nAB	18	0.53	0.13	0.16	0.29	18.21
E_3	Topological	$pIC_{50} = -5.047(\pm2.249) + 0.002(\pm0.000)$ piPC07 $+ 30.502(\pm7.436)$X2A $+ 0.124(\pm0.043)$SEigz	18	0.81	0.09	0.10	0.71	20.47
E_4	Geometrical	$pIC_{50} = 3.167(\pm0.324) + 0.160(\pm0.065)$MAXDP	18	0.27	0.17	0.18	0.16	20.62
E_5	Functional group	$pIC_{50} = 3.817(\pm0.044) + 0.111(\pm0.024)$nCaR	18	0.56	0.13	0.16	0.30	20.62
E_6	Molecular descriptor	$pIC_{50} = 4.026(\pm0.072) + 0.167(\pm0.024)$ nCaR $- 0.588(\pm0.164)$ ASP $- 0.237(\pm0.081)$nNHRPh	18	0.82	0.08	0.12	0.61	21.94

Table 15
Correlation coefficient (r^2) matrix for the descriptors of all compounds used in the different MLR equations (*Staphylococcus aureus*).

	MPC	DMy	nDB	RBN	X1A	piPCO7	DELS	PJI3	nCONHR	pMIC
MPC	1	0.540	0.545	0.016	−0.764	0.633	0.752	0.190	0.675	0.572
DMy		1	0.368	0.022	−0.568	0.418	0.488	−0.070	0.404	0.606
nDB			1	0.377	−0.512	0.331	0.677	0.016	0.652	0.531
RBN				1	−0.053	0.243	0.317	0.522	0.288	−0.074
X1A					1	−0.631	−0.616	−0.191	−0.528	−0.466
piPCO7						1	0.780	0.310	0.369	0.192
DELS							1	0.190	0.608	0.465
PJI3								1	0.016	−0.307
nCONHR									1	0.540
pMIC										1

Table 16
Correlation coefficient (r^2) matrix for the descriptors of all compounds used in the different MLR equations (*Candida albicans*).

	SUMPC	Softness	Mass	SA	nBM	Me	BAC	SEigz	D/Dr10	J3D	ASP	nNR2	nCaR	nCoNHR	nCs	SPI	pMIC
SUMPC	1	0.033	0.755	0.697	0.727	−0.453	0.030	0.504	0.664	−0.468	−0.060	0.001	0.453	0.652	−0.240	0.627	0.364
Softness		1	0.267	−0.072	0.473	0.761	−0.597	0.702	0.180	0.788	−0.001	−0.791	0.30	0.228	−0.633	0.044	0.610
Mass			1	0.633	0.669	−0.240	−0.153	0.663	0.725	−0.633	−0.075	−0.213	0.604	0.638	−0.413	0.555	0.554
SA				1	0.593	−0.517	0.127	0.365	0.517	−0.304	0.055	0.122	0.325	0.631	−0.079	0.522	0.150
nBM					1	−0.066	−0.405	0.574	0.688	−0.807	−0.026	−0.397	0.675	0.424	−0.620	0.223	0.672
Me						1	−0.364	0.502	−0.103	−0.372	−0.123	−0.647	−0.071	−0.081	−0.361	0.103	0.373
BAC							1	−0.205	0.083	0.694	−0.227	0.420	−0.313	−0.213	0.600	0.407	−0.587
SEigz								1	0.538	−0.713	−0.165	−0.596	0.327	0.488	−0.468	0.532	0.633
D/Dr10									1	−0.447	−0.226	−0.396	0.673	0.188	−0.201	0.637	0.528
J3D										1	−0.058	0.598	−0.613	−0.440	0.640	−0.189	−0.732
ASP											1	0.097	0.023	−0.168	0.127	−0.209	−0.330
nNR2												1	−0.372	0.091	0.346	−0.128	−0.622
nCaR													1	0.038	−0.492	−0.011	0.585
nCoNHR														1	−0.366	0.316	0.341
nCs															1	0.016	−0.556
SPI																1	0.076
pMIC																	1

Table 17
Correlation coefficient (r^2) matrix for the descriptors of all compounds used in the different MLR equations (*Pseudomonas aeruginosa*).

	Hardness	AMW	RBF	nR10	SEigz	IC3	MAXDN	nNR2	pMIC
Hardness	1	−0.695	0.430	−0.282	−0.739	−0.277	−0.705	0.617	−0.555
AMW		1	−0.475	0.241	0.739	0.183	0.597	−0.729	0.618
RBF			1	−0.232	−0.270	−0.342	−0.153	0.252	0.050
nR10				1	0.591	0.629	0.310	−0.445	0.378
SEigz					1	0.640	0.627	−0.630	0.622
IC3						1	0.254	−0.117	0.102
MAXDN							1	−0.530	0.464
nNR2								1	−0.593
pMIC									1

Table 18
Correlation coefficient (r^2) matrix for the descriptors of compounds **9**, **10**, **15**–**24** used in the different MLR equations (*Candida albicans*).

