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Original article

Hansch analysis of veratric acid derivatives as antimicrobial agents

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Abstract

The synthesis, characterization and antimicrobial evaluation of a new series of veratric acid derivatives are presented. Preliminary in vitro antimicrobial activity of the title compounds was assessed against a panel of microorganisms including Gram-positive and Gram-negative bacteria and fungi. Some of the veratric acid derivatives exhibited significant in vitro antimicrobial activity. QSAR investigation applied to find a correlation between different physicochemical parameters of the veratric acid derivatives and their antimicrobial activity indicated the importance of topological parameters in describing the antimicrobial activity.

Keywords: Veratric acid; Antibacterial activity; Antifungal activity; QSAR

1. Introduction

Microbial infections are associated with rates of attributable morbidity and mortality [1]. The resistance of common pathogens to standard antibiotic therapies is rapidly becoming a major public health problem throughout the world. The incidence of multi-drug resistant Gram-positive and Gram-negative bacteria is increasing and infections caused by them are becoming problematic now-a-days [2]. There is an urgent need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms distinct from those of well known classes of antimicrobial agents to which the clinically relevant pathogens are now resistant [3].

Veratric acid isolated from the stem bark of *Tabebuia impetiginosa* [4] have been reported to have antibacterial [5], antifungal [6], antioxidant [7], anti-inflammatory [8] and antispasmodic activities [9].

Quantitative structure activity relationship (QSAR), one of the most important areas in chemistry, gives information that is useful for drug design and medicinal chemistry. QSAR models are mathematical equations relating chemical structure to a wide variety of physical, chemical and biological properties. The derived relationship between molecular descriptors and activity is used to estimate the property of other molecules and/or to find the parameters affecting the biological activity [10].

We have previously reported the synthesis, antimicrobial evaluation and QSAR studies of some simple organic acid derivatives as possible antimicrobial agents [11–16] as a part of our composite programme on rational drug design [11–23]. In view of the above in the present work we have decided to synthesize and evaluate the antimicrobial activity of veratric acid derivatives. Further, we have decided to carry out the QSAR studies to perceive the importance of molecular properties, which are critical in accentuating the antimicrobial activity of veratric acid derivatives.

2. Chemistry

Synthetic route to compounds **2**–**17** and **18**–**41** is shown in Schemes 1 and 2, respectively. Esters of veratric acid (**2**–**14** and **16**) were synthesized by its reaction with corresponding alcohol in the presence of sulphuric acid. Phenyl veratrate (**15**) and 8-quinolinyl veratrate (**17**) were synthesized by the reaction of phenol and 8-hydroxyquinoline with acid chloride of veratric acid, which is prepared by the reaction of veratric acid with thionyl chloride (Scheme 1). Amides and anilides (**18**–**41**) were prepared by the reaction of veratryl chloride

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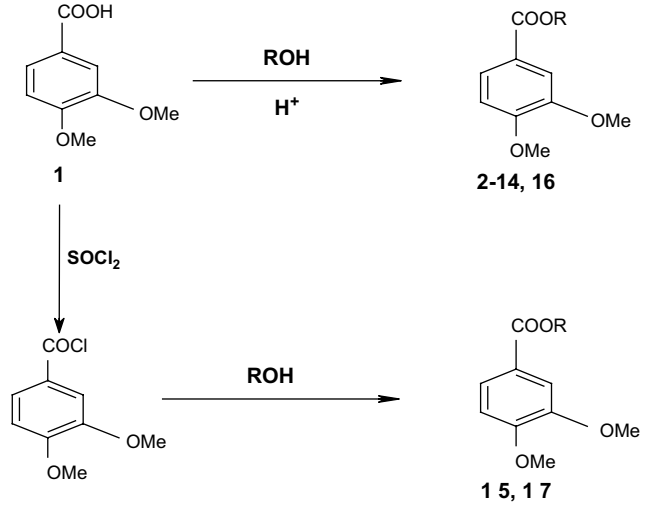
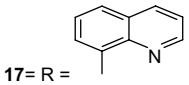
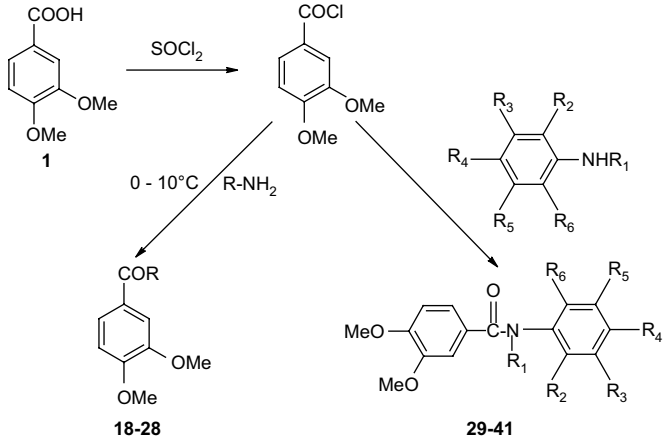


2= R = CH_3; **3**= R = C_2H_5; **4**= R = C_3H_7; **5**= R = $(CH_3)_2CH$;
6= R = CH_2=$CHCH_2$; **7**= R = C_4H_9;
8= R = $(CH_3)_2CHCH_2$; **9**= R = $CH_3CH(CH_3)CH_2$; **10**= R = $(CH_3)_3C$;
11= R = C_5H_{11}; **12**= R = $(CH_3)_2CHCH_2CH_2$; **13**= R = $CH_3(CH_2)_4CH_2$;
14= R = $CH_3(CH_2)_5CH_2$; **15**= R = C_6H_5;
16= R = $C_6H_5CH_2$



Scheme 1. Synthetic route for esters of veratric acid.



18 = R = NH_2
19 = R = CH_3NH
20 = R = $(CH_3)_2N$
21 = R = C_2H_5NH
22 = R = C_3H_7NH
23 = R = HOC_2H_4NH
24 = R = $(OHC_2H_4)_2N$
25 = R = $C_6H_5CH_2NH$
26 = R = C_4H_8NO
27 = R = $C_5H_{10}N$
28 = R = NH_2NH

29 = R_1, R_2, R_3, R_4, R_5, R_6 = H
30 = R_1, R_3, R_4, R_5, R_6 = H, R_2 = Cl
31 = R_1, R_2, R_4, R_5, R_6 = H, R_3 = Cl
32 = R_1, R_2, R_3, R_5, R_6 = H, R_4 = Cl
33 = R_1, R_3, R_4, R_5, R_6 = H, R_2 = CH_3
34 = R_1, R_2, R_4, R_5, R_6 = H, R_3 = CH_3
35 = R_1, R_2, R_3, R_5, R_6 = H, R_4 = CH_3
36 = R_1, R_3, R_4, R_5, R_6 = H, R_2 = OCH_3
37 = R_1, R_2, R_3, R_5, R_6 = H, R_4 = OCH_3
38 = R_1, R_2, R_4, R_5, R_6 = H, R_3 = NO_2
39 = R_1, R_2, R_3, R_5, R_6 = H, R_4 = NO_2
40 = R_1, R_2, R_3, R_5, R_6 = H, R_4 = Br
41 = R_1, R_2, R_4, R_5, R_6 = H, R_3 = OH

Scheme 2. Synthetic route for amides/anilides of veratric acid.

with corresponding amines/anilines (Scheme 2). The purity of compounds was checked by single-spot thin layer chromatography on silica gel G. The physicochemical properties of synthesized compounds are presented in Table 1 and the spectroanalytical data are presented in Table 2.

The formation of esters was confirmed in general by the appearance of IR bands (cm^{-1}) in the range of 3075–3085 (CH str., aromatic), 2940–2970 (CH str., aliphatic), 2825–2840 (CH str., OCH_3), 1695–1715 (C=O str., ester) and 1510–1520 (C=C skeletal str., aromatic). Further, the IR spectra of compound **6** (allyl ester) showed IR peaks at 1648 (C=C str., RCH=CH_2) and 3004 cm^{-1} (CH str., RCH=CH_2) in addition to the aforementioned general peaks for esters of veratric acid which confirmed its formation. The IR spectra of secondary amides of veratric acid (**19–41** except **20**, **24**, **26**

