SYNTHETIC, SPECTRAL, ANTIMICROBIAL AND QSAR STUDIES ON NOVEL MANNICH BASES OF GLUTARIMIDES

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ABSTRACT

A series of forty Mannich bases of glutarimides with sulfonamides and secondary amines were synthesized and evaluated *in vitro* against six pathogenic Gram-positive and Gram-negative bacteria. The synthesized Mannich bases were characterized by elemental and spectral analysis. The modeling anti-bacterial activities of these newly synthesized Mannich bases against six bacteria was attempted employing ^{1}H NMR chemical shift, physicochemical properties and topological indices as the correlating parameters. Our results, based on Quantitative Structure-Activity Relationships (QSARs), have indicated that statistically significant models are obtained for modeling the anti-bacterial activities The results are discussed critically using a variety of statistical parameters.

Keywords: Glutarimides, sulfonamides, Mannich bases, antimicrobial activity, QSAR.

INTRODUCTION

The Quantitative Structure-Activity Relationships (QSARs) are mathematical models relating measured biological activity of series of structurally related compounds / pharmacological agents to the variation in their chemical structure. In cases in which some physico-chemical properties or toxicities of such compounds are related to their structures, the methodology is called Quantitative Structure-Property relationships (QSPRs) or Quantitative Structure-Toxicity Relationships (QSTRs), respectively. Such methodologies are widely used in environmental toxicology to understand the adverse effects of chemical compounds. The QSPR /QSAR /QSTR methodology is very useful for a large number of untested chemicals present in nature, and because of the high costs of biological testing [1-19]. QSAR models are nowadays regarded as a scientifically credible tool for predicting and classifying biological activities of untested chemicals. QSAR has become inexorably embedded as an essential tool in the pharmaceutical industry, from lead discovery, optimization to lead development and computer –aided drug designing [20, 21]. A growing trend is to use QSAR early in the drug discovery process as a screening and enrichment tool to estimate from further development those chemicals lacking drug like properties [21] or those chemicals predicted to elicit a toxic response. The fundamental assumption of QSAR is that variations in the biological activity of a series of chemicals that target a common mechanism of action are correlated with variations in their structural, physical and chemical properties [22]. These biological activity of structurally related chemical compound in terms of in-*vivo* or *in-vitro* properties can be determined by experimental or more efficiently by computational mean.. Needless to state that a statistically validated QSAR model is capable of predicting the biological activity of a new chemical within the same series in lieu of the time-consuming and lab our-intensive processes of chemical synthesis and biological evaluation. Applied judiciously, QSAR can save substantial amount of time, money, and human resources.

The molecular structure and NMR chemical shift information of organic compounds acting as drugs can be combined to form powerful models of biological activity. Such data-activity relationship is now-a-day called Quantitative Structure-Data-Activity Relationship (QSDARs) in place of QSAR as it involved the use of spectroscopic data. As is well known [23-30], chemical shifts in NMR offer a powerful probe for the study of the immediate atomic environment in a molecule. It is worthy to mention that NMR spectra reflect quantum mechanical properties and that QSAR depends on local electrostatics and geometry of the molecule. The ^{13}C NMR spectrum of a compound contains a pattern of frequencies that correspond directly to the quantum mechanical properties of the carbon nuclear magnetic dipole in a magnetic field. The spectral pattern reflects the local electrostatic environment and electron orbital configuration of each atom. The resonance from different carbon orbital configurations is generally well-separated from each other, which permits the use of advantageous for ^{13}C NMR spectral directly to build the QSDAR models [23, 24].

Recently one of the authors (PVK) has initiated interesting investigations on ^{13}C NMR chemical shift [25 - 30]. His approach was two-fold: firstly to establish ^{13}C NMR chemical shift as a molecular descriptor and secondly to use the same for modeling property-activity- toxicity of organic compounds acting as drugs. One of such applications studied by Khadikar being modeling CA inhibition using ^{13}C NMR chemical shift [6]. Prompted by these results we have undertaken the present study, in that we have investigated variance of antibacterial activity using ∑NMR chemical shifts as one of the correlating parameters. We have also observed that in many cases ∑NMR chemical shifts in combination with physicochemical parameters as well as topological indices improved results are obtained. In doing so we have used maximum -R^2 method and applied variety of statistics[10]. We have, therefore, attempted modeling of antibacterial activities of the newly synthesized Mannich bases using ∑NMR chemical shifts, physicochemical parameters as well as topological indices as the correlating parameters. At this stage it is interesting to mention that some people cretised the use of topological indices and consider their use in the development of the models as quite restrictive, since these descriptors only depend on 2D molecular features and not in conformational or electronic properties that are most expected to govern the biological activity of the compounds under study than topological features. Such a criticism is due to the fact that generally no physical significance is attached to topological indices. However, there are several cases in that topological indices correlate excellently with conformational, electronic and other related properties making their judies use in QSAR.

The increasing popularity of the Mannich reaction as well as utility of Mannich bases has been fluted by the ubiquitous nature of nitrogen in drugs and natural products as well as by the potential of this multi-component reaction to generate diversity. Interest in Mannich bases has been quite attractive and wide ranged considering the enormous domain of the applications involving variant biological [31-35], pharmaceutical [36-40] and industrial[41-43] properties. Glutarimides moiety with the intact imide group is acting as the carrier molecule (vector), which transports biologically active substitutents (functional groups) through cell membranes[44]. It is also a component of newly synthesized antibiotics, which exert antiviral and antifungal activity [45, 46]. Glutarimides (2,6-piperidinedione) moiety is found in a number of antibiotics with antiviral and fungicidal activity[47-50]. Furthermore, the 2,6-piperidinedione moieties constitute an important centre in several new anticancer drugs which have recently been introduced into experimental chemotherapy[51-53]. It is also a structural part of a number of molecules with interesting biochemical activity[54]. In view this we have attempted aminoalkyaltion of various glutarimides-moieties with sulphonamides and secondary amines. The structural characterization these newly synthesized Mannich bases is made using elemental, UV, IR and ^{1}H NMR studies.