	HOMO	SA	nO	BAC	PJI3	G(N…N)	nCp	nCaH	pMIC
HOMO	1	0.629	−0.775	0.450	0.606	0.388	0.588	0.269	−0.680
SA		1	−0.823	0.747	0.694	0.680	0.778	−0.062	−0.680
nO			1	−0.478	−0.760	−0.534	−0.673	0.156	0.651
BAC				1	0.768	0.606	0.929	−0.336	−0.702
PJI3					1	0.774	0.618	−0.085	−0.739
G(N…N)						1	0.633	−0.405	−0.262
nCp							1	−0.346	−0.753
nCaH								1	−0.202
pMIC									1

Table 19
Correlation coefficient (r^2) matrix for the descriptors of compounds **9**, **10**, **15**–**24** used in the different MLR equations (*Pseudomonas aeruginosa*).

	HOMO	PHI	IDDE	pMIC
HOMO	1	0.626	0.626	−0.615
PHI		1	0.716	−0.184
IDDE			1	−0.710
pMIC				1

be noted that the chemical, constitutional, geometrical and functional group descriptors did not have significant impacts on the activity of these compounds. The first equation E_1 was found by using quantum descriptors and includes the negative effect of HOMO on the antimicrobial activity of compounds. This contribution suggests that electronic interaction plays important roles in antimicrobial activity of these compounds. Molecules with low HOMO energy values are more able to accept electrons than

Table 20
Correlation coefficient (r^2) matrix for the descriptors of compounds **25–43** used in the different MLR equations (*Staphylococcus aureus*).

	DMy	nDB	BAC	nRORPh	nCs	nCaR	nAB	RBF	VRA2	L/BW	SPAN	pMIC
DMy	1	0.319	0.609	−0.348	−0.573	0.053	0.002	−0.235	0.116	0.164	0.249	0.653
nDB		1	0.461	−0.265	−0.084	−0.373	−0.396	−0.222	0.751	0.280	0.549	0.501
BAC			1	−0.677	0.028	−0.297	−0.414	0.228	0.025	−0.156	−0.065	0.598
nRORPh				1	−0.217	0.307	0.509	−0.440	0.243	0.352	0.400	−0.478
nCs					1	−0.378	−0.429	0.607	−0.208	−0.167	−0.472	−0.347
nCaR						1	0.771	−0.606	0.107	−0.061	0.364	−0.370
nAB							1	−0.670	0.163	0.045	0.429	−0.431
RBF								1	−0.559	−0.462	−0.792	−0.075
VRA2									1	0.301	0.781	0.026
L/BW										1	0.627	−0.042
SPAN											1	0.030
pMIC												1

molecules with high HOMO energy values. As it can be seen from equation E_2, a valuable correlation is observed between topological descriptors (mean information content) on the distance degree quality (IDDE), kier flexibility index (PHI) and pMIC of these compounds. The last equation (E_3) was derived from all calculated descriptors. Stepwise selection and elimination of variables produced a two-parametric equation which is similar to what was obtained for E_2.

MLR analysis with different types of descriptors for compounds **9**, **10**, **15–24** was performed, but no relationship between the structure and the antimicrobial activity against *S. aureus* was obtained.

Table 13 describes that the QSAR models resulted for the antimicrobial activity of compounds **25–43** against *S. aureus* by using different sets of molecular descriptors. The equation E_1 shows that among quantum descriptors, DMy has a positive effect on the antimicrobial activity. This contribution suggests that electronic interaction plays an important role in inhibitory activity of these compounds. The second equation of Table 13 was found by using constitutional descriptors (E_2). This equation explained the positive effect of (nDB) on the antimicrobial activity of compounds **25–43**. The effect of topological descriptors on the antimicrobial activity of these compounds has been described by equation E_3 of Table 13. It explains the positive effect of (BAC) on the activity of these compounds. The effect of functional group descriptors on the antimicrobial activity of the studied compounds has been described by equation E_4 of Table 13. It explained the negative effect of number of ethers (aromatic) (nRORPh), number of total secondary C (SP^3) (Cs) and the number of substituted aromatic C (SP^2) (nCaR) on the antimicrobial activity of the compounds. The negative sign of the coefficient of the nRORPh, Cs and nCaR proposes that decreasing the number of the above-mentioned descriptors, enhances the activity. Equation E_5 was obtained from the all types of calculated descriptors. Stepwise selection and elimination of variables produced again a six-parametric QSAR equation. This equation, which has a high statistical quality ($R^2 = 0.93$, $Q^2 = 0.82$), demonstrates that quantum (DMy), constitutional (nAB) & (RBF), topological (average Randic-type eigenvector-based index from adjacency matrix (VRA2)) and geometrical (length-to-breath ratio by WHIM (L/BW), SPAN (Span R)) parameters are major factors that affect the antimicrobial activity of compounds **25–43**. All described parameters except DMy and SPAN have negative effect on the activity.