Table 1
Physicochemical characteristics of veratric acid derivatives

Compound	Molecular formula	M.wt.	M.P. (°C)	R_f value	Yield (%)
1	$C_9H_{10}O_4$	182	176–178	0.15	40.41
2	$C_{10}H_{12}O_4$	196	48–50	0.41	33.52
3	$C_{11}H_{14}O_4$	210	34–36	0.43	30.20
4	$C_{12}H_{16}O_4$	224	55–57	0.67	32.40
5	$C_{12}H_{16}O_4$	224	150–152	0.28	64.38
6	$C_{12}H_{14}O_4$	222	35–37	0.65	58.32
7	$C_{13}H_{18}O_4$	238	152–154	0.63	42.17
8	$C_{13}H_{18}O_4$	238	110–112	0.52	36.21
9	$C_{13}H_{18}O_4$	238	100–102	0.61	66.80
10	$C_{13}H_{18}O_4$	238	170–172	0.28	45.86
11	$C_{14}H_{20}O_4$	252	38–40	0.79	52.12
12	$C_{14}H_{20}O_4$	252	160–162	0.75	21.31
13	$C_{15}H_{22}O_4$	266	144–148	0.67	65.99
14	$C_{16}H_{24}O_4$	280	25–27	0.58	59.45
15	$C_{15}H_{14}O_4$	258	120–122	0.64	54.87
16	$C_{16}H_{16}O_4$	272	42–44	0.86	73.32
17	$C_{18}H_{15}O_4N$	309	70–72	0.71	45.89
18	$C_9H_{11}O_3N$	181	151–153	0.43	80.80
19	$C_{10}H_{13}O_3N$	195	158–160	0.52	79.02
20	$C_{11}H_{15}O_3N$	209	159–161	0.11[a]	70.13
21	$C_{11}H_{15}O_3N$	209	160–162	0.25	65.09
22	$C_{12}H_{17}O_3N$	223	140–142	0.16	81.32
23	$C_{11}H_{15}O_4N$	225	40–42	0.32	67.88
24	$C_{13}H_{19}O_5N$	269	44–46	0.25	72.35
25	$C_{16}H_{17}O_3N$	271	111–113	0.27	78.67
26	$C_{13}H_{17}O_4N$	251	38–40	0.07	54.50
27	$C_{14}H_{19}O_3N$	249	35–37	0.13	46.31
28	$C_9H_{12}O_3N_2$	196	150–152	0.31	40.44
29	$C_{15}H_{15}O_3N$	257	160–162	0.31	47.89
30	$C_{15}H_{14}O_3NCl$	292	116–118	0.40	50.67
31	$C_{15}H_{14}O_3NCl$	292	105–107	0.34	70.56
32	$C_{15}H_{14}O_3NCl$	292	200–202	0.26	88.67
33	$C_{16}H_{17}O_3N$	271	150–152	0.18	79.09
34	$C_{16}H_{17}O_3N$	271	80–82	0.16	76.77
35	$C_{16}H_{17}O_3N$	271	150–152	0.13	69.87
36	$C_{16}H_{17}O_4N$	287	70–72	0.20[a]	72.47
37	$C_{16}H_{17}O_4N$	287	170–172	0.38[b]	76.03
38	$C_{15}H_{14}O_5N_2$	302	163–165	0.28[b]	65.68
39	$C_{15}H_{14}O_5N_2$	302	120–122	0.30	56.89
40	$C_{15}H_{14}O_3NBr$	336	194–196	0.25[a]	62.45
41	$C_{15}H_{15}O_4N$	273	194–196	0.40	69.01

TLC mobile phase – toluene:chloroform (7:3).
[a] Benzene.
[b] Ethyl acetate:benzene (1:3).

Table 2
Spectroanalytical data of synthesized veratric acid derivatives

Compound	IR, ν (cm^{-1})	^1H NMR (δ, ppm)	Elemental analysis, Calc (found)
2	3086 (CH str., aromatic), 2841 (CH str., OCH_3), 1721 (C=O str., ester), 1518 (C=C str., aromatic)	6.87–7.69 (m, 3H, Ar H), 3.93 (s, 6H, 2 (OCH_3)), 3.89 (s, 3H, $COOCH_3$)	$C_{10}H_{12}O_4$: C, 61.22 (60.87); H, 6.16 (6.24); O, 32.62 (36.41)
3	3085 (CH str., aromatic), 2838 (CH str., OCH_3), 1696 (C=O str., ester), 1517 (C=C str., aromatic), 2968 (C–H str., aliphatic)	3.95 (s, 6H, OCH_3), 1.37–1.40 (t, 3H, CH_3), 4.33–4.38 (q, 2H, CH_2 of CH_2CH_3), 6.87–7.76 (m, 3H, ArH)	$C_{11}H_{14}O_4$: C, 62.85 (61.96); H, 6.71 (6.76); O, 30.44 (30.02)
4	3085 (CH str., aromatic), 2838 (CH str., OCH_3), 1709 (C=O str., ester), 1515 (C=C str., aromatic), 2965 (C–H str., aliphatic)	0.89–0.93 (t, 3H, CH_3 of CH_2CH_3), 1.76–1.81 (m, 2H, CH_2 of CH_2CH_3), 3.94 (s, 6H, OCH_3), 4.24–4.28 (t, 2H, CH_2 of OCH_2), 6.87–7.70 (m, 3H, ArH)	$C_{12}H_{16}O_4$: C, 64.27 (64.28); H, 7.19 (7.00); O, 28.54 (29.04)
5	3009.7 (CH str., aromatic), 2938 (CH str., aliphatic), 2836 (CH str., OCH_3), 1678 (C=O str., ester), 1516 (C=C str., aromatic)	1.35–1.37 (d, 6H, CH_3 of $CH(CH_3)_2$), 3.90–3.96 (s, 6H, OCH_3), 5.22–5.25 (m, 1H, CH of $CH(CH_3)_2$), 6.86–6.93 (m, 3H, ArH)	$C_{12}H_{16}O_4$: C, 64.27 (64.31); H, 7.19 (7.23); O, 28.54 (28.89)
6	3085 (CH str., aromatic), 2839 (CH str., OCH_3), 1715 (C=O str., ester), 1515 (C=C str., aromatic), 2938 (C–H str., aliphatic), 3004 (CH str., of R–CH=CH_2), 1648 (C=C str. of R–CH=CH_2)	3.94 (s, 6H, OCH_3), 4.79–4.81 (d, 2H, CH_2 adjacent to COO), 5.29–5.30, 5.37–5.38 (dd, 2H, CH_2 of CH=CH_2, $J_{cis} = 4$ Hz), 5.99–6.07 (m, 1H, CH of CH=CH_2), 6.87–7.72 (m, 33H, ArH)	$C_{12}H_{14}O_4$: C, 64.85 (64.81); H, 6.35 (6.29); O, 28.80 (29.20)
7	3084 (CH str., aromatic), 2873 (CH str., OCH_3), 1713 (C=O str., ester), 1515 (C=C str., aromatic), 2959 (C–H str., aliphatic)	0.93–0.95 (t, 3H, CH_3 of butyl), 1.41–1.52 (m, 2H, CH_2 of C_3 of butyl), 1.70–1.78 (m, 2H, CH_2 of C_2 of butyl), 3.94 (s, 6H, OCH_3), 4.29–4.32 (t, 2H, CH_2 of C_1 of butyl), 6.87–7.76 (m, 3H, ArH)	$C_{13}H_{18}O_4$: C, 65.53 (65.47); H, 7.61 (7.65); O, 26.86 (26.92)
8	3085 (CH str., aromatic), 2838 (CH str., OCH_3), 1705 (C=O str., ester), 1513 (C=C str., aromatic), 2967 (C–H str., aliphatic)	0.95–0.99 (t, 3H, CH_3 of $CHCH_2CH_3$), 1.32–1.34 (t, 3H, CH_3 of $CHCH_3$), 1.64–1.76 (m, 2H, CH_2 of $CHCH_2CH_3$), 3.93–3.95 (s, 6H, OCH_3), 5.05–5.10 (m, 1H, CH of $CHCH_3$), 6.87–7.28 (m, 3H, ArH)	$C_{13}H_{18}O_4$: C, 65.53 (65.51); H, 7.61 (7.63); O, 26.86 (26.88)
9.	3085 (CH str., aromatic), 2839 (CH str., OCH_3), 1710 (C=O str., ester), 1514 (C=C str., aromatic), 2961 (C–H str., aliphatic)	0.98–1.03 (d, 6H, CH_3), 2.04–2.11 (m, 1H, CH of $CH(CH_3)_2$), 3.94 (s, 6H, OCH_3), 4.07–4.09 (d, 2H, CH_2 adjacent to COO), 6.87–7.70 (m, 3H, ArH)	$C_{13}H_{18}O_4$: C, 65.53 (65.42); H, 7.61 (7.82); O, 26.86 (26.94)
10	3003 (CH str., aromatic), 2836 (CH str., OCH_3), 1677 (C=O str., ester), 1517 (C=C str., aromatic), 2964 (C–H str., aliphatic)	3.95 (s, 6H, OCH_3), 6.91–7.80 (m, 3H, ArH), 1.3 (s, 9H, CH_3 of *tert*-butyl)	$C_{13}H_{18}O_4$: C, 65.53 (64.98); H, 7.61 (7.34); O, 26.86 (26.48)
11	3085 (CH str., aromatic), 2871 (CH str., OCH_3), 1713 (C=O str., ester), 1515 (C=C str., aromatic), 2957 (C–H str., aliphatic)	0.93–0.95 (t, 3H, CH_3 of amyl), 1.44–1.50 (m, 2H, CH_2 of C_3 of amyl), 1.63–1.80 (m, 2H, CH_2 of C_4 of amyl), 2.16–2.18 (m, 2H, CH_2 of C_2 of amyl), 3.93 (s, 6H, OCH_3), 4.31–4.35 (t, 2H, CH_2 of C_1 of amyl), 6.87–7.69 (m, 3H, ArH)	$C_{14}H_{20}O_4$: C, 66.65 (66.27); H, 7.99 (7.72); O, 25.37 (25.21)
12	2836 (CH str., OCH_3), 1691 (C=O str., ester), 1513 (C=C str., aromatic), 2954 (C–H str., aliphatic)	0.91–0.93 (d, 6H, CH_3), 1.47–1.53 (m, 2H, CH_2 of C_2 of isoamyl), 1.63–1.75 (m, 1H, CH of C_3 of isoamyl), 3.92 (s, 6H, OCH_3), 4.31–4.35 (t, 2H, CH_2 of C_1 of isoamyl), 6.87–7.68 (m, 3H, ArH)	$C_{14}H_{20}O_4$: C, 66.65 (66.43); H, 7.99 (7.81); O, 25.37 (25.32)
13	3084 (CH str., aromatic), 2857 (CH str., OCH_3), 1713 (C=O str., ester), 1515 (C=C str., aromatic), 2931 (C–H str., aliphatic)	0.86–0.90 (t, 3H, CH_3 of hexyl), 1.26–1.32 (m, 4H, CH_2 of C_3 and C_4 of hexyl), 1.72–1.77 (m, 2H, CH_2 of C_2 of hexyl), 3.92 (s, 6H, OCH_3), 4.27–4.31 (t, 2H, CH_2 of C_1 of hexyl), 6.87–7.69 (m, 3H, ArH)	$C_{15}H_{22}O_4$: C, 67.64 (67.62); H, 8.33 (8.27); O, 24.03 (24.42)
14	3084 (CH str., aromatic), 2855 (CH str., OCH_3), 1713 (C=O str., ester), 1515 (C=C str., aromatic), 2933 (C–H str., aliphatic)	0.90 (t, 3H, CH_3), 1.26–1.29 (m, 2H, CH_2 adjacent to terminal CH_3), 1.55–1.60 (m, 6H, CH_2 of C_3–C_5 of heptyl), 1.65–1.78 (m, 2H, CH_2 of C_2 of heptyl), 3.91 (s, 6H, OCH_3), 4.27–4.30 (t, 2H, CH_2 of C_1 of heptyl), 6.85–7.68 (m, 3H, ArH)	$C_{16}H_{24}O_4$: C, 68.54 (68.29); H, 8.63 (8.57); O, 22.83 (22.63)
15	3010 (CH str., aromatic), 2977 (CH str., aliphatic), 2842 (CH str., OCH_3), 1727 (C=O str., ester), 1512 (C=C str., aromatic)	6.94–7.40 (m, 8H, Ar H), 3.96 (s, 6H, OCH_3)	$C_{15}H_{14}O_4$: C, 69.76 (69.72); H, 5.46 (5.43); O, 24.78 (24.85)
16	3082 (CH str., aromatic), 2846 (CH str., OCH_3), 1703 (C=O str., ester), 1510 (C=C str., aromatic), 2910 (C–H str., aliphatic)	3.86 (s, 6H, OCH_3), 4.50 (s, 2H, CH_2), 6.63–7.23 (m, 3H, ArH of veratric acid), 7.30–7.37 (m, 5H, ArH of benzyl alcohol)	$C_{16}H_{16}O_4$: C, 70.57 (71.12); H, 5.92 (5.85); O, 23.50 (23.98)