The anti-bacterial potential vis-à-vis QSAR study of the newly synthesized Mannich bases in the present is made in the following four different ways:

(i) QSAR study based on using ∑NMR chemical shifts as molecular descriptor;

(ii) QSAR study based on using physicochemical parameters as molecular descriptor;

(iii) QSAR study based on using topological indices as molecular

descriptor, and (iv) QSAR study based on combination of ∑NMR chemical shifts, with physicochemical properties and or topological indices.

The results obtained are presented in Tables 1-7.

EXPERIMENTAL SECTION

General information.

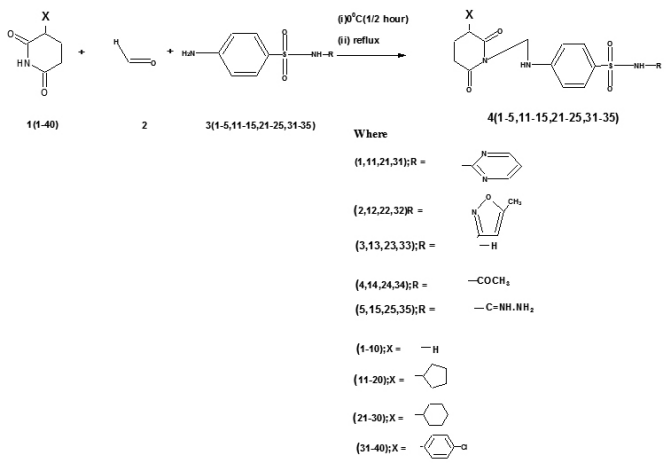
All the m.p. of the synthesized Mannich bases ware determined using Thomas Hoover capillary melting point apparatus and their purity was ascertained by TLC method. The antimicrobial screening was performed using paper disc method and the results were statistically evaluated using variety of statistical parameters. Mullar Hinton Agar was taken as media for cultivation of bacteria. The inhibitory effect of the samples were measured against each of the bacteria after incubation for 24 hours at 37^0C.The experiments were run in triplicate and the mean of readings were recorded.

Synthesis of Mannich bases from primary amines.

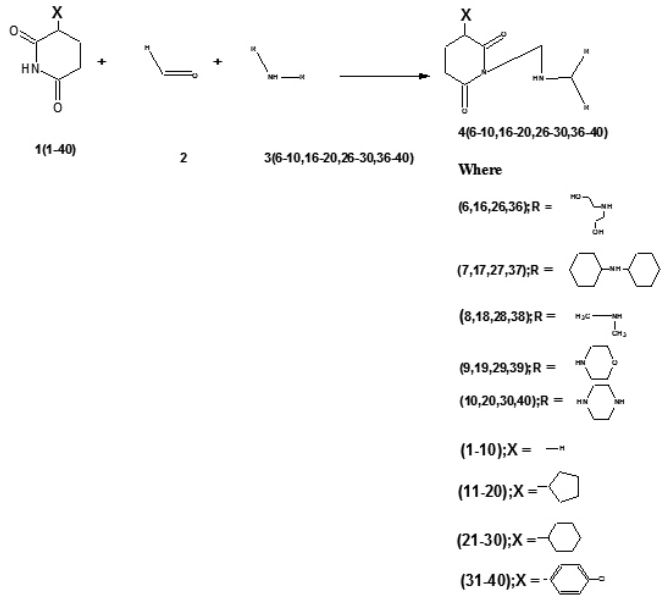
Mannich bases of glutarimides were prepared by reacting various glutarimides (0.01mol) dissolved in 20 mL of ethanol with sulfonamide (0.01mol). 2.5 mL (0.01mol) of formaldehyde solution (37%, v/v) was added slowly with constant stirring. The pH of the mixture was adjusted to 3.5 by adding 0.5 mL of 1 mol L^{-1} HCl. The mixture was kept at efficient ice cooling for half an hour, and then refluxed on water bath. Reflux time varied with the sulfonamide used. The refluxed mixture was kept at 0^0C for four days when crystalline product was obtained. The product was re-crystallized from dry distilled ethanol and dioxane-water (1:1). The compounds were characterized by elemental, UV, IR and NMR studies.

Synthesis of Mannich bases from secondary amines.

Secondary amine (0.01mol) was added to an ethanolic solution (50 mL) of glutarimides (0.01 mol) in a flat bottom flask. Amount of 0.4 mL (0.015mol) of formaldehyde solution (37%, v/v) was added slowly with constant stirring. The reaction mixture was stirred at 70-75^{0}C for 3.0 to 8.5 hours, depending upon the secondary amine. The remaining portion of formaldehyde solution was added in two installments after 1 and 2 hours, respectively. The reaction mixture was kept overnight in the refrigerator. Next day, the excess of solvent was distilled off from the reaction mixture under reduced pressure. It was again kept for crystallization in the refrigerator. The products obtained were purified by re-crystallization from dry distilled ethanol. The compounds were characterized by elemental, UV, IR and NMR studies. For details see schemes **1** and 2.



Scheme 1: Synthesis of Mannich bases from sulphonamides.



Scheme 2: Synthesis of Mannich bases from secondary amines.

Spectral studies.

The ^{1}H NMR spectra in DMSO and $CDCl_3$ solvent were recorded on Bruker DRX-300 FT NMR Spectrometer. The UV spectra were recorded on Schimadzu UV-160A, UV-visible spectrophotometer.

Antimicrobial activity .