The resulted equations for the antimicrobial activity of compounds **25–33**, **35–43** against *C. albicans* are listed in Table 14. In this series the quantum parameter did not have a significant impact on the antimicrobial activity. As it can be seen from equation E_1 (Table 14) mass of studied molecules has a positive effect on the antimicrobial activity. The equation obtained from the effect of constitutional parameter on the antimicrobial activity of the compounds **25–33**, **35–43** (E_2) shows, the positive effect of the number of aromatic bonds (nAB) on the activity of these compounds. The positive sign of the coefficient of nAB proposes that increasing the number of aromatic bonds of the molecule enhances the activity. The equation found by using topological descriptors (E_3) explains the positive effect of molecular multiple path count of order 7 (piPC07), average connectivity index chi-2 (X2A) and Eigenvalue sum from van der Waals weighted distance matrix (SEigv) on the activity of the studied compounds. This equation, which has a high statistical quality could explain and predict 0.81% and 0.71% of variance in pMIC data, respectively. The equation obtained from the effect of geometrical parameter on the antimicrobial activity of the studied compounds (E_4), shows a positive effect of maximal electrotopological positive variation (MAXDP) on the antimicrobial activity of compounds **25–33**, **35–43**. The MLR equation of Table 14 obtained from the pool of functional group descriptors (E_5) explained the positive effect of the number of substituted aromatic C (SP^2) (nCaR) on the antimicrobial activity. The last equation (E_6) was derived from all calculated descriptors. Stepwise selection and elimination of variables produced a three-parametric equation. It shows the positive effect of nCaR and the negative effect of ASP and the number of secondary amines

Table 21
Correlation coefficient (r^2) matrix for the descriptors of compounds **25–33**, **35–43** used in the different MLR equations (*Candida albicans*).

	Mass	nAB	X2A	SEigv	piPc07	MAXDP	nCaR	ASP	nNHRPH	pMIC
Mass	1	0.525	−0.634	−0.626	0.630	0.767	0.511	0.087	0.289	0.484
nAB		1	−0.61	−0.100	0.618	0.590	0.770	0.205	0.615	0.730
X2A			1	0.377	−0.715	−0.690	−0.749	−0.058	−0.648	−0.488
SEigv				1	−0.470	−0.503	−0.112	−0.104	−0.075	−0.103
piPCO7					1	0.655	0.669	0.021	0.643	0.721
MAXDP						1	0.586	−0.019	0.383	0.524
nCaR							1	0.114	0.717	0.750
ASP								1	0.069	−0.307
nNHRPH									1	0.316
pMIC										1