(*continued on next page*)

Table 2 (*continued*)

Compound	IR, ν (cm^{-1})	^1H NMR (δ, ppm)	Elemental analysis, Calc (found)
17	3006 (CH str., aromatic), 2839 (CH str., OCH_3), 1728 (C=O str., ester), 1516 (C=C str., aromatic), 1433 (C–N str., aromatic)	6.92–7.32 (m, 9H, Ar H), 3.95–3.98 (s, 6H, OCH_3)	$C_{18}H_{15}NO_4$: C, 69.89 (69.87); H, 4.89 (4.87); O, 20.69 (20.74); N, 4.53 (4.61)
18	3440 (NH str., 1° amide), 3084 (CH str., aromatic), 2836 (CH str., OCH_3), 1690 (C=O str., 1° amide), 1516 (C=C str., aromatic)	3.92 (s, 6H, OCH_3), 6.70–7.55 (m, 3H, ArH), 7.85 (s, 2H, NH_2)	$C_9H_{11}NO_3$: C, 59.66 (59.62); H, 6.12 (6.23); O, 26.49 (26.57); N, 7.73 (7.79)
19	3445 (NH str., 2° amide), 3084 (CH str., aromatic), 2830 (CH str., OCH_3), 1675 (C=O str., amide), 1514 (C=C str., aromatic)	2.87 (s, 3H, CH_3), 3.89 (s, 6H, OCH_3), 6.70–7.52 (m, 3H, ArH), 7.92 (s, 2H, NH_2)	$C_{10}H_{13}NO_3$: C, 61.53 (61.62); H, 6.71 (6.78); O, 24.59 (24.67); N, 7.18 (7.25)
20	3447 (NH str., 2° amide), 3089 (CH str., aromatic), 2836 (CH str., OCH_3), 1677 (C=O str., amide), 1516 (C=C str., aromatic)	2.10 (s, 6H, CH_3), 3.92–3.96 (s, 6H, OCH_3), 6.91–7.79 (m, 3H, ArH)	$C_{11}H_{15}NO_3$: C, 63.14 (63.57); H, 7.23 (7.42); O, 22.94 (23.21); N, 6.69 (6.41)
21	3442 (NH str., 2° amide), 3085 (CH str., aromatic), 2834 (CH str., OCH_3), 1673 (C=O str., amide), 1518 (C=C str., aromatic)	1.46–149 (t, 3H, CH_3), 3.42–3.45 (m, 2H, CH_2 of CH_2CH_3), 3.94 (s, 6H, OCH_3), 6.78–7.70 (m, 3H, ArH), 8.15 (s, 1H, NH)	$C_{11}H_{15}NO_3$: C, 63.14 (62.87); H, 7.23 (7.86); O, 22.94 (23.41); N, 6.69 (6.62)
22	3302 (NH str., amide), 3100 (CH str., aromatic), 2935 (CH str, aliphatic), 2837 (CH str., OCH_3), 1678 (C=O str., amide), 1514 (C=C str., aromatic)	6.85–7.79 (m, 3H, Ar H), 3.92–3.96 (s, 6H, OCH_3), 3.40–3.45 (m, 2H, CH_2 adjacent of CH_2CH_3), 1.62–1.67 (m, 2H, CH_3 of CH_2CH_3), 0.97–1.01 (t, 3H, CH_3 of CH_2CH_3)	$C_{12}H_{17}NO_3$: C, 64.55 (65.21); H, 7.67 (7.92); O, 21.50 (22.01); N, 6.27 (6.30)
23	3396 (NH str., 2° amide), 3080 (CH str., aromatic), 2840 (CH str., OCH_3), 1621 (C=O str., amide), 1505 (C=C str., aromatic)	3.56–3.60 (t, 2H, CH_2 of $NHCH_2$), 3.79–3.82 (t, 2H, CH_2 of CH_2OH), 8.4 (s, 1H, NH), 3.85 (s, 6H, OCH_3), 6.75–7.41 (m, 3H, ArH)	$C_{11}H_{15}NO_4$: C, 58.66 (58.25); H, 6.71 (6.43); O, 28.41 (28.21); N, 6.22 (6.15)
24	3367 (NH str., 3° amide), 2835 (CH str., OCH_3), 1602 (C=O str., amide), 1516 (C=C str., aromatic)	1.25 (s, 2H, OH), 3.15–3.30 (t, 4H, CH_2 of NCH_2), 3.65–3.75 (m, 4H, CH_2 of CH_2OH), 3.92 (s, 6H, OCH_3), 6.80–7.61 (m, 3H, ArH)	$C_{13}H_{19}NO_5$: C, 57.98 (57.87); H, 7.11 (7.24); O, 29.71 (29.48); N, 5.20 (5.20)
25	3294 (NH str., 2° amide), 3011 (CH str., aromatic), 2835 (CH str., OCH_3), 1632 (C=O str., amide), 1511 (C=C str., aromatic)	3.88–3.89 (s, 6H, OCH_3), 4.59–4.61 (d, 2H, CH_2 of benzyl), 6.60–7.33 (m, 8H, ArH), 7.45 (s, 1H, NH)	$C_{16}H_{17}NO_3$: C, 70.74 (70.81); H, 6.26 (6.15); O, 17.68 (17.80); N, 5.15 (5.25)
26	3435 (NH str., 3° amide), 2964 (CH str., aliphatic), 2856 (CH str., OCH_3), 1621 (C=O str., amide), 1515 (C=C str., aromatic) 3082 (CH str., aromatic), 2776 (C–O–C str., morpholine)	3.64–3.70 (t, 4H, CH_2 of C_2 and C_6 of morpholine), 3.20 (t, 4H, CH_2, C_3 and C_5 of morpholine), 3.90 (s, 6H, OCH_3), 6.86–7.01 (m, 3H, ArH)	$C_{13}H_{17}NO_4$: C, 62.14 (62.19); H, 6.82 (6.91); O, 25.47 (25.49); N, 5.57 (5.61)
27	3429 (NH str., 3° amide), 3001 (CH str., aromatic), 2856 (CH str., OCH_3), 1683 (C=O str., amide), 1519 (C=C str., aromatic), 1462 (CH_2 str., piperidine)	1.60–1.70 (m, 6H, CH_2 of C_3–C_5 of piperidine), 3.10 (t, 4H, CH_2 of C_1 and C_6 of piperidine), 3.92 (s, 6H, OCH_3), 6.85–7.30 (m, 3H, ArH)	$C_{14}H_{19}NO_3$: C, 67.74 (67.75); H, 7.62 (7.42); O, 19.29 (19.41); N, 5.61 (5.69)
28	3210 (NH str., 2° amide), 3050 (CH str., aromatic), 2837 (CH str., OCH_3), 1677 (C=O str., amide), 1514 (C=C str., aromatic)	7.7 (s, 1H, NH), 7.5–7.7 (m, 3H, Ar H), 3.85–3.93 (s, 6H, OCH_3)	$C_9H_{12}N_2O_3$: C, 55.05 (55.91); H, 6.16 (6.12); O, 24.46 (24.42); N, 14.28 (14.31)
29	3315 (NH str., 2° amide), 2937 (CH str., aromatic), 2838 (CH str., OCH_3), 1647 (C=O str., amide), 1510 (C=C str., aromatic)	3.94 (s, 6H, OCH_3), 6.89–7.62 (m, 8H, ArH), 7.64 (s, 1H, NH)	$C_{15}H_{15}NO_3$: C, 70.02 (70.17); H, 5.88 (5.92); O, 18.66 (18.71); N, 5.44 (5.37)
30	3280 (NH str., 2° amide), 3019 (CH str., aromatic), 2943 (CH str., aliphatic), 2839 (CH str., OCH_3), 1650 (C=O str., amide), 1512 (C=C str., aromatic), 755 (Cl str., Ar–Cl)	3.94–3.97 (s, 6H, OCH_3), 6.94–8.53 (m, 7H, ArH), 8.55 (s, 1H, NH)	$C_{15}H_{14}NO_3Cl$: C, 61.76 (61.67); H, 4.84 (4.86); O, 16.45 (16.41); N, 4.80 (4.76); Cl, 12.15 (12.21)
31	3309 (NH str., 2° amide), 3083 (CH str., aromatic), 2840 (CH str., OCH_3), 1650 (C=O str., amide), 1505 (C=C str., aromatic), 751 (Cl str., Ar–Cl)	3.92–3.94 (s, 6H, OCH_3), 6.87–7.48 (m, 7H, ArH), 7.50 (s, 1H, NH)	$C_{15}H_{14}NO_3Cl$: C, 61.76 (62.01); H, 4.84 (4.84); O, 16.45 (16.50); N, 4.80 (4.84); Cl, 12.15 (12.20)