The sum of the NMR chemical shift ,∑NMR, and the anti-bacterial activity for the set of 40 Mannich bases as given in Table 1.The useful descriptors (∑NMR, physical parameters and topological indices) from a large set were chosen using multiple variable selection and are given in Table 2. The regression analyses were performed starting from a single to ten descriptors and the regression reports are given as supplementary material. The plots of number of descriptors against R^2 (Fig. 1) indicated that eight variable model is the most appropriate model for modeling anti-bacterial activity against all the six bacteria.. This is in accordance with the rule of Thumb [63] which states that the descriptors to be used in multiple regression analysis should be one-fifth of the total number of compounds under study. The detail regression analysis of the models is given in the text under Results and Discussion section. The correlelation matrices for the parameters involved in these models are also presented separately as supplementary material. Finally, Ridge parameters for each of these models are also mention as supplementary material.

Table 1. Antibacterial activity of the Mannich bases and their and NMR-Chemical shift (1-40).

C.N.	∑NMR	*B. subtilis*	*S. typhi*	*E.coli*	*S. aureus*	*K. pneumoniae*	*P. aruginosa*
1	33.98	10.5	10.5	10.0	11.5	10.8	10.5
2	34.26	10.0	12.0	12.o	11.0	11.5	10.0
3	31.68	10.5	10.0	11.0	12.0	12.0	10.5
4	32.5	11.0	10;.0	8.5	9.5	13.0	11.0
5	34.29	12.0	11.0	7.5	10.5	13.5	12.0
6	30.42	13.0	11.5	10.0	10.0	14.2	13.0
7	36.44	11.0	10.5	10.5	10.5	15.8	11.0
8	34.36	10.5	8.5	11.0	10.5	10.8	10.5
9	30.88	10.0	10.0	11.5	10.0	10.2	10..0
10	32.48	11.2	11.0	11.5	11.5	11.8	11.2
11	30.98	11.6	11.0	11.0	12.4	13.0	11.6
12	31.28	11.8	11.0	10.0	11.0	13.5	11.8
13	36.92	13.2	11.5	10.5	11.2	12.5	13.2
14	32.22	14.8	12.5	12.0	11.5	12.0	14.8

15	30.24	15.6	13.5	12.5	12.0	12.0	15.6
16	34.60	10.5	10.0	10.5	11.0	11.5	10.5
17	34.28	8.5	10.0	10.5	11.0	11.5	8.5
18	32.60	7.5	10.0	10.5	10.5	11.6	7.5
19	34.46	10.5	10.0	10.0	11.0	11.5	10.5
20	30.92	12.0	10.0	11.0	11.5	11.5	12.0
21	30.28	13.4	11.5	11.0	11.5	11.5	13.4
22	30.60	11.0	12.5	11.5	12.0	12.0	11.0
23	32.42	15.5	13.5	12.5	12.5	11.5	15.5
24	32.72	14.8	12.5	11.5	11.0	11.0	14.8
25	27.04	-	-	-	-	-	-
26	34.42	12.0	13.5	12.5	12.0	12.5	12.0
27	30.90	12.5	12.5	10.0	12.5	12.0	12.5
28	34.90	11.5	10.5	11.0	12.5	12.5	11.5
29	32.48	11.0	10.5	10.5	11.5	12.5	11.0
30	36.5	10.0	10.0	10.5	14.5	12.5	10.0
31	30.26	10.8	10.8	12.2	13.5	11.5	10.5
32	34.62	10.4	13.8	11.2	12.5	10.5	15.5
33	34.42	10.6	11.8	13.2	16.5	12.0	12.5
34	30.24	10.8	12.8	12.2	17.5	12.5	11.5
35	32.4	10.9	13.8	13.2	12.5	12.5	11.5
36	30.92	10.6	11.8	14.2	11.5	11.5	11.5
37	30.24	10.4	14.8	15.2	10.5	10.5	11.5
38	30.2	10.4	13.8	11.2	13.5	13.0	11.5
39	34.92	10.0	12.8	10.2	12.5	13.5	11.5
40	34.9	10.0	11.8	12.2	13.5	11.5	11.5

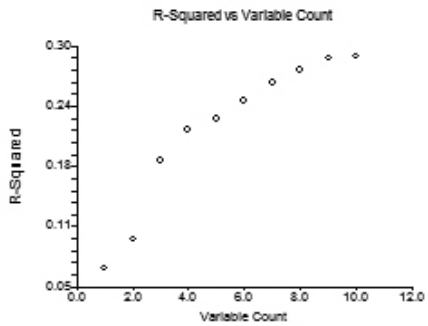
Table 2. Useful variables from the larger pool and their codes.

Code	Variable	Symbol	Name of the variable
A	C2	SNMR	Sum of NMR
B	C9	d	Density
C	C10	α	Polarizability
D	C11	MM	Monoisotopic Mass
E	C12	NM	Nominal Mass
F	C13	AM	Average Mass
G	C14	ZM1	First Zagreb index M1
H	C15	ZM2	Second Zagreb index M2
I	C16	Pol	Polarity number
J	C17	SMT1	Schultz Molecular Topological Index
K	C18	Xu	Xu Index
L	C19	SP1	Superpendentic Index
M	C20	W	Wiener Index
N	C21	Har	Harary Index
O	C22	^{0}c	Zero order randic connectivity Index
P	C23	^{1}c	First order randic connectivity Index
Q	C24	^{2}c	Second order randic connectivity Index
R	C25	^{3}c	Third order randic connectivity Index
S	C26	^{4}c	Fourth order randic connectivity Index
T	C27	$^{0}c^{v}$	Zero order randic valence connectivity Index
U	C28	$^{1}c^{v}$	First order randic valence connectivity Index
V	C29	$^{2}c^{v}$	Second order randic valence connectivity Index
W	C30	$^{3}c^{v}$	Third order randic valence connectivity Index
X	C31	$^{4}c^{v}$	Fourth order randic valence connectivity Index