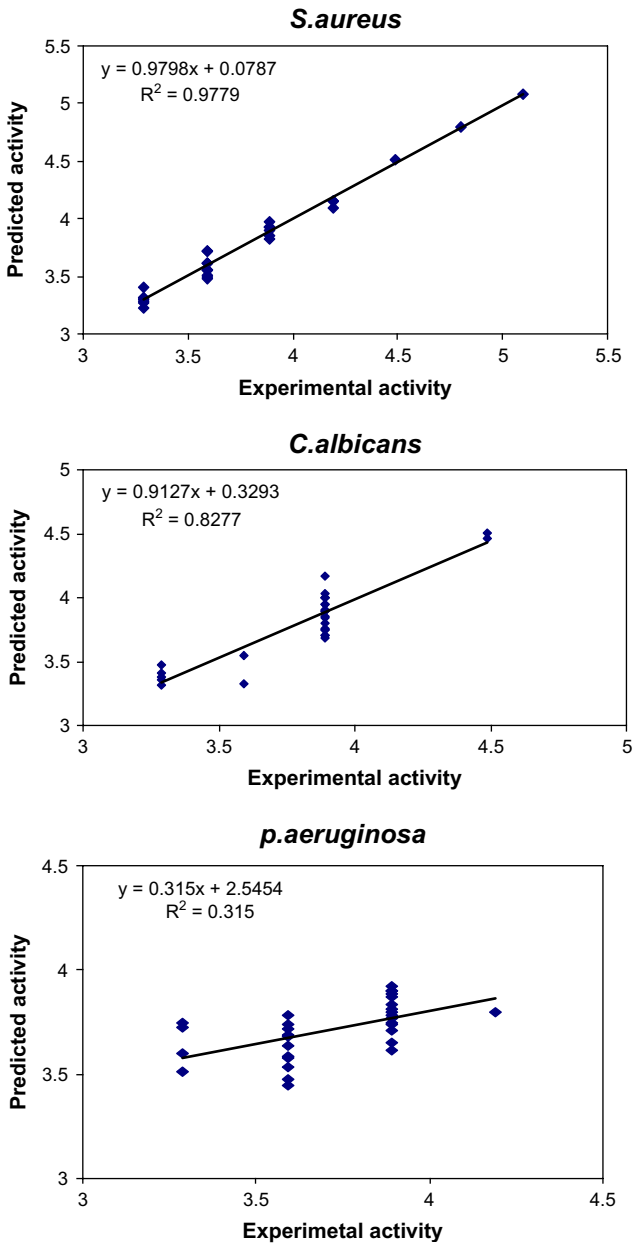


Fig. 1. Plots of the cross-validated predicted activity against the experimental activity for the MLR model obtained against three microorganisms.

(aromatic) (nNHRPh) on the antimicrobial activity of compounds **25**–**33**, **35**–**43**. This equation, which has a high statistical quality could explain and predict 0.82% and 0.61% of variance in pMIC data, respectively. MLR analysis with different types of descriptors for compounds **25**–**43** was performed, but no relationship between the structure and antimicrobial activity against *P. aeruginosa* was obtained. Plots of the cross-validated predicted activity against the experimental activity for the MLR model obtained against three microorganisms are given in Fig. 1.

3. Conclusions

A series of novel Mannich bases of 2-alkyl-3-hydroxy-pyridine-4-ones, namely 2-alkyl-3-hydroxy-5-*N*-piperidylmethyl or *N*,*N*-dialkylaminomethyl pyridine-4-ones were prepared. The synthesized compounds along with two *N*-aryl-2-methyl-3-hydroxy-pyridine-4-one derivatives and two *N*-alkyl-2-methyl-3-hydroxy-pyridine-4-one compounds were screened for their antibacterial and antifungal activities. Some of the tested compounds were moderate to good inhibitors of bacterial growth. Compounds **9**, **20** and **21** were active against *S. aureus* at 16 μg/ml concentration. Compound **9** was active against *S. enteritidis* at the same concentration. It was also a moderate inhibitor of *B. subtilis*.

Quantitative relationships between molecular structure and antimicrobial activity of a set of ten derivatives of 3-hydroxypyridin-4-one **9**, **10**, **15**–**22** which we prepared and twenty-one derivatives of 3-hydroxypyridine-4-one and 3-hydroxypyran-4-one **23**–**43** which have been reported before were discovered by MLR method. According to Table 9, geometrical parameters (J3D, ASP), functional group descriptor (nCs) and topological parameter (SPI) have important roles in the antimicrobial activity against *C. albicans*. This equation has a good statistical quality ($R^2 = 0.81$, SE = 0.14, $Q^2 = 0.73$). Results for compounds **9**, **10**, **15**–**24** revealed that nCp and nCaH parameters have significant impact on the antimicrobial activity of the compounds against *C. albicans*. The proposed equation containing this parameter could explain and predict 0.81% and 0.61% of variance in pMIC data, respectively. In compounds **25**–**43**, quantum (DMy), constitutional (nAB, RBF), topological (VRA2) and geometrical (L/BW, SPAN) parameters are major factors that affect antimicrobial activity against *S. aureus*. This equation has a high statistical quality ($R^2 = 0.93$, SE = 0.19, $Q^2 = 0.82$). As it can be seen from E_3 of Table 14 topological indices (piPC07, X2A and SEigv) have a significant role in the antimicrobial activity against *C. albicans*. This equation has a high statistical quality ($R^2 = 0.81$, SE = 0.09, $Q^2 = 0.71$). According to E_6, geometrical parameter (ASP) and functional parameter (nCaR and nNHRPh) have also significant impact on the antimicrobial activity against *C. albicans*. This equation has a good statistical quality and can explain and predict 0.82% and 0.61% of variance in pMIC data, respectively.

In summary, all of the studied compounds showed a better QSAR model for the antimicrobial activity against *C. albicans* and compounds **25**–**43** had the best QSAR model for anti *S. aureus* activity in comparison with other tested microorganisms.