Table 2 (*continued*)

Compound	IR, ν (cm^{-1})	^1H NMR (δ, ppm)	Elemental analysis, Calc (found)
32	3298 (NH str., 2° amide), 3011 (CH str., aromatic), 2844 (CH str., OCH_3), 1643 (C=O str., amide), 1508 (C=C str., aromatic), 768 (Cl str., Ar–Cl)	3.93–3.94 (s, 6H, OCH_3), 6.88–7.60 (m, 7H, ArH), 7.85 (s, 1H, NH)	$C_{15}H_{14}NO_3Cl$: C, 61.76 (61.72); H, 4.84 (4.83); O, 16.45 (16.31); N, 4.80 (4.76); Cl, 12.15 (12.20)
33	3447 (NH str., 2° amide), 3002 (CH str., aromatic), 2836 (CH str., OCH_3), 1677 (C=O str., amide), 1517 (C=C str., aromatic), 2964 (CH_3 str., Ar–CH_3)	2.10 (s, 3H, CH_3), 3.95–3.96 (s, 6H, OCH_3), 6.91–7.60 (m, 7H, ArH), 7.77–7.80 (s, 1H, NH)	$C_{16}H_{17}NO_3$: C, 70.83 (70.78); H, 6.32 (6.27); O, 17.69 (17.57); N, 5.16 (5.22)
34	3323 (NH str., 2° amide), 3080 (CH str., aromatic), 2833 (CH str., OCH_3), 1644 (C=O str., amide), 1515 (C=C str., aromatic), 2910 (CH_3 str., Ar–CH_3)	2.35 (s, 3H, CH_3), 3.91–3.92 (s, 6H, OCH_3), 6.85–7.49 (m, 7H, ArH), 7.89 (s, 1H, NH)	$C_{16}H_{17}NO_3$: C, 70.83 (70.89); H, 6.32 (6.41); O, 17.69 (17.75); N, 5.16 (5.19)
35	3317 (NH str., 2° amide), 3085 (CH str., aromatic), 2844 (CH str., OCH_3), 1646 (C=O str., amide), 1506 (C=C str., aromatic), 2943 (CH_3 str., Ar–CH_3)	2.33 (s, 3H, CH_3), 3.93 (s, 6H, OCH_3), 6.87–7.52 (m, 7H, ArH), 7.78 (s, 1H, NH)	$C_{16}H_{17}NO_3$: C, 70.83 (70.82); H, 6.32 (6.31); O, 17.69 (17.59); N, 5.16 (5.18)
36	3332 (NH str., 2° amide), 3089 (CH str., aromatic), 2837 (CH str., OCH_3), 1651 (C=O str., amide), 1514 (C=C str., aromatic)	8.7 (s, 1H, NH), 6.94–8.23 (m, 7H, Ar H), 3.88–3.93 (s, 9H, OCH_3)	$C_{16}H_{17}NO_4$: C, 66.89 (66.82); H, 5.96 (5.88); O, 22.27 (22.30); N, 4.88 (4.82)
37	3295 (NH str., 2° amide), 3080 (CH str., aromatic), 2839 (CH str., OCH_3), 1641 (C=O str., amide), 1513 (C=C str., aromatic)	3.81–3.93 (s, 9H, OCH_3), 6.87–7.54 (m, 7H, ArH), 7.76 (s, 1H, NH)	$C_{16}H_{17}NO_4$: C, 66.89 (66.76); H, 5.96 (5.91); O, 22.27 (22.31); N, 4.88 (5.12)
38	3327 (NH str., 2° amide), 3084 (CH str., aromatic), 2845 (CH str., OCH_3), 1652 (C=O str., amide), 1511 (C=C str., aromatic), 1528 (NO_2 str., Ar–NO_2)	3.96–3.97 (s, 6H, OCH_3), 6.92–8.11 (m, 7H, ArH), 8.47–8.48 (s, 1H, NH)	$C_{15}H_{14}N_2O_5$: C, 59.60 (59.48); H, 4.67 (4.72); O, 26.46 (26.51); N, 9.27 (9.32)
39	3325 (NH str., 2° amide), 3087 (CH str., aromatic), 2845 (CH str., OCH_3), 1650 (C=O str., amide), 1514 (C=C str., aromatic), 1523 (NO_2 str., Ar–NO_2)	3.93 (s, 6H, OCH_3), 6.91–8.15 (m, 7H, ArH), 8.45–8.46 (s, 1H, NH)	$C_{15}H_{14}N_2O_5$: C, 59.60 (58.87); H, 4.67 (4.62); O, 26.46 (26.41); N, 9.27 (9.17)
40	3295 (NH str., 2° amide), 3009 (CH str., aromatic), 2931 (CH str., aliphatic), 2846 (CH str., OCH_3), 1643 (C=O str., amide), 1507 (C=C str., aromatic)	7.85 (s, 1H, NH), 6.88–7.53 (m, 7H, Ar H, Ar–Br), 3.94 (s, 6H, OCH_3)	$C_{15}H_{14}NO_3Br$: C, 53.59 (53.56); H, 4.20 (4.21); O, 14.28 (14.15); N, 4.17 (4.17), Br, 23.77 (23.80)
41	3333 (NH str., 2° amide), 3147 (CH str., aromatic), 2838 (CH str., OCH_3), 1645 (C=O str., amide), 1510 (C=C str., aromatic), 1418 (OH str., Ar–OH)	3.89–3.97 (s, 6H, OCH_3), 4.90 (s, 1H, OH), 6.91–7.58 (m, 7H, ArH), 7.77 (s, 1H, NH)	$C_{15}H_{15}NO_4$: C, 65.92 (66.20); H, 5.53 (5.47); O, 23.42 (23.39); N, 5.13 (5.18)

and **27**) showed the characteristic secondary amide peak at 1605–1630 cm^{-1}. Similarly, primary (**18**) and tertiary amides (**20**, **24**, **26** and **27**) showed corresponding peaks at 1690 and 1630–1670 cm^{-1}, respectively.

In general, the ^1H NMR spectra of all synthesized veratric acid derivatives showed signals at δ (ppm) of 6.70–7.70 (Ar H), 3.86–3.95 (OCH_3) which indicated that the basic skeleton of veratric acid is unaffected by the applied reaction conditions. Further, the appearance of double doublet signal corresponding to CH=CH_2 of compound **6** at δ (ppm) 5.29–5.30, 5.37–5.38 with a J value of 4 indicated that the double bond is of *cis* character in nature. Further, formation of secondary amides is confirmed by the appearance of proton NMR spectra signals corresponding to NH at a δ of 7.8–8.2 ppm.

3. Microbiology

The veratric acid derivatives were evaluated for their in vitro antibacterial activity against Gram-positive *Staphylococcus aureus MTCC 1430*, *Bacillus subtilis MTCC 2423*, Gram-negative *Escherichia coli MTCC 739*, and antifungal activity against *Candida albicans MTCC 227* and *Aspergillus niger MTCC 2425* by standard serial dilution method [24] using ciprofloxacin and fluconazole as reference compounds in case of antibacterial and antifungal activities, respectively. Double strength nutrient broth – I.P. and Sabouraud dextrose broth – I.P. [25] have been employed as media for growth of bacterial and fungal cells, respectively. The antimicrobial screening has been performed in duplicate.