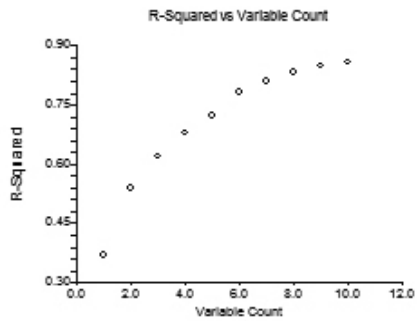
Codes for the bacteria

Code	Bacteria
C32	***B.subtilis***
C33	***S.typhi***
C34	***E.coli***
C35	***S.aureus***
C36	***K.pneumoniae***
C37	***P.aeruginosa***

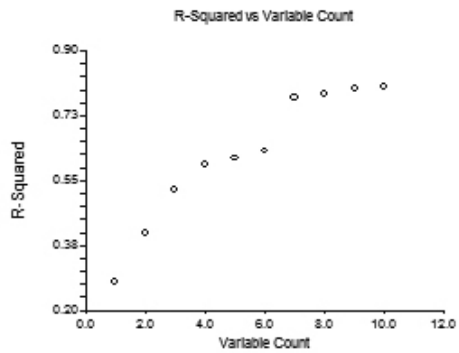
B.subtilis



S.typhi



E.coli



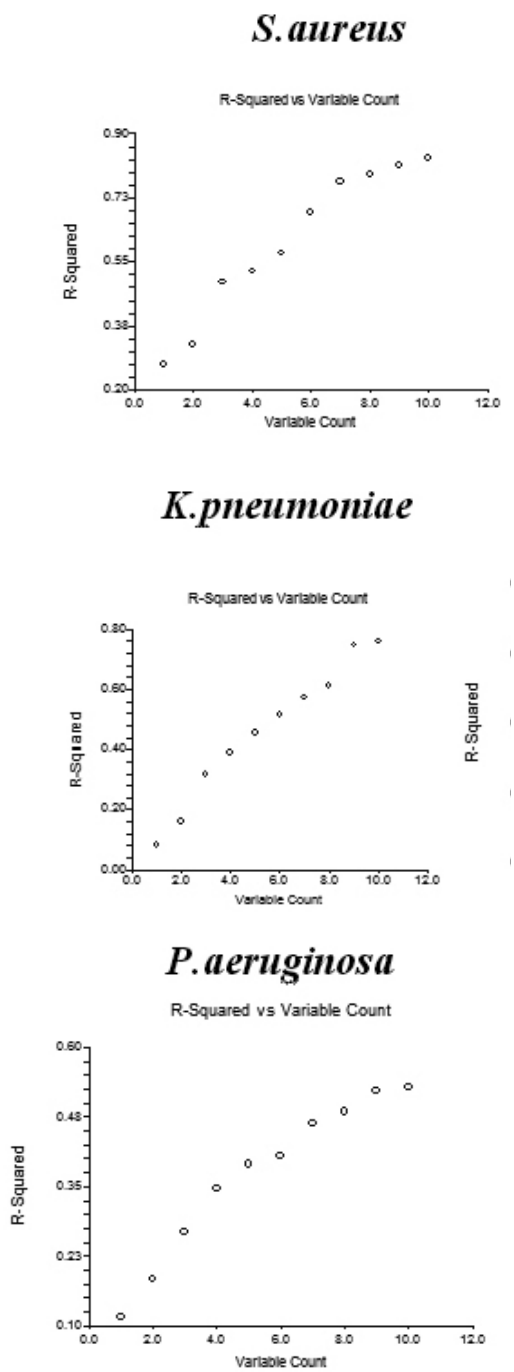


Figure 1. Correlation of number of descriptors (variable count) with R^2 Supplementary material. (1) Variable selection for multiple regression analysis.

RESULTS AND DISCUSSION

A perusal of the Table 1 demonstrates the sequence of activity in each of the bacteria used in the present study. The data presented in this Table 1 show that degeneracy in activity exists in each of the six bacteria used. Also that the sequences of activities are very different for the bacteria used. This data, therefore, do not exhibit any structure-activity relationship for the variation of antibacterial activity. This has prompted us to compute molecular descriptors and then to examine structure activity using QSAR methodology.

Preliminary regression analysis has indicated that use of ∑NMR chemical shifts alone do not give any statistically significant model. However, when ∑NMR chemical shifts are combined with physical properties and also topological indices better quality models are obtained and that most appropriate models are obtained when all the three types of molecular descriptors are used together. We observed that if ∑NMR chemical shifts are eliminated from the most appropriate model the quality of the model is decreased significantly. This exhibits the dominating role of ∑NMR chemical shifts. The proposed models are given below:

(i) M*odel for B.subtilis*

Anti-bacterial activity(*B.subtilis*) = 6.7268 + 4.6812*10^{-2}(±3.6965*10^{-2}) C2
+7.0545*10^{-3}(±1.6369*10^{-2}}C3
-3.0702*10^{-3}(±2.107*10^{-3})C17
+0.01124 (±8.949774*10^{-3})C20
+0.24859 (±0.3089584)C24
-6.4740*10^{-2}(±0.1287502)C27
+1.1076*10^{-4}(±1.4978*10^{-4})C28
+0.33949 (±0.2729939)C30
N=40,CV=0.1080532,R^2=0.2601,R^2A=0.0000,F=0.7470

(ii) *Model for S.typhi*

***Anti-bacterialactivity*(*S.typhi*)**=9.888266-4.510451*10-2 (±2.321028*10-2) C2
+1.473106(±0.2763391)C16
-1.525129(±0.4197105)C21
+0.2966534(±0.2012577)C24
+2.086771(±1.280614)C25
-0.3372007(±0.1177242) C26
-0.2197946(±6.764524*10-2) C27
+2.066341*10-4(± 9.204068*10-5) C28
N=40,CV= 6.8970,R^2=0.8328,R^2A=0.7541, F=10.5844