4. Experimental protocols

4.1. Chemistry

All chemicals used for the synthesis of the compounds were supplied by Merck or Fluka. Melting points were determined on a Mettler capillary melting point apparatus and were uncorrected. The IR spectra were recorded with a Perkin Elmer 1420 Ratio Recording IR spectrometer as a KBr disc (γ, cm^{-1}). The ^1H NMR spectra (DMSO-d_6) were recorded on a Bruker 300 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). The mass spectra were acquired with a Finnigan TSQ-70 mass spectrometer. Electron-impact ionization was performed at an ionizing energy of 70 eV. Elemental microanalyses were within ±0.4% of the theoretical values for C, H and N. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using chloroform and methanol.

4.1.1. General procedure for the synthesis of 2-alkyl-3-benzyloxy-pyridine-4-ones (*5* and *6*)

The benzyl-protected alcohols **5** and **6** were prepared by following the methodology as described by Rai and co-workers from commercially available maltol (2-methyl-3-hydroxy-pyran-4-one) **1** or ethyl maltol (2-ethyl-3-hydroxy-pyran-4-one) **2** (Scheme 1) [42].

4.1.2. General procedure for the preparation of Mannich bases of 2-alkyl-3-benzyloxy-pyridine-4-ones (*7*, *8*, *11*–*14*)

Formaldehyde solution (40%, 1.5 mL) and the proper secondary amine (10.0 mmol) were added to a solution of **5** or **6** (10 mmol) in

ethanol. The reaction mixture was refluxed for 40–80 h. The solvent was removed by rotary evaporation under vacuum to give a yellow to reddish brown oil. The product was taken into chloroform (50 mL) and washed with water (2 × 10). The organic layer fraction was dried over anhydrous sodium sulphate, filtered and concentrated to dryness under reduced pressure to give a yellow to reddish brown oil.

*4.1.2.1. 2-Methyl-3-benzyloxy-5-*N*-piperidylmethyl-pyridine-4-one (**7**).* Yellow oil (40%); IR: (KBr Disc) ν/cm^{-1}: 1655 (C=O), 1545 (C=C); ^1H NMR (DMSO-D_6): δ 1.41–2.05 (6H, m, piperidyl C_3, C_4, C_5 hydrogens), 2.25 (3H, s, **2-CH_3**), 2.85–3.65 (4H, m, piperidyl C_2, C_6, hydrogens), 4.23 (2H, s, **CH_2**-piperidyl), 5.22 (2H, s, **CH_2**–Ph), 7.41 (5H, s, **ArH**), 8.22 (1H, s, **6-H**).

*4.1.2.2. 2-Ethyl-3-benzyloxy-5-*N*-piperidylmethyl-pyridine-4-one (**8**).* Reddish brown oil (38%); IR: (KBr Disc) ν/cm^{-1}: 1655 (C=O), 1570 (C=C); ^1H NMR (DMSO-d_6): δ 1.14 (3H, t, CH_2**CH_3**, J = 6.0 Hz), 1.44–2.05 (6H, m, piperidyl C_3, C_4, C_5 hydrogens), 2.36 (2H, q, **CH_2**CH_3, J = 6.0 Hz), 2.84–3.52 (4H, m, piperidyl C_2, C_6, hydrogens), 4.32 (2H, s, **CH_2**-piperidyl), 5.24 (2H, s, **CH_2**–Ph), 7.35 (5H, s, **ArH**), 8.36 (1H, s, **6-H**).

*4.1.2.3. 2-Methyl-3-benzyloxy-5(*N,N-*dimethyl)aminomethyl-pyridine-4-one (**11**).* Reddish brown oil (48%); IR: (KBr Disc) ν/cm^{-1}: 1660 (C=O), 1550 (C=C); ^1H NMR (DMSO-D_6): δ 2.13 (3H, s, **2-CH_3**), 2.74 [6H, s, N(**CH_3**)$_2$], 4.20 [2H, s, **CH_2**–N(CH_3)$_2$], 5.24 (2H, s, **CH_2**–Ph), 7.43 (5H, s, **ArH**), 8.02 (1H, s, **6-H**).

*4.1.2.4. 2-Ethyl-3-benzyloxy-5(*N,N-*dimethyl)aminomethyl-pyridine-4-one (**12**).* Reddish brown oil (45%); IR: (KBr Disc) ν/cm^{-1}: 1650 (C=O), 1560 (C=C); ^1H NMR (DMSO-D_6): δ 1.00 (3H, t, CH_2**CH_3**, J = 6.0 Hz), 2.62–3.04 [8H, m, **CH_2**CH_3, s, N(**CH_3**)$_2$], 4.42 [2H, s, **CH_2**–N(CH_3)$_2$], 5.12 (2H, s, **CH_2**–Ph), 7.3 (5H, s, **ArH**), 8.22 (1H, s, **6-H**).