4. Results

4.1. Antimicrobial activity

The results of antimicrobial study are presented in Tables 3 and 4 which indicated that 8-quinolinyl veratrate, *p*-bromo veratranilide, and *m*-nitro veratranilide (**17**, **40**, and **38**) were the most active compounds. Compound **17** showed significant

Table 3
Antimicrobial activity of veratric acid derivatives (μg/ml)

Compound	MIC (μg/ml)				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	18.00	9.00	16.00	11.00	14.00
2	18.00	12.00	18.00	12.00	14.00
3	22.00	16.00	20.00	12.00	12.00
4	24.00	14.00	12.50	18.00	14.00
5	22.00	14.00	14.00	10.00	12.50
6	22.00	12.50	18.00	18.00	14.00
7	19.00	15.00	19.00	15.00	17.00
8	20.00	14.00	16.00	13.50	12.50
9	19.00	16.00	19.00	11.00	16.00
10	20.00	15.00	20.00	10.00	12.00
11	20.00	12.50	20.00	20.00	20.00
12	20.00	12.50	16.00	16.00	12.50
13	16.50	12.50	16.00	13.00	14.00
14	16.00	12.50	14.00	16.00	12.00
15	18.00	14.00	16.00	12.50	11.50
16	21.50	12.50	18.00	13.50	14.00
17	11.00	9.00	8.50	13.50	14.00
18	23.00	15.00	20.00	10.00	11.00
19	22.00	13.00	17.00	13.00	12.50
20	17.00	13.00	15.00	10.00	10.00
21	23.50	13.00	20.00	10.00	11.50
22	19.00	13.00	19.00	12.50	12.00
23	21.00	15.00	19.00	11.50	11.50
24	16.50	12.50	16.50	12.50	12.00
25	19.00	13.00	19.00	13.00	12.00
26	21.00	13.00	17.00	11.00	12.00
27	17.50	12.00	20.00	14.00	13.00
28	23.00	14.00	23.00	14.00	15.00
29	23.00	14.00	23.00	16.00	13.00
30	15.00	12.00	17.00	13.50	13.50
31	20.00	12.00	17.00	15.00	14.00
32	19.00	15.00	17.00	13.50	14.50
33	17.00	15.00	18.50	13.00	11.00
34	17.00	13.00	17.00	10.00	12.50
35	18.50	13.00	17.00	13.00	12.50
36	17.00	13.00	17.00	15.00	14.00
37	18.00	12.00	19.00	12.50	11.50
38	18.00	10.50	13.00	16.00	13.00
39	17.00	11.50	21.00	14.00	13.00
40	19.00	13.00	19.00	15.00	12.50
41	17.00	15.00	19.00	12.00	12.00

Table 4
Antimicrobial activity of veratric acid derivatives (pMIC in μM/ml)

Compound	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
1	1.01	1.31	1.06	1.22	1.11
2	1.04	1.20	1.04	1.20	1.15
3	0.98	1.12	1.02	1.23	1.23
4	0.98	1.20	1.25	1.10	1.20
5	1.01	1.20	1.20	1.35	1.25
6	1.00	1.25	1.10	1.10	1.20
7	1.10	1.20	1.10	1.20	1.15
8	1.08	1.23	1.17	1.25	1.28
9	1.10	1.17	1.10	1.34	1.17
10	1.08	1.20	1.08	1.38	1.30
11	1.10	1.31	1.10	1.10	1.10
12	1.10	1.31	1.20	1.20	1.30
13	1.20	1.33	1.22	1.30	1.28
14	1.25	1.35	1.30	1.25	1.37
15	1.15	1.27	1.21	1.30	1.34
16	1.10	1.34	1.18	1.30	1.29
17	1.45	1.53	1.56	1.35	1.34
18	0.90	1.09	0.95	1.25	1.20
19	0.95	1.18	1.05	1.28	1.18
20	1.08	1.21	1.15	1.31	1.31
21	0.95	1.21	1.01	1.31	1.26
22	1.08	1.23	1.08	1.25	1.28
23	1.04	1.18	1.08	1.29	1.29
24	1.21	1.31	1.21	1.31	1.36
25	1.15	1.32	1.15	1.32	1.36
26	1.08	1.28	1.17	1.37	1.33
27	1.15	1.33	1.10	1.25	1.28
28	0.93	1.15	0.93	1.15	1.12
29	1.05	1.25	1.04	1.20	1.29
30	1.28	1.39	1.23	1.34	1.34
31	1.17	1.39	1.23	1.29	1.32
32	1.19	1.28	1.39	1.34	1.30
33	1.20	1.26	1.16	1.31	1.40
34	1.20	1.31	1.20	1.42	1.35
35	1.15	1.31	1.20	1.32	1.35
36	1.23	1.34	1.23	1.29	1.32
37	1.20	1.38	1.18	1.36	1.40
38	1.22	1.46	1.36	1.27	1.36
39	1.25	1.41	1.16	1.32	1.35
40	1.25	1.40	1.25	1.34	1.44
41	1.20	1.26	1.16	1.36	1.36
SD[a]	0.11	0.09	0.12	0.08	0.08
Standard	3.33[b]	3.33[b]	3.33[b]	2.64[c]	2.64[c]

[a] Standard deviation.
[b] Ciprofloxacin.
[c] Fluconazole.

antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *C. albicans* and *A. niger* with pMIC values 1.45, 1.53, 1.56, 1.35 and 1.34, respectively. Compounds **17**, **30**, **39** and **40** were found to be more active against *S. aureus* having $pMIC_{sa}$ value more than 1.24, than other synthesized veratric acid derivatives. Compounds **17**, **38**, **39**, **40**, **30** and **31** were found to be more active against *B. subtilis* having $pMIC_{bs}$ more than 1.38, than other compounds. Compounds **17**, **32** and **38** were found to be more active against *E. coli* having $pMIC_{ec}$ more than 1.35, in comparison to other synthesized veratric acid derivatives. Compounds **34**, **10**, **26** and **37** were found to be more active against *C. albicans* having $pMIC_{ca}$ more than 1.35. Compounds **40**, **33** and **37** were more active with $pMIC_{an} > 1.39$ when compared to the antifungal activity of other compounds against *A. niger*.

4.2. Quantitative structure activity relationship studies

In order to understand the experimental antimicrobial data on theoretical basis, we established a quantitative structure activity relationship (QSAR) between the in vitro antimicrobial activity of 41 veratric acid derivatives and descriptors coding for lipophilic, electronic, steric and topological properties of the molecules under consideration using the linear free energy relationship model (LFER) described by Hansch and Fujita [10]. Biological activity data determined as MIC values were first transformed into pMIC values on molar basis, which were used as dependent variables in QSAR study

and are listed in Table 4. The different molecular descriptors (independent variables) calculated for the present study are logarithm of octanol–water partition coefficient (log *P*), molar refractivity (MR), Kier's molecular connectivity ($^0\chi$, $^0\chi^v$, $^1\chi$, $^1\chi^v$, χ^2, $^2\chi^v$) and shape (κ_1, $\kappa\alpha_1$) topological indices, Randic topological index (*R*), Balaban topological index (*J*), Wiener topological index (*W*), total energy (Te), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment (μ), electronic energy (Ele.E), nuclear energy (Nu.E) and molecular surface area (SA) [26–31]. The values of selected molecular descriptors of veratric acid derivatives used in linear regression analysis are depicted in Table 5. Table 6 presents the correlation matrix of the antimicrobial activity and Table 7 represents the correlation of different molecular descriptors with antimicrobial activity of veratric acid derivatives.

5. Discussion

5.1. Antimicrobial evaluation

From the observed antimicrobial results of veratric acid derivatives, some structure activity relationship (SAR) can be derived as follows:

1. The presence of electron withdrawing groups like Cl, NO_2, and Br improved the antibacterial activity of veratric acid derivatives which is evidenced by the antibacterial data of compounds **17**, **30**, **32**, **38**, and **40** in Table 4 against different representative bacteria. The role of electron withdrawing group in improving the antimicrobial activities is supported by the studies of Sharma et al. [32].