(iii) *Model for E.Coli*

***Anti-bacterial activity*(*E.coli*)** = 17.3471-8.5342*10^{-2}(±3.2712*10^{-2})C2
-8.2224(±4.0231C9
+12.3122 (±8.025793) C11
+12.1831 (±8.015417) C12
-4.3494*10^{-2}(±4.455554*10^{-2}) C15
-1.3177 (±0.5166374)C18
+2.6913*10^{-4}(±1.0817*10^{-4})C28
-0.3951 (±0.1142)C29
N=40,CV= 7.9712,R^2=0.7836,R^2A=0.6817, F=7.6935

(iv) *Model for S.aureus*

***Anti-bacterialactivity*(*S.aureus*)**=32.7323+0.1425 (±3.5039*10^{-2})C2
+1.8960 (±0.2853)C16
-1.0472*10^{-2}(±2.1726*10^{-3})C17
+4.9660*10^{-2}(±9.5501*10^{-3})C20
- 5.5463 (±0.8328)C22
-0.3017 (±0.2427)C24
-3.7687*10^{-4}(±1.1822*10^{-4})C28
-0.5538 (±0.1980) C31
N=40,CV= 7.7740,R^2=0.7898,R^2A=0.6909, F=7.9863

(v) *Model for K.pneumoniae*

***Anti-bacterialactivity*(*K.pneumoniae*)** =6.3773+ 0.7490 (±0.2062) C2
-15.4731 (±7.2948) C10
+15.4189(±7.2829) C11
-5.4125*10^{-3}(±1.9237*10^{-3}) C12
+2.0276*10^{-2}(±8.3083*10^{-3})C17
+0.3188 (±0.2348)C20
-3.1490*10^{-4}(±1.1616*10^{-4}) C28
N=40,CV= 7.1296,R^2=0.6142,R^2A=0.4326, F=3.3825

(vi) *Model for P.aeruginosa*

***Anti-bacterialactivity*(*P.aeruginosa*)** =6.8045+10.9606 (±0.3556)C16
+1.0584 (±0.4314)C18
-1.9102 (±0.6176)C21
+3.8562 (±1.7120)C25
-0.6298 (±0.3388)C26
-0.1285 (±0.1361) C27
+2.0391*10^{-4}(±1.3594*10^{-4}) C28
+0.6882 (±0.4046)C31
N=40, CV= 9.0854*10^{-2}, R^2=0.4846,R^2A=0.2420, F=1.9978

Physicochemical significances of the proposed models

It will be interesting to discuss the physicochemical significances of the proposed models. We observed that invariably all the models contain C2 (∑NMR) as the correlating parameter. It means, therefore, that ∑NMR play a dominating role in modeling antibacterial activity against all the bacteria. That is electronic effect is the main parameter for the exhibition of the activity.

In case of models -3 and model-5, in addition to ∑NMR, physicochemical parameters along with topological indices are involved in modeling the antibacterial activities. The physicochemical parameters involved indicate that the size and shape are responsible for the exhibition o the antibacterial activities against these bacteria. The topological indices involved in these and other models are mainly connectivity indices. This means that the extent of connectivity is the responsible for the exhibition of anti bacterial activity against all the bacteria used.

It is also worth mentioning that all the proposed models, except model-5 (which has 7 correlating parameters), all other models contain 8 correlating parameters. Looking to the size of the sample i.e. the number of compounds used this is accordance with the rule of thumb. Furthermore, the plot of number of variables used against R2

(Fig.1) also favors the use of 7 to 8 correlating parameters.

The aforementioned models were further examined employing Ridge statistics.

Ridge statistics.

Application of Ridge statistics provides important statistical parameters namely variance inflation factors (*VIF*s) for each of the parameters involved in the model. The *VIF* is defined for each variable in the equation, and not for the equation as a whole, so there should be as many *VIF*s, as there are correlating parameters. The *VIF* is defined as:

$$VIF = 1(1\text{-}R^2_i) \qquad (19)$$

Where R_i is the multiple correlation coefficient of the i^{th} independent variable on all of the other independent variables. In the proposed models, all these *VIF*s should be less than 10 indicating that no co- linearity problem exists in the model.

The *VIF*s values for the parameters involved in the aforementioned models are given supplementary material; which shows that each of the models contain one or more descriptors having *VIF*s values larger than 10. Therefore, based on *VIF*s values a major problem of co- linearity exists for them. However, the magnitude of condition number indicates the existing of mild co- linearity. In such cases Randic recommendations [59-60] are used to arrive at the final decision.

Randic recommendations

Randic [59-60] stated that if a descriptor strongly correlates with another descriptor already used in a regression, such a descriptor in most studies should be discarded. For example $^1\chi$ and $^2\chi$, $^1\chi$ often strongly correlate and in many structure-property-activity studies $^2\chi$ has been discarded. This is not theoretically justified and despite the widespread practice should be stopped. Although two highly correlated descriptors overall depict the same features of molecular structure, it is important to recognize that even highly interrelated descriptors differ in some other structural traits. The difference between them may be relatively small but nevertheless very important for structure-property regression.

The criteria for inclusion or exclusion of descriptors should not be based on parallelism between descriptors even if overwhelming, but should be based on whether the part in which two descriptors disagree is or is not relevant for the characterization of the property considered .If the part in which the second descriptor differ from the first, regardless of how small it is, is relevant for the property under consideration, then the descriptor should be included. Randic [59, 60] further stated that the selection of descriptors to be used in structure-property-activity studies should not be delegated solely to computers, although statistical criteria will continue to be useful for preliminary screening of descriptors taken from a large pool. Often in an automated selection of descriptors, a descriptor will be discarded because it is highly correlated with another descriptor already selected. But what is important is not whether two descriptors parallel one another; i. e. duplicates much of the same structural information, but whether they are complementary in those parts that are important for structure-property-activity correlations. Hence, the residual of the correlation between two descriptors should be examined and kept or discarded depending on how well it can improve the correlation based on already selected descriptors.