*4.1.2.5. 2-Methyl-3-benzyloxy-5(*N,N-*diethyl)aminomethyl-pyridine-4-one (**13**).* Yellow oil (42%); IR: (KBr Disc) ν/cm^{-1}: 1655 (C=O), 1560 (C=C); ^1H NMR (DMSO-D_6): δ 1.31 [6H, t, J = 8.0 Hz, N(CH_2**CH_3**)$_2$], 2.24 (3H, s, **2-CH_3**), 3.25 [4H, q, J = 8.0 Hz, N(**CH_2**CH_3)$_2$], 4.41[2H, s, **CH_2**–N(CH_2CH_3)$_2$], 5.12 (2H, s, **CH_2**–Ph), 7.42 (5H, s, **ArH**), 8.40 (1H, s, **6-H**).

*4.1.2.6. 2-Ethyl-3-benzyloxy-5(*N,N-*diethyl)aminomethyl-pyridine-4-one (**14**).* Reddish brown oil (38%); IR: (KBr Disc) ν/cm^{-1}: 1650 (C=O), 1550 (C=C); ^1H NMR (DMSO-D_6): δ 0.83–1.32 [9H, m, N(CH_2–**CH_3**)$_2$, 2-CH_2**CH_3**], 2.31 (2H, q, J = 6.0 Hz, 2-**CH_2**CH_3), 3.13 [4H, q, J = 8.0 Hz, N(**CH_2**–CH_3)$_2$], 4.31 [2H, s, **CH_2**–N(CH_2CH_3)$_2$], 5.11 (2H, s, **CH_2**–Ph), 7.42 (5H, s, **ArH**), 8.11 (1H, s, **6-H**).

*4.1.3. General procedure for the preparation of Mannich base derivatives of 2-alkyl-3-hydroxy-pyridine-4-ones (**9**, **10**, **15**–**18**)*

The benzyl-protected Mannich base derivatives **7**, **8**, **11**–**14** (0.5 mmol) were dissolved in dimethylformamide (5 mL) and Pd/C catalyst (5%) was added. The solution was stirred at room temperature under a constant stream of hydrogen for 2 h [43]. The reaction mixture was then filtered and the solvent was removed under vacuum. Recrystallization from ethanol/diethylether afforded white to pale yellow crystals.

*4.1.3.1. 2-Methyl-3-hydroxy-5-N -piperidylmethyl-pyridine-4-one (**9**).* M.p. 269–270 °C (22%); IR: (KBr Disc) ν/cm^{-1}: 3100–3400 (broad, NH and OH), 1635 (C=O), 1540 (C=C); ^1H NMR (DMSO-d_6): δ 1.32–1.95 (6H, m, piperidyl C_3, C_4, C_5 hydrogens), 2.45 (3H, s, **2-CH_3**), 2.93–3.55 (4H, m, piperidyl C_2, C_6, hydrogens), 4.36 (2H, s, **CH_2**-piperidyl), 5.80–6.75 (1H, br, OH), 8.23 (1H, s, **6-H**); MS (EI): m/z = 222 [$M^{+\bullet}$], 221 ($M^{+\bullet}$ − H), 223 ($M^{+\bullet}$ + H, 100%).

*4.1.3.2. 2-Ethyl-3-hydroxy-5-*N*-piperidylmethyl-pyridine-4-one (**10**).* M.p. 270–272 °C (25%); IR: (KBr Disc) ν/cm^{-1}: 3200–3500 (broad, NH and OH), 1635 (C=O), 1565 (C=C); ^1H NMR (DMSO-D_6): δ 1.35 (3H, t, CH_2**CH_3**, J = 6.0 Hz), 1.41–2.04 (6H, m, piperidyl C_3, C_4, C_5 hydrogens), 2.92 (2H, q, **CH_2**CH_3, J = 6.0 Hz), 3.41–3.52 (4H, m, piperidyl C_2, C_6, hydrogens), 4.35 (2H, s, **CH_2**-piperidyl), 5.54–6.25 (1H, br, **OH**), 8.31 (1H, s, **6-H**); MS (EI): m/z = 236 [$M^{+\bullet}$], 235 ($M^{+\bullet}$ − H), 237 ($M^{+\bullet}$ + H, 100%).