Table 5
Values of selected descriptors of veratric acid derivatives used in LR analysis

Compound	Log *P*	MR	$^0\chi$	$^1\chi$	$^1\chi^v$	$^2\chi$	$^2\chi^v$	*W*	Te
1	1.24	45.74	9.84	6.19	3.64	5.15	2.37	246.00	−2571.42
2	1.27	50.51	10.55	6.72	4.03	5.30	2.56	307.00	−2726.66
3	1.61	55.26	11.26	7.22	4.62	5.68	2.79	382.00	−2882.45
4	2.08	59.78	11.97	7.72	5.12	6.03	3.21	472.00	−3038.28
5	2.03	59.68	12.13	7.58	5.01	6.52	3.52	459.00	−3038.16
6	2.01	59.67	11.97	7.72	4.73	6.03	2.96	472.00	−3009.57
7	2.48	64.39	12.67	8.22	5.62	6.38	3.56	578.00	−3194.11
8	2.49	64.26	12.84	8.08	5.47	6.86	4.06	564.00	−3193.97
9	2.50	64.20	12.84	8.12	5.55	6.64	3.69	552.00	−3193.96
10	2.11	64.32	13.05	7.87	5.33	7.65	4.57	538.00	−3193.73
11	2.88	68.99	13.38	8.72	6.12	6.74	3.91	701.00	−3349.95
12	2.81	68.93	13.54	8.58	5.97	7.21	4.39	686.00	−3349.84
13	3.27	73.59	14.09	9.22	6.62	7.09	4.27	842.00	−3505.78
14	3.67	78.19	14.79	9.72	7.12	7.44	4.62	1002.00	−3661.62
15	2.95	70.29	13.66	9.24	5.74	7.63	3.77	756.00	−3393.29
16	3.05	75.12	14.37	9.74	6.17	7.97	4.13	910.00	−3549.38
17	3.04	84.21	16.23	11.22	7.01	9.52	4.79	1238.00	−3997.55
18	0.37	47.57	9.84	6.19	3.71	5.15	2.43	246.00	−2471.44
19	0.62	52.46	10.55	6.72	4.17	5.30	2.65	307.00	−2626.77
20	0.87	57.36	11.42	7.10	4.53	6.01	3.27	370.00	−2782.05
21	0.96	57.21	11.26	7.22	4.73	5.68	2.93	382.00	−2782.59
22	1.43	61.73	11.97	7.72	5.23	6.03	3.33	472.00	−2938.41
23	0.18	58.75	11.97	7.72	4.84	6.03	3.05	472.00	−3103.24
24	−0.02	69.94	14.25	9.17	5.91	7.11	3.81	734.00	−3734.94
25	2.40	77.07	14.37	9.74	6.28	7.97	4.24	910.00	−3449.60
26	0.53	66.43	12.96	8.76	5.85	7.17	3.97	621.00	−3386.32
27	1.59	69.50	12.96	8.76	6.27	7.17	4.42	621.00	−3221.97
28	0.36	52.05	10.55	6.72	3.96	5.30	2.54	307.00	−2691.27
29	2.30	72.24	13.66	9.24	5.83	7.63	3.89	756.00	−3293.88
30	2.82	77.04	14.54	9.65	6.34	8.14	4.44	858.00	−3654.00
31	2.82	77.04	14.54	9.63	6.34	8.26	4.51	871.00	−3653.97
32	2.82	77.04	14.54	9.63	6.34	8.25	4.50	884.00	−3654.00
33	2.77	77.28	14.54	9.65	6.24	8.14	4.34	858.00	−3449.73
34	2.77	77.28	14.54	9.63	6.24	8.26	4.39	871.00	−3449.75
35	2.77	77.28	14.54	9.63	6.24	8.25	4.39	884.00	−3449.75
36	2.05	78.70	15.24	10.19	6.36	8.34	4.22	980.00	−3769.74
37	2.05	78.70	15.24	10.17	6.35	8.42	4.25	1032.00	−3769.72
38	2.26	79.56	16.11	10.56	6.33	9.08	4.30	1104.00	−4124.81
39	2.26	79.56	16.11	10.55	6.33	9.15	4.33	1182.00	−4124.81
40	3.09	79.86	14.54	9.63	6.74	8.25	4.97	884.00	−3633.51
41	2.02	73.93	14.54	9.63	5.96	8.26	4.07	871.00	−3614.51

Table 6
Correlation matrix for the antibacterial activity of veratric acid derivatives against *S. aureus*

	$pMIC_{sa}$	Log *P*	MR	$^0\chi$	$^1\chi$	$^1\chi^v$	$^2\chi$	$^2\chi^v$	*R*	*W*	Te
$pMIC_{sa}$	1.000										
Log *P*	0.590	1.000									
MR	0.882	0.696	1.000								
$^0\chi$	0.891	0.649	0.981	1.000							
$^1\chi$	0.890	0.640	0.984	0.988	1.000						
$^1\chi^v$	0.865	0.726	0.967	0.937	0.938	1.000					
$^2\chi$	0.867	0.630	0.957	0.969	0.963	0.882	1.000				
$^2\chi^v$	0.834	0.714	0.928	0.898	0.876	0.945	0.902	1.000			
R	0.890	0.640	0.984	0.988	1.000	0.938	0.963	0.876	1.000		
W	0.892	0.659	0.971	0.985	0.989	0.919	0.957	0.858	0.989	1.000	
Te	−0.882	−0.574	−0.941	−0.981	−0.967	−0.905	−0.938	−0.853	−0.967	−0.964	1.000

2. In contrary to the antibacterial activity, the presence of electron donating substituents like OCH_3, R groups (compounds **10**, **26**, **33**, **34** and **37** in Table 4) improved the antifungal activity. This indicates that there may be a difference in the structural requirements for antifungal activity of synthesized veratric acid derivatives against *C. albicans* and *A. niger*. The different structural requirements for activity against different microorganisms are similar to the results observed by Sortino et al. [33].
3. In general the amides and anilides of veratric acid have more antimicrobial activity than its esters. The exceptionally high antibacterial activity shown by compound **17**, i.e. 8-hydroxyquinoline ester of veratric acid may be due to the presence of fused heterocyclic ring in its structure. Further, the anilides with electron withdrawing group were generally more active than simple amides which may be attributed to the presence of aromatic ring in case of anilides. The positive contribution of aromatic ring may be due to the involvement of aromatic ring in enhancing the binding of molecules with the target.

The above facts are summarized in Fig. 1.

Table 7
Correlation of different molecular descriptors with antimicrobial activity of veratric acid derivatives

	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
Log *P*	0.590	0.542	0.583	0.110	0.335
MR	0.882	0.800	0.733	0.447	0.766
$^0\chi$	0.891	0.813	0.751	0.445	0.746
$^0\chi^v$	0.878	0.771	0.750	0.442	0.742
$^1\chi$	0.890	0.827	0.749	0.428	0.740
$^1\chi^v$	0.865	0.770	0.732	0.389	0.705
$^2\chi$	0.867	0.804	0.734	0.533	0.768
$^2\chi^v$	0.834	0.731	0.707	0.492	0.741
R	0.890	0.827	0.749	0.428	0.740
Te	−0.882	−0.826	−0.758	−0.410	−0.706
W	0.892	0.845	0.752	0.418	0.721
κ_1	0.860	0.769	0.728	0.366	0.698
$\kappa\alpha_1$	0.839	0.730	0.718	0.333	0.660

5.2. QSAR analysis

In the present work a set of veratric acid derivatives consisting of 41 molecules were used for linear regression model generation. The reference drugs were not included in model generation as they belong to a different structural series. Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity of veratric acid derivatives against *S. aureus* is presented in Table 6. The high interrelationship was observed between *W* and *R* ($r = 0.989$), $^1\chi$ and $^0\chi$ ($r = 0.988$), *R* and $^0\chi$ ($r = 0.988$) and low interrelationship was observed between Te and log *P* ($r = -0.574$).

The correlation of different parameters with antimicrobial activity of veratric acid derivatives is presented in Table 7. The above table indicated the predominance of topological parameter (*W*) in describing the antimicrobial activity of veratric acid derivatives against *S. aureus* [Eq. (1)].

The linear regression equations developed using the highly correlated topological indices are reported in Eqs. (1)–(5) together with statistical parameters of regression. Apart from these equations, i.e. Eqs. (1)–(5), the other statistically significant equations derived for antimicrobial activity of veratric acid derivatives are presented in Table 8. Further, it is important to note that all these models were developed by using the entire set ($n = 41$), since no outliers were identified.

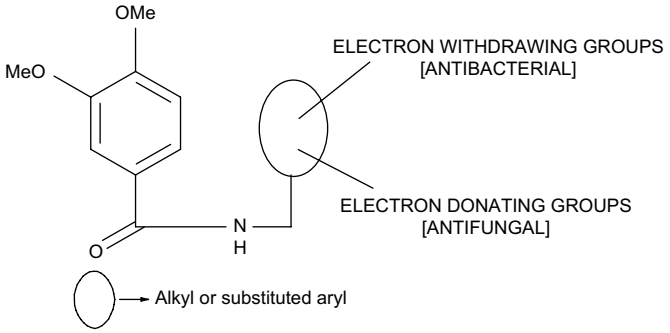


Fig. 1. SAR of antimicrobial activity of synthesized veratric acid derivatives.