CONCLUSIONS

The newly synthesized Mannich bases appeared to be very potent and outstanding antibacterial agents with promising activity and found safer. These novel Mannich bases could be used as useful drug. Our findings will prove helpful to those who are engrossed in the synthesis of potential Mannich bases as drugs with minimum side effects and also having comparatively low cost. Thus, the result presented in this paper is valuable in constructing pharmacologically imperative heterocyclic as new exotic drugs. Efforts are continuing to synthesize new amino-methyl derivatives of various active hydrogen compounds, that the derived compounds may have enhanced pharmacological activity. Our results also show that the antibacterial activity of the Mannich bases could be modeled using sum of the NMR chemical shifts, physicochemical parameters, and topological indices. The combination of these parameters gives statistically significant models for modeling antibacterial activity against ***S.typhi, E.coli***, ***S.aureus***, and ***K.pneumoniae***.

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(i) B.subtilis

(i)Using variables: C2, C9-C13. (ii) Using variables : C2,C14-C31. (iii)Using variables : C2,C9-C31.

Model Size	R^2	Coded Variables
1	0.1718	A
2	0.1722	AC
3	0.2495	BDF
4	0.2535	BDEF
5	0.2595	ABDEF
6	0.2611	ABCDEF

Model Size	R^2	Coded Variables
1	0.0630	A
2	0.1113	AR
3	0.2379	FIR
4	0.2727	FIPR
5	0.2929	FIJPR
6	0.3062	DFIJPR
7	0.3189	DFILPQR
8	0.3277	DFILOPQR
9	0.3576	DEHIJLOQR
10	0.3863	DEHIJLOPQR

Model Size	R^2	Coded Variables
1	0.0693	B
2	0.0993	BJ
3	0.1812	BJW
4	0.2136	ABJW
5	0.2241	ABJUW
6	0.2433	AJMQUW
7	0.2623	ABJMUVW
8	0.2748	ABJMQTUW
9	0.2876	ABJMQTUVW
10	0.2896	ABJMQSTUVW

(ii) S.typhi

(i)Using variables: C2, C9-C13(ii) Using variables: C2, C14-C31. (iii) Using variables: C2, C9-C31.

Model Size	R^2	Coded Variables
1	0.2866	C
2	0.4293	EF
3	0.4508	AEF
4	0.4549	ABEF
5	0.4592	ABDEF
6	0.4592	ABCDEF

Model Size	R^2	Coded Variables
1	0.3687	I
2	0.5385	IN
3	0.6190	INU
4	0.6797	AINX
5	0.7241	AFINU
6	0.7823	INRSTU
7	0.8114	AINRSTU
8	0.8328	AINQRSTU
9	0.8493	AINQRSTUX
10	0.8584	ABINQRSTUX

Model Size	R^2	Coded Variables
1	0.3687	D
2	0.5385	DI
3	0.6190	DIP
4	0.6797	ADIS
5	0.7140	ADIPS
6	0.7646	DIOPRS
7	0.8110	DIMOPRS
8	0.8255	ADIMOPRS
9	0.8493	ADILMNOPS
10	0.8580	ADFILMNOPS

(3) E.coli

(i)Using variables: C2, C9-C13. (ii) Using variables: C2, C14-C31. (iii)Using variables: C2, C9-C31.

Model Size	R^2	Coded Variables
1	0.0790	C
2	0.3151	EF
4	0.3378	ABEF
3	0.3328	AEF
5	0.3400	ABCEF
6	0.3400	ABCDEF

Model Size	R^2	Coded Variables
1	0.2784	U
2	0.4101	AM
3	0.5224	AMW
4	0.5922	AMUW
5	0.6099	AJMUV
6	0.6294	ABJMUV
7	0.7714	ABDEKUV
8	0.7836	ABDEHKUV
9	0.7993	ABDEGKRUW
10	0.8047	ABCDEHKRUW

Model Size	R^2	Coded Variables
1	0.2784	P
2	0.4101	AH
3	0.5224	AHR
4	0.5922	AHPR
5	0.6099	AEHPQ
6	0.6197	AEHPRS
7	0.7214	ADEGHJQ
8	0.7577	ACDEGHJQ
9	0.7877	ACDEGHJOQ
10	0.8675	BCDEGHJLOQ

(4)S.aureus

(i)Using variables: C2, C9-C13. (ii) Using variables: C2, C14-C31. (iii)Using variables: C2, C9-C31.

Model Size	R^2	Coded Variables
1	0.2691	G
2	0.3229	GN
3	0.4946	DKP
4	0.5242	DKPQ
5	0.6138	ADFGP
6	0.6514	ADFGPQ
7	0.7389	ABDGJPS
8	0.7763	ABCDGJPS
9	0.8240	ABCDEHJPS
10	0.8701	ABCDEFHJPS

Model Size	R^2	Coded Variables
1	0.1118	F
2	0.3602	EF
3	0.4866	AEF
4	0.5640	ADEF
5	0.5719	ABDEF
6	0.5733	ABCDEF

Model Size	R^2	Coded Variables
1	0.2691	L
2	0.3229	LS
3	0.4946	IPU
4	0.5242	IPUV
5	0.5737	DIOPU
6	0.6869	AIJMOU
7	0.7707	AIJMOUX
8	0.7898	AIJMOQUX
9	0.8118	ACIJMOQUX
10	0.8335	ACIJMOQRUX

(5) K.pneumoniae

(i)Using variables: C2, C9-C13 (ii) Using variables: C2, C14-C31 (iii) Using variables: C2, C9-C31.