*4.1.3.3. 2-Methyl-3-hydroxy-5(*N,N-*dimethyl)aminomethyl-pyridine-4-one (**15**).* M.p. 252–253 °C (25%); IR: (KBr Disc) ν/cm^{-1}: 3100–3400 (broad, NH and OH), 1640 (C=O), 1545 (C=C); ^1H NMR (DMSO-D_6): δ 2.30 (3H, s, **2-CH_3**), 2.81 [6H, s, N(**CH_3**)$_2$], 4.22 [2H, s, **CH_2**–N(CH_3)$_2$], 4.51–5.54 (1H, br, **OH**), 8.05 (1H, s, **6-H**); MS (EI): m/z = 182 [$M^{+\bullet}$], 181 ($M^{+\bullet}$ − H), 183 ($M^{+\bullet}$ + H, 100%).

*4.1.3.4. 2-Ethyl-3-hydroxy-5(*N,N-*dimethyl)aminomethyl-pyridine-4-one (**16**).* M.p. 254–255 °C (30%); IR: (KBr Disc) ν/cm^{-1}: 3100–3300 (broad, NH and OH), 1635 (C=O), 1555 (C=C); ^1H NMR (DMSO-D_6): δ 1.22 (3H, t, CH_2**CH_3**, J = 6.0 Hz), 2.60–3.00 [8H, m, **CH_2**CH_3, s, N(**CH_3**)$_2$], 4.42 [2H, s, **CH_2**– N(CH_3)$_2$], 4.65–5.55(1H, br, **OH**), 8.25 (1H, s, **6-H**),; MS (EI): m/z = 196 [$M^{+\bullet}$], 197 ($M^{+\bullet}$ + H, 100%).

*4.1.3.5. 2-Methyl-3-hydroxy-5(*N,N-*diethyl) aminomethyl-pyridine-4-one (**17**).* M.p. 254–255 °C (23%); IR: (KBr Disc) ν/cm^{-1}: 3100–3300 (broad, NH and OH), 1640 (C=O), 1555 (C=C); ^1H NMR (DMSO-D_6): δ 1.3 [6H, t, J = 8.0 Hz, N(CH_2**CH_3**)$_2$], 2.5 (3H, s, **2-CH_3**), 3.2 [4H, q, J = 8.0 Hz, N(**CH_2**CH_3)$_2$], 4.4 [2H, s, **CH_2**–N(CH_2CH_3)$_2$], 6.1–7.2 (broad, -OH), 8.4 (1H, s, **6-H**); MS (EI): m/z = 210 [$M^{+\bullet}$], 209 ($M^{+\bullet}$ − H), 211 ($M^{+\bullet}$ + H, 100%).

*4.1.3.6. 2-Ethyl-3-hydroxy-5(*N,N-*diethyl) aminomethyl-pyridine-4-one (**18**).* M.p. 256–257 °C (23%); IR: (KBr Disc) ν/cm^{-1}: 3100–3400 (broad, NH and OH), 1630 (C=O), 1545 (C=C); ^1H NMR (DMSO-D_6): δ 1.0–1.4 [9H, m, N(CH_2**CH_3**)$_2$, 2-CH_2**CH_3**], 2.7 (2H, q, J = 6.0 Hz, 2-**CH_2**CH_3), 3.1 [4H, q, J = 8.0 Hz, N(**CH_2**CH_3)$_2$], 4.3 [2H, s, **CH_2**–N(CH_2CH_3)$_2$], 4.9–5.6 (broad, –OH), 8.1 (1H, s, **6-H**); MS (EI): m/z = 224 [$M^{+\bullet}$], 223 ($M^{+\bullet}$ − H).

4.1.4. General procedure for the synthesis of N*-aryl-2-methyl-3-hydroxy-pyridine-4-ones (**19** and **20**)*

Synthesis of *N*-aryl-2-methyl-3-hydroxy-pyridine-4-ones **19** and **20** was achieved via a single step synthetic pathway. Maltol (**1**) (5 mmol) was refluxed with an excess of the suitable primary aryl amines (7.5 mmol) in an acidic solution of 9.0 mL water, 0.2 mL HCl and 1.0 mL ethanol (pH = 5) for 50–60 h. After the completion of the reaction the reaction mixture was adjusted to pH = 7 using sodium hydroxide solution (2 N) and the product were collected by filtration. Purification was achieved by re-crystallization from hot methanol.

*4.1.4.1. 1-Phenyl-2-methyl-3-hydroxy-pyridine-4-one (**19**).* M.p. 221–222 °C (35%); IR: (KBr Disc) ν/cm^{-1}: 3200 (broad, OH), 1630 (C=O), 1580 (C=C); ^1H NMR (DMSO-D_6): δ 2.0 (s, 3H, 2-**CH_3**), 5.7 (bs, **3-OH**), 6.2 (d, 1H, **5-H**, J = 8.0 Hz), 7.3–7.7 (m, 6H, **ArH** & **6-H**). MS (EI): m/z = 201 [$M^{+\bullet}$], 200 (M − H), 184 (M − OH), 124 (M − C_6H_5).