Table 8
Other statistically significant QSAR models for antimicrobial activity of veratric acid derivatives

S. no.	QSAR model (pMIC)	n	r	r^2_{cv}	F	S
B. subtilis						
1	0.059 (±0.006)$^1\chi$ + 0.765 (±0.056)	41	0.827	0.631	84.701	0.053
2	0.059 (±0.006)R + 0.765 (±0.056)	41	0.827	0.631	84.701	0.053
3	−0.0002 (±0.000)Te + 0.670 (±0.067)	41	0.826	0.636	84.040	0.053
4	0.044 (± 0.0005)$^0\chi$ + 0.698(±0.067)	41	0.813	0.519	76.03	0.055
S. aureus						
5	0.058 (±0.005)$^0\chi$ + 0.346 (±0.064)	41	0.891	0.763	150.25	0.052
6	0.077 (± 0.006)$^1\chi$ + 0.447 (±0.056)	41	0.890	0.761	148.33	0.052
7	0.077 (± 0.006)R + 0.447 (±0.056)	41	0.890	0.762	148.33	0.052
8	−0.0002 (± 0.000)Te + 0.329 (±0.068)	41	0.882	0.745	137.21	0.054
E. coli						
9	0.00033 (±0.000)W + 0.934 (±0.034)	41	0.752	0.494	50.61	0.078
10	0.51 (±0.007)$^0\chi$ + 0.483 (±0.096)	41	0.751	0.502	50.57	0.078
11	0.061 (±0.009)$^0\chi^v$ + 0.51 (±0.093)	41	0.750	0.508	50.18	0.078
12	0.068 (± 0.010)$^1\chi$ + 0.574 (±0.084)	41	0.749	0.496	49.80	0.078
C. albicans						
13	0.052 (±0.015)$^2\chi^v$ + 1.079 (±0.057)	41	0.492	0.174	12.48	0.067
14	0.0033 (±0.001)MR + 1.053 (±0.073)	41	0.447	0.133	9.72	0.069
15	0.020 (±0.006)$^0\chi$ + 1.016 (±0.085)	41	0.445	0.131	9.62	0.069
16	0.023 (±0.008)$^0\chi^v$ + 1.027 (±0.082)	41	0.442	0.128	9.50	0.069
A. niger						
17	0.0063 (±0.001)MR + 0.856 (±0.058)	41	0.766	0.551	55.31	0.055
18	0.037 (±0.005)$^0\chi$ + 0.797 (±0.070)	41	0.746	0.517	48.96	0.057
19	0.043 (±0.006)$^0\chi^v$ + 0.817 (±0.0068)	41	0.742	0.513	47.88	0.057
20	0.087 (±0.013)$^2\chi^v$ + 0.951 (±0.491)	41	0.741	0.512	47.59	0.057

The quality of the models is indicated by the following parameters: r − correlation coefficient; F − Fisher's statistics; s − standard error of estimation; and r^2_{cv} − cross-validated r^2 obtained by 'leave one out' (LOO) method.

QSAR model for antibacterial activity against *S. aureus*

$$\mathrm{pMIC_{sa}} = 0.00038(\pm 0.000)W + 0.860(\pm 0.022)$$
$$n = 41 \quad r = 0.892 \quad r^2_{cv} = 0.766 \quad s = 0.052 \quad F = 151.22 \qquad (1)$$

QSAR model for antibacterial activity against *B. subtilis*

$$\mathrm{pMIC_{bs}} = 0.000293(\pm 0.000)W + 1.077(\pm 0.022)$$
$$n = 41 \quad r = 0.845 \quad r^2_{cv} = 0.673 \quad s = 0.050 \quad F = 97.705 \qquad (2)$$

QSAR model for antibacterial activity against *E. coli*

$$\mathrm{pMIC_{ec}} = -0.00021(\pm 0.000)\mathrm{Te} + 0.456(\pm 0.098)$$
$$n = 41 \quad r = 0.758 \quad r^2_{cv} = 0.508 \quad s = 0.077 \quad F = 52.62 \qquad (3)$$

QSAR model for antifungal activity against *C. albicans*

$$\mathrm{pMIC_{ca}} = 0.034(\pm 0.009)^2\chi + 1.037(\pm 0.062)$$
$$n = 41 \quad r = 0.533 \quad r^2_{cv} = 0.219 \quad s = 0.066 \quad F = 15.47 \qquad (4)$$

QSAR model for antifungal activity against *A. niger*

$$\mathrm{pMIC_{an}} = 0.054(\pm 0.007)^2\chi + 0.898(\pm 0.052)$$
$$n = 41 \quad r = 0.768 \quad r^2_{cv} = 0.549 \quad s = 0.055 \quad F = 56.08 \qquad (5)$$

The coefficient of W in the mono-parametric model in Eq. (1) is positive indicating thereby that the antibacterial activity of veratric acid derivatives against *S. aureus* is directly proportional to the magnitude of W. The antibacterial activity increases with increase in magnitude of W. This is evidenced by the values of W in Table 5. The values of W for compounds **17** and **39** are 1238.00 and 1182.00 (Table 5), respectively, which are higher than the W values of other compounds and thus make them to be the most effective one against *S. aureus* with $\mathrm{pMIC_{sa}}$ values of 1.45 and 1.25, respectively (Table 4). Similarly, compounds **1** and **18** having minimum W values (246.0 and 246.0, Table 5) have minimum activity against *S. aureus* with $\mathrm{pMIC_{sa}}$ values of 1.01 and 0.90, respectively (Table 4). Similar trend was observed in case of *B. subtilis*, *E. coli*, *C. albicans* and *A. niger* with W, Te, $^2\chi$ and $^2\chi$, respectively.

The topological index, $^2\chi$, signifies the degree of branching, connectivity of atoms and the unsaturation in the molecule which accounts for variation in the activity [34]. The other significant parameter Wiener index (W) was introduced by Wiener [30] to demonstrate the correlations between the physicochemical properties of organic compounds and the topological structure of their molecular graphs in terms of sum of distances between any two carbon atoms in the molecule, in terms of carbon−carbon bonds. The total energy (Te) calculated by semiempirical methods can be used as a measure of non-specific interactions of a drug with its target site i.e. the total energies of the protonated and neutral forms of the molecule, can be considered as a good measure of the strength of

hydrogen bonds (the higher the energy, the stronger the bond) and can be used to determine the correct localization of the most favorable hydrogen bond acceptor site [35].

In order to confirm our results we have predicted the activities of veratric acid derivatives using the model expressed by Eqs. (1)–(5) and compared them with the observed values (Figs. 2–6), which indicated that the values are close to each other.

The cross-validation of the models was also done by leave one out (LOO) technique [36]. The cross-validated correlation coefficient ($r^2_{cv} > 0.5$) values obtained for the best QSAR models indicated their reliability in predicting the antimicrobial activity of veratric acid derivatives. In case of *C. albicans* the r^2_{cv} value is less than 0.5, which shows that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha [37] who have recently reported that the only way to estimate the true predictive power of a model is to test their ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other, the QSAR model for *C. albicans* [Eq. (4)] is a valid one.

Even though the sample size and the 'Rule of Thumb' [19] allowed us to go for the development of multi-parametric model in multiple linear regression analysis, the high interrelationship among the parameters restricted us for mono-parametric model. The multi-colinearity occurs when two independent variables are correlated with each other. One should note that the change in signs of the coefficients, a change in the values of previous coefficient, change of significant variable into insignificant one or an increase in standard error of the estimate on addition of an additional parameter to the model are indications of high interrelationship among descriptors [13].

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of antimicrobial activities of the synthesized compounds is within 1 order of magnitude. But it is important to note that the predictability of the QSAR models developed in the present study is high, which is evidenced by the low residual values (Figs. 2–6). This is in accordance with the results suggested by Bajaj et al. [38], who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range. Further, recent literature reveals that the QSAR has been applied to describe the relationship between the narrow range of biological activity and the physicochemical properties of the molecules [16,39–41]. When biological activity data lie in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies [16,21]. The minimum standard deviation (Table 4) observed in the antimicrobial activity data justifies its use in QSAR studies.

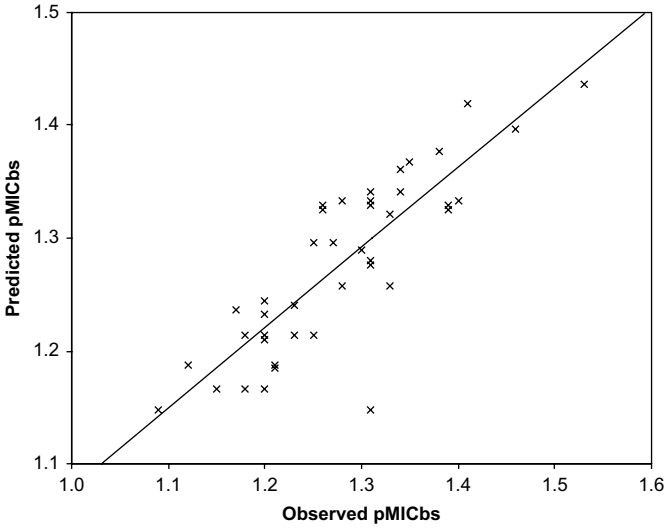


Fig. 3. Plot of predicted $pMIC_{bs}$ values against experimental $pMIC_{bs}$ values for the linear regression developed model by Eq. (2).

6. Conclusion

Summarizing, a number of veratric acid derivatives have been synthesized in efficient yields. The synthesized compounds

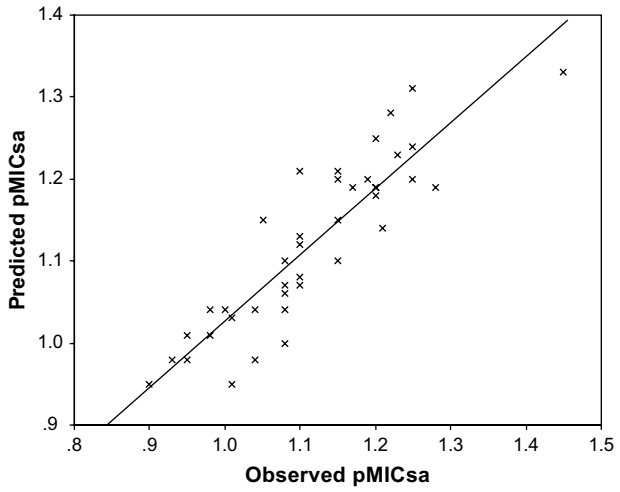


Fig. 2. Plot of predicted $pMIC_{sa}$ values against experimental $pMIC_{sa}$ values for the linear regression developed model by Eq. (1).