Model Size	R^2	Coded Variables
1	0.0058	F
2	0.0184	BF
3	0.0474	BCF
4	0.0567	BCEF
5	0.0597	ABCEF
6	0.0631	ABCDEF

Model Size	R^2	Coded Variables
1	0.0807	P
2	0.1521	DP
3	0.3034	DEP
4	0.4448	GHJL
5	0.4753	GHJLP
6	0.4851	GHJLOP
7	0.5093	FGHJLNP
8	0.6532	BCEGHJOQ
9	0.7020	BCEGHJOQR
10	0.7271	BCEGHJNOQR

Model Size	R^2	Coded Variables
1	0.0807	U
2	0.1603	CU
3	0.3181	CJU
4	0.3884	CJMU
5	0.4576	CJMQU
6	0.5170	CDEJMU
7	0.5723	ACDEJMU
8	0.6142	ACDEJMQU
9	0.7483	CDGHLMTVX
10	0.7604	CFGHLMTVWX

(6) *P.aeruginosa*
(i)Using variables: C2, C9-C13. (ii) Using variables: C2, C14-C31 (iii)Using variables: C2, C9-C31.

Model Size	R^2	Coded Variables
1	0.1143	B
2	0.1830	BV
3	0.2670	BJV
4	0.3463	FMNV
5	0.3899	EMNUW
6	0.4040	EJNRUV
7	0.4633	CIKNRUW
8	0.4846	IKNRSTUX
9	0.5220	CIKNRSTUX
10	0.5293	CFIKNRSTU

Model Size	R^2	Coded Variables
1	0.1758	E
2	0.2032	AF
3	0.2085	ACD
4	0.2144	ACDE
5	0.2181	ACDEF
6	0.2192	ABCDEF

Model Size	R^2	Coded Variables
1	0.1031	Q
2	0.1227	QS
3	0.1395	AQS
4	0.3097	DFIM
5	0.3603	DFIMQ
6	0.4273	DFIMPR
7	0.4579	DFIMNPR
8	0.4846	DFIMNOPS
9	0.4978	CDFIMNOPS
10	0.5124	CDHIJMNOPS

(2). Correlation matrix for the parameters of most appropriate model for modeling antibacterial activity.

B.subtilis

	C2	C3	C17	C20	C24	C27	C28	C30	C32
C2	1.0000	0.4219	0.4146	0.4270	0.3689	-0.0481	-0.1762	-0.1112	0.2509
C3	0.4219	1.0000	0.9726	0.9745	0.9061	0.5369	0.2669	0.4923	0.1334
C17	0.4146	0.9726	1.0000	0.9988	0.9153	0.4974	0.2964	0.4745	0.0777
C20	0.4270	0.9745	0.9988	1.0000	0.9111	0.5021	0.2772	0.4702	0.0918
C24	0.3689	0.9061	0.9153	0.9111	1.00001	0.4814	0.2737	0.44`0	0.1228
C27	-0.0481	0.5369	0.4974	0.5021	0.4814	1.0000	0.2610	0.8064	0.1670
C28	-0.1762	0.2669	0.2964	0.2772	0.2737	0.2610	1.0000	0.1793	-0.0432
C30	-0.1112	0.4923	0.4745	0.4702	0.4410	0.8064	0.1793	1.0000	0.1906
C32	0.2509	0.1334	0.0777	0.0918	0.1228	0.1670	-0.0432	0.1906	1.0000

S.typhi

	C2	C16	C21	C24	C25	C26	C27	C28	C33
C2	1.0000	0.3080	0.3315	0.3688	0.3297	0.2483	-0.0481	-0.1762	-0.1436
C16	0.3080	1.0000	0.9937	0.8849	0.9807	0.6253	0.5789	0.3521	0.6072
C21	0.3315	0.9937	1.0000	0.9011	0.9943	0.6212	0.5378	0.3518	0.5575
C24	0.3688	0.8849	0.9011	1.0000	0.9023	0.4646	0.4813	0.2736	0.5252
C25	0.3297	0.9807	0.9943	0.9023	1.0000	0.6412	0.5009	0.3536	0.5323
C26	0.2483	0.6253	0.6212	0.4646	0.6412	1.0000	0.1160	0.1982	0.2289
C27	-0.0481	0.5789	0.5378	0.4813	0.5009	0.1160	1.0000	0.2609	0.3838
C28	-0.1762	0.3521	0.3518	0.2736	0.3536	0.1982	0.2609	1.0000	0.4722
C33	-0.1436	0.6072	0.5575	0.5252	0.5323	0.2289	0.3838	0.4722	1.0000

E.coli

	C2	**C9**	**C11**	**C12**	**C15**	**C18**	**C28**	**C29**	**C34**
C2	1.0000	0.6249	0.4232	0.4233	0.3286	0.4322	-0.1762	-0.1071	-0.4132
C9	0.6249	1.0000	0.7893	0.7895	0.6337	0.7448	-0.0007	0.1882	-0.0267
C11	0.4232	0.7893	1.0000	1.0000	0.9628	0.9900	0.2663	0.4913	0.2523
C12	0.4233	0.7895	1.0000	1.0000	0.9627	0.9899	0.2662	0.4911	0.2523
C15	0.3286	0.6337	0.9628	0.9627	1.0000	0.9791	0.3389	0.5314	0.2559
C18	0.4322	0.7448	0.9900	0.9899	0.9791	1.0000	0.2765	0.4800	0.2080
C28	-0.1761	-0.0006	0.2663	0.2662	0.3389	0.2765	1.0000	0.1830	0.5276
C29	-0.1071	0.1882	0.4913	0.4911	0.5314	0.4800	0.1830	1.0000	0.0675
C34	-0.4132	-0.0267	0.2523	0.2523	0.2559	0.2080	0.9791	1.0000	0.9791