*4.1.4.2. 1-(3-hydroxyphenyl)-2-methyl-3-hydroxy-pyridine-4-one (**20**).* M.p. 268–269 °C (40%); IR: (KBr Disc) ν/cm^{-1}: 3100 (broad, OH), 1630 (C=O), 1600 (C=C); ^1H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-**CH_3**), 4.5 (bs, Ar–**OH**), 6.3 (d, 1H, **5-H**, J = 8.0 Hz), 6.8–7.7 (m, 5H, **ArH** & **6-H**). MS (EI): m/z = 217 [$M^{+\bullet}$], 216 ($M^{+\bullet}$ − H), 200 ($M^{+\bullet}$ − OH).

4.1.5. General procedure for the synthesis of N-alkyl-2-methyl-3-hydroxy-pyridine-4-ones (**21** and **22**)

The *N*-alkyl derivatives of maltol **21** and **22** (Scheme 3).were prepared following the methodology as described by Harris from commercially available maltol **1** [38].

4.2. Antimicrobial activity determination

Muller–Hinton broth and RPMI 1640 medium buffered with MOPS at pH 7.0 was used for bacteria and fungi respectively [41]. The inocula of microorganism (10^6 c.f.u ml^{-1}) were prepared from culture and suspensions were adjusted to 0.5 McFarland standard turbidity. The final size of inocula was 1.5×10^4 for bacteria. The test compound dissolved in dimethyl sulfoxide (DMSO) was first diluted to the highest concentration to be tested and DMSO had no effect on the microorganisms in the concentrations studied. Serial two-fold dilutions were made in concentration range from 8 μg ml^{-1} to 512 μg ml^{-1} in sterile 96 well microplates. 20 μl of Alamar Blue® reagent was added to each well. Plates were covered and sealed with parafilm and incubated for 24 h at 37 °C. The MIC was defined as the lowest concentration, which prevented a color change from blue to pink. Ciprofloxacin was used as standard antibacterial drug. The same method except some modifications was used for the antifungal studies. The final size of inocula of microorganism was 1.5×10^5 for fungi. The incubation time was 48 h at 25 °C. Ketoconazole was used as standard antifungal agent.

4.3. QSAR analysis

4.3.1. Software and descriptor generation

A Pentium IV personal computer (CPU at 3.06 GHz) with windows XP operating system was used. The two-dimensional structures of molecules were drawn using Hyperchem 7.0 software. The final geometries were obtained with the semi-empirical AM1 method in Hyperchem program. The molecular structures were optimized using the Polak–Ribiere algorithm until the root mean square gradient was 0.01 kcal mol^{-1}. The resulted geometry was transferred into Dragon program package, which was developed by Milano chemometrics and QSAR Group [44]. The *z*-matrix of the structures were provided by the software and transferred to the Gaussian 98 program [45].

Complete geometry optimization was performed taking the most extended conformation as starting geometries. Semi-empirical molecular orbital calculation (AM1) of the structures was performed using Gaussian 98 program [45].

A large number of molecular descriptors were calculated using Hyperchem, Gaussian 98 and Dragon package [44]. Some chemical parameters including molecular volume (V), molecular surface area (SA), hydrophobicity (log P), hydration energy (HE) and molecular polarizability (MP) were calculated using Hyperchem Software. Gaussian 98 was employed for calculation of different quantum chemical descriptors including, dipole moment (DM), local charges, HOMO and LUMO energies, hardness (η), softness (S), electronegativity (χ) and electrophilicity (ω) according to the method proposed by Thanikaivelan et al. [46]. Dragon software calculated different functional groups, topological, geometrical and constitutional descriptors for each molecule.

4.3.2. Data processing and modeling

The selection of significant descriptors, which related the antimicrobial data to the molecular structures, is an important step in QSAR modeling. Selection of significant descriptors was performed through the following steps:

i) The calculated descriptors were collected in a data matrix, **D**. First the descriptors were checked for constant or near constant values and those detected were removed from the original data matrix. The correlation of descriptors with each others and with the activity data was determined.
ii) The input variable in MLR must not be highly correlated. Among the collinear descriptors detected ($r > 0.9$) one with the highest correlation with the activity was retained and the rest were omitted.
iii) The selected descriptors from each class and the experimentally antimicrobial data were analyzed by the stepwise regression SPSS (version 12.0) software.

For the development of QSAR equations, Stepwise-MLR method was used.

In present study, MLR with stepwise selection and elimination of variables was applied for developing QSAR models using SPSS software (SPSS Inc., version 12.0). The resulted models were validated by leave-one out cross-validation procedure (using MATLAB software) to check their predictability and robustness.

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