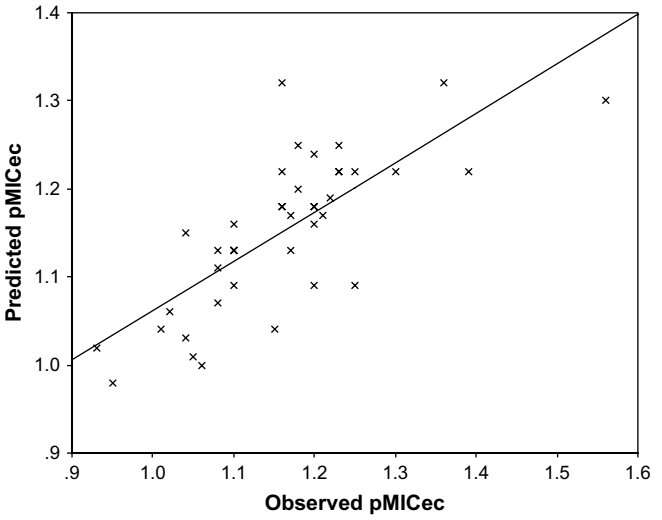


Fig. 4. Plot of predicted $pMIC_{ec}$ values against experimental $pMIC_{ec}$ values for the linear regression developed model by Eq. (3).

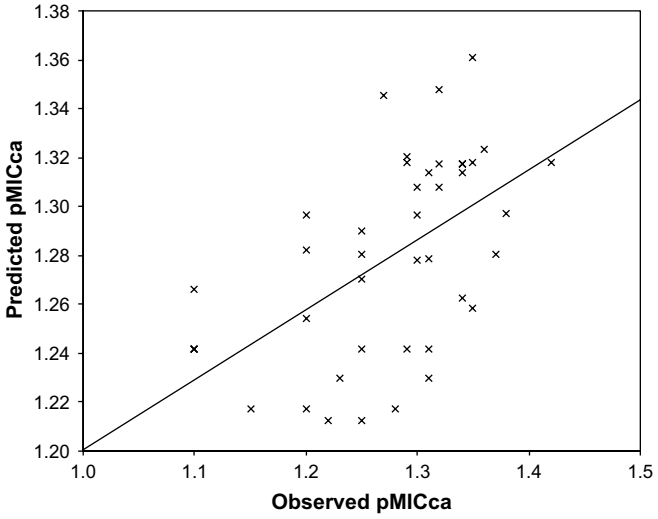


Fig. 5. Plot of predicted $pMIC_{ca}$ values against experimental $pMIC_{ca}$ values for the linear regression developed model by Eq. (4).

exhibited good in vitro antibacterial and antifungal profiles. The trend of antimicrobial studies showed that these title compounds were highly effective against Gram-positive bacteria. Moreover, quantitative structure activity relationship study revealed that the antimicrobial activity of these synthesized derivatives against microorganisms under test is mainly governed by the topological parameters, namely, Wiener topological index (W) and the second order molecular connectivity index ($^2\chi$).

7. Experimental

Melting points in degree Celsius were determined with Elico melting point apparatus and are uncorrected. The FTIR spectra were recorded in KBr pellets on Perkin Elmer IR spectrophotometer. ^{1}H NMR was recorded on Bruker Avance II 400 NMR spectrophotometer using $CDCl_3$ as a solvent and TMS as an internal standard (chemical shift in δ, ppm). The purity of compounds was checked by thin layer chromatography (TLC) on silica gel G plates. The spots were detected by exposure to iodine vapours. The elemental analysis of compounds was performed on Vario EL instrument.

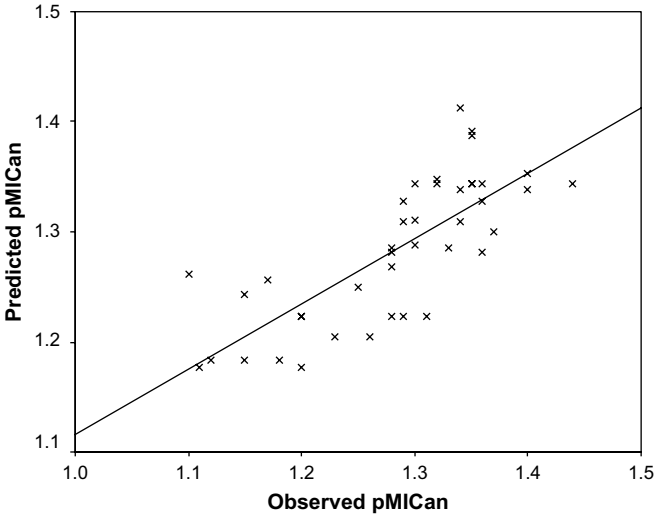


Fig. 6. Plot of predicted $pMIC_{an}$ values against experimental $pMIC_{an}$ values for the linear regression developed model by Eq. (5).

7.1. General procedure for preparation of esters of veratric acid derivatives (**2–17**)

A mixture of veratric acid (0.08 mol) and appropriate alcohol (0.74 mol) was heated under reflux in the presence of sulphuric acid (0.2 ml) till the completion of reaction. Once the reaction has been completed, the reaction mixture was added to 200 ml ice cold water and the resultant ester was extracted with ether (50 ml). The ether layer on evaporation yielded the crude ester, which was then recrystallized from alcohol.

7.2. General procedure for synthesis of amides/anilides (**18–28**/**29–41**)

The acid chloride of veratric acid was prepared by the reaction of veratric acid with thionyl chloride. The solution of corresponding amine (0.1 mol)/aniline (0.1 mol) in ether (50 ml) was added drop-wise to a solution of acid chloride (0.1 mol) in ether (50 ml) maintained at 0–10 °C at room temperature. The solution was stirred for 30 min and the precipitated amide was separated by filtration. The crude amide was recrystallized from alcohol. In case of anilides, the precipitates of crude anilide were treated with water and the ether layer was separated, washed successively with 5% hydrochloric acid, 4% sodium carbonate and water to remove residual aniline. Evaporation of ether layer yielded anilides, which were then recrystallized from alcohol.

7.3. Antimicrobial evaluation

7.3.1. Antibacterial assay

A 24-h fresh culture was obtained by inoculation of respective bacteria in double strength nutrient broth − I.P. followed by incubation at 37 ± 1 °C. The stock solution of synthesized veratric acid derivatives was serially diluted in tube containing 1 ml of sterile double strength nutrient broth − I.P. to get a concentration of 50–3.125 μg/ml and then inoculated with 100 μl of suspension (with a count of 10^5 cfu/ml) of respective microorganisms (Gram-positive *S. aureus MTCC 1430*, *B. subtilis MTCC 2423*, Gram-negative *E. coli MTCC 739*) in sterile saline. The inoculated tubes were incubated at 37 ± 1 °C for 24 h and minimum inhibitory concentrations (MICs) were determined. From the observed MIC values, the exact MIC values were determined by making suitable dilution of the stock solution.

7.3.2. Antifungal assay

The antifungal activity of synthesized veratric acid derivatives against the fungal species *C. albicans MTCC 227* and *A. niger MTCC 2425* was determined by serial dilution method similar to antibacterial assay (Section 7.3.1) using Sabouraud

dextrose broth – I.P. The inoculated tubes were incubated at 37 ± 1 °C and 25 ± 1 °C for a period of 2 and 7 days in case of *C. albicans* and *A. niger*, respectively.

7.4. QSAR studies

Data set is the set of molecules whose biological activity is regressed with its molecular descriptor values. Our data set consisted of 41 veratric acid analogs, synthesized and biologically evaluated, for antimicrobial activity by tube dilution method. Descriptor is any molecular property which is characteristic of a molecule and can be utilized to determine new QSAR. The number of descriptors selected for the present study fell into four categories, viz. electronic, steric, lipophilic, and topological [28–33] (Table 5). The structure of veratric acid derivatives was optimized by energy minimization. The lowest energy structure was used for each molecule to calculate the physicochemical properties using TSAR 3.3 software for Windows [42]. Further, the regression analysis was performed using the SPSS software package [43].

7.4.1. Cross-validation

The predictive powers of the equations were validated by leave one out (LOO) cross-validation method [44], where a model is built with $N-1$ compounds and Nth compound is predicted. Each compound is left out of the model derivation and predicted in turn. An indication of the performance is obtained from cross-validated (or predictive q^2) method which is defined as

$$q^2 = 1 - \sum \left(Y_{\text{predicted}} - Y_{\text{actual}}\right)^2 / \sum \left(Y_{\text{actual}} - Y_{\text{mean}}\right)^2$$

where $Y_{\text{predicted}}$, Y_{actual} and Y_{mean} are the predicted, actual and mean values of target property (pMIC), respectively. $\sum (Y_{\text{predicted}} - Y_{\text{actual}})^2$ is the predictive residual error sum of squares.

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