S.aureus

	C2	**C16**	**C17**	**C20**	**C22**	**C24**	**C28**	**C31**	**C35**
C2	1.0000	0.3080	0.4145	0.4269	0.4237	0.3688	-0.1762	-0.0529	0.1437
C16	0.3080	1.0000	0.9520	0.9467	0.9796	0.8849	0.3521	0.3764	0.3216
C17	0.4145	0.9520	1.0000	0.9987	0.9729	0.9153	0.2963	0.2350	0.2868
C20	0.4269	0.9467	0.9987	1.0000	0.9745	0.9110	0.2772	0.2215	0.2912
C22	0.4237	0.9796	0.9729	0.9745	1.0000	0.8961	0.2782	0.2603	0.2652
C24	0.3688	0.8849	0.9153	0.9110	0.8961	1.0000	0.2736	0.1839	0.2162
C28	-0.1761	0.3521	0.2963	0.2772	0.2782	0.2736	1.0000	0.0927	-0.1530
C31	-0.0529	0.3764	0.2350	0.2215	0.2603	0.1839	0.0927	1.0000	0.3186
C35	0.1430	0.3216	0.2868	0.2912	0.2652	0.2162	-0.1530	0.3182	1.0000

K.pneumoniae

	C2	**C10**	**C11**	**C12**	**C17**	**C20**	**C24**	**C28**	**C36**
C2	1.0000	0.2764	0.4232	0.4233	0.4145	0.4269	0.3688	-0.1762	0.1127
C10	0.2768	1.0000	0.9609	0.9608	0.9426	0.9346	0.8934	0.3878	0.1498
C11	0.4232	0.9609	1.0000	1.0000	0.9727	0.9746	0.9063	0.2663	0.1297
C12	0.4233	0.9608	1.0000	1.0000	0.9727	0.9746	0.9063	0.2662	0.1297
C17	0.4145	0.9426	0.9727	0.9727	1.0000	0.9987	0.9153	0.2963	0.0399
C20	0.4269	0.9346	0.9746	0.9746	0.9987	1.0000	0.9110	0.2772	0.0504
C24	0.3688	0.8934	0.9063	0.9063	0.9153	0.9110	1.0000	0.2736	0.1732
C28	-0.1761	0.3878	0.2663	0.2662	0.2963	0.2772	0.2736	1.0000	-0.2846
C36	0.1124	0.1498	0.1297	0.1297	0.0399	0.0504	0.1732	-0.2846	1.0000

P.aeruginosa

	C16	**C18**	**C21**	**C25**	**C26**	**C27**	**C28**	**C31**	**C37**
C16	1.0000	0.9818	0.9937	0.9807	0.6253	0.5789	0.3521	0.3764	0.2268
C18	0.9818	1.0000	0.9875	0.9768	0.5828	0.5147	0.2765	0.2871	0.2197
C21	0.9937	0.9875	1.0000	0.9943	0.6212	0.5378	0.3518	0.3461	0.1836
C25	0.9807	0.9768	0.9943	1.0000	0.6412	0.5009	0.3536	0.3479	0.1675
C26	0.6253	0.5828	0.6212	0.6412	1.0000	0.1160	0.1982	0.7700	0.0159
C27	0.5789	0.5147	0.5378	0.5009	0.1160	1.0000	0.2609	0.3795	0.3184
C28	0.3521	0.2765	0.3518	0.3536	0.1982	0.2609	1.0000	0.0927	0.1042
C31	0.3764	0.2871	0.3461	0.3479	0.7700	0.3795	0.0927	1.0000	0.1169
C37	0.2268	0.2197	0.1836	0.1675	0.0159	0.3184	0.1042	0.1169	1.0000

(3) Ridge regression parameters: VIF, Tolerance, Eigen values, Condition number for the most appropriate model for modeling antibacterial activity.

B.subtilis S.typhi

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C2	1.3667	0.6783	4.806702	1.00
C16	24.7792	0.0048	1.288230	3.73
C21	19.6221	0.0016	0.831546	5.78
C24	5.7580	0.1558	0.588309	8.17
C25	25.3768	0.0050	0.361899	13.28
C26	2.0528	0.3649	0.108315	44.38
C27	1.9469	0.4307	0.013966	344.16

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C2	1.6190	0.5921	4.758598	1.00
C3	16.5952	0.0446	1.540658	3.09
C17	16.0823	0.0015	0.930304	5.12
C20	15.8720	0.0015	0.432212	11.01
C24	5.7972	0.1464	0.185510	25.65
C27	3.0755	0.2633	0.119560	39.80
C24	10.2254	0.0701	0.002257	3148.50
C25	17.6452	0.0366	0.000001	6525191.27

E.coli S.aureus

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C2	1.5640	0.4156	5.131911	1.00
C16	25.2418	0.0063	1.242010	4.13
C17	14.0883	0.0009	0.923066	5.56
C20	11.8939	0.0008	0.510967	10.04
C22	28.7419	0.0049	0.131963	38.89
C24	5.8384	0.1495	0.053840	95.32
C28	1.3123	0.6546	0.005835	879.56

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C2	1.9409	0.4508	5.092069	1.00
C9	5.7861	0.1059	1.466185	3.47
C11	11.1715	0.0000	0.802052	6.35
C12	11.2217	0.0000	0.366760	13.88
C15	25.4340	0.0181	0.251692	20.23
C18	36.8436	0.0071	0.015330	332.15
C28	1.2886	0.7392	0.005911	861.46
C29	1.6359	0.5153	0.000000	91622650.97

K.pneumoniae
P.aeruginosa

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C16	31.4770	0.0048	5.073812	1.00
C18	29.4035	0.0127	1.206077	4.21
C21	19.4545	0.0012	0.859504	5.90
C25	24.2164	0.0046	0.788952	6.43
C26	9.4800	0.0727	0.043209	117.42
C27	4.1585	0.1756	0.018254	277.95
C28	1.3646	0.5851	0.009341	543.20

Variable	VIF	Tolerance	Eigenvalue	Condition Number
C2	1.5844	0.5515	6.019250	1.00
C10	16.2188	0.0165	1.196181	5.03
C11	7.0243	0.0000	0.552880	10.89
C12	7.0662	0.0000	0.134921	44.61
C17	13.4693	0.0010	0.069433	86.69
C20	12.6196	0.0010	0.026828	224.36
C24	6.0028	0.1428	0.000506	11896.45
C28	1.3852	0.6056	0.000000	94674753.04